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RESEARCH PAPER

Efficacy of different isolates of bacterial antagonist against *Aloe vera* soft rot pathogen (*Erwinia chrysanthemi*) under *in vitro*

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Abstract : Fifteen pathogenic isolates of *Erwinia chrysanthemi*, the soft rot of pathogen of *Aloe vera* were established from 15 different areas of Southern Tamil Nadu and their identity was confirmed by Microbial Type Culture Collection (MTCC) and Gene Bank, Chandigarh. Among the different *Pseudomonas* isolates tested *in vitro*, Pf 32 and Pf 45 was the most effective against the pathogen followed by Pf 4 and of the ten *Bacillus subtilis* tested *in vitro*, Bs5 was the most effective against *E. chrysanthemi.*

Key Words : Soft rot, *Aloe vera*, *Pseudomonas* isolates, *Bacillus subtilis*

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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). Plants of the genus *Aloe vera* belong to old world and are indigenous to eastern and southern Africa. The plant is found in the tropics and introduced to India for ornamental and medicinal purpose. Aloe genus consists of about 325 species all of which grow in rosette shape (Anselm, 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. Diseases are the greatest enemies of *Aloe vera* accounting for huge losses varying from 25-75 per cent.

Among the various diseases, soft rot caused by *Erwinia Chrysanthemi* is an important disease in *Aloe vera* growing areas of the world. The survey conducted in this study revealed the occurrence of bacterial soft rot disease of *Aloe vera* in different *Aloe vera* growing areas of southern Tamil Nadu. The disease is serious when abundant moisture is available

through irrigation and rain. The disease symptoms are manifested in the form of small, water soaked lesions at the base of the leaves, stems, and underground parts. The rotting progresses very fast and the whole plant dies out within two to three days. The leaf epidermis bulges out due to gas formation and the leaf content is converted into a slimy mass while the gas is eventually released (Mandal and Maiti, 2005). It is a serious disease in Madurai, Tuticorin, Tirunelveli and Kanyakumari districts, where the cultivation of cultivar *Aloe barbadensis* occupies major area.

MATERIAL AND METHODS

Screening of antagonistic bacteria under *in vitro* conditions :

Bacterial antagonists were screened by the method explained by Chakarvarthi *et al.* (1972). Different strains of *Bacillus* and of *Pseudomonas* were tested against *E. chrysanthemi.* Twenty four h old culture (10^8 CFU/ml) of each antagonistic bacterium was streaked at one side of a petri plate containing nutrient agar (NA). After two days, several streaks of suspension of *E. chrysanthemi* were made perpendicularly to the growth produced by antagonistic bacteria at 24ºC. The extent of pathogen inhibition was measured after two days of incubation.

***In vitro* efficacy of biocontrol agents against *E. chrysanthemi* (paper disc assay) :**

Bacterial antagonists suspended in sterile water from an actively growing 24 h old culture were inoculated into nutrient broth and incubated at room temperature (28 $\pm$ 1ºC) for 48 h with constant shaking. Centrifugation was done at 6000 g for 10 minutes, and the cells were resuspended in 0.01M-Phosphate buffer, pH-7.0, cell free culture filtrate was obtained by passing through seitz filter and used for the bioassay against the pathogen. The sterilized filter paper discs were dipped in the antagonistic bacterial cultures filtrate and uniformly mopped the excess in the disc. These were air dried and then placed onto the *Erwinia* seeded nutrient agar medium equidistantly at the rate of three discs per Petri plate. The plates were incubated at room temperature and the inhibition zone was measured after 72 h. The filter paper discs treated with sterile distilled water served as control.

RESULTS AND DISCUSSION

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

Management of the disease :

Isolation of rhizosphere and phylloplane bacteria :

Sixty bacterial isolates (50 isolates of *Pseudomonas* sp. and 10 isolates of *Bacillus subtilis*) were isolated from the rhizosphere soils and phylloplane collected from different crops grown in different parts of southern Tamil Nadu and one isolate of *Pseudomonas fluorescens* was obtained from Department of Plant Pathology, Agricultural college and Research Institute, Coimbatore (Table 1).

Characterization of bacterial antagonists :

Biochemical characteristics of Pseudomonas sp. isolates :

All the fifty one isolates of *Pseudomonas* sp. gave positive result to KOH test, arginine dihydrolase, anerobic growth, and gelatin liquefication, growth at 4ºC and in producing fluorescent pigment. These gave negative result to levan formation from sucrose, starch hydrolysis and growth at 45ºC in all the isolates.

Biochemical characteristics of Bacillus subtilis isolates :

Ten isolates were identified as *Bacillus subtilis* and all of which showed positive reaction to starch hydrolysis, utilization of citrate, catalase test, levan production, Gram staining, growth in three per cent NaCl and negative reaction to growth at 4ºC and anerobic growth.

