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RESEARCH ARTICLE

Management of bacterial wilt of tomato caused by *Ralstonia solanacearum* by bacterial antagonists and botanicals

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SUMMARY

Study was undertaken to know the effective management strategy through novel bio control agents and botanicals against *Ralstonia solanacearum* a dreaded pathogen causing bacterial wilt of tomato. The study revealed that *Bacillus subtilis* was found to be most effective in inhibiting the growth of the pathogen followed by *Pseudomonas fluorescens*, by producing an inhibition zone of 22.50 mm and 18.00 mm in diameter, respectively under *in vitro* conditions. However, among plant extract tested Isabgol seed extract was found to be highly inhibitory to the growth of the pathogen in which inhibition of 22.00, 19.50 and 18.00 mm was observed at 1:0, 1:1 and 1:5 dilutions. Similarly under field condition, Bacteriophages and *Bacillus subtilis* were found to be very effective in reducing the disease incidence by 72.50 per cent and 64.58 per cent with reduced soil population of the pathogen both in soil and rhizosphere

Key Words : *Ralstonia solanacearum*, Bacterial antagonists, Botanicals

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Tomato (*Lycopersicon esculentum*) is one of the important vegetable crop, which suffers badly from a most destructive bacterial disease caused by *Ralstonia solanacearum* (E.F. Smith) (Yabuuchi *et al.*, 1995). The pathogen is most widespread in tropical, subtropical and warm temperate regions of the world. This disease is a major constraint in the production of tomato and many other important vegetables, fruit, cash-crops *viz*., potato, brinjal, ginger, groundnut, tobacco, banana etc. In extreme cases loss in yield due to the disease in eggplant and tomato has been reported to be as high as 80 and 90 per cent, respectively (Rao, 1976). Representatives of 50 families comprising of more than 350 host plants are affected by this disease and that number of new species continue to increase (Hayward,

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1991). The disease is wide spread, affecting many solanaceous crops in India, especially in Karnataka, which is one of the leading vegetable growing states in the country. Biological control has been regarded as an important component of integrated management strategy for the control of bacterial wilt. There are conflicting reports about efficiency of various biological agents in controlling bacterial wilt under field conditions. Therefore, an attempt was made to evaluate various plant extracts obtained from different plant species under *in vitro* conditions. While choosing the botanicals care has been taken to see that medicinal plants and plant parts which are known to possess antimicrobial property are explored as the potential antibacterial agent in management of this important dreaded disease.

MATERIAL AND METHODS

In vitro and field experiments have been designed and carried out to develop suitable control measures for bacterial wilt of tomato so as to identify the effective control and develop sustainable management strategy.

In vitro evaluation of antagonistic bacteria on the growth of *R. solanacearum:*

Four antagonistic bacteria namely *Pseudomonas fluorescens* (Trevisan) Migula, *Bacillus megatherium* (Prazmowski) *Pseudomonas aeroginosa* Mace and *Bacillus subtilis* Ehrenberg) Cohn. maintained in Plant Bacteriology Laboratory, Department of Plant Pathology, University of Agricultural Sciences were used in the studies.

Pseudomonas fluorescens was grown and maintained on King's B broth. *Pseudomonas aeroginosa, Bacillus subtilis* and *B. megatherium* were grown and maintained in sterilized distilled water in vials.

A heavy suspension of 48-hour-old culture *Ralstonia solanacearum* (7 x 10^8 cfu/ml) was mixed with molten (50^0C) nutrient agar contained in 500 ml conical flask so as to get a thick lawn of the bacteria. The seeded medium was then poured into sterilized Petri plates and allowed to solidify.

A loopful of each of the antagonistic bacteria was placed in the center of seeded medium contained in the Petri dishes. The inoculated plates were incubated at 32^0C for 48 hours. Observations were recorded for the production of zone of inhibition around the antagonistic bacteria, and the diameter of the inhibition zone was measured after 48 hr incubation.

