Mycopath (2014) 12(1): 1-8 **Research Article**

Incidence of Fusarium wilt of chilli (*Capsicum annum* L.) in Kashmir valley and its management by *Trichoderma* spp.

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Abstract

An extensive field survey was conducted in five major vegetable growing areas of district Anantnag and Kulgam of Kashmir valley (temperate region of India) during April to May 2013. The Fusarium wilt disease was first noticed in nursery during transplantation and was found maximum during flowering/fruiting stage. Redwani Payeen village of district Kulgam showed maximum disease incidence of 6% and 40% during transplanting and flowering/fruiting stage, respectively. Bangidar village of Anantnag district showed 4% and 24% disease incidence during transplanting and flowering/fruiting stages, respectively. During the survey, eleven isolates of *Fusarium solani* viz., C1-C11 and ten of *Trichoderma harzianum* viz. Tr 1, Tr 2, Tr 3, Tr 4, Tr 6, Tr 8 Tr 10, Tr 11, Tr 12 and Tr 16 were isolated from infected roots, shoots and rhizopheric soils of healthy plants. All the isolates of *Trichoderma* species significantly inhibited the growth of *F. solani*, but isolate Tr16 exhibited the maximum inhibition of 77% followed by Tr11 (72%) and Tr08 (68%) in dual culture technique, whereas Tr 8 showed maximum inhibition of 89% followed by Tr 2 (78%) and Tr 3 (56%), respectively, in volatile tests.

Keywords: Biological management, chilli wilt, disease incidence, *Trichoderma* spp.

Introduction

Chilli (*Capsicum annum* L.) is considered as one of the most important vegetable and spice crop belongs to the genus *Capsicum*, popularly known as "red pepper while belongs to nightshade family Solanaceae" (Sahi and Khalid, 2007). *Fusarium* wilt is the most important soil borne disease caused by *Fusarium* spp. in chilli plant. *Fusarium solani* causing wilt in chilli was reported to cause great losses in pepper production in different countries in the world (Madhavi *et al.*, 2006; Rani *et al.*, 2009). Although chemicals viz., Ridomil Gold, Carbandazim, Metalaxyl and Mancozeb are being used to control the *Fusarium* wilt (Sitara and Hasan, 2011). Since chemicals are hazardous and causes environmental pollution and biological control is the best alternative for the management of wilt diseases (Mohiddin *et al.*, 2010).

Trichoderma is most commonly used biological control agent and have long been known as effective antagonistic against plant pathogenic fungi (Chet *et al.*, 1981; Papavizas, 1985; Chet, 1987; Kumar and Mukharjii, 1996). *Trichoderma* as a potent fungal biocontrol agent against a range of plant pathogens has attracted considerable scientific attention during last few decades (Tewari and Mukhopadhyay, 2001; Rini and Sulochana, 2007). The antifungal action of *Trichoderma* spp. is well documented against number of foliar and soil borne fungi like. *Fusarium* sp., *Pythium* sp., *Rhizoctonia solani*, *Sclerotium rolfsii*, in vegetables, field, fruit and industrial crops (Tran, 1998; Ngo *et al.*, 2006). Therefore, the present study was conducted to manage *Fusarium* wilt of chilli by locally available *Trichoderma* isolates.

Materials and Methods

Survey

An extensive field survey was conducted from major chilli growing areas of district Anantnag and Kulgam of Kashmir valley for the isolation of wilt pathogen from infected chilies and for the isolation of *Trichoderma* spp. from the rhizopheric soils of chilli fields. Three fields in every village were selected randomly and sampling procedure was based on standardized sampling techniques. Survey was done at three different stages viz., seedling stage (15-25 days

after seed sowing), transplanting stage, and at the time of flowering/fruiting of plants.

