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Advances in *Trichoderma* seed and soil application for Eco-sustainable Agriculture

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Abstract

Trichoderma is a large group of microorganisms that plays an important role in our eco-environment. Trichoderma has a positive impact on plant system by stimulating plant growth and protecting plants from fungal and bacterial infection. They play an important role in bioremediation and are also used as biofungicides. Trichoderma are utilized in industry for production of enzymes, antibiotics, metabolites, and biofuel. Use of chemical fertilizers and pesticides has led to high level of soil toxicity and thus the demand of biofertilizers has increased manifold. The advancement of agricultural technologies including use of fertilizers and pesticides has undeniably ameliorated production, yet these practices pose environmental challenges viz., soil and ecosystem pollution. A pressing need in modern organic agriculture is thus the quest for eco-friendly practices for elevated yields of crop plants. Consequently, there is an urgent demand for environment-friendly agricultural practices for which use of Trichoderma in an innovative manner is a modern approach for their widespread adoption in biological control for enhanced agricultural productivity. Thus, Trichoderma stands out for its biocontrol ability on one hand and also to produce diverse biochemicals crucial for its biocontrol activities in the soil microbiome. These activities encompass the degradation of cell walls as well as tolerance to both biotic and abiotic stresses for protection of soilborne and seedborne diseases and also various crop disorders. The present brief review is a futuristic vision for modern agriculture.

Keywords: *Trichoderma, Plant protection, Biocontrol agent, agriculture*

Introduction

Over the last four decades, the comprehension of filamentous fungi belonging to the *Trichoderma* genus has undergone a significant transformation, especially in molecular genetics. Initially perceived merely as a fungus in biocontrol, *Trichoderma* spp. are now recognized for their established role as symbionts manifesting beneficial effects on plants. This highlights the importance of *Trichoderma* spp. in exploring and implementing eco-friendly solutions for harnessing the abilities of *Trichoderma* to strike a balance between agricultural productivity, environmental preservation, and mitigating losses against various biotic stresses, especially insect pests and diseases. Biotic stresses leads to global crop loss estimates per crop up to 21.5, 30.0, 22.6, 17.2, and 21.4% for wheat, rice, maize, potato, and soybean, respectively (Savary et al., 2019). Keeping this in view, the present review highlights the usefulness of *Trichoderma* for amelioration of soil and eco-environment with a view to mitigate losses in different crops.

Habitat of *Trichoderma*

Trichoderma spp. are prevalent in all climatic zones as per their climatic requirements. Samuel et al., (1996) described it in habitats of soil and rotting wood. These fungi produce conidial spores and several pigments ranging from greenish-yellow to reddish colouration. Howell (2003) and Beintez et al., (2004) has explained ability of *Trichoderma* to antagonise plant pathogens. It has also been isolated from marine, bivalves, shellfish and termites. In the classification of *Trichoderma* species, morphological features have been widely utilized (Rifai and Webster, 1966). According to Waghunde et al. (2016), the *Trichoderma* genus comprises approximately 10,000 species, making it one of the most rapidly growing genera.

Taxonomy of *Trichoderma*

Name *Trichoderma* was given by Persoon (1794). When initially observed, *Trichoderma* typically exhibit a white and cottonish appearance. As they develop, these strains transform into yellowish-green to deep-green compact tufts, particularly noticeable at the canter of a growing spot or in concentric zones on the agar surface. This information suggests that the morphological changes in *Trichoderma* spp., viz., the colouration of colonies and the formation of compact tufts serve as important characteristics for species identification within the genus *Trichoderma*. The arrangement of conidiophores, phialides, and conidia as well as cultural characteristics including growth pattern, pigment production, etc., has also been highlighted and reviewed by various workers. It belong to phylum Ascomycetes,class Sordariomycetes ,order Hypocreales, and family Hypocreaceae. Tulasne and Tulasne (1860) described *Hypocrea rufa* as a telomorph of *Trichoderma viride.* Rifai (1969) on the basis of morphological characters found distinguished nine species of *Trichoderma* like *T.koningii, T.polysporum, T.piluliferum, T.aureoviride, T.longibrachiatum* and *T.pseudokoningii.* Year 1990 to 2002 was considered with an increase in *Trichoderma* number to forty-seven. *Trichoderma* belonging to the family Hypocreaceae is prevalent in all types of soils. They have the ability to fix mutualistic endophytic association with a number of plant species. It is included in Ascomycota division of class Sordariomycetes. The variation effect is based on DNA sequence analysis of internal transcribed spacers (ITS) 1 and 2 of the rDNA gene cluster and fragments of the tef 1, rpb2, chi18-5, and actin or calmodulin genes. Most of the species include *T. atroviride* (38%), *T. harzianum* (21%), *T. lentiforme* (9%), *T. virens* (9%) and *T. simmonsii* (6%). Others include *T. atrobrunneum, T. citrinoviride, T. crassum, T. gamsii, T. hamatum, T, spirale, T. tomentosum and T. viridescens.* MIST was discussed by Dou et al., (2020).

Isolation

Trichoderma is isolated from soil samples especially rhizosphere soil. From these soil samples, five gram is dissolved in 100 mL of sterile water, shaken well, and made into stock solution which is further diluted to 1/10, 1/100, and 1/1000. Each concentration of the solution approx. 200 µL is plated on to potato dextrose agar medium in a Petriplate and cultured at 28^0C for 24-96h. During this period, the Petri plates are observed after every 24 h, and the *Trichoderma* colonies are transferred to a new PDA medium for purification. The purified culture is inoculated on a PDA plate medium and cultured in the dark for 7 days at 28^0C to observe their macroscopic morphology by staining with blue dye.

