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A research article on *Trichoderma* spp: An effective biocontrol agent for management of plant diseases and enhance the sustainability

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Abstract
Trichoderma spp. are free living asexual fungi that are present in all types of agricultural soils, root ecosystem and also in decaying wood. The fungus is also a decomposer of cellulosic waste materials and antagonistic activity showed that it is parasitic on many soil-borne and foliage pathogens *viz.*, bacteria, fungus, virus, nematodes and higher parasitic plants and effectively control collar rot, root rot, foot rot, stem rot, sheath blight, gray mold, damping off, mildew and other disease like nematode causing problems etc. The traditional method (chemical-based pesticides) control plant diseases very effectively and suppress plant pathogens but these are not eco-friendly. Continuous use of agro-chemical and pesticides caused the resistance in pathogens towards these chemicals. It not only develops resistance among pathogens but also causes environmental pollution, this hazardous chemical pollutes water body and pollute our food chain too. It is harming fish and other aquatic lives, beneficial insects like honeybee, predator insects and other non-targeted organisms like plant growth promoting rhizobacteria-fungus (PGPR & PGPF). For overcome with these unsafe methods researchers are searching some other alternative methods that are significantly eco-friendly. Biological control agents, plants extract, crop rotation with potential crop/varieties, repellent plant, natural enemies, PGPR and PGPF are some of the most effective control measure in which, Trichoderma is the most versatile fungus genus that can control and manage phytopathogenic diseases and so increase plant growth. So, this review was summarised that *Trichoderma* spp. act as effective bio-agent can control many plant pathogenic fungus like *Fusarium, Phytophthora, Pythium, Colletotrichum, Fulvia, Rhizoctonia, Rhizopus, Alternaria, Verticillium,* and *Sclerotinia* etc.

Keywords: Trichoderma spp, bioagent, diseases management

Introduction
Instead of above this it also very useful for plants because it improve plant growth by solubilising mineral nutrients (especially phosphorus and micronutrient) that improve root system and drought resistance in plant, secondary metabolites production that compounds induce ethylene production, hypersensitive responses and other survival reactions in plant and so improve or stimulate plant defence mechanism, produce growth regulating compounds and production of siderophores, *Trichoderma* gene (*Endochitinase* gene) used in biotechnology for developing resistant transgenic crop (tobacco and tomato plants), also used in bioremediation of soil (contaminated with pesticides and herbicides) because of its ability to degrade a wide range of insecticides: Organochlorines, Organophosphates and Carbonates etc.

Mechanisms of *Trichoderma* spp.
Now a day, these are several strains of *Trichoderma* spp have been isolated as potential biocontrol agents effective against fungal phytopathogens. These all species of *Trichoderma viz., Trichoderma asperellum, T. harzianum, T. viride* and *T. hamatum* are inhibits or supress the fungal phytopathogens. In general, bio-control agent grows in its natural habitat on the root surface, and so affects root disease in particular, but it can also be effective against foliar diseases (Leaf rot) and bark diseases (Citrus gummosis).They can act indirectly, by competing for nutrients and space, modifying environmental conditions, or promoting plant growth and plant defensive mechanisms and antibiosis, or directly, by mechanisms such as mycoparasitism (Vinale *et al.*, 2008) [17].

Competition for Nutrients and Living Space: *Trichoderma* spp. are rapidly growing fungi that have persistent conidia and a broad spectrum of substrate utilization. They are very efficient competitors for nutrition and living space. In addition, *Trichoderma* spp., are naturally resistant to many toxic compounds, including herbicides, fungicides, and phenolic compounds. Therefore, they can grow rapidly and impact pathogens by producing metabolic compounds that impede spore germination (Fungistasis), kill the cells (antibiosis), or modify the rhizosphere, e.g. by acidifying the soil so that the pathogens cannot grow (Benitez *et al.*, 2004) [3]. Starvation is the most common cause of death for microorganisms, so competition for limited nutrients is especially important in the biocontrol of phytopathogens. Iron uptake is essential for filamentous fungi and under iron starvation; fungi excrete low-molecular weight ferric-iron specific chelators, termed siderophores. *Trichoderma* spp. produce highly efficient siderophores that chelate iron and stop the growth of other fungi (Benitez *et al.*, 2004) [3].

