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Management of *Alternaria brassicae* through some plants extract

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ABSTRACT

Among all the extracts tested, Eucalyptus leaf extract showed significant reduction in radial growth, sporulation and spore germination. Under laboratory condition, leaf extracts of Eucalyptus, Ocimum and Anagallis showed maximum reduction (92.74, 91.93 and 91.53 per cent decrease over check, respectively) in radial growth whereas Ocimum, Eucalyptus and Utrica showed minimum sporulation intensity 91.66, 89.90 and 71.29 per cent, respectively. Significantly lowest reduction of spore germination was observed with Utrica followed with Ocimum and Eucalyptus by 86.76, 79.56 and 79.11 per cent, respectively. Under glasshouse condition, Eucalyptus spray gave significant lesser number of spot/leaf (2.05), minimum size of spot (1.28mm), minimum sporulation intensity (1.22) and minimum disease index (13.6) followed by Calotropis, Ocimum and Polyanthai extract spray.

***Keywords**: Efficacy, Alternaria brassicae, Natural products, Management.*

INTRODUCTION

There are hundreds of plants that have a long history of antimicrobial properties against various plant diseases. However, screening of plants for their antimicrobial activity are essential and needs urgent attention in order to know the real value of plant genetic resources, which is eroding very fast. The screening of plants for their biologically active principals is done on the basis of either their chemotaxonomic investigation or ethnabotanical knowledge for a particular disease[1]. Alternaria leaf blight caused by *Alternaria brassicae* is one of the most sever yield establishing factors. The disease can cause yield reduction up to 45% in mustard[2]. The toxic substances obtained from various plant species, manage a number of fungal diseases of crop plants. Keeping this in view, an effort was made to evaluate some plants extracts against the Alternaria blight pathogen, *A. brassicae* under laboratory and glasshouse conditions. The screening of the different parts of the plants was tested for their antifungal activities to ascertain the distribution of the inhibiting principles.

MATERIALS AND METHODS

Leaves of *Anagallis arvensis*, *Azadirachta indica*, *Calotropis procera*, *Eucalyptus globules*, *Ocimum sanctum*, *Polyanthai longifolia* and *Utrica dioeca* were collected. Leaves were thoroughly washed with sterilized distilled water. These were kept into oven 38 ºC for 2 hrs. when the leaves were dried and then crushed in mortar and pestle for preparing dry powder. Plant extracts were prepared with the help of soxhlet assembly and methanol was used as a solvent. The plant extracts were collected in methanol, which is a highly polar solvent.

Extra methanol of plant extracts was evaporated by keeping the plant extracts in the oven at 35 ºC. After the evaporation of the methanol a paste of leaf extract was obtain, stored in the refrigerator prior to use. Leaf concentrations were prepared by dissolving 0.5, 1.0, 2.0 and 5.0 g of plant extracts in 100ml of sterilized distilled water. For bioassay double strength botanical concentrations were used.

The pathogenic fungus, *Alternaria brassicae* was used in the study for screening antifungal activity of the leaf extracts. Infected leaves exhibiting different types of typical symptoms of *Alternaria* spots, usually with concentric rings with grey center were collected separately from most susceptible genotype of Varuna verity. Fresh isolations from the above respective category of lesions from tested plants were obtained separately as generation of single conidial culture on radish root mannitol agar supplemented with rose Bengal (50 µg/l). The isolate was further maintained on the above medium at 20 ± 2 ºC. For this study, Poisoned food technique was used. After pouring, solidification and cooling of different concentrations of extract amended PDA, plates (9 cm dia.) were inoculated at the center by a 5 mm disc of the fungus, placed with a sterilized needle from the edge of a 10 days old fungus culture maintained on PDA with five replicates. After 15 days, fungal growth was measured with the help of mean colony dia. To get spore in suspension, two discs of 5 mm each were cut from the center of the colony of test fungus, grown on plant extracts amended PDA. Vigorously shaking of these discs was done with two ml of lectophenol plus water solution (1:9) in vials. The number of spores/ml was counted with a haemocytometer. The spores of the fungus, growing on PDA for 15 days were removed with the help of a sterilized needle and brush in sterilized water. One drop each of spore suspension and different concentrations of different plant extracts, were put separately into cavity slides under aseptic conditions with five replicates. The slides were placed in Petri plates, lined with moist blotter paper to serve as mist chamber. For control, the spores were added to sterilized water. Per cent germination of spores was recorded after 24 hrs. of incubation at 24 ± 2 ºC. The percentage of inhibition of spore germination was calculated.

