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Isolation and Identification of Antagonistic Fungi from Phylloplane and Rhizosphere as Biocontrol Agents for Chilli Twig Blight Disease

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ABSTRACT

The present study was conducted to know the antagonistic activity of the fungal isolates which were isolated from phylloplane and rhizosphere mycoflora of chilli by serial dilution method. The fungal antagonist obtained from the phylloplane were identified as Aspergillus niger, Aspergillus flavus, Penicillium spp., Paecilomyces spp., Fusarium spp., Alternaria spp., Mucor spp and Rhizopus spp where as from the rhizosphere four Trichoderma isolates was evaluated against Choanephora cucurbitarum under in vitro conditions. The results revealed that among the fungal isolates from phylloplane, Fusarium spp showed maximum inhibition (57.77%) and minimum inhibition was obtained in case of Alternaria spp (47.00%). Similarly from rhizosphere Trichoderma viride isolate 1, Trichoderma harzianum isolate 2, Trichoderma viride isolate 2 and Trichoderma harzianum isolate 1(62.44, 55.33, 62.22 and 40.11 per cent, respectively) recorded significantly highest per cent inhibition of mycelial growth of C. cucurbitarum.

Key words: *chilli, phylloplane, rhizosphere, mycoflora, Choanephora cucurbitarum, Fusarium spp, Trichoderma viride, Trichoderma harzianum*

INTRODUCTION

Chilli is an annual herbaceous spice/vegetable/cash crop grown in both tropical and sub-tropical regions and belongs to family Solanaceae. India is the leading country in the production of chillies contributing 41.11% of the world's production. India is well known as the land of spices the world over. Chilli is one of the most important spices cultivated all over the world except in colder parts. India stands first in production and consumption of chilli globally. The crop is gaining popularity among farmers as a cash crop. Export of chillies during 1999-2000 was 64776 metric tonnes valued at Rupees 250.66 crores. However, chilli is the second commodity in our export basket earning nearly 13% of foreign exchange from spices[8]. Chilli suffers from many diseases caused by fungi, bacteria, viruses, nematodes and also abiotic stresses. Among the fungal diseases *Choanephora*, anthracnose or fruit rot, *Alternaria*, powdery mildew and leaf spots are the most prevalent ones.

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During a survey 2014-2015, Fresh leaves from healthy and diseased plants were collected randomly in a polythene bag and brought to the laboratory and isolated different antagonistic pathogen from phylloplane and rhizosphere.

MATERIAL AND METHODS

The present investigation was carried out in the Department of Plant Pathology, College of Agriculture, Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad, Ranga Reddy District, Telangana.

Isolation of phylloplane mycoflora

During survey, Fresh leaves from healthy and diseased plants were collected randomly in a polythene bag and brought to the laboratory. The mycoflora associated with chilli leaves was isolated following modified leaf washing technique[1].

Five discs were cut from each leaf with the help of 5 mm sterile cork borer. As such 50 leaf discs were cut and transferred to a conical flask containing 100 ml sterile water blank. The leaf discs were thoroughly agitated for 20 minutes using a Neolab Vortex mixer. Serial dilutions were prepared in the standard way and the dilutions (10^{-3} and 10^{-4}) were plated in Petri dishes containing potato dextrose agar and the inoculated Petri dishes were incubated in a BOD incubator at 25 $\pm$ 2^{o}C. Four replicates were maintained for all the dilutions tested. The Petri plates were observed daily and the fungal colonies obtained were transferred to PDA slants for further use.

Isolation of rhizosphere mycoflora

Diseased and healthy plant samples with rhizosphere soil of chilli plants were collected in polythene bags from each survey plots. *Trichoderma* was isolated from the rhizosphere soil, using Serial dilution plate method. The collected soil was dried under shade and ground to powder with a mortar and pestle and passed through 2mm mesh sieve.

One gram of soil from each sample is taken in a 250 ml conical flask with 99 ml of sterile distilled water and agitated for 5 minutes to prepare 10^{-1}dilution. This suspension was used for serial dilutions up to 10^{-4}. One ml of the suspension from $10^{-2.}$, 10^{-3} and 10^{-4} were plated separately on sterilized Petri plates containing sterilized media and incubated at room temperature (25^{o}C). Three plates were inoculated for each dilution from a particular sample. The suspension was then distributed uniformly on medium surface by horizontal shaking and was incubated for seven days. Isolated fungal antagonists from rhizosphere soil were transferred to culture tubes containing appropriate media for further examination. The green colonies of the antagonists usually appeared at 4th or 5th day of incubation. Each colony was studied carefully under microscope, using 0.1 % lactophenol- cotton blue stain (0.1g cotton blue mixed in 100ml of standard lactophenol solution) and the isolated fungi were compared up to the level of genus or species by illustrations given by Raper and Fennel[4], Raper and Thom[5], Subramanian[7] and Rifai[6]. The fungi isolated from leaves were further purified by single spore isolation. Pure cultures were maintained in culture tubes containing the PDA for further examination and identification. Four isolates of *Trichoderma* spp. were identified and selected for the study (Plate 1).

