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Doi: HTTPS://DOI.ORG/10.23910/IJEP/2019.6.4.0339

Prevalence and Incidence of Fusarium Wilt (*Fusarium oxysporum* f.sp. *dianthi*) of Carnation and its Management through Microbial Antagonists

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Article History

Article ID: IJEP0339
Received in 12th November, 2019
Received in revised form 18th November, 2019
Accepted in final form 22nd November, 2019

Abstract

In the present study, field survey was carried out in different carnation growing areas of Himachal Pradesh to record the incidence of wilt in polyhouses. During the survey of the disease, 12.9-37.35% incidence of wilt was recorded. Different soil antagonists were tested against the Fusarium wilt of carnation caused by *Fusarium oxysporum* f.sp. *dianthi.* Native fungal antagonistic species of *Trichoderma* viz., *T. harzianum, and T. viride* and four bacterial antagonists i.e. *Bacillus subtilis, Brevibacillus brevis, Azotobacter chroococcum* and *Pseudomonas fluorescens* were evaluated under *in vitro* conditions and all exhibited their inhibitory effect against the test pathogen. Both the species of *Trichoderma* were found effective against the wilt pathogen with 67.5 and 65.6% inhibition, respectively. Among bacterial antagonists, *A. chroococcum* was found most effective with 57.7% inhibition. In field evaluation, *T. viride* was found most effective among all the treatments which resulted in reduction of the incidence of carnation wilt to 14.6% in comparison to 45.3% in control.

Keywords: Antagonists, *Azotobacter chroococcum,* Fusarium wilt, *T. viride*

1. Introduction

Carnation (*Dianthus caryophyllus* L.) is one of the major cut flower grown all over the world. Fusarium wilt is most prevalent disease in carnation caused by *Fusarium oxysporum* f. sp. *dianthi* (*Fod*) and up to 79% incidence has been recorded in different parts of the Himachal Pradesh (Chandel and Katoch, 2001). Eight races have been reported within this forma specialis by Garibaldi (Garibaldi, 1983) and Race 2 is found worldwide (Manicom et al., 1990). The fungus is soil inhabitant and has the potential ability to cause significant yield reduction and loss in flower quality (Jacob and Krebs, 1985). Wilt of carnation is a soil- borne disease and these diseases are difficult to control due to complex soil eco-system. Attempts have been made to control the pathogen by drenching the affected field with chemical fungicides (Biswas et al., 2004). However, the continuous use of fungicides adversely affects the soil eco-system. The present study was undertaken to record the incidence of Fusarium wilt of carnation in different carnation growing areas of Himachal Pradesh and to determine *in vitro* and *in vivo* efficacy of fungal and bacterial antagonists against the wilt pathogen (*F. oxysporum* f.sp. *dianthi*).

2. Materials and Methods

A survey of different polyhouses in carnation growing areas in Solan, Sirmaur, Bilaspur, Kullu, Mandi and Chamba districts was carried out to record the incidence of Fusarium wilt in the crop. The survey was conducted during July in 2010 and 2011. The areas surveyed were Mahog, Solan and Nauni in district Solan; Rajgarh in district Sirmaur; Namhol and Chandpur in district Bilaspur; Tisa in district Chamba and Sundernagar in district Mandi. Disease incidence (%) was calculated by the formula given below:

Disease incidence (%)=(Number of diseased plants÷Total number of plants observed)×100

During survey, the diseased samples i.e. wilted plants along with roots and rhizosphere soil were collected from the disease surveyed areas and were kept in the paper bags and these samples were then brought to laboratory for the isolation of associated pathogen.

The isolations were taken from roots and collar region of those infected plants which were showing the characteristic wilt symptoms of the disease. Small bits of 1 to 2 mm size were taken from the juncture of diseased and healthy portion of roots, with the help of a sterilized blade. These bits were surface sterilized with sodium hypochlorite (1%) for 10 to 20 seconds and were then washed thrice with sterilized distilled water under aseptic conditions. The bits were then placed on the sterilized filter paper to remove the excess moisture and were subsequently transferred to sterilized Petri plates containing Potato Dextrose Agar (PDA) medium. The medium was supplemented with streptocyclin (30 mg l^{-1}) while pouring it in Petri plates after sterilization (autoclaved at 1.05 kg cm^{-2} for 20 minutes). The inoculated Petri plates were incubated at 27±1 °C in BOD incubator and examined daily for the growth of the mycelial growth. The fungal growth developed in Petri plates was purified by hyphal tip technique and was further cultured on test tube slants containing PDA. The isolated fungus was identified as *Fusarium oxysporum* f.sp. *dianthi* in accordance with key "The Genus *Fusarium*" given by Booth (1971).

