

Review

Trichoderma- a potential and effective bio fungicide and alternative source against notable phytopathogens: A review

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Trichoderma, is a useful, filamentous fungi and they are cosmopolitan in nature which have attracted the attention because of their multi prong action against various plant pathogens. There are several biocontrol mechanism employed by *Trichoderma* against plant pathogens, these include release of hydrolytic enzymes that degrade cell wall of phytopathogenic fungi, competition for nutrients, parasitism, and antibiotics. *Trichoderma* spp. generally grows in its natural habitat on plant root surface and therefore it controls root diseases in particular.

Key words: *Trichoderma*, anatagonism, phytopathogens.

INTRODUCTION

Modern agricultural practices are getting affected by various problems such as disease, pest, drought, decreased soil fertility due to use of hazardous chemical pesticides, bio-magnification and various environmental problem. The fungal disease is one of the major constraints for agriculture productivity in India losses is about 22 to 25% of its crops due to fungal diseases each year. This damage is estimated to be Rs.50,000 crores annually. Although, use of fungicides in crop protection has certainly contributed for minimizing yield losses. So, indiscriminate and unscientific use chemical pesticides for prevention of various fungal disease leads to cause of resurgence of new pest, fungicidal resistance, ultimately affecting the land, soil fertility and remains toxic when consumed by humans and animals. Their application needs care and must only be used when the threshold

limits exceeds. As a result, there is a need for some eco-friendly biocontrol agents that help in resolving problems. Hence, there is a need to look forward to develop practices and eco-friendly bio-control in order to reduce the dependence on synthetic agrochemicals. Therefore, recent trends favor the use of alternative substances derived from natural plant extracts, biological control and several others components. The various types of biological control agents such as bacteria and fungi are involved in biocontrol activity. Biological control is an essential part of these strategies as a substitute of agrochemicals.

Biological control agents (BCAs) offer an alternative to the chemical control of fungal phyto-pathogens as they can kill or limit the growth of pathogens without harming the host. Various bacteria, fungi and protists are known to

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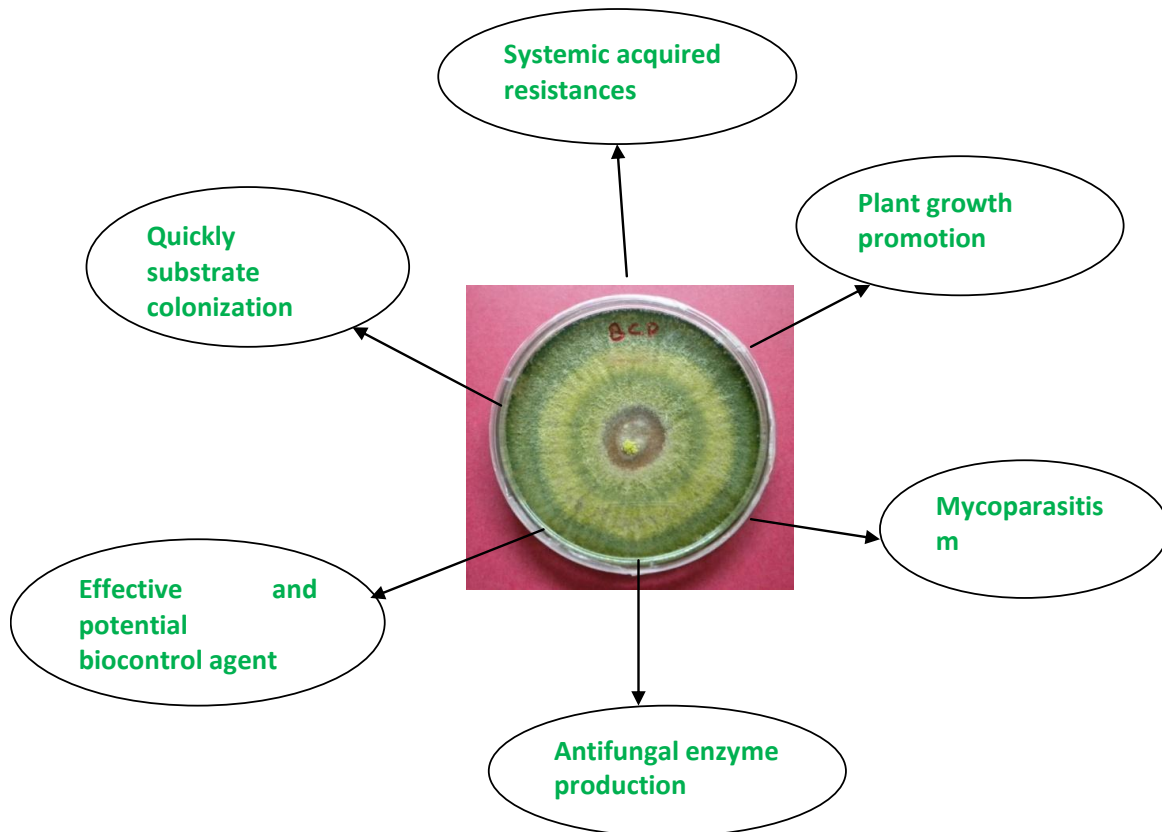


