Volume 5 - Issue 03 - March,2025

SABUJEEMA
An International Multidisciplinary e-Magazine

Article ID: SIMM0514 Popular Article

TRICHODERMA AS POTENTIAL BIOCONTROL AGENT, ITS EXPLOITATION IN AGRICULTURE

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Abstract

The novel technologies in all areas of agriculture have improved agricultural Production, but some modern practices affect the environment. The recent challenge faced by advanced farming is to achieve higher yields in an environment-friendlymanner. Thus, there is an immediate need to find eco-friendly solutions. Among Various types of species being used as biocontrol agents, *Trichoderma* is widely used as biocontrol agent against different kinds of plant pathogens. *Trichoderma spp*. are asexual fungi that are present in all types of agricultural soils and also in decaying Wood. The hostile activity of *Trichoderma* species showed that it is parasitic on many Soil-borne and foliar plant pathogens. Recent studies showed that this fungus not only acts as biocontrol agent but also stimulates plant resistance, plant growth and development resulting in an increase in crop production. The antagonistic activity Involves mycoparasitism, antibiotics, competition for nutrients and also induces systemic resistance in plants. Currently, *Trichoderma spp.* are being used to control Plant diseases in sustainable disease management system. This paper reviews the already published information on *Trichoderma* as biocontrol agent, its biocontrol Activity and its commercial production and application in plant disease management Programs.

INTRODUCTION

The term 'biocontrol or biological control' was introduced for the first time in 1914 by Tubeuf and Smith in 1919 with special concern to plant pathogens and Insects respectively.Biocontrol refers to the reduction in Plant pest population by naturally occurring organisms that are part of IDM. A diversity of biocontrol agents (BCAs) or bio-fungicides are present in the ecosystem and there is need to isolate for bringing into play because BCAs have a low cost of production, long lasting effect on the growth of pathogen and no effect on human health. Only between 1-10% microbes show the ability to inhibit. Pathogen growth in vitro when fungal and bacterial isolates tested for biocontrol activities. Some of these broad-spectrum have the ability to suppress plant pathogen under in vivo Favorable conditions while few have broad-spectrum activities against miscellaneous pathogens texa.

Some important microbes belonging to different genera that are currently being marketed worldwide as biocontrol agent / biofertilizer are Agrobacterium, Ampelomyces Candida, Bacillus, Coniothyrium, Pseudomonas (Haas and Défago, 2005), Streptomyces and Trichoderma. These BCAs interact with plant and pathogens that suppress the Pathogen growth by direct and indirect mechanisms.

Trichoderma as biological control agent:-

In the early 1930s, *Trichoderma* was firstly reported as biocontrol Agent and species of genus *Trichoderma* are free-living and cosmopolitan fungi in Soils, decaying organic and vegetable matter. Richoderma attacked other plant pathogenic fungi and Promotes plant and root growth. It uses different Mechanisms for the control of plant pathogenic Pathogens including antibiosis, mycoparasitism, the Induced resistance of host cell and competition for Nutrient and space

Trichoderma biology:

Mycoflora belonging to genus *Trichoderma* and is classified as imperfect fungi belonging to orderorder Hypocrites of Ascomycota .The salient feature of this genus is the ability to parasitize other pathogenic fungal mycoflora specially associated with root rot and Wilt diseases .

Morphological characteristics:

conidia shape is ellipsoidal to oblong and some Trichoderma species have globose to subglobose with the length/width ratio 1.4 and 1-3 respectively. while few species have smooth conidia Conidia color morphology varies from species to species but typically green or may be gray, white and yellow.

