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Antifungal Activity from Water Extracts of Some Common Weeds

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Abstract: The water extracts from the weed species *(Ageratum conyzoides, Oxalis corniculata, Phyllanthus debilis, Vernonia cinerea* and *Desmodium trifolium)* were assayed for their antifungal activity against some plant pathogenic fungi. The extract from *Ageratum conyzoides* inhibited the mycelial growth of *Rhizoctonia solani, Aspergillus niger* and *Phomopsis theae*. The extract from *Oxalis corniculata* was active against *A. niger* while, *Phyllanthus debilis* suppressed the growth of *P. theae*. The activity generally declined after three days of incubation, while *A. conyzoides* remained active nine days after incubation.

Key words: Antifungal activity, water extract, weeds, *Ageratum conyzoides*, plant pathogenic fungi.

Introduction

A major factor for the survival and persistence of weeds is their ability to resist pests and pathogens in their environment. Thus they could be a potential source of anti-microbial compounds. Their identification is necessary to develop cheap alternatives from commonly available plants.

Plants are an important source of natural chemicals and are important in controlling agricultural pests. The antifungal activity of plant extracts using different solvent systems has been extensively reported (Grayer and Harborne, 1994). However, few studies have used water as a solvent system (Qasem and Abu-Balan, 1995) to determine the efficacy of plant extracts against fungal growth.

The use of chemical fungicides to control disease and maintain agricultural productivity is necessary in the context of modern agriculture. With the development of resistance to chemical fungicides and the demand for safer products, alternatives such as plant extracts offer a viable choice which are non-persistent in the environment and safer to use.

We selected weed species based on their extensive occurrence and the absence of visible fungal infestations on their shoots. With these criteria, five plant species commonly found in the wet zone of Sri Lanka were selected. These species also occur in the tropical regions of India, Thailand, Philippines, China and also in Africa and N. America (Dassanayake, 1980 and Jayaweera, 1980a).

The antifungal activity of aqueous extracts of these species has not been previously reported and our objective was to determine the ability of these species to inhibit mycelial growth of common plant pathogens *In vitro*.

Material and methods

The five species in this study *Ageratum conyzoides, Oxalis corniculata, Phyllanthus debilis, Vernonia cinerea* and *Desmodium trifolium* were identified by comparing with herbarium specimens in the National Herbarium, Royal Botanical gardens Peradeniya, Sri Lanka. Plants were collected in the Kandy district during July – September 1999. Fresh shoots were washed in running tap water followed by distilled water. Each sample of 300 g fresh shoots was homogenized in a Warding blender with one liter distilled water for 5 min and allowed to stand. The mixture was filtered through muslin cloth and filter paper to remove the debris. The extract was sterilized by autoclaving at 121°C and 15 psi for 20 min. Sterile filtration was done aseptically by filtering the extract through a Sartorius Minisart, 0.2-micron membrane filter.

The fungi *Aspergillus niger, Rhizoctonia solani, Botryodiploidia theobromae* and *Pestalotiopsis theae* were obtained from the department of Agricultural Biology, University of Peradeniya and Department of Agriculture, Peradeniya. Cultures were maintained by regular sub-culture on potatoes dextrose agar (PDA) medium. A 10 mm disc was taken using a sterile cork-borer from the edge of actively growing colonies and placed in the center of an 85 mm petri dish containing 20 ml of PDA medium and 3 ml of the sterile plant extract. The control petri dishes contained 3 ml of sterile distilled water. Petri dishes were incubated in the dark at 27° C and colony diameter measured. Inhibition of mycelia growth was determined by the following formula:

$$\% \text{ Mycelia inhibition} = \frac{\text{Control (area)} - \text{Treatment (area)}}{\text{Control (area)}} \times 100$$

The area of the inoculum was deducted from the control and treatment areas.

Results

The aqueous extracts from the five plant species were assayed for their ability to suppress the growth of fungal plant pathogens *In vitro*.

