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Antifungal Potential of Plant Extracts against Seed-borne Fungi Isolated from Barley Seeds (*Hordeum vulgare* L.)

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Abstract

A laboratory experiment was conducted to study the efficacy of some botanicals against seed-borne fungi isolated from barley. *Alternaria alternata* was the most frequently isolated fungi followed by *Rhizopus* spp., and *Mucor* spp., determined by plating the seeds on both standard blotter and agar plate method. Leaf extracts of five plants viz., *Eucalyptus globulus*, *Calotropis procera, Melia azedarach, Datura stramonium* and *Acalypha indica* @ 5%, 10% and 20% concentration were evaluated against *A. alternata*. The results revealed that all the plant extracts significantly inhibited the mycelial growth of *A. alternata*. Effect of these five plant extracts varied with the concentrations. Leaf extract of *E. globulus* at 20% concentration caused highest inhibition of mycelial growth of *A. alternata* (52.6%) followed by *C. procera* (50.88%), *M. azedarach* (48.21%) and *D. stramonium* (47.42%), whereas the lowest inhibition (37.52) of mycelial growth was recorded at 5% leaf extract concentration in case of *A. indica* as compared to control. However, seed treatment at 20% concentration of all the tested plant extracts was also found to be effective in eliminating majority of fungi and reducing the relative frequency of seed-borne fungi occurring on the seeds and also results in percent germination increase in both standard blotter and agar plate method over control.

Keywords: Seed-borne fungi; Antifungal activity; *A. alternate;* Plant extracts; Seed treatment

Introduction

Barley is an important rabi season crop of India and plays a major role in barley production all over the world. Ranking of barley is next to the maize, wheat and rice both in acreage and in production of grain. It is very nutritious and a rich source of Vitamin 'B' complex and Protein. The cultivated crops are infected by one or more fungal pathogens causing economic losses. The majority of the diseases are caused by seed-borne fungi. These seed-borne pathogens are resulting into losses or death of crop plants. The damage caused by plant parasitic pathogens and seed-borne fungi considered as worldwide and has extensive host range. Due to this they cause potentially serious constrains to crop productivity.

The most important unit of the crop is the seed; which should be of high quality and pathogen free. Healthy seeds free from pathogens are used for sowing to achieve desired germination, emergence, healthy seedlings and plant population [1]. Seed-borne fungi result in heavy losses in crop yield and seed quality. The most abundant seed-borne fungi, *Alternaria* spp. is ubiquitous and includes both plant pathogenic and saprophytic species that may damage crops in the field and cause post-harvest decay. Certain grains are frequently reported to be infected by several species of *Alternaria*, in particular *A. alternata* Keisler, cause a disease called black point consisting of a discoloration of the germ and of the seed which is due to its mycelium and spore mass [2]. The disease can be a major problem in barley in those areas where heavy rainfall occurs during the early stages of kernel development [3]. *A. alternata* the most frequently isolated fungi were determined by plating the seeds on agar medium or on moistened filter paper [4]. The presence of fungal mycelium in different parts of black spotted kernels has been determined by microscopical observations [5]. Biological control of plant pathogens is preferred in comparison with the hazardous chemical based products. More use of fungicides like organo-mercurials, carbamates etc. have posed serious health problems to human and environment so there is need to search of natural biodegradable source of bio-fungicides have always been quest for the researchers for control of fungal diseases of plants. Phyto-metabolites and plant based pesticides appear to be one of the better alternatives for the control fungal diseases, as they are known to have minimal environmental problems and less danger to consumers in contrast to synthetic pesticides [6]. Exploitation of phyto-metabolites in crop protection and prevention of biodeterioration grains caused by fungi appear to be promising. Research on a more sustainable agriculture system and eco-friendly is the need of the hour, as there is a growing concern on the deteriorating quality of the environment as a result of intensive agriculture. So with this view, the present study was undertaken to select plants that could be effective in the development of new mechanism for the control of diseases caused by fungi to the plants of economic importance.

