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Trichoderma Spp.: AS POTENTIAL BIO-CONTROL AGENTS (BCAs) AGAINST FUNGAL PLANT PATHOGENS

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ABSTRACT

Bio-control agents (BCAs) are in great demand for replacing the chemical pesticides to control fungal plant pathogens. About 70% of fungal BCAs market comprises of *Trichoderma* spp. On 26th March 1999, *Trichoderma* has been included in the gazette of India as potential bio-control agents. *Trichoderma* spp is extensively used for controlling several soil and seed borne fungal plant pathogens. *Trichoderma* spp is mainly preferred to other fungal bio-control agents owing to the antagonistic properties include the competition for key nutrients, inducing host-plant resistance, production of antibiotics and hydrolytic enzymes, viz. hemicellulases, cellulases, chitinases, proteases and β-1, 3-glucanase that are involved in the process of mycoparasitism.

KEYWORDS : *Trichoderma spp.*, BCAs, Fungal Plant Pathogens

The genus *Trichoderma* Pers. Ex Fr. exists in nearly all agricultural soils and in other environments and is very common in diverse habitats (Samuels, 2006). The species of genus *Trichoderma* has been reported as most potential biocontrol agents (BCAs) against a wide range of soil-borne plant pathogens (Lewis and Papavizas, 1991; Haran et al., 1996a; Haran et al., 1996b; Elad, 2000; Joshi et al., 2010; Hermosa et al., 2012) due to their ability to successfully antagonize other fungi. Establishment of the *Trichoderma* and other biocontrol agents in the soil ecosystem has greatly affected by numerous biotic (nature of the target organism and of the host plant, presence of predators, parasites or antagonistic microorganisms among the resident microflora) and abiotic (nature of the soil or substrate, humidity, availability of nutrients, temperature, radiations, salinity and pH) factors (Dandurand and Knudsen, 1993; Eastburn and Butler, 1988a, b; Hubbard et al., 1983; Knudsen and Bin, 1990). There are several mechanisms involved in antagonism of *Trichoderma* species namely antibiosis, enzyme secretion, substrate competition, hyphal interactions and mycoparasitism (Haran et al., 1996b). In order to solve the national and global problems of environmental hazards due to application of chemicals for disease control, antagonistic microbes have been considered as prospective agents for the purpose (Cook, 1985).

According to Baker and Cook (1974) Biological control is the reduction of inoculum density or disease producing activities of a pathogen or parasite in its active or dominant state, by one or more organisms, accomplished naturally or through manipulation of the environment, host or antagonist, or by mass introducing of one or more antagonists. Biological control is the adjustment in cultural practices, which either terminates a niche, or encourages competitions of the pathogens to fill the niche and also provide the crop with a better means to resist, tolerate or escape the pathogen or in other words biocontrol of disease provide the crop a better and healthy atmosphere for survival.

Now a major challenge is felt in the field of plant pathology to introduce or develop some new disease control strategies, as the more traditional chemical control measures have become obsolete. The release, accumulation and persistence of chemicals (generally fungicides and pesticides) into the environment over a period of time especially in the soil and aquatic ecosystem have hazardous effect on plant and animal life. The greatest overall potential for an increased role of biological control in crop disease management list not only in commercial biological control agents, but in exploiting or ever-increasing understanding of the role of various cultural practices on general biological activities in crop ecosystems. The biological control of plant diseases has received significant attention, since it promises to offer a more sustainable food supply. Moreover, a successful biological management strategy of a crop disease can offer a marketable products at considerably lower cost compared to conventional measures (Chung, 1994). In the present review paper, the *Trichoderma* spp. is

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discussed and its role as potential biocontrol agents (BCAs) against fungal plant pathogens is reviewed and summarized.

The application or delivery of the biocontrol agents into the soil is done in the following manner:

a. Apply suspension of agent propagules to soil directly or in a substrate.
b. Substrate or some other medium reading the antagonists is directly mixed with soil. Several soil-borne diseases have been controlled by this method, e.g. wheat bran colonized by *Trichoderma* viride when incorporated in a strawberry nursery; it decreased the disease severity of damping-off of seedlings caused by *Rhizoctonia solani*, thereby increasing the yield (Elad et al., 1981).
c. Synthetic substrates such as sodium alginate can be used to form pellets in which biocontrol agent's spores are incorporated. This is an effective method of the delivery of *Trichoderma* spp.
d. For foliar pathogens, biocontrol agents are applied to the aerial surfaces of the plants.
e. In other cases, biocontrol agents are applied directly to bulbs, seeds, potato tubers, pieces or harvested fruits, before sowing in the fields.

BIO-CONTROL MECHANISMS OF *Trichoderma Spp.*

The scientific evidence signifies that several soil and seed borne diseases of fungal pathogen can be controlled by *Trichoderma* spp. The various modes of antagonism include competition, mycoparasitism and antibiosis.