The ability of the aqueous extracts to suppress the fungi varied between the weed species and over the incubation period. The weeds *A. conyzoides, O. corniculata* and *P. debilis* suppressed the fungal mycelial growth by 71 to 86% after three days incubation (Table 1). *A. conyzoides* successfully controlled the growth of *R. solani, A. niger* and *P. theae* by at least 70% after three days of incubation (Fig. 1).

The suppression of mycelia growth decreased with the number of days of incubation. The extract of *A. conyzoides* was active up to 9 days and it was particularly effective against *R. solani* (Table 1).

The extract from *A. conyzoides* was also sterile filtered and incorporated in the PDA medium to determine the heat stability of the fungal inhibitory factor. There was no significant difference between the sterile filtered and autoclaved extracts (Fig. 2).

The extract of *O. corniculata* was effective against *A. niger*. It was moderately active against *R. solani* and *P. theae* three days after incubation. The activity rapidly declined by nine days. The extract of *P. debilis* inhibited the growth of *P. theae* by 72% after three days of incubation, which declined thereafter. A similar effect was produced by *A. conyzoides*. *P. debilis* was also moderately active against *R. solani* and *A. niger*, 3 days after incubation.

The extracts of *V. cinerea* and *D. triflorum* suppressed the

Table 1: Inhibition of mycelial growth (%) by the water extracts of five weed species on some plant pathogenic fungi

Days after incubation	*R. solani*			*B. theobromae*			*A. niger*			*P. theae*		
	3	6	9	3	6	9	3	6	9	3	6	9
Species												
A. conyzoides	85.4	71	42	12.7ab	11.2	0	73.0bc	52.5	22.3	71.3b	41.5	12.3
V. cinerea	33.3	0	0	20.3b	8.0	0	23.9a	18.6	0.0	29.6a	17.0	0.0
D. triflorum	29.5	11	0	18.0ab	0.0	0	20.2a	12.0	0.0	31.0a	14.0	0.0
O. corniculata	46.7	21	0	29.9b	13.6	0	76.5c	62.4	31.0	35.4a	18.2	10.7
P. debilis	57.6	19	0	21.6b	8.4	0	39.3ab	27.7	0.0	72.2b	41.0	16.8
Water	0	0	0	0	0.0	0	0.0	0.0	0.0	0.0	0.0	0.0

Values followed by similar letters down the columns are not significantly different (P = 0.05)

Fig. 1: Mycelia inhibition of four fungi by aqueous water extract of *Ageratum conyzoides* after three days of incubation. Vertical bars represent ±SE.

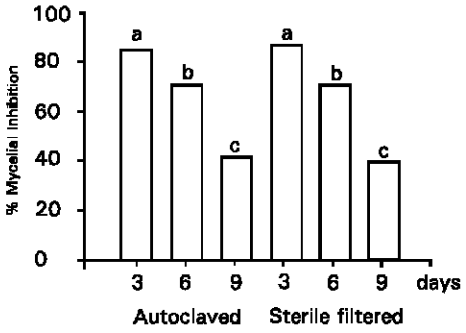

Fig. 2: Mycelia inhibition by autoclaved and filter sterilized extracts of *Ageratum conyzoids* on *Rhizoctonia solani*. Bars with similar letters are not significantly different (P=0.05)

mycelial growth by 20-30% only which rapidly declined. Whereas of the four fungi tested three were inhibited by one or more of the five plant species, *B. theobromae* was particularly resistant to all the extracts.

Discussion

Antifungal compounds are broadly classified by Kuhn and Hargreaves (1987) into active and constitutive compounds. Active compounds are only expressed during a fungal infection, while constitutive compounds are already present in healthy plants prior to infection. In this experiment since only healthy plants were chosen for extraction, the antifungal activity observed can be regarded as constitutive.

Of the five weed species, *A. conyzoides* was the most effective against the *R. solani*, *A. niger* and *P. theae*. It inhibited the growth of mycelium by at least 70%. Almost all members of the family Asteraceae (to which the genus *Ageratum* belongs) are known to contain sesquiterpene lactones, which are cytotoxic while *A. conyzoides* contains hydrocyanic acid, coumarin and an alkaloid (Lewis and Elvin-Lewis, 1977). The latter compounds also probably inhibit grazing of the plant by herbivores. The active compounds in *A. conyzoides* are heat stable (at 121°C, 15 psi) as there was no significant difference from the filter sterilized samples of the same aqueous extract.

