



RESEARCH ARTICLE

PLANT PATHOLOGY

**BIOCONTROL OF *FUSARIUM UDUM* THROUGH *TRICHODERMA*****S. I. PATEL<sup>\*1</sup>, R. L. PATEL<sup>2</sup>, A. G. DESAI<sup>3</sup> AND D.S.PATEL<sup>2</sup>**<sup>1</sup>Centre of Excellence for Research on Wheat, S. D. Agricultural University, Vijapur, Gujarat, India.<sup>2</sup>Department of Plant Pathology, S. D. Agricultural University, Sardarkrushinagar, Gujarat, India.<sup>3</sup>Castor Research Station, S. D. Agricultural University, Sardarkrushinagar, Gujarat, India.**S. I. PATEL**Centre of Excellence for Research on Wheat, S. D. Agricultural University, Vijapur,  
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**ABSTRACT**

Management of wilt of pigeonpea by soil application of fungicides are economically non-viable. The potential role of biocontrol agent, *Trichoderma*, in the management of many soil borne plant pathogens are well documented. Hence, eleven different isolates of *Trichoderma* were assessed *in vitro* for their efficacy against *Fusarium udum*. The local strain of *Trichoderma* was equally efficacious against all the *Fusarium udum* isolates. Soil application of talc based preparation of this potential isolates was proved better to its seed treatment. The highest reduction in wilt was resulted in talc based preparation of promising local strain of *Trichoderma* compared to market products.



## KEYWORDS

Pigeonpea, wilt, *Fusarium udum*, *Trichoderma*

## INTRODUCTION

Pigeonpea [*Cajanus cajan* (L.) Millspaugh] wilt caused by *Fusarium udum* Butler is an important disease and responsible for lethal damage to crop. Management of wilt by use of fungicides in soil is difficult and economically non-viable. Biological pesticides have the potential to replace or augment conventional plant disease management. Several studies have demonstrated the potential of using fungal antagonists in general and *Trichoderma* in particular. Isolates of *Trichoderma* spp. are well documented as effective biocontrol agent in managing many soil-borne pathogens. The antagonistic activity of *Trichoderma* is highly variable across the locations and growing situations. Now-a-days, the commercial formulations of *Trichoderma* have already become available in the market. But, inadequate information about the performance of such preparations may some times create problems to the disease management strategy on large scale. The research on application of *Trichoderma* isolates in the management of pigeonpea wilt pathogen, *F. udum* is limited<sup>1</sup>. The present investigations were, therefore, carried out to evaluate the *in vitro* efficacy of *Trichoderma* against various isolates of *F. udum*, assessing the efficacy of efficient strain of *Trichoderma* by its application through seed treatment as well as soil applications in varying proportions as well as relative efficacy of promising isolate of *Trichoderma* vis-à-vis *Trichoderma* based bio-fungicides available in the market for the management of wilt disease of pigeonpea.

## MATERIALS AND METHODS

### (i) Collection of *F. udum* isolates :

Pigeonpea plants showing typical wilt symptoms were collected from different pigeonpea growing areas of Gujarat state and brought to the laboratory for tissue isolation. After isolation, 21 isolates of *F. udum* with distinct cultural and morphological characters were established by single spore culture of individual isolates on potato dextrose agar medium. On the basis of colony growth, the isolates were classified as fast growing (> 85.00 mm), moderately growing (80-85 mm) and slow growing (< 80 mm). From each growing category, two isolates were selected for screening against different isolates of *Trichoderma* in order to determine the promising strain for further study.

### (ii) *In vitro* screening of different strains of *Trichoderma* :

One culture of *Trichoderma* spp. was established by collecting rhizosphere soil samples of wilted pigeonpea plants obtained from Main Pulses Research Station, Sardarkrushinagar, Gujarat after isolation in the medium specific for *Trichoderma* by soil plate technique<sup>2</sup>. The other cultures of *Trichoderma* were obtained from different institutes. Six representative isolates of *F. udum*, two each selected on the basis of different growth habit were evaluated against eleven *Trichoderma* isolates using dual culture technique on PDA<sup>3</sup>. The percentage of inhibition in growth of the fungus in each treatment in comparison to control was calculated after seven days of incubation<sup>4</sup>.



