

Original Research Article

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Screening of Antagonistic Bacteria and Organic Amendments against Bacterial Soft Rot of *Aloe vera* Disease Incidence in the Pot

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ABSTRACT

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Aloe vera soft rot *Erwinia chrysanthemi* is a bacterial disease that affects the aloe plant worldwide and causes serious economic losses in southern Tamil Nadu. The objective of this study is to study the effect of Antagonistic Bacteria and organic amendments on *Aloe vera* soft rot and rhizosphere population. Methods in the present study, pot experiments were conducted to evaluate the *Pseudomonas fluorescens* 32 and 45 and *Bacillus subtilis* strains and their derived bio organic amendments as potential biocontrol agents against aloe vera soft rot disease. In the pot, Pf isolates Pf 32, Pf 45 and *Bacillus* isolate of Bs 5 were compatible with each other. The shelf life of the talc based formulation of Pf 32, Pf 45 and Bs5 was more than 90 days. In the pot culture experiment, pre inoculation spraying of Pf32 + Pf 45 + Bs5 talk based formulation (1g/l) was the most effective against *Aloe vera* soft rot disease.

Introduction

Aloe vera (L) Burm is one of the medicinal plants widely used throughout the world (Sofowora, 1984). It is a well-known medicinal plant of India and is one of the world most demanded crop. It is naturalized throughout the country, more common along the west coast (Robert and Hentry, 2004). It has been established that the inner gel of the leaf contains most of its beneficial part (Swaminathan and Kochhar, 1992). *Aloe vera* has many medicinal and cosmetic usages and hence has growing demand in the market. The plant is a rich source of amino acids and enzymes (Blumenthal and Mark, 2000). The gel of the leaf of *Aloe vera* contains 96 per

cent of water and the remaining different elements such as vitamins and minerals (Tyler, 1994). The plant also contains essential oil components (Davis *et al.*, 2000). It is referred to as miracle plant for its numerous uses, particularly in the area of man's health (Hect, 1981). Among the diseases affecting this crop, pest viz., ants, mealy bug, aloe scale, weevil aphid, shield bug, root bug and mites and diseases viz., leaf spot, basal stem rot, aloe rust sooty mould and bacterial soft rot. Among these bacterial soft rot of *Aloe vera* caused by *Erwinia chrysanthemi* is economically important as it causes yield loss upto 80 per cent. Besides,

chemicals pose serious health hazards to an applicator as well as to a consumer of the treated material.

In addition to target organism, pesticides also kill various beneficial organisms. Their toxic forms persist in soil and contaminate the whole environment. Prospects of biological control of soil-borne plant pathogens using most promising biocontrol agent, the genus *Pseudomonas* has been described. Successful reductions of soft rot in many crops with application of different species of have been found. However, it is also reported that all the isolates of *Pseudomonas* spp. are not equally effective in control of pathogen in vitro and in vivo conditions to control diseases. Therefore, specific isolates are needed for successful control of a particular pathogen. Therefore the objectives of the present study were to assess the ability of isolates of *Pseudomonas* species in suppressing the populations of soft rot of aloe vera under in vitro and in vivo conditions.

The PGPR formulation was also effective under field conditions for the management of rice sheath blight and mango anthracnose (Vidhyasekaran and Muthamilan, 1999; Vivekanandan *et al.*, 2004). A peat-based formulation of PGPR was also found to be effective against the rice sheath blight disease under field conditions (Rabindran and Vidhyasekaran, 1996). Rajkumar (2006) reported that the ability of survival of fluorescent *Pseudomonas* in talc was differed with the isolates. Seed treatment followed by foliar spray of talc based formulation combined application of $BBs_1 + BBs_2 + BPF_1$ on two, four and six months after planting increased *viz.*, chlorophyll content, nitrate reductase activity, stomatal resistance, transpiration rate, number of leaves and leaf area index. Management of these diseases by cultural methods and by using toxic chemicals as bactericides has reduced the biodiversity of soil microbes and lead to the

development of resistant strains of the pathogen. Hence, the present investigation was taken under to develop eco-friendly management strategies like exploiting induced resistance by using native bacterial antagonistics and botanicals.

