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SCREENING OF *TRICHODERMA VIRIDE* AS A BIO-CONTROL AGENT AGAINST DIFFERENT SOIL-BORNE INFECTIOUS AND PHYTOPATHOGENIC FUNGI

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ABSTRACT

Phytopathogenic fungi such as *Fusarium solani*, *F. oxysporum*, *Alternaria alternata*, *Rhizoctonia solani*, *Drechslera biseptata*, *Penicillium purpurogenum* etc. were isolated from wastewater irrigated agricultural soil at the site of Malir river near Quaidabad and Lyari river near old Sabzi mandi. Soil samples were collected from the rhizosphere of various vegetable crops i.e. Ridged gourd, Round gourd, Bottle gourd, Corn, Okra, Chili, Tomato, Eggplant and Cabbage etc. Besides these fungal pathogens, bio-control agent was also sequestered from soil samples. *In-vitro*, *Trichoderma viride* was tested against all isolated infectious and pathogenic fungi of plant by dual culture technique. In present study, *T. viride* significantly suppressed the pathogens by inhibiting its mycelial growth. It showed the antagonistic activity on most common and economically important phytopathogenic fungi i.e. *F.solani* (82.91%), *F. oxysporum* (82.38%), *A. alternata* (77.19%), *R. solani* (62.25%), and *P. purpurogenum* (50.79%). It was also very effective against various infectious soil-borne fungi such as *Aspergillus fumigatus* (79.92%), *A. parasiticus* (74.49%) *Humicola* sp. (66.67%) and *A. terreus* (47.62%). With the help of present studies, it is evaluated that *T. viride* comprises the higher inhibitory response towards several soil-borne infectious and pathogenic fungi that harm economically important food crops.

KEY-WORDS: Antagonism, Soil-borne fungi, pathogens

INTRODUCTION

Several fungal and bacterial phytopathogens cause serious diseases in crops that lead to massive losses to the economy of any country. To conquer this problem farmers use various methods such as crop rotation (Ikeda *et al*. 2015), disease-resistant varieties of crops (Witek *et al*. 2016) and many other strategies. Beside these, farmers use different agrochemicals against phytopathogens to prevent crop loses (Srivastava and Sharma 2014), but these chemical pesticides that are used against various plant diseases cause serious environmental hazards. They not only pollute our land but also inhibit the growth of beneficial microorganisms of our environment, cause reproductive toxicity, respiratory disorders, and carcinogenesis in mammals and adversely affect the health of humans when they consume the affected mammals. Due to these reasons, various biological control agents such as plant materials, antagonistic microbes etc. are gaining more importance against phytopathogens.

The bio-control agents not only inhibit phytopathogens but they also act as growth promoter of plant (Sharon *et al*., 2011). Various fungi and bacteria have been scrutinized as biological control for many years as they have antagonistic properties against several phytopathogenic microbes. Antagonistic microorganisms produce some antibiotics (Glick *et al*., 2007) to inhibit the growth of various phytopathogens (Dilantha *et al*., 2005). The biotic agents include numerous plant growth promoting microorganisms such as *Pseudomonas* sp. (Bakker *et al*., 2007), *Bacillus* sp. (Jourdan *et al*., 2009; Kloepper *et al*., 2004), *Serratia* sp. (Press *et al*., 1997), *Piriformospora indica* (Shoresh *et al*., 2010), *Trichoderma* sp. (Koike *et al*., 2001; Segarra *et al*., 2009), *Penicillium simplicissimum* (Elsharkawy *et al*., 2012), *Phoma* sp. (Sultana *et al*., 2009), non-pathogenic *Fusarium oxysporum* (Fravel *et al*., 2003) and Arbuscular mycorrhizal fungi (Pozo *et al*., 2009).

Bio-control agents compete with pathogen for space and nutrition to grow. Some antagonists are also able to produce enzymes, antibiotics and toxic compounds by which they impede the growth of pathogen. In case of *Trichoderma* sp., it obstructs pathogen propagules to germinate and execute the pathogen cells by producing metabolites. It also acidify the medium to inhibit the growth of pathogen. Correspondingly, *Trichoderma* sp. can use as bio-fertilizer that it produce positive effects on plant growth by stimulating the mechanism of plant-defense. According to Vyas and Vyas (1995), *Trichoderma* spp. are very effective to control numerous phytopathogens like *Rhizoctonia solani*, *Sclerotium rolfsii*, *Pythium ultimum*, *Fusarium oxysporum* and *Macrophomina phaseolina* etc. so that it alter the use of chemical fungicides such as captan, benomyl, methyl bromide.

