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Eco-friendly management of bacterial wilt (*Ralstonia solanacearum*) of brinjal in Arunachal Pradesh

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ABSTRACT

An experiment was conducted for two consecutive years (2011-12 and 2012-13) to identify resistant brinjal genotypes and evaluate the bio-efficacy of *Pseudomonas fluorescens* against bacterial wilt incited by *Ralstonia solanacearum.* None of the tested genotypes was found highly resistant against pathogen. Swarna Shyamali recorded the lowest wilt incidence (2.50% wilted plants) among all the genotypes. Hybrid-41, Swarna Pratibha, Arka Keshav and VNR-60 also showed resistant reaction. Anamika and BE-706 were found highly susceptible to bacterial wilt. Combination of seedling root dip treatment at the time of transplanting and soil drenching at 20 days after transplanting with *P. fluorescens* gave the lowest wilt incidence (14.75%), highest fruit yield (244.55 q ha^{-1}) and the highest economic returns. However, it was comparable with soil drenching alone with *P. fluorescens.* The chemical treatments with bleaching powder and streptomycine (streptomicin sulphate 90% + tetracycline hydrochloride 10%) were found inferior to *P. fluorescens* soil drenching. Wilt incidence by using soil amendment mustard oil cake @ 5 q ha^{-1} and seedling root dip with *P. fluorescens* @ 25 g l^{-1} was at par with the chemical treatments.

Key words: Brinjal, bacterial wilt, *Pseudomonas fluorescens*, *Ralstonia solanacearum.*

INTRODUCTION

Brinjal, aubergine or eggplant (*Solanum melongena* L.) a member of Solanaceae family is a common vegetable crop grown in tropical and sub-tropical region of the world. In India, It is a major source of income for the small and marginal vegetable growers. One of the major constraint in the successful cultivation of brinjal is bacterial wilt disease caused by *Ralstonia solanacearum* (Yabuuchi *et al*., 15). The pathogen is primarily a soil borne with wide distribution in the tropics, sub-tropics and warm temperate regions of the world (Buddenhagen *et al*., 1). *R. solanacearum* is a rod shaped, gram negative and β-proteobacterium. Approximately, 450 crop species were reported as host of this pathogen (Swanson *et al*., 13). It survives for long time in water, soil and latently infected plants and can be transmitted by soil, water, equipment and infected plant materials (Lwin and Ranamukhaarachchi, 9). The pathogen invades through wounds in roots, lateral root emergence points or stomata and colonizes the intercellular spaces of the root cortex and vascular parenchyma, eventually entering the xylem vessels (Islam and Koki, 7). Among the various options of disease management, use of resistant varieties is an easily adoptable and effective approach. However, the resistance in the variety against disease is also influenced by the environmental factors and the strain of the pathogen. This necessitates the screening of the resistant varieties in different agro-climatic regions. Due to the breakdown of resistance in a variety, different eco-friendly management tactics are needed. Bleaching powder and antibiotic chemicals are suggested for management of the bacterial wilt in brinjal. However, continuous use of such chemicals may leads to development of resistant strains (Ritchie and Dittapongpitch, 12). On the other hand, reports on the possibility for biological management of bacterial wilt using several bacteria and actinomycetes are also available. Keeping in view the above facts, the present study was conducted to identify bacterial wilt resistant varieties of brinjal in the agro-climatic conditions of Arunachal Pradesh and also evaluate the efficacy of a local strain of *Pseudomonas fluorescens* (CHF*Pf*-1) for an effective integrated disease management programme against bacterial wilt in brinjal.

MATERIALS AND METHODS

The experiment was conducted for two consecutive years during 2011-12 and 2012-13, in the research farm of Department of Plant Protection, CAU, Pasighat, Arunachal Pradesh. The soil texture of the experimental field was sandy with sick bed

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condition. The experimental station is situated at an altitude of 155 m above msl and lying between 27° 43' and 29° 20' North latitudes and 94° 42' and 95° 35' East latitudes. It has warm and humid climate with distinct rainy season spread over 5 months from May to September. Average annual temperature, relative humidity and rainfall ranges from 16-35°C, 60-100 per cent and 4000 mm, respectively.

