



Available Online at EScience Press

## Plant Protection

 ISSN: 2617-1287 (Online), 2617-1279 (Print)  
<http://esciencepress.net/journals/PP>

### A COMPREHENSIVE NOTE ON *TRICHODERMA* AS A POTENTIAL BIOCONTROL AGENT AGAINST SOIL BORNE FUNGAL PATHOGENS: A REVIEW

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#### ARTICLE INFO

##### Article history

Received: 23<sup>rd</sup> October, 2021

Revised: 28<sup>th</sup> November, 2021

Accepted: 19<sup>th</sup> December, 2021

##### Keywords

*Trichoderma* spp.

Soil born fungi

Synthetic pesticides

Antagonistic microbes

Bio-fungicide formulation

#### ABSTRACT

The extensive use of synthetic pesticides has a harmful impact on the environment, plants and animal health. It is a big challenge for all farming systems to develop novel approaches, which are eco-friendly and improve food quality. As compared to synthetic pesticides, the use of beneficial microbes is the best option to maintain the environmental condition because they are cost-effective and ecofriendly. In the recent era, biological antagonistic microorganisms (*Trichoderma* spp.) are the best approach to control the soil-borne fungal pathogens associated with plant roots of agriculturally important crops. Due to fast growth and rhizospheric colonization ability, this fungus competes with other pathogenic soil-borne fungi by producing different metabolites (volatile and non-volatile). *Trichoderma* protected the plants from pathogenic fungi through mycoparasitic and antibiosis capability. Furthermore, it has the ability to improve plant health by inducing SAR (Systemic acquired resistance), ISR (Induce systemic resistance), producing antifungal enzymes ( $\alpha$ -1, 3-glucanases, *Trichoderma* ketone, and trichodermin) and antioxidant enzymes that strengthen the immune system by increasing activities of guaiacol peroxidase (GPX), ascorbate peroxidase (APX), catalase (CAT) and superoxide dismutase (SOD) after pathogen attack. Development of bio-fungicide formulation by using the spore of *Trichoderma* species (*T. harzianum*, *T. viride*, and *T. virens*) are most effective against soil-borne pathogenic fungi at different concentrations and temperatures. This review article has significantly focused on gathering and summarizing the most recent literature to highlight the visible production and application of *Trichoderma* as a biomonitoring and biocontrol agent in plant diseases management program.

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#### INTRODUCTION

The imperfect and green spore-producing filamentous

fungi, *Trichoderma* species, are well-known bio-control agents against soil-borne fungal pathogens (Baron et al.,

2019; Waghunde et al., 2016). This fungus has been found in all types of soils, on plant roots, and in decaying woods (Chohan et al., 2019; Puyam, 2016). In soil, the fungus *Trichoderma* takes part in the absorption of nutrients and decomposition of plant residues (Govarthanan et al., 2018; Sharma et al., 2012). Various strains of this fungus were associated with diseases of plants, while a few violent strains attack mushroom crops and cause a significant loss. *Trichoderma* has great importance in biotechnology industries due to the production of cellulase (Tiwari et al., 2013). Most *Trichoderma* species have antagonistic ability to control different fungal plant pathogens e.g. *Alternaria*, *Pythium*, *Sclerotinia*, *Fusarium*, and *Botrytis* (Srivastava et al., 2016; Win et al., 2021). *Trichoderma* has different modes of action to control other fungal plant pathogens under *in vitro* and *in vivo* conditions. It produces antifungal metabolic compounds. The other mechanisms include mycoparasitism, antibiosis, competition to acquire nutrients and space, cell wall degrading enzymes (proteases, chitinases, and glucanases), systemic acquired resistance, plant growth-promoting hormones, and decrease activities of soil fungal pathogens (Bader et al., 2020; Gajera et al., 2013; Harman, 2000; Harman et al., 2004a). *Trichoderma harzianum*, *T. reesei*, *T. viride*, *T. hamatum*, *T. atroviride*, and *T. longibrachiatum* are most commonly used as biocontrol agents (Chohan et al., 2019; Rai et al., 2016; Sallam et al., 2019; Srivastava et al., 2016; Srivastava et al., 2014). Genes found in living organisms perform a specific function in the body of an organism. It encodes all information of DNA and shows different expressions to control the diseases. These genes play a vital role in biological controls by activating the signals and secretion of enzymes. A few genes of *Trichoderma* have been used to provide genetic resistance in stresses (biotic and abiotic) such as drought, salt, and heat (Mastouri et al., 2012; Montero-Barrientos et al., 2010; Poveda et al., 2020; Zaidi et al., 2014). Amongst all the *Trichoderma* species, *Trichoderma harzianum* is the most effective biocontrol agent (Kexiang et al., 2002). In factories, it was used to produce a lytic enzyme, and this lytic enzyme is applied as biocontrol mediators (Monfil and Casas-Flores, 2014). The use of this fungus in bioremediation is limited but nowadays due to its antagonistic ability, the fungus is used as biotransform conservation of noxious waste (Su et al., 2011; Zafra and Cortés-Espinosa, 2015). Due to two different physiological and morphological stages of this fungus, the nomenclature of *Trichoderma* is complex.

Hypocrea is the generic name of the teleomorph stage; on the other hand, the anamorphic stage is *Trichoderma*. The genus of *Trichoderma* is collectively Hypocrea/*Trichoderma*. Proper classification is necessary to study the ecology of this fungus (Srivastava et al., 2014). Antagonistic activity of *Trichoderma* species was not only for soil-borne fungi but it was also applied against foliage fungal pathogens. It produced volatile and non-volatile enzymatic compounds to inhibit or reduce the growth of foliage fungal pathogens such as *Botrytis cinerea* (Amin and Razdan, 2010).

#### **Taxonomic History of *Trichoderma***

In the 19<sup>th</sup> century, the famous genus of fungal plant microbes *Trichoderma* was reported. The link was established with sexual stage Hypocrea, accepted in 1865 by Tulasne brothers, so taxonomy remains opaque until recent decades (Bisby, 1939). Morphological differences attributed to single species *T. viride*. The scientist, Rifai, made thoughtful attempts for species aggregate. Nine taxa that were not biological entities after association with teleomorph distinguished were also discussed (Rifai, 1969). The network of teleomorphs were recognized in form of ascospore isolates discussed by Dingley (Bissett, 1991; Dingley, 1957; Domsch, 1980). The prosperity of teleomorph debated in Japan along with cultural and anamorph types described by Doi (1972) unluckily no one culture remain preserved.

Further, no struggle brought about by Doi and Doi related to the diversity of anamorph (Doi, 1969). Bissett (1991) studied the morphological details of anamorphs, who discriminates approximately 21 species in the *Pachybasium* group and seven species in the *Longibrachiatum* group, but other sections were not needed to enter into the group. Some taxonomic groups of morphology contain information related to secondary metabolites, due to which diversity is more in the genus (Okuda et al., 1982). In microtiter plates, functional features were noticeable and ultimately gave information related to the identification. A specific sequence of ITS region belongs to molecular information, fingerprinting technique applicable in modern years helps in effective studies at taxonomic level (Anzai et al., 1984; Muthumeenakshi et al., 1994).

#### **Genetic features of *Trichoderma***

*Trichoderma* is a filamentous, haploid nucleus with small genomic size fungi belonging to division Ascomycota. More than 100 filamentous fungi are included in this genus (Druzhinina et al., 2006). These fungus species

have been found in all different types of soils, on plant roots, decaying parts of wood, and other carbon-containing ingredients (Zafra and Cortés-Espinosa, 2015). The genome size of all *Trichoderma* species varies from species to species, and most effective antagonistic species are present with their respective genomic sizes such as 34.1 MB of *T. reesei*, 39MB of *T. virens*, 36.1 MB of *T. atroviride*, 40.98MB of *T. harzianum*, 37.46MB of *T. asperellum*, 32.24MB of *T. Longibrachiatum* and 33.48 of *T. ctrinoviride*. As compared to genomic size, the number of genes in each species is also different (Table 1). The maximum number of genes (14095) are found in *T. harzianum* as compared to *T. asperellum* 12566, *T. virens* 12427, *T. atroviride* 11863, *T. Longibrachiatum* 10792, *T. ctrinoviride* 9397, and *T. reesei* 9129 respectively. These genes performed different functions when they expressed and help in an antagonistic activity. A few

major genes that participate in biological control are tubulins, chitinase, xylanase, protease, and glucanase genes. All these genes play a vital role in cell wall degradation, such as a glycosidic bond is broken down by chitinases gene, and hemicellulose is broken by the xylanases gene (Sharma et al., 2011b). The mating type of filamentous fungi depends on loci genes that are homothallic or heterothallic (MAT 1-1 or MAT 1-2). In the homothallic mating-type, the organisms of this species reproduce themselves but in the heterothallic mating-type required two different partners for mating. Most of the *Trichoderma* species are heterothallic such as MAT 1-2 in *T. reesei*, MAT 1-2 in *T. virens*, MAT 1-2 in *T. atroviride*, MAT 1-2 in *T. harzianum*, MAT 1-1 in *T. asperellum*, MAT 1-1 in *T. Longibrachiatum* and MAT 1-2 in *T. ctrinoviride* (Debuchy et al., 2010; Linke et al., 2015).

