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EFFECT OF VOLATILE AND NON VOLATILE COMPOUNDS OF *TRICHODERMA* SPP. AGAINST *FUSARIUM* ISOLATES CAUSING CHICKPEA WILT IN PUNJAB

Mohit Kumar, Vipul Kumar, Meenakshi Rana and Seweta Srivastava*

School of Agriculture, Lovely Professional University, Phagwara - 1 44 411, Punjab, India

Abstract

Background and Objective: Antagonistic strains belonging to the *Trichoderma* genera were able to produce various secondary metabolites which can play a role in the mechanism of their biological activity. So, the aim of this study was to assess the potential of volatile and non-volatile metabolites released from three selected species of *Trichoderma viz*. *Trichoderma viride*, *Trichoderma harzianum* and *Trichoderma konigii* isolates against three isolates of *Fusarium oxysporum* f. sp. *ciceri* which caused wilting of chickpea.

Materials and Methods: After isolation and identification of the experimental microbes, the effect of selected antagonists was checked against the test pathogen. *In vitro* bio-efficacy test of antagonists' *viz*. *Trichoderma viride*, *Trichoderma harzianum* and *Trichoderma konigii* had been done against *Fusarium oxysporum* f. sp. *ciceri* with the help of dual culture and their effects were studied by Zone of Inhibition technique. The production of volatile compounds by the same isolates of antagonists against *Fusarium oxysporum* f. sp. *ciceri* was studied by using the inverted plate technique.

Results & Conclusion: Data proved that *Trichoderma harzianum* produced maximum inhibition zone (76.90%) against FOC strain of *Fusarium oxysporum* f. sp. *ciceri* followed by *Trichoderma viridae* (70.10%). *In vitro* studies have demonstrate that volatile compounds produced by *Trichoderma harzianum* showed strong inhibitory effect on the mycelial growth of all isolates of test pathogen but maximum against FOC2 isolate *i.e.,* 79.25% followed by the *Trichoderma viridae i.e.,* 64.16% against FOC1 isolate.

Key words: Antagonist, chickpea, *Trichoderma*, strain, metabolites, wilt

Introduction

Chickpea (*Cicer arietinum* L.) is an annual grain legume, grown mainly for human consumption. Susceptibility to several fungal, bacterial and viral diseases are the limiting factor contributing for the low yield of chickpea (Rehman *et al.,* 2013). Among the economically important diseases, wilt (*Fusarium oxysporum* f. sp. *ciceris*), dry root rot (*Rhizoctonia bataticola*) and collar rot (*Sclerotium rolfsii*) are the major and widespread diseases affecting chickpea cultivation and production (Nene and Sheila 1999). Use of bio-control agents to manage the disease represents a viable alternative in place of chemical fungicides because it is considered to be a safe without having any residual effect, cost effective and eco-friendly method for plant disease management (Benitez *et al.,* 2004).

Among all beneficial microbes, *Trichoderma* has long been considered as one of the most promising biocontrol agent uses to control several plant pathogens because it is produced many antifungal secondary metabolites that have an adverse affect on the growth of different fungal and bacterial phytopathogens (Barakat *et al.,* 2014; Li *et al.,* 2016). Production of antibiotic and cell wall degrading enzymes, competition for key nutrients, parasitism and stimulation of plant defense mechanisms are numerous modes of action have been proposed to explain the bio-control of plant pathogens by *Trichoderma* (Cook, 1985). *Trichoderma* spp. generally grows in its natural habit around the rhizosphere zone of host plant and therefore it controls root or soil borne diseases in particular (Faruk *et al.,* 2002; Kamlesh and Gujar, 2002, Monte 2001). The species of *Trichoderma* have been evaluated against

***Author for correspondence :** E-mail : seweta.21896@lpu.co.in

the wilt pathogen and have exhibited greater potential in managing chickpea wilt under field condition (Podder *et al.,* 2004).

