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Effect of Six plant extracts on the mycelial growth of *Alternaria alternata* causing leaf spot of *Aloe vera*

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Abstract:

The experiment was conducted on Plant Pathology Laboratory of SHIATS, Allahabad by collecting the infected leaf of *Aloe vera* found around at the garden of Department of Plant protection of university. Pathogen was isolated, identified and cultured in the Potato Dextrose Agar (PDA) medium. The Pathogen was identified as *Alternaria alternata.* Leaf of six plants viz. *Eucalyptus deglupta*, *Annona squamosa*, *Ocimum sanctum*, *Cannabis sativa*, *Madhuca longifolia*, *Tagetes patula* was collected and its fresh leaf extract was prepared on weight by weight basis so as to test the efficacy of these extracts against the pathogen by Poison food techniques. The leaf extracts were evaluated against *A. alternata* at 50% of concentration i*n vitro*. The mycelial growth of the fungus at different concentrations was measured after 48 and 72 hours of inoculation. The results revealed that all the extracts significantly inhibited the mycelial growth at this concentrations wherever *Madhuca longifolia* and *Tagetes patula* showed least mycelial growth . However, the leaf extract of *Eucalyptus* was found to be most effective as compared to other including control for controlling the disease caused by *Alternaria alternata*. An intense study on these leaf extract may help to use them as an effective biopesticides in commercial scale.

Key words: *Aloe vera* , *Alternaria alternata* , leaf extracts, Poison food techniques, biopesticide

Introduction:

Aloe vera is a stemless or very short-stemmed succulent plant growing to 60-100 cm tall, spreading by offsets. *Aloe vera* belong to the family Aloeceae and division Magnoliophyta is a Monocotyledonous and flowering plant. The leaves are grey to grey-green, with some varieties showing white flecks on their upper and their lower stem surfaces. The margin of leaf is serrated and has small white teeth (www.en.wikipedia.org/wiki/*Aloe_Vera*). Besides, *Aloe vera* is used for treating x-ray burns, dermatitis, cutaneous and disorders of skin. Drug from its juice is tonic and used in jaundice, ameneorrhoea, atonic and piles and also used as effective pain killer (www.naturecare.in/aloevera.html). *Aloe vera* is used to make the antiseptic, which can kill mold, bacteria, fungus and viruses (www.drgranny.com/2011/03/10/uses-of-aloe-vera).

Alternaria alternata is a naturally occurring fungus and have the capability to survive in many kinds of soil throughout the world. *Alternaria alternata* was classified with the fungi Imperfecti or Deuteromycetes and is one of the most important among the allergenic fungi. Brown segmented mycelia give rise to simple or solitary conidiophores, which may produce solitary apical spores, or a string of spores. The spores produced by imperfect fungi vary in shape, size, texture, colour, number of cells, and thickness of the cell wall (www.en.wikipedia.org/wiki/*Alternaria-alternata*).

Many plants have been found as an insecticidal and pesticidal properties and being used. Some of the economically important plants used in this study are as follows:

Eucalyptus deglupta: It belongs to the family Myrtaceae. It has desirable traits such as being fast-growing sources of wood, producing oil that can be used for cleaning and as a natural fungicides (Hardel *et al.,* 2011). Extract of *Eucalyptus* is used against *Alternaria alternata* causing leaf spot of *Aloe vera.*

Ocimum sanctum: It belongs to family lamiaceae. *Ocimum sanctum* leaves possessed antifungal activity against clinically isolated dermatophytic fungi (Kumar *et al.*, 2013).

Annona squamosa: It belongs to the family Annonaceae. Extract of *Annona sps* may be used for protection of Anthracnose disease caused by *Colletotrichum sps.* and has broad anti-fungal capacity (Rajni and Jothi Nisha, 2013).

Cannabis sativa: It belongs to family cannabaceae. Leaf extract of *cannabis* is most affective to control the growth of all the pathogen and has capacity to inhibit 100% mycelial growth of the fungi (*C. lunata*).

Madhuca longifolia: It belongs to the family Sapotaceae. It contains sapogenins, triterpenoids, saponins, steroids and flavonoids which leads this plant extract to fungicide (Sarwey *et al.,* 2013).

Tegetes patula: It belongs to family Asteraceae. Methanol extract obtained from the plant have adverse affect on the growth of fungus but don't have inhibitory action as successful as above plants have.

Keeping in view the importance of botanical pesticides, the present study was done to study effect of six plant extracts on the mycelial growth of *Alternaria alternata* causing leaf spot of *Aloe vera.*

MATERIALS AND METHODS

Collection of plants materials

For the purpose of isolation of leaf extracts six plants were selected. The plants were collected in an around SHIATS, Allahabad. The taxonomic identification of the specimens was performed based on various morphological characters. The six plants selected were *Eucalyptus deglupta, Annona squamosa, Ocimum sanctum, Cannabis sativa, Madhuca longifolia* and *Tagetes patula.*

Plant extracts

Extracts were prepared from leaves of selected medicinal plants. The leaves were thoroughly washed in running tap water and sterile distilled water, air dried at 27^0 C and ground to obtained extracts of each plant species the extraction was done by means of pestle and mortar. Water extract was obtained by adding each 30g of leaves to 30 ml of distilled water (1:1 w/v).

Preparation of media

The fungus was grown on potato dextrose agar (PDA) media, which was prepared by using the following preparation.