Table 1: Identified and reported *Trichoderma* genes and their biocontrol function.

<i>Trichoderma</i> spp.	Gene	Function of genes	References
<i>T. virens</i>	<i>Tvsp-1</i>	By producing serine <i>protease</i> , Used to control <i>Rhizocotonia solani</i> which causes disease in cotton seedling	(Sandhu, 2014)
<i>T. virens</i>	<i>TvGST</i>	Provide tolerance in stress condition	(Dixit et al., 2011)
<i>T. virens</i>	<i>Tac-1</i>	Inhibit the growth of <i>Rhizocotonia solani</i> and <i>Pencilliummultimum</i> by Mycoparasitism mode of action	(Mukherjee et al., 2008)
<i>T. reesei</i>	<i>TrCCD1</i>	Improve conidiophore and hyphae growth of <i>Trichodermareesei</i> in vitro	(Zhong et al., 2009)
<i>T. harzianum</i>	<i>tri5</i>	Inhibit pathogen growth by a synthesis of <i>trichothecene</i> enzymes	(Malmierca et al., 2013)
<i>T. harzianum</i>	<i>ThPG1</i>	By producing cell wall degradation enzyme <i>endopolygalacturonase</i> it inhibit the growth of <i>Penciliu ultimum</i> and <i>Rhizocotonia solani</i>	(Morán-Diez et al., 2009)
<i>T. virens</i>	<i>Tga A-B</i>	Inhibit the growth of <i>Sclerotium rolfsii</i> and <i>Rhizocotonia solani</i>	(Munir et al., 2014; Nicolás et al., 2014)
<i>T. harzianum</i>	<i>Erg-1</i>	This gene produces <i>Epoxidase</i> enzyme which synthesis of ergosterol, it also works as gene silencing	(Cardoza et al., 2006)
<i>T. harzianum</i>	<i>Th-Chit</i>	Produce antifungal compound in transgenic tobacco plant for long term	(de las Mercedes Dana et al., 2006)
<i>T. harzianum</i>	<i>Thkel1</i>	In <i>Arabidopsis thaliana</i> provide tolerance in salt stress	(Hermosa et al.,

		and standardize <i>glucosidase</i> movement	2011)
<i>T. brevicompactum</i>	<i>Tri-5</i>	Produce antifungal <i>Trichodermin</i> against <i>Aspergillus fumigates</i> , <i>Candida albicans</i> , <i>Candidatropicalis</i> and <i>S. cerevisiae</i>	(Özkale, 2017; Tijerino et al., 2011)
<i>T. longibrachiatum</i>	<i>Egl-1.</i>	Inhibit the activities of <i>Pencillium ultimum</i> in the control condition	(Munir et al., 2014)
<i>T. harzianum</i>	<i>Qid-74</i>	Inhibit the growth of <i>Rhizocotonia solani</i> by Mycoparasitism mode of action	(Rosado et al., 2007)
<i>T. atroviride</i>	<i>Taabc2</i>	This gene plays a vital role in ABC (ATP Binding Cassette) this gene has an antifungal role against different soil borne fungal pathogen	(Cortes et al., 1998; Ruocco et al., 2009)
<i>T. asperellum</i>	<i>Sm1</i>	Good antagonistic activities against different pathogens	(Buensanteai et al., 2010)
<i>T. harzianum</i>	<i>Tubulin</i>	This gene has good antagonistic activities against fungal pathogens	(Munir et al., 2014)

### Biology of *Trichoderma* species

As a cosmopolitan, the filamentous fungi *Trichoderma* has found every type of soil in the world. Genetic diversity between the *Trichoderma* species varies according to their environment (Zhang et al., 2007). Different culture media was used for the isolation of *Trichoderma* from soil samples, plant materials, and organic matters. The growth of this fungus on culture media is fast as compared to pathogenic fungal species belonging to other genera. *Trichoderma* specific media (TSM) for the isolation of *Trichoderma* species from soil sample was used (Kale et al., 2018). The growth and multiplication of this fungus are also fast on other culture media named PDA (Potato Dextrose Agar), MA (Malt Agar), and CDA (Czapek Dox Agar) (Ghazanfar et al., 2018b). The spore produced by these filamentous fungi on culture media is a different color and characterized by green thick-walled chlamydospore. Due to the biocontrol feature, "parasitize" this fungus used to control other pathogenic fungal species associated with wilting and root rot diseases. This fungus is also important due to opportunistic plant symbionts and competition with other pathogenic fungi for space and nutrients (Chaverri et al., 2011; Kim et al., 2012).

### Morphological and ecological feature of *Trichoderma* species

The study of morphological character is a primary method for the identification of any pathogen species. Based on the morphological character, the *Trichoderma* species were

identified, but the morphological study is not a good method to understand the diversity between the species. Potato Dextrose Agar Media (PDA) used for the study of morphological character and this fungus grows well at an optimum temperature between 25-30 °C (Latifian et al., 2007; Zhang et al., 2019). Besides, to increase the sporulation of this fungus the other carbon and nitrogen compounds were also added in growth media and after sporulation, the fungus produces green conidia on the PDA plate. These green conidia were used as diagnostic tools for the identification of *Trichoderma* species and the differentiation of *Trichoderma* species from other related and unrelated pathogenic fungus species *Penicillium*, *Aspergillus*, and *Myrothecium* (Alvandia and Hirooka, 2011). On PDA plate, the conidiophores of this fungus appeared after one week of incubation, after one week the conidia and phialides appeared at the tip of the branched hyphae. Normally the ellipsoidal to oblong shapes of conidia were identified but a few *Trichoderma* members showed spherical, subspherical, and flat shape conidia respectively. Morphological study of conidia showed the green, gray, white, and yellow color of conidia on PDA plate of different species of *Trichoderma* collected from soil, decaying wood, and organic sources (Ghazanfar et al., 2018b).

Ecological features of *Trichoderma* species showed that this fungus distributes worldwide in all types of soil, on plants root and decaying bark of plants (Singh et al., 2014). Due to fast-growing saprophyte, it produces

coconut type smell reason behind the coconut smell is 6PP (6-pentyl-2pyrone) volatile compound. As compared to total *Trichoderma* species 3-15% propagules from pasture and forest soil (Brotman et al., 2013). According to their climatic condition, *Trichoderma* species grow and spread well in all types of soil. In the case of *T. harzianum*, this fungus grows best in warm temperatures, but on the other hand, *T. polysporum* and *T. viride* were grown best in a cool climate (Brotman et al., 2013). A few *Trichoderma* species were not reported from Indo-Pak but identified and reported from South Africa such as *T. citrinoviride* reported from South Africa (Zhang et al., 2007). Commonly, the *Trichoderma* has found more in acidic soil (Carreras-Villaseñor et al., 2012).

#### Molecular diagnostic tools for the characterization of *Trichoderma* species

Different molecular diagnostic techniques were used to check the variation between the species of *Trichoderma*. In the start, the protein markers and DNA based markers were applied to identify the species. PCR is a simple technique to make multiple copies of DNA and used for the detection purposes of *Trichoderma*. Based on PCR detection method, AFLP (Amplified Fragment Length Polymorphism), Nested PCR, RFLP (Restriction Fragment Length Polymorphism), MS (Microsatellite markers),

RAPD (Random Amplification of Polymorphic DNA), SCAR (Sequence Characterized Amplified Region) and ISSR (Inter-Simple Sequence Repeat) techniques were applied for molecular characterization of *Trichoderma* (Hassan et al., 2019). Other molecular techniques such as SA (Sequence Analyses) (Sagar et al., 2011), DGGE (Denaturing Gradient Gel Electrophoresis) (Ganuza et al., 2019) and GFE (Green Fluorescent Protein) (Hermosa et al., 2001; Parmar et al., 2015) were also used for molecular characterization. Molecular characterization of two filamentous species named *T. longibrachiatum* and *T. koningiopsis* PCR with ITS primers, SCAR, and ISSR techniques were applied by Hassan et al. (2019). To check the genetic diversity between the species using ITS1 and ITS4 primers during the PCR technique and amplified the 5.8SITS region of few species. Sequencing analyses of two species *T. harzianum* and *T. longibrachiatum* are showing a maximum identity of 99% with an already submitted sequence in GenBank (Fahmi et al., 2016). RT-qPCR technique was done for the gene expression of nine *Trichoderma* spp. collected from soil samples (Saravanakumar and Wang, 2020). The molecular diagnostic PCR based markers used for the detection and differentiation of *Trichoderma* species have been listed in Table 2.

