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ANTIFUNGAL ACTIVITY OF SOME COMMON WEED EXTRACTS AGAINST SEED-BORNE PHYTOPATHOGENIC FUNGI *ALTERNARIA* SPP

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ABSTRACT

Herbal fungicides are mostly using to control plant disease of fungi because of their ecofriendly nature and their cost effectiveness. The present investigation focuses on the antifungal activity of solvent based extracts extracted from some common weeds *Achyranthes aspera*, *Parthenium hysterophorus*, *Cannabis sativa*, *Calotropis gigantean*, *Chenopodium album*, *Canada thistle*, *Phalaris minor*, *Cynoden dactylon*, *Argemone maxicana*, *Ageratum conyzoides*, and *Lantana camera* were screened against seed-borne phytopathogenic fungus *Alternaria* SPP. by modified food poison method. The acetone, methanol, benzene, ethyl acetate and chloroform extracts of different parts of plants were evaluated for this study; the antifungal activity was more effect in extracts of *Ageratum conyzoides* and *Parthenium hysterophorus*, against phytopathogenic fungus *Alternaria* SPP. The present study suggests that chloroform and methnol extracts of *Ageratum conyzoides* and methanol extract of *Parthenium hysterophorus*, can form the basis for the development of novel broad spectrum herbal fungicidal formulations. We conclude from this that these extracts exhibit amazing fungicidal properties that support the notion that plant extracts may be used as herbal fungicides.

INTRODUCTION

A major factor for the revival of weeds is their ability to resist pests and pathogens in their environment. Thus, they could be a potential source of antimicrobial compounds and their identification is necessary to develop cheaper pesticides. The developments of resistance in weeds to the common pesticides and the increasing restrictions on the use of toxic material in the environment have given an impetus to search for novel plant protectants that interfere with the pathogenicity factors. Herbal fungicides are gaining growing interest because of their eco-friendly attributes (Dwivedi and Singh, 1998; Karnwal and Singh, 2006). Pathogenic fungi are the main infectious agents in plants, causing alterations during developmental stages including post-harvest. Fungi are ubiquitous in the environment, and infection due to fungal pathogens has become more common. The genus *Alternaria* is widely distributed in nature and its species are among the most common fungi on the phyllosphere (Lopes and Martins, 2008). More than 800 million people in the developing countries do not have adequate food supplies and at least 10% of food is lost due to plant diseases (Strange and Scott, 2005). In fruit and vegetables, there is a wide variety of fungal genera causing quality problems related to aspect, nutritional value, organoleptic characteristics, and limited shelf life (Agrios, 2004). Fungal species of the genera *Alternaria*, *Aspergillus* and other species have been considered to be major plant pathogens world wide (Ghafoor and Khan, 1976; Mirza and Kureshi, 1978). For farmers and Gardner, *Alternaria* is a common concern because it can cause plant blights. Controlling *Alternaria* can be difficult because it spreads so readily and it is estimated that nearly 20% of the crops damage worldwide is caused by these busy fungi (Agrios, 2000).

As compared to other plant parasites, fungi cause the greatest impact with regard to diseases and crop production losses. The most important method of protecting the plants against the fungal attack is the use of fungicides. However, many fungicidal agents available in the market are toxic and have undesirable effects on other organisms present in the environment. Some synthetic fungicides are non-biodegradable, and hence can accumulate in the soil, plants and water, and consequently effect the humans through the food chain. The development of resistance of pathogenic fungi towards the synthetic fungicides is of great concern. Therefore, it is desirable to use some ecofriendly measures for the management of diseases.

Many of the earlier pesticides were the extracts of plants, and several plants have been exploited more widely as sources of commercial insecticides. But, from 1940s, synthetic agrochemicals

largely replaced the plant-derived products as the key commercial pesticides research on plant derived natural products for the use in agriculture went into decline for a number of years. But this trend is now reversed as it becomes evident that plant natural products still have enormous potential to inspire and influence the modern agrochemical research (Choi *et al.*, 2004).

Natural products seem to be a viable solution to the environmental problems caused by the synthetic pesticides and many researchers are trying to identify the effective natural products to replace the synthetic pesticides (Kim *et al.*, 2005). Similarly, the use of natural products for the control of diseases in plants is considered as an alternative source to synthetic pesticide due to their lower negative impacts on the environment. Besides being harmless and non-phytotoxic it has been proved that plant extracts exhibit inhibitory effect on pathogens. Several higher plants and their constituents have been successful in plant disease control and have proved to be harmless and non phytotoxic, unlike chemical fungicides. The plant based fungicides are cheap, locally available, non-toxic, and easily biodegradable (Singh *et al.*, 1986; Dubey, 1991; Alam *et al.*, 2002). Although there is a growing interest in the use of medicinal plants to control the plant diseases, only about 2,400 plant species among more than 250,000 higher plants have been screened for the phytoactivity (Oluwalana and Adekunle, 1998; Oluwalana *et al.*, 1999; Khafagi and Dewedar, 2000).

