

Antifungal activity of plant extracts against by *Alternaria helianthi*

P. Ahila Devi, S.Mohan and G.Thiribhuvanamala

ABSTRACT

Twenty plant leaf extracts viz., *Acalypha indica*, *Azardirecta indica*, *Alternanthera sessilis*, *Aloe vera*, *Vitex negundo*, *Wedelia calendulaceae*, *Centella asiatica*, *Ocimum tenuiflorum*, *Gilircidia maculate*, *Nila nirgundi*, *Leucas aspera*, *Lantana camera*, *Solanum trilobatum*, *Tephrosia purpurea*, *Hibiscus cannabinus*, *Cissus quadrangularis*, *Mentha arvensis* *Polyanthes tuberosa*, *Polygala elata* and *Solanum xanthocarpum* were tested against the growth of a sunflower leaf blight causing pathogen *Alternaria helianthi* by poisoned food technique under *in vitro* conditions. Among them, leaf extracts of *Acalypha indica* at 10 per cent concentration inhibited the mycelial growth, sporulation and spore germination to about 78.38 per cent, 85.90 per cent and 52.48 per cent respectively. The *A. indica* leaf extract was very effective against *A. helianthi* and can be used to manage this fungus under field condition.

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Key words: Sunflower, leaf blight, *Alternaria helianthi*, plant extracts, *Acalypha indica***INTRODUCTION**

Sunflower *Helianthus annuus* L. is one of the important oil seed crop in India affected by several diseases like leaf spot, rust, downy mildew, root and collar rot, leaf blight that leads to enormous losses. Among these diseases, leaf blight caused by *Alternaria helianthi* is one of the major diseases (Prathibha *et al.*, 2008). Sunflower is most susceptible to *A. helianthi* during anthesis and seed filling stage of growth. However, *A. helianthi* can cause seedling blight, which reduces crop stand and can infect both leaves and stems of 10–32 days old plants (Jeffrey *et al.*, 1984). The disease symptoms appear more frequently on older leaves than on young and expanding ones (Balasubramanyam and Kolte, 1980). The disease is usually controlled by conventional fungicides applied as foliar spray however, high cost of fungicides and the problem of environmental pollution have stimulated investigation of alternatives strategies for the control of pest and pathogens (Kuc, 1987; Lyon *et al.*, 1995).

Apart from conventional fungicides and microbial agents, plant extracts have been found to be effective against a wide range of pathogens (Amadioha, 2003; Bowers and Locke, 2004; Sahayaraj *et al.*, 2009). Furthermore, plant products based biofungicides are systemic, specific in action,

non phytotoxic and does not pose environmental pollution (Singh,1994).The extracts of many plants possess active constituents which have either direct antimicrobial activity (Ansari,1995; Amadioha, 2000) or induce host defense response thereby resulting in reduction of disease development (Schneider and Ullrich, 1994). The aim of the present study was to compare the effect of some selected medicinal plant extract and fungicides on *Alternaria helianthi* mycelial growth and spore germination in-vitro and identify the concentration of plant extract that have fungicidal properties against sunflower leaf blight pathogen.

MATERIALS AND METHODS**Isolation of different isolates of *A. helianthi***

The diseased sunflower leaf showing the typical symptoms of leaf blight were collected from sunflower from different places of Tamil Nadu and designated as different isolates of *Alternaria helianthi*. The pathogen was isolated on Potato Dextrose Agar (PDA) medium from diseased specimen showing the typical symptoms. The plates were incubated at room temperature ($28 \pm 2^{\circ}\text{C}$) for five days the purified isolates were maintained on PDA slants and used for further studies.

