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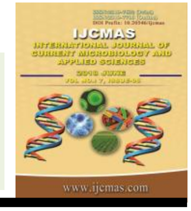

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Prospects of *Trichoderma* in Agriculture-Fundamentals and Applications

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A B S T R A C T

The role of microorganisms does not just start with nutrient mineralization, organic matter decomposition and ends with it. There is much more a particular microbe can do in soil and its ecosystem. One of such is *Trichoderma*, a fungus found in almost all soil ecosystem and a varied environment conditions covering normal soil to harsh conditions as marshy land, desert soil and other degraded environment. With the ill effects of chemical fertilizers becoming more and more obvious and the increase cost of production that follows, chances have to be given to biocontrol agents like *Trichoderma*, that has multiple usage in agriculture and beyond besides its ability to control disease incidence. *Trichoderma* are highly interactive in soil and plant roots and its growth is more in acidic condition compared to alkaline. They are the best known for its role in controlling diseases by acting as mycoparasite. Other roles include bioremediation, waste decomposer offering benefits like solubilizing nutrients, promoting plant growth and resistance to stresses, which ultimately leads to enhanced plant growth.

Introduction

Plant and soil related problems have been with us since the beginning of agriculture affecting the overall production of crops. Although the use of chemical fertilizers and pesticides has made us achieved higher yield, considerable damages has been caused to the environment disturbing its ecological balance, raising concerns of both the environment and human health. Under such circumstances, use of biological methods or agents involving microorganisms comes to rescue and application of such biological agents is attracting researchers from different fields.

One of the most effectively and widely adopted biological agent is the fungus Trichoderma. Trichoderma is a genus of filamentous Ascomycetes fungi that are among the most frequently isolated soil microorganisms; tropical soils contain 101–103 culturable propagules per gram (Harman *et al.*, 2004; Etschmann *et al.*, 2015). They are widely distributed all over the world (Domsch *et al.*, 1980), and found in all soils including forest humus layer (Wardle *et al.*, 1993) as well as in agricultural orchard soils (Roiger *et al.*, 1991) and natural habitats, especially in those containing or consisting of organic matter (Papavizas, 1985). They are highly reproductive, ability to increase plant growth, efficient in nutrient utilization and offer plant defense mechanisms. The most useful strains exhibit a characteristic called 'rhizosphere competence', meaning the ability to colonize plant roots and live in association with it. Apart from its role in parasitizing fungi and reducing diseases, Trichoderma species are also used in decomposing waste and organic matter, to remediate polluted soil, in weed control and various other applications in the fields of medical, industries and food. They are reliable, environmental friendly and cost effective.

Taxonomy and evolution of trichoderma

Trichoderma belongs to the kingdom fungi, division Ascomycota, class Sordariomycetes, order Hypocreales, family Hypocreaceae and genus *Trichoderma*. To date, at least 1100 *Hypocrea* (sexual telemorphic stage)/*Trichoderma* (asexual anamorphic stage) strains have been identified from 75 molecularly characterized species and many new species are being recognized (Druzhinina *et al.*, 2011).

The history of Trichoderma can be date back to 1794 when Persoon first introduced the name *Trichoderma*. It was until 1927 that Gilman and Abbott recognized four species under the genus *Trichoderma.* The first move on development of a particular protocol for species identification was made in 1969 (Rifai, 1969; Samuels, 2006). Subsequently, many novel species of *Trichoderma* were revealed and by 2013, the genus already consists of more than 200 phylogenetically defined species based on rpb2 sequence (Atanasova *et al.,* 2013). Presently, the International Sub commission on *Trichoderma* lists 104 species (http://www.isth.info/biodiversity/index.php) characterized at the molecular level. Although numerous species have been identified, the most important *Trichoderma* species used in the fields of agriculture, industrial and medical are: *Trichoderma hamatum*, *T. viride*, *T. harzianum*, *T. koningii*, *T. longibrachiatum*, *T. reesei* and *T. virens*.

