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Environmentally benign management of bacterial wilt of brinjal incited by *Ralstonia solanacearum* (Smith)

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ABSTRACT

A field experiment was conducted to study the effect of *Pseudomonas fluorescens* on wilt disease of brinjal incited by *Ralstonia solanacearum*, during three consecutive *Kharif* seasons of 2010, 2011 and 2012 at the experimental farm of Zonal Agricultural Research Station, Sub-montane Zone, Kolhapur (Maharashtra) India. Pooled data of the three years revealed that the wilting was reduced remarkably in the plots, where the plants were drenched with copper oxychloride 40 g + streptocycline 2 g (per 10 L water). This treatment gave outstanding disease control and was the best amongst all the treatments tried, in reducing wilt incidence by 62.77 per cent and yield increase by 71.93 per cent compared to untreated control. However, maximum benefit-cost (B: C) ratio of 3.11 was recorded in the treatment wherein *P. fluorescens* was inoculated to seed + seedling roots + soil. Consequently, on the basis of relative efficacy of *P. fluorescens* in terms of degree of disease control, additional yield, net profit per hectare and benefit: cost ratio, seed treatment with talc based culture of *P. fluorescens* @ 10 g kg^{-1} seed before sowing and seedling root dip (2.5 kg of talc based formulation of *P. fluorescens* in 40 L water) as well as soil application (2.5 kg of talc based formulation of *P. fluorescens* mixed in 50 kg of FYM $acre^{-1}$ soil), at the time of transplanting may be recommended to farmers for management of bacterial wilt of brinjal and thereby gaining higher fruit yield.

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INTRODUCTION

Bacterial wilt of brinjal and other solanaceous vegetables caused by *Ralstonia solanacearum* (Smith) is the most destructive disease in the tropical, subtropical and temperate regions of the world, causing heavy economic loss. The disease affects wide host range of economically important crops such as tomato, potato, brinjal, chilli and non-solanaceous crops such as ginger, banana and groundnut in India. It causes heavy losses varying from 2-90 per cent in different climates and seasons in India (Mishra *et al.*, 1995). This devastating soil borne plant pathogen has a global distribution and an unusually wide host range. This pathogen causes wilt in over 450 host species in 54 botanical families (Hayward, 1991), which may give the pathogen an evolutionary advantage. Because of wide host range of the pathogen, it is difficult to manage the disease. Other than chemical fumigants, there is no commercial pesticide available for control of the disease (Ramesh, 2008). Furthermore, chemicals used for management of plant diseases have adverse effects on the environment and non-target organisms. The chemicals cause soil and water pollution due to residues, which affects human health as well as development of resistant mutant by pathogen against pesticides. Hence, non-chemical methods including cultural methods, resistant cultivars and biocontrol with antagonistic bacterial agents have been used successfully to manage bacterial diseases (Marten *et al*., 2000 and Almoneafy *et al*., 2012). However, resistant cultivar is not completely effective due to lack of stability or durability (Boucher *et al*., 1992). Resistant cultivars are limited to locations, climate and to the strains of the pathogen. Only a few varieties show stable resistance, but are not generally preferred by the growers (Ramesh, 2008). Moreover, genetic diversity of the pathogens often overcomes the resistance of the crops. In biocontrol method, various fungal and bacterial antagonists are used to control plant diseases successfully. In recent years plant growth promoting rhizobacteria (PGPR) have been reported to be potential biological control agents, as they are known for growth promotion as well as causing disease reduction in crops (Jetiyanon and Kloepper, 2002). Among PGPRs, fluorescent pseudomonads have been reported to be effective against broad spectrum of plant pathogens. Recent studies have indicated that biological control of bacterial wilt could be a sustainable and eco-friendly strategy (Ramesh, 2008).

Bacterial wilt caused by *Ralstonia solanacearum* is one of the major stumbling blocks in the cultivation of brinjal crop in Kolhapur district of Maharashtra. This pathogen causes extensive economic loss to the crop. The disease becomes visible in the field when the plants are in flowering and begin to bear fruits. Sudden wilting is followed by death of the plants within 3 to 5 days of the infection that is the first recognizable symptom of the disease. The disease is more severe in the year of heavy monsoon, when the fields become very frequently water-logged (Das and Chattopadhyay, 1955). Considering foregoing facts, the biological control of *R. solanacearum* would be ideal if a suitable antagonist is identified, which is very effective, economical and eco-friendly and highly specific in the context of the bacterial diseases such as bacterial wilt.

MATERIAL AND METHODS

A field experiment was conducted to study the effect of *Pseudomonas fluorescens* on wilt disease of brinjal incited by *Ralstonia solanacearum*, during three consecutive *Kharif* seasons of 2010, 2011 and 2012 at the experimental farm of Zonal Agricultural Research Station, Sub-montane Zone, Kolhapur (Maharashtra) India.

