Pest Management in Horticultural Ecosystems
Vol. 26, No.2 pp 245-250 (2020)

DOI number: 10.5958/0974-4541.2020.00001.6

Efficacy of bioagents and fungicides against *Fusarium oxysporum* f. sp. *dianthi*

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ABSTRACT: Studies were carried out to evaluate the bio-efficacy of twelve fungicides and nine bio-agents against *Fusarium oxysporum* f. sp. *dianthi* under *in vitro* conditions. Among fungal and bacterial bioagents tested, *Trichoderma viride, Trichoderma harzianum, Streptomyces viridobrunneus* and *Bacillus amyloliquefaciens* were found to be most effective in limiting radially growth of pathogen. In case of different fungicides tested, Tricyclazole 75 WP in all concentration was found to be the effective fungicide with complete inhibition of the radial growth.

Keywords: Carnation, bio-agents, *Fusarium oxysporum* f. sp. *dianthi,* fungicides

INTRODUCTION

Carnation (*Dianthus caryophyllus* L.) belongs to family Caryophyllaceae is one of the important commercially grown flowers in the world. Wilt caused by *Fusarium oxysporum* f.sp. *dianthi* is the most prevalent disease occurring around the world. There are eleven races of pathogen within this forma special is reported from around the world, of which Race 2 was found worldwide causing serious damage to the carnation cultivation (Castano *et al.,* 2014, Manicom *et al.,* 1990). The fungus is primary soil inhabitant and has potential ability to cause significant reduction in yield and flower quality (Jacob and Krebs, 1985). Generally carnation is cultivated under polyhouse conditions, which is more congenial environmental conditions for fungal inoculums development due to continuous availability of host plant throughout the year making it difficult for disease management. There are several control measures such as soil disinfestation, use of partially resistant cultivar, raised bed cultivation, fungicides and bioagents application that has been well documented for management of wilt disease in carnation, but there are no labelled fungicides registered for Fusarium management in carnation (http://ppqs.gov.in/sites/approved use of fungicide.pdf). Whatever, few fungicides are used regularly for control of wilt disease management reports of fungicide resistance developed in Fusarium in polyhouses (Chen *et al.,* 2014; Xu *et al.,* 2019). Other than fungicides use of bioagents and crop rotation is more useful in keeping the pathogen inoculum under check. However, in case of carnation cultivation, crop rotation is difficult as the crop remains in polyhouse for 2-3 years. Use of bioagents during land preparation can help not only reducing the inoculums build up but they also help in increasing immunity of the host. Currently, a total of 14 bacteria and 12 fungi have been registered with the EPA for the control of plant diseases (Fravel, 2005). Major fungal and bacterial bioagents widely used are *Trichoderma harzianum, Trichoderma viride, Bacillus subtilis and Pseudomonas flourescens*. Though there is a gap to explore other bioagents which can be utilized in different management strategies. Hence, the aim of the present study is to test the *in vitro* efficacy of new fungicides and bioagents against Fusarium wilt which can be used to contain the disease.

MATERIALS AND METHODS

Fusarium oxysporum f.sp. *dianthi* was isolated from infected carnation stem and root samples collected from poly-house near Bengaluru. For isolation of pathogen standard plant pathological procedures were followed. After isolation the pathogen was incubated at 25°C. The pure culture of the fungus was further transferred to PDA plates and maintained until further use. The pathogen associated with wilt disease of carnation was confirmed based on morphological and PCR based *Tef-1* sequencing. The sequence was submitted in NCBI database under accession number MT707624.

Collection of fungal and bacterial bio-agents

The fungal bioagents included *T. harzianum* and *T. viride*; Bacterial bioagents: *Pseudomonas fluorescens*, *Bacillus subtilis*, *Bacillus pumulis*, *Bacillus amyloliquifaciens*, *B. aryabhattai, Pseudomonas taiwanensis* and three species of actinomycetes *Streptomyces viridobrunneus, S. griseorubens* and *S. bulli* against pathogen by dual culture plate method. All the pure cultures of the bioagents were collected from ICAR-Indian Institute of Horticultural Research, Bengaluru (Table 1). The fungal bioagents were cultured on Potato Dextrose Agar and bacterial bioagents on Nutrient Agar and actinomycetes on Kenknight media incubated at 25°C for further use (Kenknight and Muncie, 1939).

