Biocontrol potential of *Trichoderma* species against wilt disease of carnation (*Dianthus caryophyllus* L.) caused by *Fusarium oxysporum* f.sp. *dianthi*

P. Mahalakshmi and I. Yesu Raja

ABSTRACT

A study was carried out to test the antagonistic potential of four isolates of *Trichoderma viride* and eight isolates of *T. harzianum* and five isolates of *T. reesei* against *F. oxysporum* f. sp. *dianthi,* the cause of Fusarium wilt in carnation. *T. harzianum* (Th2) showed the maximum inhibition of mycelial growth of *F. oxysporum* f. sp. *dianthi.* (45.33%) over control followed by *T. harzianum* (Th6) and *T. viride* (Tv3) with 42.11 and 38.66 per cent inhibition respectively. The Competitive Saprophytic Ability (CSA) and colonization by each isolates of *Trichoderma* spp. was assessed. Among the 17 isolates, Th2 registered 61.84 per cent colonization followed by Th6 with 59.12 per cent colonization. All other isolates of *Trichoderma* spp. were inferior to Th2 in its saprophytic ability. The antagonistic isolates of *Trichoderma* spp. were also tested for production of antifungal volatiles and their effect on the growth of *F. oxysporum* f. sp. *dianthi.* All the isolates were found to produce volatile metabolites, which inhibited the mycelial growth of test pathogen at varing levels from 16.33 to 50.11 per cent.

Key words: *Trichoderma,* carnation Fusarium wilt, competitive saprophytic ability, volatile compounds

INTRODUCTION

Carnation *Dianthus caryophyllus* L. is one among the most popular commercial cut flowers of the world, ranking second in commercial importance next only to rose. Carnation is preferred for export owing to its excellent keeping quality, wide range of forms, colours and ability to withstand long distance transportation. Cut carnations, roses and chrysanthemums contribute close to 50% of the world cut flower trade (Jawaharlal *et al.,* 2009). The most important phytopathological problem affecting carnation in most areas of the world is *Fusarium* wilt, caused by *Fusarium oxysporum* f. sp. *dianthi*. *Fusarium* wilt is prevalent in 79 % of the national production areas of carnation and affects 45 % of its total production (Anonymous, 2006).Wilt disease is being controlled through systemic fungicides, it leads to health hazards, environmental pollution and toxicity besides reducing the population of beneficial microorganisms in soil. Hence it is obligatory to find out some alternate methods to reduce the usage of chemical fungicides. The present day global interest in control of plant pathogens by biocontrol agents has direct impact on economic assistance to farmers. Many research efforts have been made to demonstrate the significance of biocontrol agents in the rhizosphere and on the phyllosphere. *Trichoderma* spp. are economically important, in part because of their mycoparasitic ability and volatiles which makes them suitable for application as biocontrol agents against soil-borne plant-pathogenic fungi (Benitez *et al*., 1998; Manczinger *et al*., 2000).*Trichoderma* spp., are effective in control of soil/seed borne fungal diseases in several crop plants (Kubicek *et al.,* 2001). Major mechanisms involved in the biocontrol activity of *Trichoderma* spp., are competition for space and nutrients, production of diffusible and/or volatile antibiotics, and hydrolytic enzymes like chitinase and â-1, 3-glucanase. These hydrolytic enzymes partially degrade the pathogen cell wall and leads to its parasitization (Kubicek *et al*., 2001). Species of *Trichoderma* species *viz*., *T. harzianum*, *T. viride*, *T. koningii* and *T. virens* showed more mycelial inhibition of pathogenic organisms (Mishra *et al.,* 2004) due to their higher competitive ability. In the present work, use of entophytic fungi *Trichoderma* spp for bioconrol of *Fusarium oxysporum* f. sp. *lentis* (FOL) was studied.