Figure 1. Biocontrol activities exhibited by *Trichoderma*.

have such features. They do so either by one or many of the following mechanisms: Competition for nutrients, secretion of lytic enzymes, secretion of toxic metabolites and direct parasitism on host (Agrios, 2005). Members of the genus *Trichoderma* have shown promise as BCAs (Elad et al. 1983; Chet 1987). The first practical control of crop disease with *Trichoderma* was in the control of root rot causing (*Armillaria mellea*) in citrus. It has since been very effectively used for the control of large number of soil borne plant pathogen like *Phytophthora*, *Rhizoctonia*, *Sclerotium*, *Phythium*, *Fusarium*, *Sclerotinia*, and *Galumannomyces*. Presently, entrepreneurs are mass producing its three main species viz, *Trichoderma harzianum*, *Trichoderma viride* and *Trichoderma koningii*.

They also have important economic impact, due to their ability to produce hydrolytic enzymes, secondary metabolites and degradation of xenobiotics (Harman et al., 2004). This versatile fungus is also used commercially in food and textile industry and as a source of transgene to develop disease resistance transgenic plants. This ecological and economic relevance of the genus *Trichoderma* makes it an ideal candidate as a model organism in various studies, spanning from molecular plant pathology, plant protection, biological control, and microbial ecology. Due to their rapid growth and capability of utilizing different substrates, species of

this genus are often predominant components of the soil mycoflora in various ecosystems, such as agricultural, pasture, forest, salt marsh, prairie and desert soils over a wide range of climatic zones.

Trichoderma spp, native isolates must therefore be isolated and characterized for BCA activity so that they could be integrated into integrated pest management.

Fungal species belonging to the genus *Trichoderma* are worldwide spreads and easily isolated from soil by serial dilution technique. They are known as imperfect fungi, because they have no sexual stage. Faster growth rate in culture and production of conidia that are varying shades of green characterize fungi in the genus (Chaverri and Samuel, 2003). *Trichoderma* species are fungi with telomorphs belonging to the *Hypocreales* order of Ascomycotina division.

Trichoderma species are being shown to be the anamorphs of hypocreale species. Hypocrea was first described by Elias Fries in 1825 based on *sphaeria rufa* pers.: fr., a species with hyaline, ascospores.

In order to make the most effective use of biological control agent we must understand the mechanism of biological control. The various biocontrol mechanisms employed by *Trichoderma* are as follows (Figure 1):

1. Induced systemic resistances: Induced systemic

Table 1. Secondary metabolites release by *Trichoderma* species.

Name of Secondary metabolites	Release by	References
(Mannitol)	<i>T. hamatum</i>	hussain et al. (1975)
2- hydroxymalonic acid	<i>T. pseudokonigii</i>	kamal et al. (1971)
Trichodermaol	<i>Trichoderma species</i>	Adachi et al. (1983)
p-hydroxybenzyl alcohol	<i>T. konigii</i>	hung et al. (1995a)
1-hydroxy-3-methylantraquinone	<i>T. viride</i> PrL 2233	slater et al. (1967)
Pencolide	<i>T. album</i>	Ren 1977
Viridifungin A	<i>T. viride</i> ATCC74084	Harris et al. (1993)
Trichodermene A	<i>T. pseudokonigii</i>	Kamal et al. (1971)
Carbolic acid	<i>Trichoderma species</i>	Turner and Aldridge (1983)
Dermadin methyl ester	<i>T. polysporum</i>	Jin and jin (1989)
Isonitrin A	<i>T. harzianum</i> IMI 3198	Baldwin et al. (1991)
Spirolactone	<i>T. hamatum</i> HLX 1379	Baldwin et al. (1985a)
Dermadin	<i>T. viride</i> uc 4875	Pyke and Dietz (1966)

