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# Enzyme activity by different isolates of *E. chrysanthemi* in *Aloe vera*

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**A**BSTRACT :

*Aloe vera* soft rot disease, which also induced activity of Pectate lyase activity, Pectin methyl esterase, Polygalacturonase, pectin trans eliminase activity. The enzyme activity of inoculated aloe plants increased from the two day till the six DAI and slowly declined thereafter in all the 15 isolates.

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**K**EY **W**ORDS : Soft rot, Pectate lyase activity, Pectin methyl esterase, Polygalacturonase, Pectin trans eliminase activity, *Aloe vera*

## 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. 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 (Kunal and Satyabrata, 2005). It is a various disease in Madurai, Tuticorin, Tirunelveli and Kanyakumari districts, where the cultivation of cultivar *Aloe barbadensis* occupies major area. PGPR include fluorescent pseudomonads, *viz.*, *P. fluorescens* and *P. putida*, non-fluorescent pseudomonads, *B.subtilis* and *Serratia* spp. PGPR induce systemic resistance against fungi, bacteria, viruses, nematodes and even to insects on a wide variety of crops (Van Loon *et al.*, 1998). There are major differences in ISR as compared to other mechanisms. Fluroscent pseudomonas appear to be ideal for this purpose because some strains can induce systemic resistance in plants against fungal, bacterial and viral diseases (Van Loon *et al*., 1998; Zehnder *et al*., 1997 and Wei *et al*., 1996). If ISR once activated, it activates multiple defense mechanisms that include increased activity of chitinases, β-1,3– glucanases and peroxidases (Xue *et al*., 1998; Dalisay and Kuc,1995) and accumulation of low molecular weight substances phytoalexins (Van Peer and Schippers, 1992 and Loganathan (2002) reported that induction of defense related proteins *viz.*, phenylalanine ammonia lyase, peroxidase, polyphenol oxidase, phenol, chitinase and β-1, 3- glucanase were found to be in higher levels in treatments involving bioformulation mixture containing *P. fluorescens* (Pf1) against fungal pathogens and root knot nematode in cabbage and cauliflower. The reduction of wilt incidence in pigeonpea was due to the increased activity of PAL and PO. *P. fluorescens* isolate 4-92 treated chickpea plants exhibited the time course accumulation of PR proteins like chitinase and glucanase. The level of chitinase and glucanase increased by 6.6 to 7.0 fold upto four days after post inoculation. Thereafter a little decrease in the activity of PR protein was observed. (Rajkumar, 2006) reported that induction of defense related proteins *viz.*, phenylalanine ammonia lyase, peroxidase, polyphenol oxidase, phenol, chitinase and β-1, 3- glucanase was found to be in higher levels in treatments involving bioformulation mixture containing *P. fluorescens* (Pf1) and *B. subtilis* against *E. carotovora* var. *carotovora.*

## MATERIAL AND METHODS

**Pathophysiology:**

The bio-chemical changes taking place in the leaves at one, two, three, four and five days after inoculation of *E. chrysanthemi* was studied as described below. Leaf without pathogen inoculation was kept as control.

**Estimation of enzymes involved in pathogenesis:**

*Erwinia* culture cells was collected from each isolates by centrifugation at 1200 r/minute and the supernatants were sterilized by filtration through a 0.22 (pore size) milliphore filter. For extracellular enzymes, supernatant was collected in early stationary phase because the extracellular enzymes are produced in a growth phase dependent manner. All the supernatants were from at -20°C in aliquots were maintained until required for further experiments (Pirhonen *et al*., 1993).

**Assay of pectate lyase (PL) activity:**

PL activity was determined by monitoring the formation of $C_4$ and $C_5$ unsaturated products spectrophotometrically at 235 nm (Laurent *et al.*, 2000). Five hundred µl of 0.1 M TrisHC1 (pH 9) buffer containing 0.5 mM $CaCl_2$ was rapidly mixed in a 1.5 ml cuvette with 370 µl of distilled water, 100 µl of a polygalacturonic acid solution (1% w/v) in water and 30 µl of supernatant obtained by grinding infected *Aloe vera* leaf tissue in a pestle and mortar using 0.1M Tris Hcl (pH9) buffer. The reaction mixture was incubated at 30°C. One unit was defined as the amount of enzyme which produced 1 µm of unsaturated product. Activity was expressed in micromoles of unsaturated product liberated per minute per ml of supernatant and specific activity was expressed in µm of unsaturated product liberated per minute per ml of OD at 580 nm.