Materials and Methods

Efficacy of biocontrol agents against soft rot of *Aloe vera* in glasshouse

To study the biocontrol potential of the antagonists *Pseudomonas* and *Bacillus*, pot culture experiment was conducted in a completely randomized design (CRD). The talc based formulation of the antagonistic bacteria was delivered as seedlings dip 25 g/plant. The pathogen (*Erwinia*) mass multiplied on nutrient agar medium was incorporated in the pots at five per cent (w/w) under glass house conditions. The observations on the percent disease incidence of soft rot were recorded upto harvest. In addition growth parameters like height, number of leaves and leaf weight were recorded at bimonthly intervals.

Each treatment was replicated thrice and nine plants per replication were maintained. Potting mixture used was Red earth: River sand: Farm yard manure -1:1:1. Each 30cm pot contained three kg of this potting mixture.

These pots were maintained in the glasshouse by regular and uniform watering. There were 25 treatments as detailed bellows.

T1-*Pseudomonas fluorescence*-32 (1kg/l)

T2 -Pf45 (1kg/l)

T3-*Bacillus subtilis* 5(1kg/l)

T4-BS9 (1kg/l)

T5-T1 + T2 (1kg/l)

T6-T1 + T3 (1kg/l)

T7-T1 + T4 (1kg/l)

T8-T2 + T3 (1kg/l)

T9-T2 + T4 (1kg/l)

T10-T3 + T4 (1kg/l)
T11-T1 + T2 + T3 (1kg/l)
T12-T1 + T2 + T3 + T4 (1kg/l)
T13-NSKE (10%)
T14-Groundnut cake extracts (10%)
T15-Mahua cake extracts (5%)
T16-Neem oil (3%)
T17-Mahua oil (3%)
T18-Soap oil (3%)
T19-Vermicompost extracts (3%)
T20-Coirpith compost extracts (3%)
T21-Zimmu leaf extracts (10%)
T22-Aswaganadha (10%)
T23-Streptomycin (0.01%)
T24-Copper oxy chloride (0.1%) and
T25-Control

Results and Discussion

Glasshouse studies

Efficacy of spraying of talc based antagonistic bacterial formulations against soft rot of *Aloe vera* in pot culture

The results of the pot culture experiment conducted on the efficacy of spraying the formulations bacterial biocontrol agents (*P. fluorescens* and *B. subtilis*) on *Aloe vera* plants and dipping the suckers before planting in the suspension of the biocontrol agents in by giving the first spray 45 days after planting followed by six monthly sprays, against soft rot of *Aloe vera* are furnished in (Table 1).

Among the 25 treatments comprising of four antagonistic bacteria in talc formulation viz., *P. aeruginosa* Pf 32 (1g/l) *Pseudomonas* 45 (1g /lit) and *Bacillus subtilis* 5 and *B. subtilis* 9 (1 g/l), mixture formulations of Pf 32 + Pf 45, Pf 32 + Bs 5, Pf 32 + Bs 9, Pf 45 + Bs 5, Pf 45 + Bs 9, Bs 5 + Bs 9, Pf 32 + Ps 45 + Bs 5 + Bs 9, Pf 32 + Pf 45 + Bs 5, three oil cake extracts in water (5%) viz., NSKE, groundnut cake, Mahua cake, three plant oil formulations viz., neem oil (3%) Mahua

oil(3% and 5%) viz., decomposed banana, Pulses leaves, sheep manure, two leaf extracts in water (10%) viz., Zimmu (*Allium sp*) and neem tested against *Aloe vera* bacterial soft rot disease in pot culture as pre inoculation spray (24 before inoculation of *E. chrysanthemi*) spraying of Pf 32 + Pf 45 + Bs 5 recorded the least (11.11%) disease incidence as against (55.77%) in the unsprayed control thus accounting for the highest disease reduction (80.07%) while streptomycin @ 0.01 per cent recorded the minimum (20.58%) disease incidence with 63.10 per cent disease reduction followed by copper oxychloride @ 0.1 per cent which recorded the minimum (21.42%) disease incidence with 61.59 per cent disease reduction.