Effect of environment on Trichoderma:

The environmental and nutritional parameters play an important role in enhancing mycelial growth and biomass production of Trichoderma species and growth and multiplication of biocontrol agents varies with the substrates . Species of Trichoderma spp. Are reported to be more Sensitive to light and nutrient media. All microorganisms growth including Trichoderma species Is influenced by environmental parameters that affect Antagonistic potential of biocontrol agents. Among the Environmental parameters pH is the most important factor That affects mycelial growth of Trichoderma. Maximum Growth and sporulation of T. viride was observed at 4.5-6.0 pH. The influence of pH on biomass and mycelial growth demonstrated that acidic pH is the most important key Factor for biomass production of all Trichoderma species.

Importance of *Trichoderma spp*:

Trichoderma species are opportunistic, avirulent, and Plant symbionts that can compete as well as survive in the Complex ecosystem (Harman et al., 2004b). Although, These are capable of successful root colonizer and their Number increases when abundant healthy roots are Present in the ecosystem (Brotman et al., 2008) and Protect the roots and plants from pathogens as well as Diseases (Howell, 2003). They increase plant resistant Ability against drought conditions and promote the Growth of a plant by phosphate, micro-nutrients, andSolubilization (Kumar, 2013). Some species of Trichoderma are efficient producer of extracellular Enzymes that degrade complex compounds of Polysaccharides and also used commercially (Samanta et Al., 2012). Trichoderma species are environmental Friendly (Singh et al., 2008) and an alternative to Synthetic chemicals (Gupta and Dikshit, 2010) that Developed symbiotic relationship with plants rather than Parasitic relationship reduced chances of behavioral Changes in human caused by the use of synthetic Chemicals (Brimner and Boland, 2003).

Efficiency of *Trichoderma spp*

Uses of *Trichoderma spp*.:

The discovery of Cellulase production by Trichoderma reesei, which Was isolated by Reese (1976), led to it becoming a Very important cellulase or enzyme producer. The cellulase produced by Trichoderma spp. is used mainly for malting, baking, and grain alcohol production. The filamentous cellulolytic Trichoderma spp., produce a broad range of cellulases and hemicellulases. The main application of lignocellulosic biomass is the

Table 1: Efficacy of *Trichoderma* species against soil-borne fungal pathogens.

Trichoderma strains	Pathogen(s)	Plant/ Crop	Disease	Efficacy (Inhibition)	Experiment	Reference
T. harzianum TH-H-3 *T. virens* TV-K-3	*Rhizoctonia solani*	Tomato	Wilt	5 %	Pot Exp.	Kumar (2013)
T. harzianum *T. viride*	*Fusarium solani*	Tomato	Root rot	70-72%	*In vitro*	Haggag and El-Gamal, 2012
T. harzianum *T. viride*	*R. solani*	Tomato	Damping off	51% 39%	*In vitro*	Haggag and El-Gamal (2012)
T. harzianum Mutants	*R. solani*	Tomato	Damping off	40% in greenhouse 100% in field	Greenhouse and field	Montealegre et al. (2010)
T. viride (Tv-R)	*M. phaseolina*	Chickpea	Dry root rot	62%	laboratory conditions	Manjunatha et al. (2013)
T. harzianum *T. viride* *T. virens*	*R. bataticola*	Mungbean	Dry root rot	87%	Pot and field conditions	Dubey et al. (2009)
T. harzianum T22	*Pythium ultimum*	Tomato	Wilt	74%	*In vitro*	Mastouri et al. (2010)
T. harzianum T-22	*F. verticillioides*	Maize	Ear and kernel rot	65% reduce size of necrotic area		Ferrigo et al. (2014)
T. harzianum *T. viride*	*F. oxysporum* f. sp. *Ciceris*	Chickpea	Chickpea wilt	44-60%	Field exp.	Dubey et al. (2007)
T. harzianum	*F. oxysporum* f. sp. *Radicis cucumerinum* *Botrytis cinerea*	Cucumber *Arabidopsis thaliana*	Stem and root rot	12-79%	Pots experiments	Alizadeh et al. (2013)
T. viride	*F. oxysporum* f. sp. *adzuki*	Soybean	Root rot	-	*In vitro*	John et al. (2010)
Trichoderma spp.	*Pythium arrhenomanes* *Phytophthora cactorum*	Strawberry	Damping off Leather rot	88% in 2001 97.6% in 2002 99.0% in 2003	Field Exp.	Porras et al. (2007)
T. harzianum *T. viride* *T. virens* *T. koningii* *T. pseudokoningii*	*Alternaria tenuissima*	Sorrel	Leaf spot	67-76% 78-80% 72-77% 77-80% 72-80%	*In vitro*	Ambuse et al. (2012)
T. harzianum 1	*M. phaseolina* *F. solani* *R. solani*	Cotton	Charcoal rot Wilt and boll rot Boll rot and leaf spot	72% 71% 58%	*In vitro*	Asran-Amal et al. (2010)
T. atrovirde *T. Longibrachiaum* *T. virens* *T. hazianum.*	*F. sambucinum*	Potato	Potato dry rot	*T. longibrachiatum* showed the strongest inhibition	*In vitro*	Ru and Di (2012)
T. harzianum	*A. alternate*	Tobacco	Brown spot	Diffusible metabolites more effective than volatile diffusible	*In vitro*	Gveroska and Ziberoski (2012)
T. harzianum T39	*B. cinerea,* *Pseuperonospora cubensis,* *Sclerotinia sclerotiorum* *Sphaerotheca fusca*	Cucumber	Grey mould Downy mildew white mould Powdery mildew	35-44% on fruit and 43-64% on stem 48-78% 64 on fruit and 30-35% on stem, 45-71 %	Greenhouse	Elad, 2000

