

TRICHODERMA: ITS ECOPHYSIOLOGY, MECHANISM OF BIOCONTROL AND APPLICATION METHODS

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ABSTRACT

Chemically managed plants impose environmental risks to humans and environment. Using biological approaches to control plant diseases is a more effective and environment friendly alternative. The biological approach to plant disease refers to controlling disease by using organisms like fungi, bacteria and viruses. This can be done by introduction or utilization of resident antagonistic living organisms. Biological control can be achieved through different forms of interactions between organisms and the interactions includes hyperparasitism, antibiosis, commensalism, neutralism, and competition. Various biocontrol agents commonly used against plant pathogens are *Trichoderma harzianum*, *Trichoderma hamatum*, *Trichoderma viride*, *Trichoderma koningii*, *Gliocladium virens*, *Gliocladium roseum*, *Paecilomyces liacinus*, *Coniothyrium minitans*, *Bacillus subtilis*, *Bacillus polymyxa*, and *Pseudomonas fluorescens*.

1. INTRODUCTION

Chemically managed plants impose environmental risks to humans and environment. Using biological approaches to control plant diseases is a more effective and environment friendly alternative. The biological approach to plant disease refers to controlling disease by using organisms like fungi, bacteria and viruses (O'Brien, 2017). This can be done by introduction or utilization of resident antagonistic living organisms. Biological control can be achieved through different forms of interactions between organisms and the interactions includes hyperparasitism, antibiosis, commensalism, neutralism, and competition. Various biocontrol agents commonly used against plant pathogens are *Trichoderma harzianum*, *Trichoderma hamatum*, *Trichoderma viride*, *Trichoderma koningii*, *Gliocladium virens*, *Gliocladium roseum*, *Paecilomyces liacinus*, *Coniothyrium minitans*, *Bacillus subtilis*, *Bacillus polymyxa*, and *Pseudomonas fluorescens* (Tyśkiewicz *et al.*, 2022).

The most important genera against soil pathogens are *Trichoderma*, *Pseudomonas* and *Bacillus* (Guo *et al.*, 2004; Huang *et al.*, 2015). *Trichoderma* is found to be an effective biocontrol agent especially for soil borne

pathogens like *Rhizoctonia solani*, *Sclerotium rolfsii*, *Phythium aphanidermatum*, *Fusarium oxysporum*, and *Gaeumannomyces graminis* under both field and greenhouse conditions (Chet & Inbar, 1994; Basim *et al.*, 1999). The *Trichoderma* spp. have a positive effect on plant growth through hydrolysis of cellulose in soil, increases plant defense mechanism, mineral solubilization and improvement in root morphology, enabling roots to cover large volume of soil (Junaid *et al.*, 2013; Timila *et al.*, 2015).

2. TRICHODERMA: ITS BIOCONTROL POTENTIAL AND ECOPHYSIOLOGY

Trichoderma spp., the promising antagonistic fungi are established in agriculture use. These are classified as an anamorphic Hypocreales, belonging to Ascomycetes (Esposito & Da Silva, 1998). Conidiophores are highly branched and produce lateral side branches that may be paired or not. Phialides are formed on the conidiophore main axis or at the tip. The conidiophore with paired branches takes a shape of pyramid in some species (Samuels & Hebbbar, 2015). Watanabe (1985) found *T. hamatum*, *T. harzianum*, *T. koningii*, *T. pseudokoningii*

