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 80 tons 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. This soil application of fungicides is hazardous to applicator and also affects beneficial soil microflora. Apart from this the chemical will persist in soil for long duration and contaminates soil 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 new pathogenic 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 worldwide for 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 can be 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 and are 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 diseases like wilts (Fusarium spp.), root rots (Rhizoctonia spp.), collar rots (Sclerotium spp.), damping off (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 frequently enhances 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 successful biocontrol agents as more than 60% of the registered bio-fungicides used in today’s agriculture belongs 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 production of 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-parasitism is the mechanism which involves hyphal supercoiling and formation of appresorium like structure (Ponnurugan 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 or systemic 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 for their 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 isolates and untreated control (60.98 cm 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%) (Akrani 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. breviconstrictum (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. breviconstrictum 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%), 60 days 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-392433 and 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, Cu, Mg, S, Zn, Cu, Mn and Fe) was also higher (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) than the control, by consortium.

Inorganic phosphorus was released from tri-calcium phosphate by all Trichoderma isolates and are consistent 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 waste the 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 and promoting 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 plant growth compared to the control. The efficacy of this formulation resulted in increased growth of 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. are correctly 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 multitaled 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.

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