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Bio-efficacy of Trichoderma species against Pigeonpea wilt pathogen

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Abstract: Three biocontrol agent *viz., Trichoderma viride*, *Trichoderma virens* and *Trichoderma harzianum* were evaluated to test the antagonism against *Fusarium udum* under *in vitro* conditions. All the three biocontrol agents have the potential of parasitizing the growth of *Fusarium udum in vitro*. The rate of parasitism was found fastest in *T. viride* (61.12% over growth in 96 hrs) than *T. virens* and *T. harzianum*. The volatile compounds from *Trichoderma viride* suppressed the mycelial growth of *Fusarium udum by* 43.13% and found effective when compared to *Trichoderma virens* and *Trichoderma harzianum*. Non-volatile compounds or culture filtrate from *Trichoderma virens* at 15% concentration shows complete mycelial inhibition of the test fungi. The antagonist *T. virens* was chosen to be the most promising bio-control agent for *F. udum.*

Keywords: Bio-efficacy, *Fusarium udum,* Pigeonpea, Trichoderma, Wilt

INTRODUCTION

Pigeonpea **(***Cajanus cajan* (L.) Millsp.) commonly known as tur or arhar is an important pulse crop grown in northern plains, central and eastern parts of India. The total area under Pigeonpea in India was 36.3 lakh ha with a total production of 27.6 lakh tonne and 760 Kg/ha productivity. In Madhya Pradesh, during 2015-2016, Pigeonpea was cultivated in an area about 3.50 lakh hectares with production of 2.17 lakh tones and 737 kg/ha productivity (Shrivastava *et al*., 2016). Pigeonnpea provides quality food, fuel wood, and fodder. Its soil rejuvenation qualities such as release of soil-bound phosphorous, fixation of atmospheric nitrogen, recycling of soil nutrients, and addition of organic matter and other nutrients make pigeonpea an ideal crop of sustainable agriculture in the tropical and subtropical regions of India. Though several factors are known to affect pigeonpea cultivation, the most important being the diseases. Some of the important diseases are Fusarium wilt, Phytophthora blight, Cercospora leaf spot, collar rot, dry root rot, Alternaria leaf spot, powdery mildew, sterility mosaic and phyllody. Incidentally, only a few of them causes economic losses in India (Kannaiyan et al., 1984). Fusarium wilt (FW), caused by fungal pathogen *Fusarium udum*, is one of the major disease widely prevalent in north and central parts of the India causing yield loss ranging from 30 to 100% (Reddy et al., 1990). The yield loss due to this disease also depends upon the stage at which the plant wilt and it can approach over 50% and even up to 100% when wilt occurs at the pre pod stage (Okiror, 2002). Saxena et al. (2010) reported that Fusarium wilt disease in pigeon pea is so devastating that it can cause production loss up to 97000 tonnes per year in India alone. The disease is soil and seed borne therefore, difficult to manage through fungicide alone. Continuous use of fungicides results in detrimental effect on environment and development of resistant strains of the pathogen, health hazards to an applicator as well as to a consumer of the treated material. Their toxic forms persist in soil and contaminate the whole environment (Hemanth *et al*., 2016). One of the best possible ways to reduce yield losses due to FW is to grow resistant pigeonpea varieties. Therefore, enhancement of resistance to FW in pigeonpea is a major challenge. Several studies have been conducted to understand the genetic systems that control wilt disease in pigeonpea but, conclusive evidence is yet to arrive about genetics of FW resistance in pigeonpea (Singh *et al,* 2016). Prospects of biological management of soil-borne plant pathogens using most promising biocontrol has been described (Kumar. 2013; Sabalpara *et al*. 2009). Successful reductions of Fusarium wilt in many crops with application of different species of Trichoderma have been found (Kumar *et al.,* 2009; Sundaramoorthy and Balabaskar, 2013). However, it is also reported that all the isolates of Trichoderma spp. are not equally effective in management of pathogen *in vitro* (Biswas and Das., 1999; Ramezani, 2008). Therefore, specific isolates are needed for successful management of a particular pathogen. Therefore, the objectives of the present study were to assess the ability of three Trichoderma species in suppressing the *Fusarium udum* in pigeonpea under *in vitro* conditions.

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MATERIALS AND METHODS

Three biocontrol agent *viz., Trichoderma viride*, *Trichoderma virens* and *Trichoderma harzianum* were evaluated to test the antagonism against *Fusarium udum* the Department of Plant Pathology J.N.K.V.V. Jabalpur (M.P.) during 2015-16.