Fourteen brinjal genotypes, *viz.* Anamika, Green Long-183, Pusa Purple Long (PPL)-74, Pusa Kranti (PK)-123, Hybrid-41 and Shekhar collected from Sungro Seeds Ltd., Delhi; BE-706 and Chhaya of Nunhems India Pvt. Ltd., Bengaluru; Kiran of Team Seeds Pvt. Ltd., Sirsa, Haryana; VNR- 60 of VNR Seeds Pvt. Ltd., Raipur; Debgiri of Debgiri Agro Products Pvt. Ltd. Kolkata; Arka Keshav of Indian Institute of Horticultural Research (IIHR), Bengaluru; Swarna Shyamali and Swarna Pratibha of ICAR Regional Centre for Eastern Region, Patna, India, were evaluated for their reaction against the strain of *R. solanacearum* available in the region. Seedlings were raised during July, 2011 and 2012 and 35-day-old seedlings were transplanted in the month of August during both the years. The experimental field was laid out in randomized block design (RBD) in a plot of 12.6 m × 6.6 m. Each genotype was transplanted at 60 cm × 60 cm spacing in three replications. A total 200 plants were transplanted per plot and reared initially but subsequently per cent wilted plants were recorded everyday and bacterial wilt infection was confirmed by dipping the lower cut end of the wilted plant in a glass of water for checking bacterial ooze. Based on the per cent wilted plants, each varieties were graded on a 0-5 scale given by Winstead and Kelman (14) with some modifications (Hussain *et al*., 6). The modified rating scale used as: 0 = Highly resistant (HR): Plants did not show any wilt symptom; 1 = Resistant (R): 1-20% plants wilted; 2 = Moderately resistant (MR): 21-40% plants wilted; 3 = Moderately susceptible (MS): 41-60% plants wilted; 4 = Susceptible (S): 61-80% plants wilted; and 5 = Highly susceptible (HS): More than 80% plants wilted.

The susceptible variety Anamika was used in the experiment to enhance the disease pressure in epiphytotic condition so that the effectiveness of different treatments could be evaluated. The seedlings were raised in the month of September, 2011 & 2012. 35-day-old seedlings were transplanted in the first fortnight of October. Eight treatments including control, *viz*., intercropping with marigold (one row after every nine rows of brinjal and border); mustard oil cakes @ 5 q ha^{-1} as soil amendments; seedlings root dip with CHF*Pf*-1 (a formulation prepared with a local strain of *P. fluorescens* with 2 × 10^8 cfu g^{-1}) @ 25 g l^{-1} of water for 30 min. before transplanting; soil drenching with CHF*Pf*-1 @ 25 kg ha^{-1} at 20 days after transplanting (DAT); seedlings root dip + soil drenching with CHF*Pf*-1; soil drenching with streptocycline (streptomycin sulphate 90% + tetracycline hydrochloride 10%) of Hindustan Antibiotics Ltd, Pune, India @ 2 kg ha^{-1} at 20 DAT; soil drenching of bleaching powder (J. Industries, Guwahati, India) @ 20 kg ha^{-1} at 20 DAT and control were evaluated. For soil drenching 10,000 l of water was used for one hectare. The observations on bacterial wilt incidence were recorded for every 10 days after transplantation. The wilted plants were first confirmed with ooze test and the confirmed plants were recorded and converted into per cent wilted plants. Plant height was recorded at 60 and 80 days after transplanting while number of fruits and weight of fruits in each plucking were recorded from 10 marked plants in each plot and average number and weight of fruit per plant was worked out. The yield ha^{-1} for each treatment was calculated based on number and weight of fruits per plant. The recorded data were subjected for analysis of variance using AGRES version 7.01 © 1994 Pascal International Software Solutions. The benefit: cost ratios were calculated based on the cost involved in crop protection measures. The cost of CHF*Pf*-1 was considered as Rs. 280 kg^{-1}, which is the price of Su-Mona®, a formulation marketed by Pest Control India Ltd., Bengaluru prepared with *P. fluorescens* 2 × 10^8 cfu g^{-1}.