Preparation of plant extracts

Fresh plant leaves of selected plant species viz., *Acalypha indica*, *Azardiracta indica*, *Alternanthera sessilis*, *Aloe vera*, *Vitex negundo*, *Wedelia calendulaceae*, *Centella asiatica*, *Ocimum tenuiflorum*, *Gilircidia maculate*, *Nilanirgundi*, *Leucasaspera*, *Lantana camera*, *Solanum trilobatum*, *Tephrosia purpurea*, *Hibiscus canabinus*, *Cissus quadrangularis*, *Mentha arvensis*, *Polyanthes tuberosa*, *Polygala elata*, *Solanum xanthocarpum* were used for extracting antifungal principles as per the method of Shekawat and Prasada (1971). The freshly collected plant leaf materials were washed separately with tap water, and finally with repeated changes of sterile distilled water. They were separately ground in sterile distilled water at the rate of one mL/g of the leaf tissue in a sterilized pestle and mortar. The extract was strained through two layers of muslin cloth subsequently filtered through Whatman No: 1 filter paper and finally passed through Seitz filter to eliminate bacterial contamination. This formed the standard plant extract solution (100 %).

Inhibition of mycelial growth of *A. helianthi*

All the plant extracts mentioned above were used at 10 per cent concentration. The standard plant extract solution (100 %) and the medium were prepared as already described. Ten ml of the plant extracts was added through membrane filter to 90 ml of the sterilized warm PDA medium each separately and poured in to the sterilized petridishes / plates under aseptic conditions. A nine mm disc of 15 day old culture of the pathogen was cut by means of a sterilized cork borer and placed in to the medium at the center of the petriplate. Three replications were maintained. The plates were incubated at room temperature ($28 \pm 2^{\circ}\text{C}$). The medium without incorporating the plant extract served as control. The fungicide, Dithane M 45 (0.2 per cent) was used as standard check. The mycelial growth of the pathogen was measured when the control treatment with pathogen reached full growth. Three plates per replication were maintained for each treatment. The percent inhibition of mycelial growth was calculated

Inhibition of spore germination of *A. helianthi*

This extract was further diluted to 10 per cent concentration by adding requisite quantities of

sterile distilled water. The plant extracts at 10% concentration were prepared by using sterile distilled water. A drop of extract was separately placed in the depression of the cavity slides and allowed to dry. Then a drop of the spore suspension of *A. helianthi* with a concentration of 10^5 / mL in sterile distilled water was placed in the cavity and mixed thoroughly. Spore suspension in sterile distilled water served as control. Then the slides were kept in a Petri plate-glass bridge moist chamber. Sterilized cotton wool was spread inside a sterile 15 cm petriplate and moistened by pouring sterile distilled water and the excess water was decanted. The depression (cavity) slides were placed on a glass bridge over the moistened cotton. The petri dish lid was replaced and the plates were incubated at room temperature. Observations on spore germination were recorded 24 hrs after incubation by counting the total number of spores and the number of spores germinated in each microscopic field (Anonemous, 1943). Three such microscopic fields were observed and the mean per cent germination and per cent germination inhibition were worked out as described earlier. Three replications were maintained in each treatment.

Inhibition of sporulation of *A. helianthi*

To assess the efficacy of plant extracts against sporulation of the fungus, the fungal discs were taken from different leaf extract treatments and transferred to a test tube containing 10 mL of sterile distilled water and shaken for 10 min to dislodge the spores of *A. helianthi* and the number of spores per ml was determined using a haemocytometer. The per cent reduction in sporulation caused by the plant extracts was calculated as described earlier.

RESULTS

Mycelial growth of *A. helianthi*

To ascertain the role of plant extract against *A. helianthi* mycelial growth an experiment was conducted with 20 plant extracts. Among the plant extract tested *Acalypha indica* at 10 per cent concentration recorded the minimum mycelial growth of 1.15 cm which accounted 78.38 per cent reduction as against control followed to this *Lantana camera* showed its efficacy and recorded