The experiment was laid out in Randomized Block Design (RBD) having nine treatments and three replications, in plots of 4.50 m x 3.75 m with 90 cm × 75 cm spacing, using brinjal cv. MANJARI GOTA. The treatments consisted of seed, seedling roots and soil application of *Pseudomonas fluorescens* singly and in combinations there of. The treatments were seed treatment with *P. fluorescens,* seedling root treatment with *P. fluorescens,* soil application of *P. fluorescens,* seed treatment + seedling root treatment with *P. fluorescens,* seed treatment + seedling root treatment + soil application of *P. fluorescens,* seedling root treatment + soil application of *P. fluorescens,* seed treatment + soil application of *P. fluorescens,* drenching of soil with copper oxychloride 40 g + streptocycline 2 g (per 10 lit water) and untreated control. Seed treatment with talc (sodium ammonium silicate) based formulation of *P. fluorescens* (2.5 x 10^{10} cfu g^{-1}) was done @ 10 g per kg seed prior to sowing in nursery seedbeds. For seedling root treatment, roots of brinjal seedlings were dipped in a solution prepared by mixing 2.5 kg of talc based formulation of *P. fluorescens* in 40 L of water. Soil application of *P. fluorescens* was done by broadcasting mixture of talc based formulation of *P. fluorescens* (2.5 kg) and farm yard manure (50 kg) in furrows at the time of transplanting. In the treatment of soil drenching with copper oxychloride 40 g + streptocycline 2 g (per 10 lit water), soil around the brinjal plants was first drenched 25 days after transplanting and subsequent drenching were followed at an interval of 3 weeks. Brinjal plants in all the treatments were inoculated with bacterial suspension of the pathogen at the concentration of 5×10^8 cfu ml^{-1} on the third leaf axils from the top of the plant after transplanting in the sick plot. The crop was raised as per the recommended package of practices. Observations on death count due to bacterial wilt at peak flowering and at the end of crop season stages were recorded. The fruit yield per plot was recorded in kg $plot^{-1}$ and converted in to t ha^{-1}.

RESULTS AND DISCUSSION

The findings of the present study as well as relevant discussion are presented under following heads:

Wilt incidence:

Pooled data of three years revealed that all the treatments caused significant reduction in incidence of bacterial wilt disease over untreated control, which ranged from 19.71 to 62.77 per cent (Table1). However, maximum reduction in wilting was observed in the plots, where the plants were drenched with copper oxychloride 40 g + streptocycline 2 g (per 10 lit water). This treatment gave outstanding disease control and was the best treatment amongst all the treatments tried, in reducing wilt incidence by 62.77 per cent compared to untreated control. This treatment was followed by the treatment

Table 1: Effect of *Pseudomonas fluorescens* on incidence of bacterial wilt and fruit yield of brinjal (Pooled data for *Kharif*, 2010 to *Kharif*, 2012)

Treatments*	Bacterial wilt		Fruit yield	
	Incidence (%)	Disease reduction over control (%)	Yield (t ha^{-1})	Yield increased over control (%)
T_1- ST	48.89 (44.34)	19.71	24.15	21.72
T_2- SRT	44.44 (41.76)	27.02	24.91	25.55
T_3- SA	43.11 (41.00)	29.20	25.16	26.81
T_4- ST + SRT	33.78 (35.47)	44.52	27.58	39.01
T_5- ST + SRT + SA	31.11 (33.83)	48.91	29.98	51.11
T_6- SRT + SA	37.78 (37.88)	37.95	26.99	36.04
T_7- ST + SA	35.56 (36.55)	41.60	27.51	38.66
T_8- Drenching with COC + streptocycline	22.67 (28.27)	62.77	34.11	71.93
T_9- Control	60.89 (51.29)	--	19.84	--
S.E. ±	0.873		0.275	
C.D. (P=0.05)	2.419		0.833	

Figures in parentheses are angular transformed values * ST = Seed treatment with *P. fluorescens*,
SRT = Seedling root treatment with *P. fluorescens*, SA = Soil application of *P. fluorescens*, COC = Copper oxychloride

Table 2: Net return and benefit-cost ratio due to various treatments for controlling bacterial wilt of brinjal

Treatments*	Yield (t ha^{-1})	Cost of cultivation** (Rs. ha^{-1})	Gross monetary returns (Rs. ha^{-1})	Profit (Rs. ha^{-1})	Benefit: Cost ratio
T_1- ST	24.153	1,92,166.75	4,83,060.00	290893.25	2.51
T_2- SRT	24.913	1,92,541.00	4,98,260.00	305719.00	2.59
T_3- SA	25.160	1,92,541.00	5,03,200.00	310659.00	2.61
T_4- ST + SRT	27.580	1,92,541.75	5,51,600.00	359058.25	2.86
T_5- ST + SRT + SA	29.980	1,92,916.75	5,99,600.00	40668.25	3.11
T_6- SRT + SA	26.987	1,92,916.00	5,39,740.00	346824.00	2.80
T_7- ST + SA	27.507	1,92,541.75	5,50,140.00	357598.25	2.86
Drenching with COC + streptocycline	34.107	2,33,606.00	6,82,140.00	448534.00	2.92
T_9 - Control	19.840	1,92,166.00	3,96,800.00	204634.00	2.06