Seeds of tested plant were grown in plastic pots having steam sterilized soil (loam soil, sand and FYM in ratio of 3:1:1). For this purpose, surface sterilized seeds with 0.5% $NaCL_2$ solution were sown in these pots and kept inside the glasshouse. Five plants/pot with ten replications of each genotype were maintained after 20 days of sowing. Conidial suspension was passed through four layers of cheese cloth, to remove mycelium fragments and centrifuged at 3000 rpm for 5 min for removal of unwanted materials. The conidial concentration was maintained at 1.5×10^5 conidial ml^{-1} with the help of haemocytometer.

Thirty days old plants were inoculated separately with *A. brassicae* with the inoculum as prepared above. The plants were kept in the moist chamber with 95% Rh and temperature 15 ± 2 ºC. The pots were well marked/tagged and kept inside the moist chamber for symptoms development. Spraying of different concentrations of plant extracts was started one week after the inoculation and weekly spraying was done up to one month. Appropriate control was also maintained by inoculating the leaves with sterilized distilled water.

RESULTS AND DISCUSSION

On the basis of data presented in Table 1, significant reduction in radial growth of the fungus was observed in all plant extracts except *Polyanthai*, over the control in all the concentrations. Among the extracts tested, *Eucalyptus* leaf extract was found most effective in all the concentrations and it gave significant reduction of radial growth of tested pathogen. It gave 80.64 per cent reduction in mycelium growth at 0.5 per cent and90.33 per cent at 1.0 per cent concentration. It also resulted, 100 per cent reduction in growth at 2.0 and 5.0 per cent concentrations. Leaf extract of *Polyanthai longifolia*, *Urtica dioeca* and *Anagallis arvensis* also results of 100 per cent reduction in growth at 2.0 and 5.0 per cent concentrations.

Table 1. Effect of leaf extracts on radial growth of *A. brassicae* after 15 days [a]

Treatment	0.5	1.0	2.0	5.0	Mean
A. arensis	77.42[b]	88.71	100.00	100.00	91.53
A. indica	45.16	45.16	30.65	35.48	54.95
C. procera	20.97	41.93	58.07	87.10	52.02
E. globules	80.64	90.33	100.00	100.00	92.74
O. sanctum	80.64	88.71	98.39	100.00	91.93
P. longifolia	46.77	82.23	100.00	100.00	82.25
U. dioeca	53.22	59.68	100.00	100.00	87.22

[a] values is an average of five replicates,[b]per cent reduction over control, CD (p= 0.05, Treatment= 0.11, Interaction= 0.22,Concentrations= 0.81, CV= 3.55)

On an average *Eucalyptus* extract was found most effective and it gave 92.74 per cent reduction over control followed by *Ocimum sanctum* (91.93 per cent) and *Anagallis arvensis* (91.53 per cent). Prasad *et al.* (2010) also recorded overall radial growth of fungus was in the order or ashok ≤ *Eucalyptus* ≤ neem dust ≤ neem leaf. Similar results of suppression of fungal disease by the use of neem and its components[3].

Table 2. Effect of leaf extracts on sporulation of *A. brassicae* after 15 days [a]

Treatment	0.5	1.0	2.0	5.0	Mean
A. arensis	14.81[b]	37.04	62.96	81.48	49.07
A. indica	22.22	25.92	33.34	55.56	34.26
C. procera	25.92	40.75	62.96	96.30	56.48
E. globules	77.78	85.52	96.30	100.00	89.90
O. sanctum	81.48	88.88	96.30	100.00	91.66
P. longifolia	25.92	44.45	70.37	81.48	55.56
U. dioeca	40.74	70.37	85.18	88.89	71.29

[a] values is an average of five replicates, [b]per cent reduction over control, CD (p= 0.05, Treatment= 0.06, Interaction= 0.15, Concentrations= 0.41, CV= 3.00)

Significant reduction in sporulation of the fungus was observed in all leaf extracts over the control in all the concentrations (Table 2). At 0.5 per cent concentration, maximum inhibition of spore formation was recorded in *Ocimum* and *Eucalyptus* (100.00 per cent). Significant reduction in sporulation of the fungus was also recorded at 2.0and 5.0 per cent concentrations of all leaf extracts. On an average *Ocimum sanctum* extract was found best which showed 91.66 per cent reduction followed by *E. globulus* which showed 89.90 per cent reduction over control. Similar trends in the results were also observed[4].

