

Original Research Article

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Antagonism of Native and Commercial *Trichoderma* spp. against *Fusarium solani* Isolates Causing Root Rot of Papaya (*Carica papaya* L.)

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ABSTRACT

Fusarium solani listed inmost notorious among the top ten plant pathogen. It is a soil-borne plant pathogen, caused root rot of the papaya which is responsible for estimate losses upto 40-95 percent. In this study we had collected different isolates of *Fusarium solani* from different districts of Bihar and the five isolates of *Fusarium solani* were selected on the basis of cultural, morphological and disease potential studies then further evaluated the fungus against native and commercial *Trichoderma viride* and *Trichoderma harzianum* by dual culture technique in the laboratory of the Department of Plant Pathology, DRPCA U Pusa, Samastipur in 2016. Observations were taken at 120 hours and 240 hours for the assessment of their inhibitory effects. In the presence of native *T. viride* and *T. harzianum* the variability in the growth inhibition over control is observed among five isolates, however the maximum growth inhibition was observed in the isolates FS-V(78.1%, 72.5%) and minimum in FS-II (73.1%, 68.1%) respectively, likewise in the presence of commercial *T. viride* and *T. harzianum* spp. maximum growth inhibition observed in FS-V (60.9%, 53.2%) and minimum in FS-II(53.8%, 49.2%) respectively were significantly reduced in comparison to control. Both the native and commercial *Trichoderma* spp. significantly reduced the mycelial growth of *Fusarium solani* isolates in comparison to control.

Keywords

Fusarium solani isolates, *Trichoderma* spp., Variability, Root rot

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Introduction

Papaya (*Carica papaya* L.) is a tropical fruit plant (Langdon, 1989) which belongs to the family Caricaceae. The papaya fruit is a rich source of vitamin A and C and its papain utilize in many industries for manufacturing the cosmetics items, silk, and clarification of beer (Ko, 1982; SPC, 1980). India is the

largest producer of papaya (5.94MT) in the world (FAOSTAT2018-19). The papaya plant attacked by various plant pathogenic diseases which are considered as the limiting factor due to causes several economic losses during cultivation. There are over 17 diseases reported which affect the papaya plants all over the world (Singh, 2003). The major disease of papaya includes root rot, foot rot,

damping off, phytophthora blight, stemphylium fruit spot, and bunchy top, etc. In India over the last four years; a new disease of papaya with the symptom of root rot has emerged as a serious threat to the crop in the Bihar causing 90-95 percent crop failure and thus inflicting a heavy loss to the growers. Most of the varieties have been found to be highly susceptible to this disease. The disease has been found to affect the crop round the year at all growth stages of the plants. However, the development of disease becomes fast after rain occurring in any month. Singh and Kumar (2015) first time established the etiology of Papaya root rot in agro-ecological conditions of Bihar and has informed that root rot disease was caused by the fungus *Fusarium solani* (Mart.) Sacc. Due to its devastating nature, there is an urgent need to manage the disease for better returns from papaya cultivation (AICRP (Fruits), 2012-2017). As per Bapat and Shar (2000) the adverse effect of fungicides on the environment and increased interest in sustainable agriculture, biological control has been tried as an effective approach for the management of soil-borne plant pathogens. *Trichoderma harzianum* and *Trichoderma viride* gave effective management of *Fusarium* spp described by Gurjar *et al.*, (2004).

Materials and Methods

Isolation and purification of fungus

For isolation, the diseased plant part (root) of papaya was collected from different district of Bihar. After collection, infected plant parts were brought in the lab and washed gently in several changes of tap water for 5 minutes to remove dirt and cut into small bits of 2-5mm dimension. These bits were surface sterilized by 0.1 percent mercuric chloride ($HgCl_2$) solution for 30 seconds followed by three washing by sterilized distal water then drying

on blotting paper. After drying, infected plant parts placed aseptically on PDA slants with the help of inoculating needle under aseptic condition and incubate at $28\pm 2^\circ C$ for complete growth of the fungi (Hemalatha *et al.*, 2018). After 6 days of incubation, a bit of hyphal growth from growing tips was transferred aseptically to fresh PDA slants. The fungus was purified by employing the hyphal tip method (Singh, 1988). Then after purification, the collected isolates of *Fusarium solani* were categorized in five different groups viz; Fs-I, Fs-II, Fs-III, Fs-IV, and Fs-V on the basis of their cultural, morphological and pathogenicity characters. However, the culture is maintained by periodically transfer on PDA slants for further studies.