Evaluation of bioagents against *Fusarium oxysporum* f.sp. *dianthi*

The antagonistic activity of bioagents was tested against *Fusarium oxysporum* f.sp. *dianthi* by following dual culture technique (Dennis and Webster, 1971) for fungal bioagents Potato Dextrose Agar was used as the medium. The culture plugs (7 days old) were placed in opposite end of the petriplates and each treatment was replicated four times for each bioagent with suitable control incubated at 25±2 °C for 7 days. The radial growth of the pathogen was recorded and the percent inhibition was calculated by using following formula (Vincent, 1927).

$$R= T_0-T_1/T_0X100$$

R = Per cent growth reduction of test pathogen

T_0 = Radial growth of test pathogen in control (mm)

T_1 = Radial growth of test pathogen in treatment (mm)

The antagonistic activity of the six bacterial biocontrol agents was tested against *Fusarium oxysporum* f.sp. *dianthi*. A gentle superficial streak was made at one side of the sterilized petriplate on Nutrient Agar by means of a sterilized inoculation needle. 9mm mycelial disc of virulent strain of the pathogen was placed on the opposite side of the petridish perpendicular to the bacterial streak. Three replications were maintained for each bacterial antagonists and a control was maintained by inoculating the pathogen alone containing NA medium. The plates were incubated at 25±2˚C for seven days. The per cent reduction over control was calculated by using the formula mentioned above. *In vitro* evaluation of actinomycetes was also carried out by following the same method described for bacterial bioagents on Kenknight media.

Evaluation of fungicides against *Fusarium oxysporum* f. sp. *dianthi*

Twelve systemic fungicides were assayed for their efficacy against *Fusarium oxysporum* f.sp. *dianthi* under *in vitro* condition by poisoned food technique at 3 concentrations (500 ppm, 1000 ppm and 1500 ppm). Three replications were maintained for each concentration. Required quantity of fungicides were thoroughly mixed in Potato Dextrose Agar media before pouring in 90mm sterilized petriplates and allowed to solidify. The plates were inoculated at centre with 5 mm mycelia disc of 7 days old culture of *Fusarium oxysporum* f.sp. *dianthi*. Control without fungicide in media was also maintained. The inoculated petriplates were incubated at 25±2°C. The colony diameters were measured after 10 days when the control plates were full of fungal growth. Per cent inhibition of growth was calculated by using formula given by Vincent (1927).

I = C-T/C X 100

I = Percent inhibition of mycelia growth

C = Colony diameter in control (mm)

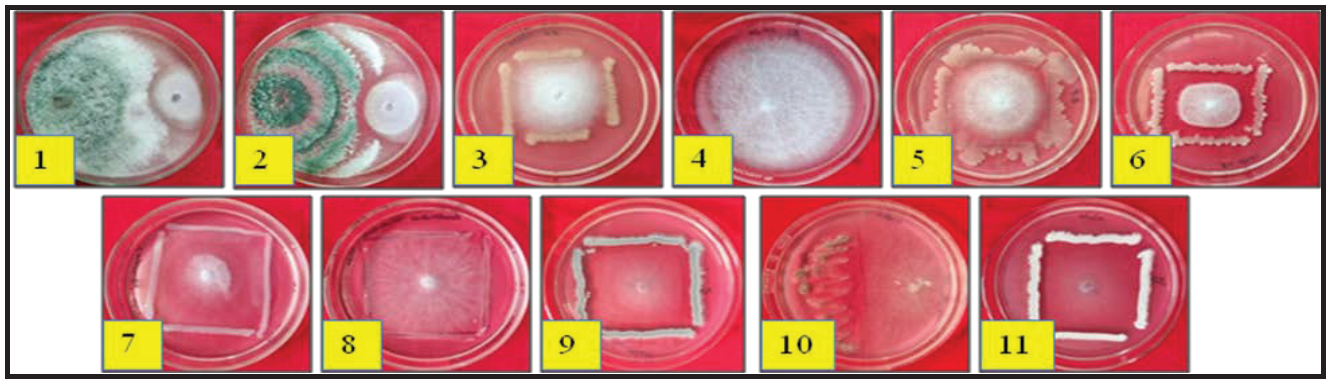
T = Colony diameter in treatment (mm)