Agar agar - 20gm

Dextrose - 20gm

Pealed potato - 200gm

Distilled water - 1000 ml

In vitro test

In vitro test were carried out in sterile petridishes containing PDA. The effect of plant extracts on spore formation and radial growth of pathogen was determined using poisoned food technique (Nene and Thapliyal, 1979). Now different concentration is prepared by adding the plant extract to the PDA. Solution so obtained was autoclaved at 15 psi for about 30 min. Inoculation of *Alternaria alternata* in petridishes was done by gently touching the needle tip with a 7 days old *Alternaria alternata* culture grown on PDA. The inoculated petridishes were kept in incubator at 27˚Celsius for the growth of fungus (Mehrotra and Aggarwal, 2010). The petridishes were observed at regular interval of 48 and 72 hours of time to check the colony formation of fungus. The diameter of fungal colony was measured in mm by colony count method.

Results and discussion

In the present study the efficacy of six leaf extracts was evaluated against *Alternaria alternata*. Table 1 Reveals that among the six plant leaf extracts tested all the leaf extracts showed an inhibitory effects on *Alternaria alternata.* After 48 and 72 hrs, the minimum growth of mycelium was observed in *Eucalyptus deglupta* (15mm), followed by *Ocimum sanctum* (23mm), *Annona squamosa* (28mm), *Cannabis sativa* (30mm), *Madhuca longifolia* (40mm). The maximum growth of 50mm after 48hrs was observed in *Tagetes patula.*

Table:1 In vitro efficacy of six plant extracts against mycelial growth of *Alternaria alternata*

Treatments	Mycelium growth	
	48 hrs(mean)	72 hrs(mean)
Control	58.20^{a}	77.60^{a}
E. deglupta	17.80^{f}	26.40^{g}
O. sanctum	25.60^{e}	33.60^{f}
A. squamosa	30.40^{d}	39.80^{e}
C. sativa	33.60^{d}	43.40^{d}
M. longifolia	42.00^{c}	52.00^{c}
T. patula	51.80^{b}	63.40^{b}
Grand mean	37.06	48.03
CV(%)	6.9	5.7
SE(d)	1.606	1.717
LSD(p(0.05))	3.314^{**}	3.545^{**}

** significant p<0.01 level of significance

Furthermore, it was observed that colony formation varied with time interval. In general, it was observed that growth of colony formation increases with increase in inoculation period. After 72 hrs the maximum growth was observed in *Tagetes patula* (64mm). The minimum mycelia growth was observed in

Eucalyptus deglupta (32mm) followed by *Ocimum sanctum* (36mm), *Annona squamosa* (41mm), *Cannabis sativa* (48mm) and *Madhuca longifolia* (55mm).

Further more maximum percentage inhibition were observed in *Eucalyptus deglupta* (69.41%) followed by *Ocimum sanctum* (56.01%), *Annona squamosa* (47.76%), *Cannabis sativa* (42.26%), *Madhuca longifolia* (27.83%), *Tagetes patula* (10.99%) after 24 hrs of observance and after 72 hrs of observation it was found as *Eucalyptus deglupta* (65.97%), *Ocimum sanctum* (57.08%), *Annona squamosa* (48.71%), *Cannabis sativa* (44.072%), *Madhuca longifolia* (32.98%) and *Tagetes patula* (18.29%) (Table 1).

Figure 1 depicts about the mycelium growth in *in vitro* testing of six plants extract against *Alternaria alternate*. It showed that the percentage inhibition of mycelium growth after 48 and 72 hrs was highest in the *E. deglupta* leaf extracts followed by *O. sanctum*. The least was found in *T. patula*.

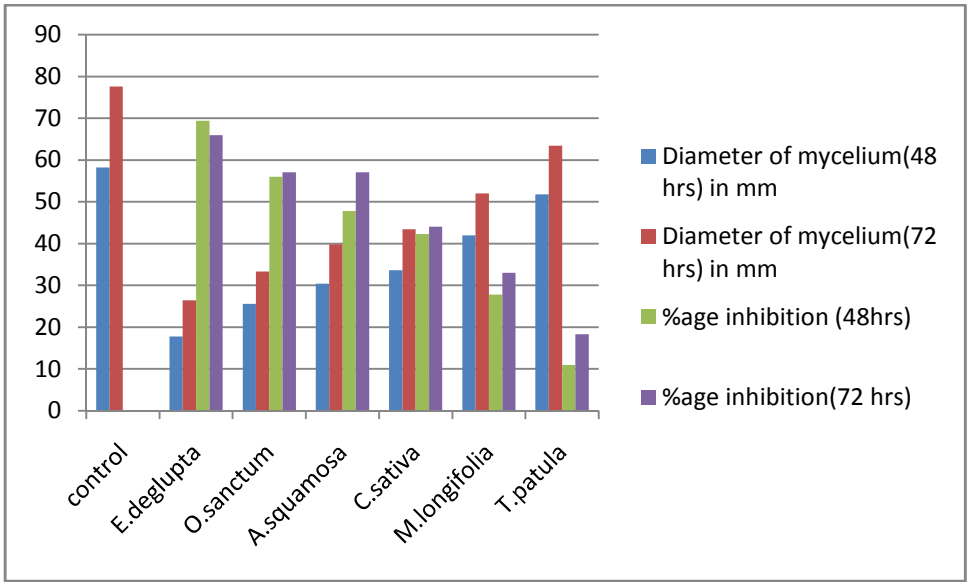

Fig 1: *In vitro* testing of six plants extract against *Alternaria alternata*

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