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In vitro antagonistic activity of *Trichoderma* species against *Fusarium oxysporum* f. sp. *melongenae*

Ramaraju Cherkupally*[1], Hindumathi Amballa[1] and Bhumi Narasimha Reddy[2]

**Department of Botany, K.R.R. Government Arts and Science College, Kodad, Telangana State, India.*
[1&2] Department of Botany, Osmania University, Hyderabad. 500 007. Telangana State, India.
**Corresponding author E mail: praveenaramaraju@gmail.com*

Abstract

A study was undertaken to evaluate the antagonistic activity of seven *Trichoderma* species, against brinjal vascular wilt causing pathogen, *Fusarium oxysporum* f. sp. *melongenae* (Fom) under *in vitro* conditions. The antagonistic activities of seven *Trichoderma* species were screened *in vitro* against Fom by dual culture plate technique and production of volatile and non-volatile metabolites. All the biocontrol agents showed considerable reduction in the growth of the pathogen. Out of the seven fungal antagonists studied for their efficacy, *T. harzianum* showed maximum extent of inhibition 81.11%, followed by *T. koningii* 80.00%, *T. pseudokoningii and T. viride* 78.88% each, *T. virens*, *T. atroviride*, and *T. reesei* 77.77% each by nonvolatile compounds. The results of the present study suggest that *T. harzianum* has a highly antagonistic potential against the Fom by production of both volatile and nonvolatile compounds. *T. koningii* showed least antagonistic efficacy of 28.88% by the production of volatile compounds.

Keywords: antagonism, biocontrol, brinjal, inhibition, rhizosphere, *Trichoderma*.

INTRODUCTION

Plant diseases caused by a variety of fungi may cause significant losses on agricultural crops. All plants are attacked by some kinds of fungi, and each of parasitic fungi can attack one or many kinds of plants. More than 10,000 spp. of fungi

cause disease in plants [1]. Brinjal (*Solanum melongina* L.) is grown as a vegetable crop in India and the plant is affected by various fungal diseases which in turn produces low crop yield. Pathogens being soil borne, causes a huge problem in controlling the disease. Soil borne diseases are difficult to control and seed treatment with fungicides has low impact. The use of chemical pesticides has been known to cause various environmental and health problems. Intensive use of fungicides for the control of diseases has resulted in the accumulation of toxins to human beings as well as to the environment. Restrictions on the use of chemical pesticides have been increasing. Knowing the ill effects of these chemical residues found in eatables, plant growers are being challenged to maintain the plant health with reduced input from agricultural chemicals.

Microorganisms that can grow in the rhizosphere are ideal for use as biocontrol agents, since the rhizosphere provides the front line defence for root against attack by pathogens. *Trichoderma*, a filamentous soil borne mycoparasitic fungus, has been shown to be effective against many soil borne plant pathogens as they have more than one mechanism of action [2]. Characterisation for the antagonistic potential of *Trichoderma* species is the first step in utilising the full potential of *Trichoderma* spp. for specific applications [3]. *Trichoderma* spp. has the potential to control *Macrophomina phaseolina in vitro* to the extent of 77.77% [4]. Therefore, the present study was conducted to evaluate the antagonistic potential of different *Trichoderma* spp. in inhibiting the growth of *Fusarium oxysporum* f. sp. *melongenae*. To determine the antagonistic property of *Trichoderma* spp. against *Fusarium oxysporum* f. sp. *melongenae* (Fom), isolates were compared on a medium and at temperature where both antagonist and Fom can grow well in the laboratory. The present study was undertaken, to find out the biocontrol efficacy of *Trichoderma* spp. against Fom.

MATERIALS AND METHODS

Isolation of pathogenic fungi

Evaluation of infected parts of the brinjal plant resulted in isolation and identification of *Fusarium oxysporum* f sp. *melogenae* (Fom) based on the examination under microscope. Parts of plants with symptoms of Fusarium wilt infection were surface sterilised by immersion in 0.3% sodium hypochlorite for 10 minutes, and then in 70% ethanol and later rinsed thoroughly with sterile distilled water. They were transferred to potato dextrose agar (PDA) medium in petri plates and incubated at 26 ± 2^{o}C for seven days [5]. The characteristic growth of the fungus with morphological characters of micro conidia and macro conidia and chlamydospores were observed [1]. Pure cultures were maintained on PDA slants and stored at 4^{o}C in the refrigerator.