Two native fungal antagonistic species of *Trichoderma* viz., *T. harzianum* and *T. viride* and four bacterial antagonistic species viz., *Bacillus subtilis, Brevibacillus brevis, Azotobacter chroococcum* and *Pseudomonas fluorescens* were procured from the Department of Mycology and Plant Pathology, Nauni, Solan (H.P.). Fungal antagonists were tested for their antagonistic activities against *F. oxysporum* f.sp. *dianthi* by dual culture technique (Huang and Hoes, 1976). Culture discs (4 mm diameter) of each of the antagonists and the pathogen were taken from margin of their vigorously growing culture and transferred aseptically to solidified PDA contained in Petri plates (90 mm) with the opposite side facing each other at a distance of 1 cm from the margin of the plate. The Petri plate containing only mycelial bit of the pathogen served as control. Each treatment was replicated thrice and the Petri plates were incubated at 27±1 °C in BOD incubator. The colony diameter of test fungus was recorded till the control plates were full with mycelium of the test pathogen.

Antagonistic activity of *Bacillus subtilis, Brevibacillus brevis, Azotobacter chroococcum* and *Pseudomonas fluorescens* against the wilt pathogen was observed by streak plate method (Utkhede and Rahe, 1983). The Petri plates containing sterilized PDA were streaked at the c entre with 48 hours old colonies of bacteria with the help of bacterial loop. Mycelial bit (4 mm diameter) of the test pathogen was placed on opposite sides of the streak at a distance of 1 cm from the margin of the plate. Petri plate without bacterial streak but with only mycelia bits served as control for comparison. Each treatment was replicated thrice and incubated at 27±1 °C in BOD incubator. Mycelial inhibition (%) in the growth of the test pathogen was calculated according to formula given by Vincent (1947):

$I=(C-T)\div C\times 100$

Where,

I - % inhibition of mycelial growth

C - Linear mycelial growth in control (mm)

T - Linear mycelial growth in treatment (mm)

The experiment was also laid out in Randomized Block Design in the polyhouse of the experimental farm to know the effect of these fungal and bacterial antagonists on disease incidence, important plant growth characteristics and quality parameters of the flowers. All the biocontrol agents were used and applied before planting at the rate of 1% i.e. by mixing 10 g of the formulation in 1 kg FYM per bed of 1×1 m^2 size by thoroughly mixing into the soil. Carnation cuttings of variety 'Master' were planted at a distance of 20×20 cm with 25 cuttings per bed. Data pertaining to wilt incidence (%) and plant growth and quality parameters viz., plant height (cm), number of days taken for first flowering (days), number of flowers per $plant^{-1}$, length of flowering stem (cm), flower size (cm) and flowering period (days) were recorded by selecting 5 plants per replication in each treatment.

3. Results and Discussion

3.1. Disease survey

In the present study, incidence of wilt was found serious in almost all the locations surveyed. In 2010, maximum incidence of 36.4% of the wilt was recorded at Chandpur in district Bilaspur followed by Rajgarh 30.0% in district Sirmaur in contrast to lowest incidence 11.2% at Tisa in district Chamba. Similarly in 2011, maximum incidence (38.3%) of wilt was recorded at Chandpur in district Bilaspur followed by Rajgarh (32.67%) in district Sirmaur in contrast to lowest incidence (14.7%) at Tisa in district Chamba (Table 1).

Table 1: Incidence of Fusarium wilt of carnation in different parts of Himachal Pradesh during 2010 and 2011

District	Locality	Disease incidence (%)		Mean	Over all mean
		2010	2011		
Solan	Mahog	20.7	25.11	22.90	24.63
	Nauni	26.1	23.88	24.99	
	Solan	24.7	27.3	26.00	
	Mean	23.83	25.43		
Sirmour	Rajgarh	30.00	32.67	31.33	31.33
Chamba	Tisa	11.2	14.7	12.95	12.95
Bilaspur	Chandpur	36.4	38.3	37.35	33.90
	Namhol	28.3	32.6	30.45	
	Mean	32.35	35.45		
Kullu	Kullu	15.4	17.2	16.3	16.30
Mandi	Sundar nagar	32.3	29.4	30.85	30.85

Wilt of carnation was more severe in low sub-mountainous and warmer regions of the state. The incidence of the wilt at higher elevations like Tisa in district Chamba was recorded lower (12.95%) and this can be attributed to prevailing of comparatively lower temperature in the area, which must have restricted the multiplication and spread of the pathogen in soil resulting in slow progress of the disease. Temperature of 25-30 °C has been reported optimum for the growth of the *F. oxysporum* f.sp. *dianthi* on PDA and also spread of the disease (Etebarian, 1996). Ben-Yephet and Shtienberg (1994) reported that soil temperature of 25-26 °C resulted in severe wilt epidemics in carnation and at temperature below 18 °C plants remained symptomless. Negi (2009) has also reported serious incidence of wilt carnation at Berthin (34.7%) in district Bilaspur followed by Rajgarh (31.0%) in District Sirmaur and lowest in Tisa (11.2%) in district Chamba of Himachal Pradesh.