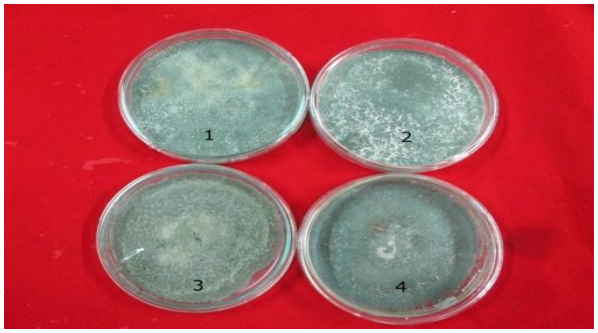

Plate 1: Pure culture of different *Trichoderma* spp.

1	Trichoderma viride isolate 1
2	Trichoderma viride isolate 2
3	Trichoderma harzianum isolate 1
4	Trichoderma harzianum isolate 2

Evaluation of phylloplane and rhizosphere mycoflora against *Choanephora cucurbitarum* under laboratory conditions

The fungi isolated from phylloplane and rhizosphere of chilli were screened for antagonism against *Choanephora cucurbitarum* under *in vitro* conditions on potato dextrose agar medium by following dual culture technique[2, 3].

Fifteen millimeter of sterilized luke warm potato dextrose agar medium was aseptically poured into 90 mm diameter sterilized Petri plates. Five mm discs of various fungal cultures and the test pathogen was cut with a sterilized cork borer from the edge of three day old cultures and was placed on the solidified medium opposite to each other. Three replicates were maintained for each of the treatment. Suitable control was maintained by placing only the pathogen on culture medium. The plates were incubated at 23 $\pm$ 1°C. Petri plates were observed daily for recording antagonistic interactions between the pathogen and biocontrol agent. The Per cent growth reduction (I) of the test pathogen was calculated when the growth of the pathogen was full in the control plates by using the formula as given below.

$$I = \frac{C - T}{C} \times 100$$

Where,

I = Per cent inhibition of mycelial growth

C = Radial growth of pathogen in control (mm)

T = Radial growth of pathogen in treatment (mm)

RESULTS AND DISCUSSION

Identification of fungal biocontrol agents from phylloplane

The antagonistic activity of isolated microorganisms from phylloplane of chilli was tested against *C. cucurbitarum* by dual culture technique. About seven fungi were isolated from the phylloplane. The data on the inhibition of growth of *C. cucurbitarum* by fungal biocontrol agents isolates are presented in Table 1 and Fig 1.

Table 1: Evaluation of phylloplane in inhibiting the radial growth of *Choanephora cucurbitarum in vitro*

Sl. No.	Treatment	Radial growth of *C. cucurbitarum* (mm)	Per cent inhibition of mycelia growth over control (%)
1	*Paecilomyces* sp	43.0	52.22
2	*Aspergillus niger*	39.5	56.11
3	*Penicillium* sp	43.3	51.88
4	*Aspergillus flavus*	39.6	56.00
5	*Fusarium* sp	38.0	57.77
6	*Alternaria* sp	47.7	47.00
7	*Mucor* sp	42.0	53.33
8	*Rhizopus* sp	41.7	53.66
9	Control	90.0	0.00
CD (P=0.05)		1.41	
SE (m)		0.57	

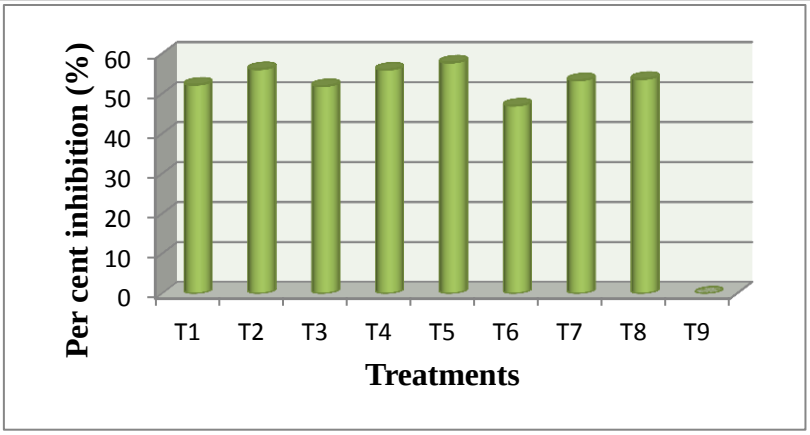

Fig. 1: *In vitro* evaluation of phylloplane in inhibiting the radial growth of *Choanephora cucurbitarum*