Isolation of antagonist

The rhizosphere soil samples were collected from the brinjal field. Seven *Trichoderma* species viz., *Trichoderma viride, Trichoderma harzianum, T. virens, T. atroviride, T. koningii, T. pseudokoningii, T. reesei* were isolated by soil dilution

technique [6] on Trichoderma specific medium. The green coloured colonies were identified by comparing with taxonomic key [7]. They were purified by single spore isolation method and maintained on potato dextrose agar (PDA) slants. The cultures were stored in the refrigerator at 4^{o}C.

Antagonism activity of *Trichoderma* against Fom

The antagonistic activity of seven *Trichoderma* spp. was screened *in vitro* against Fom by dual culture plate technique [8]. The antagonistic efficacy against test pathogen was evaluated on PDA medium. Both pathogen and antagonists were grown on sterilized PDA plates separately for 5 days. For testing antagonism in dual culture method a mycelial disk of 5 mm in diameter of antagonist was excised from the edge of an actively growing 5 day old culture plate and inoculated opposite to the pathogenic fungi in the same plate 1cm away from the edge inoculated similarly. For each treatment three replicates were maintained and incubated at 26 ± 2^{o}C. The test pathogen was inoculated in the middle of the plate in triplicates. These paired cultures of antagonist and test pathogen were placed equidistant from the periphery so that they would get equal opportunity for their growth (**Plate 1**).

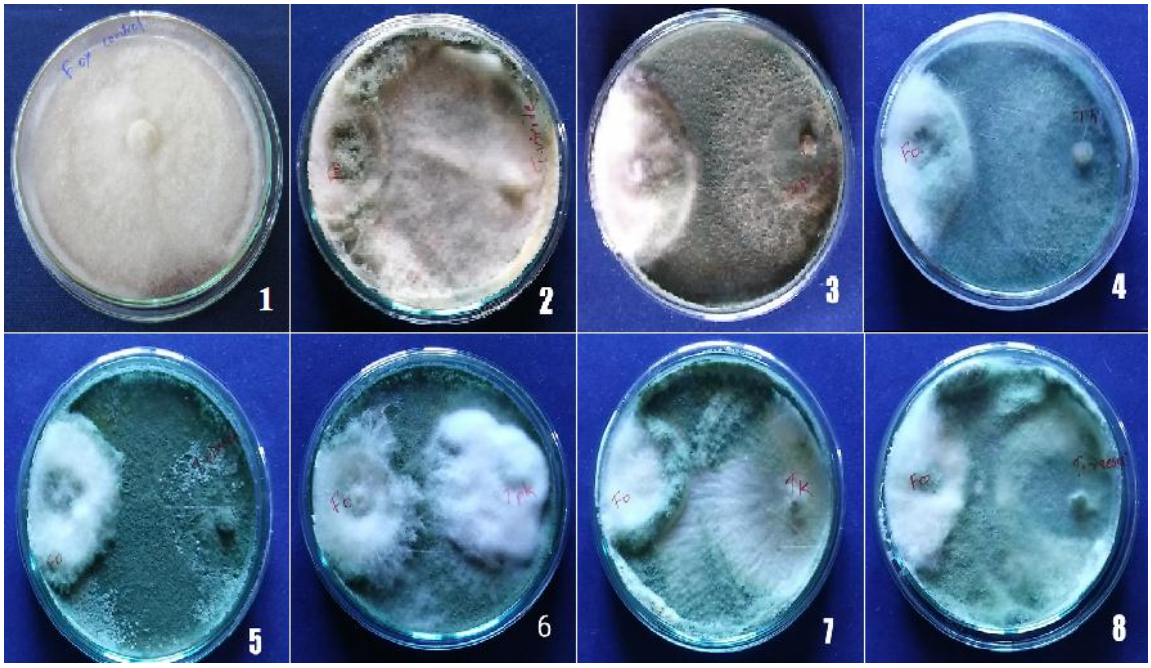

Plate 1. Antagonistic efficacies of *Trichoderma* spp. against brinjal wilt pathogen *Fusarium oxysporum* f. sp. *melongenae* (Fom).