General characteristics of Trichoderma

Trichoderma strains have long been recognized as biological agents, for the control of plant diseases and for their ability to increase root growth and development, crop productivity, resistance to abiotic stresses, and uptake and utilization of nutrients. *Trichoderma* species are ubiquitous in the environment, especially in soils. The optimum temperature of most *Trichoderma* species is between 25-30^{0}C. They are strong opportunistic invaders, fast growing, prolific producers of spores and also powerful antibiotic producers even under highly competitive environment for space, nutrients, and light (Schuster and Schmoll, 2010; Montero-Barrientos *et al.*, 2011). Because of their ability to colonize cellulosic materials, they are found in areas where there is decaying plant material and their population increases with more abundant healthy vegetation (Jaklitsch, 2009). This fungus grows easily on media such as potato dextrose agar (PDA), Malt agar (MA), Blakeslee's agar (BLA) and Czapek Dox agar (CDA) (Bissett, 1991). *Trichoderma* sporulates profusely on most media producing masses of powder, or in a few species produce slimy, green conidia. The green conidia produced are generally diagnostic feature for identification of the genus. They are prolific producers of extracellular proteins, and are best known for their ability to produce enzymes that degrade cellulose and chitin, although they also produce other useful enzymes. They also show a high level of genetic diversity, and can be used to produce a wide range of products of commercial and ecological interest.

Genetic approaches

With the advancement in science, there also comes the need to combine genetic approaches in fungal Trichoderma to help achieve strains that performs better than the former. Screening of diverse population of biocontrol agents is an important requirement for developing efficient biocontrol agents. Recent research shows three species been sequenced, namely *T. reesei*, *T. atroviride* and *T. virens.* The smallest genome size (34 Mb) was found in the weakly mycoparasitic *T. reesei* whereas the T. virens was found to have the largest genome (38.8 Mb) (Mukherjee, 2011). Highly parasitic *Trichoderma* species contain numerous genes that encode production of different enzymes and other compounds involve in the attack against other microbes (Druzhinina *et al.,* 2011). Hundreds of genes are found to be involved in the process of biocontrol mechanisms like mycoparasitism, antibiosis, resistance to stresses, competition and production of enzymes (Monte, 2001). These genes from *Trichoderma* species are identified, cloned to achieve greater promise that offers more resistance to diseases, higher enzymes production.

Protoplast fusion is a technique of genetic modification where two distinct species are fused together to form new species having characteristics of both. The various enzymes produced by *Trichoderma* which serve in biocontrol can be combined to give out better strain than the individual strain. This is achieved through the process of protoplast fusion. Use of protoplast fusion technique for Trichoderma yielded improved biocontrol action against *Fusarium oxysporum, Rhizoctonia solani, Venturia inaequalis, and Cochliobolus miyabeanus* (Kumari, 2000). Mutation is another way to enhance biocontrol ability genetically. It is utilized to bring about variability in populations in order to choose the most desirable trait (Kumar, 2013).

Ecological functions

Root colonization

Trichoderma species are known to colonize plant roots upon coming in contact with it and forms a symbiotic relationship. The major advantage of this relationship is that root colonization increases root growth and length, ultimately leading to an increase in plant growth and productivity as well. When the root system is colonized, a robust root system is developed improving nutrient and water uptake and also providing protection against pathogenic microorganisms (Harman 2000; Benıtez *et al.,* 2004; Contreras-Cornejo 2015). Benitez *et al.,* (2004) claimed that the mechanisms involved in root colonization by *Trichoderma* species are similar to those in mycorrhizal fungi. There are certain toxic compounds like phytoalexins, flavonoids, terpenoids and phenols elicited by plants upon infection and *Trichoderma* is said to be resistant to these compounds and hence can colonize root successfully.

Plant growth promotion

The interaction of *Trichoderma* with plant has an advantageous effect on plants. Among the many, promotion of plant growth is one of the beneficial traits of *Trichoderma* species (Shukla *et al.,* 2012). Plant growth promotion by *Trichoderma* has been observed in several crops. This can be seen in results shown by Harman (2000) that there was an increase of 123% in soybean yield when inoculated with *T. harzianum*. In yet another report using 10-day-old seedlings of the inbred maize line Mo17 grown from untreated or T-22-treated seeds, an increased in growth of shoot and root was observed (Bj ¨orkman *et al.,* 1998; Harman *et al.,* 2004). *Trichoderma* fungi are also capable of producing zeaxanthin and gibberellin, i.e. compounds accelerating seed germination. They induces root branching and increase shoot biomass because of cell division, expansion and differentiation by the presence of fungal auxins compound.