Table 2: Molecular diagnostic PCR based marker used for the detection and differentiation of *Trichoderma* species.

Sr. No.	Sample collection	<i>Trichoderma</i> spp.	Diagnostic technique	References
1	Forest and agriculture soil	<i>T. viride</i> and <i>T. harzianum</i>	ITS-PCR and RFLP	(Chakraborty et al., 2010)
2	substrates of oyster	<i>T. atroviride</i> , <i>T. harzianum</i> , <i>T. virens</i> and <i>T. citrinoviride</i>	PCR, RFLP	(Park et al., 2005)
3	Rhizosphere soil	<i>T. longibrachiatum</i>	ITS-PCR	(Shahid et al., 2013)
4	Rhizosphere soil	<i>T. viride</i>	PCR, ISSR	(Shahid et al., 2014)
5	Rhizosphere soil	<i>T. harzianum</i>	RAPD	(Sharma et al., 2009)
6	Rhizosphere soil	<i>T. viride</i> , <i>T. harzianum</i>	PCR with ITS primers	(Cumagun et al., 2000)
7	Rhizosphere soil	<i>T. harzianum</i> , <i>T. virens</i> , <i>T. viride</i>	RAPD-PCR	(Kredics et al., 2018)
8	Rhizosphere soils	<i>Trichoderma</i> spp.	PCR	(Kasa et al., 2015)
9	Tobacco Rhizosphere soils	<i>T. harzianum</i> , <i>T. viride</i> ,	PCR, RAPD	(Kredics et al., 2018)
10	Tomato rhizosphere soil	<i>Trichoderma</i> spp.	PCR, RAPD	(Rai et al., 2016)
11	Rhizosphere soil	<i>T. harzianum</i> , <i>T. viride</i> ,	RAPD	(Ranga et al., 2017)
12	Rhizosphere soil	<i>T. harzianum</i> , <i>T. atroviride</i>	multiplex PCR	(Oskiera et al., 2017)
13	Soil	<i>T. harzianum</i> T22	Multiplex Q-PCR	(Horn et al., 2016)
14	Soil	<i>T. atroviride</i> Ta040	SCAR and RT-PCR	(Feng et al., 2011)

15	Soil	<i>T. virens</i>	SSR Marker	(Geistlinger et al., 2015)
16	Soil	<i>T. atroviride T1</i>	SCAR and RT-PCR	(Cordier et al., 2007)
17	Rhizosphere soil	<i>T. atroviride</i>	PCR, RAPD	(Skoneczny et al., 2015)
18	Rhizosphere soil	<i>T. harzianum</i>	SCAR marker	(Pérez et al., 2014)
19	Banana rhizosphere	<i>Trichodermaspp.</i>	AFLP and PCR	(Xia et al., 2011)
20	Tomato rhizosphere	<i>T. harzianum</i>	ITS-PCR and ISSR	(Mazrou et al., 2020)

**Secondary metabolite produced by *Trichoderma* species and their functions**

Bioactive secondary metabolite compounds are produced by filamentous fungi *Trichoderma* that showed their vital role in agriculture, food industries, and the medical field (Figure 1). This fungus produces major two types of compounds, volatile and nonvolatile. Volatile compounds are involved in the environmental process and biological controlling process. These volatile compounds are found in the form of ketones, sesquiterpenes, alcohols, lactone, and C8. 6PP (6-pentyl-2H-pyran-2-one) is a volatile compound that improves plant growth at different conditions produced by *Trichoderma* spp. (Salwan et al., 2019; Shah and Afiya, 2019; Shakoor et al., 2015). The non-volatile compounds are also produced by many *Trichoderma* species and these compounds produce biocontrol activates against fungus, bacteria, nematode, and human diseases. Volatile and non-volatile metabolites with their function have been given in Table 3. Nowadays, most secondary

metabolites produced by *Trichoderma* play roles in improving plant growth and inhibit the growth of many pathogenic microbes in a different way. Therefore, this fungus works directly or indirectly to manage the disease and plant health.

**Interaction of *Trichoderma* spp. with plants**

A bundle of microbes has been found in the rhizosphere, those attached directly or indirectly with the plant through a different connection. *Trichoderma* is one of them that attached to the roots of plants by symbiosis and protected the plants from other harmful pathogens. *Trichoderma* is a good biocontrol agent against different soil-borne fungal pathogen and works as a plant growth promoter (Chohan et al., 2019). The beneficial association of this biocontrol agent with plants are found in the form of growth-promoting, increase tolerance during stress condition, phytoalexins accumulation in a plant, accumulation of jasmonic acid and salicylic acid, activate and enhance defense system of plants (Mendoza-Mendoza et al., 2018).

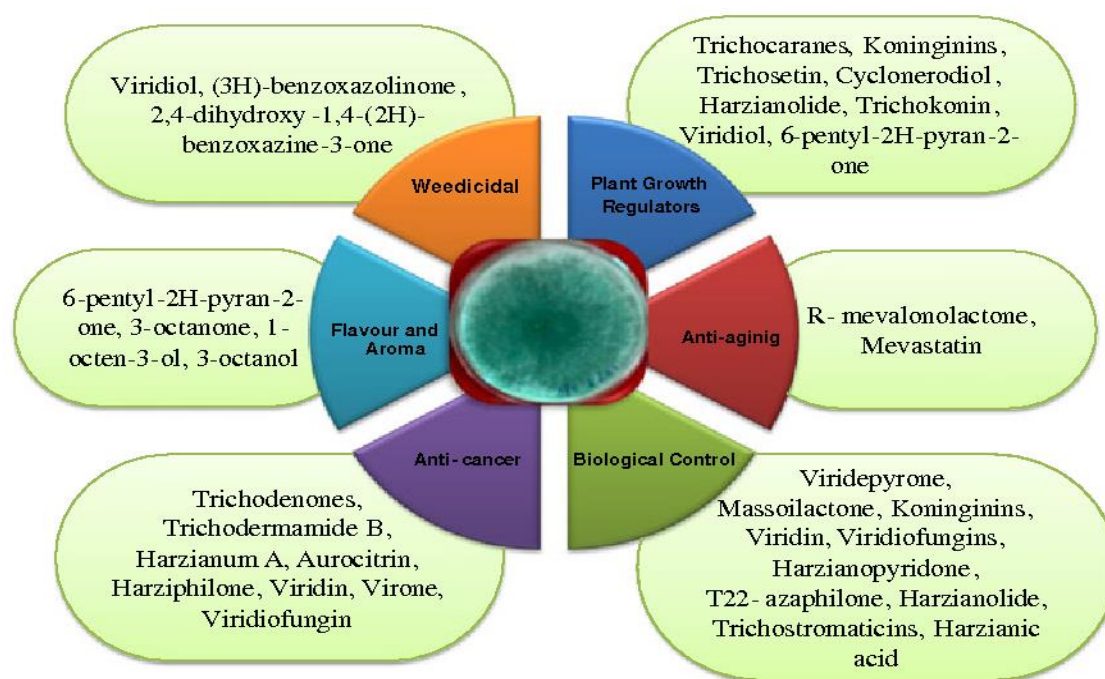


Figure 1: Role of Secondary metabolite produce by *Trichoderma* species in different field.

Table 3: Volatile and non-Volatile secondary metabolites of *Trichoderma* spp. and their function (Lee et al., 2016; Li et al., 2019b).

