Int.J.Curr.Microbiol.App.Sci (2017) *6*(12): 196-203

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 6 Number 12 (2017) pp. 196-203**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2017.612.025

Biocontrol Potential of Local *Trichoderma atroviride* Isolated from Bihar, India against Fungal Plant Pathogens *Aspergillus flavus* and *Fusarium solani*

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Keywords

Aspergillus flavus, Fusarium solani, Trichoderma atroviride.

Article Info

Accepted:
04 October 2017
Available Online:
10 December 2017

A B S T R A C T

In the present investigation, the *Trichoderma atroviride* spp. were isolated from the soil samples and potent antagonists of these isolates were selected for screening against the fungal phytopathogens *Aspergillus flavus* and *Fusarium solani* causing various diseases of crops. The soil samples were collected from various local rhizosphere of Bihar state and were inoculated on the Trichoderma Specific Media (TSM) and Potato Dextrose Agar (PDA) media using the serial dilution technique. After a series of inoculations, 18 different *Trichoderma* isolates were obtained in which the isolates of *Trichoderma atroviride* Ta_2, Ta_5 and Ta_7 having a higher radial growth at optimum temperature were selected, for screening its antagonistic potential. These isolates of *T. atroviride* were identified using authentic manual of fungi for morphological, microscopic and further identification was done based on molecular techniques using D1/D2 region of LSU (Large SubUnit:28S rDNA). These selected antagonists were checked for screening its antagonistic potential against the two fungal phytopathogens namely *Aspergillus flavus* and *Fusarium solani*. Both the pathogens were isolated from rotten papaya (*Carica papaya*) collected from local markets of Samastipur district of Bihar in India and were confirmed by microscopic identification. The screening was done using three antagonistic techniques namely Dual culture, Culture Filtrate Assay and Slide culture Assay. In the dual culture technique, the isolate Ta_5 showed maximum 85.4% and 73.1% growth inhibition against *A. flavus* and *F. solani* respectively. The cell free culture filtrate which was extracted from Ta_2, Ta_5 and Ta_7 isolates were screened at three different concentrations *i.e.*, 20%, 40% and 60%. The screening of culture filtrate against these fungal phytopathogens shows significant inhibition of radial growth especially at 40% and 60% concentration. Similarly, the Ta_2, Ta_5 and Ta_7 isolated were grown with both fungal pathogens on slide culture and the growth of mycelia was measured. The impact of slide culture method was marked as reduced mycelial growth of both fungal pathogens. These three techniques used for antagonistic effect of *Trichoderma atroviride* isolates against the selected fungal pathogens confirmed the antagonistic potential of the local *Trichoderma atroviride* isolates.

Introduction

Crops are the essential food components of a balanced diet for human beings. Apart from basic building blocks like amino acids, carbohydrates, fats etc., these provide a lot of enzymes, nutrients and antioxidants. However, many fungal phytopathogens all

over India are reported to cause diseases on the crops but *Aspergillus flavus* and *Fusarium solani* are much prominent fungal phytopathogens which reduce its yield up to a considerable extent. In recent times, biological control of diseases is given priority over chemical sprays which are already proved harmful in different ways. Now a day, efforts are being made to explore the biocontrol potential of local microbial inoculants like *Trichoderma* spp. as bio-control agents as effective and attractive alternatives to chemical fungicides.

Similarly, in the present investigation some local species of *Trichoderma atroviride* isolated from local soil samples of Bihar were exploited as antagonists against these two fungal phytopathogens. This work explores the antagonistic potential of local isolates of *Trichoderma atroviride* spp. for its use in the biological control of various diseases in Bihar, India.

Materials and Methods

Isolation of *Aspergillus flavus* and *Fusarium solani*

Samples of few infected papaya fruits (*Carica papaya*) collected from local markets of Samastipur district of Bihar were brought to the laboratory. After undergoing processes of sterilization and culturing of root samples, these two fungal pathogens were identified using authentic manual of fungi. These isolated pathogens were maintained on Potato Dextrose Agar (PDA) medium at 4^{o}C.