RESULTS AND DISCUSSION

The varieties/ hybrids of brinjal showed different reactions (Table 1) in terms of bacterial wilt incidence. None of the varieties/ hybrids was found to be free from the wilt incidence. The first appearance of wilt incidence ranged from 20.67 DAT in Anamika to 49.33 DAT in Hybrid-41. Among all the genotypes, Swarna Shyamali with 2.50% wilted plants (WP) recorded the lowest wilt incidence and showed resistance reaction. The varieties/ hybrids *viz.* Hybrid-41 (3.08% WP), Swarna Pratibha (8.67% WP), Arka Keshav (9.08% WP) and VNR-60 (9.25% WP) also showed resistance reaction to bacterial wilt. PPL-74 and PK-123 with 42.33 and 56.33 per cent wilt plants, respectively, showed moderately susceptible reaction. Other genotypes, *viz.* Shekhar (60.67% WP), Debgiri (62.58% WP), Kiran (64.50% WP), Green Long-183 (66.50 % WP) and Chhaya (78.00% WP), were found susceptible to bacterial wilt, whereas, BE-706 (85.75% WP) and Anamika (91.50% WP) showed highly susceptible reaction. According to Hoque *et al*. (5), the first symptom of wilting caused by *R. solanacearum* observed on the

Table 1. Reactions of brinjal genotypes against *Ralstonia solanacearum.*

Genotype	First appearance of wilt symptoms (DAT#)	50% wilt (DAT)	Per cent wilt incidence*	Reaction
Anamika	20.67	51.17	91.50 (75.12)^{g}	HS
BE-706	31.00	52.17	85.75 (69.41)^{fg}	HS
Chhaya	25.67	48.50	78.00 (62.49)^{ef}	S
Green Long-183	22.00	54.50	66.50 (54.92)^{de}	S
Kiran	27.83	54.50	64.50 (53.95) ^{d}	S
Debgiri	22.17	58.67	62.58 (55.95)^{d}	S
Shekhar	24.17	72.67	60.67 (51.46)^{d}	S
PK-123	35.00	81.50	56.33 (48.83)^{d}	MS
PPL-74	36.83	-	42.33 (40.54)^{c}	MS
VNR 60	42.17	-	9.25 (17.42)^{b}	R
Arka Keshav	40.16	-	9.08 (17.19)^{b}	R
Swarna Pratibha	40.50	-	8.67 (16.40)^{ab}	R
Hybrid-41	49.33	-	3.08 (9.64)^{a}	R
Swarna Shyamali	48.17	-	2.50 (8.23)^{a}	R
$CD_{0.05}$	8.33	7.67	8.26	
CV (%)	14.92	13.52	11.91	

*Figures in parentheses are angular transformed values; # DAT=days after transplanting ; R = Resistant, MR = Moderately resistant, MS = Moderately susceptible, S = Susceptible, HS = Highly susceptible

15th days after inoculation of the bacterium. In our study, the genotypes showing highly susceptible and susceptible reactions showed first average wilting symptom from 20.67 to 31.00 DAT. However, the varieties/ hybrids with resistance reaction delayed in expressing the wilt symptom starting from 40.16 DAT. The results of present study were in agreement with Hussain *et al.* (6). They also recorded longer incubation period of bacterial wilt in resistant and moderately susceptible genotypes.

Data on efficacy of different eco-friendly treatments are shown in Table 2. All the treatments showed a significantly lower wilt incidence of bacterial wilt disease than the untreated control. The lowest incidence of bacterial wilt with 14.75% WP was recorded in the plot treated with seedling root dip + soil drenching with *P. fluorescens* and it was at par with soil drenching with *P. fluorescens* (18.25% WP). Soil drenching with *P. fluorescens* was comparable with soil drenching with streptomycine (19.83% WP), soil application with mustard oil cake (20.59% WP), soil drenching with bleaching powder (21.00% WP) and seedling root dip with *P. fluorescens* (23.58% WP). Intercropping with marigold recorded a highest 42.39% WP among all treatments except untreated control. The highest average plant height (68. 29 cm), highest average number of fruit per plant (9.14 fruits) and average fruit weight (114.58 g fruit^{-1}) was recorded in seedling root dip + soil drenching with *P. fluorescens* and it was closely followed by soil drenching with *P. fluorescens* with 67.84 cm plant height, 8.75 fruits and 113.26 g fruit^{-1}, in terms of average plant height, average number of fruits per plant and average weight of fruit, respectively. The highest yield ha^{-1} was recorded in treatment with seedling root dip + soil drenching with *P. fluorescens* (244.55 q ha^{-1}) and it was comparable with soil drenching with *P. fluorescens* (222.14 q ha^{-1}). The yield of soil application with mustard oil cake (190.60 q ha^{-1}), seedling root dip with *P. fluorescens* (185.94 q ha^{-1}), soil drenching with bleaching powder (181.27 q ha^{-1}) and soil drenching with streptomycine (178.67 q ha^{-1}) were at par. The intercropping with marigold recorded only 96.74 q ha^{-1}. While, considering the net profit of the different treatments, seedling root dip + soil drenching with *P. fluorescens* gave the highest profit with ₹ 63,235 ha^{-1} and it was followed by soil drenching with *P. fluorescens* (₹ 53,730 ha^{-1}), seedling root dip with *P. fluorescens* (₹ 48,430 ha^{-1}), soil application with mustard oil cake (₹ 39,360 ha^{-1}), soil drenching with bleaching powder (₹ 38,495), soil drenching with streptomycine (₹ 24,995) and intercropping with marigold (₹ 3,530) in descending order. In terms of benefit: cost ratio, only soil drenching with *P. fluorescens* gave the highest benefit (29.49:1).