**(iii) Efficacy of promising strain of *Trichoderma* against *F. udum* by different methods of application :**

The *Trichoderma* strain found promising after *in vitro* study was evaluated further by different method of application in pots. The inoculum of *F. udum* multiplied on sand pigeonpea meal medium for 20 days at  $27 \pm 2$  °C temperature was thoroughly mixed with the sterilized soil in the proportion of 1 : 10 w/w and filled in the sterilized pots. The pots were watered and kept for a week for uniform spread of the pathogen. The talc based formulation<sup>5</sup> of promising isolate of *Trichoderma* spp. was used as seed treatment as well as soil application. The pigeonpea cv. ICP 2376 seeds were treated with this formulation at 2, 4 and 6 g per kg seed. For soil application, the talc based preparation was mixed at 10, 20 and 30 g per kg soil in the pots which were previously inoculated with the inoculum of *F. udum*. In these pots also, the pigeonpea cv. ICP 2376 seeds were sown. The control pots without application of talc based preparation of *Trichoderma* were also maintained for comparison. Ten seeds were sown in each pot and the pots were watered regularly. The experiment was laid out in completely randomized design with four replications of each treatment. The observations on number of seeds germinated were recorded after seven days of sowing and wilt incidence was recoded 30 days after sowing in each treatment. The per cent reduction in wilt in comparison to control was calculated.

**(iv) Comparative efficacy of efficient strain of *Trichoderma* vis-à-vis market products :**

Five different *Trichoderma* preparations commonly available in the market along with talc based preparation of local isolate

were ascertained against *F. udum* in pots. After evaluating the efficient method for application of bio-control agent, the efficacy of local isolate of *Trichoderma* was further compared with the different *Trichoderma* based bio-fungicides viz., Current WP, Nisarg liquid, Nisarg WP, Monitor WP and Nicoderma WP commonly available in the local market. The inoculum of *F. udum* was thoroughly mixed with the sterilized soil as described earlier. Talc based formulation of local isolate of *Trichoderma* along with bio-fungicides as mentioned above were determined by soil application. Ten (10) g wet powder formulation of each product was mixed thoroughly in upper 7.5 cm layer of the pot. For liquid formulation, 10 ml of the preparation was mixed with 90 ml water and uniformly drenched in soil just after sowing. The control pots without application of *Trichoderma* were also maintained for comparison. Ten seeds of pigeonpea cv. ICP 2376 were sown in each pot and the pots were watered regularly. The experiment was laid out in completely randomized design with four replications of each treatment. The observations on number of seeds germinated were recorded after seven days of sowing and wilt incidence was recoded 30 days after sowing in each treatment. The percentage of reduction in wilt in comparison to control was calculated.

## RESULTS AND DISCUSSION

**(i) *In vitro* screening of different strains of *Trichoderma* :**

The results of antagonism of different isolates of *F. udum* revealed significant difference in the growth inhibition (%) (Table 1). All the *Trichoderma* isolates significantly inhibited the growth of different isolates of *F. udum*. Against the slow growing isolates, per cent growth inhibition

ranged from 74.77 to 87.68 in FU-10 isolate and 75.83 to 88.61 in FU-14 isolate by *T. harzianum* (Navsari) and *Trichoderma* spp. (Sardarkrushinagar), respectively. Similarly, against the moderately growing isolates (FU-6 and FU-9), the data indicated that the per cent growth inhibition ranged from 76.06 to 87.83 in FU-6 and 77.28 to 89.19 in FU-9 by *T. harzianum* (Navsari) and *Trichoderma* spp. (Sardarkrushinagar), respectively. Antagonism of fast growing isolates (FU-2 and FU-15) of *F. udum* by

various *Trichoderma* isolates also revealed significant difference in the growth inhibition. The growth inhibition of FU-2 isolate of *F. udum* ranged from 74.22 per cent by *T. harzianum* (Navsari) to 86.89 per cent by *Trichoderma* spp. (Sardarkrushinagar). Against the most virulent isolate FU-15 used in various investigations, *T. viride* (Hyderabad) proved least effective with growth inhibition of 76.58 per cent whereas maximum inhibition of growth