<i>Trichoderma</i> species	Metabolites (Non-Volatile)	Function of metabolite	<i>Trichoderma</i> species	Metabolites (Volatile)	**Function of metabolites
<i>T. longibrachiatum</i> YM311505	sohironone A	Antifungal	<i>T. asperellum</i> GJS	ethyl 2-methylbutyrate	IPG, RFG
<i>T. asperellum</i> dl-34	3 $\beta$ ,5 $\alpha$ ,,	Antifungal	<i>T. asperellum</i> 02-65	Octadecane	IPG
<i>T. harzianum</i> dl-36	9 $\alpha$ -trihydroxyergosta-7	Antifungal	<i>T. longibrachiatum</i> TR 97	3-methylbutyl propanoate	IPG
<i>Trichoderma</i> sp. YM311505	22-dien- 6-one	Antifungal	<i>T. longibrachiatum</i> CBS	2,4-heptadienal	IPG, RFG
<i>T. virens</i> ITC-4777	Gliotoxin	Antifungal	<i>T. virens</i>	2-butanone	IPG, RFG
<i>T. virens</i> Y13	Trichorenin B	Antifungal	<i>T. stromaticum</i>	3-methyl-1-butanol	IPG, RFG
<i>T. atroviride</i> S361	Catenioblin C	Antifungal	<i>T. pseudokoningii</i>	2-methyl-1-propanol	IPG, RFG
<i>T. harzianum</i> T77	6-pentyl- $\alpha$ -pyrone	Antifungal	<i>T. pseudokoningii</i>	Acetone	IPG,
<i>T. harzianum</i>	Trichodermin	Antifungal	<i>T. virens</i>	Limonene	IPG, RFG
<i>T. virens</i> Y13-3	Chromone	Antifungal	<i>T. pseudokoningii</i>	$\beta$ -caryophyllene	IPG, RFG
<i>T. reesei</i>	Cyclonerodiol	Antifungal	<i>T. longibrachiatum</i>	$\beta$ -farnesene	IPG
<i>T. harzianum</i> T39	Harzianolide	Antifungal	<i>T. longibrachiatum</i>	2-norpinene	IPG
<i>T. viride</i>	Trichodimerol	Antifungal	<i>Trichoderma</i> spp.	$\beta$ -acoradiene	IPG, RFG
<i>T. harzianum</i> T-4	Harzianopyridone	Antifungal	<i>Trichoderma</i> spp.	$\beta$ -cubebene	IPG, RFG
<i>T. harzianum</i> T39	T22azaphilone	Antifungal	<i>Trichoderma</i> spp.	$\beta$ -cedrene	IPG
<i>Trichoderma</i> spp.T3	1,1-dimethylethyl	Antifungal	<i>Trichoderma</i> spp.	$\beta$ -bisabolene	IPG, RFG
<i>T. harzianum</i> T39	T39butenolide	Antifungal	<i>Trichoderma</i> spp.	$\beta$ -himachalene	IPG, RFG
<i>T. harzianum</i>	6-n-pentyl-pyrone	Antifungal	<i>Trichoderma</i> spp.	$\gamma$ -himachalene	IPG, RFG
<i>T. koningii</i>	<i>Trichoderma</i> ketone	Antifungal			
<i>T. koningii</i> T-11	6-pentyl- $\alpha$ -pyranone	Antifungal			

*Trichoderma* spp. suppressor inhibits the growth of other pathogenic fungal pathogens *in vivo* and *in vitro* through different modes of action such as mycoparasitism, antibiosis, competence, induce systemic acquired resistance, induce systemic resistance, and as bio-fungicide. The molecular interaction of *Trichoderma* spp. with the plant is still anonymous, so a few proteins work as effectors and develop interaction between them (Plett et al., 2015). An example of

this protein is TgSWO, synthesized by *T. guizhouense* in cucumber plant, which helps the plant to promote growth and modified the morphology of plant roots (Meng et al., 2020). Due to the presence of sucrose in plant roots, all-fungal pathogens are attracted toward the roots and interconnect with the root by secreting an enzyme that degrades plant cell wall and hydrophobin proteins (Mendoza-Mendoza et al., 2018). *T. harzianum* and *T. virens* suppress or

inhibit the growth of rhizospheric bacteria and pathogenic fungus *Alternaria solani* up to 80% during soil treatment in tomato field (Li et al., 2019a). Pathogenic fungus species present in the soil become the reason for reducing crop yield. To control the pathogenic fungus in soil by chemical a traditional rule, these chemicals nowadays become a great threat due to their bad impact on plant health, animal and human health (Bastakoti et al., 2017).

It is time to develop a beneficial strategy that is cost-effective and environmentally friendly to control the soil borne pathogen. *Trichoderma* spp. is a good biocontrol agent against different soil-borne pathogens. This biocontrol fungus not just kills the pathogenic microbes but also at the same time improves plant health. Bastakoti et al. (2017) conducted an experiment *in vitro* and revealed that *Trichoderma* spp. inhibit the mycelial growth of soil-borne pathogenic fungal species by 100%, 68%, and 62% of *Sclerotium rolfii*, *Fusarium solani*, and *Rhizoctonia solani* respectively. *In vitro* dual culture on PDA plates, the biocontrol fungus *T. spirale* inhibits the growth by 79% of *Fusarium* species collected from tomato fields (Vargas-Inciarte et al., 2019). *T. harzianum* isolates ET4 suppress or inhibit the mycelial growth of *Alternaria* spp. up to 67.74% in dual culture technique (Tekiner et al., 2019). Seed treatment of radish with effective biocontrol strain of *Trichoderma* vz. *T. harzianum*, *T. viride*, *T. asperellum*, *T. spirale*, *T. atroviride*, and *T. virens* improve the shoot and root growth with yield up to 96% against *R. solani* in both *in vivo* and *in vitro* conditions (Lee et al., 2016). Another experiment was conducted with four isolates of *T. harzianum* and five isolates of *T. Longibrachiatum* with Ridomil gold plus fungicide against purple blotch of onion pathogen, *Alternaria porri*. Biocontrol agent showed good results as compared to fungicide in *in vitro* condition. *T.*

*harzianum* control (73.12%) maximum mycelial growth of associated pathogenic pathogen as compared to *T. Longibrachiatum* (71%) and fungicide (70%) respectively (Abo-Elyousr et al., 2014).

#### **Interaction of filamentous fungi (*Trichoderma*) with other pathogenic microorganisms**

Due to fast growth, these biocontrol agents compete for space and nutrients with other pathogenic fungus species and colonized around the roots of the host plant. All pathogenic fungi also have a good, well-developed mechanism of colonization, but the genus *Trichoderma* has a diverse mechanism than others (Vinale et al., 2008). *Trichoderma* creates a direct connection with the targeted pathogen by hyperparasitism mode of action. This mode of action consisted of various stages named recognition stage, attack mode, and penetration with pathogenic fungal hyphae to kill the pathogen (Vinale et al., 2008). To degrade the cell wall of pathogenic fungi, *Trichoderma* synthesizes cell wall degrading enzymes (CWDE) which include xylanases, glucanases, cellulases, amylases, proteases, and lipases. The enzyme chitinases, play a major and vital role in cell wall degradation of the targeted pathogen. Celluloses enzyme having the ability to degrade lignocellulose by hydrolyzing. Volatile metabolites such as 6-PAP produced by *Trichoderma* to inhibit the mycelia growth of targeted pathogens (Ghazanfar et al., 2018b). The control of different soil-borne pathogenic fungi by using different *Trichoderma* species as biocontrol agent has been given in Table 4.

Table 4: Control of soil-borne pathogenic fungi by using different *Trichoderma* species as biocontrol agent.

Crop name	Soil borne fungal pathogen	<i>Trichoderma</i> spp.	References
Tomato	<i>F. oxysporum</i>	<i>T. harzianum</i>	(Arenas et al., 2018)
Brinjal	<i>F. oxysporum</i>	<i>T. harzianum</i>	(Balaji and Ahir, 2011)
		<i>T. harzianum</i>	
Tomato	<i>A. solani</i>	<i>T. viride</i>	(Sarfraz et al., 2018)
		<i>T. hamatum</i>	
		<i>T. harzianum</i>	
Mung bean	<i>M. phaseolina</i>	<i>T. hamatum</i>	(Khan et al., 2019)
		<i>T. koningii</i>	
Brinjal	<i>R. solani</i>	<i>T. harzianum</i>	(Faruk and Rahman, 2017)
Ginger	<i>Pythium aphanidermatum</i>	<i>T. harzianum</i>	(Gupta et al., 2010)
Tomato	<i>R. solani</i>	<i>Trichodermaspp.</i>	(Kashyap et al., 2019)
		<i>T. harzianum</i>	
Tomato	<i>A. solani</i>	<i>T. viride</i>	(Ramakrishna et al., 2017)
Fenugreek	<i>S. sclerotiorum</i>	<i>T. harzianum</i>	(Sharma et al., 2014)
Tomato	<i>A. solani</i>	<i>T. harzianum</i>	(Lakhdari et al., 2018)