Table 2. Bio-efficacy of *Pseudomonas fluorescens* combinations against bacterial wilt of brinjal.

Treatment	Per cent wilt incidence*	Plant height (cm)	No. of fruits plant^{-1}	Fruit wt. (g)	Yield (q ha^{-1})
Marigold (after every 9 rows of brinjal and borders)	42.39 (40.58)^{c}	64.44^{e}	6.99^{d}	108.16^{de}	96.74e
Mustard oil cakes @ 5 q ha^{-1} as soil amendment	20.59 (26.87)^{b}	67.24^{abc}	7.93^{b}	110.78^{bcd}	190.60b
Soil drenching with *P. fluorescens* @ 25 kg ha^{-1}	18.25 (25.19)^{ab}	67.84^{ab}	8.75^{a}	113.26^{ab}	222.14a
Seedlings root dip with *P. fluorescens* @ 25 g l^{-1} of water	23.58 (28.95)^{b}	66.64^{bc}	7.97^{b}	111.46^{bc}	185.94b
Seedlings root dip with *P. fluorescens* @ 25 g l^{-1} of water + soil drenching with *P. fluorescens* @ 25 kg ha^{-1} at 20 DAT	14.75 (22.40)^{a}	68.29^{a}	9.14^{a}	114.58^{a}	244.55a
Streptomycine (streptomycin sulphate 90% + tetracycline hydrochloride 10%) @ 2 kg ha^{-1} soil drenching at 20 DAT	19.83 (26.41)^{b}	65.71^{cde}	7.44^{bcd}	109.42^{cde}	178.67b
Bleaching powder @ 20 kg ha^{-1} soil drenching at 20 DAT	21.00 (27.07)^{b}	66.18^{cd}	7.69^{bc}	109.44^{cde}	181.27b
Untreated control	60.17 (50.02)^{d}	64.90^{de}	7.27^{cd}	107.74^{e}	85.68c
$CD_{0.05}$	5.67	1.62	0.57	2.88	22.43
CV (%)	9.97	1.41	4.19	1.50	7.48

*Figures in parentheses are angular transformed values

The present findings of reduction in wilt incidence with the root and soil application of *P. fluorescens* were in conformity with Chakravarty and Kalita (3). Root zone application of *P. fluorescens* increased rhizosphere population of the bacteria thus; increase in the population of the antagonists might have suppressed the population of the *R. solanacearum*. In addition to the environmental factor,wilting of the plants is mainly dependent on the population of *R. solanacearum* in the soil, when the pathogen population is reduced corresponding to the higher level of the antagonists, the wilt will also decrease simultaneously (Chakravarty and Kalita, 3). Biological control of soil borne pathogens by soil application of certain strains of fluorescent pseudomonads was also reported by Burr and Caesar (2) as they have the ability to colonize on the roots. Higher wilt incidence in the treatment with root dip alone as compare to soil drenching and root dip + soil drenching may be due to lesser population of *P. fluorescens.* In the present investigation, it is also observed that soil application of *P. fluorescens* also increases the plant height, average fruit weight per plant and number of fruits per plant. *P. fluorescens* giving positive effect on the plant growth characters like plant height, fruit weight per plant and number of fruits per plant was also reported by Jinnah *et al.* (8). Higher yield observed in soil drenching and root dip + soil drenching of *P. fluorescens* is the combined effect of *P. fluorescens* acting as biocontrol agent and plant growth promoting rhizobacteria. Ramesh *et al.* (11) also reported that *P. fluorescens* act as a biocontrol agent of bacterial wilt in brinjal as well as plant growth promoting rhizobacteria. The efficacy of soil amendment with mustard oil cake in terms of reducing wilt incidence was comparable with the chemical treatments. This may be the result of their effectiveness in controlling plant parasitic nematodes in the soil. The nematodes make wounds through which the bacteria may enter and also release metabolites useful for bacterial growth (Hayward, 4). Thus, galling by root knot nematode increases the susceptibility of tomatoes to bacterial wilt. Lower wilt incidence in intercropping with marigold may also possibly due to lower population of nematode in the marigold intercropped field compared to untreated control. Crop rotation or mixed cropping including marigold suppressed nematode population in the soil, however, effectiveness varied with the varieties of the marigold (Ploeg, 10).