**Assay of pectin methyl esterase (PME) activity:**

The enzyme activity was assayed as per the procedure given by Mahadevan and Sridhar (1982). Twenty ml of pectin solution was pipetted in a 50 ml beaker and pH was adjusted to 7.0. Ten ml of the enzyme solution was then added and the pH was immediately adjusted to 7.0 by adding 1N NaOH. This was kept as zero time. At every 15 min the pH was checked and alkali was added from the burette when the pH falls

below the reference point, while stirring. To adjust the pH, 0.02 N NaOH was used and the volume of alkali consumed was noted at each interval. The enzyme activity was expressed as mm of hydrogen ion per min per ml of the enzyme preparation.

$$\text{PME } Z \frac{(Vs \text{ J } Vb)\, NaOH}{Vt} x\, 100$$

where,

Vs = Titre value

Vb = Volume of 1N sodium hydroxide consumed to adjust the pH to 7.0

V = Volume of incubation mixture (ml)

t = Time period (minute).

**Assay of pectin trans eliminase (PTE) activity :**

PTE activity was estimated by the viscometric method described by Mahadevan and Sridhar (1982). Four ml of the substrate and one ml of the enzyme were pipetted into the viscometer. The loss in viscosity of the pectin solution was determined by using of Vinsell Viscometer of size 300. The activity was expressed as per cent reduction in viscosity.

$$V = \frac{T_0 - T}{T_0 - T_{H_2 0}} x\, 100$$

where,

V - Per cent loss in viscosity

$T_o$ - Flow time in seconds at zero time

T - Flow time of reaction mixture at time T

$T_{H_2O}$ - Flow time of distilled water.

**Assay of polygalacturonase (PG) activity :**

The activity of PG was assayed as per the method described by Mahadevan and Sridhar (1982). One g of tissue was transferred to a wearing blender and five ml of 0.1M chilled phosphate buffer (pH 6.6) was added. The material was blended for five min, filtered through two layers of cheese cloth and centrifuged at 2000 rpm for 30 min at 4°C. The supernatant was decanted and the clear extract was taken as enzyme source. Four ml of the substrate, one ml of acetate buffer (pH 5.2) and two ml of enzyme source were taken in a viscometer and the contents were mixed gently by drawing air rapidly through the large arm of the viscometer by suction. The efflux time of the mixture was determined by suction through small arm (zero time). The efflux time of the mixture after 30 min was measured. From this, the enzyme activity was calculated as per cent reduction in viscosity of the substrate from the following formula :

$$V = \frac{T_0 - T}{T_0 - T_{H_2 0}} x\, 100$$

where,

$T_o$ - Flow time in seconds at zero time

T - Flow time of reaction mixture at time T

$T_{H_2O}$ - Flow time of distilled water.

## RESULTS AND DISCUSSION

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

**Pectate lyase activity (PL) by different isolates of *E. chrysanthemi* in *Aloe vera*:**

The results revealed that the pectate lyase activity of all the aloe plants inoculated increased from one minute to three min time interval and after words slowly declined in the case of all isolates. The enzyme activity was the maximum in $I_9$ (Melur) isolate (OD 3.010) at three min of incubation as compared to the other isolates of *E. chrysanthemi*. The pectate lyase activity in three min ranged from 2.645 to 3.010OD. The least pectate lyase activity was observed in $I_2$ (Tenkasi) (OD 2.645) (Table 1). The production of extra cellular endo poly-galacturanase and endo pectin trans eliminases by*E. carotovora* subsp. *carotovora* inhibiting soft rot of banana was reported by Rajkumar (2006). Pectate lyases are known to be virulence factors in many plant pathogenic soft-rotting micro-organisms (Bruhlmann, 1995).