Isolation of *Trichoderma* isolates

Trichoderma spp. were isolated from the local soil samples of various native areas of Bihar, India using serial dilution technique on Trichoderma specific medium (TSH) (Askew and Laing, 1993). These soil samples were spread over Trichoderma Specific Media (TSM) and Potato Dextrose Agar media (PDA). Total 08 isolates of *Trichoderma* spp. were identified using authentic manual of fungi. *Trichoderma atroviride* was identified based on molecular techniques using D1/D2 region of LSU (Large SubUnit: 28S rDNA). The isolate Ta_2, Ta_5 and Ta_7 (*Trichoderma atroviride*) were selected as antagonists based on their relative mycelial growth at different temperatures. The isolates were further maintained on PDA medium.

In vitro screening for antagonistic activities of the Biocontrol agents

Inhibition of the fungal phytopathogens growth by the test antagonist Ta_2, Ta_5 and Ta_7 were carried out on PDA medium using dual culture technique. The experiment was laid down with four treatments and their three replicates. The treatments were made by three bio-agents versus the pathogen and fourth were the control. In control experiment, the test antagonist was replaced by sterile agar disc. Two different assessments were made following the dual culture techniques. The first was to obtain the percentage inhibition of radial growth (PIRG) and secondly the number of days taken for *Trichoderma atroviride* isolates to totally overgrow onto the *Aspergillus flavus and Fusarium solani.*

First assessment

Five millimeter diameter mycelia disc of each test antagonist were paired against *Fusarium solani* on PDA contained in 90 mm diameter Petri dishes following method of Evans *et al.*, (2003). Five millimeter plugs of each of the Bio-agents were placed at 20 mm away from the edge of Petri dish and after 24 h *Fusarium* spp was placed at 20 mm away from other edge of the same Petri dish. The set up was incubated at $28+2^{o}$C for ten days enquire. The growth of the pathogen in both the test and

control experiments were recorded. Data were obtained for the percentage inhibition of radial growth (PIRG) = $(R_1-R_2)/ R_1$ X 100. When R_1 =radial growth of pathogen in control, R_2= radial growth of pathogen in dual culture experiment with the antagonist.

Second assessment

The number of days taken by *Trichoderma atroviride* isolates to completely overlap the pathogen colony was recorded. The pathogen taken the least number of days was counted for signifying good antagonistic properties.

Culture filtrate assay

100 ml of Potato Dextrose Broth (PDB) were dispensed into 250 ml- Erlenmeyer flasks (ten) and inoculated with 5mm diameter disc from edge of 7 days old culture of the three test antagonists already maintained on PDA. Each flask was inoculated with three discs of each in triplicate and set up was inoculated at 28+2^{o}C for 15 days (Kept on mechanical shaker to maintain homogenous growth in liquid medium). After optimum incubation, the cultures were filtered through Whatman No. 1 filter paper and stored at 4^{o}C for further use. The sterilized filtrate was amended in PDA to make three concentrations (20% 40% and 60%) in petri plates. The solidified agar plates in triplicates were inoculated at the centre with 5 mm diameter mycelial disc of test pathogen and inoculated at optimum temperature for 7days. The plates devoid of culture filtrate served as control. The radial growth of *A. flavus* and *F. solani* was measured.

Results and Discussion

Identification of isolates of *Trichoderma atroviride* spp

Trichoderma atroviride isolates were initially observed as white colony on agar which then enlarged to 5-6 mm within 5-6 days. By this time, the white colony turned off white yellowish and then greenish on TSM (Fig. 1) which aided in their identification from other soil borne fungi. Two techniques i.e. visual observations on petridishes and micro-morphological studies in slide culture were adopted for identification of *Trichoderma atroviride* species. Shape, size, arrangement and development of conidiophores or phialides provided a tentative identification. Samples were compared to a taxonomic key by Rifai MA C 1969) (Table 1).

