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Research Article

Biological control of stem rot disease of carnation caused by *Rhizoctonia solani* Kuhn

SUSHMA SHARMA and SUNITA CHANDEL

Department of Plant Pathology. Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni,Solan (H.P.) 173 230, India. Email: sushmasharma1989@gmail.com

ABSTRACT: Five fungal antagonists *viz., Trichoderma harzianum, T. hamatum, T. viride, T. polysporum* and *T. virens* and two bacterial antagonistic species namely *Bacillus subtilis* and *Pseudomonas fluorescens* were evaluated under *in vitro* and field condition against stem rot of carnation variety Rubesco during 2009 and 2010. Under *in vitro* conditions biocontrol agents significantly inhibited the growth of *Rhizoctonia solani* upto 65.08 per cent in case of *Trichoderma viride* and under field conditions they caused significant reduction in stem rot incidence, increase plant growth and quality parameters as compared to untreated control.

KEY WORDS: *Rhizoctonia solani,* fungicides, *Trichoderma* spp., carnation, bacterial antagonists, fungal antagonists

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INTRODUCTION

Rhizoctonia solani is a major pathogen of both greenhouse and field conditions in the warm ornamental growing areas of the world. *R. solani* is essentially a soil-borne pathogen which inflicts heavy crop losses under favourable conditions (Mathur *et al*., 1995). The management of this disease is difficult owing to the long saprophytic survival ability of pathogen in soil. Fungicidal application as seed or soil treatment, however, has been found to be ineffective against these pathogens as the propagules are capriciously distributed in the soil and often beyond the reach of chemicals. Biological control, therefore, holds promise as a strategy for disease management and it is environment friendly too. Antagonist fungi especially *Trichoderma* spp. and the bacteria, fluorescent *Pseudomonas* have been widely used against a number of phytopathogens (Rini and Sulochana, 2007). In recent years, attempts were also made to use a consortium of biological agents to get persistent control of plant pathogens. Keeping this in view and the growing importance of biological control agents, the present study was carried out the objective to evaluate the biocontrol efficiency of native isolates of *Trichoderma* spp., fluorescent *Pseudomonas* and *B. subtilis* against stem rot pathogen and to study their impact on disease and growth parameters.

MATERIALS AND METHODS

Five different native fungal antagonist species of *Trichoderma viz., T. harzianum, T. hamatum, T. viride, T. polysporum* and *T. virens* and two bacterial antagonistic species namely *Bacillus subtilis* and *Pseudomonas fluorescens* were procured from the Department of Plant Pathology and Department of Basic Science of Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan (H.P.). Fungal antagonists (*Trichoderma* spp.) were tested for their antagonistic activities against stem rot by dual culture technique as adopted by Huang and Hoes (1976). Culture discs (4 mm diameter) of each of antagonists and the pathogen were taken from margin of their vigorously growing culture and transferred aseptically to solidified PDA (Potato Dextrose Agar) contained in Petri plates (90 mm) on the opposite side facing each other at a distance of 1 cm from the margin of the plate. The Petri plates containing only culture of the pathogen served as control. The experiment was laid out in CRD and 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 achieved full growth of the test fungus.

The antagonistic activity of *Bacillus subtilis* and *Pseudomonas fluorescens* against the stem rot pathogen was observed by streak plate method (Utkhede and

Rahe, 1983). The Petri plates containing sterilized PDA were streaked at the centre 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 plates without bacterial streak served as control for comparison. Each treatment was replicated thrice under Completely Randomized Design (CRD) and incubated at 27±1°C in BOD incubator. Per cent mycelial inhibition in the growth of test pathogen was calculated as per Vincent (1947).

All the effective fungal bio-control agents except *Bacillus subtilis* and *P. fluorescens* were applied before planting at the rate of 10 per cent i.e. by mixing 10 g of the formulation in 1 kg FYM per bed of 1m × 1m size. Fungal antagonists were applied in solid form by mixing properly in soil before planting of cutting whereas, *Bacillus subtilis* and *P. fluorescens* were used as broth culture at the rate of 1 per cent by mixing 10 ml of the broth culture in 1 litre of sterilized distilled water and applied by dip method. The rooted cuttings of carnation variety 'Rubesco' were dipped in this culture for 30 minutes prior to planting.

After treatment with antagonists, the carnation cuttings were planted at a distance of 20 × 20 cm in 1m × 1m bed with 25 cuttings per bed. The beds without application of any bio-control agents were kept as control. Each treatment was replicated thrice in (RBD) Randomized Block Design. The application of antagonists was repeated at monthly intervals maximum to three times till the bud formation of the crop was achieved with same concentration. The data pertaining to stem rot incidence (%), average plant growth and flower parameters *viz*., plant height (cm), number of flowers per plant, stem length (cm), day of 1st flowering and flower size (cm) were recorded at 10 days intervals as specified earlier and analysed statistically.

