J. Hort. Sci.
Vol. 1 (1): 44-47, 2006

Management of bacterial wilt of tomato caused by *Ralstonia solanacearum* (Smith) Yabuuchi *et al* using biological control agents

A. Manoj Kumar and Girija Ganesan[1]
Department of Crop Physiology
University of Agricultural Sciences
G. K. V. K., Bangalore-560 065, Karnataka, India.
E-mail: manojarthik@rediffmail.com

ABSTRACT

Biological control agents, *Glomus mosseae* IIHR, *Bacillus subtilis* IIHR-1, *Pseudomonas fluorescens* IIHR+3, *Trichoderma harzianum* IIHR P_1 and *T. viride* IIHR P_{22} were evaluated against tomato wilt caused by *Ralstonia solanacearum* in pathogen-infested plots during 2003 -2004. Microbial preparations were applied either as transplant root dips or root dip plus soil drench 30 days after transplanting. Per cent survivability increased with the use of all biological control agents tested. However, *G. mosseae* treated plants resulted in better survival (25.75 and 28.79% in root dip alone and 60 and 66.67% in root dip plus drench against untreated control 0 and 1.5% during 2003 and 2004 respectively), compared to the rest of the treatments, suggesting *G. mosseae* amendment to pathogen-infested soil would result in substantially higher plant survival against the untreated controls.

Key words: Biocontrol microorganisms, bacterial wilt, survivability, *Ralstonia solanacearum,* tomato

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill. Nom. Cons.) is one of the widely distributed vegetable crops grown worldwide. Tomatoes are a key food crop throughout the tropics and subtropics for low-income group of farmers and are also a valuable cash crop. In India, tomato occupies 4180 ha with annual production of 6.4 million tonnes (Anon, 1998). Bacterial wilt caused by *Ralstonia* (formerly *Pseudomonas*) *solanacearum* (Smith) Yabuuchi *et al.*, (Yabuuchi *et al.,* 1995) is a soil borne plant disease of great economic importance. It is endemic in most tropical, subtropical and warm temperate regions of the world (Hayward, 1991), proving a major limitation to tomato production in Karnataka, Andhra Pradesh, Kerala, Maharashtra, Assam, Orissa, Jharkhand and North Eastern states.

The pathogen's host range is rapidly expanding and means of control of the disease seems limited. However, due to the oligogenic nature of plant resistance and because of the variation in aggressiveness of bacterial isolates from different locations around the world, alternative control measures such as biological control have been receiving increased interest. None of the chemicals is effective in achieving desired levels of disease control. Hence, biological control is gaining more importance as an eco-friendly means of disease management. Bacteria, which are antagonistic towards *P. solanacearum* have been reported by many workers earlier (Anuratha and Gnanamanickam, 1990; Ciampi Panno *et al*, 1989; Fucikovsky *et al*, 1989 and Gallardo *et al*, 1989). In most cases, field experiments were limited and the degree of protection was in-sufficient for commercial use (Tanaka *et al,* 1990). In many cases protection failed because root colonization of the biological control agent was poor (Chen and Echandi, 1984). Hence, in the present study different biological control agents *viz.*, *Glomus mosseae* IIHR, *Trichoderma harzianum* IIHR-P_1, *T. viride* IIHR-P_{22}, *Bacillus subtilis* IIHR-1 and *Pseudomonas fluorescens* IIHR+3 were collected from Indian Institute of Horticultural Research, Bangalore and tested against wilt causing bacteria of tomato under field conditions and results obtained are presented.

MATERIAL AND METHODS

A field experiment was conducted in the *R. solanacearum* infested plot during 2003 and 2004. Twenty five day old tomato seedlings of cv. PKM-1 were transplanted in pathogen-infested soil. Three replications were maintained for each treatment with 22 plants/ replication. Plants were spaced 75 cm x 50 cm in a plot size of 7.5 m^2. Untreated plots served as control. Randomly, ten pathogen-infested soil samples were picked f-orm each

[1]Division of Plant Pathology, Indian Institute of Horticultural Research, Hesseraghatta Lake Post, Bangalore-560 089, India

plot, and were pooled, i.e., 'a composite sample'. Similarly, 33 composite samples were collected from 33 plots. The bacterial population of the pathogen-infested plots were determined for all 33 plots by serial dilution on TTZ (2, 3, 5, triphenyl tetrazolium chloride) medium. The mean populations of *R. solanacearum* were $2.4\pm0.4x10^7$ cfu g^{-1} and $2.9\pm0.35x10^7$ cfu g^{-1} during the year 2003 and 2004, respectively.

Fungal antagonists *viz.*, *T. harzianum* and *T. viride* were multiplied on Potato Dextrose Broth at 27°C for 7 days in B.O.D incubator and the mycelium was blended thoroughly. Spore count of $7x10^9$conidial spores/ml in *T. harzianum* and $7.4x10^9$conidial spores/ml in *T. viride* was recorded by using heamocytometer. The bacterial antagonists *viz.*, *B. subtilis* and *P. fluorescens* were multiplied on nutrient broth at 35°C for 72h at 120 rpm in the incubator shaker. Bacterial antagonist population was determined by serial dilution method on Nutrient Agar medium and were found to be $4x10^9$ c.f.u/ ml (*P. fluorescens*) and $4.3x10^9$ c.f.u/ ml (*B. subtilis*). However, *Glomus mosseae* was maintained on *Eleusine coracana* for six months showing 60-70% of root colonization with 20 spores/g soil.

At the time of transplanting, roots were dipped in broth containing biological control agents *viz.*, *T. harzianum, T. viride, B. subtilis* and *P. fluorescens* for 30 min. and transplanted to the sick plots, in randomized block design (RBD). In case of *Glomus mosseae*, the seedlings were grown on *G. mosseae* soils and transplanted.