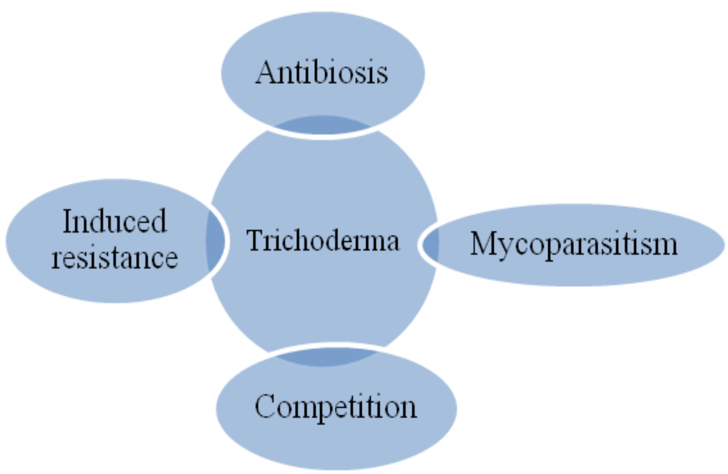
Nutrient solubilization

Nutrient solubilization and plant growth promotion are complimentary to each other. The ability of *Trichoderma* to increase plant growth involves solubilization of nutrients and making it available for plant uptake. Many *Trichoderma* strains produce acids, e.g. gluconic, citric, and coumaric acids, causing the release of phosphorus ions and microelements, which subsequently become available to plants (Harman *et al.,* 2004). Trichoderma species like *T. harzianum* T22 can solubilize various plant nutrients, such as rock phosphate and micronutrients like Fe^{3+}, Cu^{2+}, Mn^{4+} and Zn that can be limiting to plants in certain soils (Altomare *et al.*, 1999).

Resistance to abiotic stresses

Another yet superstar function of *Trichoderma* is its ability to offer resistance to abiotic stresses. The effect of *T. harzianum* T-22 been evaluated on the germination of tomato seeds under osmotic stress. Seed treatment with T-22 resulted in faster and more uniform germination compared with untreated seeds at all water deficit levels (Mastouri, Bj ¨orkman and Harman 2010). *Trichoderma* are known to produce several secondary metabolites, among which IAA has a key role in saline stress adaptation and is considered to be a new mechanism by which microorganisms induce salt stress tolerance, altering the hormonal pathway (Waqas *et al.*, 2012). (Brotman *et al.,* 2013).

Fig.1 Mode of biocontrol action by *Trichoderma*



Enzyme production

One of the most interesting aspects of *Trichoderma* species lies in its ability to produce a number of enzymes which are involved in different mechanisms of promoting plant growth as well as in reducing disease incidence. Secretion of these enzymes results in the suppression of plant pathogen activities directly and indirectly.

Cell wall degrading enzymes such as chitinases and glucanases produced by *Trichoderma* are responsible for suppression of the plant pathogen. These enzymes function by breaking down the polysaccharides, chitin, and β- glucans that are responsible for the rigidity of fungal cell walls, thereby destroying cell wall integrity (Howell, 2003).

Trichoderma spp. attach to the host hyphae by coiling, hooks or apressorium-like structures and penetrate the host cell walls by secreting hydrolytic enzymes such as a basic proteinase (Geremia *et al.,* 1993), β-1,3-glucanase and chitinase (Elad *et al.,* 1993). Chitin, β -1,3-glucan and protein are the main structural components of most fungal cell walls (Peberdy, 1990). This serves as the basis for the suggestion that hydrolytic enzymes produced by *Trichoderma* spp. play an important role in destruction of plant pathogens (Chet and Baker, 1981).

Prospects of Trichoderma as a biocontrol agent

The potential use of *Trichoderma* species as a biocontrol agent was suggested by Weindling in 1932. Estimated proportion of about 90% of antagonistic fungi used in plant protection is by *Trichoderma* (Benitez *et al.,* 2004). Most of these biocontrol belongs to the species *T. harzianum*, *T. viride* and *T. hamatum*. The different modes of actions are mycoparasitism, antibiosis, induced resistance and competition.

Mycoparasitism

Mycoparasitism is an antagonistic interaction where one fungi parasitize the other fungi. The process is a complex one and includes growth of *Trichoderma* towards the host, recognition of the host, secretion of extra cellular enzymes, penetrations of the hyphae and lysis of the host (Zeilinger *et al.,* 1999). The process of mycoparasitism is under the control of enzymes secretion including β- 1,3-glucanase and proteases. Upon reaching the host, its hyphae coils around it forming a hook-like structure called appressoria (Elad *et al.,* 1983). The step consists of the production of cell-wall degrading enzymes (CWDEs) and peptaibols (Howell, 2003), which facilitate both the entry of *Trichoderma* hyphae into the lumen of the parasitized fungus and the assimilation of the cell-wall content.