Fusarium sp. is a soil borne fungal pathogen that attacks plants through roots at all stages of plant growth, is considered as one of the main soil-borne systemic diseases and the major limiting factor in the production of many crops both in greenhouse and field-grown (Srivastava *et al.,* 2010; Borrero *et al.* 2004). In India, chickpea is ranked first in terms of production and consumption in the world (Patole *et al.,* 2017). *Fusarium* wilt caused by the soilborne fungus *Fusarium oxysporum* f. sp. *ciceri*, has become a major limiting factor of chickpea production worldwide (Jiménez-Díaz *et al.,* 2015).

Considering these points, the aim of this study was to assess the potential of volatile and non-volatile metabolites released from three selected species of *Trichoderma viz*. *Trichoderma viride*, *Trichoderma harzianum* and *Trichoderma konigii* isolates against three isolates of *Fusarium oxysporum* f. sp. *ciceri* which causes wilting of chickpea.

Materials and Methods:

Cultural and morphological characters of *Fusarium* isolates

Isolations of *F. oxysporum* f. sp. *ciceri* was isolated from chickpea wilt samples collected from adjoining farmer's field. Isolation was made from infected root samples and pure culture was maintained on Potato Dextrose Agar medium slants (Aneja, 2005).

Effect of non-volatile compounds produced by *Trichoderma* species on the mycelial growth of *Fusarium* isolates

Bio-efficacy test of antagonists has been done against *Fusarium oxysporum* f. sp. *ciceri* with the help of dual culture[15]. The effects of antagonists were studied by Zone of Inhibition technique (*in vitro*). The three antagonists were *Trichoderma viride*, *Trichoderma harzianum* and *Trichoderma konigii*.

Formula to calculate I.O.C. % is:

$$I.O.C(\%)=\frac{Redial\ growth\ in\ control\ plate-Redial\ growth\ in\ treate\ plate}{Redial\ growth\ in\ control\ plate}\times 100$$

Effect of volatile compounds produced by *Trichoderma* species on the mycelial growth of *Fusarium* isolates

The production of volatile compounds by three *Trichoderma* spp. *viz*. *Trichoderma viride*, *Trichoderma harzianum* and *Trichoderma konigii* against *Fusarium oxysporum* f. sp. *ciceri* was studied using the inverted plate technique described by Dennis and Webster (Dennis and Webster 1971). The mycelial disk (5 mm) of *Trichoderma* excised from the edge of 5 days old cultures was inoculated into the center of a Petri dish which containing PDA medium. The lid of each plate was replaced by the bottom of a plate containing PDA medium inoculated with a 5-mm-diameter mycelial disk of *Fusarium* isolates so as test pathogens were directly exposed to antagonistic environment created by *Trichoderma*. Then, the two plates were sealed together with parafilm and incubated at 28°C for 6 days in the dark. The control sets did not contain the antagonist. Radial growth of pathogens was recorded after 6 days of incubation and percentage inhibition was calculated in relation to control. The percentage inhibition was calculated in relation to the control by the formula:

L = C-T/Cx100

where: L – inhibition of radial mycelial growth; C – the radial growth measurement of the pathogen in the control; T – the radial growth measurement of the pathogen in the presence of antagonists.

Results

Effect of Volatile compounds:

The results for volatile metabolites activity against *Fusarium* isolates were presented in table 1. After 7 days of incubation, the effect of volatile compounds was

Table 1: Effect of different species of *Trichoderma* on the growth of *Fusarium oxysporum* f. sp. *ciceri*

Trichoderma spp.	*Fusarium oxysporum* f.sp. *ciceri*					
	FOC		FOC1		FOC2	
	Radial average of growth pathogen	*Per cent inhibitions of mycelial growth %*	*Radial average growth of pathogen*	*Percent inhibitions of mycelial growth %*	*Radial average growth of pathogen*	*Percent inhibitions of mycelial growth %*
Trichoderma viride	26.7	70.1	29.4	45.14	33.6	37.07
Trichoderma harzianum	20.6	76.9	23.6	55.97	25.2	52.80
Trichoderma konigii	36.7	58.9	27.3	49.06	27.2	49.06
Control	**89.4**		**53.6**		**53.4**	

Table 2: To check the secondary metabolites of *Trichoderma* spp. against FOC, FOC 1, and FOC 2

