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Efficacy of Plant Extracts Against Alternaria Leaf Blight (*Alternaria helianthi*) of Sunflower

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ABSTRACT

Alternaria leaf blight caused by *Alternaria helianthi* (Hansf.) Tubaki and Nishihara is one of the major diseases of Sunflower. In the present study different plant extracts, i.e., *Allium sativum* (garlic), *Azadirachta indica* (neem), *Pongamia pinata, Eucalyptus globules, Catheranthus roseus, Jatropha multifida, Polyalthia longifolia, Tridax procumbense, Calotropis jelodona* and *Prosopis julifera* were evaluated against Alternaria leaf blight under *in vitro* and *in vivo* conditions. Plant extracts were prepared with sterile water at concentrations of 0.5, 1.0, 2.0 and 5.0 %. Among different concentrations tested, reduction in spore germination of *A. helianthi* was recorded with 0.5 % of all plant extracts. Garlic extract was found to be effective with 85.1% reduction in spore germination over control followed by neem (83.3%) and *P. pinata* (75.9 %.). In pot culture studies, these extracts were sprayed three times at different intervals on sunflower plants artificially inoculated with *A. helianthi*. Garlic showed disease reduction of 52.0% over pathogen check followed by neem.

Key words : *Alternaria helianthi,* Botanicals, Disease severity, Spore germination, Whole plant assay method

Sunflower (*Helianthus annuus* L.) is an important oilseed crop in India with high quality edible oil and wider adaptability. In India the crop is cultivated over an area of 1813 thousand ha with a production of 1158 thousand tonnes with productivity of 639 kg/ha. Karnataka occupies first position accounting 53 per cent of total area and 35 per cent of total production of India. Several diseases are known to cause yield loss in sunflower and some of important diseases are sunflower necrosis disease (SND), *Alternaria* leaf blight, downy mildew, rust, powdery mildew and head rot. *Alternaria* blight is an important fungal disease of sunflower and estimates of yield losses range from 27 to 80% (Balasubrahmanyam and Kolte 1980). Resistant sources are not available against this disease. *Alternaria* leaf blight is known to infect all aerial parts of plant *viz*., leaf, petiole, stem, floral parts and seeds. The fungus causes leaf spot, seedling blight, stem spot and head rot.

Usage of fungicides gives effective management of *Alternaria* leaf blight but their constant use may lead to environmental pollution and also increased cost of plant protection. Environmental and health concerns about the extended usage of pesticides in agriculture necessite finding of alternative approaches for controlling plant pathogens. There are hundreds of plant products that have a long history of antimicrobial properties against various plant pathogens. Screening of plant products for these antimicrobial activities is very essential and needs urgent attention in order to know the real value of our national plant genetic resources. The toxic substances obtained from various plant species, manage a number of fungal diseases of crop plants as given by Raghav (2003). Several authors (Buckingham, 1993; Dev Maji *et al.,* 2005; Patni *et al*., 2005) have reported use of botanicals as biorationals. Few workers (Mesta *et al.,* 2009) evaluated different botanicals against *Alternaria* leaf blight of sunflower under *in vitro* conditions. The screening of plants for their biologically active principles is done on the basis of either their chemotaxonomic investigation or ethno-botanical knowledge and literature for *Alternaria* blight disease. In the present study, an effort was made to evaluate some plant extracts against the *Alternaria helianthi* of sunflower under *in vitro* and *in vivo* conditions.

MATERIAL AND METHODS

Pathogen

For isolation of sunflower leaf blight pathogen, naturally infected sunflower leaves showing the leaf blight symptoms were collected

Table 1. Plants used for evaluation against *Alternaria helianthi*.