In order to isolate the *Trichoderma* spp. about 250 to 300 g of soil around roots (rhizopheric) was collected in a polythene bags and data on host, locality, soil type, etc. were tagged. Care was taken to ensure that soil samples moist during transit. In order to isolate the wilt pathogen about 100 g of diseased sample (root/shoot) were collected in polythene bags. The collected samples were brought to the laboratory of the division of plant pathology, SKUAST-K for further analysis.

Isolation and mass culture of the wilt fungus

The root and shoot samples of chilli plants showing characteristic wilt symptoms were collected from naturally infected farmers fields in district Anantnag and Kulgam to isolate wilt fungus *Fusarium* spp. Fungal pathogens were isolated by tissue segment method. Location wise chilli samples were separated. The chilli samples were disinfected by dipping in sterilized distilled water until all dust particles were removed and dried with sterilized blotting paper. Diseased pieces of chilli samples were separated and cut into small pieces (2-5 mm), surface sterilized in 1% mercuric chloride up to 40 seconds, rinsed three times in sterilized distilled water, dried on sterile blotting paper. The pieces were placed aseptically to a Petri plates containing solidified potato dextrose agar (PDA) with 5% lactic acid to inhibit bacterial growth. The inoculated plates were incubated in an incubator at 25 ± 2 ºC. The fungal colonies developed in the plates were sub cultured on PDA slants. The fungi developed on slants were examined microscopically to identify and isolate the pure culture of *Fusarium* spp. The established pure cultures of *Fusarium* spp. were multiplied on maize seeds mixed with sand. First the seeds were transferred to conical flasks of 500 mL capacity. The flasks were autoclaved twice for 15-20 minutes. Thereafter, the flasks were inoculated with the pure culture of *Fusarium* spp. and incubated for 8-10 days in an incubator at 25 ± 2 ºC. During incubation, the flasks were shaken manually for a few minutes daily for uniform colonization of seeds. The inoculum so prepared was incorporated in pots containing sterilized soil mixed with vermi-compost. The pots were mixed thoroughly. Three replicates were maintained and in each pot surface sterilized plantlets were grown. The pots were irrigated with tap water regularly to maintain adequate moisture. After 15 days plants were observed for wilt disease symtoms (Agrios, 1988).

Isolation of common biocontrol fungi

Trichoderma spp. were isolated from both healthy and diseased rhizospheric soils of irrigated and non irrigated fields collected from the major vegetable growing areas of district Anantnag and Kulgam. From the rhizospheric soil samples, 10 isolates of *Trichoderma* species were isolated by dilution plate technique (Johnson, 1957). *Trichoderma* selective medium (TSM) was used for isolation (Elad *et al.*, 1991). After few days of incubation, colonies appear in varying densities, depending upon the amount of dilution from the original material. The fungal colonies developed in the plates were sub cultured on TSM slants. The isolated strains of *Trichoderma* spp. were maintained throughout the study by periodical transfers on TSM slants under aseptic conditions to keep the culture fresh and viable.

Screening of biocontrol fungi against the wilt fungus

In vitro tests were carried to screen the effect of biocontrol fungi *Trichoderma* against wilt fungi *Fusarium* by using following tests:

Monoculture growth rates of *Fusarium* and *Trichoderma* isolates

Isolates of *Trichoderma* and wilt fungus *Fusarium* were cut (6 mm PDA discs) from seven days old culture plates with a sterilized cork borer. The discs were placed individually at the centre in 9 cm Petri plates containing solidified PDA. The plates were incubated at 25 ± 2 ºC for 7-13 days and radial growth was measured.

Dual culture test

The isolates of biocontrol were evaluated against *Fusarium* spp. in the laboratory by dual culture technique (Morton and Stroube, 1955). Six mm discs of *Fusarium* and each of *Trichoderma* spp. were cut from seven days old culture plates with sterilized cork borer and placed on a solidified PDA towards two ends of Petri plates. The plates were incubated at 25 ± 2 ºC. The observation on the growth and ability of biocontrol agents to restrict and colonies *Fusarium* spp. were recorded on 3rd, 4th, 5th and 7th day of inoculation.