resistances (ISR) of plants against plant pathogens is a widespread phenomenon with respect to the underlying signaling pathways. After primary pathogen infection, plants are capable of producing an immune response which is known as systemic acquired resistances, the expression of pathogenesis genes such as acidic and basic β -1, 3 glucanase and chitinase and endochitinase are involved for the activation of SAR. The peptides, proteins and low molecular weight compounds produced by *Trichoderma* and these compounds induce ethylene production, hypersensitive response and other defence related reactions in plants.

2. Fungistasis: Most soils inhibit fungal germination and growth to a certain extent, a phenomenon known as soil fungistasis. *Trichoderma* grow rapidly when applied in soil, because they are resistant to many toxic compounds, including fungicides, herbicides and pesticide such as DDT.

3. Production of cell wall degrading enzymes (CWDES): Chitinase, β -1, 3 glucanase produced by some *Trichoderma* species they are key enzymes in lysis of cell wall during their mycoparasitic action against plant pathogenic fungi.

4. Competition with other fungi: *Trichoderma* and other plant fungi feed on the waste products of the plant. Because *Trichoderma* can colonize the roots very rapidly it has a benefit in the competition for these nutrients.

5. Mycoparasitism of other fungi: *Trichoderma* can parasitize other fungi and release a digestive enzyme which breaks down the chitin in these fungi. In this way nutrients will be extracted from them.

6. Secondary metabolites: They are producers of extracellular proteins, and are best known for their ability to produce enzymes that degrade cellulose and chitin. They also produce other useful enzymes such as endochitinase, chitinase, glucanase, cellulase play an important role in biocontrol activity. Different strains produce more than 100 different metabolites that have

known antibiotic activities. It is found that large variety of volatile secondary metabolites produced by *Trichoderma* such as ethylene, hydrogen cyanide, aldehydes and ketons which play an important role in controlling the plant pathogens (Table 1).

MAAS PRODUCTION OF TRICHODERMA

In India talc based formulation was first of all developed in Tamil Nadu Agriculture university, Coimbatore (Jeyarajan et al., 1994). Talc based formulation is formed by both solid and liquid state fermentation.

Solid state fermentation

This is the most common method for *Trichoderma* mass production. Several grains like sorghum, corn, rye and millets are used as substrates for *Trichoderma* mass production. These grains are moistened, sterilized and inoculated with *Trichoderma* spp and incubated for 10 to 15 days. *Trichoderma* produces dark green coloured spores covering the whole grain. These grains can be powdered and mixed in sterilized talc powder in 1:9. The finally processed product can be used for soil and seed treatment. Solid state fermentation is used for commercial level production; the main disadvantage associated with this technique is that it is very tedious and time consuming.

Liquid state fermentation

In liquid fermentation system *Trichoderma* is grown in liquid medium in shaker and fermenter and after the incubation period completes the fermented media is mixed in sterilized talcum powder in 1:9 ratio. Maximum

Table 2. Growth media used for production of *Trichoderma* in liquid state fermentation.

Growth media	References
Molasses and brewers yeast	Sankar and Jeyarajan (1996)
Potato dextrose broth, V -8 juice and Molasses-yeast medium	Prasad and Rangeshwaran (1998)
Molasses soy medium	Prasad and Rangeshwaran (2000)
Jaggery-soy medium	Prasad et al. (2002)

Table 3. Agro domestic wastes used to produce *Trichoderma* sp. by solid state fermentation.