production of biofuels such as ethanol although it is also used in the pulp, paper and textile industries. Trichoderma is also used for safe industrial enzyme production. Macerating enzymes are used to improve the brewing process for fruit juice production and as a feed additive for livestock and pet food. Trichoderma also used for seed germination, for example, a study showed that sunflower seeds germination significantly increased in T. viride or T. resei treated plants compared to control plants. The commercial use of several Trichoderma species for the protection and growth enhancement of a number of crops is ongoing (Samuels, 2006). Currently, the commercially available formulations are RootShield TM, BioTrek 22 TM, T- 22G TM, and T-22 HBTM (Bio-works, USA); Suprevisit TM (Borregaard BioPlant, Denmark); Binab TM (Bio-Innovation Sweden); Trichopel TM, Trichojet TM, Trichodowels TM, and Trichoseal TM (Agimm, New Zealand); Trieco TM (Ecosense Labs, India), and Tricho-green (Mycology Lab, Malaysia).

Mechanisms of trichoderma:

The most important and Fascinating feature of Trichoderma is the study of Mechanisms varying for management of phytopathogens And plant diseases in which pathogen antagonized by Biocontrol agent results from different types of Interaction between organisms (Pal and Gardener, 2006). The followings are direct and indirect biocontrol Mechanisms to control plant pathogens (Figure 1).