Volatile test

PDA discs (6mm) of seven days old culture of isolates of *Trichoderma* spp. were placed aseptically on solidified PDA at the center in the Petri plates (Dennis and Webster, 1971). The lids of plates were replaced by a plate with solidified PDA inoculated with *Fusarium* spp. A set of

plates with PDA medium without biocontrol agent at lower side and with *Fusarium* spp. inoculated at upper side of plate served as a control. The plates in pairs were sealed together with cellophane adhesive tape and incubated at 25 ± 2 ºC. The growth inhibition was recorded on 3rd 4th and 8th day and growth inhibition was calculated according to the formula:

$$I = C\text{-}T/C \times 100$$

Where, I = percentage growth inhibition; C = Radial growth in control (mm); T = Radial growth in treated plates.

Results and Discussion

During the survey the disease incidence was found highest during flowering/fruiting stage in Redwani Payeen village of Kulgam district (40%) followed by Bangidar village of Anantnag District (24%) (Fig. 1). Disease incidence in surveyed areas at flowering/fruiting stage in decreasing order was as follows: Redwani Payeen Kulgam (40%), Bangidar Anantnag (24%), Bagi-Wonpoh Anantnag (23%), Wazirpora Kulgam (20%), Khudwani Kulgam (12%). At transplanting stage the disease incidence was observed highest in Redwani Payeen village of Kulgam district (6%) followed by (4%) at Bangidar locality of Anantnag District. Among the surveyed area the disease incidence at transplanting stage in decreasing order was as follows: Redwani Payeen Kulgam (8%), Bangidar Anantnag (4%), Bagi-Wonpoh Anatnag (3%), Wazirpora Kulgam (1%), Khudwani Kulgam (1%). Ten isolates of *Trichoderma* spp. (Tr 1, Tr 2, Tr 3, Tr 4, Tr 6, Tr 8 Tr 10, Tr 11, Tr 12 and Tr 16) and eleven isolates of *F. solani* (C1- C11) were isolated from rhizopheric soils of healthy chilli plants and diseased roots and shoots of chilli samples (Table 1 and 2).

Monoculture study of *Fusarium* and *Trichoderma* isolates

Radial growth of *Fusarium* isolates was determined so as to select the best among eleven isolates. Although all the isolates grew well the Petri plates, but the isolates C8 and C9 showed complete growth in the Petri plates within 9 days while as other isolates C1, C2, C3, C4, C5, C6, C7, C10 and C11compleatly covered the plate in 10th day (Fig. 2). From this study isolate C9 identified as *F. solani* was found superior and was selected for dual culture and volatile tests studies against 10 antagonistic isolates of *Trichoderma.* Radial growth of *Trichoderma* isolates was studied to get the most efficient isolate among the ten isolates. Almost all the isolates showed similar growth covering the Petri plates completely at 5th day, but Tr02 and Tr03 were exceptional which cover the Petri plates completely at 6th day and Tr08 was best which completely grew over Petri plates on 3rd day (Fig. 3). Hence all the ten *Trichoderma* isolates were selected to determine their antagonistic efficacy against *Fusarium* spp. in dual culture and volatile tests.

Dual culture test

The ten fungal *Trichoderma* isolates were screened against the fungus *F. solani* C9 *in vitro* for their antagonistic effect by using dual culture test (Morton and Stroube, 1955). The observation on the growth and ability of biocontrol agents to restrict and colonize *F. solani* were recorded on 3rd, 4th, 5th and 7th day of inoculation. Dual culture experiment reveals that all the isolates of *Trichoderma* had a marked significant inhibitory effect at $P \leq 0.05$ on the growth of *F. solani* as compared to that of control, but the isolate Tr16 exhibited maximum inhibition growth (77%) followed by Tr11 (72%) and Tr08 (68%) respectively (Fig. 4). Growth inhibition in decreasing order on 7th day of ten isolates was as follows: Tr 16 (77%) > Tr 11 (71%) > Tr 8 (68%) > Tr 10 (68%) > Tr 12 (66%) > Tr 6 (66%) > Tr 1 (66%) > Tr 3 (65%) > Tr 4 (62%) > Tr 2 (52%).

