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EFFECT OF AQUEOUS LEAF EXTRACTS OF ALLELOPATHIC TREES ON GERMINATION AND SEED-BORNE MYCOFLORA OF WHEAT

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Abstract

Effect of aqueous leaf extracts of 8 allelopathic tree species viz., *Accacia nilotica* (L.) Willd ex Del., *Alstonia scholaris* (L.) R. Br., *Azadirachta indica* (L.) A. Juss., *Eucalyptus citriodora* Hook, *Ficus bengalensis* L., *Mangifera indica* L., *Melia azedarach* L. and *Syzygium cumini* (L.) Skeels was studied on germination and seed-borne mycoflora of wheat (*Triticum aestivum* L.). Wheat seeds were soaked in 20% aqueous leaf extract of test allelopathic tree species for 10 and 20 minutes. Surface sterilization of seeds with 1% Sodium hypochlorite for 2 minutes was used as reference. Five fungal species were isolated from untreated seeds. *Alternaria alternata* (Fr.) Keissler was recorded with highest percentage (43%) followed by *Fusarium solani* (Mart.) Appel & Wr. (30%), *Cladosporium* sp. (17%) *Rhizopus arrhizus* A. Fischer (7%) and *Aspergillus niger* Van Tieghem (3.3%). Surface sterilization with $HgCl_2$ significantly reduced *A. alternata* (13%) and completely checked the growth of all other fungi. Aqueous extracts of all the test tree species significantly reduced the frequency of occurrence of the two most frequent seed-borne fungi viz., *A. alternata* and *F. solani*. Generally there was not any pronounced difference between effectiveness of 10 and 20 minutes treatments. Aqueous treatment for 10 minutes generally enhanced seed germination as compared to control.

Introduction

The extensive use of agrochemicals especially fungicides, which to pose more of carcinogenic risk than other pesticides (Anonymous, 1987) may give rise to undesirable biological effects on animals and human beings (Osman & Al-Rehiayani, 2003). Therefore, the development of biopesticides has been focused as a viable pest control strategy in recent years. One source of potential new pesticides is natural products produced by plants. Plant extracts and essential oils show antifungal activity against a wide range of fungi (Grane & Ahmad, 1988; Wilson *et al*., 1997; Abd-Alla *et al.*, 2001). Recently Alkhail (2005) showed that aqueous extracts of plants viz., *Allium sativum, Cymbopogon proxims, Carum carvi, Azadirachta indica* and *Eugenia caryophyllus* had strong antifungal activity against fungi viz., *Fusarium oxysporum, Botrytis cinerea* and *Rhizoctonia solani.* Similar effects of *Magnolia grandiflora* L., extracts against *Alternaria alternata, Helminthosporium* spp., *Fusarium oxysprum, F. culmorium* and *Rhizoctonia solani* have also been reported by Ahmad & Abdulgaleil (2005). In the present study the antifungal activity of aqueous leaf extracts of eight allelopathic trees against seed-borne mycoflora of wheat was investigated.

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Materials and Methods

Collection of plant materials: Fresh and healthy leaves of eight allelopathic tree species viz., *Acacia nilotica* (El-Khawas & Shehata, 2005), *Alstonia scholaris* (Javaid *et al.*, 2007), *Azadirachta indica* (Hussain *et al.*, 1985), *Eucalyptus citriodora* (Bajwa & Naz, 2005), *Ficus bengalensis*, *Mangifera indica* (Shafique *et al*., 2005a), *Melia azadarach* (Mushtaq *et al.*, 1993) and *Syzygium cumini* (Bajwa *et al*., 1999) were collected from University of the Punjab. Quaid-e-Azam Campus, Lahore, Pakistan. Leaves were washed thoroughly with detergent to remove any dust. Washed leaves were dried in an electric oven at 30°C for 72 hours and crushed to make powder.

Preparation of aqueous extract: Twenty grams of dried leaf powder of each of the 8 test tree species was soaked in 100 ml of sterilized distilled water for 24 hours. Extract was filtered through a double layered muslin cloth followed by Whatman No. 1 filter paper and used in the further experiment immediately.