T_1 - *Paecilomyces* sp
T_2 - *Aspergillus niger*
T_3 - *Penicillium* sp
T_4 - *Aspergillus flavus*
T_5 - *Fusarium* sp
T_6 - *Alternaria* sp
T_7 - *Mucor* sp
T_8 - *Rhizopus* sp
T_9 - Control

The data revealed that all the fungal isolates inhibited radial growth of *C. cucurbitarum*, but varied in their efficacy. The fungal isolates isolated from phylloplane of chilli leaves significantly inhibited radial growth of *C. cucurbitarum* vary from 57.77% (*Fusarium* sp) to 47.00% (*Alternaria* sp) (Plate 2a and Plate 2b). The fungal isolates of *Aspergillus* genera such as *Aspergillus niger* and *Aspergillus flavus* showed more than 56.00% of inhibition where as *Mucor*, *Rhizopus* which belongs to same taxonomic position of *Choanephora* showed nearly similar per cent inhibition (53.3, 53.66) respectively. *Penicillium* sp and *Paecilomyces* sp recorded similar per cent of inhibition (51.88, 52.2).

Plate 2a: Photomicrograph of *Fusarium* spp. isolated from chilli leaves

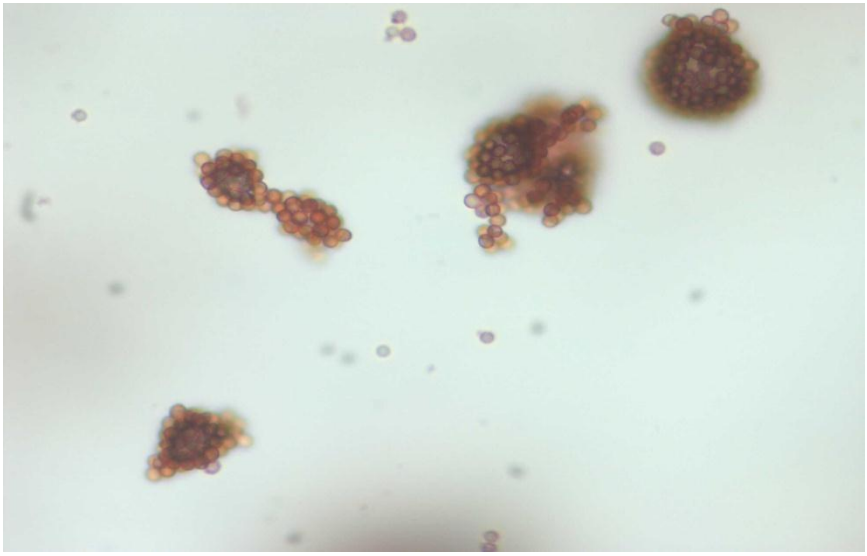

Plate 2b: Photomicrograph of *Aspergillus niger* isolated from chilli leaves

Identification of fungal bio control agents from rhizosphere

Antagonistic potential of *Trichoderma* spp. were measured through dual culture technique against the test pathogen *Choanephora cucurbitarum* by dual culture technique. Based on the key characteristics provided by Rifai[6] the morphological characters were studied and the isolates were identified (Table 2 and Plate 3).

Table 2: Characteristic feature of the *Trichoderma* spp.

S.No.	*Trichoderma* isolate	Characteristics of the isolate
1	*Trichoderma viride* isolate- 1	Plain green colony with distinct regular growth and profuse sporulation
2	*Trichoderma viride* isolate- 2	Slightly fluffy growth, light green colony with profuse sporulation.
3	*Trichoderma harzianum* isolate-1	Fluffy growth, colour greenish yellow, later turns yellowish green.
4	*Trichoderma harzianum* isolate-2	Fluffy with sparsely rhythmic growth, yellow colony, profuse sporulation.