RESULTS AND DISCUSSION

Evaluation of Bioagents against *Fusarium oxysporum* f.sp. *dianthi*

Eleven bioagents *viz. Trichoderma harzianum*, *T. viride, Pseudomonas fluorescens*, *Bacillus subtilis, Bacillus pumulis, Bacillus amyloliquefaciens, B. aryabhattai*,*P. taiwanensis*,*Streptomyces viridobrunneus*, *Streptomyces griseorubens* and *Streptomyces bulli* were evaluated *in vitro* for their antifungal activities against Fusarium wilt disease of carnation, caused by *Fusarium oxysporum* f. sp. *dianthi* by using dual culture technique (Fig.1 and Table 1).

Results revealed that only few bioagents evaluated, exhibited fungistatic/antifungal activity against *Fusarium oxysporum* f. sp. *dianthi* and significantly inhibited its growth, over untreated control. Of the bioagents/ antagonists tested, *Trichoderma viride* was found most effective with mycelial growth inhibition of (86.50%). In later stage *Fusarium oxysporum* f. sp. *dianthi* mycelia growth was completely covered by fungal bioagents. *Trichoderma harzianum* was also found to suppress radial growth with inhibition percentage of 82.60 percent. In the study undertaken with bacterial bioagents, *Bacillus amyloliquefaciens* was found to be highly infective in inhibiting radial growth (85.14 per cent) compared to other bioagents. Whereas other, *Bacillus* and *Pseudomonas* species were found to less or ineffective in suppressing the mycelial growth of the pathogen. In three species of actinomycetes tested *Streptomyces viridobrunneus* (62.24) was found to be most effective in limiting radially growth of pathogen but other two species *i.e. Streptomyces griseorubens* and *Streptomyces bulli* were less effective. The results are in accordance with the findings of Kishore and Kulkarni, (2008) they reported that *T. viride* (73.89) and *T. harzianum* (73.66) as best treatment in inhibiting mycelial growth of *Fusarium oxysporum f. sp. dianthi* in *in vitro* studies. Krishna *et al.,* 2019 reported *Trichoderma harzianum* as significantly superior over other bioagents in arresting the growth



T. harzianum, , 2. *T. viride*, 3. *P. fluorescens*, 4. *B. subtilis*, 5. *B. pumilus*, 6. *B. amyloliquefaciens*, 7. *B. aryabhattai*, 8. *P. taiwanensis*, 9. *S. viridobrunneus*, 10. *S. griseorubens*, 11. *S. bulli*

Fig. 1. *In vitro* studies on efficacy of Bioagents against *Fusarium oxysporum* f.sp. *dianthi*