**Table 1**  
**Antagonism of slow, moderately and fast growing isolates of *F. udum* by various isolates of *Trichoderma* in vitro**

Trichoderma isolate	Growth inhibition (%)					
	Slow growing		Moderately growing		Fast growing	
	FU-10	FU-14	FU-6	FU-9	FU-2	FU-15
<i>Trichoderma harzianum</i> (Anand)	62.12 <sup>e</sup> (77.66) <sup>*</sup>	62.71 <sup>g</sup> (78.51)	61.67 <sup>e</sup> (77.15) <sup>*</sup>	63.07 <sup>e</sup> (79.01)	60.36 <sup>d</sup> (75.05) <sup>*</sup>	63.01 <sup>de</sup> (78.92)
<i>Trichoderma harzianum</i> (Vadodara)	67.89 <sup>b</sup> (85.36)	67.69 <sup>c</sup> (85.12)	69.31 <sup>ab</sup> (87.05)	69.15 <sup>b</sup> (86.86)	65.70 <sup>c</sup> (82.60)	66.57 <sup>bc</sup> (83.72)
<i>Trichoderma harzianum</i> (Savli)	63.62 <sup>d</sup> (79.79)	63.36 <sup>ef</sup> (79.42)	64.75 <sup>d</sup> (81.33)	65.31 <sup>cd</sup> (82.08)	61.24 <sup>d</sup> (76.38)	63.36 <sup>d</sup> (79.42)
<i>Trichoderma</i> spp. (Sardarkrushinagar)	69.86 <sup>a</sup> (87.68)	70.70 <sup>a</sup> (88.61)	70.00 <sup>a</sup> (87.83)	71.25 <sup>a</sup> (89.19)	69.18 <sup>a</sup> (86.89)	70.85 <sup>a</sup> (88.77)
<i>Trichoderma harzianum</i> (Sardarkrushinagar)	64.92 <sup>c</sup> (81.56)	65.87 <sup>d</sup> (82.82)	64.97 <sup>d</sup> (81.59)	66.09 <sup>cd</sup> (83.08)	64.75 <sup>c</sup> (81.32)	65.56 <sup>c</sup> (82.41)
<i>Trichoderma harzianum</i> (Navsari)	60.16 <sup>f</sup> (74.77)	60.87 <sup>h</sup> (75.83)	61.02 <sup>e</sup> (76.06)	61.86 <sup>e</sup> (77.28)	59.80 <sup>d</sup> (74.22)	62.24 <sup>def</sup> (77.36)
<i>Trichoderma harzianum</i> (GSFC, Vadodara)	60.77 <sup>f</sup> (75.68)	61.71 <sup>gh</sup> (77.06)	61.66 <sup>e</sup> (77.00)	62.15 <sup>e</sup> (77.71)	60.16 <sup>d</sup> (74.77)	61.78 <sup>ef</sup> (77.17)
<i>Trichoderma harzianum</i> (Kanpur)	64.26 <sup>cd</sup> (80.67)	64.33 <sup>e</sup> (80.76)	64.30 <sup>d</sup> (80.73)	64.76 <sup>d</sup> (81.35)	61.10 <sup>d</sup> (76.18)	62.87 <sup>de</sup> (78.74)
<i>Trichoderma harzianum</i> (Pantnagar)	68.46 <sup>b</sup> (86.04)	69.14 <sup>b</sup> (86.85)	67.55 <sup>c</sup> (84.94)	68.22 <sup>b</sup> (85.76)	67.92 <sup>ab</sup> (85.39)	66.82 <sup>bc</sup> (84.03)
<i>Trichoderma viride</i> (Anand)	67.33 <sup>b</sup> (84.67)	68.16 <sup>bc</sup> (85.68)	68.14 <sup>bc</sup> (85.65)	68.61 <sup>b</sup> (86.23)	67.27 <sup>b</sup> (84.60)	67.11 <sup>b</sup> (84.40)
<i>Trichoderma viride</i> (Hyderabad)	64.33 <sup>cd</sup> (80.77)	64.36 <sup>bc</sup> (80.81)	65.73 <sup>d</sup> (82.64)	66.41 <sup>c</sup> (83.52)	60.05 <sup>d</sup> (74.61)	61.38 <sup>f</sup> (76.58)