In vitro evaluation of botanicals against *R. solanacearum :*

The efficiency of various plant extracts were tested against *R. solanacearum,* by inhibition zone assay method. In all 5 botanicals that were used to test the efficiency of the water extracts of different plant species are presented in Table A.

The seeds, twigs and leaves constituting economic parts of the plants were used for the purpose of extraction. 50 g of leaves/seeds/twigs as the case were taken and cut into small pieces under aseptic conditions. The sample was put into warring blender containing 50ml sterilized distilled water at a ratio of 1:1 (water: plant material). The blended material was then squeezed through a sterilized muslin cloth so as to get a crude liquid extract. The crude extract was filtered through Whatmen No. 1 filter paper followed by sterilized Seitz filter. The sterilized filterate was collected in sterilized glass tubes and the tubes were sealed under aseptic conditions and labelled. The water extract was kept at 5^0C in refrigerator for further use.

A heavy suspension of *R. solanacearum* (7 x 10^8 cfu/ml) was mixed with molten (50^0C) CPG agar contained in 500 ml Erlenmeyer flask so as to get a thick growth of bacteria on the medium. The seeded medium was poured in sterilized Petridishes and allowed to solidify. Sterilized filter paper disc (Whatman No. 1) measuring 8 mm diameter were soaked for 10 min in plant extracts and placed on the surface of seeded CPG agar medium contained in the Petridishes. The inoculated plates were

Table A : Botanicals used for *in vitro* evaluation against *Ralstonia solanacearum*

Sr. No.	Botanicals	Dilutions
1.	Agro boom (Herbal product)	1:0, 1:1, 1:5
2.	Isabgol seed extract (*Plantago ovata* Forsk.)	1:0, 1:1, 1:5
3.	Maize root extract *(Zea mays*)	1:0, 1:1, 1:5
4.	Miswak (*Salvadora persica*)	1:0, 1:1, 1:5
5.	Ragi root extract (*Eleusine coracana*)	1:0, 1:1, 1:5

incubated first at 5^0C for 23 hr so as to allow the diffusion of the extract into the medium. The plates were then transferred to incubator maintained at 32^0C and incubated for 48 hours.

Observations were recorded by measuring the zone of inhibition produced around the filter paper disc, against *R. solanacearum* by measuring the inhibition zone in diameter.

Field evaluation of bacterial antagonists and botanicals in the management of bacterial wilt of tomato caused by *R. solanacearum* :

A field experiment was laid out to find out the efficiency of various biocontrol agents including antagonistic bacteria, botanicals and bacteriophages in red sandy loam soils at ZARS, UAS, GKVK, Bangalore in naturally wilt infested soils, in which about 80 per cent bacterial wilt incidence was recorded during the previous cropping.

The bioagents both antagonistic bacteria and botanicals, which were found effective in inhibiting the *R. solanacearum* under *in vitro* was taken to the field. Each treatment was replicated thrice under RCBD design and in each treatment 16 plants were kept. The following treatments were imposed.

Treatments imposed in the field trial conducted :

T_1 : Seedling dip in *Bacillus subtilis*+ soil drench with *Bacillus subtilis* at 30 and 60 DAT

T_2 : Seedling dip in *Bacillus megatherium*+ soil drench with *Bacillus megatherium* at 30 and 60 DAT

T_3 : Seedling dip in *Pseudomonas fluorescens* + soil drench with *Pseudomonas fluorescens* at 30 and 60 DAT

T_4 : Seedling dip in *Pseudomonas aeruginosa* + soil drench with *Pseudomonas aeruginosa* at 30 and 60 DAT

T_5 : Seedling dip in miswak @1:1 dilution+ soil drench with miswak @1:1 dilution at 30 and 60 DAT

T_6 : Seedling dip in agroboom @ 0.5 per cent+ soil drench with agroboom @ 0.5 per cent at 30 and 60 DAT