and *T. viride* having strong antagonistic potential against soil borne pathogens. They show antagonistic activity under both *in vitro* and *in vivo* conditions by competing for nutrients and space, antibiosis, mycoparasitism, promoting plant growth and plant defense responses (Brotman *et al.*, 2010). Seema and Devaki (2012) reported antagonistic effects of *T. harzianum* and *T. viride* and found significant suppression of mycelial growth and sclerotia formation of pathogen, *R. solani*. Similarly, the seedling mortality of groundnut was also significantly reduced by seed treatment with various isolates of *Trichoderma* spp. (Biswas & Sen, 2000). Freeman *et al.* (2004) reported various isolates of *Trichoderma*, including *T. harzianum* isolate T-39 significantly reduced anthracnose (*Colletotrichum acutatum*) and grey mould (*Botrytis cinerea*) in strawberry under greenhouse and *in vitro* conditions. Pastrana *et al.* (2016) reported preventive and curative application of *T. asperellum* totally avoided incidence of crown and root rot caused by *F. solani* at the same level as application of carbendazim. Similarly, incidence of charcoal rot caused by *M. phaseolina* reduced up to 65% under field conditions and up to 44% in a growth chamber with application of *Trichoderma*. Amin *et al.* (2010) tested six isolates of *Trichoderma* against three different soil borne pathogens namely *R. solani* (isolates from tomato), *Sclerotinia sclerotiorum* (causing web blight of beans) and *S. rolfii* (causing collar rot of tomato) under *in vitro* conditions and found that maximum inhibition (71.41%) in *R. solani* by *T. viride* (Tv-2) followed by *T. viride* (Tv-1) and *T. harzianum* (Th-1). *T. viride* (Tv-1) showed best antagonist inhibiting 67.91 and 66.21% over control in *S. rolfii* and *S. sclerotiorum*, respectively. They also found that all the *Trichoderma* isolates significantly inhibited sclerotia production in all three tested pathogens. *Trichoderma* is also effective against Stemphylium blight of lentil. Subedi *et al.* (2014) reported percentage disease control and percent yield increase were higher in *T. viride* i.e. 42.14% and 58.80% respectively. *T. viride* also showed high compatibility with fungicides (potassium phosphonate and fosetyl aluminium) and incompatibility with fungicides (carbendazim, hexaconazole, potassium phosphonate + hexaconazole mixture and captan + hexaconazole mixture) (Dhanya *et al.*, 2016).

2.1 Isolation from soil

In 1981, Papavizas used V-8 juice agar as the basal medium for isolation of *Trichoderma* from soil (Papavizas, 1982). Later Papavizas and Lumsden,

(1982) developed *Trichoderma* medium E (TME) for isolation of *Trichoderma* from soil. Elad *et al.* (1981) developed a *Trichoderma* selective medium (TSM) for isolation of *Trichoderma* from soil. They used chloramphenicol, pentachloronitrobenzene, p-dimethylaminobenzenediazo, sodium sulfonate and rose-bengal as selective fungal inhibitors. Elad and Chet (1983) further improved TSM medium by adding Captan 50% 2µl/ml which helped to avoid *Fusarium* species. Later, Askew and Laing (1993) modified TSM by adding metalaxyl which suppressed oomycete. However, Williams *et al.* (2003) found captan, was inhibitory to conidial germination of *T. harzianum*.

2.2 Survival in the soil

Trichoderma can abundantly proliferate in various soils and can degrade various organic matters (Papavizas, 1982; Lewis & Papavizas, 1984). They observe that survival of chlamydospore was better than conidia in soil. Lewis and Papavizas (1984) reported that even freshly added conidia declined in the soil and the stable population densities of *Trichoderma* remained is due to survival of chlamydospores. According to Kim *et al.* (1992) with increase in soil depth, the rhizosphere colonizing ability of propagules get reduced. According to Poosapati *et al.* (2014) to adapt to extreme conditions such as high temperatures, some *Trichoderma* produce trehalose, mannose, and raffinose. These sugars are stress protectant and thus *Trichoderma* are able to grow well up to 37-40°C. Some *Trichoderma* spp. are reported to grow in extreme high or low pH, low oxygen (Chovanec *et al.*, 2005) and high salinity conditions (Gal-Hemed *et al.*, 2011).