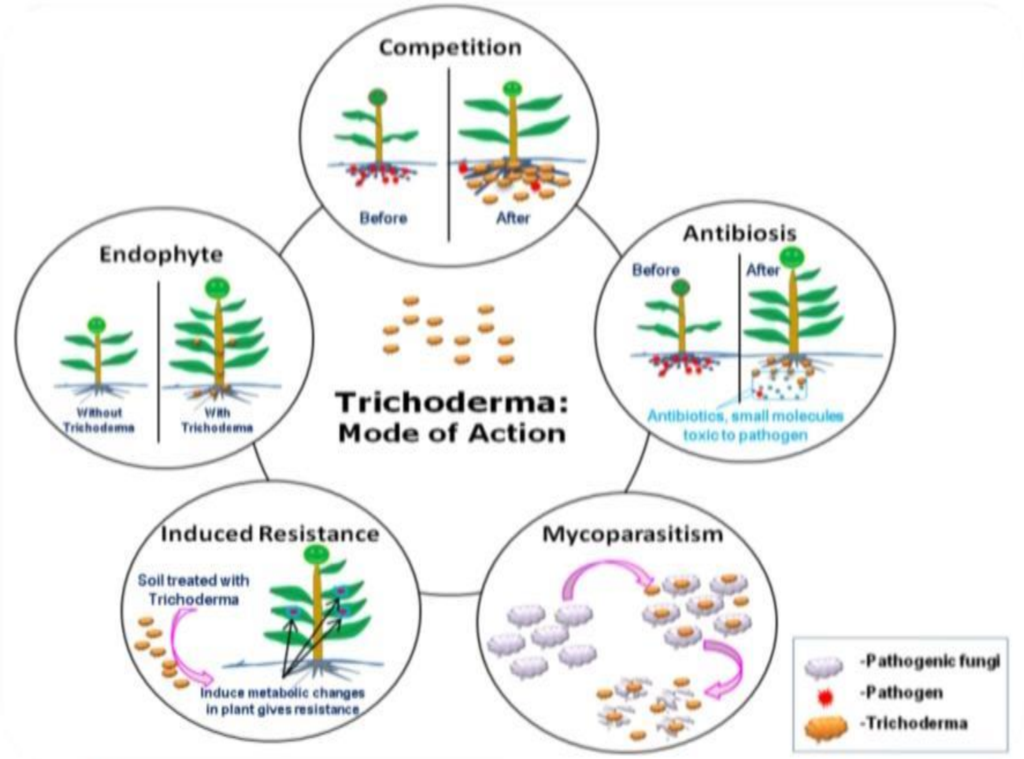

Figure 1. Model depicting mode of action of Trichoderma spp. against pathogen and plant growth improvement.

Direct Antagonism:

Direct antagonism results in the physical contact of Biocontrol agent with the pathogen. It includes;

Mycoparasitism: Mycoparasitisim or hyperparasitism is a complex process (Harman et al., 2004b) which involves The parasitic interaction of two or more fungi in which One parasitize mycelia of other (Druzhinina et al., 2011)And species of Trichoderma parasitize a wide range of Mycoparaites especially soil-borne pathogens. In this process, firstly Trichoderma species sense the pathogen and come into Contact with host involves morphological changes such as Coiling and appressorium formation which developed Hole on the surface of host or target pathogen (Omann And Zeilinger, 2010). Secondly, Trichoderma species Recognize signals from host fungus that activate Penetration of Trichoderma hyphae into the lumen of Target parasitized fungus (Kubicek and Druzhinina, 2013). Thirdly, active multiplication takes place inside The hyphae of target fungi. Trichoderma and pathogen Attachment mediated by binding of carbohydrates and Lectin that are present in Trichoderma cell wall and target Pathogen respectively.

Mixed- path Antagonism

Antibiosis and secondary metabolites

Trichoderma Species cause decay of phytopathogenic fungi without any Physical contact between microorganisms by producing The antimicrobial compounds. This process generally Called as “antibiosis” and term secondary metabolites is a Group of heterogeneous chemically divergent natural Compounds might be associated with survival functions Such as symbiosis, differentiation, and competition Against organisms etc. for the producing organism. Most of the Trichoderma Species produced volatile and non-volatile metabolic Compounds including tricholin, massoilactone, heptelidic Acid, gliovirin, 6-penthyl-□-pyrone, harzianic acid, Glisoprenins, peptaibols, alamethicins, and others have Been studied (Qualhato et al., 2013) which are toxic to Target pathogen. The synergetic effect of antibiotics and Hydrolytic enzymes achieve maximum level of Antagonism rather than alone mechanism (Monte, 2001). T. harzianum and T. virens are the most effective Biocontrol agents with respect to antibiotics that produce Gliovirin and pyrone respectively.