Trichoderma spp.	Growth inhibition (%) of *Fusarium oxysporum* f. sp. *ciceri*			Control
	FOC	FOC 1	FOC 2	
Trichoderma viride	56.61	64.16	62.27	5.3
Trichoderma harzianum	64.16	67.93	79.25	5.3
Trichoderma konigii	44.23	51.93	50.00	5.2

observed among three species of *Trichoderma viz. Trichoderma harzianum, Trichoderm viridie* and *Trichoderma koningi*. *Trichoderma harzianum* exhibited maximum growth inhibition (79.25%) against the tested three isolates of *Fusarium* when compare to the others. The *Trichoderm viridie* and *Trichoderma koningi* exhibited growth inhibition of 62.27% and 50%, respectively.

Evaluation of antagonistic activity through production of antifungal non-volatile metabolites:

The ability of *Trichoderma* species to produce the non-volatile substances was found most efficient in reducing the highest mycelial growth of tested *Fusarium* isolates by 76.9% and least recorded in *T. viride* (70.1%) followed by *T. koningi* (58.1%). From our results it is evident that among the three species of *Trichoderma*, *Trichoderma harzianum* is most potential species which could be better for soil borne pathogens for control the disease (table 2).

Discussion

Maximum growth inhibition (79.25%) against the tested three isolates of *Fusarium oxysporum* f.sp. *ciceri* was shown by the volatile compounds secreted by *T. harzianum*. A large variety of volatile secondary metabolites could be produced by *Trichoderma* spp. such as ethylene, hydrogen cyanide, aldehydes and ketones, which play an important role in controlling various plant pathogens (Faheem *et al.,* 2010; Siddiquee *et al.,* 2012; Chen *et al.,* 2015). Several researches of Calistru *et al.* (1997) revealed that volatile metabolites produced by *Trichoderma harzianum* species can significantly suppress the growth of *Aspergillus flavus* and *Fusarium moniliforme* rather than mycoparasitism (Calistru *et al.,* 1997; Srivastava *et al.,* 2011). Volatile metabolites produced by *Trichoderma* strains displayed inhibitory effects on *R. solani* and *P. ultimum* pathogens growth (Raut *et al.,* 2014). 'Chickpea wilt complex', an important disease of chickpea, was effectively controlled by a biological agent *Trichoderma harzianum* and its integration with fungicides (Kaur and Mukhopadhyay 1992).

The non-volatile substances secreted by *Trichoderma harzianum* during the experiment was found most efficient in reducing the highest mycelial growth of tested *Fusarium* isolates by 76.9%. Due to the presence of non-volatile substances, soil application with *T. hamatum, T. harzianum* or *T. viride* checked the severity of wilt and root rot disease as effectively as carbendazim (Khan *et al.* (2014). This *Trichoderma* spp. are active colonizers in soil (Akrami *et al.* 2009) and produce antibiotics like trichodermin, gliotoxins, viridin, cell wall-degrading enzymes (Bruckner and Przybylski 1984), and certain biologically active heat-stable metabolites like ethyl acetate (Mohiddin *et al.,* 2010). These substances may inhibit the activity of soil-borne pathogens (Chet and Baker 1981; Khan *et al.,* 2004; Khan *et al.,* 2011).

Further, in addition to biological control of soil borne fungal pathogens seed inoculation of *Trichoderma* spp. also found to increase growth and yield of chickpea (*Cicer arietinum* L.) under greenhouse conditions (Rudresh *et al.,* 2005).

Conclusion

As we know that use of bio-control in plant disease management is more effective, cheap without any residual effect. So, from the above findings it was concluded that non-volatile substances produced by the isolates of *Trichoderma* was found most efficient than volatile substances in reducing the highest mycelial growth of tested *Fusarium* isolates. It is also evident from above results that among the three species of *Trichoderma* used in present experiment; *Trichoderma harzianum* is most potential species which could be better for the management of soil borne pathogens like *Fusarium oxysporum* f. sp. *ciceri*. The non-volatile compounds of *Trichoderma harzianum* is found as effective as carbendazim or other systemic fungicide used to manage wilt.

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