In another set of experiment, one soil drench of bio control agents (root dip+drench) in the root zone @ 20 ml per plant was given at 30 days after transplanting (DAT) in addition to root dip. Assessment of treatment effects were recorded as per cent wilt incidence at 90 DAT and the wilted plants examined in laboratory for confirmation of the presence of *R. solanacearum*. Plant height was recorded at 30 DAT. Data were normalized through angular transformation and subjected to analysis of variance (ANOVA) followed by mean separation by the Student-Newman-Keuls' test (p=0.05). All the analysis was performed using the SAS (1996) package.

RESULTS AND DISCUSSION

Of the five biological control agents used in the investigation, the highest per cent wilt incidence of 94% and 84.9% in tomato in seedling root dip experiment was recorded in *T. harzianum* and minimum of 74.25% and 71.21% in *G. mosseae* as against 100% and 98.5% in untreated control for the year 2003 and 2004, respectively, these results indicate that the protection offered by *G. mosseae* was better in both the years as compared to other treatments (Table 1).

Further, the incidence of bacterial wilt in root dip plus soil drench treated with *G. mosseae* was much lower (40 & 33.33%) as compared to 100 and 98.5% in untreated control. However, the maximum wilt incidence of 86.7 and 66.7% was observed in *T. harzianum* during the successive years 2003 and 2004. Decreasing trend of wilt incidence was observed in *G. mosseae* and *T. harzianum* treated plots, in both the experiments, i.e., seedling root dip and seedling root dip plus soil drench. *Bacillus subtilis* and *P. fluorescens* treatments showed no change in wilt incidence for both the years, i.e., 80.31% in root dip and 66.67% in root dip plus soil drench. However, wilt incidence was increased in 2004 in both the experiments in *T. viride* treated plots.

Glomus mosseae treated plots recorded higher plant growth of 46.17cm and 50.67cm followed by *T. harzianum* during both the years as against all other treatments (Table 1). In the present investigation, some of the tested biological control agents had antagonistic effect on *R. solanacearum.* Among these, bacterial antagonists (*B. subtilis* & *P. fluorescens*) performed better than fungal antagonists. However, *G. mosseae* amended infested soil recorded less wilt incidence compared to control and other treatments. The biological control agent must compete with complex biological and physical factors, including soil composition and structure, moisture and pH, which influence the structure of the microbial population (Nesmith and Jenkins, 1985; Weller, 1988), in order to show its better performance under field conditions. Anuratha and Gnanamanickam (1990) reported that *P. fluorescens* str. Pfcp protected tomato plants from bacterial wilt up to 95% in greenhouse and 36% in the field. Similarly, *Bacillius* sp. strain BA-46 controlled the bacterial wilt disease by 21.13% in tomato under greenhouse conditions (Silveira *et al,* 1995). *T. harzianum*, isolated from Sikkim was found effective in controlling ginger wilt caused by *R. solanacearum* (Rajan *et al,* 2002). Arbuscular mycorrhiza forms a beneficial symbiotic association with roots that increased plant ability to absorb phosphorous, minor elements and water, thus enhancing the tolerance of the plant against the pathogen damage (Harley and Smith, 1983). Kobayashi, *et al* (1991) reported that VAM + charcoal compost proved effective in reducing the level of bacterial wilt of tomato in greenhouse tests. The extracts from mycorrhizal tomato roots infected with *Glomus fasciculatum* reduced populations of *P.*

Table1. Effect of different microorganisms on survival and growth in tomato in *R. solanacearum* infected soil

Treatment	Wilt incidence (%)				Plant height (cm)	
	2003		2004		2003	2004
	Root dip alone	Root dip + Drench	Root dip alone	Root dip + Drench		
Glomus mosseae	74.25	40.00	71.21	33.33	46.17	50.67
	(60.04)	(38.85)	(57.56)	(35.01)		
T. harzianum	94.00	86.67	84.85	66.67	36.92	39.17
	(75.98)	(75.71)	(63.68)	(54.99)		
T. viride	75.76	46.67	83.33	60.00	32.08	33.83
	(61.59)	(43.07)	(62.61)	(50.77)		
B. subtilis	77.28	53.33	78.79	53.33	32.58	35.25
	(62.47)	(46.92)	(67.31)	(47.29)		
P. fluorescens	80.31	66.67	80.31	66.67	36.83	38.00
	(67.45)	(54.99)	(66.25)	(54.99)		
Control	100.0	100.0	98.50	98.50	31.97	32.83
	(88.19)	(88.19)	(84.71)	(79.93)		
CD (p=0.05)	NS	31.63	7.35	21.69	-	-
		(22.44)	(7.32)	(15.11)		

* DAT – Days after transplanting; NS- non significant; Figures in parentheses are angular transformed values

solanacearum in nutrient broth, however, the active principle constituent of VAM fungi inhibiting *P. solanacearum* are yet to be studied (Suresh *et al,* 1991). It could be concluded from the present investigation that, the *G. mosseae* amended pathogen-infested soil resulted in substantially higher plant survival in *R. solanacearum* infected soil. In addition to the transplant root dip an additional drench of bioagents at 30 DAT was of an added advantage in protecting the plant from bacterial wilt pathogen.

ACKNOWLEDGEMENTS

The authors are grateful to the Director, I.I.H.R., Bangalore, for providing facilities and Mr. Puttamada Shetty, Technician, and Ms. N. Rama, Research Associate, for assistance rendered during the study. The authors are also thankful to Prof. M. Udaya Kumar, and Dr. Rohini Sreevathsa, GKVK, Bangalore for their support.

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(MS Received 4 May, 2006 Revised 13 July, 2006)