Induced systemic host resistance:

Induction of host Resistance in the plant is a complex mechanism. Generally, There are three pathways to induce resistance in the host Plant. Two of these involve direct assembly of Pathogenesis-related (PR) proteins that can be induced By the mechanism of other organisms. In one pathway PR Proteins production results by an attack of pathogenic Microbe while in other pathway PR proteins production As a result of necrosis or wound i.e. herbivory by insects. In the third type pathway of resistant induced by root Associated with non-pathogenic bacteria such as Rhizobacteria referred to as Rhizobacteria-Induced Systemic Resistance (RISR).

Species	Plant species	Pathogens	Outcome	References
T. virens	Cotton	*Rhizoctonia solani*	Protected plant by inducing terpenoid phytoalexins toxic to fungi	Kumar et al. (2009)
T. harzianum	Pepper	Phytophthora capsici	Improved production of the phytoalexins capsidiol toxic to pathogen	Ahamed and Vermette (2009)
T. virens	Tomato	*Pseudomonas syringe*	Secreted proteins-Sm1 and Ep11 both induced systemic acquired resistance	Salas-Marina et al. (2015)
T. asperellum	Cucumber	*Pseudomonas syringe*	Modulated the expression of proteins related to jasmonic acid/ethylene signaling	Shoresh et al. (2005)

Endophytic activity:

Endophytic activity of many Microorganisms (growth inside plant tissue without any Harm) may useful to host plant by stimulating of plant Growth, a postponement to the beginning of drought Stress and the obstruction to pathogens. Endosymbiotic Species are capable of establishing colonies in plant roots And triggers the expression of many plant genes affecting Stress responses. Recently, there are reports showing Trichoderma isolates acting as endophytic plant Symbionts in some woody plants (Chaverri et al., 2011).

Commercialization of Trichoderma products:

Commercialization of Trichoderma or biocontrol agents Depends upon the screening process of biocontrol Microorganism and its efficacy against pathogenic Mycoflora. The first species of Trichoderma (*Trichoderma Harzianum*) registered with EPA in 1989 for control of Plant pathogens and diseases (Fravel, 2005).Commercialization of biocontrol products is a multi step

Process and includes :

- Isolation of microorganisms
- Evaluation of antagonists in lab and field Conditions
- Selecting best isolate in field conditions
- Mass production
- Formulation
- Delivery
- Compatibility
- Registration and release

Commercial Product/ Trade name	*Trichoderma* species	Target disease	Company/ Manufacturer or distributor
Anti-Fungus	*Trichoderma* spp.	Root rot	Grondoontsmettingen De Ceuster, Belgium
Binab	*Trichoderma* spp.	Root rot and wilt	Binab, Sweden
Biofungus, Superesivit	*Trichoderma* spp.	Root rot and wilt	Bioplant, Denmark
Root Pro	*T. harzianum*	Root rot	Efal Agri, Israel
Root Shield, Plant Shield, T-22 Planter box	*T. harzianum* T-22	Root rot	Bioworks, Geneva, USA
T-22B, T-22G	*T. harzianum T-22*	Root rot	TGT Inc. New York, USA
T35	*T. harzianum*	Wilt	Makhteshim-Agan Chemical Israel
ECO T/T22	*T. harzianum*	Root rot	PHP Ltd., South-Africa
GlioGard and SoilGard	*T. virens*	Root rot	Grace-Sierra Co. Maryland
Harzian 20, Harzian 10	*T. harzianum*	Wilt	Natural Plant Protection, Noguerres, France
Biospark Trichoderma	*T. parceramosum* *T. pseudokoningii*	Wilt	Biospark Corporation, Phillipines
F-stop	*T. harzianum*	Root rot	Eastman Kodal Co. TGT Inc., New York, USA
Soil Gard	*T. virens* GL-21	Root rot	Certis, USA
Tricho-X	*T. viride*	Root rot	Excel Industries Ltd., ,India
Trichodex	*T. harzianum*	Grey mold	Makhteshim Chemical Works, Israel
Trichopel	*Trichoderma* spp.	Root rot	Agrimm Technologies, New Zealand
Bip T	*T. viride*	Wilt	Poland
Trieco	*T. viride*	Root rot, wilt	Ecosense Laboratories, India
Pant biocontrol agent-1	*T. harzianum*	Root rot, wilt	Deptt. of Plant Pathology, GB plant University of Agriculture & Technology, Panatnagar, Uttarakhand
Plant helper	*T. atroviride*	Root rot	Ampac, California

