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Biological control of *Fusarium* wilt of chillies using *Trichoderma* spp.

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ABSTRACT

Chilli wilt caused by *Fusarium solani* is a serious menace in black cotton soils of Karnataka. Wilt incidence of 19.8 to 31.5 and 7.5 to 12.3% was recorded in major chilli growing regions of Karnataka during 2011-12 and 2012-13 respectively. *Trichoderma harzianum* isolates 1, 2 and 5 and *T. viride* isolate 2 completely overgrew *F. solani* and inhibited mycelia growth by 40-50% *in vitro*. *T. viride* gave moderate effect against wilt and increased yield upto 30% in Haveri district, while at Bellary, *T. harzianum* isolate 1 reduced wilt incidence by 39.7%.

Key words: Bio-control, *Capsicum annuum*, *Fusarium* wilt, Karnataka, *Pseudomonas* fluorescens, Trichoderma.

INTRODUCTION

Chilli (*Capsicum annum*) is a major commercial crop grown as vegetable and spice having value addition in pharmaceuticals, cosmetics and beverages. India is a major producer, exporter and consumer of chilli with a cultivated area of 7.94 lakh ha and annual production of 13.04 lakh tonne (Anon, 2). It is grown primarily in Andhra Pradesh, Gujarat, Karnataka, Odisha and Maharashtra. In the recent decades, fungal wilts and dry root rots caused by *Fusarium oxysporum*, *F. solani*, *Rhizoctonia solani* and *Sclerotium rolfsii* have caused severe crop losses in chilli (Devika Rani *et al*., 5; Singh, 15). Area under chilli is dwindling due to *Fusarium* wilt in the intermediate hill zone of Jammu and Kashmir (Nayeem *et al*., 10). Yield loss to the tune of 50-80% was reported under heavy incidence (Madhavi *et al*., 7). In Karnataka, incidence is particularly high in the irrigated tracts of black cotton soil (Devika Rani *et al*., 5). At experimental level, chemicals gave promising results against chilli wilt (Singh, 15; Singh *et al*., 14). However, application of fungicides under field conditions results in high cost, environment pollution and inconsistency in efficacy. Chilli varieties / hybrids that are popular are highly susceptible to wilt and resistant accessions fail to make any dent with available strategies having little impact against chilli wilt. Bio-control agents also have been tested and successfully employed against soil borne pathogens including *Fusarium* (Mukhopadhyay, 8). Present study was undertaken to identify effective bio-agents against chilli wilt for irrigated (Haveri) and rain-fed/ irrigated (Bellary) chilli growing tracts in Karnataka during 2011-12 and 2012-13.

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MATERIALS AND METHODS

A roving survey for the incidence of chilli wilt was undertaken during January, 2012 and 2013 in the major chilli growing regions of Haveri (Haveri, Hangal, Hirekerur, Byadgi and Ranebennur), Bellary (Hospet, Sirugoppa and Bellary), Raichur (Raichur and Deodurga), Koppal (Gangavathi) and Yadgir (Shahapur) districts of Karnataka. In each location, 10 villages with ten fields/ village were surveyed. Occurrence of wilt in 15 plants in each of 10 spots per field was recorded and incidence per cent was calculated. Average incidence for the locations was calculated and means incidence for each district was also obtained. Samples of affected plants were brought to the laboratory and isolated on potato dextrose agar (Aneja, 3). Based on morphological and microscopic observations, identification of the fungus was made and pure culture of the pathogen deposited in ITCC, New Delhi after proof of pathogenicity (Fiume and Fiume, 6).

Twelve *Trichoderma* isolates isolated by serial dilution (Aneja, 3) from the rhizosphere of potato, chilli and bell pepper and five isolates of *Pseudomonas fluorescens* (Division of Plant Pathology, IARI, New Delhi) were evaluated for their efficacy against *Fusarium solani* by dual culture technique. Five mm discs from seven-day-old cultures of *F. solani* (slow growing) were placed at one end of the petridishes and exactly after 86 h, five mm discs of *Trichoderma* (fast growing) was placed at the other end of petridish to compensate for their growth behavior. In all, three replications were maintained for each treatment with suitable control for *Trichoderma* and *Fusarium.* Petridishes were incubated at 27°C and observations were made at regular intervals for mycelia inhibition by each of the *Fusarium* and *Trichoderma* isolates on one another (Sharma, 13) and overgrowth if any

was recorded. Five mm plug from the leading edge of freshly growing culture of *F. solani* was placed at the centre of the plate and after 86 h, 48-h-old culture of *P. fluorescens* was streaked at both ends equidistant point along the perimeter of the plate. In all, three replications were maintained for each treatment and plates without bacteria served as control. Plates were incubated at 27°C till 12 days and per cent inhibition was calculated based on the radial growth of *F. solani* (Dennis and Webster, 4).