Growth of antagonist and the pathogen in monoculture and dual culture: To study the growth of antagonists and the test fungus in monoculture, 5 mm mycelial discs of *Trichoderma viride*, *Trichoderma virens, Trichoderma harzianum* and *Fusarium udum* were inoculated centrally on sterilized potato dextrose agar in Petri-dishes. Then plates were incubated in BOD incubator at 28 + 1^0C. Observations on colony diameter of individual antagonist and the pathogen were recorded after 72 hrs of incubation. For screening of the antagonists against *Fusarium udum,* dual culture technique developed by Morton and Straube, (1955) was adopted. Observation on colony diameter of bioagents and test fungus was recorded. Inhibition of mycelial growth of test pathogen over check was calculated by formula given by Vincent (1947). Re isolation was done by transferring 5 mm mycelial disc cut by cork borer from the zone where the test fungus was already overgrown by the antagonist on PDA medium to study the viability of test fungus.

Effect of volatile and non volatile compounds from antagonist(s) on the radial growth of *Fusarium udum*: The effect of volatile compounds from *Trichoderma viride, Trichoderma virens and Trichoderma harzianum* on radial growth of *Fusarium udum* was followed as per the method given by (Dennis and Webster, 1971a and b). The two bottom portion of petriplates containing PDA were inoculated with a 5 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 serves as control. The observations on the radial growth of the test fungus were recorded after 5 days of incubation at 28 ± 1ºC. To study the effect of non volatile compounds, the bio-control agents were grown in Potato dextrose broth at 27ºC with intermittent shaking at 150 rpm. The metabolites were collected after 15 days and filtered. The sterilized filtrate was amended in PDA to make 5, 10 and 15% concentration in petriplates. The solidified agar plates in triplicates were inoculated at the centre with 5 mm diameter mycelial disc of pathogen and incubated at 28ºC for 5 days. The Plates without filtrate served as control. The Colony diameter was measured and percent inhibition of radial growth was calculated using the formula given by Vincent 1947**.**

RESULTS AND DISCUSSION

In monoculture, *Trichoderma viride* showed 90 mm growth on PDA after 72 hrs of incubation followed by *Trichoderma virens* and *Trichoderma harzianum* which exhibited 86.83 mm and 84.66 mm colony diameter respectively. *Fusarium udum* showed 36.66

Table 1. Growth of antagonistic and pathogen in mono and dual culture.

Treatment	Monoculture	Dual culture		
	Colony diameter (mm)*	Colony diameter of antagonist (mm)*	Colony diameter of Pathogen (mm)*	Growth Inhibition (%)
Trichoderma viride	90.00	75.67	14.33	61.12
Trichoderma virens	86.83	70.00	20.00	45.74
Trichoderma harzianum	84.66	67.17	22.83	38.06
Fusarium udum	36.66		36.86	-
CD (0.05)	1.723		1.773	

*Average of 3 replications

Table 2. Effect of volatile and non-volatile compounds from *Trichoderma* on radial growth of *Fusarium udum* after five days of incubation.

Treatment	Volatile compounds		Non - volatile compounds					
			5%		10%		15%	
	Radial growth of mycelium (mm)*	Growth inhibi-tion (%)	Myceli-algrowth of pathogen (mm)*	Growth Inhibi-tion (%)	Mycelial growth of pathogen (mm)*	Growth Inhibi-tion (%)	Mycelial growth of pathogen (mm)*	Growth Inhibi-tion (%)
Trichoderma virens	42.17	31.79	26.17	57.67	20.50	66.84	0.0	100
Trichoderma viride	35.16	43.13	27.83	54.98	26.86	56.16	10.83	82.50
Trichoderma harzianum	51.00	17.52	32.00	48.24	30.17	51.20	25.16	59.31
Fusarium udum	61.83	--	61.83	--	61.83	--	61.83	--
CD (0.05)	1.851		1.912		1.971		1.851	