**Pectin methyl esterase (PME) by different isolates of *E. chrysanthemi Aloe vera***

All the 15 isolates of *E. chrysanthemi* were assessed for their ability to produce PME in *Aloe vera*. The accumulation of PME increased two days after inoculation (DAI) and attained a peak six days after inoculation and slowly declined thereafter in all the isolates. The PME activity six DAI ranged from 0.27 to 1.14µ mole hydrogen ion $min^{-1}$ $ml^{-1}$ in the isolates. Enhanced activity of PME was recorded in the leaves inoculated with $I_9$ (Melur) isolate (1.14 µ mole hydrogen ion $min^{-1}$ $ml^{-1}$) six days after inoculation. The isolate $I_{11}$ exhibited the least amount of PME activity (0.28 µ mole hydrogen ion $min^{-1}$ $ml^{-1}$) (Table 2). The present study

showed an enhanced activity of PME in the aloe leaves inoculated with the most virulent isolate. Similar observations made in carrot roots inoculated with *Fusarium solani* f. sp. *radicicola* by Abraham (1999).

**Polygalacturanase (PG) activity of different isolates of *E. chrysanthemi Aloe vera*:**

The PG activity of inoculated aloe plants increased from the two day till the six DAI and slowly declined thereafter in all the 15 isolates. The isolate $I_9$ (Melur) showed higher activity of Polygalacturonase (40.30 % in viscosity) on eight DAI as compared to the other isolates. The PG activity six DAI was ranged from 23.50 to 40.30 per cent reduction in viscosity. The PG activity was minimum (23.10 % reduction in viscosity) in the isolate $I_3$ (kadambur) six DAI. (Table 3). By the above studies, the most virulent isolate $I_9$ showed the highest

**Table 1: Pectate lyase activity (PL) by different *E. chrysanthemi* isolates in *Aloe vera***

| Isolates | OD value at 580nm* | | | |
|---|---|---|---|---|
| | 1 min | 2 min | 3 min | 4 min |
| $I_1$ | 2.604$^{g}$ | 2.724$^{f}$ | 2.808$^{g}$ | 2.687$^{h}$ |
| $I_2$ | 2.496$^{m}$ | 2.564$^{m}$ | 2.645$^{l}$ | 2.504$^{o}$ |
| $I_3$ | 2.549$^{k}$ | 2.655$^{k}$ | 2.741$^{j}$ | 2.655$^{j}$ |
| $I_4$ | 2.688$^{b}$ | 2.769$^{c}$ | 2.854$^{c}$ | 2.725$^{c}$ |
| $I_5$ | 2.613$^{e}$ | 2.716$^{g}$ | 2.825$^{e}$ | 2.714$^{e}$ |
| $I_6$ | 2.587$^{i}$ | 2.691$^{i}$ | 2.801$^{h}$ | 2.596$^{m}$ |
| $I_7$ | 2.609$^{f}$ | 2.723$^{f}$ | 2.739$^{k}$ | 2.634$^{k}$ |
| $I_8$ | 2.541$^{l}$ | 2.652$^{l}$ | 2.738$^{k}$ | 2.632$^{i}$ |
| $I_9$ | 2.715$^{a}$ | 2.897$^{a}$ | 3.010$^{a}$ | 2.879$^{a}$ |
| $I_{10}$ | 2.689$^{b}$ | 2.803$^{b}$ | 2.924$^{b}$ | 2.575$^{n}$ |
| $I_{11}$ | 2.626$^{c}$ | 2.713$^{h}$ | 2.802$^{h}$ | 2.754$^{b}$ |
| $I_{12}$ | 2.562$^{j}$ | 2.689$^{j}$ | 2.794$^{i}$ | 2.671$^{i}$ |
| $I_{13}$ | 2.613$^{e}$ | 2.738$^{d}$ | 2.826$^{e}$ | 2.703$^{f}$ |
| $I_{14}$ | 2.621$^{d}$ | 2.734$^{e}$ | 2.831$^{d}$ | 2.720$^{d}$ |
| $I_{15}$ | 2.597$^{h}$ | 2.715$^{h}$ | 2.813$^{f}$ | 2.699$^{g}$ |
| Control | 0.006 | 0.009 | 0.008 | 0.008 |

* Mean of three replications  In a column, means followed by common letters are not significantly different at 5 per cent level by DMRT