Materials and Methods

Collection of seed sample

Barley (*Hordeum vulgare* L.) variety Narendra barley-2 was collected from the quarsi agricultural farm of Aligarh district of (UP) India. The sample was collected in gunny bag and stored at room temperature 28°C till the processing. The sample was examined for the seed-borne mycoflora according to the international rules for seed testing (ISTA).

Standard blotter and agar plate methods are usually followed where seeds are incubated for a definite period under specific conditions. The associated fungi are identified based on their morphological and habit characters on seed surface and colony characters on the medium.

Standard blotter method

Usually glass transparent petri-plates of 9 cm diameter are used

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in the test. Three blotters of the size of the petri-plate are dipped in sterilized water and placed in the petri-plate after dropping off extra water. Untreated 10 seeds are plated at equal distance in each petri-plate; three hundred seeds of barley sample were examined. The plates are incubated for alternate periods of 12 hours light and 12 hours darkness at 28 ± 2°C. The plates are removed on the eighth day. After incubation, the fungi developed on seeds are examined under different magnification of a stereomicroscope and identified.

Agar-plate method

The agar plate method is another popular method for the detection of seed-borne mycoflora, in which seeds are plated on an agar medium and sterilized petri-plates of 9 cm diameter containing potato dextrose agar media were used. In each petri-plate 10 seeds were placed. Three hundred seeds of barley were examined. The plated seeds are usually incubated for 8 days at 28 ± 2°C under 12th alternating cycles of light and darkness. At the end of the incubation period, fungi growing out from the seeds on the medium are examined and identified. Identification is based on colony characters and morphology of sporulating structures under a compound microscope.

Preparation of aqueous plant extracts

For the study, fresh leaves were collected, detached and surface sterilized with 1% mercuric chloride and washed first in tap water then in distilled water and blotter dried. 100 g of fresh sample was chopped and then crushed in a surface sterilized mortar and pestle by adding 100 ml distilled water (1:1 w/v). The extract was filtered through two layers of muslin cloth and was used as stock solution in the above experiment.

Antifungal activity of botanicals on the growth of fungi by poison food method

Leaf extracts of five plants viz., *Eucalyptus globulus, Calotropis procera*, *Melia azedarach*, *Datura stramonium, Acalypha indica* were evaluated against mycelial growth of *Alternaria alternata.* These plant extracts were used at 5, 10 and 20% concentrations for which 5, 10 and 20 ml of stock solution was mixed with 95, 90 and 80 ml of sterilized molten PDA media. The medium was thoroughly shaken for uniform mixing of leaf extract. 20 ml of agar media was poured into sterile petri-plates and allowed to solidify. Five mm of agar disk of test fungi were cut from 8-10 days old culture plate by using sterile cork borer and placed in the centre of petri-plate containing different concentration of plant extract. There were three replicates of each treatment. The petri-plates without plant extracts serve as control. The inoculated plates were incubated at 28°C for seven days. The percentage inhibition of mycelial growth was calculated as per formula given by Vincent.

$$1 = \frac{C\text{-}T}{C}$$

Where

I= Percent Inhibition; C= Growth of pathogen in control and T= Growth of pathogen in treatment.

Seed treatment with plant extracts

For the seed treatment with five plant extracts, three hundred moderately infected barley seeds were treated with each of the plant extracts involving soaking the seeds in the 20% concentration (as it was found to be most effective in poison food technique) for 12 hours. Treated seeds were dried on blotter sheets overnight under room temperature and then placed on the standard blotter and on agar-plate method and incubated for 8 days under 12th alternating cycles of light and darkness at 28 ± 2°C. After incubation seeds were examined for fungal development. Seeds soaked in distilled water served as control. Relative frequencies of seed-borne fungi and percent germination of seeds were calculated.