Potato	<i>P. infestans</i>	<i>Trichodermaspp.</i>	(Yao et al., 2016)
Potato	<i>R. solani</i>	<i>T. harzianum</i>	(Ibrahim, 2017)
Guava	<i>F. solani</i>	<i>T. viride</i>	(Dwivedi and Dwivedi, 2012)
Tobacco	<i>R. solani</i>	<i>T. harzianum</i>	(Sumana and Devaki, 2012)
Sunflower	<i>R. solani</i>	<i>T. harzianum</i>	(Singh et al., 2011)
Sunflower	<i>F. moniliforme</i>	<i>T. viride</i>	(Jat and Agalave, 2013)
	<i>Sclerotinia</i>		
Cotton	<i>Fusarium</i>	<i>T. harzianum</i> CCM 341	(Hassanein, 2012)
	<i>Pythium</i>	<i>T. koningii</i> CCM	
Sesame	<i>F. moniliforme</i>	<i>T. harzianum</i>	(Jeyalakshmi et al., 2013)
Sugarcane	<i>S. scitamineum</i>	<i>T. viride</i>	(Joshi and Misra, 2013)
	<i>Colletotrichum falcatum</i>		
Soybean	<i>S. rolfsii</i>	<i>T. viride</i>	(Sharma et al., 2014)
	<i>A. alternata</i>	<i>T. harzianum</i>	
Tomato	<i>F. oxysporum</i>	<i>T. asperellum</i>	(Li et al., 2018)
Tomato	<i>F. oxysporum</i>	<i>T. virens</i>	(Jogaiah et al., 2018)
Tomato	<i>Botrytis cinerea</i>	<i>T. harzianum</i>	(You et al., 2016)
		<i>T. koningiopsis</i>	
Tomato	<i>R. solani</i>	<i>Trichoderma hamatum</i>	(Mohammed et al., 2020)
Tomato	<i>F. oxysporum</i>		
	<i>R. solani</i>	<i>Trichodermaspp.</i>	(Al-Mekhlafi et al., 2019)
Onion	<i>F. oxysporum</i>	<i>T. harzianum</i> KUEN 1585	(Ghanbarzadeh et al., 2016)
Onion	<i>A. porri</i>	<i>T. harzianum</i> th-3	(Sharma et al., 2014)
		<i>T. virens</i>	
Sorrel	<i>Alternaria tenuissima</i>	<i>T. pseudokoningii</i>	(Ambuse et al., 2012)
Grapevine	<i>Plasmopara</i>	<i>T. harzianum</i> T- 39	(Banani et al., 2014)
Sugar beet	<i>S. rolfsii</i>	<i>T. viride</i>	(Paramasivan et al., 2014)
		<i>T. reesei</i>	
Sugar beet	<i>R. solani</i>	<i>T. harzianum</i>	(Kakvan et al., 2013)
		<i>T. viride</i>	
Maize	<i>F. verticillioides</i>	<i>T. harzianum</i> T-22	(Ferrigo et al., 2014)
Soybean	<i>F. oxysporum f. sp. adzuki</i>	<i>T. viride</i>	(John et al., 2010)
Chickpea	<i>M. phaseolina</i>	<i>T. viride</i>	(Manjunatha et al., 2013)
Chickpea	<i>F. oxysporum</i>	<i>T. harzianum</i>	(Verma et al., 2014)
Chilli	<i>Colletotrichum capsici</i>	<i>T. viride</i>	(Sangeetha et al., 2011)
Soybean	<i>S. sclerotiorum</i>	<i>T. citrinoviride</i>	(Thakkar and Saraf, 2015)
Chilli	<i>Phytophthora capsici</i>	<i>T. harzianum</i>	(Sriram et al., 2010)
Cotton	<i>S. delphinii</i>	<i>T. harzianum</i>	(Ghazanfar et al., 2018a)
		<i>T. viride</i>	
Maize	<i>F. moniliforme</i>	<i>T. aurepviride</i>	(Harleen and Chander, 2011)
Tomato	<i>F. oxysporum</i>	<i>T. harzianum</i> Th908	(Marzano et al., 2013)
Cucumber	<i>F. oxysporum f. sp. Radicis</i>	<i>T. harzianum</i>	(Alizadeh et al., 2013)
	<i>Pythium spp.</i>		
Green bean	<i>F. oxysporum</i>	<i>T. harzianum</i>	(Ghazanfar et al., 2018a)
		<i>T. viride</i>	
Tomato	<i>R. solani</i>		(Karima and Nadia, 2012)
Yam	<i>Botryodiplodia theobromae</i>	<i>T. harzianum</i>	(Okigbo and Emeka, 2010)

### ***Trichoderma*: Mode of action against phytopathogenic soil-borne fungi**

The important modes of action of *Trichoderma* species include mycoparasitism, antibiosis, competition and ISR (Induce systemic resistance).

#### **Mycoparasitism**

The term mycoparasitism or hyperparasitism is an antagonistic association of two fungal species in which one fungal species suppresses the growth of other fungal species by producing enzymes, metabolites, cell wall degrading, and by penetrating the hyphae of apposite fungal specie (Druzhinina et al., 2011). A high level of histochemical and ultrastructural tactics were applied to observe the special effects of enzymes used during cell wall-degrading of the target pathogen. To check the cell wall bursting of pathogenic fungi stained the hyphae or mycelial growth with a blue fluorescein isothiocyanate calcofluor (Ghazanfar et al., 2018b). Recently an electron microscope operates to observe the cell wall lysis of *R. solani* by the antagonistic secreted enzyme of *Trichoderma*. *Trichoderma* produces chitinases, proteases,  $\beta$ -1,3-glucanases, and lipases enzymes to degrade the cell wall of targeted pathogenic fungi. On the other hand, to parasitize the target pathogen, first of all, the *Trichoderma* changes the morphology of the opposite pathogen by coiling around the hyphae and by producing appressorium development. Then, this biocontrol agent produces a signal against the target pathogen and penetrates the hyphae into the lumen of a pathogen. The presence of lectin and carbohydrates in the cell wall of both *Trichoderma* and targeted pathogen help in binding into each other of both pathogens (Ghazanfar et al., 2018b). At this time, 1100 strains of *Trichoderma* from 75 species recognized molecularly and morphologically as biocontrol through mycoparasitic way (Druzhinina et al., 2011). Benítez et al. (2004) described that the MAP and cAMP kinase (a signaling movement) play a vital role in *T. atroviride*, also having a G-  $\alpha$  protein to control the enzyme activities, production of antibiotics, and coiling direction of hyphae (Benítez et al., 2004). The addition of mastoparan protein and flouroaluminatate protein, also called G-protein during biochemical reaction increases the coiling capacity of *Trichoderma* nearby nylon fibers. Proteinase (prb1 gene) increases the coiling capacity of *T. atroviride* and *T. viride* in the presence of tga1 gene (G-  $\alpha$  gene) against *Rhizoctonia* spp. (Gajera et al., 2013). Five *T. harzianum* isolates (*Trichoderma* 31, 32, 30, 78,

and 57) were-encoded for a gene (chit 33 and chit 42, prb1, exc 2 and exc 1, bgn 13.1) and expressed their mycoparasitism activities against soil-borne pathogenic fungus *F. oxysporum*. The dual culture technique used to test the antagonistic activity of *Trichoderma* and RT-PCR was done to confirm the gene expression (López-Mondéjar et al., 2011) and isolated encoding gene *tca1* from *T. virens* showing his mycoparasitism activities against *P. ultimum* and *R. solani*. A transporter gene (*ThPTR2*) of *T. harzianum* suppresses the growth of *B. cinerea* through mycoparasitism (Sharma et al., 2011a). For cloning and gene expression, the cDNA of the *ThPTR2* gene was synthesized by using the RT-PCR technique. The final product after cloning was tested by the dual culture method (Munir et al., 2014). Mycoparasitic activities of *T. atroviride* against *P. ultimum*, *B. cinerea*, and *R. solani* were also studied through dual culture assay. A *T. harzianum* gene *qid74* showing his mycoparasitic activities by cell defense and afford adherence to aqua-phobic layer against soil-borne pathogen *R. solani* (Rosado et al., 2007). The mycoparasitic activities of *Trichoderma* spp. against *R. solani* have been shown in Figure 2.

### ***Trichoderma* release cell-wall-degrading enzymes**

#### **a) Chitinases**

Based on their function, chitinases are divided into three main categories exochitinases, endochitinases, and 1,4- $\beta$ acetylglucosaminidases. 1,4- $\beta$ acetylglucosaminidases also called GlcNAcases, different GlcNAcases gene isolated and identified from *Trichoderma* spp. such as *nag1*, *tvnag1,exc2*, and *tvnag2* isolated from *T. harzianum* (Harman et al., 2004a). GlcNAcase Nag1 has been isolated from *T. atroviride* and this gene necessary for chitinases gene manifestation. A *Trichoderma* strain 2413 produces three endochitinases gene named *chit42*, *chit37*, and *chit33* these genes were helped in cell wall degradation of pathogenic soil-borne fungi (Viterbo et al., 2001).

#### **b) Glucanases**

$\beta$ -1,3-glucanases combined with antibiotic and chitinases inhibit the growth and germination of spores of many soil-borne pathogenic fungi (El-Katatny et al., 2001). Different glucanases were isolated from different *Trichoderma* spp. such as *Tv-bgn1* and *Tv-bgn2* isolated from *T. virens*, *lam1.3*, and *bgn13.1* isolated from *T. harzianum* and *glu7* isolated from *T. atroviride* (Benítez et al., 2004). A transformant *BGN13* was identified and reported as a growth inhibitor against *P. citrophthora*, *B. cinerea*, and *R. solani* (El-Katatny et al., 2001). In

addition, *BGN16* is showing its synergistic effect with chitinases to control the fungal pathogens.