2.3 Establishment and proliferation in the soil or rhizosphere

The plant root exudates various organic compounds that affect ecological and biological processes in rhizosphere (Barman *et al.*, 2021). Several *Trichoderma* species compete with pathogens for nutrients, space and infection sites on plant roots (Blaszczyk *et al.*, 2014). The effectiveness of *Trichoderma* is affected by their biocontrol qualities and their competency in the rhizosphere and plant root colonization (Barman *et al.*, 2021). *Trichoderma* introduced on seed must multiply first in the rhizosphere of the host plant to inhibit the pathogen. Biswas and Sen (2000) reported seed treatment with conidial suspension was more effective in reducing disease incidence than soil application. Juliatti *et al.* (2019) stated that seed microbiolization is an important method of application of biocontrol agents

since it requires significantly less amount of biological material as compared to the quantity needed for soil application.

2.4 Environmental effects on growth of *Trichoderma*

2.4.1 Effect of soil type

Trichoderma species are affected by soil pH, moisture and electrical conductivity. According to Wong *et al.* (2002); Singh *et al.* (1998) moist soil conditions is favorable for the antagonistic activity and growth of *Trichoderma*. Similarly, *Trichoderma* spp. are favored by the acidic soils condition. Amir-Ahmadi *et al.* (2017) loam and clay loam reported the best performance of *T. harzianum* in sandy loam soil and loam soil containing 2% organic matter. Soil texture affect the ability of antagonistic fungi to suppress the plant pathogens (Moosavi & Zare, 2015).

2.4.2 Effect of temperature

Temperature is among the important parameters that affect the biomass production of *Trichoderma*. According to Sharma *et al.* (2005) incubation temperature had profound effect on growth of *Trichoderma*. Domingues *et al.* (2016) isolates of *Trichoderma asperellum* (IBLF 897, IBLF 904 and IBLF 914) found that the mycelium of *Trichoderma* grew well at 27 to 32°C and inhibited at the temperature of 7°C and 42°C. Carro-Huerga *et al.* (2021); Adhikari *et al.* (2022) observed that the studied *Trichoderma* strains grew well at 25°C while, some strains showed good response at 35-40°C as well. Di Lelio *et al.* (2021) observed different growth for *T. afroharzianum* and *T. atroviride* at 20 and 25°C, both in *in vitro* and *in vivo* conditions. The lower temperature i.e. 20°C encouraged *T. afroharzianum* growth, whereas *T. atroviride* was abundant at 25°C. According to Klein and Eveleigh (2002); Carro-Huerga *et al.* (2021) the optimum growth temperature for most *Trichoderma* spp. is in the range between 25-30°C and thus are mesophilic in nature. Poosapati *et al.* (2014) observed *T. asperellum*, and *T. harzianum* survived the heat stress, however, with increase of temperature to 37°C, the germination rates of all isolates reduced. The tolerance of *Trichoderma* towards high temperature can be attributed to its ability to accumulate stress protectants. Under stress conditions accumulation of trehalose, mannose and raffinose increase in the cells exposed to stress conditions (Poosapati *et al.*, 2014). Under heat stress condition, accumulation of trehalose and mannitol are reported to be necessary for fungal survival and cell protein and structure stability (Daryaei

et al., 2016) trehalose is accumulated in response to a heat shock or to an oxidative shock. The authors have characterized the *A. nidulans* *tpsA* gene encoding trehalose-6-phosphate synthase, which catalyses the first step in trehalose biosynthesis. Expression of *tpsA* in a *Saccharomyces cerevisiae* *tps1* mutant revealed that the *tpsA* gene product is a functional equivalent of the yeast *Tps1* trehalose-6-phosphate synthase. The *A. nidulans* *tpsA*-null mutant does not produce trehalose during conidiation or in response to various stress conditions. While germlings of the *tpsA* mutant show an increased sensitivity to moderate stress conditions (growth at 45 °C or in the presence of 2 mM H₂O₂).