Mycoparasitism: The direct interaction between *Trichoderma* and pathogen is called mycoparasitism the first to recognize that *Trichoderma* spp., is a biocontrol agent and at the same time he also noticed mycoparasitism of *T. viridae* hyphae coiling and killing *R. solani* (Wells, 1988) [18]. Mycoparasitism is a complex type mechanism that generally involves the production of a cell wall lytic enzyme. It involves four sequential steps

1. Chemotropism and recognition.
2. Attachment and coiling.
3. Cell wall penetration.
4. Digestion of host cell.

Trichoderma strains detect other fungi, grow straight towards them, and its sequentially produce hydrolytic cell wall degrading enzymes. *Trichoderma* attach to the host, and coil hyphae around the host, form appressoria on the host surface, penetrate the host cell, and collapse the host hyphae (Steyaert *et al.*, 2003) [16]. The molecular level induction of mycoparasitism was first reported in 1994 (Carsolio *et al.*, 1999) [6]. Based on the study of regulation of an endochitinase-encoding gene (Ech42). Ech 42 was expressed during the mycoparasitic interaction between *T. harzianum* and *Rhizoctonia solani.* Another study showed that in the P1 mutant strain of *T. atroviride*, the expression of exo chitinase nagI or endochitinase Ech42 gene was needed to induce mycoparasitism in treatments containing purified colloidal chitin from the fungal cell walls (Vinale *et al.*, 2008) [17]. Production and regulation of lytic enzymes such as chitinases, glucanases, and proteases by Trichoderma spp. also play key roles in the mycoparasitism/biocontrol process (Mukherjee *et al.*, 2008) [12].

Induction of plant defence through *Trichoderma* spp: *Trichoderma* spp. induce gene expression of proteins in plants such as chitinase, glucanase, and peroxidase against antagonistic microbes (Hanson *et al.*, 2004) [8]. It has also been shown that pre-treatment of plants with *Trichoderma* spp. increased plant resistance to pathogen attack (Harman, 2004) [9]. Trichoderma spp. are opportunistic invaders, fast growers and large spore producers. They contain cell wall degrading enzymes (e.g., Celluloses, Chitinases, and Glucanases) and produce antibiotics. Moreover, the presence of *Trichoderma* spp. stimulates the induction of the hypersensitive response, systemic acquired resistance (SAR), and induced systemic resistance (ISR) in plants (Vinale *et al.*, 2008) [17]. For example, tomato plants colonized by *T. hamatum* actively induced systemic changes in plant physiology and disease resistance (Alfano *et al.*, 2007) [2]. In a study of cucumber plants, *T. asperellum* induced a systemic response of two defence genes encoding phenylalanine and hydroperoxidase lyase and systemic accumulation of phytoalexins against *Pseudomonas syringae pv. Lachrymans* between the host plant, the pathogen, the biocontrol agent and several environmental factors.

Table 1: Diseases management by using biological control agents *Trichoderma* spp.