Trichoderma spp. are accompanied with the rhizosphere and rhizoplane of plants as they are capable to colonize the roots of plants and make symbiotic association like mycorrhizae so that they protect the roots of plant against pathogenic infection by stimulating the plant-defense mechanism as well as enhance the growth of plant (Harman *et al*., 2004). Certain species of *Trichoderma* colonize the roots of plants on long-term basis and enter the epidermal tissues where, they secretes some compounds inside the plant body (such as non-ribosomal peptides, terpenoids, pyrones and indolic-derived compounds) that stimulate the resistance of plant against pathogens attack (Harman *et al*., 2004; Vinale *et al*., 2008; Contreras-Cornejo *et al*., 2016). According to McIntyre *et al*. (2004), some antagonists change the morphology of pathogen body by coiling and form appressorium-like structures that penetrate the host body and comprise the high concentrations of osmotic solutes like glycerol. In case of *Trichoderma* sp., it attaches to the pathogen lectins by its cell-wall carbohydrates. After binding, it starts coiling around the body of pathogen and develop its appressoria to infect (Howell, 2003). The aim of the present study was to determine inhibition of various phytopathogenic soil-borne fungi via *Trichoderma viride* under *in-vitro* conditions.

MATERIALS AND METHODS

In the month of May 2018, the soil samples were collected from the rhizosphere and rhizoplane of different vegetable crops which were irrigated with wastewater at the site of Malir and Lyari River. The fungal species were isolated from soil samples by serial dilution plate method with the incubation period of 5-7 days at 28 ± 2 ^{o}C. Isolated fungal species were identified by the standard references and manual books of Barnett and Hunter (1972), Raper and Fennell (1965) and Ellis (1971; 1976). The isolated phytopathogens and bio-control agent were multiplied on PDA (Potato Dextrose Agar) plate for further study.

T. viride was selected as bio-control agent from isolated fungal species of soil samples to evaluate its antagonistic activity against different sequestered phytopathogens. The agar disc of 6mm of each pathogen from pure culture was inoculated at the periphery of the PDA plates 0.5cm away from edge of the plate, as well as same sized disc of antagonist was placed opposite to the different pathogen inoculated plates. In the same manner agar disc of test pathogens were placed near the edge of fresh PDA plate for each pathogen lonely and marked as control. Plates were inoculated for 5-7 days at 28 ± 2 ^{o}C to evaluate the interaction of antagonist with each pathogen. The interaction was determined by growing colonies of antagonist and pathogen towards each other as shown in Fig. 1. The radius of each colony with control plate were measured in cm. The inhibition percentage of pathogens by antagonist was calculated by following formula (Royse and Ries, 1977; Whips, 1987; Reddy and Hynes, 1993).

$$\text{Inhibition percentage (\%)} = \frac{R_1 - R_2}{R_1} \times 100$$

Where; R_1 was symbolized as the radius of pathogen from control plate and R_2 was the radius of treated pathogen with antagonist.

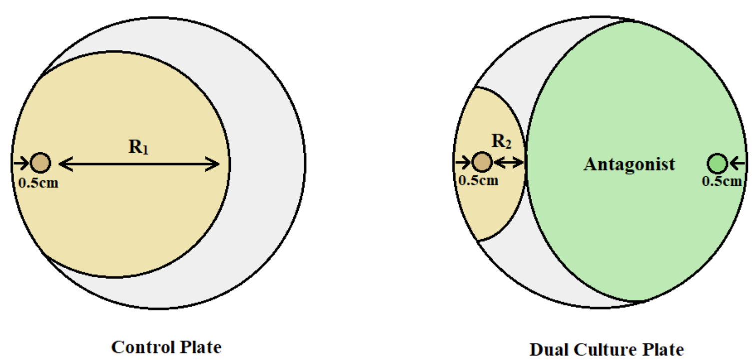

Fig. 1. R_1 indicates mycelial growth of pathogen alone as control (Control Plate) and R_2 is the inhibited growth of pathogen by antagonist (Dual Culture Plate, Skidmore and Dickinson, 1976).).

Analysis of variance was performed by Minitab 17.

RESULTS

The growth of the soil borne phytopathogenic fungal species tested was significantly and differentially suppressed by the *T. viride* (Table 1). The inhibition of various fungi by *T. viride* is presented in Fig. 2 and 3. *F.*

solani and *F. oxysporum* were suppressed with the highest inhibition percentage that is 82.90 ± 0.43% and 82.38 ± 0.48%, these are economically important pathogenic fungi to cause disease in many plants. *A. alternata* was controlled by 77.20 ± 0.88%. *R. solani* was inhibited by 62.2 ± 1.06% of inhibition as well as colony growth of *D. biseptata* was suppressed with the 60.90±0.64% of inhibition percentage by *T. viride*. It was also effective against the colony growth of *P. purpogenum* with the inhibition percentage of 50.79 ± 1.59%. Besides, *P. digitatum* was controlled by the percentage of 47.62 ± 2.28%.