Table 3. Effect of leaf extracts on spore germination of *A. brassicae* after 15 days [a]

Treatment	0.5	1.0	2.0	5.0	Mean
A. arensis	52.35[b]	62.94	91.76	93.53	75.15
A. indica	34.70	45.29	48.82	58.23	44.26
C. procera	56.47	64.12	68.82	81.18	67.65
E. globules	48.23	68.23	100.00	100.00	79.11
O. sanctum	44.70	73.53	100.00	100.00	79.56
P. longifolia	56.47	68.82	91.76	93.53	77.64
U. dioeca	66.47	87.65	92.94	100.00	86.76

[a] values is an average of five replicates, [b]per cent reduction over control, CD (p= 0.05, Treatment= 0.19, Interaction= 0.30, Concentrations= 0.81, CV= 3.70)

Significant reduction over control was observed in spore germination of pathogen. At 0.5 per cent concentration, maximum reduction was recorded in *U. dioeca* (100.00 per cent) followed by *P. longifolia* (93.53 per cent). On an average, *U. dioeca* leaf extract was found best which showed 86.76 per cent reduction over control followed by *O. sanctum* (79.56 per cent), *E. globules* (79.11 per cent) and 75.15 per cent by *A. arvensis* (Table 3).

Table 4. Disease components on leaves under glasshouse condition [a]

Treatment	A	B	C	D
A. arensis	4.10	3.73	3.06	21.47
A. indica	6.43	7.43	5.89	32.71
C. procera	2.80	2.18	1.79	16.30
E. globules	2.05	1.28	1.22	13.96
O. sanctum	3.07	1.96	2.10	17.90
P. longifolia	3.22	2.21	2.00	16.68
U. dioeca	3.51	2.54	2.52	19.83
Control	7.76	4.53	8.42	24.57

[a] values is an average of ten replicates, A= No. of spots, B= Size of spots (mm), C= Sporulation intensity, D= Disease index

Significant lowest number of spots was observed in *Eucalyptus* spray (2.05) followed by *Calotropis* (2.80), *Ocimum* (3.07). Highest number of spots was observed in control with an average of 7.76 (Table 4). *Eucalyptus* spray had minimum sporulation which was significantly different from others, followed by *Calotropis*, whereas, control had the maximum sporulation. *Eucalyptus* spray had also the minimum disease severity by 13.96 which were significantly different from others, followed by *Calotropis* by 16.30. Observations revealed that there was a significant different in the disease severity of tested plant by the application of different concentrations of different botanicals sprays (Table 4).

The plant protection workers all over world are aiming at non-chemical means to undertake the pathogens and ultimately disease problems[5-8]. A study was made and revealed that the extracts of *O. tenuiflorum* having antifungal properties against some important vegetable pathogenic fungi[9]. In the other investigation, aqueous leaf extract of four different species of *Datura* were tested against *A. solani* and *Fusarium oxysporum* f.sp. *udum* at different concentrations. Further, they have pointed that *D. stramonium* and *D. innoxia* at 20 per cent concentration was found more inhibitory activity against *F. o.* f.sp. *udum*, while the extract of *D. stramonium* was inhibitory against *A. solani*[10]. The growth and disease intensity of *Fusarium* wilt was significantly impaired by all extracts except wild sunflower and creeping wood sorrel. However, he documented at 25 per cent concentration the most active extracts were aromatic ginger, wild basil and neem[11]. Incidence of brown rust disease of wheat was suppressed due to use of extracts of some botanical plants and ultimately increased of wheat yield under field condition using foliarspray of these biopesticides[12]. Among the extracts tested, *E. citriodora* was highly affected in inhibiting the growth of *A. solani*, as it produced 67.60 and 85.98 per cent growth inhibition at 2 and 10 per cent concentrations. By spraying of *E. citriodora* extract by 1.9 of 5 per cent concentration in field condition as compared to the unspraying plot, the disease intensity was easily managed[8].

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