



Effect of bacterial antagonists against tuber rot of tuberose caused by *Fusarium oxysporum*

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ABSTRACT: Three bacterial antagonists, viz. *Pseudomonas fluorescens*, *Serratia marcescens* and *Bacillus subtilis*, were evaluated against *Fusarium oxysporum*, the cause of tuber rot in tuberose (*Polyanthes tuberosa* L.) *in vitro* and in glasshouse conditions. In dual culture *P. fluorescens* reduced the growth of pathogen to an extent of 51.78 per cent over control, while *S. marcescens* and *B. subtilis* reduced the growth of the pathogen to the extent of 48.75 and 41.76 per cent over control, respectively. Tuber treatment with talc based formulation of *P. fluorescens*, *S. marcescens* and *B. subtilis* @ 10g kg⁻¹ significantly enhanced the germination percentage of tuberose under glasshouse conditions. Among the bacterial antagonists tested, *P. fluorescens* @ 10g kg⁻¹ significantly reduced the incidence of tuber rot (67.81 % over control) at 80 days after planting and increased the shoot and root length by 127.67 per cent and 128.13 per cent over control.

KEY WORDS: *Bacillus subtilis*, *Fusarium oxysporum*, *Pseudomonas fluorescens*, *Serratia marcescens*, tuberose, tuber rot

Tuberose (*Polyanthes tuberosa* L.) is an important ornamental crop widely cultivated in Tamil Nadu for its flowers. Tuber rot of tuberose caused by *Fusarium oxysporum* is a serious disease causing considerable economic loss to the farmers. The disease becomes severe during warmer period coupled with rains. Biological control is now gaining more importance as a potential alternative to chemical control as an eco-friendly means of disease management. An experiment was conducted at Tamil Nadu Agricultural University, Coimbatore, during the year 2003 for the management of tuber rot disease of tuberose using bacterial antagonists.

Isolation of pathogen and biocontrol agents

Fusarium oxysporum was isolated from infected tuberose tubers onto potato dextrose agar (PDA) medium by tissue segment method. The cultures of *Pseudomonas fluorescens*, *Serratia marcescens* and *Bacillus subtilis* were obtained from the Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, India. *Bacillus subtilis* culture was maintained on nutrient agar medium (Difco Manual, 1953) and *P. fluorescens* and *Serratia marcescens* cultures were maintained on King's B medium (Kings *et al.*, 1954).

Effect of bacterial antagonists on the growth of *Fusarium oxysporum* *in vitro*

The antagonists, *viz.* *P. fluorescens*, *B. subtilis* and *S. marcescens*, were evaluated *in vitro* against *F. oxysporum* by dual culture technique to screen the most efficacious one. One loopful of each bacterial antagonist was streaked at one end of the Petri plate separately and in the opposite end, a mycelial disc of *F. oxysporum* was placed. Then the plates were incubated at room temperature ($28 \pm 2^\circ\text{C}$). The radial growth of pathogen was recorded after 48 hrs of incubation and per cent inhibition was worked out.

Effect of bacterial antagonists on the incidence of tuber rot and growth parameters under glasshouse conditions

The biocontrol agents tested *in vitro* were evaluated under glasshouse conditions with the following treatments.

T₁ – Tuber treatment with talc based formulation of *P. fluorescens* @ 10g/kg of tuber

T₂ – Tuber treatment with talc based formulation of *S. marcescens* @ 10g/kg of tuber

T₃ – Tuber treatment with talc based formulation of *B. subtilis* @ 10g/kg of tuber

T₄ – Control (without any treatment)

The pots (30cm) were filled with sterilized pot

mixture and inoculated with the pathogen that was multiplied on sand maize medium @ 100g kg⁻¹ of soil. Ten days after inoculations, treated tubers were planted in these pots @ five tubers / pot. The germination percentage was recorded at 25 days after planting. Similarly the incidence of tuber rot and growth parameters, *viz.*, shoot length and root length, were recorded at 80 days after planting.

The experiment on *in vitro* antagonism of bacterial antagonists against *F. oxysporum* revealed that *P. fluorescens* significantly reduced the growth of *F. oxysporum* to an extent of 51.78 per cent over control, followed by *Serratia marcescens* with a per cent inhibition of 48.75 per cent over control (Table 1). Cipriano *et al.* (1989) reported that dual inoculation of *Pseudomonas* spp. with *F. oxysporum* f. sp. *lycopersici* in Petri-plate inhibited the mycelial growth of *Fusarium* and produced inhibition zones. Rangeshwaran and Prasad (2000) reported that *P. putida* (PDBCAB 19) and *P. fluorescens* (PDBCAB 2) were the most effective antagonists against *F. oxysporum* f. sp. *ciceri*.