**Table 2 : Pectin methyl / esterase (PME) by different isolates of *E. chrysanthemi* in *Aloe vera***

| Isolates | PME ($\mu$ mole hydrogen ion $min^{-1}$ $ml^{-1}$) | | | |
|---|---|---|---|---|
| | Days after inoculation(DAI)* | | | |
| | 2 | 4 | 6 | 8 |
| $I_1$ | 0.16$^{d}$ | 0.21$^{e}$ | 0.48$^{d}$ | 0.36$^{cd}$ |
| $I_2$ | 0.14$^{f}$ | 0.25$^{d}$ | 0.45$^{g}$ | 0.38$^{b}$ |
| $I_3$ | 0.07$^{k}$ | 0.17$^{j}$ | 0.38$^{k}$ | 0.27$^{def}$ |
| $I_4$ | 0.18$^{c}$ | 0.29$^{c}$ | 0.53$^{c}$ | 0.40$^{c}$ |
| $I_5$ | 0.12$^{h}$ | 0.23$^{f}$ | 0.41$^{j}$ | 0.33$^{cd}$ |
| $I_6$ | 0.15$^{e}$ | 0.24$^{e}$ | 0.47$^{e}$ | 0.36$^{cd}$ |
| $I_7$ | 0.11$^{i}$ | 0.20$^{i}$ | 0.42$^{i}$ | 0.31$^{cde}$ |
| $I_8$ | 0.14$^{f}$ | 0.22$^{g}$ | 0.43$^{h}$ | 0.30$^{cde}$ |
| $I_9$ | 0.27$^{a}$ | 0.54$^{a}$ | 1.14$^{a}$ | 0.93$^{a}$ |
| $I_{10}$ | 0.25$^{b}$ | 0.43$^{b}$ | 0.81$^{b}$ | 0.62$^{b}$ |
| $I_{11}$ | 0.13$^{g}$ | 0.21$^{h}$ | 0.28$^{m}$ | 0.19$^{f}$ |
| $I_{12}$ | 0.15$^{e}$ | 0.24$^{e}$ | 0.33$^{l}$ | 0.21$^{ef}$ |
| $I_{13}$ | 0.08$^{l}$ | 0.16$^{k}$ | 0.45$^{g}$ | 0.34$^{cd}$ |
| $I_{14}$ | 0.10$^{j}$ | 0.21$^{h}$ | 0.43$^{h}$ | 0.32$^{cd}$ |
| $I_{15}$ | 0.14$^{f}$ | 0.25$^{d}$ | 0.47$^{f}$ | 0.35$^{cd}$ |
| Control | 0.02 | 0.03 | 0.05 | 0.04 |

* Mean of three replications  In a column, means followed by common letters are not significantly different at 5 per cent level by DMRT

PG activity in the present study. The poly galacturonase activity of *Erwinia carotovora* sub sp. *carotovora* in carrot was documented by Parthiban (2004).

**Pectin trans eliminase (PTE) by different isolates of *E. chrysanthemi* in *Aloe vera*:**

Assay for pectin trans eliminase activity indicated that, there was no pectin trans eliminase activity in healthy plants. While pectin trans eliminase of all the inoculated aloe plants increased from two DAI till the six DAI and thereafter slowly declined in all the isolates. The plants inoculated with *E. chrysanthemi* isolate $I_9$ (Melur) showed higher activity of pectin trans eliminase (64.60 % reduction in viscosity) on six DAI (Table 4). The enzyme activity six DAI ranged from 39.70 to 64.60 (% reduction in viscosity). The enzyme activity while it was