3. MODE OF ACTION BY *TRICHODERMA*

Trichoderma spp. are free-living, commonly growing fungi in soil and rhizosphere of various crops (Harman *et al.*, 2004). *Trichoderma* can control plant pathogens directly by competition for nutrients, mycoparasitism, production of cell wall degrading enzymes, and production of antibiotics (Harman, 2006) and indirectly by stimulation of plant defense systems (Benítez *et al.*, 2004).

3.1. Direct mechanism

3.1.1 Competition for nutrients and space

According to Chet (1987) competition between antagonist and plant pathogen for space and nutrients is a classical mechanism of biological control. One of the most common causes of death of microorganisms are due to starvation. *Trichoderma* are competent for getting limited nutrients resulting in control of fungal plant pathogens (Benítez *et al.*, 2004). Generally, filamentous fungi require iron for spore viability and under iron starvation condition, low-molecular-weight ferric-iron specific chelators are excreted to mobilize environmental iron (Eisendle *et al.*, 2004). These ferric-iron specific chelators are known as siderophores. Some *Trichoderma* species produce high amount of effective siderophores that chelate iron and absorb them causing starvation of iron to other fungi and stopping their growth (Benítez *et al.*, 2004). Based on the chemical nature siderophores are divided into three major groups i.e hydroxamate, catecholate, and carboxylate (Tyśkiewicz *et al.*, 2022).

Trichoderma are proficient in mobilization of immobile nutrients and their utilization (Singh *et al.*, 2018). Root exude excess amount of sugar, amino acids, iron, vitamins, organic acids and *Trichoderma* compete for these carbon source with other fungi such as *R. solani*

and *F. oxysporum* (Sarrocco *et al.*, 2009). *Trichoderma* can displace pathogens from a common habitat such as plant tissues, rhizospheres, and phyllospheres by colonizing them (Ghorbanpour *et al.*, 2018). The degree of colonization of the host plant by the biocontrol agents depend upon their adaptation to the environmental conditions in which they live and plant colonization strategies (Ghorbanpour *et al.*, 2018). Rapid growth and proliferation of *Trichoderma* on substrate determine their aggressiveness (Tyśkiewicz *et al.*, 2022). The strain quick in colonizing substrate can quickly eliminate slow growing pathogens (Oszust *et al.*, 2020).

3.1.2 Mycoparasitism and cell wall degrading enzymes

Trichoderma is a mycoparasite and has ability to parasitize other fungi such as *R. solani*, *S. rolfsii*, *S. sclerotiorum* etc. The mycoparasitic activity of *Trichoderma* on fungal pathogens starts with prey sensing and growing toward them, adhesion to the host, and intense branching and coiling around them. Also, they can form appressoria-like structure for penetration of the host cells (Moreno-Ruiz *et al.*, 2020; Mukherjee *et al.*, 2012). After penetration of the host mycelium, *Trichoderma* utilize the intracellular contents of the host (Saba *et al.*, 2012). *Trichoderma* species when come in contact with the pathogen cell wall, are triggered to produce many chitinolytic enzymes such as endochitinases, 1,4- β -acetylglucosaminidases, and exochitinases (Benítez *et al.*, 2004). β 1,3 glucanases produced by *Trichoderma* hydrolyse β 1,3 glucan present in cell wall of fungal pathogens (Druzhinina *et al.*, 2011). According to Vinale *et al.* (2008) volatile secondary metabolites produced by *Trichoderma* species also play a key role in mycoparasitism of fungal pathogens. Contreras-Cornejo *et al.* (2016) reported *T. atroviride* produced 6-pentyl- α -pyrone and *T. virens* produced mono- and sesquiterpenes with antimicrobial functions. According to Karlsson *et al.* (2017) lectins from the fungus cell wall and release of secondary metabolites play important roles in recognition and signaling pathways such as MAPK and cAMP pathway in *Trichoderma*. After recognition of hosts, expression of molecular weapons involved in host lysis and parasitization occur. During mycoparasitism genes families such as *ech42* and *prb1* are upregulated in *Trichoderma* (Köhl *et al.*, 2019). Nature of antagonism by *Trichoderma* species also dependent upon target pathogen. Against *R. solani*, parasitism is more common mode of antagonism while, against *Fusarium*, antibiosis is the predominant one.