Efficacy of different isolates of *Pseudomonas* sp. and *Bacillus subtilis* against *E. chrysanthemi in vitro* :

Among the 61 bacterial isolates screened *in vitro*, 51 *Pseudomonas* sp. and ten *Bacillus subtilis* isolates showed inhibitory action against *E. chrysanthemi* of *Aloe ver,* the 51 isolates of *P. aeruginosa* recorded inhibition zone ranging from 3.13 to 15.64 mm (Table 2). It was found that *Pseudomonas* isolates Pf 4 (6.39 mm), Pf 16 (6.30 mm), Pf 26 (8.42 mm), Pf 32 (15.64mm) and Pf 45 (14.31mm) exhibited the inhibition against the growth of the pathogen. Among the isolates Pf 32 showed significantly the maximum (15.64 mm) inhibition of the growth of the pathogen followed by Pf45 (14.31). Pf 19 showed the minimum (3.13mm) growth inhibition. *P. aeruginosa* (Anjaiah *et al.,* 2003) had been used as seed inoculants on crop plants to suppress pathogens as well as to promote plant growth and yield. The development of wilt disease in apple and peach plants was reduced due to competition between *P. fluorescens* and *Erwinia amylovora* (Stellies and Senft, 1998).

Among the ten isolates of *B. subtilis* screened, the isolate Bs_5 effectively (13.25 mm) inhibited the growth of the pathogen followed by Bs_1 (10mm). The rest of the isolates exerted growth inhibition ranging from 7.68 to 9.50 mm. The least (6.93mm) inhibition zone was recorded in Bs_8 isolate (Table 3). Sendhilvel (2000) also documented the efficacy of *Pseudomonas* strain FP 7 and *B. subtilis* in reducing the growth of soft rot bacteria of onion under *in vitro* conditions. Of the 25 *Bacillus* isolates screened *in vitro,* on by two isolates were found to inhibit the growth of*E. carotovora*, *Pseudomonas syringae*, *Xanthomonas campestris* pv. *campestris* (Foldes *et al.,* 2000). *B. subtilis* BS 107 showed microbial activity *in vitro* and on the plants against *E.*

Table 1 : Bacterial antagonists isolated from rhizosphere soils of different crops in southern Tamil Nadu

Districts and places	Crop	Code no. of the isolate	Antagonists
Madurai district			
Melur	*Aloe vera*	*Pf 1*	*Pseudomonas fluorescens*
-do-		*Bs 1*	*Bacillus subtilis*
-do-		*Pf 2*	*P. fluorescens*
Palamadu		*Pf 3*	*P. fluorescens*
-do-		*Bs2*	*B.subtilis*
-do-		*Pf 4*	*P. fluorescens*
AC&RI		*Pf 5*	*P. fluorescens*
(Madurai)			
-do-		*Pf 6*	*P. fluorescens*
Chekkanurani	Onion	*Bs 3*	*B.subtilis*
-do-		*Pf 7*	*P. fluorescens*
-do-	Tomato	*Pf 8*	*P. fluorescens*
-do-		*Pf 9*	*P. fluorescens*
Tirunelveli district			
Kelekalangal	*Aloe vera*	*Pf10*	*P. fluorescens*
-do-		*Pf 11*	*P. fluorescens*
Tenkasi		*Bs 4*	*B.subtilis*
-do-		*Pf 12*	*P. fluorescens*
-do-		*Pf 13*	*P. fluorescens*
Kadambur	Rice	*Pf 14*	*P. fluorescens*
-do-		*Pf 15*	*P. fluorescens*
Tuticorin district			
Kayattar	*Aloe vera*	*Pf 16*	*Serratia*
-do-		*Pf 17*	*P. fluorescens*
-do-		*Bs 5*	*B.subtilis*
-do-		*Pf 18*	*P. fluorescens*
Duraisamypuram		*Pf 19*	*P. fluorescens*
-do-		*Pf 20*	*P. fluorescens*
-do-	Green gram	*Pf 21*	*P. fluorescens*
-do-		*Pf 22*	*P. fluorescens*
-do-	Sunflower	*Pf 23*	*P. fluorescens*
Muthulapuram		*Pf 24*	*P. fluorescens*
-do-	Blackgram	*Pf 25*	*P. fulva*
-do-		*Pf 26*	*P. fluorescens*

Table 1 : Contd.....