MATERIALS AND METHODS

Isolation of the pathogen

The wilt pathogen *F. oxysporum* f.sp. *dianthi* was isolated from the diseased carnation plants collected during the survey. The infected plant tissues were surface sterilized with 80 per

cent ethanol and transferred on to the potato dextrose agar medium (Peeled potato -250 g, Dextrose -20g, Distilled water1000 mL at pH-7.0) in Petri plate. The Petri plates were incubated at room temperature (28 ± 2^{0}C) and observed periodically for the growth of pure colonies. The pure colonies which developed from the bits were transferred to PDA slants for the maintenance of the culture. The *Fusarium* culture isolated from carnation was identified based on the morphological and cultural characters by Booth (1971).

Isolation of antagonists from the rhizosphere region

Antagonistic fungi were isolated from the rhizosphere soil collected from different carnation growing areas of Tamil Nadu. The plants were pulled out gently with intact roots and the excess soil adhering on roots was removed gently.

Ten gram of rhizosphere soil was transferred to 250 mL ErlenMeyer flask containing 100 mL of sterile distilled water. After thorough shaking, the suspension was used for making further dilutions.From the dilutions of 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}, one ml of aliquot was transferred to sterile petri plates separately followed by pour of *Trichoderma* special medium (TSM). These plates they were gently rotated clockwise and anti -clockwise for uniform distribution and incubated at room temperature (28 $\pm$ 2^{0}C) for 5 days. *Trichoderma* sp. isolated from TSM medium and the culture purified in PDA medium. The pure cultures were maintained on PDA slants at 4^{o}C.

Screening of the fungal antagonists against *F. oxysporum* f. sp. *dianthi in vitro*

Four isolates of *T. viride*, eight isolates *T. harzianum* and five isolates of *T. reesei* were screened against *F. oxysporum* f. sp. *dianthi* by dual culture method (Dennis and Webster, 1971a). A nine mm mycelial disc of *F. oxysporum* f. sp. *dianthi* and each isolate of *Trichoderma* spp. were placed opposite to each other near the periphery of the Petri plate and incubated at room temperature (28 ± 2^{0}C). After five days of incubation, mycelial growth of pathogen and inhibition zone were measured in all the petriplates including control plates. The Per cent Inhibition (PI) of mycelial growth was calculated using the formula suggested by Pandey *et al.* (2000).

Competitive Saprophytic Ability (CSA)

To test the competitive saprophytic ability of *Trichoderma* spp. isolates (Tv1, Tv2, Tv3, Tv4, Th1, Th2, Th3, Th4, Th5, Th6, Th7, Th8, Tr1, Tr2, Tr3, Tr4 and Tr5) the method suggested by Ahmed and Baker (1987) was followed. The isolates of *Trichoderma* spp. grown on molasses yeast medium at room temperature were mixed with unsterilized soil, so as to get a conidial concentration of 10^6 per g of soil. The soil was mixed thoroughly and filled in plastic pots of 10 cm diar. Matured and clean paddy straw was cut into one cm segments. Twenty pieces were buried randomly in each pot. The pots were arranged in a completely randomized design, covered with plastic cover to conserve moisture and incubated in dark. After seven days, all the 20 pieces from each isolate including control were removed from the pots washed in tap water to remove the adhering soil and debris and surface disinfected in a mixture of one per cent sodium hypochloride solution and five per cent ethanol for two min. Segments were placed on a *Trichoderma* selective medium and incubated at room temperature for five days. The per cent colonization of paddy straw pieces by the antagonist was determined. Three replications were maintained per isolate.

Volatile compounds

Effect of volatile metabolites produced by *Trichoderma* spp. (Th1, Th2, Th3, Th4, Th5, Th6, Th7, Th8, Tv1, Tv2, Tv3, Tv4, Tr1, Tr2, Tr3, Tr4 and Tr5) on mycelial growth of *F. oxysporum* f. sp. *dianthi* was studied by Paired Petri dish technique (Laha *et al,*. 1996). A nine mm mycelial disc of four day old culture of *Trichoderma* isolates was placed on PDA medium in a Petri dish. The top of each Petri plate was replaced with bottom of PDA plates inoculated centrally with a nine mm dia mycelial disc of *F. oxysporum* f. sp. *dianthi* and inverted over the antagonist (*Trichoderma* spp.) plates and sealed with parafilm and incubated at room temperature for four days. The PDA plates inoculated with the pathogen alone and paired with PDA plate without biocontrol agents served as control. Three replications were maintained for each isolate. The mycelia growth of the pathogen was measured after incubation period and expressed as per cent inhibition over control.