The extract from other species were active against specific fungi. Although *A. conyzoides* was active against *R. solani*, *A. niger* and *P. theae*, it hardly inhibited the growth of *B. theobromae*. In fact none of the extracts were able to suppress this fungus. *O. corniculata* was effective against *A. niger* and moderately against *R. solani* and *P. theae*. The extract of *P. debilis* suppressed the growth of *P. theae* but was moderately active against *R. solani*. The different degrees of activity by the extracts and the specificity of the extract to the fungus precludes the possibility that the inhibition of fungal growth is due to alterations in the culture medium environment as may be possible through changes in the pH, osmotic pressure etc. as suggested by Kuhn and Hargreaves (1987). In a similar study, aqueous extracts from the weeds *Ranunculus asiaticus*, *Chenopodium murale*, *Inula viscosa* and *Solanum nigrum* inhibited the fungi *Penicillium digitatum*, *Sclerotinia sclerotiorum* and *Verticillium dahliae* (Qasem and Abu-Blan, 1995).

Present study was restricted to aqueous extracts from the mature shoots of weed species. Thus only the water-soluble compounds would be present in the extract. Plant tissues contain many products of secondary metabolism. It is possible that some of these metabolites suppress fungal growth. Their concentration in the plant tissues could vary with developmental stages and environmental conditions (e.g., rainy or arid climates). The synthesis of these secondary metabolites could also be a function of the genotype, which offers a potential for selection.

The active aqueous extracts suppressed fungal growth for three days after incubation and thereafter gradually declined. With *A. conyzoides* the antifungal activity was maintained up to 9 days. The decline and difference of activity could be due to breakdown of the active components or even metabolizing the fungi to non-toxic products. The latter is possible if the initial inoculum is in excess of the concentration of the antifungal compound or compounds. Qasem and Abu-Balan (1995) also reported a similar decline of the antifungal effect with incubation period of weed extracts. They suggest this may be due to transformation of these materials to non-toxic forms or the loss of volatile inhibitors. Chaturvedi *et al.* (1987) reported a similar decline in antifungal effect from loss of volatile inhibitors.

Similar studies with crude plant extracts were reported for controlling post harvest losses in fruits and seeds. In tomato, fruit rot caused by *A. niger* was reduced when the fruits were treated with leaf extract of *Argemone mexicana* (Saxena and Saxena, 1990) and the latex of *Euphorbia hirta* (Sinha and

Saxena, 1989). In fruit-rot of grapes caused by *Phomopsis* species satisfactory control was achieved with Eucalyptus and *Aegle marmelos* leaf extracts (Arun, 1988). Aqueous leaf extracts of *Cycas revoluta* and *Thuja orientalis* reduced the fungal incidence in stored rice seeds (Kumar, 1990).

The constitutive antifungal compounds present in healthy plant tissues belong to a broad range of structural classes such as phenols, isoflavones, terpenoids, alkaloids, triterpenoid saponins etc. (Harborne and Ingham, 1978; Mansfield, 1983; Schoenbeck and Schloesser, 1976).

An implication of this study is to investigate the effect on soil fungi by incorporating the weeds, especially *Ageratum*, in the soil. The degradation of the plant tissues can be expected to similarly inhibit fungal growth. Angus *et al.* (1994) studied the incorporation of *Brassica* plants in the soil. The breakdown of *Brassica* tissues in the soil and the subsequent build up of isothiocyanates controlled pathogenic fungi.

This study shows the existence of specific inhibition of fungi by chemical compounds present in aqueous extracts from certain weeds. It is necessary to increase the efficacy of extraction by polar and non-polar organic solvents and eventually characterize their chemical nature. It should enable the improvement of their stability by alteration of the compound and provide non-hazardous pesticides to control specific fungi.

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