Isolation and identification of native and commercial fungal bioagents

Soil was collected from the rhizosphere of healthy papaya plants in poly-ethylene bags and brought in the laboratory. Mostly serial dilution methods (Johnson and Curl, 1972) were used for the isolation of fungal antagonists which are abundant in rhizospheric soil of healthy plants. The mycoflora from the soil was isolated on a suitable rose Bengal agar medium by using a dilution of 10^{-3} and 10^{-4} . Normally 100 μl of prepared soil suspension from the test tube was poured into sterilized Petri a plate containing the liquefied and cooled medium was poured in it and then spread with help of L-spreader (PW1085, HiMedia Laboratories Pvt. Ltd.) so that the soil suspension gets uniformly distributed into the medium. Then, the plates were incubated in B.O.D at an optimum temperature of $27\pm 2^\circ C$ and frequently observed for the growth of colonies. The fully grown colonies were picked with the help of a needle and identified on the basis of mycological keys described by Gilman (1957); Nelson *et al.*, (1983) and

Barnett and Hunter (1986) for the identification of bioagents *T. viride* and *T. harzianum*.

Bioagents that are available in a commercial formulation, *i.e.* Nisarga (Multiplex Agricare Pvt. Ltd. 1%W.P.) and Antagon (Arihant Nature crop Pvt. Ltd. 1%W.P.) were also isolated as per the method described in native bioagents isolation.

Screening by dual culture methods

Dual culture technique (Sabalpara, 2009, Karunanithi and Usman, 1999) usually used to evaluate the antagonistic effect of *Trichoderma spp.* against the *Fusarium solani*. Initially, a 5 mm disc of *Trichoderma spp.* (7 days old culture) and agar disc of *Fusarium solani* of the same size were kept opposite to each other normally near the edge of Petriplate (90mm) containing PDA. For control *Fusarium solani* placed in a similar manner on the PDA plate. The experiment carried out three replications and then incubated at 25°C in a B.O.D. incubator. The radial growth and percent inhibition of fungus mycelial growth were recorded. The percentages of reduction of linear mycelial growth of pathogenic fungi were calculated using formula as given by Vincent (1927)

$$I = \left(\frac{C - T}{C} \right) \times 100$$

Where,

I = Percent inhibition over control.

T = Growth of test pathogen in the presence of antagonist (mm). The percent inhibition data were analyzed statistically using a completely randomized design (C.R.D)

C = Growth of test pathogen without the antagonist (mm).

Snedecor and Cochran (1980) stated that the data undergo proper statistical analysis of the

variance. Mean of treatments at a level of 0.05% were match with the F test and L.S.D.

Results and Discussion

In the present investigation, both biocontrol agents *i.e.* *T. viride* and *T. harzianum* found effective in suppression of radial growth of all five isolates of *Fusarium solani*.

Effect of a native *Trichoderma viride* and *Trichoderma harzianum* species on different isolates of *Fusarium solani* in vitro

The antagonistic activity of native *Trichoderma viride* and *Trichoderma harzianum* species against the five isolates of *Fusarium solani* (Fs-I, Fs-II, Fs-III, Fs-IV, and Fs-V) were recorded and presented in Table 1. In the case of native *T. viride* lowest colony diameter was recorded in isolate Fs-V (18.5mm) followed by Fs-I (19.5mm), Fs-III (20.5mm), Fs-IV (21.0mm) and the highest growth was recorded in isolate Fs- II (24.0mm). The results of dual culture indicated that *T. viride* significantly inhibited the growth of *F. solani* at varying degrees across the duration of incubation. However, maximum inhibition was recorded in isolate Fs-V (78.1%), followed by Fs-I (77.2%), Fs-III (76.4%), Fs-IV (76.1%) while, the minimum inhibition was recorded in isolate Fs-II (73.1%) over control after 240 hrs respectively. While in the case of native *T. harzianum*, minimum colony diameter was recorded in isolate Fs-V (23.mm) followed by Fs-I (25.0mm), Fs-III (25.8mm), Fs-IV (26.9mm) and the maximum growth were recorded in isolate Fs- II (28.5mm). However, the maximum percentage of inhibition was recorded in isolate Fs-V (72.5%) followed by Fs-I (70.8%), Fs-III (70.4%) and Fs-IV (69.4%). while minimum inhibition was recorded in Fs-II 68.1%. after 240 hours over control respectively.