Fig. 1: Fom (control); Fig. 2: Fom/ *T. viride*; Fig. 3: Fom/ *T. atroviride*;

Fig. 4: Fom/ *T.harzianum*; Fig. 5: Fom/ *T. virens*; Fig. 6: Fom/ *T. pseudokoningii*;

Fig. 7: Fom/ *T. koningii*; Fig. 8: Fom/ *T. reesei*

After the incubation period, the radial growth of Fom in control, as well as in treatment plate was measured and the per cent inhibition was calculated using the formula [9]:

$$L = \frac{(C - T)}{C} X\ 100$$

Where,

L = Per cent inhibition of radial growth of pathogen (%)

C = Radial growth of the pathogen (mm) in control

T = Radial growth of the pathogen (mm) in treatment

In dual cultures, *Trichoderma* spp. efficacy was categorized based on their ability to over grow and inhibit the growth of the pathogen by giving them a score as per modified Bell's scale [10]. Where R1 = 100% over growth, R2 = 75% over growth, R3 = 50% over growth, R4 = locked at the point of contact. The mycelial mats from zone of interaction in dual culture plate between pathogen and antagonist were placed on glass slide. The glass slides were stained with lacto phenol cotton blue (HiMedia) to improve the visibility of the hyphae and then observed under a light microscope. The hyphal interaction between the mycelia of the opposite colonies was studied.

Efficacy of volatile antibiotics

Production of volatile antibiotics was studied by sealing agar plate method [11]. Seven spp. of *Trichoderma* were inoculated in the centre of the petri plate having solidified sterilised PDA medium by placing 5 mm disk (5 day old culture) cut from the margin of the actively growing region of *Trichoderma* spp. and incubated for 2 days at 26 ± 2ºC. After that the top lid of each petri plate was replaced with bottom part of another petri plate with same size containing PDA medium duly inoculated with a 5 mm mycelia disks of the test pathogen after 2 days of incubation. The pairs of each plate were sealed with parafilm and incubated at 26 ± 2ºC. The PDA medium without *Trichoderma* isolate in the bottom part of the petri plate with respective test pathogen on the upper lid of plate served as control. Three replicates were maintained for each treatment. This assemble was opened after 7 days and the observations were recorded by measuring colony diameter of the test pathogen in mm in each plate and that of control plates (**Plate -2**).