RESULTS AND DISCUSSION

A total of 17 isolates of *Trichoderma* spp. were isolated from the rhizosphere regions of carnation grown in different areas of Tamil Nadu. *Trichoderma* spp. are effective biological control agents against plant diseases caused by soil borne fungi (Sivan and Chet, 1994; Basim *et al.,* 1999) and attracted considerable scientific attention (Rini and Sulochana, 2007). In the present study, eight isolates of *T. harzianum,* four isolates of *T. viride* and five isolates of *T. reesei* were tested for their antifungal activity against *F. oxysporum* f. sp. *dianthi*. Varying degrees of antagonism were exhibited by these isolates against pathogen in dual culture plates. Among these isolates tested, *T.harzianum* (Th2) significantly exerted the highest per cent mycelial inhibition of 45.33% over control and this was followed by *T. harzianum* (Th6) and *T. viride* (Tv3) which recorded of 42.11 and 38.66 per cent inhibition of mycelial growth over control respectively. The isolate *T. reesei* (Tr5) showed the least mycelial growth inhibition of

Table 1. Antifungal activity of *Trichoderma* sp. against growth of *F. oxysporum* f. sp. *dianthi in vitro*

Treatments	Mycelial growth (cm)*	Per cent reduction over control
T.harzianum(Th1)	6.68	25.77
T.harzianum (Th2)	4.92	45.33
T.harzianum (Th3)	6.80	24.44
T.harzianum (Th4)	6.21	31.00
T.harzianum (Th5)	8.05	10.55
T.harzianum (Th6)	5.21	42.11
T.harzianum (Th7)	7.21	19.88
T.harzianum (Th8)	7.34	18.44
T.viride (Tv1)	7.17	20.33
T.viride (Tv2)	7.63	15.22
T.viride (Tv3)	5.52	38.66
T.viride (Tv4)	7.47	17.00
T.ressei (Tr1)	7.84	12.88
T.ressei (Tr2)	6.94	22.88
T.ressei (Tr3)	7.71	14.33
T.ressei (Tr4)	8.16	9.33
T.ressei (Tr5)	8.22	8.66
Control	9.0	-
0.23	**0.79**	

* Mean of three replications

8.66 per cent (Table 1). The results are in line with the results of Manka and Fruzynska's (1989) who reported the effect of *T. viride* and *T. harzianum* against growth of wilt pathogen in carnation. Pratibha Sharma (2000) also reported the efficacy of *T. harzianum* against *F. oxysporum* f. sp. *dianthi*. *In vitro* evaluation of *Trichoderma* spp. against *F. oxysporum* f. sp. *ciceri* revealed positive cumulative effect of *T. viride* + *T. harzianum* + *T.hamatum* in respect to the per cent inhibition of the test fungus (Nikam *et al.,* 2007). Gupta and Misra (2009) reported that *Trichoderma* spp. gave maximum mycelial inhibition of *F. solani.*

Competitive Saprophytic Ability (CSA)

The isolate, Th2 recorded maximum per cent colonization of 61.84 followed by Th6, which recorded of 59.12 per cent colonization (Table 1).The per cent colonization of *Trichoderma* spp. was positively correlated with CSA activity. Therefore, it inferred that Th2 and Th6 were found to have higher level of Competitive Saprophytic Ability. Isolation of *Trichoderma* from baits of plant material buried in field soil provided direct evidence that recovered fungi could colonize these substrates as competitive saprophytes. The increased antagonistic potentiality of the isolates of *T. viride* was attributed to the increased CSA (Locke *et al*., 1984). Dinakaran (1997) reported that *T. viride* mutant M_3 colonized the paddy straw pieces actively at all population levels and it had higher CSA index than other mutants. Thiruvudainambi *et al.* (2010) reported that *T. viride* reduced the mycelial growth of *S. rolfsii* due to its highest competitive saprophytic ability.