Si. No.	*Trichoderma* species	Target Pathogens	Specific comments	Reference
1	*T. viride*	*Drechslera sorokiniana*	Cause root rot, seedling and foliar blights of wheat	Prasad *et al.*, 1978 [13].
2	*T. viride*	*Nivotia indica*	Reduction in germination of *teliospores* and *sporidia* on wheat crop	Singh *et al.*, 1991 [14].
3	*T. viride*	*Ustilago segetum tritici*	Inhibition of germination of chlamydospores and inhibits of mycelial growth of loose smut of wheat and also tested in field condition	Aggarwal *et al.*, 1993 [1].
4	*T. harzianum*	*S. rolfsii*	Root rot of various crops	Wells *et al.* 1972 [18].
5	*T. harzianum*	*Macrophomina phaseolina*	Seedling blight of cotton	Elad *et al.*, 1986 [7].
6	*T. harzianum*	*F. oxysporum* f. sp. vasinfectum	Seedling blight of melon and wheat	Siven and Chet, 1986 [15].
7	*T. harzianum*	*F. oxysporum* f. sp. *vasinfectum*	Seedling blight of wheat	Sivan *et al.*, 1986 [15].
8	*T. harzianum*	*F. oxysporum* f. sp. *radius lycopersici*	Crown rot of tomato	Sivan *et al.*, 1986 [15].
9	*T. harzianum*	*F. solani*	Brown root rot of peanut	Calvet *et al.*, 1990 [5].
10	*T. harzianum*	*F. oxysporum* f. sp. *cicer*	Wilt of chickpea	Mukherjee and Mukhopadhyay, 2008 [12].
11	*T. harzianum*	*Pythium aphanidermatum*	Cause various crop of root rot and damping off disease	Bhardwaj and Gupta, 1987[4].
12	*T. harzianum*	*Rhizoctonia solani*	Sheath blight of rice	Wilson *et al.*, 1988[19].
13	*T. pseudokoningii*	*N. indica*	Karnal bunt wheat	Aggarwal *et al.*, 1992 [1].
14	*T. reesi*	*D. sorokiniana*	Spot blotch of wheat and barley	Mandal, 1995.
15	*T. harzianum & T. viridae*	*S. rolfsii.*	Root rot of sugar beet	Mathur and Sarbhoy 1978 [11].
16	*T. hamatum, T. viridae and T. harzianum*	*R. solanii Pythium* spp, and *Fusarium* spp.	Damping off of cotton	Elad *et al.*, 1982[7].

(Yedidia *et al.,* 2003) [20]. Harman *et al.,* (2004) [9] reported that the induction of localized or systemic resistance is an important component for plant disease control by *Trichoderma* spp. Thus, disease control by root-colonizing *Trichoderma* spp. involves a complex interaction.

Antibiosis: It is involved in releasing of secondary metabolites that inhibit the effect on other parasitic fungi and prevents them to become active parasites or kills them. It is not only effective against other forms of fungi but also active against various bacteria and virus that may cause threat to the fungi. The secondary metabolites released by the fungi are trichorovins and trichodecenins.

1. **Competition:** In order to dominance, it also competes with other fungi in a given ecosystem. For this Trichoderma species release different compounds like *T. longibrachiatum* produce iron chelating compounds siderophores that inhibit the activity and growth of its competitor from surroundings.
2. **Infection:** It causes infection in other organism for his survival and growth that leads them in various ecosystems.
3. **Host plant resistance:** It association with higher plants root by rhizosphere the fungi helps in increase root hair, biomass and develop resistance in plant root against various surrounding fungi and microbes. As *Trichoderma* hyphae either grow along the host hyphae or coil around it they secrete different lytic enzymes (Chitinase, Glucanase and Pectinase) that cause mycoparasitism.

Benefits of Trichoderma: The *Trichoderma* genus fungi are a very large group of microorganisms that play a vital role in environment protection. They perform a variety of mechanisms to colonise in various ecosystems and positively affect various plant by stimulating plant growth, and protecting them from various bacteria, fungi, virus, and other phytopathogens. Due to its very unique bio controlling nature it is largely used in agriculture. Some of its beneficial properties are following:

1. **Disease Control:** *Trichoderma* is a potential bio-control agent and it is used extensively for soil borne diseases as well as foliar diseases. It also produces cell wall degrading enzymes against the pathogens.
2. **Antibiotics**: It produces antibiotics that can kill the plant pathogens.
3. **Plant Growth Promoter:** it solubilises phosphorus and micronutrients, increases the root length and help them to increase availability of nutrient and avoid moisture stress during dry season.
4. **Biochemical Elicitors of Disease:** It induces resistance in plants by releasing various chemicals.
5. **Transgenic Plants:** *Trichoderma* gene endochitinase are successfully introduced with tobacco and tomato plants and it shows a great resistance to fungal growth and selected transgenic lines are highly resistance to foliar pathogens and soil borne pathogen.
6. **Bioremediation**: It plays a great role in bio remediation of soil contaminated with high pesticides and herbicides. As it degrades a wide range of pesticides in nature and free soil from various contaminations.
7. **Colonizing the Soil:** It grow faster near root region and occupying space and show dominance against other microorganisms.
8. **Part of Plants:** It starts symbiosis with plants root and covers root hair and occupy physical space or root rhizosphere that avoids the multiplication of other plant pathogens in rhizosphere. It also increases the length and depth of root.
9. **Nutrient Solubilising:** It solubilising the phosphorus and micronutrient and increases the availability to the plant.
10. It is cheaper than all chemical pesticides and significantly effective against various plant pathogens, so economic for farmers and for environmental safety.