Substrate	References
Sorghum grain	Upadhyay and Mukhopadhyay (1986)
Wheat bran	Mukhopadhyay (1986)
FYM,Rice straw	Kousalya (1990), Sangeetha et al. (1993)
Spent compost of mushroom	Singh et al. (2012)
spent tea leaf waste and coffe husk	Bhai et al. (1994)
Decomposed coconut coir pith spent malt	Gopalkrishanan et al. (2003)
Neem cake + wheat flour 10%	Singh et al. (2012)

biomass of *Trichoderma* can be produced in short time by using appropriate fermentation media and fermentation conditions such as aeration, agitation, temperature, pH and antifoam control. Project Directorate of Biological Control (PDBC) Bangalore revealed that maximum amount of biomass and viable propagules of *T. harzium*/ *T. viride* can be obtained within 96 h of fermentation in a fermentor with aeration, agitation, temperature control (Prasad and Rangeshwaran 1998) (Table 2)

AGRO DOMESTIC WASTES AS SUBSTRATES FOR THE MASS MULTIPLICATION OF *TRICHODERMA*

For mass production the first step is to identify a suitable substrate which should be cheap, stable and easily available. The various agro domestic wastes used for *Trichoderma* mass production are Corn flour, sorghum grain, wheat bran, farm yard manure, Tea waste, wheat straw rice bran vegetable waste.

Various agricultural and domestic wastes such as: Corn flour, sorghum grain, wheat bran, farm yard manure, tea waste, wheat straw, rice bran, vegetable waste, had been evaluated for the mass multiplication of *Trichoderma* (Table 3). For formulation purpose all the waste materials are dried and grounded to a fine powder and after this they are filled in autoclavable bags. The materials were subjected to 45% of moisture and sterilized twice at 121°C. Seven day old culture of *Trichoderma* sp. was used to inoculate the waste medium. The spore suspension of the *Trichoderma* in DDW was made (1×10^6 conidia per ml). The plastic bags were then inoculated with the 10 ml of *Trichoderma* spore suspension and incubated at 28°C. After four days of incubation the

inoculated bags were transferred in clean and sterilized plastic trays and the trays were covered with thin plastic film. After ten days of incubation the substrate is mixed with sterilized talcum powder and CFU of *Trichoderma* was calculated on every substrate (Figure 2).

SELF LIFE OF *TRICHODERMA* FORMULATIONS

Shelf life is the most important factor which determines the quality of *Trichoderma* bioformulation. Talc, peat, lignite and kaolin based formulations of *Trichoderma* have shelf life of 3 to 4 months. CFU count of *Trichoderma* in talc formulation has been found to reduced to 50% after 120 days. At PDBC in Bangalore, it was found that the shelf life of *Trichoderma* formulation was increased using various ingredients (chitin and glycerol) and heat shock at the end of log phase of fermentation was carried out which extends the shelf life of talc based formulation up to one year (Sriram et al., 2010; Sriram et al., 2011).

EFFECT OF THE ADDITION OF COLLOIDAL CHITIN EXTRACTED FROM SEA SHELLS ON THE SHELF LIFE OF *TRICHODERMA* SP.

To study the effect of colloidal chitin in on the shelf-life of talc formulations of *Trichoderma* sp. Colloidal chitin was prepared and added at the rate of 1, 2 and 3% (w/v) in PDB (Potato Dextrose Broth) and Sorghum Based Medium used in liquid as well as solid state fermentation. And CFU was counted regularly to check the effect of colloidal chitin on shelf life of *Trichoderma* bioformulation.



Figure 2. Effect of different agro and domestic based substrates on the population of *Trichoderma harzianum* (Th. Azad).



Figure 3. Different Concentration (1, 2 and 3%) of colloidal chitin in potato dextrose broth base medium.

Addition of colloidal chitin at 2% in production medium enhanced the shelf-life as compared to control (Figures 3 and 4)

Application of *Trichoderma* formulations

Several methods are recommended for the application of Bio fungicides for the successful management of plant diseases. Delivery and establishment of *Trichoderma* (bio fungicide) also play a very important role to the site of action (Table 4). The most common methods of application of *Trichoderma* are, by seed bio priming, Seedling dip, soil application and wound dressing, and foliar spray is rare.