T_7 : Seedling dip in isabgol @1:1 dilution+ soil drench with isabgol @1:1 dilution at 30 and 60 DAT

T_8 : Seedling dip in Bacteriophages + soil drench with Bacteriophages at 30 and 60 DAT

T_9 : Seedling dip in ragi root extract @1:1 dilution+ soil drench with ragi root extract @1:1 dilution at 30 and 60 DAT

T_{10} : Seedling dip in maize root extract @1:1 dilution+ soil drench with maize root extract @1:1 dilution at 30 and 60 DAT

T_{11} : Untreated control

Observation on percentage of wilt incidence was recorded. The population of *R. solanacearum* in the rhizosphere and soil was recorded at flowering and at harvest stage, initial population was $10x10^8$ cfu/g soil before transplanting. The statistical analysis was done for the data by RCBD design.

The soil samples for the estimation of the pathogen was obtained by drawing soil plugs with the help of soil sampler of one inch diameter. Soil 3-4" away from the base of the plants to a depth of 3-4" were drawn. Rhizosphere soil sticking to the roots were collected from each plant. The population was enumerated by dilution plate technique on Modified semi-selective SMSA (Englebrecht, 1994)

RESULTS AND DISCUSSION

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

***In vitro* evaluation *of* antagonistic bacteria on the growth of *R. solanacearum*:**

The antagonistic bacteria namely *Pseudomonas fluorescens, P. aeroginosa, Bacillus subtilis* and *B. megatherium* were tested for their efficiency in inhibiting the growth of *Ralstonia solanacearum in vitro.* The data is presented in Table 1.

Table 1 : Effect of various bioagents against *Ralstonia solanacearum*

Sr. No.	Bioagent used	Mean inhibition zone (mm)
1.	*Bacillus subtilis*	22.50
2.	*Pseudomonas fluorescens*	18.00
3.	*Bacillus megatherium*	12.25
4.	*Pseudomonas aeroginosa*	9.50
S.E.±		0.386
C.D. (P=0.01)		1.162

Of the four antagonistic bacteria tested, *Bacillus subtilis* was found to be most effective in inhibiting the growth of the pathogen followed by *Pseudomonas fluorescens,* by producing an inhibition zone of 22.50 mm and 18.00 mm in diameter, respectively. However, *B. megatherium* and *P. aeroginosa* were found to

produce an inhibition zone of 12.25 and 9.50mm in diameter, on the lawn of *Ralstonia solanacearum* grown on TTC medium without tetrazolium salt. The four antagonistic bacteria *viz., Pseudomonas fluorescens, P. aeroginosa, Bacillus subtilis* and *B. megatherium* differed significantly in their ability to inhibit the pathogen on seeded media. Karuna and Khan (1994) found that *Pseudomonas fluorescens* was very effective in inhibiting the growth of the pathogen followed by *Pseudomonas aeroginosa*. However, they observed *Bacillus polymyxa* was not at all effective in inhibiting the growth of the bacteria. While in present study, *Bacillus polymyxa* produced inhibiting zone of 5.0 mm, which was found to be moderately effective. *Bacillus subtilis* also was found to be inhibitory for *Ralstonia solanacearum* (Karuna and Khan, 1994).

In vitro evaluation of botanicals against *R. solanacearum :*

The *in vitro* evaluation was done to study the inhibitory effect of various plant extracts on the growth of *R. solanacearum* and the data are presented in the Table 2, plant extracts were tested at 1:0, 1:1 and 1:5 dilutions, Isabgol seed extract was found to be highly inhibitory to the growth of the pathogen in which inhibition of 22.00, 19.50 and 18.00 mm was observed. The next best plant extract was Miswak twig extract with inhibition zone of 20.00, 19.00 and 16.50 mm at 1:0, 1:1 and 1:5 dilutions, respectively followed by agroboom, ragi root extract and maize root extracts with inhibition zone measuring about 12.50,8.60 and 7.00 mm at 1:0 dilution. Several workers have reported that the extracts of medicinal plants possess inhibitory effect on wide range of microorganisms (Sivasankara rao and Nigam, 1987). Essential oil extracts from *Calendula officinalis* and *Thymus serphyllum* was highest against *Corynebacterium michiganense* pv. *michiganense* (Mishenkova *et al.,* 1983).