Efficacy of volatile compounds produced by *Trichoderma* spp. against *F. oxysporum* f. sp. *dianthi*

Many isolates of *Trichoderma* produced both volatile and non-volatile metabolites that adversely affected growth of different fungi (Dennis and Webster, 1971a, 1971b; Claydon *et al*., 1987; Corley *et al*., 1994). A comparison between the inhibitory effects of volatile metabolites in the present study revealed that the maximum inhibition of the radial growth of *F. oxysporum* f. sp. *dianthi* was due to the volatile metabolites of Th2 (69.11%), which was followed by Th6 (67.88%) and Tv3 (65.33%). The isolate Tr5 showed minimum inhibition of radial growth of the pathogen (39.77%) as compared to other isolates (Table 2). The results indicated that the volatile metabolites seemed to be more effective in the antagonism mechanism. Such findings was also reported by El-Katatny *et al*. (2001) who explained the biocontrol efficacy of *T. harzianum* (T24) against *Sclerotium rolfsii* might be due to overproduction of volatile metabolites.

Table 2. Competitive saprophytic ability of *Trichoderma* sp.

Sl.No	Isolates	Per cent colonization *
1	*T.harzianum* (Th1)	49.35
2	*T.harzianum* (Th2)	61.84
3.	*T.harzianum* (Th3)	46.12
4.	*T.harzianum* (Th4)	52.78
5.	*T.harzianum* (Th5)	34.71
6.	*T.harzianum* (Th6)	59.12
7	*T.harzianum* (Th7)	43.02
8	*T.harzianum* (Th8)	41.71
9	*T.viride* (Tv1)	43.52
10	*T.viride* (Tv2)	37.85
11	*T.viride* (Tv3)	56.75
12	*T.viride* (Tv4)	41.56
13	*T.ressei* (Tr1)	35.41
14	*T.ressei* (Tr2)	45.21
15	*T.ressei* (Tr3)	36.12
16	*T.ressei* (Tr4)	32.85
17	*T.ressei* (Tr5)	30.59
18	Control	0.0
CD (P=0.05)		**0.84**

* Mean of three replications

Table 3. Efficacy of volatile compounds production by *Trichoderma* sp. against the growth of *F. oxysporum* f. sp. *dianthi in vitro*

Isolates	5 DAI		10 DAI	
	Mycelial growth (cm)*	Per cent reduction over control	Mycelial growth (cm)*	Per cent reduction over control
T.harzianum (Th1)	3.65	59.44	5.14	42.88
T.harzianum (Th2)	2.78	69.11	4.49	50.11
T.harzianum (Th3)	3.81	57.66	5.65	37.22
T.harzianum (Th4)	3.36	62.66	5.26	41.55
T.harzianum (Th5)	5.10	43.33	6.75	25.00
T.harzianum (Th6)	2.89	67.88	4.78	46.88
T.harzianum (Th7)	4.01	55.44	6.01	33.22
T.harzianum (Th8)	4.12	54.22	5.98	33.55
T.viride (Tv1)	3.98	55.77	5.89	34.55
T.viride (Tv2)	4.57	49.22	6.78	24.66
T.viride (Tv3)	3.12	65.33	5.07	43.66
T.viride (Tv4)	4.49	50.11	6.23	30.77
T.ressei (Tr1)	4.68	48.00	6.49	27.88
T.ressei (Tr2)	3.87	57.00	5.36	40.44
T.ressei (Tr3)	4.66	48.22	6.85	23.88
T.ressei (Tr4)	5.24	41.77	7.01	22.11
T.ressei (Tr5)	5.42	39.77	7.53	16.33
Control	9.0	0.0	9.0	0.0
CD(P=0.05)	**0.23**		**0.20**	

* Mean of three replications; DAI-Days After Incubation

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*** P. Mahalakshmi and I. Yesu Raja**
Department of Plant Pathology, Agricultural College and Research Institute, Madurai -625 104, Tamil Nadu, India.
*Communication author e-mail: magaagri_19@rediffmail.com.

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