Antagonistic potential of *Trichoderma atroviride* isolates using dual culture method

PIRG and colony overgrowth assessment

The identified isolates of *Trichoderma* viz., *T atroviride* (*Ta*$_2$, *Ta*$_5$ and *Ta*$_7$) inhibited the mycelial growth of *A. flavus* and *F. solani* at different degree of inhibition (Table 2 and 3). PIRG by *Trichoderma atroviride* spp. ranged from minimum 71% to maximum 82%. Highest PIGR value recorded was that of *Ta*$_5$ (Table 2). *Ta*$_5$ overgrew the fungal pathogen colony fully at 7 days after incubation, whereas *Ta*$_2$ grew over after 10 days. *Ta*$_7$ took 14 days to overlap the pathogens. Thus *Trichoderma atroviride* according to both the assessments revealed the best antagonistic potential as compared to the other two isolates (Fig. 2).

The current investigation confirms the competency of *Trichoderma atroviride* for its availability in the soil rhizosphere and expresses the biocontrol ability on various fungal phytopathogens. This result matches with the research finding by McLean *et al.*, (2005). The isolates of *Trichoderma atroviride* showed excellent antagonistic activity against the fungal plant pathogens *Aspergillus flavus* and *Fusarium solani* causing rot diseases in various crops.

Table.1 Identification of *Trichoderma* species and its isolates from different soil samples

Sl. No.	*Trichoderma* species	*Trichoderma* isolates
1.	*Trichoderma atroviride*	Ta_2, Ta_5 and Ta_7
2.	Other species of *Trichoderma*	*T-1, T-3, T-4* and *T-6*

Table.2 Showing antagonistic potential of *T. atroviride* against *Aspergillus flavus* in dual culture

Sl. No.	Antagonist strain	Radial growth (cm)	% inhibition of radial growth (PIRG)	Time of overgrowth (days)
1.	Ta_2	1.75	80.5 %	8
2.	Ta_5	1.31	85.4%	7
3.	Ta_7	2.49	72.3%	10
4.	Control (Fungal pathogen alone)	9.00		

Table.3 Showing antagonistic potential of *T. atroviride* against *Fusarium solani* in dual culture

Sl. No.	Antagonist strain	Radial growth (cm)	% inhibition of radial growth (PIRG)	Time of overgrowth (days)
1.	Ta_2	2.58	71.3 %	9
2.	Ta_5	2.42	73.1%	7
3.	Ta_7	2.48	72.4%	10
4.	Control (Fungal pathogen alone)	9.00		

Table.4 Radial growth of *A. flavus* treated with different concentration of *T. atroviride* culture filtrate

Antagonistic microbial agents	Radial mycelium of *Aspergillus flavus* (in mm)			
	Concentration of culture filtrate (in %)			
	0 % (Control)	20%	40%	60%
Ta_2	90	78.4	48.6	29.9
Ta_5	90	74.5	46.2	26.5
Ta_7	90	79.2	56.7	38.5

Table.5 Radial growth of *F. solani* treated with different concentration of *T. atroviride* culture filtrate

Antagonistic microbial agents	Radial mycelium of *Fusarium solani* (in mm)			
	Concentration of culture filtrate (in %)			
	0 % (Control)	20%	40%	60%
Ta_2	90	80.2	55.7	33.8
Ta_5	90	76.5	51.1	31.0
Ta_7	90	81.6	57.4	38.3

Fig.1 Cultural characteristics of *Trichoderma atroviride* isolates (A) *Trichoderma atroviride* isolates in solid medium (B) *Trichoderma atroviride* isolates (C) *Trichoderma atroviride* isolates in liquid medium (D) Microscopic view of *Trichoderma atroviride* isolates