3.1.3 Antibiosis

The antagonistic organisms release low molecular weight diffusible secondary metabolites and antifungal antibiotics that are detrimental for the pathogen and inhibit their growth. Majority of *Trichoderma* species produce different types of volatile and nonvolatile toxic such as harzianic acid, alamethicins, tricholin, peptaibols, antibiotics, 6-pentyl- α -pyrone, massoialactone, viridin, gliovirin, glisoprenins and heptelidic acid (Benítez *et al.*, 2004). In general, antibiotic production is directly correlated with biocontrol ability of *Trichoderma* strains. According to Shi *et al.* (2012) *T. pseudokoningii* SMF2, exhibited antibiotic activities by producing TrichokoninsVI, a type of peptaibol causing extensive programmed cell death in fungal pathogens. Siddiquee (2017) found *T. harzianum* synthesized trichorzins, harzianins, trichotoxin, and trichokindins. Several *Trichoderma* species are also capable of producing polyketides, tetracyclines, macrolides and mycotoxins (Zeilinger *et al.*, 2015) terpenoids, pyrones and anthraquinones (Siddiquee, 2014). However, a particular antibiotic may play important role in antibiosis of one strain and another strain may not even produce it.

3.1.4 Sclerotial parasitization

The soil borne pathogens *R. solani*, *S. rolfsii* and *S. sclerotiorum*, have the ability to survive in soil for prolonged duration due to formation of sclerotia, a resting structure. These sclerotia play important role in disease cycle of the pathogen. *Trichoderma* species have ability to parasitize the mycelium and sclerotia of these soil borne plant pathogens. Papavizas and Lewis (1989); Bhagat and Pan (2011); Sarrocco *et al.* (2006); Adhikari *et al.* (2022) reported that the different strains of *Trichoderma* spp. varied in their ability to colonize the sclerotia of *S. rolfsii*. According to Sarrocco *et al.* (2006); Köhl *et al.* (2019), some *Trichoderma* species are able to penetrate the rind and colonize the inner cell layers of sclerotia, leading to destroying and leaving them not viable. Rawat and Tewari (2010) reported *T. harzianum* parasitization on sclerotia of *S. rolfsii* caused deformation, lysis and degradation and disappearance of cytoplasmic granules of cell wall causing loss of cellular integrity in sclerotia. Sarrocco *et al.* (2006) reported the uniformly distribution of *T. virens* mycelium beneath the rind of sclerotia but not in cortex and medulla. *Trichoderma* entered the sclerotia without any preferential point and caused infection. *Trichoderma* can induce enzymatic degradation of rind

walls and degrade melanin of the sclerotia (Sarrocco *et al.*, 2006). Liu *et al.* (2009) found the uniform distribution of *T. virens* mycelium in inner and outer layer of sclerotia as intercellular fungal growth. Tsahouridou and Thanassouloupoulos (2001) observed the presence of hyphae, conidia and chlamydospores of *T. koningii* in the medullar tissues of the sclerotia. Ibarra-Medina *et al.* (2010) reported deformations, collapsing, cracking and increment or diminishing sclerotia size of sclerotia by different strains of *Trichoderma*. According to Butler *et al.* (2005) among different mechanism of action in biocontrol, destruction of the melanin or inhibition of its synthesis is an important one.

