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In vitro effect of non volatile and volatile compounds produced by antagonists against *Fusarium udum*

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ABSTRACT

Six native isolates of antagonists viz, Trichoderma viride -1, Trichoderma viride-2, Trichoderma harzianum- 1, Trichoderma harzianum -2, Trichoderma harzianum-3, Gliocladium virens and one commercial antagonist, T-35(Trichoderma harzianum) were evaluated for their efficacy to manage the pigeonpea wilt pathogen (Fusarium udum) by production of non volatile and volatile compounds. The highest reduction in growth of Fusarium udum was recorded with Trichoderma harzianum-1 followed by T. harzianum-3 and T. harzianum-2. However, the lowest reduction of growth of Fusarium udum was noticed with Gliocladium virens. The inhibition of growth of Fusarium udum significantly increased with ratios of culture filtrates of antagonists. Similarly, conidia production of Fusarium udum was mostly reduced by T. harzianum-1 followed by T. harzianum-3 and T. harzianum-2. It was further observed that sporulation of Fusarium udum decreasd with increasing concentrations of culture filtrates of different bioagents. The degree of inhibition varied from one strain to another.With regards to volatile compounds, Trichoderma viride-1 showed lowest radial growth (24.00 mm) of Fusarium udum as compared to radial growth in control (41.00mm). The antagonists T. harzianum-2, T. harzianum-3, Gliocladium virens and T-35 (T. harzianum) differed significantly from each other pertaining to radial growth of Fusarium udum. The inhibition of growth was recorded highest with Tricoderma viride-1 and it declined subsequently going down to 18.1% in case of T-35(T. harzianum). All the bioagents except T-35 (T. harzianum) reduced significantly the sporulation of Fusarium udum as compared to control. T. viride-1 produced volatiles which were most effective in reducing the sporulation of Fusarium udum.

Key words:*Antagonists, Fusarium udum, pigeon pea* wilt*, non volatile and volatile compounds.*

INTRODUCTION

Pigeonpea *(Cajanus cajan* (L.) Mill sp) commonly known as Arhar is an important pulse crop grown in northern plains, central and eastern parts of India. *Fusarium* wilt (*Fusarium udum*) is one of the most important diseases of pigeonpea. Prospects of biological management of soil borne plant pathogens using most promising bio-control has been described (Kumar,2013). Successful reductions of *Fusarium* wilt in many crop with application of different species of *Trichoderma* have been found (Sundaramoorthy and Balabaskar, 2013). During process of biological control of the plant pathogens, the mechanism like competition for space and nutrition, parasitism and antibiosis have been reported to be involved. Biological control represents a viable alternative to the use of chemical fungicides and it is considered to be a safe, effective and eco-friendly method for plant disease management. *Trichoderma* is known as one of the best candidates of bio-control agents. Modes of action of this fungus include mycoparasitism, antibiosis, competition for nutrients and space, tolerance to stress through enhanced root and plant development.The antagonistic bioagents are reported to produce some non volatiles such as hydrolytic enzymes, certain lytic extra cellular cellulytic enzymes that were present in culture filtrates and were highly pernicious to the growth of *Fusarium udum.*Volatile compounds such as carbon dioxide and ethanol etc. interfering with the growth and survival of the pathogens. Studies were therefore, undertaken to determine as to what extent the native antagonists and one commercial antagonist were effective in controlling *Fusarium udum* causing wilt of pigeonpea by producing non-volatile and volatile compounds.

MATERIALS AND METHODS

The antagonists of Fusarium *udum* were isolated from six pigeonpea growing ecosystems of the district Jaunpur (U.P) and were evaluated for their biocontrol efficacy in the research laboratories of the department of Botany, Tilak Dhari P.G. College, during 2017-2018. The

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cultures of the isolates of antagonists viz., *T. viride*-1, *T. viride*-2. *T. harzianum*-1, *T. harzianum*-2, *T. harzianum*-3, *Gliocladium virens* and one commercial bioagent T-35 (*T. harzianum*) with high bio-control efficacy were used in the present studies.

