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## Efficacy of bio-agents and plant extracts against the alternaria leaf spot of cabbage (*Alternaria brassicae*)

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### Abstract

An experiment was conducted to evaluate the effect of three plant extracts, two bio agents and one fungicide *in vitro* and *in vivo* against *Alternaria brassicae* causing leaf spot of cabbage. Among the treatments *Trichoderma harzianum* @ 2% was found effective in the inhibition of mycelial growth (65.21%) of *Alternaria brassicae* followed by *Pseudomonas fluorescens* @ 2% (62.41%), eucalyptus leaf extract @ 10% (58.69%), lantana leaf extract @ 10% (54.96%) and datura leaf extract @ 10% (52.17%). as compared to treated propiconazole (86.95%) and untreated control (00.00%). The plant extracts and bio-agents were tested under field conditions during *rabi* 2016-2017 for their efficacy against the disease. Among the treatments minimum disease intensity per cent was recorded in eucalyptus leaf extract @ 10% (25.60%) followed by datura leaf extract @ 10% (28.13%), lantana leaf extract @ 10% (25.73%), *Trichoderma harzianum* @ 2% (29.84%) and *Pseudomonas fluorescens* @ 2% (30.62%) as compared to propiconazole (treated check) @ 0.05% (25.08%) and untreated control (34.05%).

**Keywords:** *Alternaria brassicae*, bio-agents, cabbage, plant extracts

### Introduction

Cabbage is one of the most popular and widely grown vegetable in the world. Botanical name is *Brassica oleracea* var *capitata* L. It belongs to family Cruciferae. *Alternaria* spot an endemic disease caused by *Alternaria brassicae* and *Alternaria brassicicola* is the most devastating disease of cabbage. The disease occurs in all parts of the world where cabbage is grown. The disease caused by this fungus is characterized by yellow-brown spots with target-like concentric rings which appear on leaves, as well as dark brown sunken spots on heads. The causal organism is air borne and soil inhabiting. It affects every part of infected plant. Regular use of fungicide is hazardous for the soil and for humans as well. Thus utilization of plant extracts and bio agents in disease management is considered as eco-friendly, without any environmental pollution plant extracts have been used in disease management for long time as foliar spray is rarely followed approach and needs screening in different crops and under different environmental conditions. The present study was carried out to explore the efficiency of some plant extracts, under *in vitro* and in field condition at Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad, (U. P), India.

### Materials and methods

#### *In vitro* Experiment

Leaf extracts of eucalyptus (10%), lantana (10%) and datura (10%) and a fungicide as treated check [propiconazole (0.05%)] were tested for their efficacy against *Alternaria brassicae* under *in vitro* by using poison food technique on Potato dextrose agar medium.

The selected fresh leaves of healthy plants were collected and washed thoroughly with clean water and dried to drain water. About 10 g of plant leaves were ground using pestle and mortar by adding same proportion (10 ml) of sterilised distilled water in weight by volume method. The plant extract thus obtained by grounding was filtered through muslin cloth and the extract was diluted by adding sterilised distilled water to get 10 per cent concentration. Calculated concentration of plant extracts and fungicides were thoroughly mixed in the medium. Twenty ml of amended medium was poured in 90 mm sterilized Petri-plates and allowed to solidify. Mycelial disc of 5 mm from seven day old actively growing culture was inoculated at the centre of the plate and then incubated at  $28 \pm 2^\circ\text{C}$  for 4 days. Control was maintained without any treatment. Three replications were maintained for each treatment and data was recorded.

*Trichoderma harzianum* and *Pseudomonas fluorescens* were tested *in vitro* for their antagonism against *Alternaria brassicae* by dual culture technique. Twenty millilitre of PDA was poured into sterile Petri plates. Fungal antagonist was evaluated by inoculating the pathogen at one side of the Petri plate and the antagonist inoculated at exactly opposite side of

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the same plate by leaving 3 to 4 cm gap and *Pseudomonas fluorescens* was evaluated by inoculated by streaking at opposite side to test pathogen. One control was maintained where in only test fungus was grown. The plates were incubated at  $28 \pm 2^\circ\text{C}$  for four days. The experiment was conducted in completely randomized block design (CRD) with three replications in each treatment. Per cent inhibition of mycelial growth calculated using the following formula.

### In vivo Experiment

Field experiment was laid-out in Randomized block design with three replications two sprays were given at an interval of 15 days. Treatments were imposed after appearance of the first disease symptoms. Observations on per cent disease intensity was recorded by using 1-9 disease rating scale at 15 days interval and yield data were obtained after the harvest on physiological maturity.

### Results and discussions

**Table 1:** Per cent inhibition of mycelial growth of *Alternaria brassicae* as affected by treatments at 96hrs after incubation.