Common name	Botanical name	Family	Parts
Garlic	*Allium sativum*	Liliaceae	Bulb
Arandi	*Jatropha curcas*	Euphorbiaceae	Leaves
Devadaru	*Polyalthia longifolia*	Annonaceae	Leaves
Vinca rosea	*Catharanthus roseus*	Apocynaceae	Leaves
Neem	*Azadirachta indica*	Meliaceae	Leaves
Eucalyptus	*Eucalyptus citriodora*	Myrataceae	Leaves
Tridax daisy/ coat buttons	*Tridax procumbens*	Asteraceae	Leaves
Giant Milkweed	*Calotropis gigentia*	Ascelepiadaceae	Leaves
Algarroba	*Prosopis juliflora*	Fabaceae	Leaves
Indian Beech	*Pongamia Pinnata*	Fabaceae	Leaves

from field. *Alternaria helianthi* was isolated on sunflower leaf extract medium by standard method (Mesta *et al.,* 2009) and maintained at 25 ± 2^{0}C for further studies.

Preparation of plant extracts

Ten plant species were selected for the study (Table 1). The collected plant leaves/bulbs were thoroughly washed with sterilized distilled water and blotter dried. These were crushed in mortar and pestle for preparing paste. Plant extracts were prepared by adding sterile distilled water to the paste (1:1 v/w) and the extracts were filtered through double layered muslin cloth for the stock solution. The appropriate amount of stock solution of plant extract was mixed in sterilized distilled water to make the concentrations of 0.5, 1.0, 2.0 and 5.0% for experiments.

In vitro* effect of plant extracts on conidial germination of *A. helianthi

Conidia were harvested from 7 days old *A. helianthi* culture grown on sunflower leaf extract medium with the help of a sterilized needle and brush in sterilized water. One drop each of conidial suspension and various concentrations (0.5, 1.0, 2.0 and 5.0%) of different plant extracts were put separately into cavity slides under aseptic conditions. Three replications of each treatment were maintained. The slides were placed in petriplates, lined with moist blotter paper to serve as moist chambers. For check, the spores were added to sterilized water and placed in cavity slides. Germination of spores was recorded after 6, 12 and 24 hr of incubation at 24 ± 2°C. The per cent inhibition of spore germination was calculated as follows:

$$\frac{A - B}{A} \times 100$$

Where, A= No of Spores germinated in Check; B= No of Spores germinated in treatment

Evaluation of plant extracts against *Alternaria* leaf blight of sunflower

Efficacy of plant extracts was tested under green house conditions. Twenty five days old sunflower plants (cv. Morden) were raised in pots containing mixture of red soil, sand, FYM and these were sprayed with different plant extracts at 0.5% concentration. Treated plants were sprayed with conidial suspension of *A. helianthi* (1 X 10^{6}spores/ ml) with an atomizer one day after first treatment spray. The check was maintained by spraying the sterilized water only and few drops of tween -20 was added which acts as sticker to spore suspension and plant extracts. Treated and control plants were covered with polyethylene cover to provide congenial conditions for disease development for one day. The second spray of plants extracts was given after 3 days of artificial inoculation, when disease symptoms were initiated on the inoculated foliage. The spray of extracts was repeated at 7 days interval after second spray and three replicates were kept for each plant extract. Observation on disease severity was recorded at different intervals after inoculation. From this, per cent disease index was computed. From the mean per cent disease index (PDI), per cent reduction over control (RDI) was calculated using

standard formula. Plants sprayed with water served as control and the experiment was repeated twice to confirm the results.

RESULTS AND DISCUSSION

Screening of the different plant extracts was studied for their antifungal activities to ascertain the distribution of the inhibiting principles, so that the plants showing maximum reduction in disease could be used in management of disease. Among different concentrations of plant extracts tested for conidial germination at different time interval, 0.5% of plant extracts with12hrs incubation showed best results. The conidial germination of *A. helianthi* due to most of plant extracts was significantly lower than that of check at different concentrations. At 0.5 concentration, garlic (*Allium sativum*) extract was found highly effective with 85.1% reduction of conidial germination over check followed by *A. indica* (83.3%) and *P. pinata* (75.9%.) (Table 2). *T. procumbens* also showed more reduction in conidial germination (72.2%). Extracts of *J. curcas* (70.3%), *P. longifolia* (70.3%) and *C. gigentia* (68.5) were on par with each other in reducing the conidial germination at 0.5% concentration. Leaf extract of *C. roseus* had low inhibitory effect on spore germination (59.2%). Patni *et al.,* (2005) studied the inhibitory effect of different plant extracts and reported that *Euphorbia* leaf extract gave complete reduction in growth at 1, 2 and 5% concentrations where as Rumax, Urtica and Eucalyptus extracts resulted in total reduction in growth at 2 and 5% concentrations.