3.2 Indirect action of biocontrol agents

3.2.1 Induction of resistance in plants

Plants have ability to protect themselves from range of deleterious microorganisms. They are enable to recognize the invaders and produce an arsenal of antimicrobial compounds and thereby reduce the impact of pathogens invasion (Djonović *et al.*, 2007). Induction of defense response is one of the most effective resistance mechanisms and are activated only upon pathogen invasion. The induction of plant resistance to pathogens are the consequences of the action of various elicitors released from the microorganisms cells (Tyśkiewicz *et al.*, 2022). *Trichoderma* strains have ability to induce plant defense response and systemic resistance in plant. During plant - *Trichoderma* interaction, *Trichoderma* release numerous elicitors which induce salicylic acid (SA), jasmonic acid (JA) or reactive oxygen species (ROS) mediated signals in plant triggering defense proteins expression (Nawrocka & Małolepsza, 2013). After gene activation, the plant produces different enzymes that suppress the pathogen and enhance biochemical and structural barriers against the intruders (Nawrocka & Małolepsza, 2013). Perazzolli *et al.* (2011) showed that *Trichoderma* strain, T39 reduced downy mildew severity on susceptible grapevines by a direct modulation of defense-related genes and enhanced their expression after pathogen inoculation. They suggested that jasmonic acid and ethylene signals were induced by T39. Vinale *et al.* (2008) reported plant defence mechanisms were activated and plant growth in pea, tomato and canola were regulated when harzianolide, 6-pentyl-a-pyrone, and harzianopyridone were applied. Alizadeh *et al.* (2013) reported *T. harzianum* Tr6, elicit induced resistance in cucumber against *F. oxysporum* f.sp. *radicis cucumerinum* and in *A. thaliana* against *B. cinerea*. Plants immune system are trigger via

microbe-associated molecular patterns (MAMPs) when *Trichoderma* colonize root of plants (Hermosa *et al.*, 2012). According to Guo *et al.* (2021) *T. asperellum* ACCC30536 secreted xylanase that stimulated the systemic resistance in *Populus davidiana* and *P. alba* var. *pyramidalis* Louche seedlings against *Alternaria alternata*, *R. solani*, and *F. oxysporum*.

3.2.2 Growth promotion

Trichoderma spp. are hyperparasite of other fungi as well as avirulent plant symbionts (Saba *et al.*, 2012). Some strains of *Trichoderma* colonize root surfaces of plants and penetrate into the epidermal cells. Root colonization by *Trichoderma* suppress deleterious root microflora, produce growth stimulating factors and increase nutrient uptake. Mastouri, (2010) reported increase in vigour and emergence of tomato by *T. afroharzianum*. Mayo-Prieto *et al.* (2020) observed *Trichoderma* isolated from soil increased hypocotyl diameter and length of root system of bean plants. Degani *et al.* (2021) caused by *Magnaportheiopsis maydis*, is considered a major threat to commercial fields in Israel, Egypt, Spain, and India. Today's control methods include chemical and agronomical intervention but rely almost solely on resistant maize cultivars. In recent years, LWD research focused on eco-friendly biological approaches to restrain the pathogen. The current study conducted during two growing seasons explores the potential of three *Trichoderma* species as bioprotective treatments against LWD. These species excelled in preliminary assays performed previously under controlled conditions and were applied here in the field by directly adding them to each seed with the sowing. In the first field experiment, *Trichoderma longibrachiatum* successfully rescued the plants' growth indices (weight and height also reported reduction in incidence of *Magnaportheiopsis maydis* in maize and improved growth and yield by application of *T. longibrachiatum*. Nieto-Jacobo *et al.* (2017) reported increase in *Arabidopsis* fresh biomass by 72% and the number of secondary roots by 64 by *Trichoderma atroviride*. However, they found that *T. asperellum* significantly inhibited *Arabidopsis* growth up to 74% than in non-inoculated soil. They stated that not all *Trichoderma* strains promote plant growth. Baazeem *et al.* (2021) revealed that inoculation of *T. hamatum* on maize, cowpea, small millet, green gram, and black gram resulted in improved seedlings growth. They also reported that the enzyme activity of rhizosphere soil improved by 12-69%. *T. hamatum* soil treatment promote enzyme mediated nutrient recycling activity.

