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Research Note

Effect of delivery systems of *Pseudomonas fluorescens* on the rhizosphere survival and management of fusarial wilt of tomato

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ABSTRACT: Pot experiments conducted to evaluate different delivery systems for *Pseudomonas fluorescens* in the management of fusarial wilt of tomato revealed that FYM enriched with *P. fluorescens* as seed and soil application was very effective in minimizing wilt incidence to 15.53 per cent. Carbendazim at 0.1% recorded 18.56% disease incidence and the untreated control recorded the maximum disease incidence of 38.25%. Significantly highest population of *P. fluorescens* was recorded when FYM enriched with *P. fluorescens* was used for seed treatment and soil application (30×10^7 CFUs g^{-1}) on 60 days after sowing and the same treatment recorded the lowest population of *Fusarium oxysporum f. sp. lycopersici* (8.9×10^2 CFUs g^{-1} of soil).

KEY WORDS: Delivery systems, *Fusarium oxysporum* f. sp. *lycopersici*, management, *Pseudomonas fluorescens*, survival, tomato, wilt.

Fusarial wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* occurs throughout the tropical and subtropical countries (Singh, 1985). The losses due to this disease ranging from 10 to 80 per cent have been reported from different parts of India (Chand, 1965; Kapoor, 1988; De Boer, 2003). Several pathogenic strains designated as formae specialties exist within *F. oxysporum* and within *formae speciales*, races have been found to exist. These *formae speciales* cause vascular wilt. Fungicidal treatment has not been well accepted because of multiplicity of pathogens involved and its hazardous impact on environment and human beings. Biological control of plant pathogens is a promising approach in combating soil borne pathogens. Certain strains of *P. fluorescens* when applied to seed and soil could provide biological control of root pathogens (Saxena and Saxena, 1995). FYM was the best substrate for multiplication of *Pseudomonas fluorescens* in poly bags (Najam Waris Zaidi and Singh, 2004). Delivery system of the antagonists is critical for effective implementation of biocontrol of various crop diseases. The objective of this study is to evaluate the efficacy of formulation of *P.fluorescens* for the management of wilt disease of tomato under pot culture.

F. oxysporum f. sp. *lycopersici* was brought into pure culture from infected plant materials collected at Annamalainagar and multiplied on sand – maize meal (l00: 3 w/w) medium. The grains (3g); (100g) liver sand were soaked overnight in water (20ml) in conical flasks, autoclaved at 1.5 kg cm^{-2} for one and half hour for two consecutive days. After sterilization and cooling of medium each flask was inoculated with five mycelial bits of the pathogen and incubated at 22 ± 1°C for 15 days.

Soil samples were collected from different rhizosphere region of tomato plants and isolation of *P. fluroscens* was made by serial dilution method. King's broth was prepared and distributed in 50ml quantities in 250ml Erlenmeyer flasks and autoclaved. After sterilization the flasks were inoculated with cell suspension of *P. fluorescens* prepared from 48h culture @ 0.5ml. The flasks were continuously shaken in rotartory shaker (120rpm) for 3 days.

For pot experiments, sterilized soil was used. The pathogen after mass multiplication as above was incorporated into the pots @ 20g kg^{-1} soil. Tomato seeds (PKM 1) were moistened and pelleted with various formulations, *viz*., talc, FYM, flyash, gypsum and pressmud @ 10g kg^{-1} of seed and shade dried for 2h. The treated seeds were sown in three pots containing unsterilized soil. Soil application of various formulations @ 5kg ha^{-1} was done in another set of three pots. In another set of pots, combination seed treatment and soil application was done. Seed treatment with carbendazim 0.1% was tested for comparison. The incidence of wilt in tomato was assessed in each pot and it was expressed as disease incidence. Soil samples were collected from each treatment at 20, 40 and 60 DAS from rhizosphere. The survival of *P. fluorescens* and *F. oxysporum* was assessed by serial dilution plating method.