*Average of 3 replications

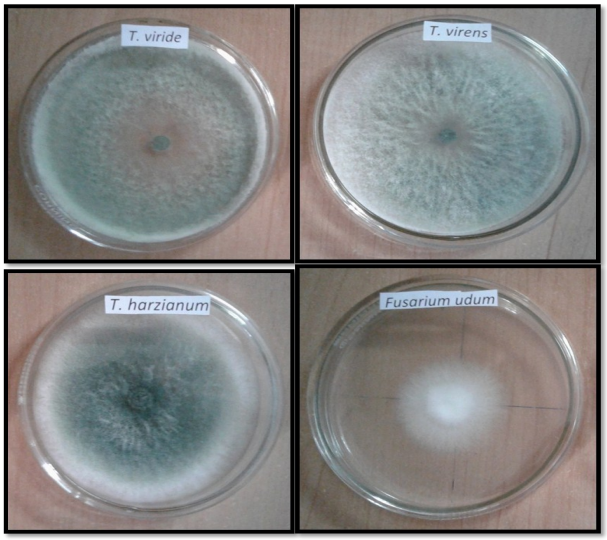

Plate 1. *Growth of Trichoderma and pathogen in monoculture.*

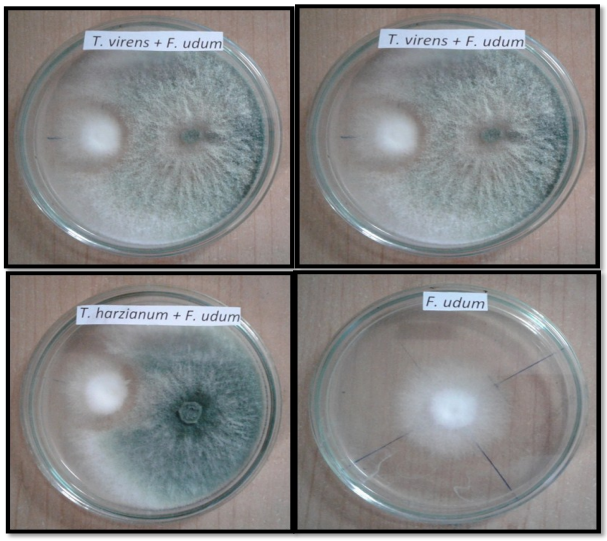

Plate 2. Growth of *Trichoderma* and pathogen in dual culture.

mm growth on PDA after 72 hrs of incubation (Table 1 and Plate 1). Singh (1991) also observed similar growth trend of bioagent. He reported that *Trichoderma viride* and *Trichoderma virens* showed 90 mm growth, while *Trichoderma harzianum* showed 76.6 mm growth after 72 hrs of incubation in monoculture at 28 ± 1ºC. In dual culture, all the three antagonists parasitized the growth of *Fusarium udum*. The rate of inhibition was fastest in *Trichoderma viride* (61.12% growth in 72 hrs) than *Trichoderma virens* (45.75 % growth in 72 hrs) and *Trichoderma harzianum* (38.06 % growth in 72 hrs) (Table 1 and Plate 2). Hence, *Trichoderma viride* proved best antagonist due to its faster and better overgrowing potential. The inhibitory effect of these bioagents against tested pathogen was probably due to competition and/or antibiosis. These results are in agreement with Chaudhary *et al*. (2004), Kappor *et al.* (2010), Kumar *et al*. (2014). Goudar and Kulkarni (2000) evaluated antagonistic potential of 8 antagonists under *in vitro* conditions, *T. viride* exhibited the maximum inhibition, followed by *T. harzianum* . Both later overgrew and completely suppressed the test pathogen. The volatile compounds from *Trichoderma viride* suppressed the mycelial growth of *Fusarium udum* by 43.13% and found effective when compared to others (Table 2 and Plate 3). The non–volatile secondary metabolites from *Trichoderma* species were found more effective in suppressing the mycelial growth of *Fusarium udum* when compared to volatile compounds. It was observed that the non–volatile compounds from *Trichoderma virens* completely inhibited the radial mycelial growth of *Fusarium udum* at a concentration of 15% as compared to *Trichoderma viride* (82.50%) and *Trichoderma harzianum* (59.31%) (Table 2 and Plate 4). It was also observed that with an increase in concentration of culture filtrates of all the *Trichoderma* species, the radial mycelial growth of test pathogen was proportionally decreased. Chakraborty and Chatterjee (2008) studied the effect of volatile and non-volatile antibiotics of *Trichoderma* origin on growth inhibition of the wilt pathogen (*Fusarium solani*) of egg plant (*Solanum melongena* L.). *T. harzianum* showed maximum growth inhibition (86.44 %) of the pathogen through mycoparasitism. The non-volatiles produced by the *Trichoderma* species exhibited 100 % growth inhibition of the pathogen under *in vitro* condition. Production of siderophores and fungal cell wall degrading enzymes, chitinase and β-1, 3-glucanase were found. Treatments with two most efficient *Trichoderma* species, *T. harzianum* and *T. viride* resulted in the decreasing population of *Fusarium solani* in soil thereby deterring disease incidence in field condition.Works on the effect of non-volatile compounds of Trichoderma on some more pathogens such as *Botrytis fabae* (Barakat *et al.,* 2014, *Fusarium moniliforme* (Kumar *et al.,* 2012) and other Fusarium species (Sain and Pandey, 2016) have been reported. But very few such