T. viride also has antagonistic property against the colony growth of *A. parasiticus* and *A. fumigatus* that is 74.48 ± 0.41% and 79.92 ± 0.40%. These are infectious soil-borne fungi for plant and animal as well that produce alfatoxins. The growth of *Humicola* sp. was controlled by the inhibition percentage of 66.67 ± 1.93% whereas *A. terreus* was suppressed by 47.62 ± 1.19%.

Table 1. F- Value derived from ANOVA for inhibition percentage of different pathogenic fungi by *T. viride*.

Source	**DF**	**SS**	**MS**	**F-Value**	**P-Value**
Pathogenic Fungi	10	5665.74	566.574	126.99	0.001***
Error	22	98.15	4.461		
Total	32	5763.89			

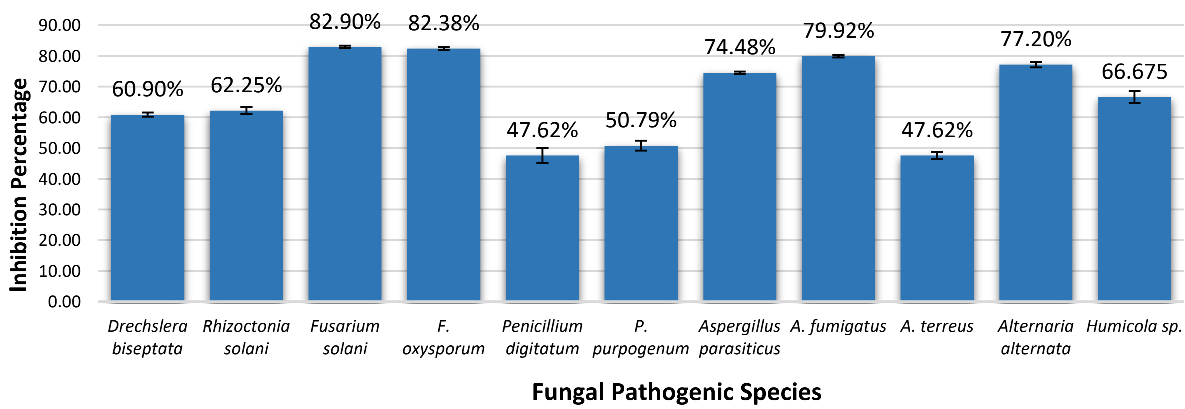

Fig 2. Inhibition percentage of various phytopathogens by *Trichoderma viride.*

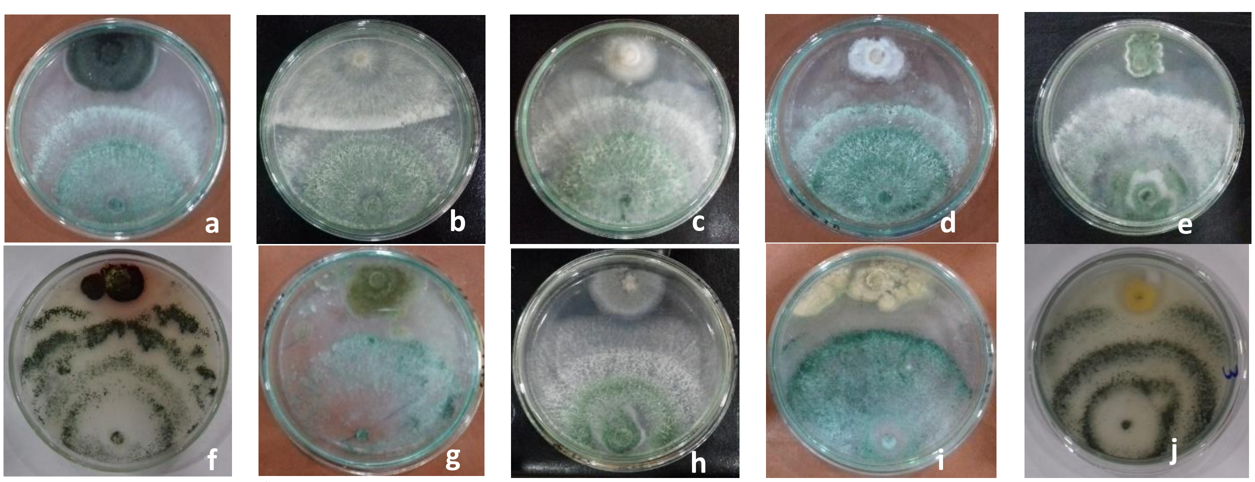

Fig. 3. Antagonistic activity of *Trichoderma viride* against (a) *Drechslera biseptata* (b) *Rhizoctonia solani* (c) *Fusarium solani* (d) *F. oxysporum* (e) *Penicillium digitatum* (f) *P. purpogenum* (g) *Aspergillus parasiticus* (h) *A. fumigatus* (i) *A. terreus* and (j) *Humicola* sp.