Formulations of selected *Trichoderma* and *P. fluorescens* were made by following the methods of Ramanujam *et al.* (12) and Nandakumar *et al.* (9). *Trichoderma* was multiplied in pre-soaked sterilized sorghum seeds for 10 days at 29°C in a BOD incubator. Sorghum seeds covered with dark green *Trichoderma* mass were shade dried and ground to a fine powder using a blender. Entire content was mixed with sterile talcum powder and calcium carbonate (10 g/kg) to get the desired population of 2-5 × 10^8 cfu/g powder. *P. fluorescens* was cultured in Kings B broth for 48 h in an orbital shaker at 150 rpm. One kg sterile talcum powder, 15 g calcium carbonate and 10 g carboxy methyl cellulose were added to the 400 ml bacterial broth, mixed and dried overnight. Formulation was packed in polypropylene bag and sealed till further use in fridge. Bacterial population at the time of application was 2.5 × 10^8 cfu/g.

Field testing of bio-agents: Based on *in vitro* studies, three isolates of *T. harzianum*, one each of *T. viride* and *P. fluorescens* were tested against *F. solani* under field conditions at two locations in Haveri and one at Bellary district, Karnataka. At Haveri, experiment was taken up during the crop season of October to May (irrigated/ rainfed) during 2011-2012 / 2012-13 using the local popular and highly susceptible variety BSS 414. At Bellary, local popular variety and highly susceptible Byadgi Kaddi was used and experiment was carried out during July to February 2011-12/ 2012-2013 under irrigated / rainfed conditions. In Haveri, chilli seeds were treated with the formulations @ 10 g/kg seeds and raised in nursery during October-November and transplanted in November, while in Bellary, treated seeds were directly sown in the main field. At the time of transplanting, seedlings in bundles were dipped in water containing talc based formulations (20 g/l) for 2 h. Carbendazim 12% + mancozeb 63% (std. check) seed treatment was given @ 2 g/kg seed. *Trichoderma* and *P. fluorescens* were applied to soil at 5 kg/ha twice, one at the time of sowing / transplanting and the other at the time of flowering mixed with well rotten FYM. Carbendazim 12% + mancozeb 63% WP was applied @ 2 g/l water through drenching at the time of transplanting and flowering. Crop was raised by following the standard package of practices. In all, there were seven treatments including five bio-control agents with untreated check and standard control (carbendazim + mancozeb). Each treatment was replicated thrice in plot of size 10 m × 10 m with spacing of 75 cm × 60 cm in RBD design. At Bellary, spacing was 60 cm × 60 cm. Observation on *Fusarium* wilt was made at the final stage of the crop and incidence (%) was calculated. Harvest of each green chilli picking at Haveri was weighed and expressed as yield / plot of 100 sq. m was calculated. At Bellary, only disease incidence was recorded. Treatment means were compared by Duncan's Multiple Range test (DMRT).

RESULTS AND DISCUSSION

Wilt incidence was high during 2011-12 compared to 2012-13 in all the regions of survey. Haveri recorded the highest mean incidence of 31.5%, while Koppal recorded the least 19.8%. During 2012-13, wilt incidence was low (1-18%) in the surveyed districts (Fig. 1). Bellary recorded the highest (12.3%) closely followed by Bellary and Raichur (upto 8.9%) while Yadgir recorded the least (5.1%). Repeated isolation yielded the fungus *Fusarium solani*, which was confirmed by ITCC Delhi (ID No. 8760.12). Pathogenicity was proven using 25% culture filtrate wherein treated seedling collapsed within 36 h. In all the twelve dual culture sets, initial counter inhibition was observed between *Trichoderma* and *Fusarium*, which posed varying degree of inhibition on each other. Inhibition of *Trichoderma* by *Fusarium* ranged from 38.9 to 66.7% by three days (data not shown). During the same period, inhibition of *Fusarium* by *Trichoderma* ranged from 0 to 28.6%. *T. harzianum* 1, 2 and 5 and *T. viride*, 2 isolates completely overgrew *Fusarium*, while *T. hamatum and Trichoderma* sp. 2 failed to overcome inhibition posed by *Fusarium* (Fig. 2). In a similar type of study, Sharma (13), reported pre-contact inhibition followed by chemo attractive and parasitic phase between *F. oxypsorum* f.sp. *pisi* and *Trichoderma* interaction. Among *P. fluorescens* isolates, pf3 isolate