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Kanyakumari district	Rice	*Bs 6*	*B.subtilis*
Nagerkoil		*Pf 27*	*P. fluorescens*
-do-	Banana	*Pf 28*	*P. fluorescens*
-do-		*Pf 29*	*P. fluorescens*
-do-	*Aloe vera*	*Bs 7*	*B.subtilis*
-do-		*Pf 30*	*P. fluorescens*
-do-		*Pf 31*	*P. fluorescens*
Coimbatore district	*Aloe vera*	*Pf 32*	*P. aeruginosa*
TNAU		*Bs 8*	*B.subtilis*
-do-		*Pf 33*	*P. fluorescens*
-do-	Rice	*Pf 34*	*P. fluorescens*
-do-		*Pf 35*	*P. fluorescens*
Virudhunagar district	*Aloe vera*	*Pf 36*	*Serratia sp*
Krishnankoil		*Pf 37*	*P. fluorescens*
-do-	Cumbu	*Pf 38*	*P. fluorescens*
-do-		*Pf 39*	*P. fluorescens*
Ramanathapurem district	*Aloe vera*	*Bs 9*	*B.subtilis*
Paramakudi		*Pf 40*	*P. fluorescens*
-do-		*Bs 10*	*B.subtilis*
-do-	Sorghum	*Pf 41*	*P. fluorescens*
-do-		*Pf 42*	*P. fluorescens*
Theni district	Chilli	*Pf 43*	*P. fluorescens*
HC&RI , Periyakulam		*Pf 44*	*P. fluorescens*
-do-	*Aloe vera*	*Pf 45*	*P. otitidis*
-do-		*Pf 46*	*P. fluorescens*
Dindugal district	*Aloe vera*	*Pf 47*	*P. fluorescens*
Gandhigram		*Pf 48*	*P. fluorescens*
-do-	Rice	*Pf 49*	*P. fluorescens*
		Pf 50	*P. fluorescens*
Department of Plant Pathology, Coimbatore	-	*Pf 51*	*P. fluorescens*

Table 2 : Efficacy of different isolates of *Pseudomonas* sp against *E.chrysanthemi in vitro*

Isolates	Inhibition zone (mm)*
Pf 1	5.97^{i}
Pf 2	4.03^{z9}
Pf 3	4.00^{z10}
Pf 4	6.39^{d}
Pf 5	4.14^{z8}
Pf 6	5.20^{l}
Pf 7	5.01st
Pf 8	6.10^{g}
Pf 9	4.33^{z4}
Pf10	6.00^{i}
Pf 11	5.40^{l}
Pf 12	3.70^{z11}
Pf 13	4.06
Pf 14	3.90^{z11}
Pf 15	4.20^{z4}
Pf 16	6.30^{e}
Pf 17	4.60^{y}
Pf 18	4.40^{z3}
Pf 19	3.13^{z12}
Pf 20	5.34^{n}
Pf 21	6.04^{h}
Pf 22	5.09^{r}
Pf 23	6.13^{f}
Pf 24	5.20^{p}
Pf 25	4.19^{z5}
Pf 26	8.42^{c}
Pf 27	4.51^{z1}
Pf 28	3.70^{z11}
Pf 29	4.36^{z2}
Pf 30	5.28^{o}
Pf 31	5.40^{m}
Pf 32	15.64^{a}
Pf 33	5.19^{q}
Pf 34	6.00^{i}
Pf 35	5.39^{m}
Pf 36	4.30^{z3}
Pf 37	6.10^{g}
Pf 38	4.70^{w}
Pf 39	3.90
Pf 40	5.42^{k}
Pf 41	4.54^{z}
Pf 42	4.69^{x}
Pf 43	5.07^{r}
Pf 44	4.83^{v}
Pf 45	14.31^{b}
Pf 46	5.17^{q}
Pf 47	4.06^{z10}
Pf 48	5.00st
Pf 49	4.16^{z9}
Pf 50	4.90^{u}
Pf 51	5.03^{s}
Streptomycin sulphate (0.01%)	14.00
Control (Sterile distilled water)	0

* Mean of three replications

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

Table 3 : Efficacy of different isolates of *B. substilis* against *E.chrysanthemi in vitro*

Sr. No.	Isolates	Inhibition zone (mm)*
1.	Bs_1	10.00^{c}
2.	Bs_2	9.50^{d}
3.	Bs_3	8.44^{f}
4.	Bs_4	8.30^{i}
5.	Bs_5	13.25^{b}
6.	Bs_6	8.33^{h}
7.	Bs_7	9.49^{e}
8.	Bs_8	6.93^{k}
9.	Bs_9	7.68^{j}
10.	Bs_{10}	8.41^{g}
11.	Streptomycin sulphate (100 ppm)	14.26^{a}
12.	Control (Sterile distilled water)	0

* Mean of three replications

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

carotovora var. *atroseptica* and *E. carotovora* subsp. *carotovora*, the causal agent of potato black leg and tuber soft rot (Sharga and Lyon, 1998).

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