RESULTS AND DISCUSSION

All the microbial antagonists evaluated under *in vitro* conditions inhibited the growth of the stem rot pathogen ranging from 51.63 to 65.08 per cent (Table 1). Out of five native species of fungal antagonists *Trichoderma viride* was found most effective and showed significant superiority among all the antagonists tested that resulted in 65.08 per cent inhibition of the stem rot pathogen followed by *T. harzianum* with 63.70 per cent inhibition. Chakraborty and Chatterjee (2008) reported 86.44 per cent growth inhibition of *Fusarium solani* causing wilt of brinjal by *T. harzianum* under *in vitro* condition. Johnson *et al*. (2008) reported *in vitro* inhibition of *F. oxysporum* by *T. viride, T. hamatum, T. harzianum* and *T. koenigi.*

Table 1. *In vitro* efficacy of antagonists against the stem rot pathogen (*Rhizoctoniasolani*)

Antagonists	Per cent inhibition in mycelial growth
Trichoderma viride	65.08
T. hamatum	56.32
T. harzianum	63.70
T. polysporum	53.18
T. virens	51.63
Bacillus subtilis	55.05
Pseudomonas fluorescens	52.26
P = 0.05	**0.38**

Out of two bacterial antagonists, *Bacillus subtilis* was found better than *Pseudomonas fluorescens* and inhibited the mycelial growth upto 55.05 per cent. While, *T. virens* and *P. fluorescens* were found least effective among all treatments with 51.63 and 52.26 per cent inhibition. Out of these antagonists, four fungal *(T. viride, T. hamatum, T. harzianum, T. polysporum)* and one bacterial *(B. subtilis)* antagonist were further tested under field conditions. Khan and Khan (2002) reported that root dip application of *B. subtilis, P. fluorescens, Aspergillus awamori, A. niger* and *Penicillium digitatum* resulted in significant decline in the rhizospheric population of *F. oxysporum* f. sp. *lycopersici* causing wilt of tomato. Karimi *et al*. (2007) found strain E121 of *B, subtilis* and strain E130 of *P,fluorescens* as most effective in inhibiting the mycelial growth of *F. oxysporum* f. sp. *dianthi* the cause of wilt of carnation by production of non volatile and volatile metabolites under laboratory conditions.

The result of field experiment during 2009-10 of different potential antagonists on disease incidence and different plant growth and flower parameters are presented in Table 2. It is evident from the data that all the treatments significantly reduced the stem rot incidence and also resulted in improvement of the different growth and flower parameters of the carnation in comparison to control. Among fungal antagonists *T. viride* and *T. harzianum* were found most effective among all the treatments which reduced the incidence of carnation stem rot to 17.33 and 20.00 per cent in comparison to 40.33 per cent in control as both the treatments were found statistically at par with each other. However,

Table 2. Effect of antagonists on incidence of stem rot, plant growth and flower parameters of carnation

Antagonists	Conc. (%)	Disease incidence (%)	Plant height (cm)	Stem length (cm)	No. of days taken for 1st flowering	No. of flowers / plant	Flower size (cm)
Trichoderma viride	1%	17.33 (24.57)	71.80	65.57	133.20	3.53	6.24
T. hamatum	1%	26.67 (31.08)	67.20	64.27	133.20	3.47	6.21
T. harzianum	1%	20.00 (26.49)	69.40	60.60	137.60	3.27	5.82
T. polysporum	1%	37.33 (37.66)	62.80	56.46	143.05	2.87	5.65
Bacillus subtilis	1%	28.00 (31.91)	66.88	59.28	137.80	3.20	5.76
Control		40.33 (40.01)	51.55	50.28	149.67	2.35	5.37
$CD_{(0.05)}$		(3.70)	5.27	5.21	10.79	0.72	0.37

Figures in parentheses are arc sine transformed values

B. subtilis antagonists resulted in 28.00 per cent reduction in the incidence of stem rot followed by *T. polysporum* (37.33%). A non significant effect was registered in *T. polysporum* with maximum disease incidence (37.33%) of stem rot pathogen. Among fungal antagonists, *T. viride* was found most effective and also resulted in giving maximum average plant height (71.80 cm), stem length (65.57 cm), number of flowers per plant (3.53), flower size (6.24 cm) and required least number of days (133.20) for first flowering compared to control where the plant had on an average significantly shorter plant height (51.55 cm), shorter stem length (50.28 cm), less number of flowers per plant (2.35), shorter flower size (5.37 cm) with maximum number of days (146.67) for first flowering. The *T. harzianum* was found next best to *T. viride* in efficacy and both were found statistically on par except for flower size whereas, *T. polysporum* was found least effective in efficacy as the average plants in the treatment were found with minimum plant height (62.80 cm), stem length (56.46), flowers per plant (2.87), flower size (5.65 cm) and 143.05 days taken for first flowering. It is also observed that none of the treatments including control had any adverse effect on calyx splitting. Elad *et al*. (1981) tested wheat bran culture of *T. harzianum* for the control of *R. solani* in carnation field pretreated with methyl bromide that could get 70 per cent reduction in disease incidence @150 g (dry weight) per square meter.

Gupta *et al*., (2006) used *T. viride* against chickpea wilt complex and found significant reduction in wilt and enhanced yield under field conditions. Rini and Sulochana (2007) reported that *P. fluorescens* isolates P28 and P51 showed the greatest inhibition against *R. solani*, *T.viride* isolates TR19 and TR22 found effective against *F. oxysporum*. Rehman and Lawrence (2010) used *T. viride* and *T. harzianum* as seed treatment and soil drench against damping off in cabbage.

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