Leaf extracts from citronella and adathoda was highly inhibitory to the growth of *R. solanacearum* and also pathogen was sensitive to neem extracts (Karuna, 1993). Venkatesh (1988) observed essential oil obtained from eucalyptus and citronella was highly inhibitory, while geranium and neem extract were moderately effective in inhibiting the growth of *R. solanacearum.*

Field evaluation of bacterial antagonists and botanicals in the management of bacterial wilt of tomato caused by *R. solanacearum :*

A field trial was conducted to study the efficiency of bio agents on the incidence of bacterial wilt, pathogen multiplication and yield is presented in the Table 3. Among the microbial antagonists tested bacteriophages and *Bacillus subtilis* were found to be very effective in reducing the disease incidence by 72.50 per cent and 64.58 per cent.The population of the pathogen in soil and rhizosphere was also suppressed drastically in plots applied with these two antagonists. The population was reduced by 1000 folds in soil and rhizosphere of bacteriophage and *Bacillus subtilis* as compared to control plots. Miswak water extract at 1:1 dilution was effective in reducing the disease incidence by 60.42 per cent and suppression of the pathogen population in soil and rhizosphere was 1000 and 100 folds at flowering and harvesting time.

The biological agents were found to suppress the multiplication of the pathogen in the infected soil. Bacteriophage (UASP) and *Bacillus subtilis* and were very effective in reducing the growth and multiplication of the pathogen by $2x10^4$, $2.33x10^5$ and $4.67x10^3$ $5.67x10^4$ cfu /g soil in soil and rhizosphere at flowering time. *Pseudomonas fluorescens, Pseudomonas aeroginosa,*

Table 2 : Effect of various botanicals on the growth of *Ralstonia solanacearum in vitro*

Botanicals / Concentrations	Inhibitory growth (mm)			Mean inhibition (mm)
	1:0	1:1	1:5	
Agro boom	12.50	9.00	4.50	8.67
Isabgol	22.00	19.50	18.00	19.84
Maize root extract	7.00	5.00	1.70	4.57
Miswak	20.00	19.00	16.50	18.5
Ragi root extract	8.60	7.30	3.00	6.3
	S.E.±	SED	C.D. (P=0.05)	
Concentration A	0.01728	0.01856	0.05196	
Botanicals B	0.03520	0.03725	0.01070	
Interactions AXB	0.05860	0.06203	0.17850	

Table 3 : Effect of various bioagents in the control of bacterial wilt of tomato under field conditions