Table 1. *In vitro* screening of plant leaf extracts on *A. helianthi*

Plant extracts	Mycelial growth (cm)	Per cent reduction over control	Sporulation (x 10 ⁵ / mL)	Per cent reduction over control	Spore germination %	Per cent reduction over control
<i>Alternanthera sessilis</i>	2.70 ^d	49.25	12.77 ^{jk}	29.68	68.03 ^f	19.88
<i>Acalypha indica</i>	1.15 ^a	78.38	6.22 ^g	85.90	40.35 ^b	52.48
<i>Azardirecta indica</i>	1.37 ^a	74.19	3.45 ^b	81.00	41.70 ^{bc}	52.07
<i>Aloe vera</i>	1.38 ^a	74.06	4.55 ^{df}	73.49	43.10 ^{bc}	49.24
<i>Vitexnegundo</i> var. <i>purpurescense</i>	1.71 ^b	67.86	5.33 ^{efg}	70.65	44.21 ^{bcd}	47.93
<i>Wedelia calendulaceae</i>	2.16 ^c	59.40	5.65 ^f	68.89	44.74 ^{bcd}	47.31
<i>Centella asiatica</i>	3.77 ^f	29.14	12.99 ^j	28.47	67.58 ^f	20.41
<i>Ocimum tenuiflorum</i>	1.29 ^a	75.75	3.13 ^b	82.76	40.35 ^b	50.48
<i>Gilircidia maculate</i>	3.05 ^e	42.67	11.00 ^{hi}	39.43	55.86 ^e	34.21
<i>Nilanirgundi</i>	2.46 ^d	53.76	10.44 ^h	42.51	76.81 ^{hi}	9.54
<i>Leucas aspera</i>	1.38 ^a	74.06	4.32 ^e	76.21	69.42 ^f	18.24
<i>Lantana camera</i>	1.18 ^a	77.82	2.98 ^b	83.50	39.10 ^{ab}	52.98
<i>Solanum trilobatum</i>	3.74 ^f	26.69	12.10 ^{ji}	33.37	71.54 ^h	15.75
<i>Tephrosia purpurea</i> .	4.16 ^h	21.80	13.44 ^k	26.00	69.73 ^f	17.00
<i>Hibiscus cannabinus</i>	3.85 ^f	27.63	16.11 ^l	11.29	71.45 ^h	15.85
<i>Cissus quadrangularis,</i>	4.91 ⁱ	7.71	17.23 ^l	5.12	79.80 ⁱ	6.01
<i>Mentha arvensis</i>	4.52 ^j	15.04	12.22 ^j	32.71	68.44 ^f	19.40
<i>Polyanthes tuberosa</i>	2.23 ^d	58.08	5.71 ^f	71.86	47.15 ^{cd}	44.47
<i>Polygala elata</i>	4.43 ^j	16.73	12.66 ^j	30.29	63.80 ^f	24.86
<i>So. xanthocarpum</i>	3.42 ^f	35.71	3.42 ^b	35.71	50.12 ^{de}	41.32
Mancozeb (0.2%)	1.07 ^a	79.89	0.00 ^a	100.00	0.00 ^a	100.00
Control	5.32 ^k		18.16 ^m		84.91 ⁱ	
CD (P=0.05)	0.92		1.13		0.90	

considerable reduction in mycelial growth of 1.18 cm which accounted 77.82 per cent respectively. *Cissus quarangularis* was least effective by recording the lowest per cent reduction 7.71 per cent reduction. The comparative check mancozeb recorded 79.89 per cent inhibition over mycelial growth remaining extract also recorded the same extent of mycelia growth reduction between the range of 15.04 per cent to 74.19 per cent over control.

Sporulation of *A. helianthi*

To ascertain the role of plant extract against *A. helianthi* sporulation an experiment was conducted with 20 plant extracts among the plant extract *Acalypha indica* @ 10% concentration recorded minimum sporulation 6.22 spores/ml which accounted 85.90 per cent reduction as against control which was on par with *Lantena camera* showed its efficacy and recorded considerable reduction in sporulation of 2.98 spores/ml which accounted 83.50 per cent respectively. *Cissus quadrangularis* was the least effective by recording lowest percent reduction 5.12 percent. The comparative check mancozeb recorded 100 per cent inhibition over sporulation. The same extent of sporulation reduction was between 11.29-81.00% over control.