Spraying of Pf 45 + Bs 5 recorded (19.44%) disease incidence and (65.14%) disease reduction, followed by Pf 32 + Pf 45 + Bs 5 + Bs 9 spray (19.51%) disease incidence, 65.01% disease reduction, Pf 32 + Bs 5 (1 g/ l) spray (22.36%) disease incidence, 59.90% disease reduction, Pf 32 + Pf 45 (1g/ l) spray (22.78%) disease incidence, 59.15% disease reduction, Pf 32 + Bs 9 (1g/lit) spray (23.07%, 58.63 disease reduction), *Allium sp* leaf extract (10%) (23.59%) disease incidence, 57.70% disease reduction, spraying neem oil at three per cent level recorded 24.00 per cent disease incidence and 59.96 per cent disease reduction, Pf 32 (1g/l) spray (24.42%) disease incidence, 55.02% disease reduction) NSKE (5%) spray (24.71% disease incidence, 56.21% disease reduction mahua oil (3%) spray (24.37%) disease incidence 56.30% disease reduction respectively. Decomposed banana and pulse leaves (5%) spray recorded 29.21% disease incidence and 47.62 per cent reduction. Spraying sheep manure (3%) soap oil (3%) spray registered (35.55%) disease incidence, 36.25 per cent disease reduction and 30.76 per cent disease incidence and 36.16 per cent disease reduction respectively.

Efficacy of spraying formulations of bacterial biocontrol agents and organic amendments on the growth parameters of *Aloe vera* in the glass house

Plant height

Spraying of biocontrol agents (1g/l) (first spray 45 days after plants followed by six monthly sprays) significantly increased the mean plant height. The treatment Pf 32 and Pf 45 and Bs5 @ 1g/l recorded the maximum height of the plant (69.80 cm) as compared to control (45.12cm) and it was followed by Pf 45 and Bs5 (68.90 cm) and Pf 32 + Bs5 (68.48 cm). The minimum plant height was recorded by 10% zimmu leaf extract (57.20 cm) compared to control.

Plant spread

The application of biocontrol agents significantly increased the plant spread. The spraying of Pf 32 +Pf 45 + Bs5 recorded the maximum plant spread (1.2m²) as compared to control (0.551m²) followed by Pf 32 +Pf 45 (0.762 m²) and Pf 45 + Bs5 (0.754m²). The minimum plant height was recorded by three per cent mahua oil (0.562m²) (Table 2).

Leaf length

There was significant increase in the mean leaf length in plants sprayed with biocontrol agents. The maximum length of the leaves was recorded in Pf 32, Pf 45 and Bs5 (55.00cm), followed by Pf 45 and Bs5 (50.26cm) while the minimum (38.80cm) length of leaves was recorded by mahua oil (3%) (Table 2).

Leaf breadth

There was significant increase in the mean leaf breadth in biocontrol treated plants. The maximum (8.18cm) breadth of leaves was

recorded in Pf 32, Pf 45 and Bs5, followed by Pf 45 + Bs5 (7.12cm) and the minimum (5.76cm) breadth of leaves were recorded by zimmu leaf extract (10%) (Table 2).

Leaf thickness

There was significant increase in the mean leaf thickness in plants sprayed with biocontrol agents. The maximum (1.76cm) thickness of leaves was recorded in Pf 32, Pf 45 and Bs5, followed with by Pf 32 + Bs5 (1.38cm) and the minimum (1.08cm) thickness of leaves was recorded by zimmu leaf extract (10%) (Table 2).

Leaf weight

There was significant increase in the mean leaf weight in treated plants using with biocontrol agents. The maximum (205.00g) weight of leaves was recorded in the case of Pf 32, Pf 45 and Bs5 sprayed plants followed by Pf 45 + Bs5 (166g) and the minimum (98g) weight of leaves were recorded by zimmu leaf extract (10%) (Table 2).