Table.1 Effect of native *Trichoderma viride* and *Trichoderma harzianum* species on different isolates of *Fusarium solani* *in vitro*

Isolates	*Radial growth(mm) of <i>Fusarium solani</i>					
	<i>Trichoderma viride</i> (native)			<i>Trichoderma harzianum</i> (native)		
	120 hrs	240 hrs	Inhibition over control (%) at 240 hrs	120 hrs	240 hrs	Inhibition over control (%) at 240 hrs
Fs-I	11.4	19.5	77.2	17.4	25.0	70.8
Control	33.1	85.7	-	33.1	85.7	-
Fs-II	15.0	24.0	73.1	21.0	28.5	68.1
Control	35.0	89.5	-	35.0	89.5	-
Fs-III	12.0	20.5	76.4	18.0	25.8	70.4
Control	33.5	87.2	-	33.5	87.2	-
Fs-IV	13.8	21.0	76.1	19.0	26.9	69.4
Control	34.0	88.0	-	34.0	88.0	-
Fs-V	10.9	18.5	78.1	17.2	23.2	72.5
Control	32.8	84.6	-	32.8	84.6	-
CD at 5%	1.79	3.52	-	1.92	2.28	-
SE(m)±	0.60	1.87	-	0.64	0.76	-
CV (%)	4.53	3.86	-	4.29	2.39	-

* Mean of three replications

Table.2 Effect of commercial *Trichoderma viride* and *Trichoderma harzianum* species on different isolates of *Fusarium solani* *in vitro*

Isolates	*Radial growth(mm) of <i>Fusarium solani</i>					
	<i>Trichoderma viride</i> (Commercial)			<i>Trichoderma harzianum</i> (Commercial)		
	120 hrs	240 hrs	Inhibition over control (%) at 240 hrs	120 hrs	240 hrs	Inhibition over control (%) at 240 hrs.
Fs-I	18.8	35.0	59.2	19.9	41.9	51.1
Control	33.5	85.8	-	33.5	85.8	-
Fs-II	22.4	41.5	53.2	23.8	45.0	49.2
Control	35.0	88.7	-	35.0	88.7	-
Fs-III	20.8	37.8	56.5	21.9	42.7	50.9
Control	33.7	87.0	-	33.7	87.0	-
Fs-IV	21.1	38.6	56.0	22.1	43.6	50.3
Control	34.0	87.9	-	34.0	87.9	-
Fs-V	18.5	33.0	60.9	19.3	39.0	53.8
Control	33.3	84.5	-	33.3	84.5	-
CDat 5%	2.07	4.17	-	2.37	3.81	-
SE(m)±	0.69	1.40	-	0.80	1.28	-
CV (%)	4.46	3.93	-	5.08	3.48	-

* Mean of three replications

Fig.1 Antagonistic activity of native species of *T. harzianum* and *T. viride* against *F. solani* (FS) isolates evaluated by dual culture assay, each value is an average of three replicates