V**olatile test**

The fungal antagonists were also screened to study the effect of volatile compounds released by the isolates of *Trichoderma* on the growth of *F. solani*. The observations on the growth and ability of biocontrol agents to restrict colonies of *F. solani* were recorded on 3rd, 4th, 5th and 7th day of inoculation. The gaseous metabolites released by *Trichoderma* isolates diffused and inhibited the growth of *F. solani* inoculated in inverted face of Petri plates. All the isolates of *Trichoderma* significantly inhibited the growth of *F. solani* but Tr08 showed the maximum inhibition (89%), followed by Tr 2 (78%) and Tr 3 (56%), respectively. Growth inhibition in decreasing order on 7th day was as follows: Tr 8 (89%) > Tr 2 (78%) > Tr 3 (56%) > Tr 16 (56%) > Tr 11 (55%) > Tr 4 (53%) > Tr 10 (51%) > Tr 6 (44%) > Tr 12 (33%) (Fig. 5).

The study indicated that the fungus responsible for wilt disease of chilli at district Anantnag and Kulgam was *F. solani.* The pathogen is probably temperature-sensitive, therefore its occurrence prevails in every May to August of the year (fruiting stage) when atmospheric temperature increases. It has been

reported that favorable conditions for the fungus are wet soils and prolonged wet periods with air and temperatures from 24–29 °C (Anonymous, 2003).

Application of fungicides is the main tool for controlling fungal diseases nevertheless fungicides has many undesirable attributes (Bastos, 1996). So a promising strategy for the replacement of chemical pesticides has been the implementation of biological control. Research has repeatedly demonstrated that phylogenetically diverse microorganisms can act as natural antagonists of various plant pathogens (Cook, 2000). So survey was conducted with aim to isolate biocontrol agent (*Trichoderma* spp.) for the management of wilt disease (*Fusarium* spp.). *Trichoderma* spp. are common saprophytic fungi which were found in almost any soil and rhizopheric microflora. They have been investigated as potential biocontrol agents because of their ability to reduce the incidence of disease caused by plant pathogenic fungi, particularly many common soil borne pathogens (Papavizas, 1985; Sivan and Chet, 1986). The results of dual culture revealed that *Trichoderma* species showed the appreciable inhibition of mycelial growth of *F. solani.* Since *Trichoderma* is a fast growing fungus, it reached the pathogen within 3-4 days. Similar findings of the interaction of *Trichoderma* spp. against *Fusarium* spp. were recorded previously (Mustafa *et al.*, 2009; Ramezani, 2010; Rajeswari and Kannabiran, 2011; Lone *et al.*, 2012; Subash *et al.*, 2013). The antagonistic nature may be due to antibiosis, nutrient competition and cell wall degrading enzymes. The gaseous metabolites released by *Trichoderma* isolates diffused and inhibited the growth of *F. solani* inoculated in inverted face of Petri plates. The volatile compounds of *Trichoderma* significantly inhibit the growth of *F. solani* with an increased growth of antagonists. The degree of effectiveness varies according to the nature, quality and quantity of antibiotics/inhibitory substances secreted by the antagonists (Dennis and Webster, 1971; Skidmore and Dickinson, 1976). Similar observation on the growth and ability of biocontrol agents to restrict and colonized *Fusarium* spp. were recorded by (Rini and Sulochana, 2007; Amin *et al.*, 2010; Rajeswari and Kannabiran, 2011; Poovendran *et al.*, 2011).

It is concluded that *Trichoderma* species have the potential to suppress the growth of *F. solani* responsible of wilt diseases in chilli. Further studies are required to develop biopesticides based on *Trichoderma* isolates for the management of chilli wilt and to enhance overall yield, and to decrease the economic crises and food shortage.

