

Biological management of wilt disease on chilli caused by *Fusarium solani*

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Abstract

Wilt disease on chilli caused by *Fusarium solani* is emerging as a serious disease for its cultivation by resource poor farmers in recent days. Chilli plants showing typical symptoms such as yellowing and drooping of leaves and vascular discoloration upon split opening the stem was observed and the *Fusarium solani* pathogen associated with the symptom has been isolated. All the 15 *Trichoderma* isolates tested *in vitro* against the pathogen showed >52% inhibition of mycelial growth over control was observed. In the field evaluation, all the isolates were found effective in reducing the wilt incidence and improving the green chilli yield compared to control. Among all Phyto 6, found to be producing consistent performance against the wilt disease on chilli and also by improving the yield compared to other isolates and chemical control.

Keywords: Chilli wilt, *Trichoderma*, Antagonistic activity, biocontrol, disease management

Introduction

Chilli, (*Capsicum annum* L), is one of the important spice cum vegetable crop cultivated under an area of 9.15 lakh ha with a total production of 3.2 million tons including both green and dried chillies in India (DACFW, 2015). Among the fungal, bacterial and viral pathogens infecting chilli crop, wilt disease caused by *Fusarium solani* is emerging as a serious menace to chilli cultivation in India. It is infecting the crop at flowering stage which leads to serious yield loss on chili crop. Several chemical fungicides are available in the markets which are being used in its management by the farmers with the risk of

environmental safety. In future biological management of plant disease will be given more emphasis using the microflora since chemical pesticides are causing hazards to environment as well as human health. Several biocontrol agents such as *Bacillus subtilis*, *Pseudomonas fluorescens*, *Trichoderma viride*, *T. harzianum*, etc were separately studied on the *Fusarium* causing wilt disease on several crop plants (Prabhukarthikeyan *et al.* 2014; Manikandan *et al.* 2010; Sundaramoorthy *et al.* 2013; Mwangi *et al.* 2011). In this study, we had made attempt to study the *in vitro* and field efficacy of *Trichoderma* isolates in the management of wilt disease on chilli caused by *Fusarium solani*.

Materials and methods

Isolation and pathogenicity of pathogen: Wilt infected chilli plant was collected from the IIVR farm and brought to the laboratory for isolation. Infected root tissues were washed with running tap water and root bits of 5 to 10 mm size were surface sterilized in 1% Sodium hypochlorite solution for 1 min followed by rinsing with sterile distilled water and dried on sterile filter paper. Sterilized tissue bits were placed on Potato Dextrose Agar (PDA) plates added with streptomycin sulphate to check the bacterial contamination. The plates were incubated at room temperature for 7–10 days for the growth of mycelium and were subcultured on PDA slants for pure culture maintenance (Norhito *et al.* 2004; Nirmaladevi *et al.* 2016). Morphological identification was done based on characteristics of the macroconidia, microconidia and chlamydospores. Isolated *Fusarium* was inoculated on susceptible cv. Pusa Jwala by root cut and dip method at the concentration of 10⁶ spore / ml for 30 min (Nirmaladevi *et al.* 2016).

***In vitro* screening of *Trichoderma* isolates:** Fifteen *Trichoderma* isolates (Phyto1 to 15) obtained from ICAR-Indian Institute of Spices Research, Kozhikode were used to screen *in vitro* against *Fusarium solani*. Antagonistic activity of 15 *Trichoderma* isolates was screened against

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F. solani through dual-culture technique (Dennis and Webster 1971). Efficiency of the *Trichoderma* isolates was determined based on their mycelial inhibition. Percent inhibition of mycelia was calculated using the formula

$$\text{Percent inhibition of mycelia (\%)} = \frac{C - T}{C} \times 100$$

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

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

Field evaluation of *Trichoderma* isolates along with chemical fungicides and plant extract:

Talc formulations of 15 *Trichoderma* isolates were prepared as per the standard protocol (Jeyarajan *et al.*, 1994) with some minor modifications. *Trichoderma* isolates were multiplied in potato dextrose broth for 7-10 days at room temperature and then mycelia are grinded using mixer grinder along with broth. Four hundred ml of culture extract was mixed with 1kg talc (sterilized at 105°C for 12 h). The bio-formulation was shade dried for 12 h and packed in polythene packs. Talc bio-formulation at the time of application should be more than 2×10^6 cfu/g. Two field trials were conducted for two consecutive years 2014-15 and 2015-16 in research farm at Indian Institute of Vegetable Research, Varanasi. The seeds of chilli (cv. Pusa Jwala) were treated with the talc formulation @ 10g /kg and shade dried for 12 h. The treated seeds were sown in nursery beds applied with talc formulation (mixed thoroughly 50 g talc formulation with 5 kg well decomposed Farm Yard Manure (FYM) and applied to the 3m² bed). Chemical and untreated control, nursery beds were treated with FYM alone @ 5 kg/3m² and seeds treated with carbendazim 50%WP (1 g/ kg), Carbendazim 8% + Mancozeb 64% 72WP (2.5g/kg) and untreated ones respectively were sown separately in the beds. After 25 days, the seedlings under bio-formulation treatments, were uprooted and the root portion was dipped in talc based bio-formulation solution (1.0 %) for 30 min and transplanted in the field applied with talc formulation @ 2.5 kg mixed in 50 kg FYM/ha. For datura and garlic extracts, only seedling dip was provided with 10% aqueous extract for 30min. Similarly, for the chemical and untreated controls, the seedlings were dipped for 30 min in carbendazim (0.1%), carbendazim + mancozeb (0.25%) solution and water respectively and transplanted separately in the main field applied with FYM alone @ 50 kg/ha. The experiment was conducted in randomized block design with three replications of each treatment. Observations on disease control and yield were recorded at the time of crop completion by split opening the stem for vascular browning. Data

obtained were analyzed using the AGRES statistical tool at 5% level of significance.

Results and discussion

Fusarium solani has been isolated on the PDA from the infected root portion of chilli plant showing typical symptoms, i.e. yellowing and drooping of leaves and vascular brown discoloration upon splitting of stem. Isolated pathogen has been inoculated on the chilli cv. Pusa Jwala under screen house conditions and the plant were exhibited wilting symptom after 25-30 days post inoculation. Similarly, Nirmaladevi *et al.* (2016) had isolated 69 strains of *Fusarium oxysporum* f.sp. *lycopersici* infecting tomato crop from Southern India and their pathogenic potential were studied on 5 different tomato cultivar.

Fifteen *Trichoderma* isolates were screened against *F. solani* for their efficacy *in vitro* (Table 1). All the isolates recorded more than 50% inhibition over the mycelial growth of *F. solani*. Phyto 7 recorded the least inhibition of 52.42% and the maximum was recorded by Phyto 4 (66.08%). There was no much significant difference was observed among the isolates tested *in vitro*. Taj *et al.* (2013) demonstrated the *in vitro* potentiality of native *Trichoderma harzianum* isolate against the major fungal plant pathogens through dual culture technique.

Under field conditions, talc based formulations of all the *Trichoderma* isolates were evaluated along with the two chemicals (Carbendazim 50%WP and Carbendazim 8%+ Mancozeb 64% 72WP and two aqueous plant extracts (datura 10% and garlic 10%). In both the trials all the 15 *Trichoderma* treated plots had shown reduced wilt disease incidence compared to untreated control

Table 1. *In vitro* efficacy of different *Trichoderma* isolates (Phyto 1-15) on mycelial inhibition of FS

S. No.	Name of the isolate	*Mycelial inhibition (%) of FS
1	Phyto-1	61.23
2	Phyto-2	61.23
3	Phyto-3	64.76
4	Phyto-4	66.08
5	Phyto-5	60.35
6	Phyto-6	62.99
7	Phyto-7	52.42
8	Phyto-8	60.80
9	Phyto-9	67.41
10	Phyto-10	58.59
11	Phyto-11	64.76
12	Phyto-12	62.12
13	Phyto-13	65.63
14	Phyto-14	62.55
15	Phyto-15	62.55
16	Control	0.00
CD @ 0.05		5.38

*Values were arc sine transformed before the analysis.