3.2. In vitro evaluation of fungal and bacterial antagonists

In the present study, antagonistic activities of the native fungal and bacterial microorganisms were determined against the wilt pathogen *F. oxysporum* f.sp. *dianthi.* All the microbial antagonists evaluated under *in vitro* conditions inhibited the growth of wilt pathogen ranging from 50.67 to 67.55% (Table 2). Out of two native species of fungal antagonists, *Trichoderma viride* was found most effective and significantly superior with 67.55% inhibition of the wilt pathogen followed by *Trichoderma harzianum* with 65.63% inhibition. Among bacterial antagonists, *Azotobacter chroococcum* was found most effective with 57.78% inhibition of the mycelial growth of the pathogen. *Brevibacillus brevis* was found least effective among all treatments with 50.67% inhibition of the mycelial growth of the test pathogen (Plate 1).

Table 2: *In vitro* efficacy of fungal and bacterial antagonists against the wilt pathogen (*F. oxysporum* f. sp. *dianthi*)

Treatments	Colony diameter of test Pathogen (mm)	Inhibition in mycelial growth (%)
Trichoderma viride	29.2	67.55
T. harzianum	32.8	65.63
Azotobacter chroococcum	39.7	57.78
Pseudomonas fluorescens	39.2	56.67
Bacillus subtilis	41.0	55.74
Bevibacillus brevis	43.2	50.67
Untreated check	90.0	0.0
CD (p=0.05)	1.52	2.69

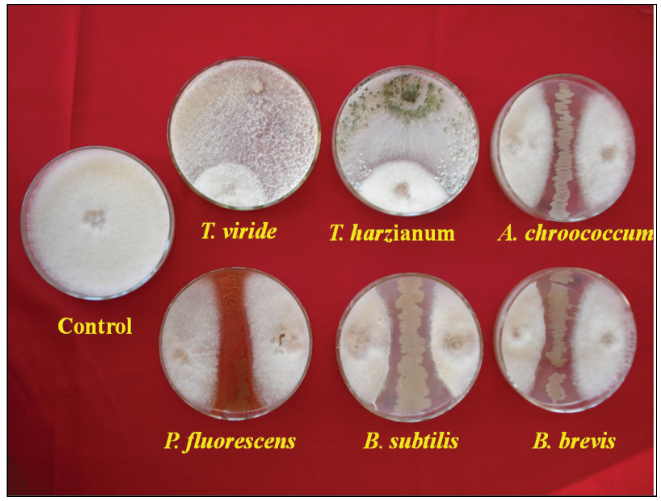

Plate 1: *In vitro* efficacy of fungal and bacterial antagonists against *Fusarium oxysporum* f. sp. *dianthi*

Chandel (2007) reported 76.0 and 70.1% inhibition by *T. harzianum* and *T. viride* in mycelial growth of *F. oxysporum* f. sp. *dianthi* causing carnation wilt, respectively. Negi (2009) reported that among different isolates of *Trichoderma* spp., *T. viride* is the most effective with 67.4% inhibition followed by *T. harzianum* with 63.7% inhibition in mycelial growth of wilt pathogen (*F. oxysporum* f. sp. *dianthi*) of carnation. Among different species of *Trichoderma*, *T. viride* is reported to cause maximum (66.3%) growth inhibition of mycelium *F. oxysporum* followed by *T. harzianum* with 57.4% inhibition (Kahkashan and Bokhari, 2012).

Mechanism for inhibition of growth of the pathogen by *Trichoderma* spp. is that it competes with the pathogen for space and nutrients, it produces the diffusible volatile antibiotics and hydrolytic enzymes like chitinase and α-1, 3- glucanase. These hydrolytic enzymes degrade the cell wall of the pathogen and leads to its parasitization (Kubicek et al., 2001). Inhibitory effect of different species of *Trichoderma* have been reported by various workers against soil-borne pathogens (Pandey and Upadhyay, 2000; Saravanam et al. 2003; Ramesh and Korikanthimath, 2004; Rajendiran et al. 2010).