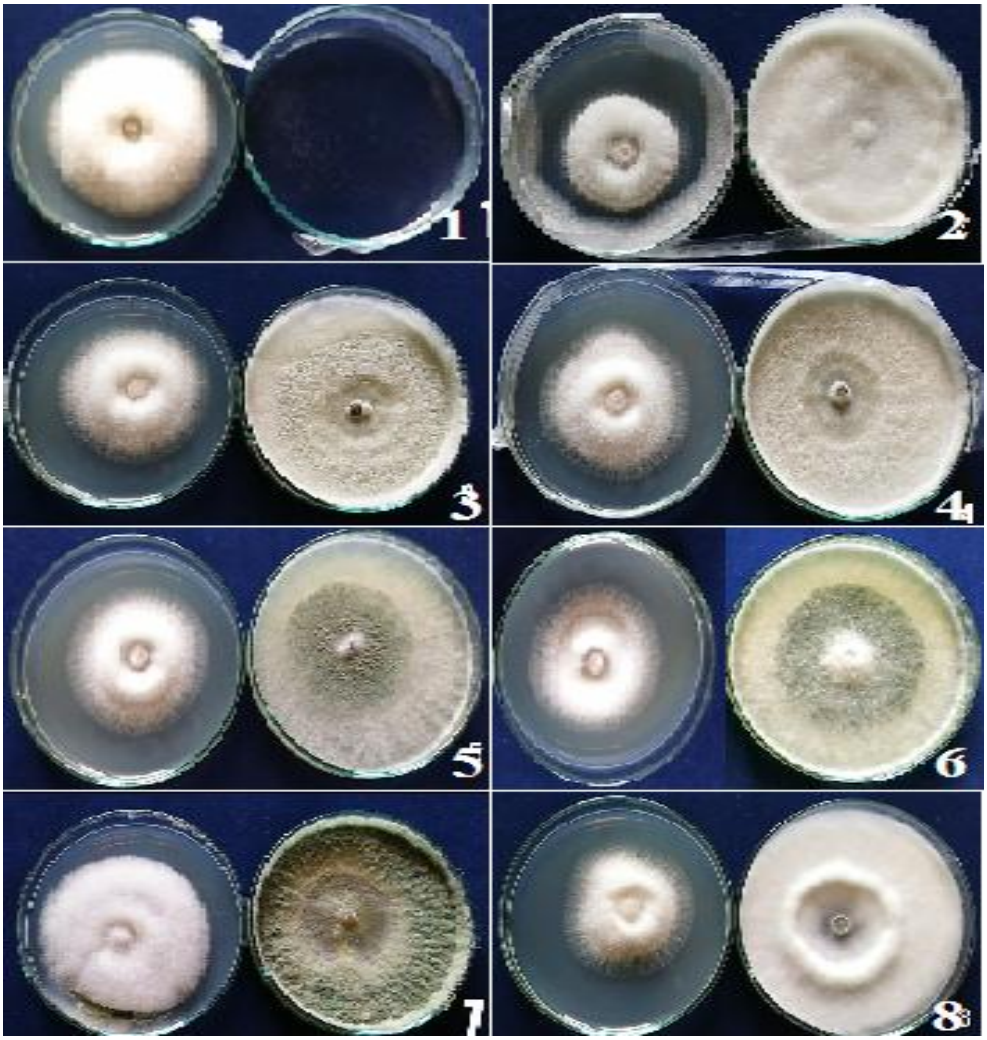

Plate 2. Efficacies of volatile antibiotics of *Trichoderma* spp. against brinjal wilt pathogen *Fusarium oxysporum* f. sp.*melongenae (*Fom).

Fig. 1: Fom (control); Fig. 2: Fom/ *T. viride*; Fig. 3: Fom/ *T. atroviride*;

Fig. 4: Fom/ *T.harzianum*; Fig. 5: Fom/ *T. virens*; Fig. 6: Fom/ *T. pseudokoningii*;

Fig. 7: Fom/ *T. koningii*; Fig. 8: Fom/ *T. reesei*

RESULTS AND DISCUSSION

Isolates of *Trichoderma* spp. were evaluated for their antifungal activity against *F. oxysporum* f. sp. *melongenae.* Of these antagonistic studies *Trichoderma* spp. showed significant reduction in terms of radial diameter after the treatment, in comparison with the control. Out of the seven fungal antagonists studied for their efficacy, *T. harzianum* showed maximum extent of inhibition 81.11%, followed by *T. koningii*

80.00%, *T. pseudokoningii and T. viride* 78.88% each, *T. virens*, *T. atroviride* and *T. reesei* 77.77% each (**Table 1**).

Table 1: Effect of non-volatile and volatile compounds of *Trichoderma* against *Fusarium oxysporum* f. sp. *melongenae* (Fom).

Trichoderma spp.	**Non volatile compounds**		**Volatile compounds**	
	Radial growth(mm)	Inhibition (%)	Radial growth(mm)	Inhibition (%)
T. virens	20	77.77	51	43.33
T. pseudokoningii	19	78.88	50	44.44
T. harzianum	17	81.11	41	54.44
T. atroviride	20	77.77	47	47.77
T. reesei	20	77.77	44	51.11
T. koningii	18	80.00	64	28.88
T. viride	19	78.88	46	48.88
Fom Control	90		90	

All the *Trichoderma* spp. tested for their efficacy against Fom come into contact with the pathogen in 2 days that infers the biocontrol agent is growing rapidly in dual cultures and occupies the space. *T. virens, T. pseudokoningii and T. reesei* were locked at the point of contact with Fom and were rated as R4 according to Bell's ranking (**Table 2**).