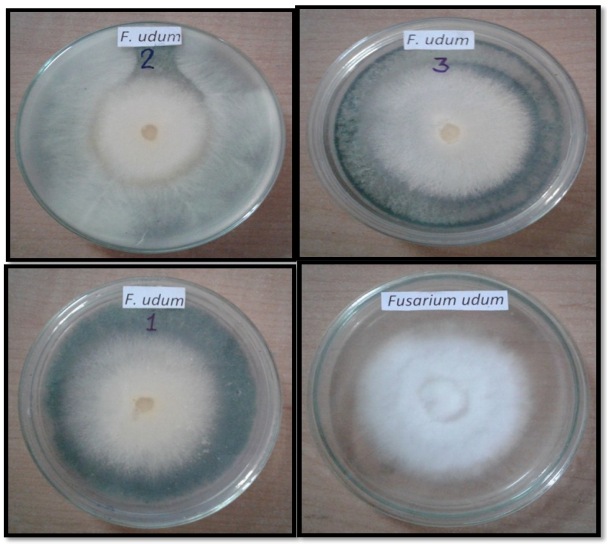

Plate 3. *Effect of volatile compounds from Trichoderma on radial growth of Fusarium udum after five days and incubation.*

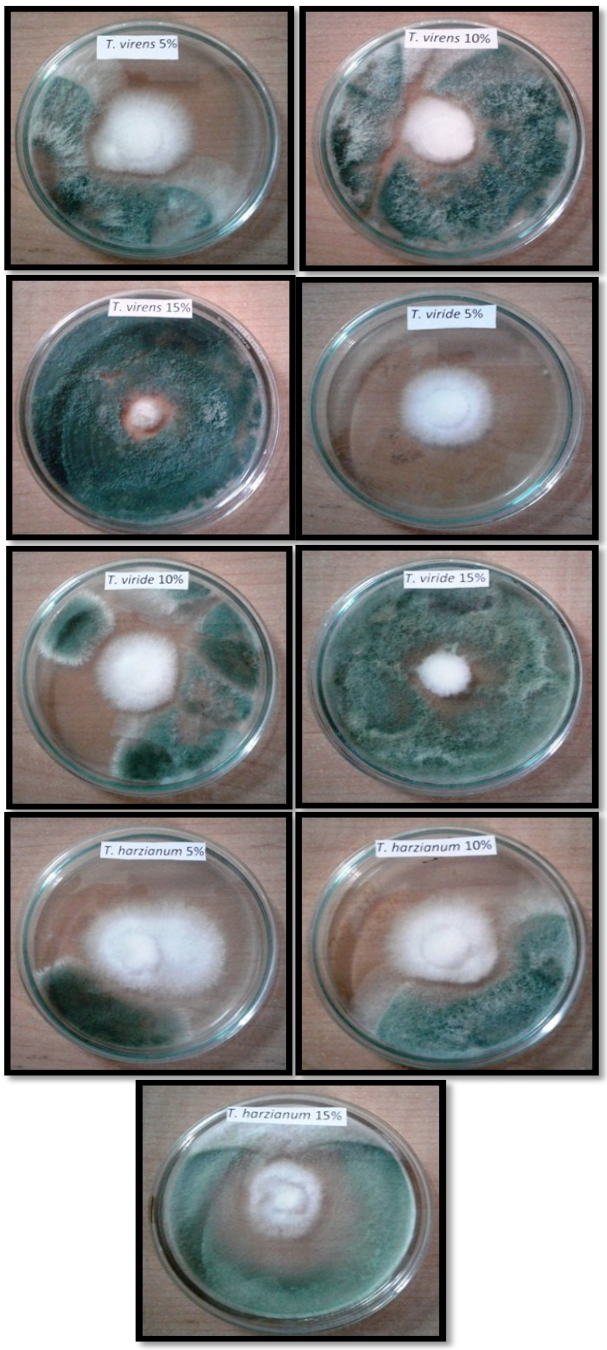

Plate 4. *Effect of non-volatile compounds from Trichoderma at 5, 10 and 15% concentration on radial growth of Fusarium udum after 5 days of incubation.*

works appears to have been done against *F. udum*, the wilt pathogen of pigeonpea. Therefore, the antagonist *T. viride* may be chosen to be the most promising biocontrol agent for *F. udum.*

Conclusion

The present evaluation thus gave clear indication that *T viride* is strong virulent antagonist, which can be effectively used in management of pigeonpea wilt.

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