### c) Proteases

*Trichoderma* spp. produced and release proteases enzyme, which are involved in the degradation of the targeted pathogen cell wall (Delgado-Jarana et al., 2000). Alkaline proteases (*Prb1*) were isolated from *T.*

*harzianum* IMI plays an imperative role to control the pathogenic fungi and improve the biocontrol proficiency of *Trichoderma* strains (El-Katatny et al., 2001). *Tra1* isolated from *T. harzianum* to degrade the pathogen cell walls. *Tvsp1* gene isolated from *T. virens* and play an aggressive role to protect the cotton plant against *R. solani* (Pozo et al., 2004).

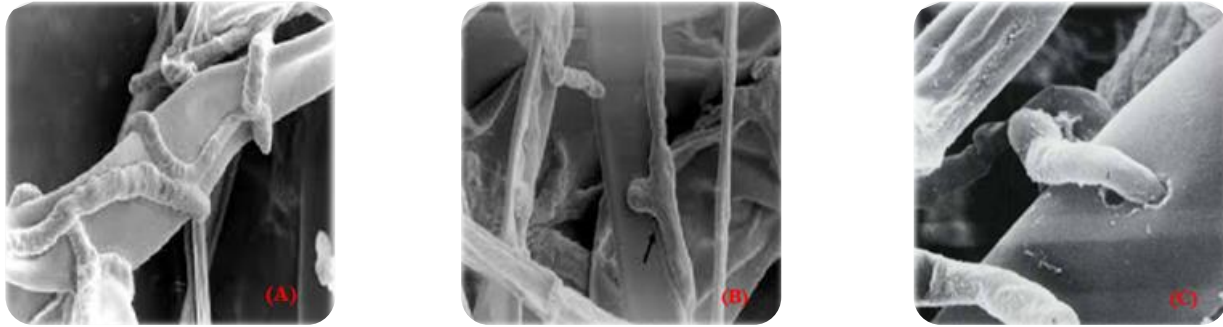


Figure 2: Mycoparasitic activities of *Trichoderma* spp. against *R. Solani* (A) coiling (B) penetration (C) cell degradation and penetration

### d) Synergism

The combined effect of *Trichoderma* lytic enzyme with an antibiotic, improve biocontrol or antagonistic effect of *Trichoderma* spp. against a soil-borne fungal pathogen. An experiment was designed to check to single and combined antagonistic effect against *R. solani*, results revealed that single *T. harzianum* control 30% growth of *R. solani* while the combination of  $\beta$ -1,3-glucanase and a  $\beta$ -1,6-glucanase with chitinase control 60% growth of *R. solani* (Benítez et al., 2004).

### Antibiosis

Antibiosis is a process in which *Trichoderma* spp. produce antimicrobial and volatile or non-volatile metabolites to kill or suppress the growth of pathogenic fungi without any physical contact. During antibiosis interaction, diffusible low molecular weight metabolites are released and stopped the pathogenic host *in vitro* condition (Gajera et al., 2013). *Trichoderma* species produced metabolites which work as antibioses such as viridian, alamethicins, gliovirin, massoiltactone, glisoprenins, 6-pentyl-  $\alpha$  -pyrone, peptaibols, peptidic acid, harmonic acid and tricholin (Qualhato et al., 2013). The mixed effect of antibiosis with cell wall degrading enzyme or hydrolytic enzymes were-showed reliable results as compared to a single one (Druzhinina et al., 2011). *T. virens* produce gliovirin while *T. harzianum* produces pyrone antibiotic. *Gaeumannomyces graminis* var. *tritici* is the reason behind "Take all disease of

wheat" is biologically controlled by *T. harzianum*, which produces an antibiotic pyrone. In *in vitro* condition, the conidial formation of *B. cinerea* is suppressed or retarded due to the combined effect of gliotoxin and endochitinases with peptaibols. *T. harzianum* 2413 produces  $\alpha$ -pyrone and extracellular enzyme which showing good antagonistic results against *R. solani*. Interestingly, metabolites were produced by *Trichoderma* used to control other pathogens rather than fungal. Such as exogenous treatment of peptaibols on tobacco plant, reduce the susceptibility of plant against TMV (*Tobacco Mosaic Virus*) (Gajera et al., 2013).

### Competition

#### 1: By fungi-stasis

Biocontrol agents or antagonistic organisms having the ability to reduce the effect of other harmful organisms without killing them by producing different metabolites. *Trichoderma* belongs to the genus filamentous fungi that have the ability to grow fast in the soil as compared to other soil-borne pathogens (Benítez et al., 2004). In soil, this fungus makes competition with other soil-borne pathogens such as *R. solani*, *F. solani*, and *Alternaria* spp. for nutrition and limited space. That is the salient feature of *Trichoderma* and it provides protection to plant roots by reducing the effect of soil-borne pathogenic fungi. Due to the well development ABC transport pathway in a few *Trichoderma* strains, this antagonistic fungus highly resists the effect of toxic

composite (Harman et al., 2004a). That is why the application of this fungus in the soil is alternating the many toxic fungicides against soil-borne pathogens (*Sclerotium rolfsii*, *R. solani*, *P. ultimum*) is successful (Waghunde et al., 2016).

## 2: Competition for space and nutrients

The reason for competition between the beneficial and harmful microorganisms in soil is "starvation". In soil, the *Trichoderma* is considering plant beneficial fungi and competes with other soil-borne fungal pathogens for limited nutrients (Ghazanfar et al., 2018b; Verma et al., 2007). Iron is a necessary element of *Trichoderma* to better survive in the soil so in the absence of this element this fungus release ferric iron chelators also called siderophore. Siderophore is the iron-mobilizing process of *Trichoderma*. As compared to *Trichoderma*, two soil-borne fungus *Aspergillus nidulans* and *A. fumigatus* were also synthesized siderophore by using carbon (Eisendle et al., 2004). Iron uptake by *Ustilago maydis* in soil decreases the plant value by an effect on its development. The most effective synthesis of siderophore by *Trichoderma* spp. is also used to retard the growth of the soil-borne fungal pathogen. *T. harzianum* strain T35 showing his competition ability against *F. oxysporium* for nutrients up taking and rhizosphere colonization (Tjamos et al., 2006). Many *Trichoderma* strains fight for space against pathogenic fungi *B. cinerea* in the rhizosphere of solanaceae crops (Prusky and Yakoby, 2003). The filamentous fungi have a higher capability to take up nutrients from the soil. For acquiring nutrients, *Trichoderma* required ATP, and different genes are involved in obtained ATP from the soil environment such as the *Gtt1* gene used to make

glucose (Benítez et al., 2004).

## Induced systemic resistance (ISR)

Nowadays, 50 types of *Trichoderma* strain formulations were prepared to control phytopathogenic fungi (Benítez et al., 2004). To identify the gene involved in this process, different tactics are applied. ISR is a complex process but *Trichoderma* strain applied and activated the immune system against different aerial and root infections. Pathogenesis-related (PR) proteins activate to protect the plant by the application of *Trichoderma* formulation in soil. As compared to *Trichoderma*, the PR proteins activated in the plant during the result of necrosis and wound by an insect. Non-pathogenic bacteria also induce and produce resistance in plants which include rhizobacteria (Ghazanfar et al., 2018b). Djonovic et al. (2007) reported that *T. virens* produce and secrete an elicitor named SM1, this elicitor induces SR (systemic resistance) in cotton plants against the pathogen *Colletotrichum graminicola* (Djonovic et al., 2007). *T. virens* induce systemic resistance in cotton plant against *R. solani* by activating the terpenoid phytoalexins (Kumar et al., 2010). *T. harzianum* induces resistance in pepper plant against *Phytophthora capsici* by activated the phytoalexin calcdiol (Vinale et al., 2008). *Trichoderma* species also induce resistance against other plant pathogenic microbes such as harmful bacteria. *T. virens* induce resistance in tomato plant against *Pseudomonas syringe* by secreted to proteins named EP11 and Sm1, these genes induce systemic acquired resistance in tomato plants (Salas-Marina et al., 2015). Induce systemic resistance by *Trichoderma* species against different soil-borne fungal pathogens has been given in Table 5.

Table 5: Induce systemic resistance by *Trichoderma* species against a different soil-borne fungal pathogen.