Treatment means with the letter/letters in common are not significant by Duncan's New Multiple Range Test at 5 % level of significance

\* Figures in parentheses are retransformed values

(88.77 %) was observed in *Trichoderma* spp. (Sardarkrushinagar) isolate. It could be seen from the above results that various isolates of *Trichoderma* differed in their efficacy against slow, moderately and fast growing isolates of *F. udum*. However, *Trichoderma* spp. (Sardarkrushinagar) was equally efficacious against all the *F. udum* isolates which indicated the uniform effectiveness of this local biocontrol agent. On the contrary, *T. harzianum* (Navsari) proved less effective against most of the *F. udum* isolates. Such differential efficacy of bioagents was reported by various research workers. Local isolate of *T. harzianum* (L1) was most promising which showed maximum inhibitory effect on mycelial growth (88.69 %) of *F. udum*<sup>6</sup>. The efficacy of 30 different *Trichoderma* isolates against *F. udum* revealed that two isolates showed highest colony growth reduction of 65.5 to 67.2 %, seven between 50 to 60 % and 21 isolates between 41-50 %<sup>7</sup>. Thus, the results obtained in the present investigations are in accordance with the earlier reports.

**(ii) Efficacy of promising strain of *Trichoderma* against *F. udum* by different methods of application :**

The results (Table 2) revealed significant difference in the seed germination (%) as

well as wilt incidence (%). The highest seed germination of 85.00 per cent was recorded in 30 g soil application; however, it was statistically at par with 20 g soil application. The seed germination of 75.00 per cent was observed in 2 and 4 g seed treatment and 77.5 per cent germination was observed in 6 g seed treatment. The wilt incidence in the different treatments ranged from 11.81 per cent to 39.73 per cent. Significantly lowest wilt incidence (11.81 %) was recorded in 30 g soil application of talc based formulation which was statistically at par with 20 g soil application, 10 g soil application and 6 g seed treatment with wilt incidence of 14.58, 14.93 and 19.64 per cent, respectively. The wilt incidence in the control was 56.70 per cent. The data further indicated that maximum reduction (79.18 %) in the wilt over control was observed in 30 g soil application followed by 20 g soil application (74.28 %), 10 g soil application (73.67 %) and 6 g seed treatment (65.35 %). These results clearly indicated the superiority of soil application over seed treatment of biocontrol agent. This might be due to the fact that the antagonists like *Trichoderma* grow rapidly when inoculated in the soil resulting in the competition for the nutritional factors and rhizosphere colonization.

**Table 2**

**Effect of method of application of local isolate of *Trichoderma* on germination and wilt incidence (%) in pigeonpea cv. ICP 2376**

Treatment	Dose	Germination (%)	Wilt incidence (%)	Reduction in wilt over control (%)
<b>SEED TREATMENT</b>				
I.	2 g	60.42 <sup>b</sup> (75.00)*	39.27 <sup>b</sup> (39.73)	29.92
II.	4 g	60.42 <sup>b</sup> (75.00)	33.40 <sup>b</sup> (29.91)	47.24

III.	6 g	62.10 <sup>ab</sup> (77.50)	26.30 <sup>c</sup> (19.64)	65.35
SOIL APPLICATION				
I.	10 g	65.83 <sup>ab</sup> (82.50)	22.96 <sup>c</sup> (14.93)	73.67
II.	20 g	67.89 <sup>a</sup> (85.00)	22.66 <sup>c</sup> (14.58)	74.28
III.	30 g	67.89 <sup>a</sup> (85.00)	20.52 <sup>c</sup> (11.81)	79.18
CONTROL	--	60.42 <sup>b</sup> (75.00)	49.13 <sup>a</sup> (56.70)	--