The results revealed that combined application (seed treatment + soil application) of *P. fluorescens* significantly

Table 1. Effect of seed treatment and soil application of *Pseudomonas fluorescens* on plant growth promotion and fusarial wilt incidence in tomato under pot culture conditions (60 DAS)

Carrier used for the formulation (5 kg ha^{-1})	Plant stand (%)	Shoot length (cm)	Root length (cm)	Vigour index	Disease incidence (%)	Per cent reduction over control	Yield kg ha^{-1}
Talc	92.33 (73.92)	36.32	17.25	4982.94	19.43 (26.15)	49.20	1221
Flyash	92.00 (73.57)	35.12	18.35	4920.16	21.20 (27.41)	44.58	1082
FYM	94.67 (76.65)	41.85	19.27	5870.4	15.53 (24.79)	54.01	1537
Gypsum	86.00 (68.02)	26.35	14.12	3562.24	28.87 (32.50)	24.52	981
Pressmud	91.33 (92.87)	31.03	16.14	4243.5	25.57 (30.38)	33.15	1051
Carbendazim	94.00 (75.82)	37.45	18.27	5297.2	18.56 (25.52)	51.47	1320
Control	81.33 (64.39)	21.12	11.57	2747.64	38.25 (38.20)	-	944
SEM	1.6330	02583	0.0288	0.5675	0.2278	-	-
CD ($P \geq 0.05$)	5.046	0.7824	0.0887	0.8026	0.3222	-	-

Figures in parentheses are arcsine transformed values

increased the plant growth and decreased the per cent wilt incidence of tomato (Table 1). Among the treatments combined application of FYM colonized *P. fluorescens* @ 10g kg^{-1} of seed plus 50g kg^{-1} of soil effectively increased the per cent plant stand (94.67), shoot length (41.85cm), root length (19.27cm) and vigour index (5870.4) and minimum per cent disease incidence (15.53) was recorded in the same treatment followed by talc (19.43%), flyash (25.57%) and pressmud based formulations (25.57%) while gypsum based formulation recorded the maximum per cent disease incidence (28.87). Carbendazim (0.1%) recorded 18.56% disease incidence, while untreated control recorded the maximum disease incidence (38.25%).

The methods of application of formulated products of biocontrol agents include seed treatment (Rosales and Mew, 1997), root dip (Maurhoer *et al.*, 1994), soil application (Vidhyasekaran*etal.*,1997) and foliar application (Jayalakshmi *et al.*, 2005; Singh and Sinha, 2005). Combination of different methods of application could be more effective in disease management than a single method of application (Meena *et al.,* 2000; Nandakumar *et al*., 2001). These reports lend support to our present findings. Seed treatment followed by soil application of talc based powder formulation has effectively checked wilt of chickpea, wilt of pigeon pea and cotton under field condition and increased the yield as reported by Srinivasan and Mathivanan (2006).

The rhizosphere survival of *P. fluorescens* with different methods of application was estimated (Table 2). Among the treatments tested, the survival of *P. fluorescens* was significantly higher in seed treatment + soil application of FYM colonized *P. fluorescens* on 60 DAS (30×10^7 CFUs g^{-1}) followed by soil application (28×10^7 CFUs g^{-1}). With regard to survival of pathogen in the rhizosphere region, the treatment with FYM colonized *P. fluorescens* as seed treatment + soil application significantly reduced population of the pathogen in rhizosphere soil when compared to control.

P. fluorescens possesses the capacity to adhere to plant root (Van Peer *et al.*, 1990). Shanmugam *et al.* (2001) observed high population of *P. fluorescens* in rhizosphere soil of blackgram due to seed treatment and soil application of *P. fluorescens* in integration with FYM and neem cake in pot culture experiments. Bunker *et al.* (2001) observed suppression of dry root rot of *Capsicum frutescens* by integration of biological agents and fungicide.

The treatment of FYM enriched with *P. fluorescens* applied as seed treatment and soil application significantly reduced the population of *F. oxysporum* with 0.89×10^3 CFUs g^{-1} of soil and 0.95×10^3 cfu g^{-1} of soil respectively. Ramamoorthy *et al.* (2001) reported that induction of defense related proteins involved in phenyl propanoid metabolic pathway and accumulation of phenolic and PR proteins might have contributed to restriction of invasion of the pathogen.

Table 2. Effect of different treatments on the rhizosphere survival of the antagonist and the pathogen in FYM colonized *P. fluorescens*

Treatment	*P. fluorescens* population ($\times 10^{-7}$ CFUs g^{-1} of soil)				*F. oxysporum* population ($\times 10^{-3}$ CFUs g^{-1} of soil)			
	20	40	60	Mean	20	40	60	Mean
Seed Treatment	15	20	24	19.67	1.12	1.24	0.87	1.08
Soil Application	19	23	28	23.33	1.02	1.14	0.70	0.95
Seed Treatment + Soil Application	21	25	30	25.33	1.00	1.11	0.56	0.89
Carbendazim (0.1%)	5	7	12	8.00	1.10	1.21	0.91	1.07
Control	1	3	5	-	4.82	5.90	7.00	5.91
Mean	11.6	15.6	19.8	-	1.812	2.12	2.01	-
	S.Ed		CD (P ≥0.05)					
Treatments	0.7957		2.5994					
Days	0.3611		1.1495					

REFERENCES

Bunker, R. N. and Mathur, K. 2001. Integration of biological agents and fungicide for suppression of dry root rot of *Capsicum frutescens*. *Journal of Mycology and Plant Pathology,* **31**: 330-334.