**Table 3 : Poly galacturonase (PG) by different isolates of *E.chrysanthemi* in *Aloe vera***

| Isolates | Polygalacturonase (% reduction in viscosity days after inoculation*) Days after inoculation (DAI)* | | | |
|---|---|---|---|---|
| | 2 | 4 | 6 | 8 |
| $I_1$ | 11.40$^{cde}$ | 16.20$^{cde}$ | 28.70$^{bc}$ | 22.40$^{cdef}$ |
| $I_2$ | 10.60$^{defg}$ | 15.40$^{cdef}$ | 26.10$^{cd}$ | 20.30$^{fg}$ |
| $I_3$ | 9.10$^{g}$ | 14.70$^{defg}$ | 23.50$^{d}$ | 19.30$^{efg}$ |
| $I_4$ | 12.70$^{c}$ | 17.10$^{c}$ | 30.00$^{b}$ | 24.20$^{c}$ |
| $I_5$ | 10.20$^{defg}$ | 13.80$^{efgh}$ | 27.60$^{bc}$ | 21.50$^{def}$ |
| $I_6$ | 10.70$^{def}$ | 11.90$^{h}$ | 26.90$^{bc}$ | 21.80$^{cdef}$ |
| $I_7$ | 10.30$^{cde}$ | 13.90$^{efgh}$ | 27.20$^{bc}$ | 20.60$^{efg}$ |
| $I_8$ | 10.40$^{defg}$ | 15.00$^{defg}$ | 28.70$^{bc}$ | 23.10$^{cdef}$ |
| $I_9$ | 23.50$^{a}$ | 36.70$^{a}$ | 40.30$^{a}$ | 39.60$^{a}$ |
| $I_{10}$ | 14.30$^{b}$ | 17.60$^{b}$ | 35.90$^{a}$ | 27.40$^{b}$ |
| $I_{11}$ | 9.70$^{efg}$ | 13.40$^{fgh}$ | 29.40$^{b}$ | 22.20$^{cdef}$ |
| $I_{12}$ | 11.50$^{cd}$ | 12.70$^{fgh}$ | 27.20$^{bc}$ | 23.70$^{cd}$ |
| $I_{13}$ | 10.20$^{defg}$ | 12.60$^{fgh}$ | 26.10$^{c}$ | 21.90$^{cdef}$ |
| $I_{14}$ | 9.80$^{efg}$ | 13.50$^{fgh}$ | 28.00$^{bc}$ | 23.30$^{cde}$ |
| $I_{15}$ | 9.10$^{fg}$ | 16.10$^{cd}$ | 27.50$^{bc}$ | 23.60$^{cd}$ |
| Control | 0.03 | 0.02 | 0.00 | 0.01 |

* Mean of three replications  In a column, means followed by common letters are not significantly different at 5 per cent level by DMRT

**Table 4 : Pectin trans eliminase (PTE) by different isolates of *E.chrysanthemi* in *Aloe vera***

| Isolates | Pectin trans – eliminase (% reduction in viscosity) days after inoculation*) Days after inoculation(DAI)* | | | |
|---|---|---|---|---|
| | 2 | 4 | 6 | 8 |
| $I_1$ | 23.40$^{h}$ | 29.70$^{l}$ | 40.60$^{k}$ | 34.20 |
| $I_2$ | 21.20$^{j}$ | 28.90$^{o}$ | 43.10g | 33.10 |
| $I_3$ | 24.10$^{e}$ | 30.30$^{j}$ | 41.70$^{i}$ | 34.30 |
| $I_4$ | 29.40$^{b}$ | 33.20$^{c}$ | 44.10$^{e}$ | 38.40 |
| $I_5$ | 26.30$^{c}$ | 31.50$^{f}$ | 40.90$^{j}$ | 36.60 |
| $I_6$ | 23.70$^{g}$ | 32.10$^{e}$ | 42.20$^{h}$ | 32.90 |
| $I_7$ | 16.30$^{n}$ | 29.60$^{m}$ | 45.30$^{d}$ | 36.10 |
| $I_8$ | 23.80$^{f}$ | 30.90$^{h}$ | 49.90$^{c}$ | 35.40 |
| $I_9$ | 33.40$^{a}$ | 46.70$^{a}$ | 64.60$^{a}$ | 56.20 |
| $I_{10}$ | 17.40$^{m}$ | 34.70$^{b}$ | 53.30$^{b}$ | 45.20 |
| $I_{11}$ | 22.60$^{i}$ | 30.80$^{i}$ | 40.80$^{l}$ | 36.20 |
| $I_{12}$ | 18.30$^{l}$ | 31.40$^{g}$ | 43.40$^{f}$ | 32.80 |
| $I_{13}$ | 15.90$^{o}$ | 30.10$^{k}$ | 40.50$^{m}$ | 30.20 |
| $I_{14}$ | 19.50$^{k}$ | 29.50$^{n}$ | 42.10$^{h}$ | 33.40 |
| $I_{15}$ | 25.10$^{d}$ | 32.20$^{d}$ | 39.70$^{n}$ | 37.10 |
| Control | 0.00 | 0.00 | 0.00 | 0.00 |

* Mean of three replications  In a column, means followed by common letters are not significantly different at 5 per cent level by DMRT

least in the isolate $I_{15}$ (39.70 % reduction in viscosity) on six DAI (Table 4). Raj kumar (2006) also observed the same trend of increase in the activity of pectin trans eliminase in the banana rhizomes inoculated with *E. carotovora* subsp. *carotovora*.

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