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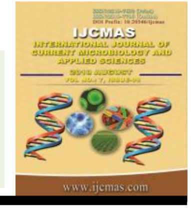

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Trichoderma in Agriculture: An Overview of Global Scenario on Research and its Application

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A B S T R A C T

The non-judicious use of agrochemicals has created a vicious impact on the environment and its inhabitants which is a major concern among society these days; although, thinking and attitude towards its use has changed and there is a growing interest to develop its alternative. On the other hand, the use of microorganisms that antagonize plant pathogens (biological control) is a risk-free approach and the species of Trichoderma are well recognized as agents for plant disease control in addition to its promontory effect on plant growth. Presently a large number of Trichoderma based products are available in the market globally which are dominated by *T. harzianum* and *T viridae*. This review is focused on various mechanisms of bio-control employed by Trichoderma, current status of research and its applications in agriculture with the objective to create awareness about this fungus in society to popularize its beneficial service to the environment and its future development.

Introduction

The organism that suppresses the pathogen or its activity is referred as biological control agent (BCA) and phenomenon known as bio-control or biological control. The species of Trichoderma are universally known for their bio-control potential in addition to plant growth and development (Vinale *et al.*, 2014). An effective and potential BCA (microbial pesticide) must have some unique characteristics for successful bio-control of plant diseases i.e. i) good shelf life and survival in the soil in its inactive or active form, ii) host specificity, iii) easy to multiply in laboratory and iv) ecofriendly and cost effective. The phenomenon of bio-control employed by Trichoderma is governed by a variety of mechanism either its direct presence at the infection site or passively due to its activities which triggers and activates the various biochemical pathways in plant system. Hence, lot of research has been focused on characterizing the unique mechanism(s) used by a particular BCA operating in different experimental situations. The variety of registered microbial agents *viz.* bacteria (*Pseudomonas aureofaciens*, *Pseudomonas fluorescens, Bacillus subtilis, Agrobacterium radiobacter, Streptomyces griseoviridis*), nematodes, protozoa viruses and fungi (*Ectomycorrhizae sp.*, *Cladosporium sp. Ampelomyces quisquallis*, *Coniothyrium minitans*, *Aspergillus niger* IARI isolate) including Trichoderma species (*T. harzianum, T. viridiae, T. virens*) are being utilized in plant disease control as the bio-pesticide (Gardener and Fravel, 2002; Koul, 2011). In this review, various mechanisms employed by Trichoderma, present scenario of research and its application are being discussed.

Direct mechanism

Mycoparasitism/Hyperparasitism

More than seventy reports on mycoparasitic ability of *Hypocrea*/*Trichoderma* species are documented against phytopathogenic fungi such as *Alternaria alternata*, *Botrytis cinerea*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *Pythium* spp., *Phytophthora* sp. and *Fusarium* spp. (Harman 2006; Druzhinina *et al.,* 2011; Atanasova *et al.*, 2013; Bae *et al.*, 2016) and it is one of the chief mechanisms involved in the antagonism and bio-control efficacy of Trichoderma spp. (Sharon *et al.,* 2001). This process involves tropic growth of the bio-control agent towards the target organism, coiling and finally enzymatic dissolution of cell wall / cell membrane of the target organism where not only an antagonist, but also target pathogen responsible to govern the nature of antagonism. In a study, it was reported that some strains of *T. harzianum* have ability to parasitize on nematodes and their egg masses also where it coiled around the second stage juveniles of *Meloidogyne javanica* and penetrated them by forming haustoria like structures (Sahebani and Hadavi, 2008). *Trichoderma longibrachiatum* was also reported to manage *Heterodera avenae* (Zhang *et. al*., 2014a) and *Meloidogyne incognita* (Zhang *et. al*., 2015) where it showed its bio-control potential.

Antibiosis

It is an antagonistic association between two microorganisms, in which one is adversely affected due to metabolites or antibiotics released by another. The chemical and analytical reports portray that 373 different secondary metabolites such as peptaibols, NRPs, volatile and non-volatile terpenes, pyrones and nitrogen containing compounds obtained from species of Trichoderma revealed their great potential to produce antibiotics and secondary metabolites (Mukherjee *et al.*, 2012a; Mukherjee *et al.*, 2012b; Crutcher *et al.*, 2013; Bae *et al.*, 2016; Contreras-Cornejo *et. al*., 2016). Gliotoxin and glyoviridin from *T. virens* (Wilhite *et al.*, 2001), viridin, alkylpyrones, isonitriles, polyketides, peptaibols, diketopiperazines and sesquiterpenes isolated from *Trichoderma* spp. (Singh *et al.*, 2001b; Reino *et al.*, 2008), trichodermin (Tijerino *et al.*, 2011; Shentu, *et al.,* 2013) and 6-Pentyl-2*H*-pyran-2-one (Garnica-Vergara *et al.*, 2015) are some of the examples which are found effective against target pathogen *in-vitro* and/or *in-situ.*