Comparing the economics of the bacterial wilt management tactics, treatment of *P. fluorescens* as combination of seedling root dip and soil drenching gave the highest economic return (Table 3). Treating alone as soil drenching or seedling root dip of *P. fluorescens* also gave better return than soil amendment and chemical treatments.

The brinjal genotypes Swarna Shyamali, Hybrid-41, Swarna Pratibha, Arka Keshav and VNR-60 can be recommended for cultivation under the agro-climatic conditions of Arunachal Pradesh considering the bacterial wilt problem in the region. As these varieties/ hybrids have different fruit shape, size, colour and yield, a desired one can be selected for cultivation. Further, to reduce the disease pressure on these varieties/ hybrids, *P. fluorescens* (2×10^8

Table 3. Economics of different eco-friendly treatments against bacterial wilt of brinjal.

Treatment	Input required (kg ha^{-1})	Cost of input (₹ ha^{-1})	Cost of input application (₹ ha^{-1})	Total cost of input (₹)	Yield (q ha^{-1})	Additional yield over control (q ha^{-1})	Gross profit (₹ ha^{-1})	Net profit (₹ ha^{-1})	B:C ratio
Marigold (after every 9 rows of brinjal and borders)	0.20	1000	1000	2000	96.74	11.06	5,530	3,530	2.77:1
Mustard oil cakes @ 5 q ha^{-1} as soil amendment	500	10000	2500	11000	190.60	104.92	52,460	39,360	4.20:1
Soil drenching with *P. fluorescens* @ 25 kg ha^{-1}	25	7000	7500	14500	222.14	136.46	68,230	53,730	4.71:1
Seedlings root dip with *P. fluorescens* @ 25 g l^{-1} of water	2.5	700	1000	1700	185.94	100.26	50,130	48,430	29.49:1
Seedlings root dip with *P. fluorescens* @ 25 g l^{-1} of water + soil drenching with *P. fluorescens* @ 25 kg ha^{-1} at 20 DAT	27.5	7700	8500	16200	244.55	158.87	79,435	63,235	4.90:1
Streptomycin (streptomycin sulphate 90% + tetracycline hydrochloride 10%) @ 2 kg ha^{-1} soil drenching at 20 DAT	2.00	14000	7500	21,500	178.67	92.99	46,495	24,995	2.16:1
Bleaching powder @ 20 kg ha^{-1} soil drenching at 20 DAT	20	1800	7500	9300	181.27	95.59	47,795	38,495	5.14:1
Untreated control	-	-	-	-	85.68	-	-	-	-

Brinjal = ₹ 500 q^{-1}; Marigold = ₹ 5000 kg^{-1}; Mustard cake = ₹ 20 kg^{-1}; *P. fluorescens* = ₹ 280 kg^{-1}; Streptocycline = ₹ 7000 kg^{-1}; Bleaching powder = ₹ 90 kg^{-1}; Labour charge = ₹ 250 per day; Sprayer charge = ₹ 50 per day; Transportation charge for mustard oil cake = ₹ 500.

cfu g^{-1}) can be applied as seedling root dip @ 25 g l^{-1} for 30 min. and/ or soil drenching @ 25 kg ha^{-1}. Even for the susceptible genotypes, combined application of *P. fluorescens* as seedling root dip and soil drenching could substantially reduce the bacterial wilt incidence in brinjal.

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