With decrease in concentration of plant extracts, from 5 to 0.5%, there was less reduction in conidial germination of *A. helianthi.* At 5% concentration of all plant extracts, there was inhibition in spore germination. In garlic and neem extracts at 5% concentration, the germ tube was produced from spore, but the growth of germ tube was stopped after 1-2mm. This may be due to more effect of phytotoxins which caused hinderance to spores to germinate. Spore germination was moderate at 2% concentration of plant extracts. In 1% concentration of plant extracts of garlic and neem had shown more reduction in spore germination. As more reduction in spore germination was observed with 0.5% concentration with all plant extracts, the same concentration was used for pot culture studies.

In pot culture studies, among ten plant extracts, garlic (*A. sativum)* extract significantly reduced disease severity by 48 to 52% over check (Table 3). The disease control may be due to the allicin, the major constituent of *Allium sativum* containing sulphur with strong toxic properties against several bacteria and fungi. Skinner (1955) reported that the active principle of *A. sativum* was a mixture of diallyl-disulphide and diallyl trisulphide which contain fungicidal properties. Slusarenko *et al.,* (2008) tested the effectiveness of garlic juice against a range of plant pathogenic bacteria, fungi and oomycetes *in vitro*. Anonymous (2002) reported that garlic bulb extract gave better control against *Alternaria* blight of sunflower which are in accordance with our results. Singh and Verma (2010) reported that garlic extract proved as the most effective treatment in checking growth and conidial germination of *Alternaria alternata* and also in controlling Alternaria leaf blight in Adusa. Two sprays of garlic extract at 15% gave 55.1% disease control on artificially inoculated Adusa plants. Chattopadhyay (1999) reported that garlic bulb extract (1% w/v) was the best among the tested botanicals and second to carbendazim, with a significant difference in their yield. Soil application of KCl @ 84 kg/ha, foliar spray of *A. indica* leaf extract (1% w/v), and azadirachtin also reduced *Alternaria* leaf blight severity and increased seed yield over the control. Extracts of *Cassia tora* and *C. sophera* completely inhibited conidial germination of *Phyllactinia corylea* and conidial germination of *Pseudocercospora mori* was completely inhibited in extracts of *Allium sativum* and *Datura metel.* Maximum inhibition of colony growth of *Morus roridum* was observed with amendment of 5% solvent extracts of *D. metel* (Dev Maji *et al.,* 2005).

Extracts of neem (*A. indica*) also recorded comparatively low disease severity when compared to check. Results were in conformity with those of Chattopadhyay (1999) who found that foliar spray of *A. indica* leaf extract and azadirachtin reduced mycelia growth of *A. alternata,* decreased disease severity and increased yield over control in tomato and sunflower. Mesta *et al.,* (2009) found that neem leaf extract (38.49%) was effective than all other plant extracts with respect to inhibition in germination and radial growth of *A. helianthi.* The fungicidal spectrum of neem has been thoroughly reviewed by Parveen and Alam (1993) and antifungal properties of *A. indica* were also studied by Ghewande (1989) and Usman *et al.,* (1991) in late leaf spot of ground nut.

Babu *et al.,* (2000) mentioned that spraying with 3% neem oil on tomato resulted in 53% reduction in disease severity over control, while Patil *et al.,* (2001) found that severity of early blight of tomato caused by *A. solani* was reduced by neem

Table 2.Effect of plant extracts on conidial germination of *Alternaria helianthi* .