Competition and rhizosphere competence

The most abundant non-pathogenic plant-associated microbes are generally thought to protect the plant by rapid colonization and thereby exhausting the limited available substrates so that none are available for pathogens to grow. Being a living organism, competition is considered as a 'classical' mechanism of Trichoderma in biological control. The omnipresence of *Trichoderma* in agricultural and natural soils throughout the world proves that it must be an excellent competitor for space and nutritional resources. Tsahouridou and Thanassoulopoulos (2002) found the *T.*

koningii as rhizosphere competent when treated tomato seeds with conidial suspension of *T. koningii* were sown which resulted in reduced damping-off pathogen.

Sclerotia colonization and parasitization

The phytopathogens like *Rhizoctonia solani*, *Sclerotium rolfsii* and *Sclerotinia* spp. have tremendous ability to survive in soil for decades through hardy structures 'sclerotia' which are highly resistant to fungicides and microbial degradation and hence, play important role in disease cycle of the pathogen (Metcalf *et al.,* 2004, Abdulla *et al.*, 2008). The idea of sclerotia parasitization or killing them using microbial antagonist may be a key point for successful biological control. However, species of *Trichoderma* are also known for their ability to degrade cellulose and chitin (Balakrishnan *et al.*, 2012, Barakat *et al.,* 2013). In previous studies it was reported that the sclerotia of *Rhizoctonia solani* and *S. rolfsii* are colonized by *Trichoderma,* completely rotted and do not germinate if parasitized by *Trichoderma* (Rawat and Tewari, 2010; Sharma, 2017).

Indirect mechanism

Induction of host resistance

Besides the ability to attack directly or inhibit the growth of plant pathogens, some of the bio-control strains of *Trichoderma* sp. are known to strongly induce systemic resistance (ISR) and host plant defense against a variety of plant pathogens which is mediated by jasmonic acid (JA)/ethylene (Harman 2004; Pal and Gardener, 2006). In a study, the isolates of endophytic *Trichoderma* from tropical environments reported to induce resistance against *Phytophthora capsici* in hot pepper and delayed the disease onset (Bae *et al.,* 2011). Yedidia *et al.,* (2003) gave evidence of induction of a systemic response by root application of *T. asperellum* which protect the cucumber plant against angular leaf spot. Induced resistance in tomato plant (cv. Sida cultivar) against bacterial leaf spot (*Xanthomonas campestris* pv. *vesicatoria*) with 69.32% reduction in disease was reported by Saksirirat *et al.,* (2009) after 14 days post inoculation of *T. harzianum* (T9). A protective effect of Trichoderma on plants was strongly inhibited when treated with an inhibitor of ethylene which confirmed the essential role of ethylene signal in ISR (Shoresh *et al.,* 2005). Moreover, there are some other reports which evidenced that species of Trichoderma are abile to induce plant defense mechanisms (Shoresh, *et. al*, 2010; Contreras-Cornejo *et al.*2011; Sharma, 2017). Research is initiated from last decade to characterize the determinants and biochemical pathways of induced resistance correlated with bio-control agents including Trichoderma and other non-pathogenic microbes.

Plant growth promotion

Bio-control agents, both fungal and bacterial, are reported to induce the growth of various crops. These responses may be due to the individual and /or cumulative effect of (i) suppression of deleterious root micro-flora, (ii) production of growth hormones or growth factors (Contreras-Cornejo *et al.*2011, 2014a; Martínez-Medina *et al.,* 2014) and/or (iii) increased nutrient uptake through solubilization and sequestration of nutrients and/or enhanced root growth (Viterbo *et al.*, 2002). Species of *Trichoderma* are unique groups of rhizospheric microorganisms associated with certain beneficial effects to enhance plant growth and development. The research by several independent research groups have observed the increased growth of various crops with the use of antagonist Trichoderma spp. (Sharma *et al.,* 2012; Contreras-Cornejo *et al.,* 2013; Hermosa *et* *al.*,2013; Samolski *et al.*, 2013; Qi and Zhao, 2013; Stewart and Hill, 2014). In a study conducted by Zhang *et al.*, (2014a) revealed that *Trichoderma longibrachiatum* was found fairly effective for promoting plant growth and nematode control in wheat. Increased shoot and roots length, weight, of pod weight and number of nodules per plant was reported in peanut with *T. harzianum* among the four *Trichoderma* as compare to control treatment (Kamaruzzaman *et al.*, 2016). The significant increase in yield (29% and 36%) of wheat (variety Raj 3765) was achieved by Sharma *et al.*, (2012) with the application of *Trichoderma harzianum* (Th3) in Jaipur and Kota respectively in the field demonstrations at farmer's field conducted during 2008-2011 Martínez-Medina *et al.*, (2014) reported that auxin production was induced and a decrease in cytokinins and abscisic acid content by Trichoderma isolates that promoted the plant growth. The biological activities of *Trichoderma* spp. in soil help in bioavailability of various nutrients and minerals either through chelation or solubilization, and thus make them available at the root surface of the plants (Menezes-Blackburn *et al.*, 2014) which results in increased plant growth.