A. Competition

Competition of the biocontrol agents with other microorganisms or fungal pathogens can be triggered by abiotic factors. The fungal pathogens (dormant propagules of fungi) living in soil and on plant surface which suffer the scarcity of certainly available nutrients which may ultimately result in nutrient competition between fungal pathogen and *Trichoderma* spp. (Adams and Ayers, 1982; Garrett 1956). Besides *Trichoderma* spp. releases compounds known as siderophores which would complex the micronutrient iron and render it as unavailable form, this leads to competition.

B. Mycoparasitism

One of the mechanism involved in the antagonistic activity of *Trichoderma* spp. against a range of economically important pathogens is the mycoparasitism (Dennis and Webster, 1971b), where the production of fungal cell wall-degrading enzymes by *Trichoderma* spp. is believed to play a role. It was shown that extracellular lytic enzymes, β-1, 3-glucanases, chitinases and proteases excreted by *Trichoderma* spp. were involved in cell wall degradation of plant pathogens. *Trichoderma* directly attacks the plant pathogen by excreting lytic enzymes such as β-1, 3-glucanases, chitinases and proteases (Haran et al., 1996 a; Hjeljord and Tronsmo, 1998). Because the skeleton of fungal cell wall contains chitin, glucan and protein, enzymes that hydrolyze these components have to be present in a successful antagonist in order to play a significant role in cell wall lysis of the pathogen (Lorito et al., 1994; Carsolio et al., 1999). β-1, 3-glucanases play in nutritional role in saprophytes and mycoparasites (Sivan and Chet, 1989). Several chitinolytic enzymes have been reported in T. harzianum (De la Cruz et al., 1992). These include endochitinases, exochitinases and 1, 4- β-N-acetylglucosaminidases. Enzymatic degradation of chitin is generally involved in many biological processes, such as autolysis (Vessey and Pegg, 1973), morphogenesis and nutrition (Griffin, 1994), and in addition to mycoparasitism plays also a role in relationships between fungi and other organisms such as plant-fungus interactions (Mauch et al., 1988). In addition to chitin and glucans, fungal cell wall contains proteins. Thus, the production of proteases also plays an important role in lysis of cell wall of fungal pathogen during mycoparasitism (Flores et al., 1997).

C. Antibiosis

This mechanism involves the secretion of both volatile and non volatile anti-microbial metabolites, which are portable in nature and thereby suppressing or killing the fungal pathogen (antibiosis) around the surrounding area (Corley et al., 1994; Horvath et al., 1995). Dennis and Webster (1971a) found that many isolates of *Trichoderma spp*. produce non volatile antibiotics, which were active against a range of pathogen. Biological activity of antagonistic fungi may partially be associated with production of antibiotic (Estebarian et al., 2000; Faull et al., 1994). The production of antibiotics; *Trichoderma* in (Godtfredsen and Vangedal, 1965), ergokonin (Kumeda et al., 1994), viridin (Chet et al., 1977; Grove et al., 1996) and viridin fungin A, B and C (Harris et al., 1993) by *Trichoderma* spp. have been reported. The principal functions of these antibiotics are cell membrane disruption, inhibition of metabolic activity and stimulation of plant defense system.

Competitive Saprophytic Ability (CSA) of *Trichoderma* Spp.

Trichoderma spp. is well known for their ability to colonize roots. Their conidia have also been applied to fruits, flowers and foliage, and plant diseases can be controlled by their application to any of these sites (Harman, 2000; Elad, 1994). Some strains of *Trichoderma* spp. can colonize only local sites on roots (Metcalf and Wilson, 2001), but rhizospere competent strains colonize entire root surface for several weeks or months (Thrane et al., 1997). Colonization implies the ability to adhere and recognize plant roots, penetrate the plant, and withstand toxic metabolites produced by the plants in response to invasion by a foreign organism (Lo et al., 1996). In the few cases that have been examined thoroughly, strains of *Trichoderma* spp. colonize root surfaces, sometimes with morphological features reminiscent of those seen during mycoparasitism and hyphal invasion of the root epidermis. There, they produce or release compounds that induces localized or systemic plant resistance responses (De Meyer et al., 1998; Harman et al., 2004; Cristina et al., 2007). Plants react against fungal invasion by synthesizing and accumulating phytoalexins, flavonoids and terpenoids, phenolic derivatives, aglycones and other microbial compounds. Root colonization by *Trichoderma* spp. frequently enhances root growth and development, crop productivity, resistance to abiotic stresses and the uptake and use of nutrients (Arora et al., 1992). The survival and subsequent dominance of introduced antagonists in the new soil microenvironment is eminent to exploit their potential against the diverse groups of pathogenic micro flora in the plant rhizosphere. After application of bio-control agents, the soil micro-environment plays the predominant role in influencing their growth and consequently the antagonistic potentiality.

Commercial Status of *Trichoderma Spp*.

