Trichoderma as a Biocontrol agent against *Fusarium* fungal diseases

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ABSTRACT:
Trichoderma spp. have been the most common fungi applied as biological control agents (BCA) as an wide range of fungal diseases of crop plants. Its use have recorded good success rate in controlling major plant diseases. *Trichoderma* mainly based on the activation of single or multiple control mechanisms. It is known that the *Trichoderma* based biocontrol mechanisms mainly rely on mycoparasitism, production of antibiotic and/or hydrolytic enzymes, and competition for nutrients as well as induced plant resistance. This chapter reviews the recent updates on published research findings on mechanisms used by *Trichoderma* as biocontrol agent of fungal diseases of crop plants particularly *Fusarium* wilt disease of tomato caused by *Fusarium oxysporum f. sp. lycopersici.*

KEYWORDS*: Trichoderma, Fusarium* wilt, Biocontrol activity

I. INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the important vegetable crops which is grown in almost all parts of India. In India, tomato was cultivated in 789.2 mil. ha. Of area, produced to a tune of 19.33 million tonnes with a productivity of 32.8 tons ha^{-1} in India. Globally, tomato production was 188 million tonnes with a productivity of 80tons ha^{-1}. It has high nutritive value and is rich in minerals like phosphorous and potassium and vitamins like vitamin- B and vitamin- C. The crop is also an important food for curing cancers like breast and prostate cancer. Tomato is normally attacked by various diseases like bacterial wilt (*Ralstonia solanacearum),* early blight (*Alternaria solani),* late blight (*Phytophthora infestans), Fusarium* wilt (*Fusarium oxysporum* f. sp. *lycopersici)* and root knot nematode (*Meloidogyne incognita)* and causes severe economic yield loss (Barari, 2016).

Fusarium wilt of tomato is one of the economically serious diseases of tomato. The disease accounts for up to 80% yield loss when incidence was severe. As the pathogen is soil borne in nature, management of this disease is very difficult. Fungicide has to be applied to soil to control the disease but the method is not practically feasible and less effective also. Thissoil application of fungicides is hazardous to applicator and also affects beneficial soilmicroflora. Apart from this the chemical will persist in soil for long duration and contaminatessoil environment (Srinon *et al.*, 2006). Even though resistant cultivars are available in the market, the crops were susceptible to other races of the same pathogen due to evolution of newpathogenic races (Margaret *et al.*, 2011).

Trichoderma (fungal BCA) are the free-living and diverse fungal microbial community, highly interactive in root, soil and foliar environments (Harman *et al.*, 2004), known worldwidefor their utility as bio-control agents in management of fungal diseases of crop plants (Ashwani*et al.*, 2015). The genus *Trichoderma* was identified long back during the early 17th century but its bio-control ability was revealed only in thirties by Weindling (1932; 1937). The *Trichoderma spp.* are investigated as an efficient antagonist over 70 years and it occupies almost 50% of fungal BCAs mark

1.Trichoderma:

Trichoderma, a filamentous fungus is cosmopolitan in nature, present in every type of soil, plant material and decaying vegetation and decomposes organic matter in the soil that canbe used as a bio fungicide. Persoon in 1794 described the genus *Trichoderma* but the first attempt to classify the genus was made by Rifai in 1969.

Trichoderma spp. have high reproductive capacity, efficient competitors for space and nutrients, have capacity to alter rhizosphere and utilize nutrients effectively, can survive in unfavourable conditions, can make unavailable portion of plant nutrients into available form andare more aggressive against plant pathogens. These features promoted *Trichoderma* as effective biocontrol agents (BCAs). There are several species of *Trichoderma* including *T. viride, T. harzianum, T. virens* etc. which are effectively used for the control of plant diseaseslike wilts (*Fusarium spp.),* root rots (*Rhizactonia spp.*), collar rots (*Sclerotium spp.*), dampingoff (*Pythium spp*.) etc. in several crop plants (Samuels, 1996).

Trichoderma are opportunistic, avirulent plant symbionts, as well as parasites of other fungi. At least some strains establish robust and long-lasting colonization's of root surfaces and penetrate into the epidermis and a few cells below this level. They produce or release a variety of compounds that induce localized or systemic resistance responses, and this explains their lack of pathogenicity to plants. These root–microorganism associations cause substantial changes to the plant proteome and metabolism (Harman *et al*., 2004).

Species morphology is mainly used for classification of this genus. In India, Thakur and Norris first isolated the genus from soils of Madras in 1928 (Kim and Hwang, 2007).