Bader *et al.* (2020) found that *Trichoderma* strains were able to produce IAA and solubilize inorganic phosphorous resulting in improved growth of tomato plant. They also found increased photosynthesis, and dry roots weight indicating profound effect of *Trichoderma* inoculation on root development and modification of root architecture. According to Contreras-Cornejo *et al.* (2009) *Trichoderma* spp. can induce growth promotion in *A. thaliana* by synthesizing IAA.

3.2.3 Germination stimulation

Seed treatment with *Trichoderma* spp. trigger seed to produce enzymes and phytohormones required for seed germination and also enhance seed germination and seedling vigor. Several researchers have observed enhanced germination percent in tomato, pea beans, chickpea, etc. (Mastouri, 2010; Singh *et al.*, 2015; Mayo-Prieto *et al.*, 2020). Bezuidenhout *et al.* (2012) observed that seeds treated with *Trichoderma* had early germination indicating the effect similar to gibberellic acid. Upon evaluation of secondary metabolites secreted by *T. harzianum*, gliotoxin was found to be mimicking the plant growth hormone. According to Howell (2002) germinating seeds release nutrients and addition of *Trichoderma*, metabolizes these nutrients rendering them unavailable to the pathogens.

3.2.4 Responses of *Trichoderma* under drought stress

Generally, plants face several abiotic stresses that affect seed germination, seedling vigor, plant establishment, growth and development, and ultimately seed yield. Several *Trichoderma* species are competent to alleviate abiotic stress and improve plant growth and vigor (Hermosa *et al.*, 2012). Bae *et al.* (2009) reported that *T. hamatum* increasing root growth of cocoa plants resulting in tolerance towards water stress condition. Mastouri *et al.* (2010) reported that *Trichoderma* strains are known to offer plant tolerance against physiological stress such as seed aging. The researcher found that peroxide levels were reduced when physiologically stressed seed of tomato treated with *T. harzianum*. Under continuous stress, Reactive oxygen species (ROS) production is increased (Schafer & Buettner, 2001). Production of ROS is important as they play a crucial role in signaling during stress condition. In response to produced ROS, plants activate the antioxidant defense systems and increased activities of ascorbate and glutathione-recycling enzymes. Mastouri *et al.* (2010) demonstrated that *T. harzianum* increases seedling vigor and ameliorates stress by inducing

physiological protection in plants against oxidative damage. Navazio *et al.* (2007) observed that when soybean cell culture was treated with culture filtrate of *T. atroviride*, intracellular ROS accumulation was detected. Such signals can induce plant ROS scavenging mechanisms which results in protection against the oxidative damage (Mastouri *et al.*, 2010). Along with the previously mentioned mechanism, *Trichoderma* can ameliorate plant growth by lowering deleterious elevated ethylene levels under abiotic stress conditions (Brotman *et al.*, 2013). In recent studies, it has been observed that *Trichoderma* spp. can reprogram plant gene expression which is a primary method of pathogen control. As a result, induced systemic resistance (ISR) occurs (Shoresh *et al.*, 2010). According to Mishra *et al.* (2020) *Trichoderma* has the ability to produce high level of phenols and proline in drought stressed rice plants leading to accumulation of chlorophyll in the plant. Ma *et al.* (2020) whereas the underlying mechanisms of *Trichoderma*-induced drought resistance of host plants remain largely elusive. Herein, the effects of a *Trichoderma harzianum* isolate on maize's responses to drought stress were investigated. Inoculation with *T. harzianum* significantly promoted the growth and enhanced drought tolerance of maize plants. The whole genome expression profiles of the *Trichoderma*-inoculated plants were examined by RNA-sequencing, showing that several differentially expressed genes were positively associated with the process of ethanol-acetic acid metabolism. Compared with non-inoculated (control reported increment in ABA level with drought stress, however, the levels of ABA was remarkably high in *T. harzianum* inoculated maize plants than the controls. Increased levels of ABA responsive gene transcripts were observed in transgenic Arabidopsis plants when exposed to abiotic and biotic stresses (Shi *et al.*, 2017).