Sr. No.	Crop	<i>Trichoderma</i> species	Target pathogen	References
1	Cucumber	<i>T. harzianum</i> T-39	<i>B. cinerea</i>	(Okon Levy et al., 2015)
2	Tomato	<i>T. harzianum</i> T-39	<i>B. cinerea</i>	(Waghunde et al., 2016)
3	Cotton	<i>T. virens</i> G-6 and G 6-5	<i>R. solani</i>	(Howell et al., 2000)
4	Tomato	<i>T. atroviride</i> and <i>T. virens</i>	<i>A. solani</i> and <i>B. cinerea</i>	(Salas-Marina et al., 2015)
5	Cucumber	<i>T. harzianum</i> Tr6	<i>F. oxysporum</i>	(Alizadeh et al., 2013)
6	Pepper	<i>T. harzianum</i>	<i>Phytophthora capsici</i>	(Ahmed et al., 2000)
7	Tomato	<i>T. harzianum</i> T-22	<i>A. solani</i>	(Waghunde et al., 2016)
8	bean	<i>T. atroviride</i> P1	<i>b. cinerea</i>	(Harman et al., 2004b)
9	Paper	<i>T. harzianum</i>	<i>P. capsici</i>	(Ghazanfar et al., 2018a)
10	Cotton	<i>T. virens</i>	<i>R. solani</i>	(Kumar et al., 2009)
11	weed	<i>T. harzianum</i> T382	<i>B. cinerea</i>	(Mathys et al., 2012)

### **Commercial formulation products and their application methods to control soil borne fungal pathogen**

To control soil-borne pathogenic fungi, different *Trichoderma* commercial formulations or biofungicides were applied in soil or seed dressing in the form of liquid and solid-state (Ha, 2010). Different commercial formulations of *Trichoderma* species used against soil-borne pathogens have been listed in Table 6. Fungal pathogen In the history of biofungicide, the *T. harzianum* used for the very first time and registered in 1989 with the name EPA to control pathogenic fungi (Fravel, 2005). Commercial biofungicide required several steps for successful biocontrol such as cost-effective and environmentally friendly, stability temperature range from -5 to 35°C, extraction or isolation of microbes, evaluation of the product *in vivo* and *in vitro*. Furthermore, selection of suitable isolate according to filed condition, mass manufacture or production, compatibility, and longer shelf life of the product and registered from the government (Ghazanfar et al., 2018b; Kumar et al., 2014). Seed dressing with biofungicide mean to control the soil-borne fungi associated with wilting and rotting diseases of the plant. One kilogram of cereal or pulses seeds treated with 10 gram of *Trichoderma* formulation before sowing or planting (Harman et al., 2004a). In soil, the biocontrol agent multiplies and reproduce, after reproducing this biocontrol agent moves around the roots of a plant and protect it from pathogenic fungi through different antagonistic mechanisms described previously. At this time, different institutes of India are working on fifteen main species of *Trichoderma*, especially *T. harzianum* and *T. viride*. The formulation as biofungicide of these two mention species was applied against about eighty seven (87) crops which include 18 against airborne fungal pathogen and 70 against the soil-borne fungal pathogen (Kumar et al., 2014; Sharma et al., 2014). A few commercial application methods of *Trichoderma* strains are given below.

#### **Seed treatment**

Seed dressing with the commercial formulation of *Trichoderma* strain in the form of dry powder or dust powder is an effective application method to control soil-borne fungal diseases. Seed dressing depended on seed size, according to Mukhopadhyay and Kumar (2020) 3 gram to 10 gram antagonistic dry powder formulation is enough for 1 kg of crop seed

(Mukhopadhyay and Kumar, 2020). The antagonistic microbes germinate and multiply on the above seed surface and protect the plant root from pathogenic soil-borne fungi such as *R. solani* and *Fusarium* spp. (Kumar et al., 2014). To control *R. solani* and *Pythium* spp. from the soil the dry commercial formulation of the three most effective antagonistic *Trichoderma* strain named *T. viride*, *T. virens* and *T. harzianum* has been applied (Mukherjee et al., 1995). Seed dressing with dry powder of *T. harzianum* and *T. viride* has been used to control sheath blight disease of rice (Das, 2000). Another study showed that the application of commercial formulation of *T. harzianum* and *T. viride* was used to control loose smut of wheat and improve the yield of wheat crop. Seed dressing with *Trichoderma* strains were used to control mustard diseases caused by *Alternaria* species. Jat and Agalave (2013) were described that the seed dressing with the most effective *Trichoderma* spp. used to suppress the growth of oil seed-borne pathogenic fungi named *A. Alternata*, *Aspergillus flavors*, *F. moniliform*, *R. nigricans*, *Curvularia lunata*, and *Penicillium notatum* which affect the crops like sesame, sunflower, and soybean respectively (Jat and Agalave, 2013).

#### **Seed biopriming**

Seed biopriming is an ecological approach to control seed and soil-borne diseases of agricultural crops; in this technique, we used the combination of seed hydration and antagonistic agent. By using this technique, first of all, we soak the seed in pre-warm water for twelve hours and after this incubation period mixed the seed with biocontrol agent *Trichoderma* and covered with jute sack to provide maximum moist and humidity to the heap. Incubate the heap at room temperature for 48 hours. During this incubation period, the biocontrol agent germinates and grows fast around the seed surface to protect. In the last step, transplant the seed on the nursery bed for germination. Seed bio priming is a good effective method with some salient features, which included decrease seed germination time, increase the germination rate, produce uniform and fast growth; inhibit the growth of soil-borne fungi. This technique is most effective in different crops such as brinjal, tomato, chickpea, and soybean respectively (Mishra and Nautiyal, 2009). Yadav et al. (2013) were applied *Trichoderma* with different combinations to control the soil-borne pathogenic fungi and improve the seed rate and growth of rajma and chickpea plants. *Trichoderma* shows the best results as compared to others (Yadav et al., 2013).

Table 6: List of a commercial formulation of *Trichoderma* species used against soil-borne fungal pathogen.

<i>Trichoderma</i> species	Product name	Formulation type	Target pathogen/Disease	Manufacture company name	Country
<i>T. harzianum</i> strain SF	Bio-Tricho	WP	<i>Rhizocotonia</i> , <i>Fusarium</i> , <i>Phytophthora</i> and <i>Botrytis</i> spp.	Agro-Organics	South Africa
<i>T. koningii</i> , <i>T. harzianum</i>	Promot	WP	Root rot and damping-off diseases	Biofa AG	Kenya
<i>T. harzianum</i> strain kd	Eco-T	WP	<i>Fusarium</i> , <i>Pythium</i> , and <i>Rhizocotonia</i> spp.	Plant Health Products, Ltd	Kenya, Morocco, India, S. Africa
<i>Trichoderma</i> spp.	Tricho Plus	WP	Soil-borne fungal diseases	Biological Control Products, Ltd	South Africa
<i>T. viride</i>	Agrigold Trichogold	Both Liquid and WP	Root and stem rot fungal diseases	AgriGold Organics Pvt. Ltd.	India
<i>T. viride</i> ,	ANOKA	WP	Rotting of the stem, Collar rot and seed rotting diseases	K N Bio Sciences Private Limited	India
<i>T. viride</i>	Bio-Shield	WP	<i>Fusarium</i> , <i>Phytophthora</i> , <i>Sclerotium</i> and <i>Pythium</i> spp.	Ambika Biotech	India
<i>T. viride</i>	Bio-Tricure	WP	<i>Fusarium</i> , <i>Alternaria</i> , <i>Sclerotinia</i> and <i>Verticillium</i> spp.	Chaitra Fertilizers & Chemicals, PLtd	India
<i>T. atroviride</i> 1237	Esquive WP	WP	<i>Botryosphaeria</i> spp.	Agrauxine, ZA de Troyalac'h	France, Australia and South Africa
<i>T. harzianum</i>	Tricone V	WP	<i>Pythium</i> spp. <i>Rhizocotonia solani</i> and <i>Fusarium</i> spp.	Neuscire Biolab	India
<i>T. atroviride</i> + <i>T. polysporum</i>	Binab T P	Pellets	Pathogenic soil borne Fungi that cause wilt, root rot and take-all.	Binab bio-innovation	USA
<i>T. viride</i>	Bioveer	WP	Against damping off, root rot, collar rot, foot Rot and stem rot.	Ambika Biotech	India
<i>T. viride</i>	Coimbatore	WP	Damping off, Wilts, Root rots, brown rot and Charcoal rot.	GreenMax Agro Tech	India
<i>T. viride</i>	Deepa Bio Tricho Plus	Both WP and Liquid	Soil borne fungal diseases	DFI Private Limited, Trivandrum	India
<i>T. asperellum</i>	Ecohope-Dry	Emulsion	Seed and root diseases of plants	Kumiai Chemical Industry Co. Ltd.	Japan
<i>T. harzianum</i> IHR-Th-2	Ecosom-TH	Both WP and Liquid	<i>Pythium</i> spp. and <i>Alternaria</i> spp.	ALSOM Phytopharma Limited	India
<i>T. viride</i> (TNAU)	Ecosom-TV	WP, Lyophilized and	<i>Pythium</i> spp, <i>Fusarium</i> spp. and <i>Rhizocotonia</i> spp.	AL SOM Phytopharma Limited	India