Trichoderma interaction with other microorganism and plants

Trichoderma colonisation competes for nutrients and space with other organisms as described by Herrera-Estrella and Chet (2004). It's interaction with other organisms is through the process of hyper parasitism, competition, and antibiosis. Vinale et al., (2008) described the process of *Trichoderma* parasitism in host species through direct contact with pathogen which is called as recognition attack, and then gradual penetration until death of pathogen occurs. A major role is played by cell wall degrading enzymes that facilitate hydrolytic degradation of pathogen cell wall. Other enzyme for cell wall degradation are Cellulase, Xylanase, Pectinase, Glucanase, Lipase, Amylase, Arabinase, and Protease as described by Strakowska et al.,(2014). Haran et al., (1996) gave importance of cell wall degrading enzymes which are known as β-N-acetyl glucosamidase, endochitinase, chitobiosidase. Certain pathogenic fungi like *Botrytis cinerea, Rhizocotina solani,* and *Fusarium culmorum* are also inhibited by proteolytic enzymes which are secreted by *Trichoderma*. *Trichoderma* has property of producing siderophores which are known as iron chelating compounds. Reino et al., (2008) noted certain compounds secreted by *Trichoderma* like koningins, viridin, dermadin, trichoviridin, lignoren, and koningic acid. Components produced by *Trichoderma* include Zeaxanthin and Gibberellin which accelerate seed germination. *Trichoderma* species produce enzyme and secondary metabolites mainly antibiotics which make them important microorganism from point of view of ecology, agriculture, and industry. Lynch and Moffat (2005) studied the importance of *T. harzianum* in detoxification of phenols, cyanides, and nitrates. Galante et al., (1998) demonstrated cellulase, hemicellulose, and pectinases produced by *Trichoderma* fungi that are used in partial hydrolysis of plant cell wall thereby enhancing the digestibility of feed and increasing nutritive value of feed. Kubicek et al., (2011) gave evidences for genomic sequence of *Trichoderma* that play a role for competition with other parasite for which *T. virens* and *T. atroviride* were taken in to consideration for its large genomic size. Druzhinina et al., (2011) studied genome of parasite *Trichoderma* that contain numerous gene encoding chitinolytic and gluconolytic enzyme as well as large array of other compounds necessary for attack against pathogens.

Trichoderma spp. as a biocontrol agent of plant diseases

Biocontrol involves utilizing living organisms to reduce pest populations, offering an environmentally friendly approach (Hajek and Eilenberg, 2018). According to Woo et al. (2014), *Trichoderma* spp. are commonly employed as biocontrol agents, effectively combating a wide range of root, shoot, and postharvest pathogens. Siemering et al. (2016) highlighted that the fungus primarily resides in plant roots, particularly along the root surfaces and beneath the outermost layer of root cells. To establish *Trichoderma* on and within plant roots, it is typically applied at the time of sowing or planting during the seeding process. Various studies including those by Gava and Pinto (2016), Xue et al. (2017), and Siddaiah et al. (2017) have demonstrated the success of seed treatment as an effective technique for ensuring the colonization of *Trichoderma* spp. on roots thus providing benefits to the plants. Up to the present time, *Trichoderma* spp. primarily employ three fundamental mechanisms for biological control against pathogens: (i) they engage in recognition and invasion towards plant pathogenic fungi by disrupting their cell walls and absorbing released nutrients, a process known as mycoparasitism (Bhat, 2017); (ii) they induce plant resistance to diseases by altering root architecture during interactions with pathogens (Kumar et al., 2019); and (iii) they combat root-knot and cyst nematodes by destroying nematode eggs, second-phase juveniles, and certain segments of adult nematodes (Heidari and Olia, 2016). The roots of plants treated with *Trichoderma* demonstrate an enhanced capacity for soil exploration and improved mineral uptake. According

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to Harman et al. (2004), various *Trichoderma* strains release several acids, including coumaric, glucuronic, and citric acids. These acids play a crucial role in facilitating the release of phosphorus ions, which are often inaccessible to plants in typical soils as depicted by Zhao et al., (2014). The presence of *T. harzianum* strain 1295-22 in soil has been found to elevate the availability of phosphorus, iron (Fe), and zinc (Zn) in liquid medium. Similarly, the application of strain T-203, also identified as *T. asperelloides*, has been shown to increase the levels of available iron and phosphorus in the rhizosphere by 30% and 90%, respectively. Additionally, *Trichoderma* inoculation leads to enhanced root and shoot growth, resulting in increased uptake of copper (Cu), sodium (Na), zinc (Zn), and other micronutrients as reported by Li, R. X et al., (2015). Xiao et al., (2023) discussed that in the control of plant diseases, *Trichoderma* has an advantage of being efficient and safe for human health and the environment.

Conclusion

Trichoderma spp. are low-cost ecofriendly biocontrol agent for different crop plants with minimal effect on the soil equilibrium. Besides, they are known to inhibit plant pathogens and promote plant growth as well as induce resistance in plants. However, there is much need to work on delivery system of *Trichoderma* spp. as these are influenced by complicated environments while their efficacy loss is determined by location, temperature, humidity, and nutrients. It is also important to work on filtering out robust *Trichoderma* those have been developed under natural mutation and selection. Research is also warranted to study the efficacy of *Trichoderma* along with *Bacillus subtilis, Pseudomonas fluorescens,* etc. in cortium.

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