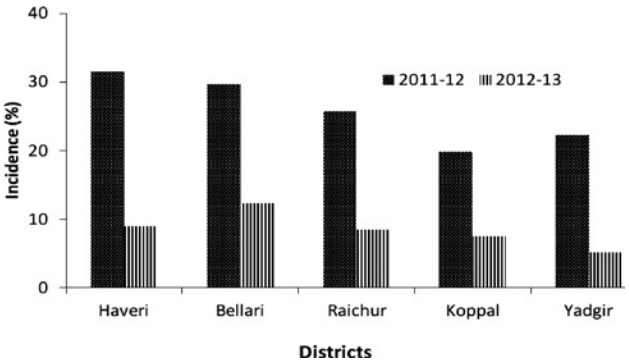

Fig. 1. *Fusarium* wilt incidence (%) in chilli in Karnataka.

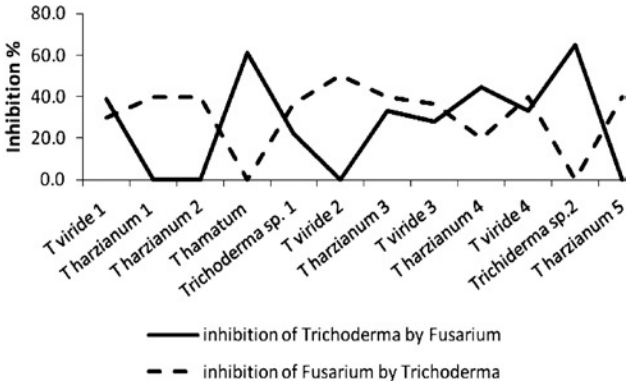

Fig. 2. Counter inhibition of *Trichoderma* and *Fusarium*.

gave the maximum inhibition (39.2%), which was at par with pf6 and pf80 isolates.

At Tavaragopppa, Haveri, *T. viride* was superior to other bio-agents in managing *Fusarium* wilt (Table 1). Incidence of 15.4 and 6.2% was recorded in *T. viride* treatment in two years as against 31.4 and 15.4% respectively in untreated control. Singh (15) reported moderate effect of *T. viride* and *T. harzianum* against chilli wilt at Himachal Pradesh. Lowest incidence (12.6 and 4.1%) was recorded in carbendazim + mancozeb treatment. At Hosalli, incidence of wilt was very high in all the treatments and none found effective including the chemical. The failure of various treatments could be due to very high load of the inoculun and favourable conditions for the pathogen. In general, *Fusarium* wilt was low during 2012-13 possibly because of dry weather. During 2011-12, highest yield (125.7 kg/ 100 sq.m) was recorded by *T. viride* treatment closely followed by carbendazim + mancozeb, which were at par. Almost a similar trend was noticed during 2012-13. *P. fluorescens* though failed to give protection, yielded 14 and 11% higher yield compared to untreated control. *Pseudomonads* are reported to be involved in growth promotion by the production of phytohormones, nitrogen fixation, phosphate solubilization (Park *et al*., 11). At Hosalli, crop yield was adversely affected due to failure of the crop. However, *T. viride* and carbendazim + mancozeb treated plots yielded almost twice the yield of untreated control. At Bellary, during 2011-12, *T. viride* and two isolates of *T. harzianum* gave moderate effect against chilli wilt with 27-35% protection over control (Table 2). During 2012-13, all treatments except control recorded < 10% incidence.