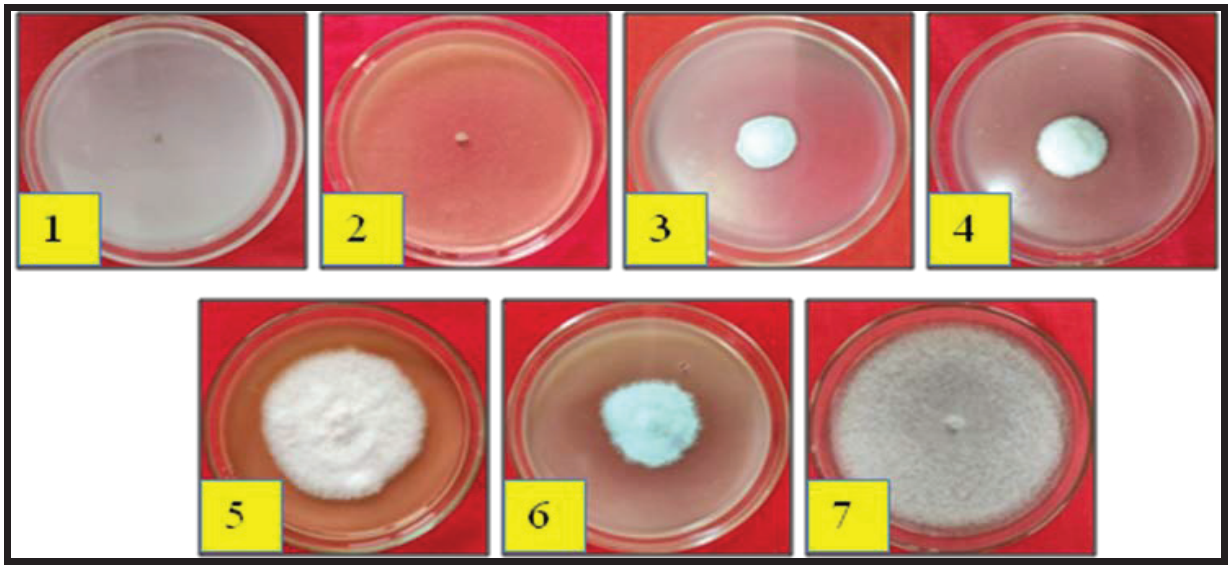
Table 1. *In vitro* studies on efficacy of Bioagents against *Fusarium oxysporum* f. sp. *dianthi*

Treatment	Strain number	Mycelial growth Inhibition (%)
Trichoderma harzianum	IIHR-Th-2	82.60 (65.41)
Trichoderma viride	IIHR-Tv-5	86.50 (68.49)
Pseudomonas fluorescens	IIHR-Pf-2	12.35 (20.57)
Bacillus subtillis	IIHR-Bs-2	12.88 (21.03)
Bacillus pumilus	IIHR-Bp-5	12.83 (20.98)
Bacillus amyloliquefaciens	IIHR-Ba-2	85.14 (67.41)
B. aryabhattai	Bel 6	11.50 (19.83)
P. taiwanensis	Mpf2	11.49 (19.78)
Streptomyces viridobrunneus	Pan Act1	62.24 (52.12)
Streptomyces griseorubens	Pan Act3	12.17 (20.37)
Streptomyces bulli	Pan Act2	14.33 (22.25)
Mean		36.73 (36.20)
S.Em.±		0.84
CD at 1%		3.36

of pathogen and exhibited 90.06 per cent inhibition against *Fusarium oxysporum* f. sp. *callistephi*. In *in vitro* studies carried out by Sharma (2019) both the species of *Trichoderma, T.viride* and *T. harzianum* were found effective against the wilt pathogen with 67.5 and 65.6% inhibition, respectively. Though there are no reports of using *Bacillus amyloliquefaciens* and *Streptomyces viridobrunneus* in managing *Fusarium oxysporum* f. sp. *dianthi* but the bioagents were evaluated against other fungal pathogens. Vitullo *et al.,* (2012) demonstrated the antifungal activity of purified surfactin from *B. amyloliquefaciens*, which suggested an important role of this molecule in the biocontrol of *F. oxysporum*. *Bacillus amyloliquefaciens* S76-3 isolated from diseased wheat spikes has strong antagonistic activity against *F. graminearum* (Gong *et al.,* 2015). (Yuan *et al.,* 2012) reported that *Bacillus amyloliquefaciens* NJN-6 produces numerous volatile compounds (VOCs) that restrict growth and spore germination of *F. oxysporum* f. sp. *cubense*. Vaidya *et al.,*

Table 2. *In vitro* studies on efficacy of Fungicides against *Fusarium oxysporum f.sp. dianthi*