Table 2. Effect of biocontrol agents, botanicals and chemicals on Fusarium wilt and yield of chilli under field conditions

S. No.	Treatment	2013-14			2015-16		
		Wilt incidence* (%)	% reduction over control	Q/ha	Wilt incidence* (%)	% reduction over control	Q/ha
1.	Phyto-1	23.34	29.04	26.38	13.70	38.37	12.9
2.	Phyto-2	23.02	30.01	22.36	16.74	24.70	13.3
3.	Phyto-3	22.46	31.71	25.31	14.02	36.93	22.5
4.	Phyto-4	16.79	48.95	28.47	15.34	30.99	18.7
5.	Phyto-5	21.68	34.08	24.12	16.85	24.20	18.3
6.	Phyto-6	20.65	37.21	28.87	15.60	29.82	21.9
7.	Phyto-7	26.30	20.04	27.98	18.17	18.26	14.8
8.	Phyto-8	25.06	23.81	23.55	15.65	29.60	17.4
9.	Phyto-9	15.19	53.82	33.33	15.35	30.95	15.3
10.	Phyto-10	22.74	30.86	28.33	15.60	29.82	15.6
11.	Phyto-11	24.65	25.05	24.44	16.70	24.88	23.7
12.	Phyto-12	21.26	35.36	30.69	15.22	31.53	15.1
13.	Phyto-13	20.17	38.67	27.36	14.19	36.17	22.8
14.	Phyto-14	22.66	31.10	25.34	17.19	22.67	19.6
15.	Phyto-15	24.34	26.00	23.59	15.11	32.03	16.3
16.	Carbendazim 8%+ Mancozeb 64% 72WP	22.94	30.25	27.33	11.99	46.06	18.4
17.	Carbendazim 50% WP	24.61	25.17	21.55	15.12	31.98	14.8
18.	Datura extract	20.91	36.42	23.13	15.94	28.30	19.7
19.	Garlic extract	23.76	27.76	21.50	17.09	23.12	12.4
20.	Control	32.89	0.00	13.33	22.23	0.00	11.5
	CD 5%	4.38		7.27	4.89		3.72
	CV	13.04		27.77	18.66		21.17

*Values were arc sine transformed before the analysis

plots (Table 2). In the trial conducted during 2013-14, phyto 9 and phyto 4 recorded highest percent reduction of wilt incidence over control of 53.82 and 48.95% respectively. These treatments were found superior to chemical plots. Maximum yield was recorded in Phyto 9, Phyto 12, Phyto 6 and Phyto 4. All treatments of *Trichoderma*, chemical and botanicals had recorded better yield compared to control plot. In case of 2015-16 trial, Phyto 1 (38.37%) and Phyto 3 (36.93%) had recorded percent maximum disease reduction over control. Phyto 11, Phyto 13, Phyto 3 and Phyto 6 were recorded superior yield than control and other treatments. In both the trials, Phyto 6 is showed consistently highest yield and reduced wilt disease incidence. Similarly, soil application of *Trichoderma harzianum* Th-10 as dried formulation had effectively controlled the fusarial wilt in banana with comparable efficacy to chemical control (Thangavelu *et al.* 2004). Several other workers were studied the efficacy of *Trichoderma* isolates against the many plant pathogenic fungus with increased economic yield (Ghazalibiglar *et al.* 2016; Figueirêdo *et al.* 2010).

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सारांश

उकटा बीमारी के कारण आजकल संसाधन विहिन कृषकों को मिर्च की खेती करना मुश्किल हो रहा है। मिर्च के पौधे पर विशेष लक्षण जैसे पीला पड़ना तथा पत्तियों का नीचे की तरफ मुड़ना एवं संक्रमित तने को दो भागों में विभक्त करने पर संवहन उत्तक बदरंग दिखते हैं तथा लक्षण आधारित फ्यूजेरियम सोलोनी रोगजन को अलग किया गया। पात्रे तकनीकी के अन्तर्गत 15 ट्राइकोडर्मा विलगों को रोग के विरुद्ध परीक्षण किया गया जिससे कवकतन्तु की वृद्धि नियन्त्रक की तुलना में 52 प्रतिशत कम हुई। प्रक्षेत्र मूल्यांकन में सभी विलग उकटा रोग को प्रभावी तरीके से कम किये और नियन्त्रक की तुलना में हरी मिर्च उपज भी बढ़े। सभी में फाइट-6 मिर्च के उकटा रोग के विरुद्ध लगातार अच्छा निष्पादन किया तथा अन्य विलगों व रसायनिक नियंत्रण की तुलना में उपज में सुधार हुआ।

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