Table 2: *In vitro* antagonism of biocontrol agents against *Fusarium oxysporum* f. sp. *melongenae* (Fom).

Trichoderma spp.	**Time taken to contact (days)**	**Time taken to overlap (days)**	**Bell's Ranking**
T. virens	2	Lkd	R4
T. pseudokoningii	2	Lkd	R4
T. harzianum	2	7	R3
T. atroviride	2	7	R2
T. reesei	2	Lkd	R4
T. koningii	2	7	R3
T. viride	2	5	R2

NC- no contact, Lkd- locked, R1- complete over growth, R2- 75 % over growth, R3- 50% over growth, R4- locked at the point of contact

The clear 2 mm zone of inhibition was observed in between antagonist and pathogen locked in plates indicates that *Trichoderma* spp. restrict further growth of Fom. *T. harzianum, T. atroviride and T. koningii*, overgrown partially over the Fom in 7 days and were rated as R3, however *T. viride* has taken 5 days to overgrow 50% and rated as R2 and showed least (28.88%) inhibition by volatile compounds. The fast growing antagonists caused more growth inhibition of the pathogens may be due to mycoparasitism and competition for space and nutrients. *Fom* was comparatively less inhibited by all *Trichoderma* species by the production of volatile compounds [3]. Observation of mycelial mats from zone of interaction in dual culture plate between pathogen and antagonist under microscope showed that *Trichoderma* spp. was interacting with Fom hyphae. Antagonist hyphae were observed to be growing towards Fom hyphae and coiled around the hyphae. The biocontrol agent was observed to produce knob like structure called as haustoria. These haustorial knob like structures with penetration pegs, penetrate the host and finally dissolve the protoplasm and shrink the hyphae which may lead to lysis [12]. Mycoparasitism as a principle mechanism of biological control is favoured by many scientists [13, 14]. Mycoparasitism includes hyphal interaction and parasitism, and is the most vital mechanism of the fungal antagonist to give protection to the plants against the pathogen attack. The variation in hyper parasitic potential of different isolates of *Trichoderma* against soil borne fungal pathogens has been reported [3, 15, 16,] and the species of *Trichoderma* were effectively selective against pathogenic fungi [3, 15]. *Trichoderma* spp. was capable of producing extra cellular lytic enzymes that are responsible for their antagonistic activity [15]. Harman et al., [17] had suggested that mycoparasitism was the principle mechanism involved in controlling Pythium damping-off of pea seed. *Trichoderma* species proved to be superior on account of their faster growth attained against Fom. This phenomenon may probably be correlated with the differences in levels of hydrolytic enzymes produced by each species or isolates when they attach the mycelium of the pathogens. Antagonism by *Trichoderma* spp. against a range of soil borne plant pathogens has been reported earlier [2, 15]. Observations on the growth and colonization of the test pathogens in dual culture screening by the antagonistic isolates proved that different species of *Trichoderm*a have variation in their ability to inhibit the growth of the pathogen Fom.

CONCLUSION

Plant diseases caused by pathogenic fungi constrain the yields. In agriculture, farmers still depend on the use of chemical fungicides to control plant diseases. However, misuse of these synthetic chemicals cause hazardous to both environment and health. The alternative method for replacement of chemical fungicides has led to the use of biological control agents. Biocontrol of soil borne pathogens is met by the introduction of microorganisms. Microorganisms that grow in the rhizosphere are ideal for use as biocontrol agents. Our studies proved that *Trichoderma* spp. have the potential to control *Fusarium oxysporum* f. sp. *melongenae in vitro* to the extent of 81.11% by non-volatile compounds and 54.44 % by volatile compounds. The

potential use of these biocontrol agents can be improved by isolation, formulation and application methods, particularly in the field.

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