Method of Application of *Trichoderma* culture

1. Rhizome, Corm, Bulb, Tuber Crop

Take one litter water and 5 to 10gm of *Trichoderma* than mixed it thoroughly and dip the rhizome/corm/bulb/tuber in it for minimum 30 minutes. Allow them to dry in shade for few minutes and then use it.

1. **Seed treatment:** For small size seed, Mix 4 gm of *Trichoderma* powder (Formulation having 2 x 10^6 cfu g^{-1} *Trichoderma* spp.) per kg of seed with water before sowing are recommended and for medium size seed, mix 8 gm of *Trichoderma* powder per kg seed with water is recommended and for large size seed, mix 10 g of *Trichoderma* powder with water per kg seed is recommended before sowing. After proper mixing of *Trichoderma* powder allow them to dry in shade.
2. **Seed Priming:** Seed priming is the process of controlled hydration of seeds to a level that permits pre-germinative metabolic activity to proceed, but prevents actual emergence of the radical. Seed priming with *Trichoderma* is performed in cereals, oil seed and pulses in which various materials (Cow Dung etc) are used with *Trichoderma* spp. powder (10 gm per kg seed).
3. **Soil Treatment:** In which *Trichoderma* spp. enriched vermicompost or compost are incorporated with field/soil before seed sowing. *Trichoderma* feed on dead and decay plant and animal body and it can be grown in acidic and basic soil having adequate amount of moisture. During composting it will grow and rapid multiply in this compost and when it applies in field it enriches the soil physical, chemical and biological properties and directly/indirectly influenced crop growth.
4. **Nursery Treatment:** Just before sowing in nursery apply 5 gm *Trichoderma* spp. powder or other *Trichoderma* spp. product in 100 m^2 of nursery bed.
5. **Cutting and Seedling root Treatment:** Mix 10 gm of *Trichoderma* spp. powder along with 100 gm vermicompost per liter of water and dip the cuttings and seedlings for 30 minutes before planting.
6. **Plant Treatment:** In perennial crop, drench the soil near root region 100 gm *Trichoderma* spp. powder are apply directly or along with compost is recommended.
7. **Foliage Apply:** it is spray over the foliage at 3 to 4 gm *Trichoderma* spp. after 8 to 10 days interval is recommended.

Precaution during Trichoderma Application

1. You should purchase *Trichoderma* formulation/powder from certified agency or store.
2. It should not be older than six month or expired or you must check the best before date on the packet.
3. It should be preserved in cool and shade place and not exposed in direct sun light and warm area.

4. Seed treatment and other treatment performed in cool and shade place and treated root; seed should not expose in direct sun light.
5. You should not apply any chemical pesticide before and after application of Trichoderma in crop field.
6. It can be used with any chemical fertilizer, bio-fertilizer (Rhizobium, PSB culture etc.) and compost.
7. You should ensure that soil/crop field have adequate amount of moisture before the application of *Trichoderma*.

Trichoderma formulations

In India, *Trichoderma viride* and *Trichoderma harzianum* are majorly used bio control agents for management of various diseases. Instead of this a large number of other species of *Trichoderma* are also have been commercialised. The potential *Trichoderma* isolates are formulated using different organic and inorganic carriers either through solid or liquid fermentation technologies.

These are examples of some solid and liquid formulation:

1. Talc based formulations
2. Vermiculite-wheat bran-based formulation
3. Pesta granules-based formulation
4. Alginate prills based formulation
5. Press mud-based formulation
6. Coffee husk-based formulation
7. Oil-based formulations
8. Banana waste-based formulations

Some of the important commercial formulations are available in the name of Sanjibani, Guard, Niprot and Bioderma. These formulations contain $3x10^6$ cfu per gram of carrier material. Generally, Talc is used as carrier for making powder formulation in India.

Trichoderma fungi is a potential bio control agent and to be used for human needs to an even greater extent than before. Nevertheless, further studies are needed to increase the efficiency and safety of the application of these fungi.

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