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Table 1: *Trichoderma* isolates isolated from different soils of district Anantnag and Kulgam

Isolate Name	Species	Area of collection
Tr 1	*Trichoderma* sp.	Bangidar Anantnag
Tr 2	*Trichoderma* sp.	Bangidar Anantnag
Tr 3	*Trichoderma* sp.	Khudwani Kulgam
Tr 4	*Trichoderma* sp.	Khudwani Kulgam
Tr 6	*Trichoderma* sp.	Redwani Payeen Kulgam
Tr 8	*Trichoderma* sp.	Redwani Payeen Kulgam
Tr 10	*Trichoderma* sp.	Bagi Wanpoh Anantnag
Tr 11	*Trichoderma* sp.	Bagi Wanpoh Anantnag
Tr 12	*Trichoderma* sp.	Bagi Wanpoh Anantnag
Tr 16	*Trichoderma* sp.	Wazirpora Kulgam

Table 2: *Fusarium solani* isolates isolated from infected chillies of district Anantnag and Kulgam

S. No.	Isolate Name	Area of collection
1	C_1	Bagi Wanpoh Anantnag
2	C_2	Bagi Wanpoh Anantnag
3	C_3	Bagi Wanpoh Anantnag
4	C_4	Bagi Wanpoh Anantnag
5	C_5	Redwani Payeen Kulgam
6	C_6	Redwani Payeen Kulgam
7	C_7	Redwani Payeen Kulgam
8	C_8	Redwani Payeen Kulgam
9	C_9	Redwani Payeen Kulgam
10	C_{10}	Bangidar Anantnag
11	C_{11}	Bangidar Anantnag

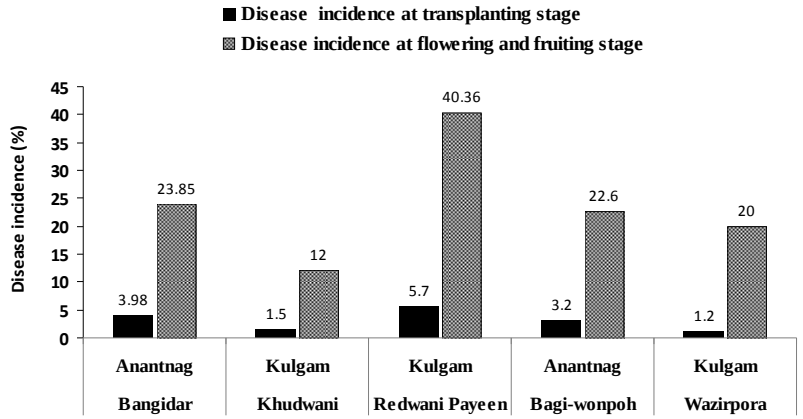

Fig. 1: Disease incidence at transplanting and flowering/fruiting stage as recorded during survey.

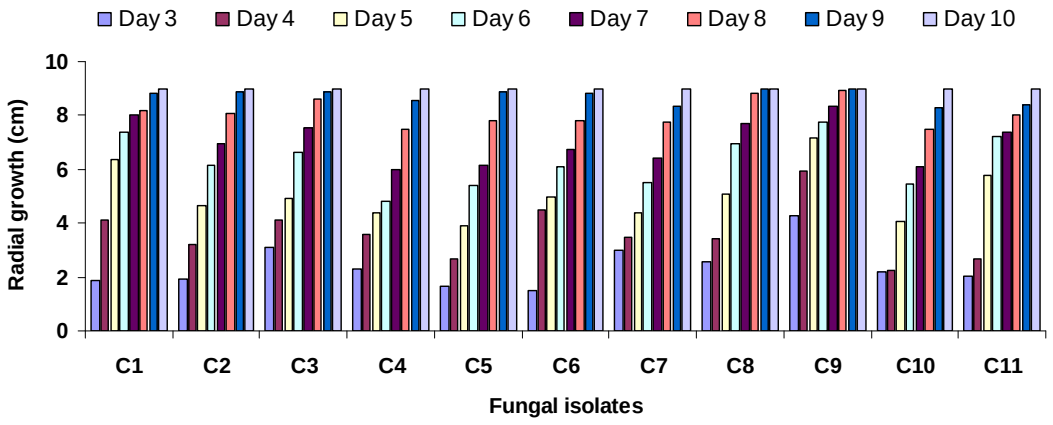

Fig. 2: Average radial growth of *Fusarium* isolates isolated from infected chilies of Anantnag and Kulgam districts.

