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Screening of biocontrol agents against *Fusarium solani* causing root rot disease in papaya

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Abstract
Research in the past few years has clearly shown that selected strains of the fungi provide an eco-friendly well balanced plant health management system through systemic resistance to diseases of biotic and a biotic nature, increasing seed vigour, improving nitrogen fertilizer use efficiency, enhancement of root area. *Fusarium solani* was established as a causal organism of root rot of papaya. Use of Bio-control agents is eco-friendly approach and a good option to manage the soil borne phyto-pathogens. These biological control agents either use the mechanism of antibiosis or mycoparasitism against the fungal pathogen. Evaluation of *Trichoderma* spp. against *Fusarium solani* showed that all the test bio- agents significantly reduced the mycelial growth of *Fusarium solani in vitro*. The highest inhibition of growth of *Fusarium solani* was exerted by *Trichoderma viride* (native isolate) after 240hrs (76.8%).

Keywords: Bio-control, *Fusarium solani* and root rot

Introduction
In view of the adverse effect of fungicides to the environment and increasing interest in sustainable agriculture, biological control has been tried as an attractive possibility for management of soil borne plant pathogens (Bapat and Shar, 2000) [2]. The value of the global biopesticide market is expected to reach $4,556.37 Million by 2019, at a compound annual growth rate of 15.30% from 2014 to 2019. The reasons for such growth can be found in: the increasing concerns on the impact of residues and overuse of synthetic chemical pesticides and the increasing relevance of pests and pathogens due to growth in food demand, the withdrawal of several chemical pesticides including soil fumigants, the appearance of new invasive species and pesticide resistant strains of pests, the effect of climate change and the specialised monoculture. Kumar (2012) [8] screened the bacterial and fungal isolates for their antagonism against *Fusarium solani*, an associated pathogen of root rot disease of mulberry. Screening revealed that eight bacterial and two fungal isolates were highly antagonistic to *F. solani*. Nine bacterial isolates and one fungal isolate showed moderately and the rest were less or not antagonistic to *F. solani*. All the bacteria which showed highly antagonistic reactions to *F. solani* were Gram negative except Bacillus sp. of these, four bacterial species were identified as *Pseudomonas*, three *Bacillus* and one *Azotobacter*. The fungi were identified as *Trichoderma harzianum* and *Trichoderma virens.* Among the bacterial isolates, highest inhibition of pathogen growth was shown by *Pseudomonas* strain-2 (65.96%) and *Pseudomonas* strain-1 (48.26%) followed by *Bacillus* strain-2 (42.61%). Similarly, among fungi the highest inhibition of pathogen was shown by, *T. harzianum* (47.74%). However, *T. virens* (23.70%) showed less inhibition. This disease has been observed as a serious threat to papaya cultivation in agro-ecological conditions of Bihar. It was not a plant-growth-stage

specific disease but affects the papaya plant at any growth stage. Up to 60- 95 per cent disease incidence was observed (Singh, and Kumar, 2105) [9]. The present study was screening the different biocontrol agents against *Fusarium solani* causing the root rot of papaya.

Material and Methods

Isolation of Pathogen: The roots of infected papaya plants were collected from Horticultural Research Station, Dr. Rajendra Prasad central Agricultural University, Pusa (Bihar) and brought to the laboratory for further study. The roots of infected papaya plants showing reddish brown-to-dark brown symptoms were collected and cut in small pieces (5 to 6 mm) of necrotic tissues with some healthy portion, were surface sterilized with 0.1% $HgCl_2$ for 20-30 seconds, washed thrice in sterile distilled water and plated onto potato dextrose agar (PDA) amended with 0.05 g liter^{-1} streptomycin sulfate. Plates were incubated at 28±2ºC for 5 days. Pure cultures with white, fluffy aerial mycelia were obtained by several subculturing of hyphal tips onto PDA (Pathak, 1987).

Isolation and identification of native and commercial fungal bio-agents

Rhizospheric soil from healthy papaya plants was collected in poly-ethylene bags and brought to the research laboratory. Serial dilution technique was used to isolate fungal antagonist from rhizospheric soil of healthy plants and shade dried. Antagonistic mycoflora were isolated on rose bengal agar medium by using a dilutions of $10^3$and 10^4. One ml of soil suspension was poured into sterilized Petriplates containing the melted and cooled medium was poured and then rotated gently to get uniform distribution of soil into the medium. Then, the plates were incubated at 27±2ºC and observed frequently for the development of colonies.

The developed colonies were picked and identified based on mycological keys described by Gilman (1957) [5]; Nelson *et al*. (1983) [11] and Barnett and Hunter (1986) [3] for identification of *Trichoderma viride* and *Trichoderma harzianum.*

Bioagents was also isolated from two commercial formulation of biocides, *i.e.* Nisarga (Multiplex Agricare Pvt. Ltd. 1%W.P.) and Antagon (Arihant Nature crop Pvt. Ltd. 1%W.P.) as per method described in native bioagents isolation.