Seed treatment

Seed treatment is one of the easy and effective method. In seed treatment seed is coated with dry powder/ dusts of *Trichoderma* just before the sowing. For commercial purpose, dry powder of antagonist is used at 3 to 10 g per kg seed based on seed size (Mukhopadhyay et al., 1992). *T. harzianum*, *T. virens* and *T. viride* were found to be effective seed protectants against *Pythium spp.* and *Rhizoctonia solani* (Mukherjee and Mukhopadhyay, 1995).

Seed biopriming

In this method seeds are treated with *Trichoderma* formulation and incubated under warm and humid conditions just prior to the emergence of radical. This technique has potential advantages over simple coating of seeds as it results in rapid and uniform seedling emergence. *Trichoderma* conidia germinate on the seed surface and form a layer around bioprimered seeds. Such seeds tolerate adverse soil conditions better.

Root treatment

Seedling roots can be treated with spore or cell suspension of antagonists either by drenching the bioagent in nursery beds or by dipping roots in bioagent suspension before transplanting. This method is generally used for the vegetable crops, rice where transplanting is practiced (Singh and Zaidi, 2002).

Soil treatment

There are several reports on the application of biocontrol agents to the soil and other growing media either before or at the time of planting for control of a wide range of

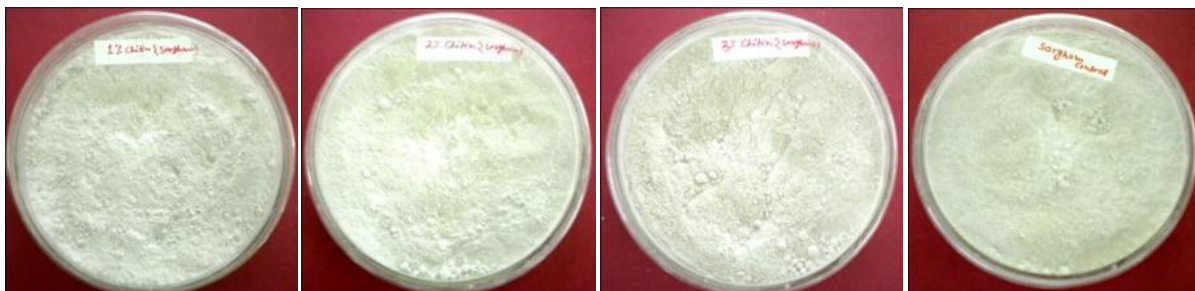


Figure 4. Different concentration (1, 2 and 3%) of colloidal chitin in sorghum base medium.

Table 4. *Trichoderma* species and its uses against different plant pathogens.

Bioagent	Plant pathogen	Crop
<i>T. viride</i> , <i>T. longibrachiatum</i> , <i>T. virens</i>	<i>Pythium</i>	Ginger
<i>T. viride</i> , <i>T. harzianum</i>	<i>Colletotrichum</i>	Chilli
<i>Trichoderma koningii</i>	<i>Ustilago segetum var tritici</i>	Wheat
<i>T. harzianum</i>	<i>Phytophthora</i>	Cardomom
<i>Trichoderma</i> spp.	<i>Cercospora moricola</i>	Mulberry
<i>T. viride</i> , <i>Trichoderma</i> species	<i>Fusarium udum</i>	Pigeon pea
<i>T. viride</i> , <i>T. harzianum</i>	<i>Botrytis cinerea</i>	Rose

soil-borne fungal pathogens (Baby and Manibhushanrao, 1996). Such applications are ideally suited for greenhouse and nursery.

Aerial spraying/wound dressing

Trichoderma has been successful applied to the aerial plant parts for the biocontrol of decay fungi in wounds on shrubs and trees (Papavizas, 1985).

CONCLUSIONS

Bio fungicide is one of the best alternatives against some notable plant pathogens. The limitations to biocontrol use are due to lack of knowledge on the ecology of the rhizosphere and use of in vitro antagonism for selection of biological control agents. But, the advantages of this method are more. *Trichoderma* spp. that are common saprophytic fungi found in soil, have been investigated. And it has been emerged as a as potential biocontrol agents because of their ability to reduces the incidences of disease caused by plant pathogenic fungi.

Conflict of Interests

The authors have not declared any conflict of interests.

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