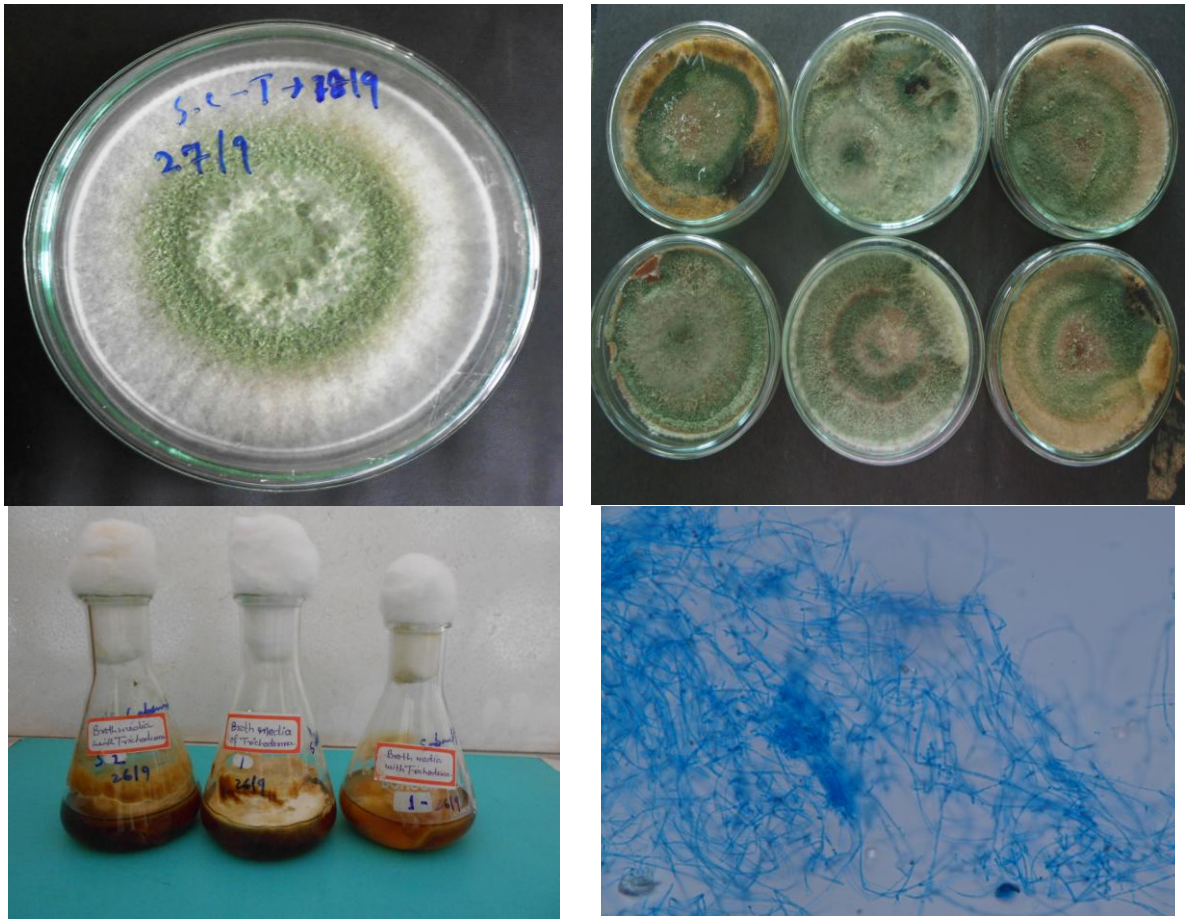


Fig.2 Showing the PIRG of the two phytopathogens due to the antagonists *T. atroviride*