Spore germination of *A. helianthi*

To ascertain the efficacy of plant extract against *A. helianthi* spore germination an experiment was conducted with 20 plant extract tested *Acalypha indica* 10% recorded minimum spore germination 40.35 per cent which accounted 52.48 per cent reduction as against control respectively which is on par with *Lantena camera* showed its efficacy and recorded considerable reduction in spore germination of 39.10 per cent which accounted 52.98 per cent reduction respectively. *Cissus quadrangularis* was the least effective by recording lowest per cent reduction 6.01 per cent respectively. The comparative check mancozeb recorded 100 per cent inhibition over spore germination. The spore germination reduction between the ranges of 9.54 per cent to 52.07 per cent over control.

DISCUSSION

It was revealed from the results that *Acalypha* plant extracts caused significant inhibition in the mycelia growth, sporulation and spore germination. Gill (1992) reported that the secondary metabolites like saponins (acalypus and acalyphine) and terpenoids characterized by strong fragrant smell and slight pungency from *Acalypha* were effective against *Claviceps purpurea* and *Persea americana*. Similarly, the crude water extracts of *Acalypha wilkesiana*, *Ocimum gratissimum*, and *Acalypha macrostachya* significantly reduced the radial growth of the post harvest diseases of avocado (Magdalene Ogbo and Oyibo, 2008).

Srivastava and Lal (1997) also reported inhibition of spore germination of *Alternaria alternate* by leaf extracts of *Ocimum basilicum* and *Ocimum canum*. Sprayin g of leaf extracts of *Delonix regia*, *Pongamia glabra* and *Acacia nilotica* inhibited the spore germination, mycelia growth and spore production of *A. helianthi*, *Fusarium solani* and *Macrophomina phaseolina* causing seed borne diseases of sunflower (Thiribhuvanamala and Narasimhan, 1998). Though many workers have reported the use of Neem extract against plant pathogens, the present study has clearly demonstrated the possibility of using extracts from *Acalypha indica* commonly called as "Kuppaimeni" which is a weed available locally in Indian gardens, backyards of houses and waste places throughout the plains of India, in plenty and can find use as an alternative control against *A. helianthi*. The root, stem and leaf of *Acalypha indica* possess therapeutic activity. Kumarasamyraja *et al.* (2012) reported that the chloroform extract of *Acalypha indica* whole plant was effective against *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and showed a promising anti bacterial activity at 300.g/ml concentration due to the presence of alkaloids, phenols, saponins and flavanoids of the chloroform extract of *Acalypha indica* was responsible for antimicrobial activity. The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. Mesta *et al.* (2009) found that among the plant extracts, neem leaf extract (38.49%) was effective than all other plant extracts with respect to

inhibition of *A. helianthi* spore germination on sunflower when compared to fungicides. Taskeen-Un- Nisa *et al.* (2011) revealed that Carbendazim, hexaconzole, bitertanol, myclobutanil, mancozeb, captan and zineb and extracts of *Allium sativum*, *Allium cepa* and *Mentha arvensis* were evaluated for their effect on the inhibition of mycelial growth and spore germination of *Fusarium oxysporum*. Raja Gopal Reddy *et al.* (2009) found that phytoextracts and plant oils were treated *in vitro* for their antifungal efficacy against the growth of *Cercospora moricola*, the incitant of leaf spot of Mulberry (*Morus alba* L.). Highest mycelial growth inhibition (72.59%) was recorded in *Eucalyptus globules* with 10% concentration. The next best plant extracts are, *Oscimum sanctum* (49.08%), *Phyllanthus emblica* (46.75%), *Aloe barbedensis* (45.75%), *Allium sativum* L. (41.08%) and *Azadirachta indica* (35.25%). There is no doubt that these *Acalypha* plant extracts possess antimicrobial principles, however further work should focus on the confirmation of the active ingredient in the plant extracts of *A.indica* for further use in management of *A.helianthi*.

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