<i>T. viride</i>	Jai Vjai	Liquid Both WP and Liquid	<i>Rhizocotonia</i> , <i>Fusarium</i> spp.and <i>Alternaria</i> spp. <i>Fusarium</i> , <i>Pythium</i> and <i>Rhizocotoniaspp.</i>	CF and CP Limited	India
<i>T. viride</i>	Jaimold	Liquid	<i>Verticillium</i> , <i>Fusarium</i> , <i>Rhizocotonia</i> , <i>Macrophomina</i> , <i>Sclerotium</i> and <i>Alternaria</i> spp.	JBI, Nashik	India
<i>T.harzianiumT. viride</i>	Neemoderma A	WP	Against soil borne fungal diseases	Bio Control Research Laboratories Pvt. Ltd Bangalore	India
<i>T. harzianum</i>	Niprot TH	Both WP and SOLID	<i>Fusarium</i> , <i>Rhizocotonia</i> , <i>Alternaria</i> and <i>Macrophomina</i> spp.	PF & CW Ltd.	India
<i>T. viride</i>	Prabhaderma	Liquid	Root, stem rots and Damping off diseases	GSF & C Ltd.	India
<i>T. harzianum</i>	Sardar Eco Green	WP	<i>Fusarium</i> , <i>Pythium</i> and <i>Rhizocotonia</i> spp.	Dr. Rajan Laboratories Green Biotech Co. Ltd., Korea	India
<i>Trichoderma</i> spp.	<i>T. viride</i> / Harzianum	WP	Against soil borne fungal diseases	Kan Biosys Pvt. Ltd.	India
<i>T. harzianum</i> GBF-0208	Tricho Gold, Green-all T WP	Both Solid and Liquid	Against soil borne fungal diseases	Boothankad Estate	Korea
<i>T. viride</i>	Tricho Shield Combat	Both Solid and Liquid	Bud rot and stem rot of fungal diseases in vegetables, <i>B. cinerea</i> , <i>Fusarium</i> , <i>Rhizocotonia</i> , <i>Sclerotinia</i> and <i>Pythium</i> spp.	Ruchi Biochemical	India
<i>Trichoderma</i> spp.	<i>Trichoderma</i>	Both Solid and Liquid	<i>B. cinerea</i> , <i>Fusarium</i> , <i>Rhizocotonia</i> , <i>Sclerotinia</i> and <i>Pythium</i> spp.	Saipan SRL	India
<i>T. viride</i>	<i>Trichoderma</i> Bio-Fungicide	WP	<i>Verticillium dahliae</i> , <i>Rhizocotonia</i> <i>solani</i> and <i>Sclerotinia</i> spp.	Isagro Spa	Italy
<i>Trichoderma</i> spp.	BioPlantguard	Liquid	Bud rot and stem rot of fungal diseases in vegetables crops. <i>Pythium</i> , <i>Fusarium</i> <i>Rhizocotonia</i> and <i>Phytophthora</i> spp.	Plant Health Products (Pty)Ltd	Spain
<i>T. asperellum</i> 012 + <i>T. gamsii</i> 080	Bioten	WP	Against soil borne fungal diseases	dragonfli	UK
<i>Trichoderma</i> spp.	CANNA Coco	Solid			UK
<i>T. harzianum</i> strain kd	Eco-T	WP			Africa, Kenya, UK and Tunisia
<i>T. harzianum</i> T-22	GROW plant	BOOST WP			UK

<i>T. harzianum</i> TH01 + <i>T. atroviride</i> TA 28,	Micosat F semi	Powder	Against soil borne fungal diseases	C.C.S Aostas.r.l.	Italy
<i>T. harzianum</i> TH01 + <i>T. atroviride</i> TA 28,	Micosat F Grano	Powder	<i>Pythium</i> , <i>Fusarium</i> <i>Rhizocotonia</i> and <i>Phytophthora</i> spp.	C.C.S Aostas.r.l.	Italy
<i>Trichoderma</i> spp.	Sani-Root	Liquid	<i>Pythium</i> spp. <i>Fusarium</i> Spp. and <i>Rhizocotonia</i> spp.	AMC Chemical, S.L. .and Trichodex, S.A.	Spain
<i>T. harzianum</i>	<i>T. harzianum</i> IAB-32	Liquid	<i>Pythium</i> spp. <i>Fusarium</i> spp.and <i>Rhizocotonia</i> spp.	IAB S.L.	Spain
<i>T. asperellum</i> T34	T34 Biocontrol	WP	<i>F. oxysporum</i>	Biocontrol Technologies S.L., Fargro Ltd.	UK
<i>T. harzianum</i> strain T-22	TRIANUM-G	Granules	Against soil borne fungal diseases	Koppert B.V.	New Zealand and Australia
<i>T. harzianum</i> strain T-22	TRIANUM-P	WP	<i>Pythium</i> spp. <i>Fusarium</i> spp.and <i>Rhizocotonia</i> spp.	Koppert B.V.	New Zealand
<i>T. harzianum</i>	<i>Trichoderma</i> s BioFlower	WP	<i>Pythium</i> spp. <i>Fusarium</i> spp. and <i>Rhizocotonia</i> spp.	Terranaturale	Spain
<i>T. asperellum</i>	Trifender	Granules	<i>Pythium</i> spp. <i>Fusarium</i> spp.and <i>Rhizocotonia</i> spp.	Bioved	Hungary
<i>T. viride</i> + <i>T.</i> <i>harzianum</i>	TUSAL WG	WG	<i>Pythium</i> spp. <i>Fusarium</i> spp. and <i>Rhizocotonia</i> spp.	NBTSA	Spain
<i>T. asperellum</i> +	Remedier WP	WP	<i>Pythium</i> spp. <i>Fusarium</i> spp.and <i>Rhizocotonia</i> spp.	Isagro USA	USA
<i>T. gamsii</i>					
<i>T. atroviride</i> 1237	Esquive WP	WP	<i>Phaeomoniella</i> , <i>Botryosphaeria</i> and <i>Phaeoacremonium</i> spp.	Agrauxine, ZA de Troyalac'h	Australia and South Africa
<i>T. harzianum</i>	Compete Plus	WP	Against soil borne fungal diseases	Plant Health Care T. Stanes and Company Limited, Coimbatore, Tamilnadu;	Spain and USA
<i>T. viride</i>	Biocure F	WP, liquid and solid	<i>Pythium</i> spp. <i>Fusarium</i> spp.and <i>Rhizocotonia</i> spp.	Binab bio-innovation	India
<i>T. atroviride</i> 206040	IMI Binab TF WP	WP	<i>Botrytis cinerea</i>		India
<i>T. atroviride</i> LC52	Trichopel	Granules	<i>Pythium</i> spp. <i>Fusarium</i> spp. and <i>Rhizocotonia</i> spp.	Agrimm Technologies Limited	New Zealand



### Root treatment

Roots of nursery seedlings before transplanting dipped in spore suspension of antagonistic *Trichoderma* spp. proved to be an effective method to control soil-borne fungal diseases (Kumar et al., 2014). The spore suspension of *Trichoderma* can also be applied on nursery beds against soil-borne fungal diseases. The method, not only protect the root from pathogenic fungi but also improve the root and plant growth. After the application of *Trichoderma* suspension on nursery beds, improvement in the growth of different crops has been reported (Singh et al., 2002). Gnanamanickam (2002) described and reported that sheath blight disease of rice is controlled by dipping the seedling roots in *Trichoderma* spore suspension before transplanting in the field.

### Soil treatment

Before planting the seed and transplanting the nursery seedling in the field treated the soil with biocontrol *Trichoderma* strain is an easy and effective way to control the soil-borne pathogenic fungi from the field (Kumar et al., 2010). These antagonistic microbes not only kill the pathogenic fungi in soil but also increase the soil fertility and improve plant growth health. Application of *T. viride* in the soil before seed sowing of jute crop has found to be best against collar rot, root rot, seedling root, and stem rot diseases (Srivastava et al., 2010). Another study shows that the application of *Trichoderma* spp. in soil control the soil-borne pathogenic fungi name *F. solani*, *F. oxysporum*, *A. Alternaria*, and *F. moniliforme* of Indian rosewood tree also called *Dalbergia sissoo* (Mustafa et al., 2009).

### Conclusions and future prospects

Nowadays bundles of studies on biocontrol agents have been published. The biocontrol agent provides an alternate method to synthetic pesticides. All biocontrol agents work good both *in vitro* and *in vivo* against targeted pathogens. The biocontrol agent almost works good and more strongly in future if we study their survival nature. By studying their survival nature we will be able to know how these beneficial microbes reproduce fastly. A lot of environmental factors affect their survival. The biopesticide application also works according to our desire but the advance application methods are not present. There is a dire need to made more efficiently *Trichoderma* based biopesticide by knowing the proper chemistry of these microbes and develop new technique to introduce these microbes in our soil.

### AUTHORS' CONTRIBUTION

All the authors equally participated in collecting, organizing, writing and editing the manuscript.

### CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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