At low to moderate level of incidence, *T. viride* and *T. harzianum* gave encouraging results and be part of management strategy. However, at high level of incidence, resistance source, bio-agents and modified cultural practices (drip irrigation wherever feasible, planting in raised ridges, level land) should be part of the management strategies.

Table 1. Effect of bio-agents against chilli wilt at Haveri, Karnataka.

Treatment	Tavaragoppa						Hosalli					
	Incidence (%)			Yield (kg/100 sq.mt)			Incidence (%)			Yield (kg/100 sq.mt)		
	2011-12	2012-13	Mean	2011-12	2012-13	Mean	2011-12	2012-13	Mean	2011-12	2012-13	Mean
T. viride 2	15.4 (3.9)^{cd}	6.2 (2.49)^{d}	10.8 (3.2)^{d}	125.7^{a}	131.3^{a}	128.5^{a}	70.1 (56.9)^{cd}	7.6 (2.74)^{d}	38.8 (38.5)^{c}	40.0^{a}	128.3^{a}	84.2^{a}
T. harzianum 1	17.6 (4.2)^{cd}	9.9 (3.15)^{c}	13.8 (3.7)^{c}	114.3^{bc}	125.7^{ab}	120.0^{bc}	77.0 (61.5)^{bc}	8.0 (2.83)^{d}	42.5 (40.6)^{b}	29.0^{b}	130.3^{a}	79.7^{b}
T. harzianum 2	16.6 (4.1)^{cd}	10.4 (3.22)^{bc}	13.5 (3.6)^{c}	113.3^{bc}	120.0^{bc}	116.7^{c}	83.0 (65.6)^{b}	9.3 (3.05)^{cd}	46.1 (42.8)^{b}	26.0^{bc}	125.3^{a}	75.7^{b}
T. harzianum 5	22.4 (4.7)^{bc}	11.1 (3.33)^{bc}	16.7 (4.0)^{b}	110.7^{c}	125.3^{ab}	118.0^{c}	79.3 (63.0)^{b}	10.8 (3.29)^{bc}	45.3 (42.3)^{b}	23.3^{c}	119.7^{b}	71.5^{d}
P. fluorescence pf 3	26.7 (5.2)^{ab}	12.4 (3.52)^{b}	19.6 (4.3)^{b}	107.7^{c}	118.3^{c}	113.0^{d}	77.0 (61.5)^{bc}	11.7 (3.42)^{b}	44.4 (41.8)^{b}	29.0^{b}	128.0^{a}	78.5^{bc}
Carbendazim + mancozeb	12.6 (3.5)^{d}	4.1 (2.03)^{e}	8.4 (2.8)^{e}	120.0^{ab}	126.3^{ab}	123.2^{b}	62.1 (52.0)^{d}	4.6 (2.14)^{e}	33.3 (34.6)^{d}	38.3^{a}	129.3^{a}	83.8^{a}
Control	31.4 (5.6)^{a}	15.4 (3.92)^{a}	23.4 (4.8)^{a}	91.7d	106.0^{d}	98.8^{e}	89.6 (71.4)^{a}	17.5 (4.18)^{a}	53.6 (47.1)^{a}	22.0^{c}	110.0^{c}	66.0^{e}

Data followed by the same letter(s) in column are not significantly different at 5% level

Table 2. Effect of bio-agents against chilli wilt at Bellary, Karnataka.

Treatment	2011-12	2012-13	Mean
T. viride 2	44.3 (41.7)^{bc}	9.3 (3.1)^{b}	26.8 (31.1)^{cd}
T. harzianum 1	39.5 (38.9)^{c}	7.1 (2.7)^{c}	23.3 (28.8)^{d}
T. harzianum 2	41.2 (39.9)^{c}	8.4 (2.9)^{b}	24.8 (29.9)^{d}
T. harzianum 5	52.1 (46.2)^{ab}	9.0 (3.0)^{b}	30.5 (33.3)^{bc}
P. fluorescence pf3	55.8 (48.3)^{a}	8.8 (3.0)^{b}	32.3(34.6)^{b}
Carbendazim + mancozeb	37.1 (37.4)^{c}	6.6 (2.6)^{c}	21.8(27.9)^{d}
Control	61.3 (51.4)^{a}	16.1 (4.0)^{a}	38.7(38.4)^{a}

Data followed by the same letter(s) in column are not significantly different at 5% level

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