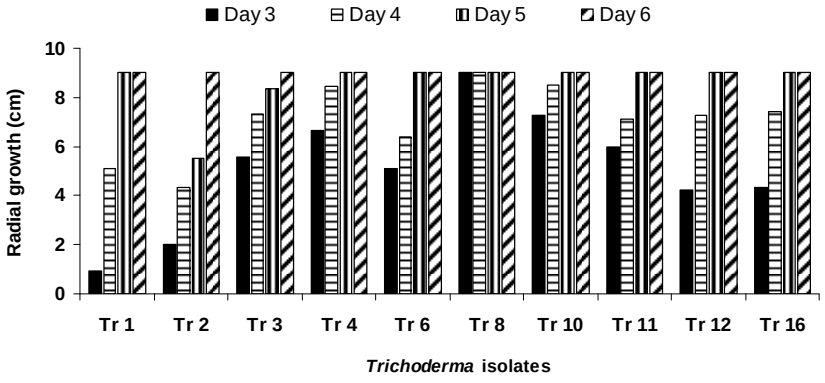

Fig. 3: Average radial growth of *Trichoderma* isolates isolated from soils of Anantnag and Kulgam districts.

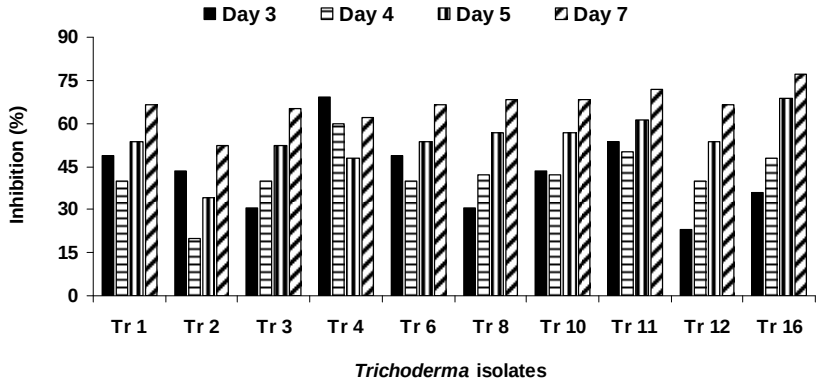

Fig. 4: Growth inhibition of *Fusarium solani* by *Trichoderma* isolates in dual culture test.

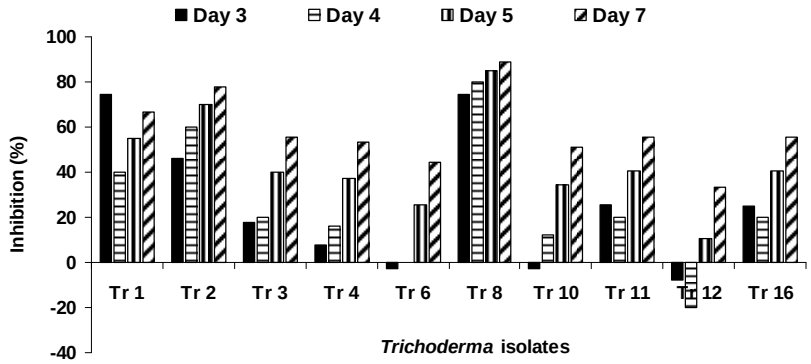

Fig. 5: Inhibition percent of *Fusarium solani* by *Trichoderma* isolates in volatile test.

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