Sucker production /year

The mean sucker production per plant per year differed significantly due to different treatments. The highest number of suckers (24.30) produce was recorded by Pf 32 +Pf 45 + Bs5 followed by Pf 45 and Bs5 (21.00) and Pf 32 + Bs5 (20.21). The lowest number of suckers produced was recorded by zimmu leaf extract (10%) (10.30) compared to control (Table 2).

In the pot culture experiment, pre inoculation (24 h later) spraying of *Aloe vera* plants *Pseudomonas* 32 + Pf 45 + Bs 5 recorded the least bacterial soft rot incidence (11.11%, 80.07% reduction while streptomycin (0.01%) recorded minimum disease incidence of 20.58 per cent and 63.10 per cent disease reduction

followed by Pf 45 + Bs 5 recorded (19.44%, 65.14%) disease reduction, Pf 32 + Pf 45 + Bs 5 + Bs 9 recorded (19.51%, 65.01%) disease

reduction and Pf 32 + Bs 5 (22.36%, 59.90%) disease reduction.

Table.1 Efficacy of spraying of talc based antagonistic bacterial formulations soft rot of *Aloe vera* in pot culture

Treatments	Soft rot incidence (%)*	Percent disease reduction *
T ₁ - <i>Pseudomonas aeruginosa</i> 32 (1g/lit)	24.42 (29.68) ⁿ	55.02 (53.74) ^c
T ₂ - <i>P. fluorescens</i> 45(1g/lit)	25.00 (30.00) ^l	55.17 (47.97) ⁿ
T ₃ - <i>Bacillus subtilis</i> 5 (1g/lit)	26.58 (31.03) ^k	52.33 (46.34) ^o
T ₄ - Bs ₉ (1g/lit)	29.21 (32.74) ⁱ	47.62 (43.64) ^q
T ₅ -T ₁ +T ₂ (1g/lit)	22.78 (28.51) ^s	59.15 (50.28) ^g
T ₆ - T ₁ + T ₃ (1g/lit)	22.36 (28.22) ^t	59.90 (50.72) ^f
T ₇ - T ₁ + T ₄ (1g/lit)	23.07 (28.70) ^r	58.63 (49.97) ^h
T ₈ - T ₂ + T ₃ (1g/lit)	19.44 (26.16) ^x	65.14 (53.81) ^b
T ₉ - T ₂ + T ₄ (1g/lit)	28.30 (32.14) ^j	49.25 (44.57) ^p
T ₁₀ - T ₃ + T ₄ (1g/lit)	31.11 (33.90) ^g	44.22 (41.68) ^t
T ₁₁ - T ₁ + T ₂ + T ₃ (1g/lit)	11.11 (19.47) ^y	80.07 (63.49) ^a
T ₁₂ - T ₁ + T ₂ + T ₃ + T ₄ (1g/lit)	19.51 (26.21) ^w	65.01 (52.57) ^m
T ₁₃ - NSKE (5%)	24.71 (29.80) ^m	56.21 (48.57) ^l
T ₁₄ - Groundnut cake (5%)	31.52 (34.18) ^c	43.48 (41.25) ^u
T ₁₅ - Mahua cake (5%)	31.41 (34.05) ^f	43.67 (41.37) ⁱ
T ₁₆ - Neem oil (3%)	24.00 (29.33) ^p	56.96 (49.00) ^j
T ₁₇ - Mahua oil (3%)	24.37 (29.58) ^o	56.30 (48.62) ^k
T ₁₈ - Soap oil (3%)	30.76 (33.68) ^h	44.84 (42.03) ^f
T ₁₉ - Decomposed banana and pulses leaves (5%)	35.60 (36.63) ^b	36.16 (36.96) ^x
T ₂₀ - Sheep manure (3%)	35.55 (36.60) ^c	36.25 (37.02) ^w
T ₂₁ - <i>Allium sp</i> leaf extract (10%)	23.59 (29.05) ^q	57.70 (49.43) ⁱ
T ₂₂ - <i>Withania somnifera</i> leaf extract (10%)	32.22 (34.58) ^d	42.22 (40.52) ^y
T ₂₃ - Streptomycin (0.01%)	20.58 (27.56) ^u	63.10 (52.59) ^d
T ₂₄ - Copper oxy chloride (0.1%)	21.42 (26.97) ^v	61.59 (51.70) ^e
T ₂₅ - Control(Sterile distilled water)	55.77 (48.31) ^a	0