Fungicide	Per cent inhibition at concentration (%)			
	500ppm	**1000ppm**	**1500ppm**	**Mean**
Carbendazim 50 WP	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
Tricyclazole 75 WP	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)
Propiconazole 25 EC	72.07 (58.14)	72.70 (58.55)	74.50 (59.72)	73.09 (58.80)
Tebuconazole 25 EC	85.03 (82.78)	86.71 (84.14)	86.95 (84.32)	86.23 (83.74)
Thiophanate methyl 70 WP	25.93 (30.63)	31.14 (33.95)	38. 07 (38.12)	31.71 (34.23)
Captan 50 WP	71.37 (69.63)	71.78 (70.06)	73.39 (71.47)	71.02 (70.38)
Pyraclostrobin 20 WG	27.22 (31.46)	30.95 (33.82)	34.97 (36.27)	31.04 (33.85)
Mean	68.80 (64.67)	70.46 (65.80)	72.55 (67.14)	70.44 (65.87)
	Fungicides (F)	Con.(C)	F x C	
SEm±	16.60	4.13	0.47	
CD 1 %	2.24	1.37	3.38	



1. Carbendazim 50 WP, 2. Tricyclazole 75 WP, 3. Propiconazole 25 EC, 4. Tebuconazole 25 EC, 5. Thiophanate methyl 70 WP, 6.Captan 50 WP, 7. Control

Fig. 2. *In vitro* studies on efficacy of Fungicides against *Fusarium oxysporum* f.sp. *dianthi*

Table 2. *In vitro* studies on efficacy of Fungicides against *Fusarium oxysporum f.sp. dianthi*

2004 reported *Streptomyces* spp. and *Trichoderma* spp., isolated from the rhizosphere soils of various crops, were screened by dual culture and cell free culture filtrate techniques against *Fusarium oxysporum* f. sp. *dianthi* and *F. oxysporum* f. sp. *gladioli,* and found that most of the isolates exhibited considerable difference in their antagonism against *Fusarium* sp.

Evaluation of different fungicides against *Fusarium oxysporum f.sp. dianthi*

A total of 12 systemic fungicides (@ 500 ppm, 1000 ppm and 1500 ppm) Carbendazim 50 WP, Tricyclazole 75 WP, Propiconazole 25 EC, Difenconazole 25 EC, Tebuconazole 25 EC, Thiophanate methyl 70 WP, Captan 50 WP, Pyraclostrobin 20 WG, Chlorothalonil 75% WP, Hexaconazole 5% EC, Kresoxim-methyl 44.3% SC and Thifluzamide 24% SC were evaluated *in vitro* by poisoned food technique (Fig.2 and Table 2). It is evident from the result that most of the fungicides were found to be insignificant in inhibited the radial growth and sporulation of *Fusarium oxysporum* f. sp. *dianthi*. However, Tricyclazole 75 WP in all concentration was found to be the best fungicide which completely inhibited the radial growth of *Fusarium oxysporum* f. sp. *dianthi* after incubation. Carbendazim 50 WP is widely used for management of Fusarium wilt therefore it was used as negative check. Tebuconazole 25 EC (86.23), Propiconazole 25 EC (73.09), and Captan 50 WP (71.02) were second next in order for percent inhibition of radial growth. There was no inhibition recorded in Difenconazole 25 EC, Kresoxim methyl 44.3 SC, Thifluzamide 24 SC fungicides.

The result is in accordance with work carried out by other workers Hegde, *et al.,* 2017 reported Carbendazim, Propiconazole, Difenconazole as best systemic fungicides against *Fusarium oxysporum* f. sp. dianthi in an experiment carried out under in vitro conditions. Barhate *et al*., (2015) reported Mancozeb + Carbendazim (0.125 + 0.05 %) had completely inhibited (100 %) mycelial growth of the pathogen followed by Thiram + Carbendazim (0.15 + 0.05 %), Carbendazim (0.1 %), Thiram (0.3 %), Carboxin (0.2 %), Captan (0.25 %), Propiconazole (0.2 %), Mancozeb (0.25 %) with 93.75, 92.50, 90.00, 87.50, 81.25, 67.50 and 62.50 per cent growth inhibition over control, respectively. In an experiment conducted by Sharma and Raj, 2019 combination of (Carbendazim 12% + Mancozeb 63%) was found most effective with average inhibition of 60.19 per cent in mycelial growth.

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MS Received 28September 2020
MS Accepted 30ctober 2020