Bacterial antagonists have also been found very effective in controlling different type of soil-borne pathogens. Agrawal and Singh (2002) reported effectiveness of fourteen strains of *Azotobacter* against *Fusarium oxysporum* under *in vitro* condition. Juber et al. (2014) also reported that the disease management potential of tested control agents i.e. *Tchigarine, A. chroococcum* and *P. fluorescens*, have shown high efficiency against *F. oxysporum* f. sp. *fragariae* on culture media (PDA). These treatments show a significant inhibition in the pathogen multiplication. Inhibitory effects of different strains of *Azotobacter* spp. have been reported by various workers against *Fusarium oxysporum* (Suneja and Lakshinarayana, 1998; Mali and Bodhankar, 2009).

3.3. In vivo evaluation of fungal and bacterial antagonists

Present results revealed that all the bioagents exhibited antagonistic effects against the wilt pathogen *F. oxysporum* f.sp. *dianthi* in the field conditions. *T. viride* was found most effective among all the treatments which resulted in reduction of the incidence of carnation wilt to 14.6% in comparison to 45.3% in control. *T. viride* was also found most effective among all the treatments and resulted in maximum average plant height (61.3 cm), required least number of days (126.7) for 1st flowering and had least flowering period (30.3 days) in comparison to control (Table 3). Second best treatment was *T. harzianum* with 16.6% disease incidence followed by

Table 3: *In vivo* evaluation of microbial antagonists against Fusarium wilt (*Fusarium oxysporum* f.sp. *dianthi*) of carnation

Treatments	Disease incidence (%)	Plant height (cm)	No. of days taken for 1st Flowering	Flowering period (days)
Trichoderma viride	14.6 (22.5)	61.3	126.7	30.3
T. harzianum	16.6 (24.1)	58.7	127.3	31.3
Azotobacter chroococcum	20.0 (26.6)	57.7	133.7	36.0
Pseudomonas fluorescens	19.6 (26.3)	57.7	132.7	36.7
Bacillus subtilis	20.6 (27.0)	57.0	133.0	37.7
Bevibacillus brevis	21.6 (27.7)	55.3	134.7	37.3
Untreated check	45.3 (42.3)	50.3	140.3	47.0
CD (p=0.05)	3.94	2.69	2.69	1.43

A. chroococcum (19.6%). Among the bioagents *T. viride* was found most effective among all the treatments and resulted in maximum number of flowers plant^{-1} (3.16), length of flowering stem of 67.0 cm and flower size of 6.13 cm in comparison to control (Table 4). All the treatments resulted in significant improvement of the different important quality parameters of the carnation flowers in comparison to control.

Table 4: Effect of microbial antagonists on important quality parameters of flowers

Treatments	No. of flowers plant^{-1}	Flower size (cm)	Length of flowering Stem (cm)
Trichoderma viride	3.16	6.13	67.20
T. harzianum	3.08	6.03	66.37
Azotobacter chroococcum	2.78	5.85	62.50
Pseudomonas fluorescens	2.83	5.84	62.37
Bacillus subtilis	2.79	5.72	63.43
Bevibacillus brevis	2.78	5.68	61.83
Untreated check	2.09	5.03	47.43
CD (p=0.05)	0.06	0.16	1.54

These results are in conformity with the findings of several workers. During field evaluation, under normal soil and semi sick soil conditions, treatment with *Trichoderma viride* was found to exhibit minimum wilt incidence i.e. 19.24% and 37.92% and recorded maximum yield i.e. 773.78 and 531.20 kg ha^{-1}, respectively (Ghante et al., 2019). Mahmoud and Abdalla (2018) reported that the highest reduction in disease severity of Sesame wilt was achieved with *T. viride* followed by *T. harzianum* with reduction in disease severity about 77 and 74%, respectively under greenhouse conditions. Harman et al. (2004) reported that *Trichoderma* spp. enhanced root biomass production, produce antibiotics, parasitized other fungi and deleterious plant microorganisms. Similarly, many researchers have also reported inhibitory effects of *A. chroococcum* on the various soil-borne diseases (Maheshwari et al., 2012; Hilal et al., 2001).

4. Conclusion

The incidence of wilt was more severe in warmer areas in comparison to cooler areas based on field surveys conducted in different carnation growing areas of state. Antagonistic activities of the native fungal and bacterial microorganisms were also determined against the wilt pathogen *Fusarium oxysporum* f. sp. *dianthi* under *in vitro* and *in vivo* conditions and *T. viride* was found most effective and significantly superior for the management of wilt pathogen.

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