Plants are protected from numerous classes of plant pathogen by responses that are similar to systemic acquired resistance. Root colonization by *Trichoderma spp.* also frequentlyenhances root growth and development, crop productivity, resistance to abiotic stresses and the uptake and use of nutrients (Vasundara *et al*., 2015). *Trichoderma spp*. are the most successfulbiocontrol agents as more than 60% of the registered bio-fungicides used in today's agriculturebelongs to *Trichoderma*-based formulation.

1.1.Mode of biocontrol activity of *Trichoderma*:

Main mechanism employed by *Trichoderma* for controlling pathogens is competition, myco-parasitism, induced resistance of the host and inactivation of pathogen enzymes (Chet and Sivan, 1986). The mode of action of *Trichoderma* includes antibiosis, that is the productionof antibiotics from antagonist and this mechanism is called as indirect antagonism because there is no direct hyphal contact with the target pathogen or microbe (Webster, 1995).

Ethylene or terpenoid phytoalexins are secreted by *Trichoderma* which serve as stimulants to plant growth and plant resistance to pathogens (Howell, 2003). Myco-parasitismis the mechanism which involves hyphal supercoiling and formation of appresorium like structure (Ponmurugan and Baby, 2003). Cellulases, chitinase, endochitinase and chitobiosidase are the hydrolytic enzymes secreted by the *Trichoderma* (Ozbay and Newman,2004).

Biological control activity by *T. harzianum* T22 is attributed to the production of antibiotics, parasitism of pathogenic fungi, competition with plant pathogens for nutrients (including key plant exudates) and space, inhibition or degradation of key enzymes required by parasites and plant pathogens to penetrate plant surfaces, and the stimulation of localized orsystemic plant resistance as a result of *Trichoderma* infection (Adams *et al*., 2007).

When plants are inoculated with *Trichoderma* various bioactive metabolites are produced extensively which includes elicitors or plant resistance inducers (Woo and Lorito 2007).

Trichoderma spp. secretes various hydrolytic enzymes that will destroy cell wall of the target microbe. Coil formation around the pathogen is common in mycoparasitism (Anita *et al.*, 2012).

1.2. Efficiency of native *Trichoderma* isolates in managing *Fusarium* wilt of tomato:

Sundaramoorthy and Balabaskar in 2013 evaluated the native *Trichoderma* isolates fortheir efficacy to promote growth and yield of tomato and to manage *Fusarium* wilt disease caused by *Fusarium oxysporum* f. sp. *lycopersici* under in vitro and in vivo conditions. Fifteen native *Trichoderma* antagonists were isolated from healthy tomato rhizosphere soil in different geographical regions. *Trichoderma harzianum* (ANR-1) isolate effectively inhibited the radial mycelial growth of the pathogen (by 53%) when compared to all other isolates under in-vitro condition. Under greenhouse conditions, the application of *Trichoderma harzianum* (ANR-1) exhibited the least disease incidence (by 15.33%). Also tomato plants treated with *Trichoderma harzianum* (ANR-1) isolate showed a significant stimulatory effect on plant height (by 73.62 cm) and increased the dry weight (by 288.38 g) of tomato plants in comparison to other isolatesand untreated control (60.98cm plant height and 25.8% disease incidence).

The effects of *T. harzianum*, *T. asperellum*, and *T. virens* on the wilt disease complex of tomato caused by *F. oxysporum f. sp. lycopersici* and *Rhizoctonia solani* were investigated under greenhouse conditions using pot culture studies. The disease control was highest with a combination of *T. harzianum*, *T. asperellum* and *T. virens* (80-87%) followed by binary combination of *Trichoderma. spp*. (79%-82%), while the lowest control was done with *T. viride*(65%) (Akrami and Yousefi, 2015).

T. harzianum isolate N-8 effectively inhibited radial mycelial growth of *F. oxysporum* f. sp. *lycopersici* by 68.22% under laboratory conditions. Under green-house conditions N-8 application resulted in less disease incidence of 14.75%. Also, there was an increase in plant height (by 70.1%) and dry weight of tomato plants (265.42g) compared to untreated control (54.6 and 195.5g) (Barari, 2016).