There are evidences from earlier works that several plant species possess antifungal and antibacterial properties (Manoharachary and Gourinath, 1988; Bandara *et al.*, 1989; Srivastava and Lal, 1997; Maji *et al.*, 2005; Nduagu *et al.*, 2008; Yasmin *et al.*, 2008; Harlapur *et al.*, 2007 and Akinbode and Ikotun, 2008). The present investigation is therefore, undertaken to test the efficacy of these common weed extracts against the seed-borne phytopathogenic Fungus *Alternaria* SPP. fungal pathogens. As well, the smallest concentration capable of inhibiting or preventing growth was determined among the species and extracts that demonstrated inhibitory properties.

MATERIAL AND METHODS

Plant Materials

Eleven plants were collected from local areas of near the Chaudhary Charan Singh University Meerut. Table-1 shows the list of common weeds used in this study. All plants were identified with the help of various scientific literatures and by discussion with Dr. S.K. Shahi, Department of Microbiology, Chaudhary Charan Singh University Campus Meerut.

Table 1. List of weeds selected for antifungal activity

Sr. No.	Common Name	Botanical Name	Part Used	Family
1.	Chirchita	<i>Achyranthes aspera</i>	Stem , leaves	Amaranthaceae
2.	Carrot Grass	<i>Parthenium hysterophorus</i>	Leaves	Asteraceae
3.	Bhang	<i>Cannabis sativa</i>	Leaves	Cannabaceae
4.	Aak	<i>Calotropis gigantea</i>	Leaves	Apocynaceae
5.	Bathwa	<i>Chenopodium album</i>	Leaves	Amaranthaceae
6.	Corn thistle	<i>Canada thistle</i>	Leaves	Asteraceae
7.	Baluri	<i>Phalaris minor</i>	Stem,Leaves,Seed	Poaceae
8.	Doab Grass	<i>Cynoden dactylon</i>	Whole Plants	Poaceae
9.	Satyanashi	<i>Argemone maxicana</i>	Leaves	Papaveraceae
10.	Chick weed	<i>Ageratum conyzoides</i>	Whole Plants	Asteraceae
11.	Red Sage	<i>Lantana camera</i>	Leaves & Flower	Verbenaceae

Preparation of plant extracts

Different parts of the collected plants were dried for 15 days. The plants were powdered with the help of blender. One gram plant powdered was then extracted in 10 ml each of five different solvents i.e. Acetone, Benzene, Chloroform, Ethanol, and Methanol separately. The overnight extracts were filtered with a Whatman's no.1 filter paper, and then extracted liquid was subjected to rotary evaporation in order to remove the solvents. After evaporation 10 ml of DMSO (di methyl sulphoxide) were added in the extracts separately. The extracted material is stored in refrigerator for further investigation.

Fungal strain

Strains of *Alternaria* spp. were obtained from the seed of *oryza sativa*. The fungus was grown at 28±2 °C on potato dextrose agar (PDA) and Sabouraud dextrose agar (SDA). Spores of the fungus were collected from cultures on agar plates after 7days. The fungal spore suspensions were stored in 20% glycerol at -40 °C.

Antifungal activity of weed plant extracts by modified poison food assay

The given plant extracts were tested by the poison food technique with slight modifications. 800 µl of SD broth in the 2 ml micro centrifuge tube (MCT), then 100 µl of the each solvent extract were taken with the help of micropipette. Mixed well the SD broth and plant extracts separately.

100 µl of test fungal microorganism inoculums (McFarland standard) were added in the SD broth. The test micro centrifuge tubes were incubated at 28±2°C for 24 hours.

Sterile disc of 0.5 mm diameter was dipped in the test suspension (800 µl SD broth +100 µl plant extract +100 µl fungal suspension culture) in MCT. The sterile discs are placed on SDA medium on petriplate. All petriplate are incubated at 28±2°C for 48 hours. The basal media (SD broth) without any phytoextract served as the control. The control is containing the DMSO in place of phytoextracts. The mycelia growth of the test fungus was measured after 48 hours and compared with control. The percentage of mycelia growth inhibition was estimated by using following formula (Tapwal *et al.*, 2011).

$$I \% = \frac{C-T}{C} \times 100$$

Where;

I = percentage inhibition, C = colony diameter in control, T = colony diameter in treatment

Determination of Minimum Inhibitory Concentrations

Strains with inhibition zones were considered sensitive to the extract, those without such a zone were considered resistant. For MIC, two-fold serial dilutions of the extracts were performed. Each inoculum was prepared in its respective medium and density was adjusted to 0.5 Mcfarland standards (108 CFU/ml) and diluted to 1: 100 for the broth micro dilution procedure. Microtiter plates were incubated at 130 rpm and 28±2°C. The MIC was recorded after 24-48h. The MIC is the lowest concentration of the compound at which the microorganism tested does not demonstrate visible growth.

