

Effect of culture filtrate of *Alternaria alternata* on leaf, seed germination and seedling vigour of dolichos bean

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The effect of culture filtrate of *Alternaria alternata* was evaluated *in vitro* on dolichos bean leaves, seed germination and seedling vigour. All the leaves showed same natural symptoms of *Alternaria* leaf spot caused by *Alternaria alternata* after application of culture filtrate. Seed germination and seedling growth (root and shoot length) was inhibited by unheated culture filtrate, at a concentration of 1:10 dilution.

Key words: *Alternaria alternata*, dolichos bean, seed germination, seedling vigour, culture filtrate

INTRODUCTION

Dolichos bean (*Lablab purpureus* (L.) Sweet) is one of the important vegetable crops grown in India and abroad. This crop is proliferating on the thatches of farmers and fields of vegetable growers in the villages and thriving in kitchen gardens in towns and cities. It is used mostly as green pods but sometimes, it is consumed as pulse in dry form. The toxic metabolites are commonly produced by plant pathogenic fungi (Rudolph, 1976). *Alternaria alternata* causing *Alternaria* leaf spot of dolichos bean produced non-host specific toxins in culture medium. The fungus adversely affects seed germination and seedling vigour, possibly due to production of toxic metabolites. *Alternaria* leaf spot was most important and serious disease in Uttar Pradesh which was reported for the first time from Rajasthan by Goyal (1966). No information is available about the effect of fungal toxins produced by *A. alternata* on leaf, seed germination and seedling vigour of dolichos bean. Keeping above facts in view, following preliminary studies were carried out under laboratory conditions and results are reported here.

MATERIALS AND METHODS

Pure culture of *Alternaria alternata* was isolated from infected leaves of dolichos bean. The pathogen was inoculated in Richards medium in 150 ml erlenmeyer flasks and these flasks were incubated at $28 \pm 1^\circ\text{C}$ for

10 days. The mycelial mat was filtered after 10 days of inoculation through Whatman's filter paper No. 42. This filtrate is considered to contain crude toxin.

The effect of toxins of the culture filtrate on leaves of dolichos bean was studied by the technique of Braun (1955). Leaves were injured by sterilized needle. Two or three drops of culture filtrate was placed on the portion of injured leaf. Observation on development of necrotic area around the injury was recorded after 24 hs.

The culture filtrate was diluted (1:10, 1:50, 1:100, 1:500 and 1:1000) and their effect on seed germination and seedling vigour (root and shoot length) were studied by soaking the surface sterilized seeds of dolichos bean variety Kalyanpur Type-1 for 24 h in different dilutions of culture filtrate. The seeds were plated on 20 cm Petri dishes lined with filter paper soaked in sterilized water. Each petri dish contained 100 seeds. The final data on seed germination were taken after 7 days.

Ten seedlings were randomly selected from each 20 cm Petri dish and data on root and shoot length were recorded. The experiment was replicated three times. The seeds, soaked separately in sterilized Richard's medium as well as water and germinated similarly, served as control. The effect of heat on inhibitory property of the culture filtrate was also studied by autoclaving at 121°C pressure for 20 minutes before soaking the seeds in it for 24 hs and carried out the germination test.

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RESULTS AND DISCUSSION

It is evident from the results in Table 1 that all the leaves developed necrotic spots, more or less, similar to the natural symptoms of the disease after application of the toxic culture filtrate, whereas in the control, no symptom was found. In the beginning, small, light brown, circular to oval spots were produced on leaves inoculated with culture filtrate and which later became dark brown. These symptoms resembled those produced by the fungus in natural condition.

Table 1. Effect of culture filtrate of *Alternaria alternata* on leaves of dolichos bean

Treatments	Number of leaves Inoculated	Number of leaves showing leaf spot symptoms
Application of filtrate after injury	50	50
Control		
(a) Application of sterilized water after injury	50	-
(b) Application of uninoculated medium filtrate	50	-

'-' = No Symptoms

Table 2. Percentage of seed germination, average root and shoot length of dolichos bean in various dilutions and heated and unheated culture filtrate of *A. alternata*

Culture filtrate	Percentage of of seed germination	Average root length(mm)	Average shoot length (mm)
1:10	36.00	12.00	2.00
1:50	48.00	15.00	4.00
1:100	53.00	20.00	5.00
1:500	61.00	21.00	6.00
1:1000	66.00	22.00	7.00
Unheated culture filtrate	32.00	8.00	2.00
Heated culture filtrate	42.00	13.00	2.00
Sterilized distilled water	84.00	24.00	9.00
Sterilized medium filtrate	82.00	24.00	8.00

a) Data are average of 100 seeds.

b) Data are average of 10 seedlings

The results (Table 2) clearly indicated that there was a marked inhibition in seed germination as well as root and shoot length in case of different dilutions of the culture filtrate as well as heated and unheated culture filtrate in comparison to the controls. The inhibition was more pronounced in unheated culture filtrate, followed by at a dilution of 1:10. Such reductions were also recorded in treatments where the seeds were soaked in heated culture filtrate, followed by the dilutions of 1:50, 1:100, 1:500 and 1:1000. The shoots were more sensitive to the toxic effect of culture filtrate compared to roots. Maximum seed germination as well as root and shoot length was found in sterilized distilled water. In case of culture filtrate, seed germination and root and shoot length were maximum in 1:1000, followed by 1:500 dilutions. These findings are more or less similar with the observations of Janardhanan and Husain (1983) on *Datura*, Vaidihi *et al.* (1985) on oilseeds crops and Visconti *et al.* (1987) on tomato produced by culture filtrates of *A. alternata*, *Alternaria sp.* and *A. alternata*, respectively.

REFERENCES

- Braun, A.C. (1955). A study of mode of infection of wild fire toxin. *Phytopathology* **45**:659-664.
- Goyal, K.N. (1966). Studies on leaf spot of beans (*Dolichos lablab* L.). M.Sc. Thesis, University of Udaipur, Rajasthan, India.
- Janardhanan, K.K. and Husain, A. (1983). Studies on isolation, purification and identification of tenuazonic acid, a phytotoxin produced by *Alternaria alternata* causing leaf blight of *Datura innoxia* Mil, *Mycopathologia* **83**: 135-140.
- Rudolph, K. (1976). Non-specific toxins. In R. Heitefues, P.H. Williams (eds.). *Physiological Plant Pathology*, New York, pp.270-315.
- Vaidihi, B.K., Jagadamba, G.V. and Lalitha, P. (1985). Effect of culture filtrate of some fungi on germination of seeds and seedlings of some oilseeds. *Indian Bot. Repr.* **4**:92-94.
- Visconti, A., Logrieco, A., Vurro, M. and Bottalico, A. (1987). Tenuazonic acid in black mould tomatoes; Occurrence and production by associated *Alternaria* species and phytotoxic properties. *Phytopath. Mediterranea* **26**: 125-128.

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