Non volatile compounds produced by antagonists: To study the effect of non volatile compounds, the biocontrol agents were grown in Potato dextrose broth at 28±1°C with intermittent shaking at 5000 rpm. The metabolites were collected after 15 days and filtered. The culture filtrates of antagonists were mixed with the autoclaved PDA medium in the ratios of 1:3, 2:2, and 3:1 (culture filtrate : PDA v/v), separately prior to pouring and 20 ml medium was poured into each Petri dish inoculated with 7 days old culture of *Fusarium udum* (6 mm disc) kept in the centre. Suitable control was maintained without culture filtrate and replicated thrice. All these Petriplates were incubated for 7 days at 28±1°C. Periodical observations on radial growth were recorded. Percent inhibition was calculated by the formula:

$$I = \frac{C-T}{C} \times 100$$

Where,

I = % inhibition,
C = radial growth in control (mm),
T = radial growth in the treated Petriplates (mm)

VOLATILE COMPOUNDS PRODUCED BY ANTAGONISTS: The two bottom portion of petriplates containing PDA were inoculated with a 6 mm disc of pathogen and antagonist respectively and both inoculated bottom plates were placed facing each other and sealed with cellophane adhesive tape. The petriplate containing PDA without antagonist served as control. The observations on the radial growth of the test fungus were recorded after 5 days of incubation at 28 ± 1ºC. Radial growth was recorded and compared by calculating percent growth inhibition of *Fusarium udum*. Conidial production (sporulation) was recorded at 10th day using Haemocytometer. Spore suspension for counting the conidia was prepared by suspending 6 mm disc in 10 ml sterilized water.

RESULTS AND DISCUSSION

Effect of non volatile compound

The results (table 1) indicated that the highest reduction of growth of *Fusarium udum* was observed by *Trichoderma harzianum-1* followed by *Trichoderma harzianum-3* and Trichoderma harzianum-.2. However, the lowest reduction of growth of *Fusarium udum* was noticed with *Gliocladium virens*. All the antagonists except T. viride-1 and *T. harzianum-2* differed significantly from each other in respect of reduction of growth of *Fusarium udum*. The inhibition of growth of *Fusarium udum* significantly increased with increasing ratios of culture filtrates of the antagonists. The data (Table1) clearly revealed that culture filtrate of *T. harzianum-1* was most effective in reducing the conidia production of *Fusarium udum* followed by *T. harzianum*-3 and *T. harzianum*-2. The bioagent *Gliocladium virens* was noted to be least effective in respect of conidia pro-duction of *Fusarium udum*. All the treatments resulted in effective reduction in sporulation of *Fusarium udum* in comparison to control. It was further observed that sporulation of *Fusarium udum* decreased with increasing con-centrations of culture filtrates of different bioagents. The degree of inhibition varied from one strain to another. Antagonistic process might rely on the dual action of the antibiotics and hydrolytic enzymes. Isolates of *Trichoderma harzianum*, T-35 (*T. harizianum*) and Isolates of T. viride produced certain lytic extra cellular cellulytic enzymes that were present in culture filtrates and were highly pernicious to the growth of *Fusarium udum*. Several toxic metabolites were produced *in vitro* by isolates of *T. harzianum*, T-35 *(T. harzianum)* and *T. viride. Similae results on the effect of non* volatile compounds of *Trichoderma* on some more pathogens such as *Fusarium moniliforme were reported by* (Kumar *et al.* 2012), *Botrytis fabae* (Barakat *et al.*, 2014), and other *Fusarium species* (Sain and Pandey, 2016). But very little work has been done against *Fusarium udum*.

Table1: In vitro effect of non-volatile compounds produced by antagonists on colony growth inhibition and conidia production (sporulation) of *fusarium udum*