4. MODE OF APPLICATION

There are several methods for application of *Trichoderma* for the successful control of plant diseases. Mode of application of *Trichoderma* play a vital role in establishment and site of action of the antagonist.

4.1 Seed treatment

Seed treatment is one of the effective method for application of biocontrol agents. In this method seed is coated with conidia of *Trichoderma* before sowing. The ability of the antagonist to proliferate and colonize the rhizosphere determine the efficacy of seed treatment

(Nakkeeran *et al.*, 2016). Juliatti *et al.* (2019) suggested that soon after treatment, seeds should be sown to avoid drying of spore and to enhance spore germination. Seed microbiolization not only promote seed germination, seedling emergence and disease control but also protect them from other fungi during storage (Juliatti *et al.*, 2019). Biswas and Sen (2000) reported seed treatment with conidial suspension of *Trichoderma* was effective in reducing collar rot of groundnut caused by *S. rolfsii*. Under saline condition, tomato seeds treated with *Trichoderma* spp. germinated faster than control (Contreras-Cornejo *et al.*, 2016; Mastouri, 2010). Juliatti *et al.* (2019) stated that seed microbiolization as an important method of application of biocontrol agents since it requires a small amount of biological material compared to the quantity needed for soil application.

4.2 Soil treatment

For the biocontrol of soil borne plant pathogens, soil application of *Trichoderma* are also recommended. But for their effective management, high population of the antagonist is required (Nakkeeran *et al.*, 2016). Jegathambigai *et al.* (2010) reported that soil amendment with *Trichoderma* spp. provided control of Pigeonpea wilt caused by *Fusarium udum* and collar rot of Zamiodulcas caused by *S. rolfsii* by 22-30.9% whereas seed treatment was ineffective at high level of pathogen and disease control was less than 7%. As *Trichoderma* sp. is a soil inhabitant, it has an opportunity to establish and multiply more quickly in soil than on seed surface. Although soil application of bio agents is effective against plant pathogens, their feasibility for field application is low because of the cost of production and higher volume requirements.

4.3 Foliar application

It is difficult for biocontrol agents to establish and proliferate in phyllosphere as the foliar environment

is frequently fluctuating and harsh (Sawant, 2014). However, the success of antagonists on foliage depends on its ability to colonize the surfaces (Lo *et al.*, 1997) brown patch, and dollar spot of creeping bentgrass was investigated. Spray applications of conidial suspensions (SA. Panwar *et al.* (2014) reported that the foliar spray of antagonist reduced the fusarium head blight of wheat caused by *F. graminearum* under greenhouse condition. Oros and Naar (2017) also reported that the liquid formulations of *T. harzianum* were efficient against rose black spot disease caused by *Diplocarpon rosae*. According to Sawant (2014), *Trichoderma* have been successfully utilized to control foliar diseases such as *B. cinerea* in strawberry, *C. gloeosporioides* and *Plasmopara viticola* in grape, *Cladosporium fulvum* in tomato and *S. sclerotiorum* in cucumber. Foliar application of bio agents is preferred during evening hours as there is least environmental effect on colonization and efficacy (Avila & Gutierrez, 1992).

5. CONCLUSION

Trichoderma is an important component in integrated disease management strategies. It does not lead to development of resistance in plant pathogens, do not enter into the food chain and do not create any pollution problems in the environment. Along with controlling plant pathogens, *Trichoderma* strains also stimulate growth and resistance in plants. *Trichoderma* is a soil resident, thus it may quickly colonize new soil, which has a positive impact on the health of the soil as well. It can be used in a variety of ways to effectively control plant diseases. *Trichoderma* application method is crucial for the establishment and location of the antagonist's site of action.

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