Collection of wheat seeds: Stored wheat seeds of previous season were collected from 4 farmers and combined to be used in the experiment.

Aqueous extract bioassay: Wheat seeds were soaked in 20% (W/V) aqueous extracts of test tree species for 10 and 20 minutes. For control, the seeds were soaked in distilled water. For reference a treatment of surface sterilization with 1% Sodium hypochlorite (NaOCl) for 2 min., was also added. Seeds of all the treatments were placed on filter papers in 9 cm diameter Petri plates, moistened with 3ml. of distilled water each. Plates were incubated in a growth room at 25 ± 2°C for 10 days. Each treatment was replicated thrice.

After 10 days of incubation, germination percentage of wheat seeds was recorded. Fungal species found growing on the surface of seeds were identified and their percentage frequency of occurrence was calculated by applying the following formula:

$$\text{Frequency of occurrence (\%)} = \frac{\text{No. of seeds on which fungus appears}}{\text{Total No. of seeds}} \times 100$$

Statistical analysis: Data regarding germination and frequency of occurrence of various seed borne fungi were analyzed statistically by applying Duncan's Multiple Range (DMR) test (Steel & Torrie, 1980).

Results and Discussion

Effect of aqueous extracts on mycoflora: Five fungal species viz., *Alternaria alternata* (43%), *Fusarium solani* (30%), *Cladosporium* sp. (17%) *Rhizopus arrhizus* (7%) and *Aspergillus niger* (3.3%) were isolated from seeds of untreated control treatment. Surface sterilization with 1% Sodium hypochlorite completely arrested the growth of all fungal species except *A. alternata* possibly because of its internally seed-borne habit. However, $HgCl_2$ treatment significantly reduced the frequency of occurrence of this fungus by 70% (Table 1). *A. alternata* is a predominant fungus causing black point disorder of wheat seed (Ilyas *et al.*, 1998).

Aqueous extracts of all the eight allelopathic test species reduced the incidence of seed-borne mycoflora of wheat. However, the toxicity of the extract against a particular fungal species varied with the test allelopathic tree species. Toxicity is assumed to be associated with the presence of strong electrophilic and nucleophilic systems. Action by such systems on specific positions of proteins or enzymes would alter their configuration and affect their activity (Macias *et al.,* 1992). Recently Hasan *et al.,* (2005) reported that aqueous extracts of rhizome of *Zingiber officinale,* bulb of *Allium sativum, A. cepa,* leaves of *Adhatoda vestica, Lawsonia alba, Azadirachta indica, Achyranthes aspera,* stem of *Cuscuta reflexa,* root of *Vicia rosea* and seeds of *Nigella sativa* significantly reduced the incidence of seed-borne fungi of wheat viz., *Bipolaris sorokiniana, Fusarium* sp., *Aspergillus* sp., *Penicillium* sp., and *Rhizopus* sp.

A. indica (neem) extract exhibited maximum toxicity against the most frequently occurring species i.e. *A. alternata* in the present study, resulting in significant reduction of 77 and 60% in fungal incidence over control in 10 and 20 minute treatments, respectively. The incidence of other species was also markedly reduced by the extract (Table 1). Aqueous extract of *A. indica* has also been reported to cause significant growth inhibition of other fungi such as *Rhizoctonia solani, Botrytis cinera* and *Fusarium oxysporum* (Alkhail, 2005). It has also been shown to inhibit the germination of *Pestolatia psidii* (Pandey *et al.,* 2002), and suppressed the *Fusarium moniliforme* in sorghum (Rai *et al.,* 2002) and *Cercospora arachidicola* in groundnut (Aage *et al.,* 2003). The bioactivity of neem extracts has been attributed to various compounds found in seeds and leaves such as nimbin, nimbidin, salannin, but the most important of these compounds is azadirachtin (Lale & Abdulrahman, 1999).