In-vitro and in-vivo studies were conducted to study the efficacy of two species of antagonists (*T. viride* and *Trichoderma spp*.) alone and in combination against tomato *Fusarium* wilt pathogen. *T. viride* caused 52.31% inhibition of radial mycelial growth of the pathogen, whereas *Trichoderma spp.* inhibited growth up to 47.09% under in-vitro condition. Under in-vivo condition both species have reduced disease severity. Combination of both *Trichoderma spp.* resulted in highest reduction in disease severity (16.98-25.68% disease severity) and also increased tomato yield by 31% compared to control (Verma *et al*., 2017).

Under greenhouse conditions using pot culture studies, the application of *T. harzianum*, Th-TJ exhibited the least disease incidence (33%). Also, tomato plants treated with *T. harzianum*, Th-TJ isolate showed a significant stimulatory effect on plant height by (78.33cm) and the dry weight by (3.33g) of tomato plants, in comparison to untreated control (1.4g) which was statistically significant ($p<0.05$) (Romika *et al*., 2019).

Treatment with *T. harzianum*, alone or biofertilizer dual inoculation with *Azospirillum brasilense*, into potting mixture proved more powerful in decreasing disease severity % of foliar yellowing and wilt or vascular browning by about (16.25 and 16.67%) and (8.75 and 10.71%), respectively of tomato cv Super-Strain B, plants than other treatments and compared with control. Combined inoculation with *A. brasilense* and *T. harzianum*, increased growth characters, i.e. plant height (cm), number of branches per plant, plant fresh weight and plant dry weight g/plant, by about (77.6 cm, 5.75/plant, 552.9 and 87.13g.) respectively, yield and yield components of tomato plants, i.e. number of fruits/plant, weight of one fruit (g), fruits yield/plant (kg) and fruits yield/plant (ton/fed) by about (14.8/plant, 113.4g, 2.07kg and 25.13ton/fed) respectively compared with the other treatments and control (Khalil and Shimaa, 2020).

1.3. Efficacy *Trichoderma* isolates in managing pathogens other than *Fusarium oxysporum* f. sp. *lycopersici (FOL)*.

Fourteen isolates of *Trichoderma* were collected, out of them eight isolates were effective in inhibiting the growth of *Fusarium solani*, causal agent of wilt of potato in dual culture and through production of volatile and non-volatile inhibitors. *T. brevicompactum* (T1), *T. longibrachiatum* (T5) and *T. asperellum* (T2) were better than all other isolates in inhibiting the pathogen. Under green-house condition, in pot culture studies all the treatments of *Trichoderma* were reduced the disease incidence when pots were artificially inoculated with *F. solani* compared to *Fusarium* infested control. Best results were obtained in the treatment *F. solani*+ *T. longibrachiatum* (T5) with 6.25% disease incidence in comparison to pathogen infested control (75% disease incidence). Whereas *F. solani*+ *T. brevicompactum* also controlled the disease with 12.50% disease incidence (Ommati and Zaker, 2012).

Different concentrations of the fungus *T. asperellum* strain T34 applied and the incidence of disease caused by *Phytophthora capsici* in pepper was studied by Segarra in 2013. Different application methods of BCA and pathogen inoculation were examined. T34 and etridiazole (Terrazole®) were compared for their ability to suppress *P. capsici*. T34 reduced the disease in most of the cases and caused up to 71% reduction in disease incidence, whereas etridiazole was effective only when applied at the same time as the pathogen.

Inoculation with *Trichoderma* isolates 2, 4, 7 and 11 significantly increased seed

germination by 4.67, 4.67, 4.33 and 4.33%, respectively over control. *Trichoderma* isolates 2, 7 and 9 showed 33.33% suppression of growth of *Rhizoctonia solani* and observed a disease severity of 20, 15.67 and 20%, respectively (Hamdia and Nadarajah, 2013). When the pathogen was inoculated alone, disease incidence and severity were 100% and 77.67% respectively. When *Trichoderma* and *Bacillus subtilis* applied together, isolate 2 and 7 showed highest reduction of disease incidence (11% each)

and disease severity (4.33%), 60days after transplanting. When *Trichoderma* isolates used in combination with *B. subtilis,* showed 22- 33% and 15.33-22% reduction in disease incidence and severity, respectively.