Culture filtrate of antagonists	Average growth inhibition control (%)			Mean	Average No. of conidia x10^6 /5mm disc at concentrations			Mean
	1:3	2:2	3:1		1:3	2:2	3:1	
Trichoderma viride-1	13.9 (21.8)	16.6 (24.0)	30.4 (33.5)	20.3 (26.5)	9.4	8.9	8.1	8.8
Trichoderma viride-2	10.7 (19.1)	12.5 (20.7)	23.5 (28.9)	15.5 (22.9)	9.9	8.5	7.9	8.7
Trichoderma harzianum-1	26.1 (30.7)	28.5 (32.3)	37.3 (37.6)	30.6 (33.5)	5.6	5.0	4.1	4.9
Trichoderma harzianum-2	6.3 (14.5)	23.7 (29.1)	36.6 (37.2)	22.2 (26.9)	8.9	8.5	7.4	8.2
Trichoderma harzianum-3	18.1 (25.2)	26.1 (30.7)	28.0 (31.9)	24.0 (29.3)	8.2	7.5	6.5	7.4
Gliocladium-virens	6.0 (14.2)	10.3 (18.7)	17.6 (24.8)	11.3 (19.2)	10.9	9.5	9.8	10.0
T-35 (Trichoderma harzianum)	15.3 (23.0)	17.1 (24.4)	25.2 (30.1)	19.2 (25.9)	10.3	9.3	7.6	9.0
Control	-	-	-	-	-	-	-	12.8
Mean	13.8 (21.2)	19.2 (25.7)	28.4 (32.0)		9.0	8.2	7.3	
S.Em±	Bioagents 0.14, Concentr-ation (0.09), Interaction (0.247)							
C.D (P=0.05)	Bioagents (0.407) Concentration (0.266), Interaction (0.705)							

Figures in paraentheses are arc sin angular transformed values

Effect of Volatile Compounds

It is evident from the data (Table 2) that *Trichoderma viride*-1 showed lowest radial growth (24.00 mm) of *Fusarium udum* as compared to radial growth in control plate (41.00 mm). The radial growth of *Fusarium udum* due to the volatiles produced by *T. viride*-2 and *T. harzianum*-1 were noticed at par with each other. The antagonists *T. harzianum*-2, *Gliocladium virens* and T-35 (*T. harzianum*) differed significantly from each other pertaining to the radial growth of *Fusarium udum.* The inhibition of growth was recorded highest with *T. viride*-1 and it declined subsequently going down to 18.1% in case of T-35 (*T. harzianum*).

Table 2: In vitro effect of volatile compounds produced by fungal antagonists on radial growth and conidia production (sporulation) of *Fusarium udum*

Antagonists	Average radial growth of *Fusarium udum* (mm)	Average growth Inhibition over control (%)		Average No. of conidia 10^6/5mm disc
Trichoderma viride-1	24.00	41.4	(40.0)	2.6
Trichoderma viride-2	25.27	38.3	(38.2)	3.9
Trichoderma harzianum-1	26.50	35.3	(36.5)	4.3
Trichoderma harzianum-2	29.67	27.6	(31.7)	8.9
Trichoderma harzianum-3	27.00	34.1	(35.7)	5.8
Gliocladium virens	31.90	22.1	(28.0)	9.8
T-35(*Trichoderma harzianum)*	33.57	18.1	(25.2)	10.6
Control	41.00	-	-	11.5
S.Em±				0.3
C.D (P=0.05)				10

Figures in parentheses are arc sin angular transformed values

All the bioagents except T-35 (*T. harzianum*) reduced significantly the sporulation of *Fusarium udum* as compared to control. *T. viride*-1 produced volatiles which were most effective in reducing the sporulation of *Fusarium udum* followed by *T. viride*-2 and *T. harzianum*-1. The

conidia production due to effect of *T. viride*-2 and *T. harzianum*-1.did not differ significantly from each other. Similarly, conidia pruduction by *Fusarium udum* due to the volatiles produced by *G. virens* and T-35 (*T. harzianum*) were noticed to be at par with each other. It was further observed that T-35 (T. harzianum) was least effective in respect of reduction of sporulation of *Fusarium udum* which was also recorded to be at par with the control. Several volatile metabolites were produced in vitro by antagonists reducing the growth and sporulation of *Fusarium udum*. Goudar and Kulkarni (2000) evaluated antagonistic potential of 8 antagonists *in vitro.* Pan and Bhagat (2008) have also reported the effectiveness of diffusible volatile compounds by *T. viride* and *T. harzianum*. Chaudhary *et al.*, (2017) reported that volatile compounds from T. viride suppressed the mycelial growth of *F. udum* and found effective when compared to others.

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