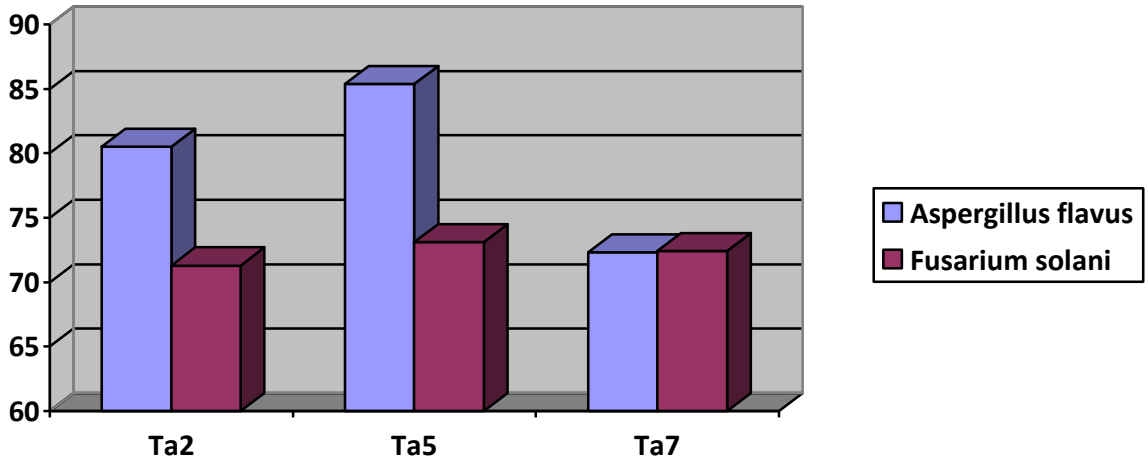
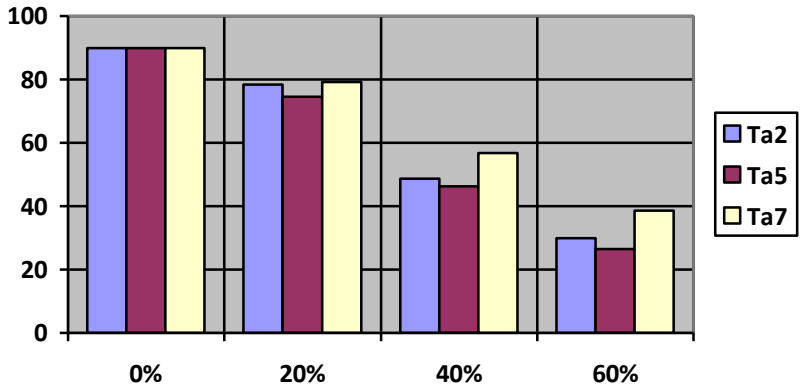
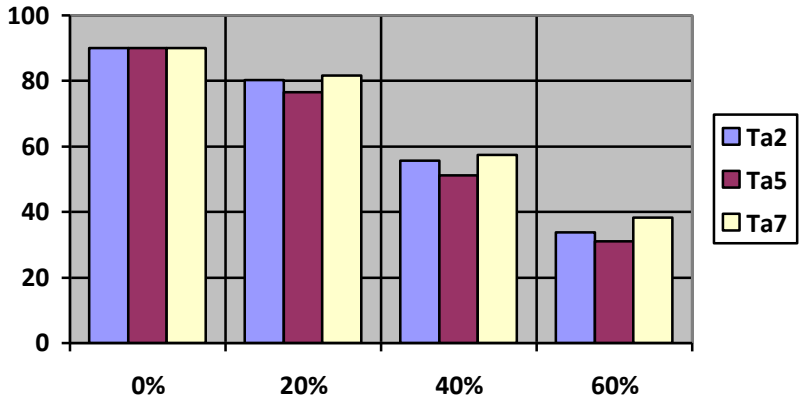


Fig.3 Inhibition of radial mycelia growth (mm) at different concentrations of culture filtrates of *T. atroviride*



Antagonistic activity of *Trichoderma atroviride* isolates against fungal pathogen *A. flavus*