DISCUSSION

Almost all the species of *Trichoderma* have been considered as prospective bio-control agents as they impede the plant pathogenic soil-borne fungi that cause severe diseases in plants (Elad and Freeman, 2002; Howell, 2003;

Ziedan *et al*., 2015). Several mechanisms that are performed by *Trichoderma* spp. to obstruct the growth of phytopathogenic fungi i.e. they compete with pathogen for nutrition and space, myco-parasitism, secretes antibiotics, stimulate the plant defense system towards pathogen (Vey *et al*., 2001; Harman *et al*., 2004; Shoresh *et al*., 2010). This study was carried out to investigate the inhibition percentage by *T. viride* on various infectious soil-borne and phytopathogenic fungi viz. *A. alternate, A. fumigatus, A. parasiticus, A. terreus, D. biseptata, F. solani, F. oxysporum, Humicola* sp., *P. digitatum, P. purpogenum*, and *R. solani*. The present research indicated that *T. viride* has caused the highest inhibition percentage against wilt pathogen *F. solani* (82.90 ± 0.43%) and *F. oxysporum* (82.38 ± 0.48%). Almost similar result was observed by Ibrahim and Abdelaziz (2017) that *in-vivo*, *Fusarium* root rot infected plants were controlled by 81% with bio-control agent *Trichoderma* sp. Correspondingly, Gil *et al*. (2008) observed the positive results in obstructing peanut root rot caused by *F. solani* with the combination of *Trichoderma* spp. and *Gliocladium* spp. Naglot *et al*. (2015) also obtained similar results of *Trichoderma* sp. against the growth of *F. solani* as well as Sundaramoorthy and Balabaskar (2013) reported antagonistic activity of *T. harzianum* towards the growth of *F. oxysporum*. Pandey and Hussain (2006) reported *T. viride* and *T. harzianum* against *R. solani* which shows similar results as those of the present study that is inhibition around 62.25 ± 1.06%. *Drechslera biseptata* was inhibited by 60.90 ± 0.64% as well as *in-vitro*, Pandey and Hussain (2010) also observed that two species of *Trichoderma* (*T. viride* and *T. harzianum*) had almost equal antagonistic activity against the growth of *D. tetramera* producing disease on capsicum.

Beside *Fusarium* spp., present study also confirmed the findings of antagonistic property of *T. viride* against *A. alternata*. Ganie *et al*. (2013) observed inhibition activity of *Trichoderma* spp. in contradiction of early blight pathogen *A. solani*. Our results are similar to Patale and Mukadam (2011) who studied the antagonistic activity of three different strains of *Trichoderm*a against A. *solani*, *P. notatum*, *R. solani*, *F. oxysporum*, *A. flavus*, *A. niger* and *Phytophthora* sp. Amongst these species, *A. alternata* was inhibited by 77.20 ± 0.88%, *P. digitatum* was inhibited by 47.62 ± 2.38% and *P. purpogenum* by 50.79 ± 1.59%.

The cosmopolitan *Aspergillus* spp. colonize the roots of many crops such as cereals, legumes, nuts, vegetables etc. It is the opportunistic pathogen for many important crops. According to Calistru *et al*. (1997), the growth of *A. flavus* and *F. moniliforme* was suppressed by culture filtrates of four different strains of *Trichoderma* spp. The results of our study also showed inhibition of A. *fumigatus* by 79.92 ± 0.40%, A. *parasiticus* by 74.48 ± 0.41% and *A. terreus* by 47.62 ± 1.19% impede by *T. viride*.

Doi and Mori (1994) have reported inhibition of fungal hyphal growth of various pathogenic fungi on culture plate, due to the volatile compounds secreting from *Trichoderma* spp. The volatile compounds secreted by *T. viride* have potential to impede the growth of wood decay fungi like *Lentinus lepidus* and *Coriolus versicolor*. Alkyl pyrones is one of the volatile compounds produced from *T. harzianum* (Fravel, 1988).

According to Elad *et al*. (1983), *Trichoderma* spp. penetrates it hypha inside pathogen, This penetration is facilitated by the enzymatic activity of Trichoderma sp. *R. solani* is parasitized by *T. harzianum* via producing chitinase (Benhamou and Chet, 1993).

CONCLUSION

It was found that *T. viride* has potential to impede the growth of several phytopathogenic fungi which cause serious diseases in important crops. This antagonistic isolate can be used as prospective bio-control agent in agriculture and forestry.

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