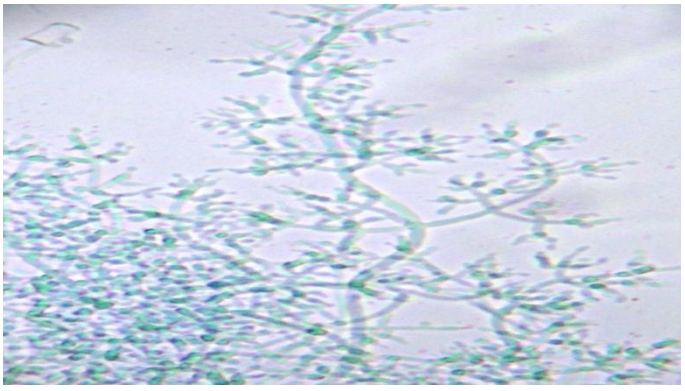

Plate 3a: Photomicrograph showing *Trichoderma viride* isolate-1

Plate 3b: Photomicrograph showing *Trichoderma harzianum* isolate-2

Four different isolates of *Trichoderma* spp were used to test antagonistic performance in dual culture with a test pathogen *C. cucurbitarum*. The data on the inhibition of growth of *C. cucurbitarum* by *Trichoderma* isolates are presented in Table 3. The data on dual culture test by *Trichoderma* spp., revealed that all the *Trichoderma* isolates inhibited radial growth of *Choanephora cucurbitarum*, but there was a variation in their inhibition. All the isolates exhibited more than 40.11% inhibition of mycelial radial growth of *Choanepora cucurbitarum* (Fig 2). However, *Trichoderma viride* isolate-1 (62.44%) showed maximum per cent inhibition of test pathogen followed by *Trichoderma viride* isolate-2 (62.22%), *Trichoderma harzianum* isolate-2 (55.33%) and *Trichoderma harzianum* isolate-1 (40.11%).

Table 3: Effect of bioagents on radial growth of *Choanephora cucurbitarum in vitro*

Sl. No.	Treatment	Radial growth (mm)	Per cent inhibition of mycelial growth over control
1	*Trichoderma viride* isolate 1	33.8	62.44
2	*Trichoderma viride* isolate 2	34.0	62.22
3	*Trichoderma harzianum* isolate 1	53.9	40.11
4	*Trichoderma harzianum* isolate 2	40.2	55.33
5	Control	90.0	0.00
CD (P=0.05)		1.47	
SE (m)		0.47	

DAI: Days after inoculation

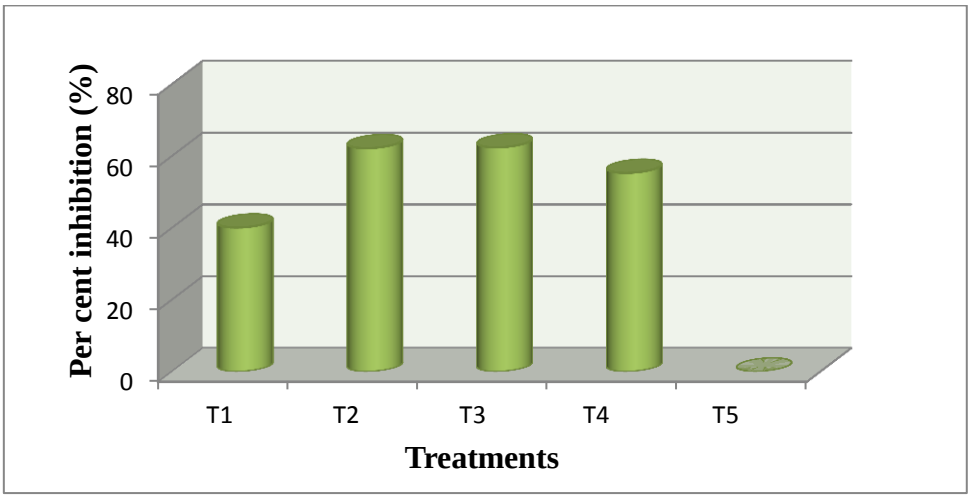

Fig. 2: Effect of bioagents on radial growth of *Choanephora cucurbitarum in vitro*

T_1 - *Trichoderma viride* isolate 1
T_2 - *Trichoderma viride* isolate 2
T_3 - *Trichoderma harzianum* isolate 1
T_4 - *Trichoderma harzianum* isolate 2
T_5 - Control

CONCLUSION

The phylloplane and rhizosphere mycoflora of chilli were isolated by serial dilution method and identified as *Aspergillus niger, Aspergillus flavus, Penicillium* spp., *Paecilomyces* spp., *Fusarium* spp., *Alternaria* spp., *Mucor* spp and *Rhizopus* spp. Among the fungal isolates, *Fusarium* spp recorded significantly highest inhibition (57.77%) of test fungus compared to all other treatments followed by *Penicillium* spp with 51.88 per cent inhibition. *Alternaria* spp recorded least (47.00%) inhibition of mycelial growth of *Choanephora cucurbitarum* compared to all other treatments. Among all *Trichoderma* isolates, *Trichoderma viride* isolate 1, *Trichoderma harzianum* isolate 2, *Trichoderma viride* isolate 2 and *Trichoderma harzianum* isolate 1(62.44, 55.33, 62.22 and 40.11 per cent, respectively) recorded significantly highest per cent inhibition of mycelial growth of *C. cucurbitarum*.

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