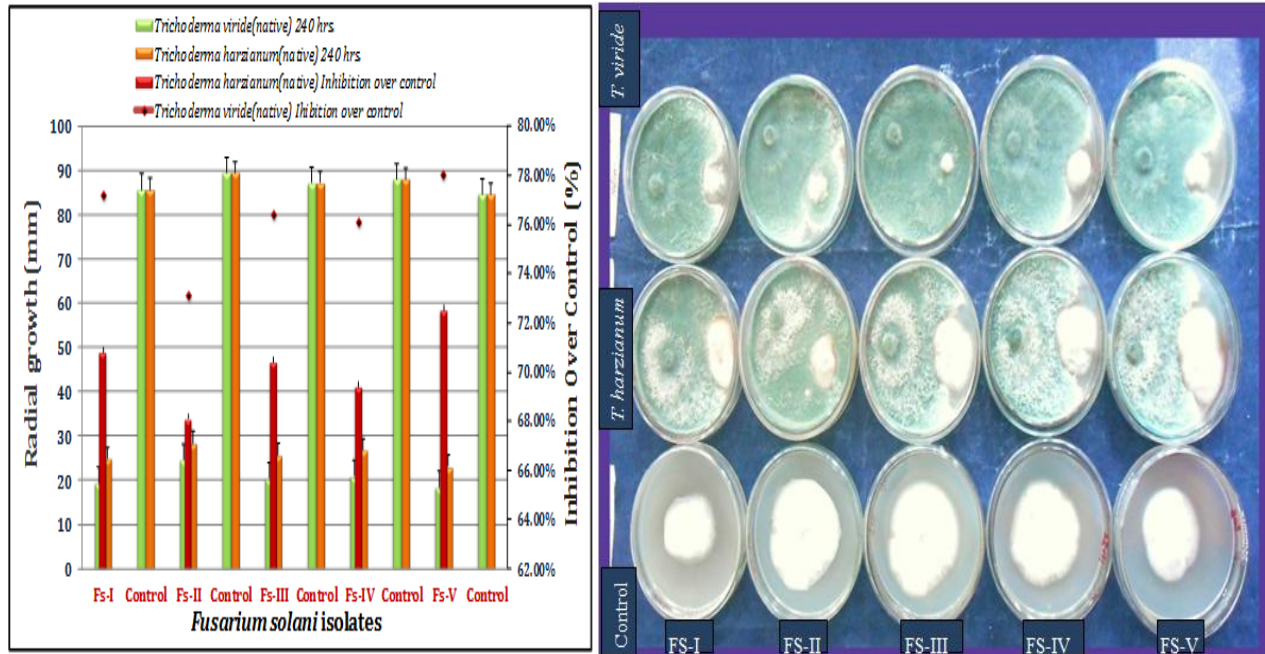
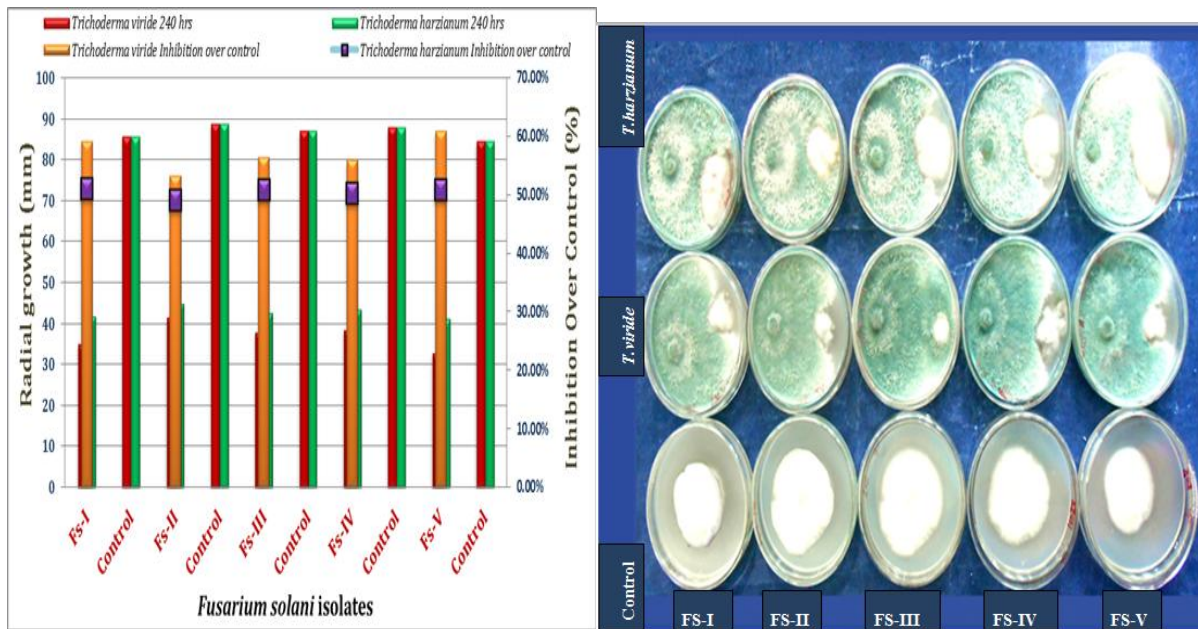