M. indica extract was found to be the second most toxic agent against *A. alternata* resulting in up to 60% suppression in fungal incidence. Incidence of other four fungal species was also markedly declined by the extract treatments (Table 1). Similar antifungal properties of ethanolic leaf extracts of *M. indica* against *Aspergillus niger, Alternaria alternata, Fusarium chlamydosporum, Rhizoctonia bataticola* and *Trichoderma viride* have recently been reported by Aqil & Ahmad (2003).

F. bengalensis extract proved highly toxic against the seed-borne mycoflora of wheat. Incidence of *A. alternata* was reduced by 37 and 47% in 10 and 20 minutes treatments, respectively. Growth of all other fungi was completely arrested (Table 1). In contrast to that, Aqil & Ahmad (2003) have reported a limited antifungal activity of ethanolic extracts of *F. bengalensis* against *A. niger, A. alternata, F. chlamydosporum, R. bataticola* and *T. viride.*

A. nilotica extract exhibited the least antifungal activity against *A. alternata* resulting in an insignificant reduction of 7 and 37% in frequency of the fungus in 10 and 20 minutes treatments, respectively. However, the growth of other fungi was markedly suppressed by the extract (Table 1). A 20 minutes treatment with extract of *S. cumini* reduced *A. alternata* frequency by 70%. However, this treatment can not be recommended as it also reduced the germination by 24% (Table 1 & Fig. 1). In the present study, aqueous extract of *A. scholaris* significantly reduced the incidence of *F. solani* by 89 & 77%, and also declined the incidence of *A. alternata* by 30 & 37% in 10 and 20 minutes treatments, respectively. However, by contrast, methnolic extracts of leaves of this tree species are reported to be ineffective against all the test fungi (Khan *et al.,* 2003).

Table1. Effect of surface sterilization with NaOCl and aqueous extracts of eight allelopathic trees on seed-borne mycoflora of wheat.

Treatments	*Alternaria alternata*	*Aspergillus niger*	*Fusarium solani*	*Rhizopus arrhizus*	*Cladosporium* sp.
Control	43 a	3.3 a	30 a	7.0 ab	17 a
NaOCl	13 cd	0.0 a	0.0 b	0.0 b	0.0 b
Acacia (10 min.)	40 ab	3.3 a	3.3 b	7.0 ab	3.3 b
Acacia (20 min.)	27 a-d	0.0 a	10 b	10 ab	3.3 b
Alstonia (10 min.)	30 a-d	3.3 a	3.3 b	7.0 ab	7 ab
Alstonia (20 min.)	27 a-d	0.0 a	7.0 b	13 ab	10 ab
Azadirachta (10 min.)	10 d	0.0 a	0.0 b	7.0 ab	0.0 b
Azadirachta (20 min.)	17 cd	0.0 a	3.3 b	7.0 ab	7.0 ab
Eucalyptus (10 min.)	27 a-d	0.0 a	13 b	0.0 b	7.0 ab
Eucalyptus (20 min.)	30 a-d	3.3 a	7.0 b	0.0 b	7.0 ab
Ficus (10 min.)	27a-d	0.0 a	0.0 b	0.0 b	0.0 b
Ficus (20 min.)	23 a-d	0.0 a	3.3 b	0.0 b	0.0 b
Mangifera (10 min.)	17 cd	3.3 a	3.3 b	0.0 b	7.0 ab
Mangifera (20 min.)	20 b-d	0.0 a	7.0 b	20 a	0.0 b
Melia (10 min.)	27 a-d	3.3 a	7.0 b	0.0 b	3.3 b
Melia (20 min.)	20 b-d	0.0 a	3.3 b	10 ab	0.0 b
Syzygium (10 min.)	33 a-c	0.0 a	0.0 b	0.0 b	0.0 b
Syzygium (20 min.)	13 d	0.0 a	10 b	13 ab	3.3 b

Values with different letters in a column show significant difference as determined by Duncan's Multiple Range Test.