Evaluation of antagonistic efficacy of Trichoderma viride and Trichoderma harzianum against *Fusarium solani*

For testing the antagonistic effect of *T. harzianum* and *T. viride* (commercial and native isolate) the PDA plate divided into equal halves (Karunanithi and Usman, 1999) [7]. The first half was separately inoculated with 7days –old culture disc (5mm in diameter) of each fungal bio-control agent, while the later was separately inoculated with one disc (5 mm in diameter) of 7 days old culture of pathogenic fungus in the opposite side. Control plate was inculcated with disc of PDA medium instead the fungal bio-control agents. Four plates were used as replicates for each treatment.

All plates were incubated for 28±2^{O}C until the growth of *Fusarium solani* in the control treatment reached to the edge of Petri dish. The percentages of reduction of linear mycelial growth of pathogenic fungi were calculated using formula as given by Vincent (1947) [15].

Data were subjected to proper statistical analysis of variance according to Snedecor and Cochran (1980) [14]. Mean of treatments were compared with F test and L.S.D. at level of 0.05%.

The radial growth of *Fusarium solani* was measured in all treatments after 240 hrs of inoculation and compared with control. The per cent inhibition was calculated by using following formula as given by Vincent (1947) [15].

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

Where,

I = Per cent growth inhibition

C=Colony diameter in control Petri plate;

T =Colony diameter in the treated Petri plate.

The per cent inhibition data were analyzed statistically using completely randomized design (C.R.D). The calculated value of F was compared with the tabulated values at 5 per cent level of significance for an appropriate degree of freedom.

General characteristics of *Trichoderma* spp.

Trichoderma viride: *Trichoderma viride* conidiophores and side branches are long and slender without sterile hyphal elongation, phialides not croweded, rather slender, colonies yellowish, bright, dull to dark green, floccose or with compact conidophore tufts. Conidia are roughened.

***Trichoderma harzianum*:** Colononies growing rapidly with most isolate 6-9 cm in diameter after 5 days. Aerial mycelium floccose, white to grayish or rarely yellowish. Hyphae hyaline, smooth walled mostly 4-6 mm in diameter or submerged mycelium occasionally up to 12 mm in diameter. Conidiophores are highly branched with primary branches arising at nearly right angles usually in whorls of 2 or 3 phialides ampulliform or subglobose to ovoid, apex broadly rounded, base more narrowly rounded and surface is smooth walled.

Mechanism of Trichoderma spp. as a fungal antagonist

In-vitro by dual technique and *in-vivo* proved that *Trichoderma spp.* is strong antagonist against mostly soil born pathogen. *Trichoderma* may directly kill or suppressed the growth of pathogen by mycoparasitism and antibiosis. It may adversely affect the growth and development of the pathogen either by antibiosis or by competing for nutrient, space for oxygen. Indirectly, it may contribute by promoting plant growth resistance to biotic and a biotic stresses and changes in the nutritional status of the plant. Many soils borne fungal pathogen like, *Rhizoctonia, Sclerotinia, Sclerotium, Macrophomina* etc. form hard resting structure called sclerotia. These sclerotia play vital role in long term survival of this pathogen in soil. It is difficult to kill these sclerotia using fungicides. In general *Trichoderma virens* colonizes and kill these sclerotia, wheres as *Trichoderma harzianum* primarily attack hyphae.

Result and discussion

A comparison of the data presented in Table 1 indicated that of the investigation of *Trichoderma viride* and *Trichoderma harzianum* native and commercial isolate. The highest inhibition of growth of *Fusarium solani* was exerted by *Trichoderma viride* (native isolate) after 240hrs (76.8%) followed by *Trichoderma harzianum* (native) after 240 hrs (70.0%), *Trichoderma viride* (commercial) after 240 hrs (52.8%) and *Trichoderma harzianum* (commercial) after 240

hrs (46.7%). The greater inhibition of *Fusarium solani* by the native isolates of *Trichoderma viride* and *Trichoderma harzianum* as compared to commercial ones might be attributed to the stronger antagonistic effect exhibited by the native isolate possibly due to their faster growth rate and enhanced antibiotic activity (plate 1).