Sl. no.	Treatments	Concentration	Per cent inhibition
T <sub>0</sub>	Control (untreated)	-	00.00
T <sub>1</sub>	<i>Trichoderma harzianum</i>	-	65.21
T <sub>2</sub>	<i>Pseudomonas fluorescens</i>	-	62.41
T <sub>3</sub>	Eucalyptus leaf extract	10 %	58.69
T <sub>4</sub>	Lantana leaf extract	10 %	54.96
T <sub>5</sub>	Datura leaf extract	10%	52.17
T <sub>6</sub>	Propiconazole (Treated check)	0.05%	86.95
	S.Em 0.52 C.D (5%) 1.55 CV(%) 3.61		

### Field evaluation of bio-agents and plant extracts against the *Alternaria* leaf spot of cabbage caused by the *Alternaria brassicae*

A field study was carried out to assess the efficacy of bio-agents and plant extracts against leaf spot (*Alternaria brassicae*) of cabbage with two sprays taken up at 45 DAT and 60 DAT during rabi 2016-2017 (Table 2). Two sprays were given at 45 DAT and 60 DAT. Results revealed that eucalyptus leaf extract (25.60%) was the most effective in minimizing the per cent disease incidence and getting higher yields and was also found effective in terms of efficacy amongst all the treatments after which was followed by datura leaf extract (25.73%), lantana leaf extract (28.13%), *Trichoderma harzianum* (29.84%), and *Pseudomonas fluorescens* (30.62%). Thus the study indicated that suitable integration of more efficient eco-friendly treatments like bio-agents and plant extracts with minimum usage of chemical fungicides may provide a better and effective management of the disease.

In the field condition, eucalyptus was found most effective

### In vitro evaluation per cent inhibition of bio-agents and plant extracts against of *Alternaria brassicae* as affected by treatments.

The data presented in table 1 for evaluation per cent inhibition of mycelial over control of bio-agents and plant extracts against *Alternaria brassicae* reveals that maximum inhibition of mycelial growth was recorded in T<sub>1</sub>- *Trichoderma harzianum* (65.21%) followed by T<sub>2</sub>- *Pseudomonas fluorescens* (62.41%), T<sub>3</sub>- eucalyptus leaf extract 10% (58.69%), T<sub>4</sub>- lantana leaf extract 10% (54.96%), T<sub>5</sub>- datura leaf extract 10% (52.17%), as compared to treated check T<sub>6</sub>- propiconazole (86.95%) and untreated control T<sub>0</sub> (00.00%). *Trichoderma harzianum* recorded maximum per cent inhibition in the mycelial growth of the pathogen. Similar findings have been reported by Chavan *et al.* (2015) [1] and Maheshwari, (2013) [5]. The probable reason for such findings may be due the antagonistic properties exhibited by *T. harzianum* such as antibiosis, mycoparasitism and food competition (Ghildyal and Pandey, 2008) [3].

amongst all the treatments for the management of *Alternaria brassicae*. Minimum disease intensity per cent (25.60%) were recorded with the same. Similar findings have been reported by Patni (2006) [6] and Sasode *et al.* (2012) [12]. *Trichoderma harzianum* which recorded maximum inhibition of the mycelial growth of the pathogen under *in vitro* conditions was not able to manage disease in the field conditions as efficiently as botanicals when used as foliar spray.

The probable reason for such findings may be about Eucalyptus Leaf extract control is due to Fungal growth inhibition by essential oils works in various ways such as, involves prevention of hyphal growth and sporulation, interruption in nutrient uptake and metabolism, induction of lysis. They are also responsible in the alternation in fungal physiology by inducing changes in cell wall composition, plasma membrane disruption, mitochondrial structure disorganization and interference with respiratory enzymatic reactions of the mitochondrial membrane (Kishore *et al.* 2007) [4].

**Table 2:** Per cent disease intensity at 30 DAT, 45 DAT and 60 DAT as affected by treatments

Sl. no	Treatments	Dosage	PDI			
			30 DAT Before spray	45 DAT after 1 <sup>st</sup> spray	60 DAT after 2 <sup>nd</sup> spray	Mean
T <sub>0</sub>	Control (untreated)	-	23.53	34.21	44.42	34.05
T <sub>1</sub>	Eucalyptus leaf	10%	20.08	26.19	30.52	25.60
T <sub>2</sub>	Datura leaf	10%	19.43	26.24	31.52	25.73
T <sub>3</sub>	Lantana leaf	10%	21.97	28.56	33.87	28.13
T <sub>4</sub>	<i>Trichoderma harzianum</i>	2.00%	22.64	30.61	36.26	29.84
T <sub>5</sub>	<i>Pseudomonas fluorescens</i>	2.00%	22.51	31.28	38.08	30.62
T <sub>6</sub>	Propiconazole (Treated check)	0.05%	21.24	25.42	28.59	25.08
	SEd± C		1.81	1.39	0.93	
	CD@5%		3.95	3.03	2.02	
	CV (%)		10.28	5.88	3.26	

FS- Foliar spray

## Conclusion

In the present study, it was found that *Trichoderma harzianum* @ 2% was found most effective against *Alternaria brassicae*, causing Alternaria leaf spot disease of cabbage under *in-vitro* condition. The treatment recorded maximum per cent inhibition of mycelia growth of the pathogen (65.21). Under field condition, leaf extract of eucalyptus @10% was found most effective against *A. brassicae*, which recorded minimum intensity (25.60%). Therefore it may be recommended for the better management of Alternaria leaf spot disease of cabbage. The results of the present experiment are limited to one crop season (Oct. 2016- Jan. 2017) under Allahabad agro-climatic conditions, as such to validate the present findings more such trials should be taken up in future.

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