* ST = Seed treatment with *P. fluorescens*, SRT = Seedling root treatment with *P. fluorescens*, SA = Soil application of *P. fluorescens*,
COC = Copper oxychloride **Cost of cultivation includes production cost and cost of *P. fluorescens*,
Copper oxychloride and Streptocycline:(Cost of *P. fluorescens*: Rs. 150 kg^{-1}, Copper oxychloride: Rs. 400 kg^{-1} and Streptocycline Rs.36 per 6 g).
(Copper oxychloride + streptocycline was drenched at an interval of 25 days, for four times during the crop period, starting first at 25 days after transplanting) Sale price of brinjal fruits: Rs. 20,000 ton^{-1}

wherein *Pseudomonas fluorescens* was inoculated to seed, seedling roots and soil (T_5) and the treatment wherein *P. fluorescens* was inoculated only to seed and seedling roots (T_4). These two treatments did not differ significantly from each other and gave disease control of 48.91 and 44.52 per cent over control, respectively. Incidence of wilt in untreated control plots was maximum, which was 60.89 per cent. Foregoing results substantiate that *P. fluorescens* was effective in holding off the onslaught of the disease. This may be due to the fact that *Pseudomonas* spp. are metabolically very active, have high growth rate and aggressively colonize root systems (Burr *et al*., 1978). Aspiras and Della Cruz (1985); Anuratha and Gnanamanickam (1990) and Gamliel and Katan (1993) reported that utilization of antagonistic rhizospheric bacteria such as *Bacillus* spp., *P. fluorescens* and *P. putida* significantly increased the survival rate of potato, tomato, eggplant and cotton by 60-90 per cent, 90 per cent and 84-90 per cent, respectively against bacterial and fusarium wilt disease. Ramesh *et al*. (2008) found that most of the selected pseudomonads produced an antibiotic 2, 4-diacetylphloroglucinol (DAPG), which inhibited *Ralstonia solanacearum* under *in vivo* conditions. They also recorded production of siderophores and IAA in the culture medium by the antagonists, which could be involved in biocontrol and growth promotion in crop plants. Reduction in wilt incidence due to *P. fluorescens*, obtained in the present investigation, can be explained on these bases.

Fruit yield:

Perusal of pooled data of three years presented in Table 1 revealed that all the treatments studied in the investigation had significant effect on fruit yield of brinjal. However, significantly highest yield of 34.11 t ha^{-1} was harvested from the brinjal plants drenched with copper oxychloride 40 g + streptocycline 2 g (per 10 lit water). This treatment gave 71.93 per cent increase in yield over control. This treatment was followed by the treatment wherein *Pseudomonas fluorescens* was inoculated to seed, seedling roots and soil (T_5). Increase in yield with this treatment over untreated control was 51.11 per cent. Lowest yield of 19.84 t ha^{-1} was harvested from untreated control plots. Maximum performance of the yield observed in these treatments can be attributed to minimum wilt incidence. Berga *et al*. (2001) registered a yield decrease due to an increase in bacterial wilt incidence. Wydra and Semrau (2005) reported comparable *Ralstonia solanacearum* wilt disease reduction and a yield increase associated with biocontrol agents *Bacillus* spp. and fluorescent *Pseudomonas*. Results of the present investigation, thus, are in agreement with those of these researchers.

Economics of wilt management:

The efficacy of *Pseudomonas fluorescens* in terms of economic viability so as to be acceptable to farmers was analyzed using net profit per hectare and benefit: cost ratio as parameters (Table 2). The mean yields obtained in most of the treatment plots were distinctly higher than the untreated control. The yield increase due to disease control through drenching of plants with copper oxychloride 40 g + streptocycline 2 g (per 10 lit water) was found to be maximum (34.11 t ha^{-1}) that derived net profit of Rs. 448534.00 ha^{-1} with a benefit: cost ratio of 2.92. However, maximum benefit-cost (B:C) ratio of 3.11 was recorded in the treatment wherein *P. fluorescens* was inoculated to seed, seedling roots and soil. Consequently, on the basis of relative efficacy of *P. fluorescens* in terms of degree of disease control, additional yield, net profit per hectare and benefit: cost ratio, seed treatment with talc based culture of *P. fluorescens* @ 10 g kg^{-1} seed before sowing and at the time of transplanting seedling root dip (2.5 kg of talc based formulation of *P. fluorescens* in 40 lit water) as well as soil application (2.5 kg of talc based formulation of *P. fluorescens* mixed in 50 kg of FYM $acre^{-1}$ soil), may be recommended to farmers for management of bacterial wilt of brinjal and thereby gaining higher fruit yield.

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