Fig.4 Inhibition of radial mycelia growth (mm) at different concentrations of culture filtrates of *T. atroviride*



Antagonistic activity of *Trichoderma atroviride* isolates against fungal pathogen *A. flavus*

In the dual culture technique, a clear zone of inhibition was observed exhibiting antibiosis between the pathogen and the antagonist. It was observed that the isolate Ta_5 (*Trichoderma atroviride*) inhibited the mycelial growth of *A. flavus* by 85.4% as high degree of inhibition followed by Ta_2 exhibiting 80.5% inhibition then Ta_7 showing 72.3% whereas the isolate Ta_5 (*Trichoderma atroviride*) inhibited the mycelial growth of *F. solani* by 73.1% as high degree of inhibition followed by Ta_7 exhibiting 72.4% inhibition then Ta_2 showing 71.3%. The maximum Percentage Inhibition of Radial Growth (PIRG) by these *Trichoderma atroviride* isolates against the fungal pathogen *A. flavus* and *F. solani* is 85.4% and 73.1% respectively. The high value of PIRG due to the antagonist *T. atroviride* shows the high antagonistic effect of the antagonist. Next, the minimum time required to overgrow the fungal pathogen colony by the isolate Ta_5,

fully after incubation and to overlap the pathogen is 7 days. Thus *Trichoderma atroviride* according to both the assessments revealed the best antagonistic potential. Chet *et al.,* (1997) reported that *Trichoderma* species are common inhabitant of rhizosphere and contribute to control of many soil borne plant diseases caused by fungi. *Trichoderma* spp. were reported by several workers as the best antagonists for growth inhibition of several soil and seed borne plant pathogens (Dubey, 2002, 2003; Poddar *et al.,* 2004) (Figs. 3 and 4).

The results indicated that the cell free culture filtrate of *T. atroviride* reduced the radial growth of *A. flavus* and *F. solani* at different concentrations (Table 4 and 5). The maximum inhibition in mycelial growth of pathogen was observed at 60% concentration of Ta_5 isolate of *Trichoderma atroviride* cell free culture filtrate. This result shows the mechanism of antibiosis with the help of its culture filtrates. The biocontrol agents are found to be managing the disease effectively as well as they are ecologically sound proof (Cook, 1983 and Mukhopadhyay, 1996). It is now widely recognized that the use of ecofriendly bio-pesticide is a distinct possibility for the future and can be successfully exploited in modern agriculture especially within the framework of integrated pest management system without affecting our precious ecosystem (Mishra *et al.*, 2000; Mishra *et al.*, 1998 and Mukhopadhyay, 1994). Hence, the results obtained from this *In Vitro* study, showed that the native isolates of *T. atroviride* can be used effectively against the soil borne prominent phytopathogens *A. flavus* and *F. solani*.

Today, the use of 'biological control' is an alternative and promising tool to maintain the quality and yield of the various crops. The biological control of the diseases caused by *Aspergillus flavus* and *Fusarium solani* will provide a promising tool to minimize the post-harvest losses of crops including fruits and vegetables while reducing the release of polluting chemical pesticides to the environment. The antagonistic interactions of *Trichoderma atroviride* isolates showed excellent activity against the fungal pathogens *Aspergillus flavus* and *Fusarium solani* causing various diseases of crops. Thus, the isolates of *Trichoderma atroviride* could be further exploited for commercial scale up under localized climatic conditions for control of various modes of infestations in crops.

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How to cite this article:

Md. Irshad Hussain, Altamash Ali Daudi and Choudhary Sharfuddin. 2017. Biocontrol Potential of Local *Trichoderma atroviride* Isolated from Bihar, India against Fungal Plant Pathogens *Aspergillus flavus* and *Fusarium solani*. *Int.J.Curr.Microbiol.App.Sci.* 6(12): 196-203. doi: https://doi.org/10.20546/ijcmas.2017.612.025