Botanicals	Time interval (h) after incubation	Reduction of conidial germination (%) over check			
		Concentration of botanicals			
		0.5%	1%	2%	5%
Allium sativum	6	67.4	60.4	53.4	37.1
	12	85.1	79.6	66.6	35.1
	24	83.0	76.4	59.1	25.3
Jatropha curcas	6	53.4	43.0	29.0	17.4
	12	70.3	62.9	48.1	7.4
	24	68.3	53.5	45.0	16.9
Polyalthia longifolia	6	63.9	52.3	30.2	13.9
	12	70.3	58.3	38.8	22.2
	24	65.4	46.4	32.8	9.8
Catharanthus roseus	6	52.3	44.1	32.5	27.9
	12	59.2	53.7	29.6	12.9
	24	63.3	46.7	30.9	14.0
Azadirachta indica	6	62.7	46.5	39.5	32.5
	12	83.3	77.7	62.0	25.9
	24	79.5	72.5	64.0	21.1
Eucalyptus citriodora	6	55.8	51.5	34.8	13.9
	12	66.6	55.5	50.0	16.6
	24	66.1	52.1	33.8	16.1
Tridax procumbens	6	55.8	43.0	29.0	18.6
	12	72.2	51.8	31.4	11.1
	24	63.2	44.3	28.1	15.4
Calotropis gigantia	6	51.5	45.3	34.8	25.5
	12	68.5	61.1	46.2	18.5
	24	64.7	50.7	36.6	18.3
Prosopis juliflora	6	58.1	51.1	34.8	9.3
	12	62.9	48.1	33.3	16.6
	24	61.9	46.4	30.2	16.9
Pongamia pinnata	6	60.4	44.1	37.2	30.2
	12	75.9	57.4	46.2	24.0
	24	77.4	61.9	47.8	19.7

CD>0.05

Treatment = 3.41; Concentration = 1.86

Treatment X Concentration = 6.34; Concentration X Treatment = 6.17

Figures were arcsine transformed values before analysis:

Table 3. Evaluation of plant extracts against *Alternaria* leaf blight under pot culture conditions.

Botanicals	Disease severity (%)	Reduction of Disease severity over control (%)
Catharanthus roseus	55.5 (48.2)	30.8
Azadirachta indica	40.6 (39.6)	49.4
Jatropha curcas	59.9 (50.7)	25.4
Polyalthia longifolia	56.5 (48.7)	29.6
Allium sativum	38.5 (38.4)	52.0
Eucalyptus globulus	62.2 (52.1)	22.5
Tridax procumbens	68.4 (55.8)	14.8
Calotropis gigantia	45.9 (42.7)	42.8
Prosopis juliflora	51.2 (45.7)	36.2
Pongamia pinnata	41.4 (40.1)	48.4
Control	70.4 (57.0)	12.3
Pathogen	80.3 (63.7)	-
CD (P e" 0.05)	5.2	-

Figures in parentheses are transformed values.

seed extract with increased fruit yield. Hassanein *et al.,* (2008) reported that tomato plants sprayed with 20% aqueous neem leaf extract reduced early blight caused by *A. solani*. Severity of early blight pathogen when plants were treated with neem leaf extract may be related to the anti fungal substance present in the extract (National Research council, 1992). Abd-El-Khair *et al.,* (2007) stated that lemon grass leaves gave the most reduction in late blight and early blight disease severity comparing with pathogen check followed by extracts of garlic bulbs , basil leaves and Marjoram leaves. The inhibitory effect of the tested extracts might be due to natural bioactive materials present in these extracts.

Among other extracts, *Pongamia pinnata* reduced the disease severity by 48% as compared to check. In our studies extract of *Prosopis juliflora* showed reduction of 36% over check but Anonymous (2006) reported that extract of *Prosopis juliflora* was highly effective in inhibiting growth of *A. helianthi* and prepared a commercial product named Prosopan from *P. juliflora*. Similarly Mesta *et al.,* (2009) reported that *P. juliflora* could inhibit less than 40% of spore germination and mycelial growth of *A. helianthi*. In case of control plants there was natural occurrence of 70.4% leaf blight, while 80.3% disease was observed in pathogen check where pathogen inoculum was sprayed.

The use of plant extracts and phytoproducts is gaining attention due to their proven nature specificity, biodegradability, low toxicity, minimal residual toxicity in the ecosystem (Adityachaudhary 1991; Pan and Deb, 1997). Most of the leaf extracts used in our studies reduced the spore germination of *A. helianthi* at 0.5% concentration and also reduced the disease severity under pot culture conditions. Among these, garlic extract is significantly effective in reduction in spore germination and also disease severity, which can be included as one of the component in integrated management of *Alternaria* leaf blight of sunflower under field conditions.

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