Methods of application Trichoderma species

Seed treatment:

For this purpose, mix 10 grams Trichoderma Formulation for 1 kg of seed to per liter of cow dung Slurry before sowing especially for pulses and cereal Crops.

Nursery treatment:

Before sowing of The crop, drench nursery bed treated with @ 5kg Trichoderma formulation per liter of water

Cutting and seedling root dip:

Dipping of cutting and seedling roots before planting for 10 minutes in a mixture of 10 g Trichoderma formulation Per liter of water.

Soil application:

Soil can also be treated With 1 kg Trichoderma formulation mix with 100 kg FYM and cover it with polythene for 7 days. Turn the position of the mixture after 4-5 days interval and apply in the field.

Application recommendation and precautions:

Recommendation:

All type of plants and vegetables can Be treated with Trichoderma for better production such As tomato, potato, pepper, tobacco, sugar beet, sugarcane, Brinjal, turmeric, ginger, betel vine, banana, eggplant, Cotton, chilies, cardamom, onion, maize, cucumber, Peanut, red gram, white gram, Lentil, chickpea, cassava, Citrus etc.

Precautions:

Some precautionary measures should be Kept in mind regarding the application of Trichoderma Inoculums in the field condition. These are given below:

- Don't settle treated Farm Yard Manure (FYM) for a Longer time.
- Don't place Trichoderma treated seed in direct sunlight.
- Don't apply chemical pesticides or fungicides after

  Application of Trichoderma for 5-6 days.
- Moisture is an important factor for Trichoderma Growth and reproduction so don't try to use it in dry soil.

Sensitivity against agrochemicals:

The efficiency of the Bioagents is hampered due to poisonous nature of Fungicides which are used simultaneously in crop Production technology. Therefore, the sensitivity and Tolerance of Trichoderma have been tested by our group And many others . Trichoderma spp. have shown greater tolerance for broad spectrum fungicides than many other soil microbes as it has the capacity to colonize the pesticides treated soil more rapidly.

Future prospects:

Sustainability is also the major driving force for the investigation of biocontrol with Trichoderma. As opportunistic plant symbionts and effective mycoparasites, numerous species of this genus have the potential to become commercial biofungicides. The challenge in this field of research will be the development of reliable screening techniques, which allow for prediction of the biocontrol efficiency of a given isolate by determination of the key factors for this process.Trichoderma As biocontrol agent is utmost important part of integrated Plant disease management that can be used against soil-Borne phytopathogens but its biocontrol potential is yet To be limited to laboratory experiments and very Diminutive attention has been paid to its commercial Formulation. Moreover, farmers also have lack of Information concerning its utilization. So, the concept of Trichoderma commercialization needs to be improved And cost-effective production formulation should be Popularized molecular tools and genetic engineering need to be Performed for improvement of BCAs that can be able to Proliferate and compete against a wide range of Phytopathogens. So, this is necessary to give the support To agencies that are engaged in this field.

CONCLUSION:

Biological control gives the impression of an alternative to chemical-based pesticides for disease suppression and control. Scientists and their research have proved that Trichoderma is non-pathogenic to plants and need to be formulated in a way that favors the activity and survival of microbes. Moreover, the novel concept of biocontrol needs a space outside the laboratory to see its fruits in present production systems.