The glasshouse experiment on the management of tuber rot revealed that all the three bacterial biocontrol agents enhanced the germination of tuberoses when compared to control. A significantly higher germination percentage of 89.07 was recorded when the tubers were treated with *P. fluorescens* @ 10g / kg of tubers (Table 2). The treatment also reduced the incidence of tuber rot significantly to an extent of 67.81 per cent over control, while the tuber treatment with *S. marcescens*

Table 1. Effect of bacterial antagonists on the growth of *Fusarium oxysporum* *in vitro*

Antagonist	Antagonism*	
	Growth of <i>F. oxysporum</i> (mm)	Per cent inhibition over control
<i>Pseudomonas fluorescens</i>	25.40	51.78
<i>Serratia marcescens</i>	27.00	48.75
<i>Bacillus subtilis</i>	30.68	41.76
Control	52.68	-
CD (P = 0.05)	4.72	

* Mean of five replications

Table 2. Effect of bacterial antagonists on germination, tuber rot incidence and growth parameters of tuberose under glasshouse conditions

Treatment*	Germination (%)	Disease severity		Shoot length		Root length	
		Disease incidence at 80 DAP	Reduction over control (%)	Shoot length (cm) at 80 DAP	Increase over control (%)	Root length (cm) at 80 DAP	Increase over control (%)
<i>Pseudomonas fluorescens</i>	89.07 (72.84)	30.18 (33.17)	67.81	36.2	127.67	7.3	128.13
<i>Serratia marcescens</i>	84.35 (67.30)	33.91 (35.40)	63.83	29.3	84.28	6.5	103.13
<i>Bacillus subtilis</i>	77.02 (61.63)	60.26 (51.00)	35.72	25.3	59.12	5.1	59.38
Control	33.33 (35.18)	93.75 (82.50)	-	15.9	-	3.2	-
CD (P = 0.05)	5.03	3.34		0.38		0.39	

* Mean of five replications; figure in parentheses are arcsine-transformed values; DAP – days after planting

and *B. subtilis* reduced the incidence to an extent of 63.83 and 35.72 per cent over control, respectively. The treatment with *P. fluorescens* recorded a higher shoot and root length by an increase of 127.67 and 128.13 per cent, respectively over control, which was followed by the treatment with *S. marcescens* with 84.28 and 103.13 per cent increase over control (Table 2).

Fusarial wilt of *Colocasia esculentum* was significantly reduced by the soil application of *P. fluorescens* (Siddiqui and Shaukat, 2003; Johansson, 2003). Similarly, Rangaswari and Prasad (2000) reported that soil application of *P. fluorescens* (PDBCAB 30) was able to control *F. oxysporum* f. sp. *ciceri*. Application of *P. fluorescens* as seedling and root dip treatments significantly increased the vegetative growth of tomato (Santhi and Sivakumar, 1995). Jayashree *et al.* (2000) reported that seed treatment and soil application of *P. fluorescens* (Pf-1) recorded the highest root and shoot length in black gram and sesame. Jagtap (2002) found that *P. fluorescens* (Bioshield) significantly increased the growth of chilli seedlings.

REFERENCES

- Cipriano, M., Travadlin, S. and Taylor, G. 1989. Antagonistic potential of *Pseudomonas* spp. against *Fusarium oxysporum* f. sp. *lycopersici*. *Journal of General Microbiology*, **135**: 684-687.
- Difco Manual, 1953. Difco Laboratories, Inc., Detroit, Michigan, USA.
- Jagtap, G. P. 2002. Bioshield[™] (*Pseudomonas fluorescens*): More than biopesticide. *Pestology*, **26**: 38-41.
- Jayashree, K., Shanmugam, V., Raguchander, T., Ramanathan, A. and Samiyappan, R. 2000. Evaluation of *Pseudomonas fluorescens* (Pf-1) against blackgram and sesame root-rot disease. *Journal of Biological Control*, **14**: 55-61.
- Johansson, P. M. 2003. Biocontrol of *Fusarium* in wheat-introducing bacteria to a system of complex interactions. Ph. D. thesis, Department of plant Pathology and Biocontrol Unit, SLU. *Acta Universitatis Agriculturae Sueciae Agraria*. 403p.
- King, E. O., Ward, W. K., and Rancy, D. E. 1954. Two

simple media for the demonstration of pyocyanine and fluorescein. *Journal of Lab Clinical Methods*, **44**: 301-307.

Rangeshwaran, R. and Prasad, R. D. 2000. Isolation and evaluation of rhizospheric bacteria for biological control of chickpea wilt pathogens. *Journal of Biological Control*, **14**: 9-15.

Santhi, A. and Sivakumar, C. V. 1995. Biocontrol potential

of *Pseudomonas fluorescens* against *Meloidogyne incognita* on tomato. *Journal of Biological Control*, **9**: 113-115.

Siddiqui, I. A. and Shaukat, S. S. 2003. Impact of biocontrol agent *Pseudomonas fluorescens* CHAO and its genetically modified derivatives on the diversity of returnable fungi in the rhizosphere of mung bean. *Journal of Applied Microbiology*, **95**: 1039.

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