Fig.2 Antagonistic activity of commercial species of *T. harzianum* and *T. viride* against *F. solani* (FS) isolates evaluated by dual culture assay, each value is an average of three replicates



The results showed that the native species *T. viride* was more effective in suppression of the radial growth of *Fusarium solani* isolates as compared to native *T. harzianum*.

Effect of commercial *Trichoderma viride* and *Trichoderma harzianum* species on different isolates of *Fusarium solani* in vitro

The antagonistic activity of commercial *Trichoderma viride* and *Trichoderma harzianum* species against the five isolates of *Fusarium solani* (Fs-I, Fs-II, Fs-III, Fs-IV, and Fs-V) were recorded and presented in Table 2. In the case of commercial *T. viride* minimum colony diameter was recorded in isolate Fs-V (33.0mm) followed by Fs-I (35.0mm), Fs-III (37.8mm), Fs-IV (38.6mm) and the maximum growth was recorded in isolate Fs- II (41.5mm). The results of dual culture indicated that *T. viride* significantly inhibited the growth of *F. solani* at varying degrees across the duration of incubation. However, maximum inhibition was recorded in isolate Fs-V (60.9%), followed by Fs-I (59.2%), Fs-III (56.5%), Fs-IV (56.0%) while, the minimum inhibition was recorded in isolate Fs-II (53.2%) over control after 240 hrs respectively. While in the case of commercial *T. harzianum*, minimum colony diameter was recorded in isolate Fs-V (39.0mm) followed by Fs-I (41.9mm), Fs-III (42.7mm), Fs-IV (43.6mm) and the maximum growth were recorded in isolate Fs- II (45.0mm). However, the maximum percentage of inhibition was recorded in isolate Fs-V (53.8%) followed by Fs-I (51.1%), Fs-III (50.9%) and Fs-IV (50.3%) while minimum inhibition was recorded in Fs-II (49.2%) after 240 hours over control respectively. The results showed that the commercial species *T. viride* was more effective in suppression of the radial growth of *Fusarium solani* isolates as compared to commercial *T. harzianum*.

Root rot disease of papaya is caused by the soil-borne fungus *Fusarium solani* (Singh and Kumar, 2015) which mainly affect the young plant, due to this fungus, the succulent seedlings become yellow followed by root rotting, which is considering primary site of infection and later entire plant turns into wilting and finally the plant dies within few days depending on the congenial environmental condition which is favors by the *F. solani*. In the soil, several types of antagonistic microorganisms are present which compete with each other with respect to food, nutrition, and space viz. *Trichoderma* spp., *Bacillus* spp. etc. (Schulz *et al.*, 2013). *Trichoderma* species are saprophytic in nature and mainly found in soil as well as in rhizospheric flora and found more effective in controlling root rot disease of papaya caused by *F. solani* by utilizing various type of mechanism viz. mycoparasitism, antibiosis, and competition, etc (Benítez *et al.*, 2004). The mycoparasitic characters of *Trichoderma* species made them effective biocontrol agents against a wide range of plant pathogens (Elad, 2000; Freeman *et al.*, 2004; Ashrafizadeh *et al.*, 2005; Dubey and Suresh, 2007) and successfully available commercially as bio fungicides as well as bio-nematicide as having potential to kill nematodes (Sharon, 2011). In dual culture interaction, *Trichoderma* spp. mainly produces extracellular exochitinases enzyme in low amount prior before interacting with the fungus mycelium and caused appreciable inhibition of mycelia growth of *F. solani* (Kullnig *et al.*, 2000; Brunner *et al.*, 2003). The penetration of exochitinases enzymes in the host cell dissolves cell fragments and influences the production of enzymes that enhance physiological changes, rapid stimulation and also help indirect growth of *Trichoderma* spp. in the host cell (Zeininger *et al.*, 1999). Once the fungi come into contact of *Trichoderma* spp., it attached to the host (fungus) cell and start coiling around it