Treatment means with the letters in common are not significant by Duncan's New Multiple Range Test at 5 % level of significance

\* Figures in parentheses are retransformed values

The seed treatment of pigeonpea with *T. harzianum* spores failed to reduce wilt whereas, augmentation of soil with *T. harzianum* in maize meal : sand medium @ 40-60 g/kg soil resulted in a significant reduction of wilt incidence up to 89 per cent<sup>8</sup>. Soil application of *T. harzianum* was more effective than seed treatment for disease suppression, thus, suggested the need to augment soil application of *T. harzianum* for obtaining effective control of pigeonpea wilt<sup>9</sup>. Therefore, the results obtained in the present investigations are in accordance with the reports of earlier research workers.

**(iii) Comparative efficacy of efficient strain of *Trichoderma vis-à-vis* market products :**

The results revealed significant difference in the seed germination (%) as well as wilt incidence (%). The highest seed germination of 85.00 per cent was recorded in talc based preparation of local isolate followed by Monitor WP (82.50 %). The seed germination in the control was 72.50 per cent. Wilt incidence (%) was also

significantly influenced by different preparations of *Trichoderma*. It ranged from 11.81 to 32.14 per cent among different bioagents preparations as against 51.34 per cent in control. Minimum wilt incidence (11.81 %) was recorded in talc based preparation of local isolate of *Trichoderma* followed by Monitor WP (14.93 %), Nisarg WP (22.32 %) and Nicoderma WP (24.26 %). Similarly, highest reduction in the wilt (77.00 %) over control was recorded in talc based preparation of local isolate followed by Monitor WP (70.92 %) (Table 3). Local isolate of *T. harzianum* (L1) was most promising, showed maximum inhibitory effect of *F. udum* as well as lowest incidence (20.37 %) in pots where seed treatment was given (Jayalakshmi *et al.*, 2003). Comparison of different products of biological control agents, *Trichoderma* spp. against wilt of pigeonpea revealed that all the products reduced wilt incidence, however, seed treatment with phule Trichokill at 8 g per kg seed recorded the highest seed germination and lowest wilt incidence<sup>10</sup>.

**Table 3**

**Efficacy of local isolate of *Trichoderma vis-à-vis* *Trichoderma* based bio-fungicides available in the market on germination and wilt incidence (%) in pigeonpea cv. ICP 2376**

Bio-fungicide	Technical specification	Germination (%)	Wilt incidence (%)	Reduction in wilt over control (%)
Current WP	<i>T. harzianum</i> (2 X 10 <sup>8</sup> cfu/g)	62.10 <sup>abc</sup> (77.50)*	32.25 <sup>bc</sup> (28.57)	44.35
Nisarg liquid	<i>Trichoderma</i> spp.	58.91 <sup>bc</sup> (72.50)	34.26 <sup>b</sup> (32.14)	37.39
Nisarg WP	<i>T. viride</i> , 1 % WP	62.10 <sup>abc</sup> (77.50)	28.39 <sup>bcd</sup> (22.32)	56.52
Local isolate	<i>Trichoderma</i> spp. (min. 10 <sup>8</sup> cfu/g)	67.89 <sup>a</sup> (85.00)	20.52 <sup>d</sup> (11.81)	77.00
Monitor WP	<i>T. viride</i> , 1 % WP (2 X 10 <sup>6</sup> to 10 <sup>8</sup> cfu/g)	65.83 <sup>ab</sup> (82.50)	22.82 <sup>cd</sup> (14.93)	70.92
Nicoderma WP	<i>T. viride</i> , 1 % WP (> 2 X 10 <sup>6</sup> cfu/g)	57.24 <sup>c</sup> (70.00)	29.43 <sup>bcd</sup> (24.26)	52.75
Control	--	58.91 <sup>bc</sup> (72.50)	46.05 <sup>a</sup> (51.34)	--

Treatment means with the letters in common are not significant by Duncan's

New Multiple Range Test at 5 % level of significance

\* Figures in parentheses are retransformed values

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