* Mean of three replications

In a column followed by common letters are not significantly different at 5% level by DMRT

Figure in parentheses are arc sine transformed values

Table.2 Efficacy of spraying antagonistic bacteria and organic amendments on the growth parameters of *Aloe vera* under glass house condition

Treatments	Growth parameters*							
	Plant height (cm)	Plant spread (m ²)	Leaf length (cm)	Leaf breath (cm)	Leaf thickness (cm)	Leaf weight (g)	Phyllocrone	Sucker production / year
T ₁ -Pf ₃₂ (1g/lit)	68.20 ^c	0.722 ^g	47.30 ^f	6.94 ^{bc}	1.30 ^{abcd}	142.40 ^c	42.00 ^c	7.00 ^{cde}
T ₂ -Pf ₄₅ (1g/lit)	63.96 ^g	0.712 ^h	46.60 ^h	6.60 ^{bcd}	1.29 ^{abcd}	125.00 ^g	32.80 ^g	6.60 ^{cdef}
T ₃ -Bs ₅ (1g/lit)	68.00 ^f	0.738 ^f	47.70 ^e	6.90 ^{bcd}	1.37 ^{abc}	147.90 ^d	39.00 ^e	7.40 ^{cd}
T ₄ -T ₁ +T ₂ (1g/lit)	68.00 ^f	0.762 ^d	47.16 ^g	7.01 ^{bc}	1.56 ^{ab}	143.80 ^e	34.80 ^f	8.00 ^c
T ₅ -T ₁ +T ₃ (1g/lit)	68.48 ^d	0.801 ^c	48.04 ^d	7.06 ^b	1.38 ^{abc}	161.40 ^c	40.80 ^d	9.39 ^b
T ₆ -T ₂ +T ₃ (1g/lit)	68.90 ^c	0.754 ^e	50.26 ^c	7.12 ^{ab}	1.32 ^{abc}	166.00 ^b	44.00 ^b	10.00 ^{ab}
T ₇ -T ₁ +T ₂ +T ₃ (1g/lit)	69.80 ^a	1.200 ^a	55.00 ^a	8.18 ^a	1.76 ^a	205.00 ^a	48.00 ^a	13.40 ^a
T ₈ -NSKE (5%)	62.28 ^h	0.708 ⁱ	45.50 ⁱ	6.44 ^{bcd}	1.24 ^{abcd}	139.80 ^e	26.40 ^j	5.80 ^{efg}
T ₉ -Neem oil (3%)	60.96 ⁱ	0.640 ^j	45.34 ^j	6.56 ^{bcd}	1.30 ^{abcd}	134.40 ^f	27.60 ⁱ	5.20 ^{fg}
T ₁₀ -Illuppai oil (3%)	60.00 ^j	0.562 ^k	38.80 ^l	5.90 ^{cde}	1.10 ^{bcd}	108.20 ^h	30.80 ^h	6.00 ^{defg}
T ₁₁ - <i>Allium sp</i> leaf extracts (10%)	57.20 ^k	0.713 ^h	39.20 ^k	5.76 ^{de}	1.08 ^{bcd}	98.00 ⁱ	32.40 ^g	5.40 ^{fg}
T ₁₂ -Streptomycin sulphate (0.01%)	69.74 ^b	0.988 ^b	52.60 ^b	7.14 ^{ab}	1.72 ^a	197.80 ^a	44.00 ^b	10.60 ^{ab}
T ₁₃ -Inoculated control	45.12 ^l	0.551 ^l	34.18 ^m	4.52 ^f	0.76 ^d	61.60 ^k	25.60 ^j	3.40 ^h
T ₁₄ -Healthy control	50.34	0.668	35.56	4.86	0.86	81.20	26.00	4.60

* Mean of three replications

In a column, means followed by common letters are not significantly different at 5% level by DMRT