Treatments	Per cent wilt incidence							Per cent disease control over check	Population at flowering cfu/g soil		Population harvest cfu/g		Yield t/ ha
	30 DAP	35 DAP	40 DAP	45 DAP	50 DAP	55 DAP	60 DAP	65 DAP	70 DAP		Soil	Rhizosphere	Soil	Rhizosphere	
T_1 *B. subtilis*	12.50	16.67	25.00	27.08	27.08	29.17	31.25	33.33	35.42	64.58	4.67×10^3	5.67×10^4	2.33×10^4	5.67×10^6	34.18
T_2 *B. megatherium*	8.33	25.00	31.25	35.42	37.50	39.58	41.67	45.83	47.92	52.08	8.0×10^3	8.0×10^4	5.67×10^4	8.33×10^5	28.57
T_3 *P. fluorescens*	10.42	18.75	29.17	31.25	33.33	35.42	35.42	37.50	41.67	58.33	5.33×10^3	6.67×10^4	5.0×10^4	7.0×10^6	25.57
T_4 *P. aeruginosa*	10.42	22.92	29.17	31.25	33.33	39.58	45.83	50.00	50.00	50.00	4.33×10^3	8.0×10^4	6.33×10^3	3.00×10^5	23.50
T_5 Miswak @ 1:1	12.50	18.75	29.17	31.25	33.33	33.33	35.42	37.50	39.58	60.42	3.67×10^3	2.00×10^4	3.00×10^3	3.33×10^4	29.36
T_6 Agroboom @ 5%	12.50	35.42	45.83	54.17	58.33	60.42	64.58	66.67	68.75	31.25	1.33×10^4	2.00×10^5	3.67×10^4	4.00×10^6	15.15
T_7 Isabogel	8.33	18.75	31.25	33.33	37.50	39.58	43.75	47.92	52.08	47.92	7.67×10^3	6.33×10^5	5.00×10^5	6.00×10^6	24.97
T_8 Bacteriophage	10.67	13.75	15.00	17.08	17.17	18.25	20.33	25.50	27.50	72.50	5.00×10^3	6.00×10^4	2.67×10^4	5.33×10^4	32.65
T_9 Ragi straw	8.33	33.33	37.50	41.67	45.83	50.00	52.08	56.25	60.42	39.58	2.00×10^4	2.33×10^5	1.00×10^5	1.33×10^6	20.49
T_{10} Maize straw	10.42	18.75	43.75	50.00	56.25	60.42	62.50	66.67	70.83	29.17	12.00×10^3	1.33×10^6	1.33×10^5	7.00×10^7	14.50
T_{11} Untreated control	16.67	31.25	62.50	66.67	79.17	87.50	95.83	100.00	100.0	-	8.33×10^5	14.00×10^6	12.00×10^5	13.33×10^7	-
S.E.±	0.004	0.007	0.009	0.008	0.006	0.014	0.018	0.031	0.042		0.004	0.018	0.007	0.009	
C.D. (P=0.05)	0.012	0.023	0.029	0.025	0.0021	0.044	0.056	0.095	0.130		0.012	0.056	0.023	0.029	

Note: DAP= Days after planting

B. megatherium recorded population of 6.67×10^4, 8×10^4 and 8×10^4 cfu/ g soil in rhizosphere at flowering, while the population in control plot was 14.00×10^6 cfu/g soil.

Bacillus subtilis and bacteriophage (UASP) treated plots recorded highest yield 34.18 and 32.65t/ha., respectively. However, Miswak extract treated plots gave yield 29.36 t/ha, which was found next best treatment.

The highest efficacy of bacteriophage indicate that the phage could be used in control of wilt disease to fit into integrated disease practices Madhiazhagon (2001) observed reduction in bacterial leaf blight of rice by 40.55 per cent in bacteriophage treated plots. When phage was applied as root dip at transplanting. Manjunath *et al.* (2003) obtained 69.78 per cent control of bacterial wilt of tomato in pot culture. Further, he studied that application of phage a day before transplantation, which reduced disease incidence by 92 per cent. Similarly pathogen population reduced in both soil and rhizosphere of the plant.

The results of present finding further revealed that *B. subtilis* and *P. fluorescens* are very good biocontrol agents and as matter of fact *B. subtilis* treated plants gave highest yield due to growth promoting activity of this bacteria. The results of our finding are in concurrence with Shekhawat (1993), Sivasankararao and Nigam (1987), Karuna and Khan (1994) who also observed highest number of transplantable seedlings with seed bacterization. Suppressing of pathogen population and increase in yield by 42.42 per cent (Sunaina *et al.*, 1997).

Better control with *P. fluorescens* and *B. subtilis*. Further he found that *Bacillus subtilis* has the growth promoting activity, which is amply evident from the higher production obtained in spite of compatibility (Venkatesh, 1988).

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