Antibiosis

Antibiosis occurs when one organism excrete compounds which are toxic to the other organisms. The antibiosis occurring during interaction involves low molecular weight diffusible antibiotics produced by *Trichoderma* that inhibit the growth of antagonistic fungi. The genus *Trichoderma* produces over 180 secondary metabolites, representing different classes of chemical compounds (Gams and Bisset 1998; Reino *et al.,* 2008). Howell and Stipanovic, in 1983 isolated from *T. virens* and described a new antibiotic called gliovirin that was found to be strongly inhibitory to *Pythium ultimum* and a *Phytophthora* species. In tobacco plants, exogenous application of peptaibols activated defense responsive genes and showed reduced susceptibility to *Tobacco mosaic virus* (Wiest *et al.,* 2002). The combination of hydrolytic enzymes and antibiotics results in a higher level of antagonism than that obtained by either mechanism alone (Monte, 2001). When combinations of antibiotics and hydrolytic enzymes were applied to propagules of *B. cinerea* and *F. oxysporum,* synergism occurred, but it was lower when the enzymes were added after the antibiotics, indicating that cell wall degradation was needed to establish the interaction (Howell, 2003).

Competition

Competition for space and nutrients always occurs where there microorganism interact and in area where nutrient is deficit. It is one of the causes for the death of many microorganisms. *Trichoderma* species are generally considered to be aggressive competitors and the ability to compete varied with species (Wardle *et al.,* 1993). Lo *et al.,* (1996) in their study found that a strain of *T.*

harzianum (T-22) was strongly rhizosphere competent and able to control several plant pathogenic fungi including *R. solani* and it reduced the initial disease severity by as much as 71% on a variety of crops. *Trichoderma* also serve as siderophore thus limiting the requirement of iron by other antagonistic fungi, ultimately leading to its death.

Induced resistance

Induction of resistance in host plant by treatment with *Trichoderma* species is another biological control mechanism (Howell, 2003). Some *Trichoderma* strains establish long-lasting colonization of plant roots and penetrate into the epidermis. There, they produce or release compounds that induce localized or systemic plant resistance responses (Harman *et al.*, 2004).

The first clear demonstration of induced resistance with *T. harzianum* strain T-39 was given by Bigirimana *et al.*, 1997. The result showed that treated soil made leaves of bean plants resistant to diseases caused by the fungal pathogens such as *B. cinerea* and *C. lindemuthianum*, even though T-39 was applied only on the roots and without any on the foliage.

Trichoderma as a biofertilizer

Trichoderma can be used for a number of crops as a biofertilizer. Application of *Trichoderma* results in the promotion of growth, yield and increases nutrient availability. *Trichoderma* biofertilizers is successfully used for various crops including cucumber, tomatoes, maize, rice, sorghum, soybeans and peppers. They offer multiple benefits over chemical fertilizers like reduces the use of chemicals, reduces pollution from overuse of NPK, improves plant growth and yield, cost effective and eco- friendly.

Bioremediation role of *Trichoderma*

Several species are frequently used in the in bioremediation of contaminated soil. Population of several *Trichoderma* species can grow rapidly because of their naturally resistant ability to many toxic compounds such as fungicides, herbicides, insecticides and phenolic compounds (Chet *et al.*, 1997). Bioremediation is often provided e.g. by *T. harzianum*, which detoxifies phenols, cyanides, and nitrates (Lynch and Moffat, 2005). They play also possess the ability to degrade a wide range of insecticides like organochlorines, organophosphates and carbonates (Harman *et al.*, 2004).

Other applications of *Trichoderma*

Trichoderma species are used in several other fields besides the applications mentioned above. They are successfully used in decomposing waste and organic matter and are known to hasten the rate of decomposition in a shorter span. Although not much is known, *Trichoderma* species are reported to be useful in control of weeds (Heraux *et al.*, 2005). Further applications of *Trichoderma* includes- in textile industry by the use of cellulase enzymes produced by Trichoderma to soften and condition the textiles. Enzymes produced are also used in paper and pulp industry to reduce lignin content. They are applied in wine making and brewery industries and animal feeds (Galante *et al.*, 1998).

It is concluded that Apart from being widely known and accepted as a successful biocontrol agent, Trichoderma has many different sides to it offering multiple advantageous effects on its usage. They boost germination rate, increases root and shoot length, solubilize nutrients, promote healthy plant growth, act against a wide range of pathogenic fungi. They possess many qualities that can be employed in different industries. Taking into account the ill effects of chemical fertilizers, they can be successfully accepted as alternative to chemical fertilizers or in combination with it.

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