and form a peg-like structure *i.e.* appressoria on the host surface lead to the production of several fungal cell wall degrading toxic substances *viz*; chitinases, glucanases (Chet *et al.*, 1998), and probably also peptaibol antibiotics (Schirmböck *et al.*, 1994) *viz*; as trichodermin, trichodermol, harzianum A and harzianolide. Further, the attachment of antagonists on the host is negotiated by the binding of carbohydrates that are present in the cell wall of *Trichoderma* (antagonist) to lectins present on the target fungus (host) (Nbar *et al.*, 1996). The overall activities of these compounds show parasitism of the target fungus by the *Trichoderma* and further result in fungal cell walls disintegration. At the sites where appressoria attached to the target fungus, a hole is formed which helps in the direct entry of *Trichoderma* hyphae into the fungus lumen (Harman, 2000). The level of efficiency of the metabolites secreted by the antagonist varies according to the nature, quality, and quantity of antibiotics (Harman and Kubicek, 1998). The research result explores the possibility of *Trichoderma* as a new organic tool that allows sustainable management of plant health problems, which nullify the detrimental impact of the use of synthetic chemical fungicides. The present results are more or less in conformity with the finding of the other workers in India and abroad. Earlier Jha and Jalali (2006) tested the efficacy of bioagents like *T. viride*, *A. terreus*, *A. sydoui*, *A. flavus* and *spicariasylyatica* against pea root rot pathogen *Fusarium solani* f. sp. *pisi* by dual culture technique and found that *T. viride* showed the strongest antagonistic activity toward pathogen. Rojoet *al.*, (2007) used *Trichoderma harzianum* isolates ITEM3636 and ITEM 3635, *T. longibranchiatum* for the management of peanut brown root rot caused by *Fusarium solani* found to be more effective than control. Sahi and Khalid (2007) tested antagonistic effect of different bioagent *viz*; *T. viride*, *T. harzianum*, *T. aureoviride*,

T.koningii and *T. pseudokoningii* against *Fusarium oxysporum*, that causes wilt in sweet pepper (*Capsicum annum*) *in vitro* condition and observed that *T. viride* gave maximum reduction (62.0%) in radial growth of fungus as compared to *T. pseudokoningii* which shows minimum reduction. Bhaliya and Jadeya (2013) evaluated five rhizospheric microflora such as *Trichoderma viride*-I, *T. viride*-II, *T. harzianum*-I, *T. harzianum* –II and *Aspergillus niger* against root rot of coriander (*Coriandrum sativum* L.) caused by *Fusarium solani* *in vitro* condition and revealed that maximum inhibition of pathogen (96.21%) was observed by *T. v* –II followed by *T. h* –II (90.58) while *Aspergillus* was less effective with 60.44% inhibition. Kumar *et al.*, (2017) evaluated native and commercial biocontrol agents (*Trichoderma viride* and *Trichoderma harzianum*) against *Fusarium solani* causing root rot of papaya and observed that native isolate of *Trichoderma harzianum* was found less effective than *Trichoderma viride* isolate (native) in order to suppress radial growth of *Fusarium solani*. Both commercial isolate of *Trichoderma viride* and *Trichoderma harzianum* also shows the same trend against the *Fusarium solani*.

It is concluded, both native and commercial biocontrol species *i.e.* *Trichoderma viride* and *T. harzianum* found effective in suppression of radial growth of five different isolates of *Fusarium solani* *viz*; (FS-I, FS-II, FS-III, FS IV, and FS-V) causing root rot of Papaya. Among both native and commercial formulated species that were used as an antagonist against *Fusarium solani* isolates, the *Trichoderma viride* in both formulations (native and commercial) was found effective as compared to *T.harzianum* in restricting the growth of pathogen but native *T. viride* which was isolated from Papaya rhizospheric soil was found to be more forcible in suppression of radial growth of *F. solani*. The research study demonstrated that *Trichoderma spp.* has

the potential to be used as a biocontrol agent to protect the papaya plant from *Fusarium solani*.

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