

## Comparative Studies of Bioagents Enhanced Protection of *Aloe vera* in the Control of *Alternaria* Leaf Spot

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### ABSTRACT

Total five bio-agents (three fungal species viz., *Trichoderma viride*, *Trichoderma harzianum* and *Trichoderma virens* and two bacterial species *Pseudomonas fluorescens* and *Bacillus subtilis*) were tested for their antagonistic effect on *A. alternata* by Dual Culture Method. Among these bio-agents *Trichoderma viride* was observed to be significantly superior and recorded least mycelial growth of *A. alternata* (15 mm) with highest inhibition (83.30 %) and thereby proved most effective antagonist against the test fungus. More mycelial growth of the test fungus was observed in case of *T. harzianum*, *P. fluorescens*, i.e. 26.00 mm and 32.00 mm with inhibition of 71.60 per cent and 64.10 per cent, respectively. Minimum inhibition was observed with *T. virens* (40.0 %) followed by *Bacillus subtilis* (44.40 %).

**Key words** *Aloe vera*, *Alternaria* leaf spot, Bio-agents, Management

*Aloe barbadensis* Mill. (*Aloe vera* Tourn. ex L.) commonly known as Indian aloe and in Sanskrit *Kumaree*, belongs to the family Liliaceae. It is indigenous to Africa and Mediterranean countries and is reported to grow wild in Island of Cyprus, Malta, Sicily, and arid tracts of India. In the world India, China, U.S.A., Mexico, Australia and some of the Latin American countries are the major producers and exporters of aloe products. In India, it is cultivated in Rajasthan, Andhra Pradesh, Gujarat and in some part of Tamil Nadu. In Rajasthan, *Aloe vera* has attained the status of a commercial crop due to its great therapeutic values and now it is cultivated extensively in Ajmer, Alwar, Sikar and Jaipur districts. *A. alternata* infected leaves become necrotic, with dark sunken areas on the upper as well as lower surface. Such leaves dry prematurely. It is therefore, a direct loss of fleshy leaves of the plant, which is having the maximum medicinal value. Therefore, this study was carried out to assess the bio-agents enhanced protection of *Aloe vera* in the control of *Alternaria* leaf spot.

### MATERIALS AND METHODS

#### *In vitro* efficacy of bio-agents against mycelial growth:

The dual culture technique (Dennis and Webster, 1971) was followed to assess the antagonistic potential of the bio-agents. The Petriplates of 90 mm diameter were poured with potato dextrose agar @ 20 ml/Petriplate. Each Petriplate was inoculated with two circular mycelial discs of equal size (each of 2 mm diameter) one each of *A. alternata* and

the test-antagonist and placed opposite to each other at equal distance (2cm) from the periphery of the Petriplate. Each treatment had four replications. Observation on zone of inhibition was recorded after seven days of incubation at  $25 \pm 1^\circ\text{C}$ .

Per cent growth inhibition was calculated by following the Vincent's (1947) formula.

$$\text{Per cent growth inhibition} = \frac{C-T}{C} \times 100$$

Where,

C = Diameter (average of the two diagonals) of the colony in check.

T = Diameter (average of the two diagonals) of the colony in treatment.

***In vivo* efficacy of bio-agents:** Efficacy of bio-agents was tested under cage house conditions. *Aloe vera* plants were raised in cage house of Department of Plant Pathology, Agriculture Research Station, Durgapura, Jaipur. Eight-week-old plants were used in the experiments. The talc based formulation of fungal bio-agents and bacterial antagonist were sprayed two times @ 5 gm/L at 15 days interval. The first spray of bio-agents was applied 7 days after inoculation, when disease symptoms initiated on the inoculated leaves. The second spray was applied after 15 days of the first spray. Three bio-agents were used at concentrations given below:

**Table 1. Concentration of bio-agents used for *in vivo* studies**

Name of bio-agent	Concentration
<i>Trichoderma viride</i>	$2 \times 10^7$ spores ml <sup>-1</sup>
<i>Trichoderma harzianum</i>	$2 \times 10^7$ spores ml <sup>-1</sup>
<i>Pseudomonas fluorescens</i>	$1 \times 10^9$ Cfu ml <sup>-1</sup>

Observations on disease intensity were recorded one month after the second spray. For this, 5 leaves were taken randomly from each plant and assessed for per cent leaf area affected with the help of disease assessment scale. From this, per cent disease intensity was computed. Plants sprayed with water served as control.