Chand, J.N. 1965. Comparative study of two isolates of *Fusarium oxysporum formae specialies lycopersici*. *Journal Research Agricultural University,* **2**: 179-182.

De Boer, M. 2003. Control of *Fusarium* wilt of radish by combining *Pseudomonas putida* strains that have different disease–suppressive mechanisms. *Phytopathology,* **93**: 626-632.

Jayalakshmi, C., Madhiazhagan, K. and Rettinasababady, C. 2005. Methods of application of *Pseudomonas fluorescens* for direct seeded rice. Paper presented at National symposium on MCPDMGT, held on December 29-30 at Annamalai Univ., India, p. 4.

Kapoor, I.J. 1988. Fungi involved in tomato wilt syndrome in Delhi, Maharashtra and Tamil Nadu. *Indian Phytopathology,* **41**: 208-213.

Maurhofer, M. Hase, C., Meuwly, P., Metraux, J. P. and Defago, G. 1994. Induction of systemic resistance of tobacco to tobacco necrosis virus by the root–colonizing *Pseudomonas fluorescens* strain CHAO: Influence of the gacA gene and of pyoverdine production. *Phytopathology,* **84**: 139-146.

Meena, B., Ramamoorthy, V., Marimuthu, T. and Velazhahan, R. 2000. *Pseudomonas fluorescens* mediated systemic resistance against late leaf spot of groundnut. *Journal of Mycology and Plant Pathology,* **30**: 151-158.

Najam, W.Z. and Singh, U.S. 2004. Development of improved technology for mass multiplication and delivery of fungal (*Trichoderma*) and bacterial (*Pseudomonas*) biocontrol agents. *Journal of Mycology and Plant Pathology,* **34**: 732-740.

Nandakumar, R., Radjacommare, R., Raguchander, T. and Samiyappan, R. 200. Ecologically sustainable rhizobacterial bio-formulation for the management of leaf folder insect, sheath blight disease and enhanced yield in rice. *Indian Phytopathology,* **54**: 515-516.

Ramamoorthy, V., Raguchander, T., Prakasam, V. and Samiyappan, R. 2001. Expression of defense-related genes in *Pseudomonas fluorescens* treated plants in response to infection by *Fusarium oxysporum* f. sp. *lycopersici. Indian Phytopathology,* **54**: 516.

Rosales, A. M. and Mew, T. W. 1997. Suppression of *Fusarium moniliforme* in rice by associated antagonistic bacteria. *Plant Disease,* **81**: 49-52.

Saxena, D. R. and Moly Saxena. 1995. Evaluation of antagonists against dry root rot pathogen of chick pea. *Indian Journal of Mycology and Plant Pathology,* **25**: 130.

Shanmugam, V., Renukadevi, P., Senthil, N., Raguchander, T. and Samiyappan, R. 2001. Interaction of *Pseudomonas fluorescens* with Rhizobium in the management of blackgram root rot incited by *Macrophomina phaseolina*. *Plant Disease Research,* **16**: 23-29.

Singh, R. S. 1985. Diseases of vegetable crops. Oxford and IBH Publishing Co., New Delhi.

Singh, R. and Sinha, A. P. 2004. Comparative efficacy of local bioagents, commercial bioformulations and fungicide for the management of sheath blight of rice, under glass house condition. *Indian Phytopathology,* **57**: 494-496.

Srinivasan, K. and Mathivanan, N. 2006. ISR mediated biological control of sunflower necrosis virus disease using consortium of plant growth promoting rhizobacteria. National symposium on BMCDM, held on March 3-4 at Annamalai University, India, p. 49.

Van Peer, R., Niemann, G. J. and Schippers. B. 1991. Induced resistance and phytoalexin accumulation in biological control of *Fusarium* wilt of carnation by *Pseudomonas* sp. strain WCS 417. *Phytopathology,* **81**: 728-734.

Vidhyasekaran, P., Sethuraman, K., Rajappan, K. and Vasumathi, K. 1997. Powder formulations of *Pseudomonas fluorescens* to control pigeonpea wilt. *Biological Control*, **8**: 166-171.

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