Tolerance to abiotic stress

Abiotic stress (salt, drought etc.) play crucial role for crop cultivation as they limit in plant growth and subsequent yield in many areas. Some rhizospheric strains of *Trichoderma* are found fairly effective to induce plant growth under abiotic stresses (Bae *et al.*, 2009; Mastouri *et al.*, 2010; Shukla *et al.*, 2012). Due to higher production and activity of ROS-scavenging enzymes (superoxide dismutase, catalase and ascorbate peroxidase) induced in tomato plants colonized with *T. harzianum* T22 under water-deficit stress condition results in enhanced plant growth (Mastouri *et al.*, 2012). Under drought conditions, the cacao plants colonized with endophytic fungus *Trichoderma hamatum* isolate DIS 219b exhibited delayed drought-induced changes in stomatal conductance and net photosynthesis (Bae *et al.*, 2009). In a glass house study where mustard plants were raised with *T. virens (Isolate PB23)* treated seeds and subjected to water stress. The plants were able to thrive well and showed delayed response under water-deficit stress (Sharma and Singh, 2014). *Trichoderma asperelloides* - T203 was found to ameliorate seed germination under saline stress conditions due to its acquired tolerance to salt stress which is supposed to link with ACCD activity (Brotman *et al.*, 2013). Inoculation of *A. thaliana* plants with *Trichoderma* resulted in significantly higher tolerance to salinity stress as compare to untreated plants which occur probably due to accumulation of antioxidant and stress hormone (Contreras-Cornejo *et al.*, 2014b).

Siderophores

Siderophores (Gr. "iron-bearers") are low molecular weight, ferric specific legends which is to supply iron to the cell (Neilands, 1981) and iron is an essential element for various enzymatic reactions occur in living organisms although; naturally abundant ferric ion is almost insoluble in oxygenated environments and thus unavailable for microbial growth. Siderophores mediate the limited amount of iron in the rhizosphere, deprive pathogens of iron which produce less siderophores or different siderophores with lower binding coefficients and finally suppress their growth. Many reports have been published showing siderophore involvement in the suppression of plant pathogenic fungi (Vinale *et al.*, 2013). *T. virens* produces three types of hydroxamate siderophores: a monohydroxamate *(cis*- and trans-fusarinines), a dipeptide of *trans*-fusarinine (dimerum acid), and a trimer disdepsipeptide (Lorito *et al.*, 1993).

Metabolism of germination stimulants

Propagules (sclerotia) of soil borne phytopathogens may germinate only in response to root exudates (germination stimulants) of host plants (Metcalf *et al.*, 2004) however, some of the non-pathogenic organisms may also prefer to utilize these compounds. Thus, a relatively new concept 'inhibition of germination stimulants' is supposed to be a indirect mechanism of bio-control and correlated with those non-pathogenic organisms where mycoparasitism / antibiosis is not established. Howell (2002) reported that control of pre-emergence damping off of cotton seedlings incited by *P. ultimum* and/or *Rhizopus oryzae* by *T. virens* (Strain G6, G6-5) or protoplast fusants of *T.virens* / *T. longibrachiatum* was due to metabolism of germination stimulants released by the cotton seed.

Application methods and factors affecting efficacy

The main problem in developing Trichoderma based bio-pesticides is that it represents a living system which should remain sufficiently viable for a period until it reaches to farmers (Vidhyasekaran and Muthamilan, 1995). Here, formulation helps to retain viability of bio-agent during preparation, storage and favours survival in the environment after application. Furthermore, it is quite essential to have an efficient, economic and ecologically viable delivery system or mode of application of bio-control agents in soil ecosystem and fairly good work has been done on this aspect under different conditions. Inoculation of seeds, corms, bulbs, tuber, etc. with antagonists prevents seed/corm decay and seedling blight. *T. harzianum*, *T. virens* and *T. viride* are effective seed protectants against *Pythium* spp. and *Rhizoctonia solani* (Jayaraj and Radhakrishana, 2003). According to Dutta and Das, (2002), soil application of *Trichoderma* spp. at the time of seedling planting reduced the collar rot in tomato as compare to seed treatment. Several reports depict the effectiveness of bio-control as foliar spray against different plant pathogens however, success of these antagonists depends largely on their ability to colonize on leaf/sheath surface (Lo *et al.*, 1997).