RESULTS

In this study, we have tested the extracts of eleven weed plants for their antifungal activity against seed-borne phytopathogenic fungi *Alternaria* SPP. All the plant extracts showed antifungal activity against *Alternaria* SPP. Extracts of *Ageratum conyzoides* and *Parthenium hysterophorus*, showed the most potential antifungal activity against the seed-borne phytopathogenic fungi *Alternaria* SPP. were the most to all the plant extracts tested. On the contrary, *Alternaria* SPP. was found to be more sensitive to chloroform extracts of *Ageratum conyzoides* (Table 2).

Table 2. Antifungal screening of weed plant extracts against seed-borne phytopathogenic fungi *Alternaria* SPP.

Plants	Percentage of mycelial growth inhibition (MGI)				
	AE	BE	CE	EAE	ME
<i>Achyranthes aspera</i>	38.57	42.85	44.28	42.85	44.28
<i>Parthenium hysterophorus</i>	50	47.14	44.28	47.14	52.85
<i>Cannabis sativa</i>	48.57	45.71	42.85	42.85	44.28
<i>Calotropis gigantea</i>	38.57	44.28	44.28	44.28	41.42
<i>Chenopodium album</i>	44.28	47.14	48.57	42.85	48.57
<i>Canada thistle</i>	41.42	44.28	50	41.42	41.42
<i>Phalaris minor</i>	44.28	47.14	50	42.85	48.57
<i>Cynoden dactylon</i>	47.14	42.85	47.14	44.28	42.85
<i>Argemone maxicana</i>	45.71	44.28	47.14	45.71	44.28
<i>Ageratum conyzoides</i>	44.28	44.28	80	44.28	52.85
<i>Lantana camera</i>	47.14	45.71	42.85	44.28	45.71

Here, **AE**=Acetone Extract, **BE**=Benzene Extract, **CE**=Chloroform extract, **EAE**=Ethyl Acetate Extract, **ME**=Methanol extract

Table 3. Minimum inhibitory concentrations of bioactive plant against *Alternaria* SPP.

Plant name	Plant extract	Minimum inhibitory concentration ($\mu\text{l/ml}$)
<i>Ageratum conyzoides</i>	Methanol	6.25×10^{-4}
<i>Parthenium hysterophorus</i>	Methanol	6.25×10^{-4}
<i>Ageratum conyzoides</i>	Chloroform	3.125×10^{-5}

Alternaria SPP was found to be the highly sensitive to the action of Chloroform extracts of *Ageratum conyzoides* (least MIC $3.125 \times 10^{-5} \mu\text{l/ml}$). Methanol extracts of *Parthenium hysterophorus* and *Ageratum conyzoides* with the least MIC being $6.25 \times 10^{-4} \mu\text{l/ml}$ (Table 3).

DISCUSSION

Natural products from many plants are known to control plant pathogens (khan *et al.*, 1979). Antifungal activity testing of weeds remains an area of interest. However not many reports are available on the exploitation of antifungal property of weeds plants and even the data regarding use of weeds as an antifungal agents are scanty. The solvent extract of *Ageratum conyzoides* and *Parthenium hysterophorus* showed a broad spectrum antifungal activity against seed-borne phytopathogenic fungi *Alternaria* SPP. The two solvents based extracts of *Ageratum conyzoides* and *Parthenium hysterophorus* showed good activity against *Alternaria* SPP. Our results also showed that the chloroform extract (*Ageratum conyzoides*) is highly active against *Alternaria*

SPP. The antimicrobial potency of plants is believed to be due to tannis, saponins, phenolic Compounds, essential oils and flavonoids (Reynolds JEF *et al.*, 1996). The antimicrobial activity of plant oils and extracts has formed the basis of many applications, including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies (Lis-Balchin *et al.*, 1997).

Thus, the extract of *Parthenium hysterophorus* and *Ageratum conyzoides* could be a possible source to obtain new and effective biofungicides to control *Alternaria* SPP caused different seed-borne diseases in various crops. Biofungicides are easily biodegradable, selective and locally produced, especially for the farmers who cannot afford expensive synthetics fungicides. By using weed plant species as raw materials for plant derived fungicides, can manage the disease, and at the same time might create economic uses for these unwanted species (Deepika *et al.*, 2011). The present investigation is an important step in developing plant based fungicides which are ecofriendly for the management of the seed borne disease of *Alternaria* SPP and development of commercial formulation of botanicals. Further investigation will be done for developing commercial formulation based on field trial and toxicological experiment.

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