Per cent disease intensity (PDI) of each plant was calculated using the formula given by Wheeler (1969).

$$\text{PDI} = \frac{\text{Sum of all numerical ratings}}{\text{Number of leaves assessed} \times \text{Maximum disease rating}} \times 100$$

**Table 2.** *In vitro* efficacy of bio-agents against mycelial growth of *A. alternata* after 7 days of incubation at  $25 \pm 1^\circ\text{C}$ .

Bio- agent	Mycelial growth* (mm)	Inhibition of growth (%)
<i>Trichoderma viride</i>	15.00	83.30 (65.88)
<i>Trichoderma harzianum</i>	26.00	71.60 (57.80)
<i>Trichoderma virens</i>	54.00	40.00 (39.23)
<i>Pseudomonas fluorescens</i>	32.00	64.10 (53.19)
<i>Bacillus subtilis</i>	50.00	44.40 (41.78)
Control	90.00	00.00
SEm±	1.30	
CD (p= 0.05)	3.86	

\* Average of four replications.

Figures in parentheses are angular transformed values.

**Table 3.** Management of leaf spot of *Aloe vera* caused by *A. alternata* through bio-agents under cage house condition.

S.No.	Treatment	Conc. (%)	PDI	PDC	PDI	PDC	Pooled	
			2006	2006	2007	2007	PDI	PDC
1.	<i>Trichoderma viride</i>	$2 \times 10^7$ spores $\text{ml}^{-1}$	37.58 (37.81)	36.52	38.50 (38.35)	38.00	39.04 (38.65)	39.75
2.	<i>Trichoderma harzianum</i>	$2 \times 10^7$ spores $\text{ml}^{-1}$	38.87 (38.56)	34.34	39.72 (39.08)	36.03	39.29 (39.17)	35.21
3.	<i>Pseudomonas fluorescens</i>	$1 \times 10^9$ cfu $\text{ml}^{-1}$	39.50 (38.94)	33.27	41.20 (39.93)	33.65	40.35 (39.41)	33.47
4.	Control		59.20 (50.30)		62.10 (52.00)		60.65 (51.12)	
	SEm±		1.06		1.09		1.10	
	CD (p=0.05)		3.27		3.34		3.28	

Figures in parentheses are angular transformed values.

PDI- Per cent disease intensity.

PDC- Per cent disease control.

## RESULT AND DISCUSSION

***In vitro* efficacy of bio-agents against mycelial growth:** Total five bio-agents (three fungal species viz., *Trichoderma viride*, *Trichoderma harzianum* and *Trichoderma virens* and two bacterial species *Pseudomonas fluorescens* and *Bacillus subtilis*) were tested for their antagonistic effect on *A. alternata* by Dual Culture Method. Results are presented in Table-2. Among bio-agents *Trichoderma viride* was observed to be significantly superior and recorded least mycelial growth of *A. alternata* (15 mm) with highest inhibition (83.30 %) and thereby

proved most effective antagonist against the test fungus. More mycelial growth of the test fungus was observed in case of *T. harzianum*, *P. fluorescens*, i.e. 26.00 mm and 32.00 mm with inhibition of 71.60 per cent and 64.10 per cent, respectively. Minimum inhibition was observed with *T. virens* (40.0 %) followed by *Bacillus subtilis* (44.40 %).

***In Vivo* Effect bio-agents on Alternaria Leaf Spot of Aloe vera:** Based on *in vitro* efficacy, three bio-agents were selected for evaluation of their efficacy in controlling *Alternaria* leaf spot of *Aloe vera* under pot conditions. The three bio-agents viz., *Trichoderma viride* ( $2 \times 10^7$ ),

*Trichoderma harzianum* ( $2 \times 10^7$ ) and *Pseudomonas fluorescens* ( $1 \times 10^9$ ) tested, caused significant reduction in the disease as compared to control (Table-3). *Trichoderma viride* was found most effective against the leaf spot disease followed by *Trichoderma harzianum* and *Pseudomonas fluorescens*. *Trichoderma viride* recorded highest per cent disease control as compared to other bio-agents. Similar results were obtained in tomato (Vadivel and Ebenezer, 2006) and sesame (Akbari and Parakhia, 2007).

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