Sagar *et al.,* (2007) [13] evaluated nine biological control agents under *in vitro* conditions against *Pythium aphanidermatum* and *Fusarium solani* which cause rhizome rot of ginger (*Bacillus subtilis, Pseudomonas fluorescens, Pseudomonas fluorescens* (Sirsi isolate), *Trichoderma koningii, Trichoderma virens (Gliocladium virens), Trichoderma viride, Trichoderma harzianum, Trichoderma harzianum* (Sirsi isolate) and *Trichoderma harzianum* (Dharwad isolate). In *Pythium aphanidermatum*, the maximum inhibition of mycelial growth was noticed with *T. harzianum* (Sirsi isolate) (77.77%), which was *at par* with *T. harzianum* (Dharwad isolate) (76.40%). Among the nine biological control agents evaluated against *F. solani*, *T. harzianum* (Sirsi and Dharwad isolates) inhibited the maximum mycelial growth (78.51 and 76.29%), which was at par with *T. virens* (77.03%). Kumar and Upadhyay (2007) [10] reported that all the three species of *Trichoderma viride, T. harzianum* and *T. virens (Gliocladium virens)* inhibited the growth of *F. udum in vitro*. *T. viride* was found more potential in inhibiting the radial growth of *F. udum*. *T. virens* was more effective in inhibiting the growth of Fu-61. Minimum disease incidence of 26.4 % and maximum disease control of 56.0 % was observed in combined application of *T. harzianum* and *T. viride* as seed treatment and soil application followed by 54 % disease control in *T. harzianum* seed treatment and soil application. Chandal and Tomer (2008) [4] reported *T. harzianum* to be superior over *T. viride* and *T. virens* in managing the wilt disease of carnation. Under field conditions all the treatments had a positive effect on the plant growth parameters. Arshad (2008) [1] reported that *Trichoderma viride, T. harzianum, T. aureoviride, T. koningii, T. pseudokoningii, Aspergillus fumigates, A. Glacus* and *A. Oryzae* were effective in controlling *in vitro* the growth of the wilt pathogen (*Fusarium solani).* Gupta and Mishra (2009) [6] reported that *Aspergillus niger* was found superior in inhibiting growth of *Fusarium solani* in dual culture techniques. In field evaluation of bioagents, the best growth was recorded in plant treated with *Aspergillus niger* to control wilt in guava.

Table 1: *In vitro* screening of native and commercial isolate of *Trichoderma viride and Trichoderma harzianum* on *Fusarium solani*

Time (hrs)	Inhibition by *Trichoderma viride* (native) over control (%)	Inhibition by *Trichoderma harzianum* (native) over control (%)	Inhibition by *Trichoderma viride* (commercial) over control (%)	Inhibition by *Trichoderma harzianum* (commercial) over control (%)
24	12.1	7.1	10.5	6.5
48	28.1	22.5	22.2	18.9
72	39.7	21.4	33.8	27.2
96	47.5	28.3	36.9	30.5
120	61.6	44.2	39.4	34.5
144	66.6	56.0	43.2	38.0
168	70.3	64.4	48.0	39.3
192	71.8	65.4	49.2	42.3
216	74.5	67.6	50.8	44.9
240	76.8	70.0	52.8	46.7
LSD(P=0.05)	3.6	3.3	2.3	2.9
C.V. (%)	4.8	4.8	4.0	6.0

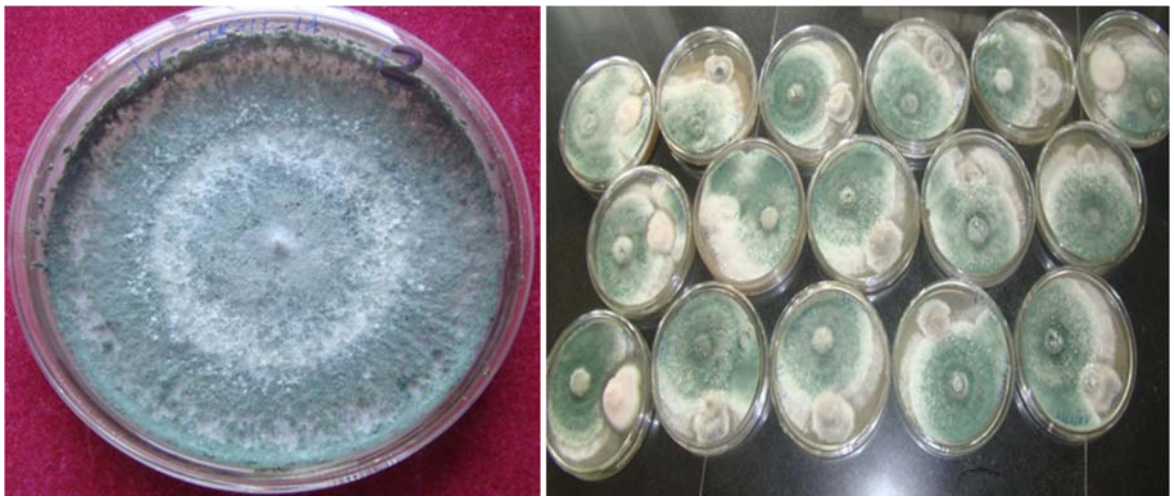

Fig A: Pure culture of *Trichoderma viride* **Fig B:** Dual culture plate *Trichoderma viride* with *Fusarium solani*

Plate 1: Screening of biocontrol agents against *Fusarium solani*

Conclusion

In present investigation, the highest inhibition of radial growth was recorded in *Trichoderma viride* (Native isolate). The native *Trichoderma harzianum* isolate was found less effective as compared to native *Trichoderma viride* in suppression of radial growth of *Fusarium solani* throughout the period of observations. The trend was same in case of commercial isolate of *Trichoderma viride* and *Trichoderma*

harzianum. Antagonistic effect of native and commercial on *Fusarium solani in vitro* was also studied by dual culture technique and found that the growth of the pathogen was much faster in the control plate than in dual culture. Thus, the native isolates of Trichoderma spp. were superior to commercial ones suppressing the pathogen growth.

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