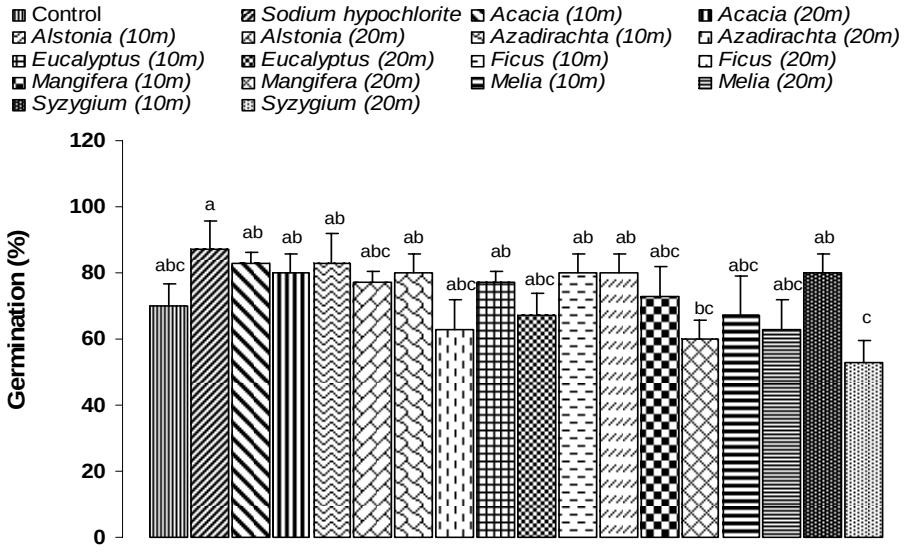

Fig. 1. Effect of surface sterilization with Mercuric chloride and aqueous extracts of allelopathic plants (for 10 and 20 minutes) on germination of wheat. Vertical bars show standard errors of means of three replicates. Values with different letters show significant difference as determined by Duncan's Multiple Range Test.

Aqueous extracts of other test allelopathic species viz., *M. azedarach*, and *E. citriodora* reduced the incidence of *A. alternata* from 37 to 53% and exhibited variable effects against other associated fungal species. Recently Shafique *et al.*, (2005b) have reported significant inhibition of seed-borne fungi of maize viz., *Aspergillus fumigatus*, *A. niger*, *R. arrhizus* and *Penicillium* sp. by aqueous extracts of *M. azedarach.* Earlier Daoud *et al.*, (1990) have reported good antifungal activity of *M. azedarach* against *Alternaria, Aspergillus* and *Penicillium* spp., Similarly Iqbal *et al.*, (2002) have reported that extract of *M. azedarach* was effective against *Fusarium chlamydosporum, A. niger* and *Hyloflora ramosa*. Carpinella *et al*., (1999) found that extract of *M. azedarach* ripe fruits showed fungicidal properties against *Aspergillus flavus, Fusarium moniliforme, Microsporum canis* and *Candida albicans*. The antifungal activity of *M. azedarach* is because of presence of fungicidal agents such as hydroxycoumarin scopoletin, vanillin, 4-hydroxy-3-methoxycinnamaldehyde, and (±) pinoresinol (Carpinella *et al*., 2003, 2005). Generally there was not any pronounced difference in fungitoxic activity of extracts between 10 and 20 min., treatments (Table 1).

Effect of aqueous extracts on germination: Sodium hypochlorite as well as extracts of *A. nilotica, A. scholaris, A. indica, F. bengalensis, S. cumini* enhanced the seed germination from 10–24%. Increase in seed germination by aqueous extracts of plants viz., *Zingiber officinale, Allium sativum, A. cepa, Adhatoda vestica, Lawsonia alba, Azadirachta indica, Achyranthes aspera, Cuscuta reflexa, Vicia rosea* and *Nigella sativa* has also been reported by Hasan *et al*., (2005). By contrast, 20 min. treatment of *S. cumini* extracts reduced the germination by 24%. However, effect of all the treatments on germination was statistically insignificant (Fig. 1).

The present study suggests that aqueous extracts of allelopathic trees especially those of *A. indica* and *M. indica* can be used to treat the wheat grains for 10 minutes before sowing or storage to reduce the fungal incidence.

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