1.4. Influence of Trichoderma isolates on nutrient uptake in tomato:

Islam *et al*., (2011), conducted an experiment to study the effect of *T. virens* IMI- 392430, *T. pseudokoningii* IMI-392431, *T. harzianum* IMI-392432, *T. harzianum* IMI-392433and *T. harzianum* IMI-392434 on seed germination and seedling parameters in chili both laboratory and field conditions. Seed coating with *Trichoderma* spore suspension added with 2% starch caused maximum seed germination. Among the five *Trichoderma* strains, *T.harzianum* IMI-392432 (100%) gave the highest germination percentage followed by *T. harzianum* IMI-392433 (98%), *T. harzianum* IMI-392434 (95%), *T. virens* IMI-392430 (80%)and *T. pseudokoningii* IMI-392431 (70%) treatment both in laboratory and field conditions, respectively while control decrease (43%) these value. Chili seeds also gave the highest vigour index values with *T. harzianum* IMI-392432 (350) which confirmed to better germination.

Singh *et al*., (2014) evaluated the effects of *T. harzianum* (BHU51, BHU105, BHU51+BHU105) on *R. solani* influence on tomato. Seed treatment of *Trichoderma* reduced damping off incidence (20-32% disease incidence) and increased vigour index (1227-1393) of the plants. Combination treatment (BHU51+BHU105) caused maximum reduction in disease incidence (20% incidence). Mineral content (N, P, K, Ca, Mg, S, Zn, Cu, Mn and Fe) was alsohigher (40-50% more than control) in *Trichoderma* treatments than untreated control. Field trials also showed increase in shoot length, chlorophyll content and yield (20-30% higher) thanthe control, by consortium.

Inorganic phosphorous was released from tri-calcium phosphate by all *Trichoderma* isolates and are consistant in producing indole-3-acetic acid (IAA) in rice. Within the species there was variation in metabolite producing capacity was observed. *T. viride* T14 isolate wasthe highest inorganic phosphate producer, IAA inducer and exhibited high plant growth promoting activity. Apart from T14, IRRI-2, IRRI-3 and IRRI-4 were the promising plant growth inducers (Kale *et al.,* 2016).

Among 58 isolates of *Trichoderma*, isolate CHF 78 (*T. asperellum*) showed several plant growth promoting traits like ability to solubilize Ca3(PO4)2 and to produce cellulases, chitinases, indole acetic acid (IAA), proteases and siderophores. Inoculation of CHF78 increased plant dry weight (33.8-67%) and plant height (20-35.9%) inoculated with or without *Fusarium* compared to those inoculated only *Fusarium.* Uptake of P, K, Mg and Zn was greatly enhanced that resulted in reduced disease incidence. There was a negative correlation between plant nutrient uptake and disease incidence in plants which was positively promoted by *Trichoderma* application (Ying *et al.,* 2018).

Agro-based wastes were evaluated as a medium for mass micropropagule production and optimal efficacy of *T. asperellum* B1092 in controlling *F. oxysporum* f. sp. *lycopersici* andpromoting tomato growth. In green-house tests of *T. asperellum* B1092 against *F. oxysporum* f. sp *lycopersici* (causing *Fusarium* wilt of cherry tomato), B1092 significantly promoted plantgrowth compared to the control. The efficacy of this formulation resulted in increased growthof roots and shoots tomato plants and total lycopene, sugar (6.66%), K (2.34%), N (2.57%), Ca(0.87%), P (0.46%) and Mg (0.42%) content after 120 days (Hasan *et al.,* 2018).

CONCLUSION

As discussed in this review, Trichoderma spp. arecorrectly renowned for their capacity to generate a biocontrol activity that have the potential to parasitize a wide array of pathogenic fungi in the rhizosphere (*Fusarium*).Further research dealing with the biochemical and physiological bases through which *Trichoderma* spp. act as biocontrol agent against several lethal fungi is necessary for a wide,in-depth knowledge of this multitalented biocontrol agent. Moreover, for the purpose of integrated disease management, the compatibility of *Trichoderma* with chemical fungicides should be evaluated.

The popularity of *Trichoderma*-based formulations among farmers for ecofriendly management of diseases should be enhanced. The ecological influence of comprehensive applications of a fungal species as well as their secondary metabolites for biocontrol should be assessed to confirm a database for the secure and sustainable usage of *Trichoderma.*

Lastly, by taking into consideration all the information provided in this review, the use of *Trichoderma* species should be promoted as a valid alternative to pesticides in the era of a green economy which aims at promoting human health and environmental safe guarding.

Acknowledgment

The authors wish to express their sincere thanks to the host institution Dr.N.G.P Arts and Science College, Management, Principal, Deans, Head of the Department, Guide and other all staffs of Department of Biochemistry for rendering all the facilities and support. Communication number: DrNGPASC 2020-21 BS057

Conflict of Interest: None

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