Currently, *Trichoderma spp.* occupies 5% of global BCAs market which also comprises viral and nematode based biopesticides comparatively younger type rather *Bacillus thuringiensis* (Bt) biopesticides and it shares about 95% of global biopesticides markets. Besides true market size is unclear and the information regarding the registered as well as non-registered biofungicides are scattered. However *Trichoderma* spp. based BCAs products are registered frequently and they share about 70% of all fungal based BCAs. Many biopesticide companies are recommending these products on regular basis and field trials throughout the world are being consented (Keswani et al., 2015). The increasing steady success of *Trichoderma* spp. based BCAs are due to its distinctive characteristics mainly simultaneous biocontrol and growth promotion activity.

Future of *Trichoderma Spp*. as Biocontrol Agents

Various researchers identified and characterized many species of *Trichoderma* but only few species seems to be active in controlling or antagonizing the plant pathogenic fungi and in contrast very few species are harmful. The ability of *Trichoderma* species against numerable plant pathogens worldwide is a promising character of the genus. The mechanisms of antagonism has been investigated at various levels still the commercial production or reliability of the biocontrol action is at infancy due to several reasons of which strain variation is most critical. The strains are dissimilar in action on enormous pathogens or on the same pathogen in different environmental conditions as these are biological entities which are easily affected by biotic and abiotic stresses. The literature clearly indicates the survival of the introduced *Trichoderma* bioagent is simply dependant on strain of the organism, host, climate, soil factors and mainly the microbial synergism or antagonism that exists naturally in the ecosystem.

Harman (2000) reviewed the myths and dogmas of biocontrol critically. Mathre and Johnston (1995) emphasized on the adaptability of the antagonist to the host plant in addition to their ability to inhibit or compete with

Table1 : Biological Control of Some Major Fungal Plant Pathogens by Different *Trichoderma spp.*

***Trichoderma* spp.**	**Plant Diseases**	**Fungal Plant Pathogens**
T. harzianum Rifai	Southern stem blight of soybean	*Sclerotium rolfsii*
T. harzianum Rifai	Root rot of blackgram	*Macrophomina phaseolina*
T. harzianum Rifai	Fusarium wilt of tomato	*Fusarium oxysporum f. sp. lycopersici*
T. harzianum Rifai	Fusarium wilt & corm rot of gladiolus	*Fusarium oxysporum f. sp. gladioli*
T. viride Pers. Ex Fr.	Pigeon pea wilt	*Fusarium udum*
T. lignorum (Tode) Harz	Damping-off of bean	*Rhizoctonia solani*
T. viride Pers. Ex Fr.	Cotton seedling disease	*Rhizopus oryzae*
T. virens (Miller, Giddens &Foster) v. Arx	Wood decay	*Serpula lacrymans*
T. virens (Miller, Giddens &Foster) v. Arx	Brown blotch disease of cowpea	*Colletotrichum truncatum*
T. harzianum Rifai	Rotting of common vegetables	*Sclerotium rolfsii*
T. harzianum Rifai	Sunflower head rot	*Sclerotinia sclerotiorum*
T. harzianum Rifai	Collar rot of lentil	*Sclerotium rolfsii*
T. koningii Oudem	White rot disease of onion roots	*Sclerotium cepivorum*

the target pathogen. Moreover, the society and farming community should accept it. The identified isolates in the past having confusion on their identity, so exact characterization of the strain is essential to use them in the disease management of plants. It is evident from the earlier work that beneficial strains are not pathogenic on mushrooms or human beings. Hence, with accurate, identification of the species, it is possible to avoid the unwanted effects in the environment if applied as bioagents. The stringent laws also control the biosafety of the *Trichoderma* and almost all countries are particular about their release into nature. Hence, the miracle organism *Trichoderma* is accepted to some extent but still not widely used. The recent idea of integration of *Trichoderma* with fungicides is gaining importance as this formulation is giving synergistic effect in integrated disease management. Hence, in future research need to be focused on accurate identification of the virulent strains of beneficial *Trichoderma* species and formulations having combination of different strains to cater the need of diverse climatic conditions of various agriculture crops against a range of plant pathogens for sustainable agriculture and chemicals pesticide free environment (Lorito et al., 1994; Kumeda and Bin, 1994; Samuels 2006).

CONCLUSION

Trichoderma spp. may suppress the growth of fungal pathogen population in the rhizosphere through competition for key nutrients and thus reduce disease development. It produces antibiotics, such as trichothecin, ergokonin, viridin and trichodermin, which have a direct effect on fungal pathogen. *Trichoderma* hyphae either grow along the host hyphae or coil around it and secrete different lytic enzymes such as proteases, chitinases and β-1, 3-glucanases that are involved in the process of mycoparasitism. Such interactions of *Trichoderma* spp. are acting against fungal plant pathogens. Thus, *Trichoderma* spp. enhances the crop yield by playing multiple roles such as promoting healthy growth in early stages of plant with its application as biofunficides. The commercial production processes should be improved in order to enhance marketability of *Trichoderma* spp. as BCAs. There is need for optimizing the operating parameters to increase sporulation or conidia production by adapting cheaper and alternative substrates to achieve economically better yield. In the near future it is projected that exploitation of *Trichoderma* spp. based BCAs would be maximized.

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