PGPR play an important role in the management of plant diseases. But, one of the major hurdles experienced with biocontrol agents is lack appropriate delivery system. The present study indicates that delivery of bacterial antagonists in talc based formulation through foliar spray was effective in reducing *Aloe vera* soft rot incidence. The application of bacterial biocontrol agent Pf 32 and Pf 45 and Bs 5 as consortial formulation was more effective in suppressing the soft rot of *Aloe vera* caused by *E. chrysanthemi* in pot culture rather than applying these individually. However a potential benefit of single biocontrol agent application was demonstrated by Raupach and Kloepper, 1998. Several approaches were used to include combined application of two or more biocontrol stains to enhance the level and consistency in disease control (Pierson and

Weller, 1994; Raupach and Kloepper, 1998). Similar observations were reported by Gasoni *et al.*, (1998). Radish seed treatment with *B. cereus* and *P. fluorescens* effectively controlled *Rhizoctonia* damping off in greenhouse studies. Saravanakumar (2002) reported that a mixture of two PGPR strains was also found to be effective by reduced the disease incidence of root rot of green gram in pot culture studies.

Sanjay and Parashar (2002) used *B. subtilis* for controlling bacterial blight of cotton under glasshouse condition. Thirukumaran (1999) reported that spraying of cotton plants with *P. fluorescens* strain as foliar application recorded the least bacterial blight incidence compared to control. Mondal (1999) found that five strains of *Pseudomonas sp* were effective in controlling the *Xam* disease. Salah

EddinKhabbaz (2002) reported that seed treatment followed by foliar application of *P. fluorescens* (Pf1 and MMP) effectively reduced the disease incidence of *Xam* under glasshouse condition. The efficacy of consortial application of biocontrol agents to reduce *E. chrysanthemi* might be due to the additive effect of the production of siderophore, antibiotics, lytic enzymes by the antagonistic bacteria and the induction of defense related enzymes viz., PO, PPO, PAL, chitinase, β -1,3, glucanase and phenol.

Growth parameters like viz., plant height, leaf size length, breadth, thickness and weight also increased when plants were sprayed with biocontrol agents. The increase in biomass production might be due to the production of plant growth promoters or through indirect stimulation of nutrient uptake and by producing siderophore or antibiotics to protect plants from deleterious rhizosphere organisms. Van peer and Schippers (1988) reported that there is increase in the root length and shoot length of tomato, cucumber, lettuce and potato as a result of bacterization with *Pseudomonas* strains.

Dubeikovsky *et al.*, (1993) suggested that the increase in plant growth might be associated with secretion of auxins, gibberellins and cytokinins. Salah Eddin Khabbaz (2002) reported that there is significant increase in seed germination, vigour index and dry weight due to treatment with *P. fluorescens* isolates (Pf 1 and MMP) under glass house conditions.

Mondal (1999) found that five strains of *Pseudomonas* sp increased the germination and vigor index of cotton seed. Safyazov *et al.*, (1995) reported that *P. fluorescens* stimulated the emergence and seedling growth and increased cotton yield besides reducing disease intensity.

Talk based formulations of different strains of Pf32+Pf45 and Bs5 formulations increased the plant growth parameters of green gram seedlings under greenhouse conditions (Saravanakumar, 2002). Many rhizobacteria were reported to stimulate plant growth (Schroth and Hancock, 1982; Ramamoorthy *et al.*, 2002b). Seed treatment with fluorescent *Pseudomonads* increased the plant growth in tomato and hot pepper (Ramamoorthy *et al.*, 2002a). Increased seedling emergence and establishment and colonization of *Pseudomonas* in peanut, resulted in the reduction in the growth of *M. phaseolina* and increased yield (Gupta *et al.*, 2002). *B. subtilis* strains were reported to inhibit several soil borne disease such as Fusarial wilt of redgram (Podile and Dube, 1985) and *R. solani* damping off of peppermint (Kamalakaran *et al.*, 2003b). Soil application of talc based formulation of *P. fluorescens* reduced the root rot caused by *M. phaseolina* and *R. solani* under pot culture experiment (Kamalakaran, 2004).

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