For successful colonization of Trichoderma in rhizhosphere / phyllosphere, the minimum inoculum is crucial and subsequent action to initiate the various mechanisms through which bio-agents inhibit, restrict even kill the pathogen directly or indirectly. Adequate amount of soil moisture, temperature near ambient and definite rage of P^H (5.5-7.5) is needed for growth and multiplication of Trichoderma species to perform their bio-control activity in soil. Against *Rhizoctonia solani* in pea and rajma *Trichoderma harzianum* is more effective in acidic P^H > pH 6.5 (Singh *et al.*, 2006). Changes of 4°C or 5 per cent RH are associated with variability in disease suppression by *Trichoderma* that may range from 25-100 per cent (Hannusch and Boland, 1996).

Global and Indian scenerio of bio-pesticides

Present share of bio-pesticides is only 5 % of the total crop protection market globally which was valued about $3 billion worldwide (Olson, 2015). Majority of Trichoderma based bio-pesticides globally are dominated by *T. harzianum* and *T. viride* (Gardener and Fravel, 2002; Koul, 2011 and Table 1). In India, only 15 types of bio-pesticides with more than 970 product are registered and more than 63 Indian private companies including Pest Control (Pvt) Ltd; Multiplex Biotech Ltd., International Panacea, Biotech International Ltd; T. Stanes; etc. have their registered products as bio-pesticide.

Table.1 List of microbial pesticides developed from Trichoderma sp.

Bio-agent	Target pest	Action	Brand name	Producer
Trichoderma harzianum	Effective against variety of soil borne pathogens and wound pathogens	Mycoparasitic, Cell wall degrading enzymes (CWDE), ISR	Root Shield	BioWorks Inc, NewYork
			BioTrek 22g	Wilbur-Ellis Co., Fresno CA
			Supresivit	Borregaard, Denmark
			T-22G	Bioworks Inc, EU
			T-22HB	
			Trianum P	Koppert Biological Systems
			Binab	*BINAB Bio Innovation* AB, Sweden
			Trichodex	Makhteshim Chemical Works LTD, Israel
Trichoderma viride	Effective against soil borne pathogens causing root rot diseases	Mycoparasitic, Anibiosis, PGP	Ecosom TV	AgriLife, India
			Tricon	Green Max, India
				AgroTech, India
			Trieco	Ecosense labs, India
			Bioderma/ Protector	Biotech International Ltd., New Delhi
			Ecofit	Eastman Kodak Company, New York
Trichoderma* sp. OR *T. harzianum* + *T. viride	Effective against *Armillaria* and, *Botryoshaeria* and other root pathogens	Mycoparasitic, Competition	Trichodry	Agrimm Technologies Ltd., New Zealand
			Vinevax	
			Trichoflow	
			Trichopel	
			Trichoject	
			Trichoseal	
Trichoderma polysporum* + *T. harzianum	Against wood decay and wounds of ornamental, shade, and forest trees		Binab T	Binab Bio-Inovation, EFTR AB, Sweden
***Gliocladium virens* GL-21**	Damping off and root rot pathogens of nursery ornamental	Mycoparasitic, Antbiosis, Competition, CWDE	SoilGard	Certis USA

(Gardener and Fravel, 2002; Koul, 2011)

Microbial pesticides market in India dominated by sale of *Trichoderma viride*, *Pseudomonas fluorescens* and *Bacillus thuringensis* and *Trichoderma* alone shared 52.5 % in the Indian bio-fungicides market in 2015 (Ken research Report, 2015). Funding for the research and production of bio pesticides and their promotion also is being led by the Department of Biotechnology (DBT) and Indian Council for Agricultural Research (ICAR) who support 22 and 31 bio-control production facilities respectively in India. For reducing complications in registration, some amendments are made in Insecticide Act of 1968 for speedy development of bio-pesticides. In India, the bio-control products are promoted by the Governments through subsidies, demonstration, training etc. and strict quality control protocols are followed to maintain its quality and efficacy. However, the major constraints for popularizing and marketing of bio-control agents (BCAs)/bio-pesticides In India can be directly correlated with factors such as lack of awareness about the use of bio-pesticides and related products, high cost and time consuming registration procedures, unreliable quality standards and product availability. Moreover, pesticide market is leaded by unorganized stakeholders and synthetic pesticides are easily availability than bio-pesticides.

Future prospects

Research findings with the use of molecular, biochemical, physiological and genetical study tools have explored a wide area of research on various mechanisms employed by Trichoderma in its biocontrol activities. Future research should be carried out efficiently on participatory mode with public-private sector approach for encouraging investment in bio-pesticide and bio-fertilizer enterprises. Additional funding for innovative R&D for improvement of a particular microbial bio-pesticide by using unique sources (nanosilica, chitosan) will be needed. Development of consortium of two or more species or strains of Trichoderma in addition to liquid formulations is another area for increasing self-life and efficient delivery system at farmer's field.

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