The Relative frequency of the fungus was calculated by the following formula:

$$\frac{\text{No. of seeds containing a particular fungus}}{\text{Total seeds used}} \times 100$$

$$\%\ \text{germination} = \frac{\text{No. of seeds germinated}}{\text{Total number of seeds}} \times 100$$

Data analysis

Data was analyzed by one-way analysis of variance (ANOVA) and LSD was calculated at p=0.05 for significance. The analysis was performed with the software R (R Development Core Team 2011).

Results

Isolation of seed-borne fungi

From blotter method 12 seed-borne fungi such as *Alternaria alternata* (65.05%), *Rhizopus* spp. (52.12%) *Mucor* spp. (48.5%) *Fusarium moniliforme* (41.4%) *Aspergillus flavus* (35.2%) *Aspergillus niger* (32.6%) *Penicillium* spp. (28.05%) *Drechslera australiensis* (19.5%) *Curvularia lunata* (16.1%) *Cladosporium* spp. (10.5%) *Stemphylium* spp. (4.8%) and *Ulocladium* spp. (2.3%) were isolated and identified. In agar plate method, 9 seed-borne fungi viz., *A. alternata* (40.5%) followed by *Rhizopus* spp. (35.2%) *Mucor* spp. (31.4%) *F. moniliforme* (28.4%) *A. flavus* (25.6%) *A. niger* (24.2%) *Penicillium* spp. (19.6%) *D. australiensis* (17.3%) *C. lunata* (12.8%) were isolated. *A. alternata* was the most frequently isolated fungi in both standard blotter and agar plate methods.

Antifungal assay

The results presented in Table 1 revealed that leaf extracts of all five plants significantly inhibited mycelial growth of *A. alternata* at all the tested concentrations. Leaf extract of *E. globulus* at 20% concentration caused highest inhibition of mycelial growth of *A. alternata* (52.6%) followed by *C. procera* (50.88%), *M. azedarach* (48.21%) and *D. stramonium* (47.42%), whereas the lowest inhibition (37.52%) of mycelial growth was recorded at 5% leaf extract of *A. indica* as compared to control.

Seed treatment

All the tested plant extracts were applied as seed treatment at 20% concentration in both agar plate and standard blotter method (Table 2). The results indicated that *E. globulus* leaf extract was found most significant against seed-borne mycoflora followed by *C. Procera, M. azedarach, D. stramonium* and *A. indica.* In case of seeds treated with the leaf extract of *E. globulus*, highest frequency was observed in *A. alternata* (26.5%) followed by *A. flavus* (18.3%), *F. moniliforme* (13.6%), *A. niger* (11%), *Penicillium* spp., (9.2%) and *D. australiensis* (4.1%) on standard blotter method as compared to untreated control. On agar plate method the highest frequency was again observed in *A. alternata* (24.3%) followed by *A. flavus* (16.2%), *F. moniliforme* (12.5%), *A. niger* (10.6%), *Penicillium* spp., (8.3%) and *D. australiensis* (3.2%) as compared to control. The germination percentage of seeds treated with *E. globulus* leaf extract was 60% in standard blotter method and 64% in agar plate method. Similarly in case of seeds treated with leaf extract of *C. procera* highest frequency was observed in *A. alternata* (30.6%) followed by *A. flavus* (21.5%), *F. moniliforme* (15.3%), *A. niger*

Botanicals	Average colony diameter (mm)			% inhibition			Mean
	5%	10%	20 %	5%	10%	20%	
Eucalyptus globulus	44.08	42.75	39.81	47.52	49.1	52.6	49.74
Calotropis procera	45.12	43.66	41.26	46.28	48.02	50.88	48.39
Melia azedarach	47.05	45.38	43.5	43.98	46.04	48.21	46.07
Datura stramonium	49.23	46.81	44.16	41.39	45.97	47.42	44.92
Acalypha indica	52.48	51.12	49.5	37.52	39.14	41.07	39.24
Control	84	84	84	0	0	0	0
LSD at 0.05	**3.44**	**3.38**	**3.27**	**-**	**-**	**-**	**-**

Table 1: *In vitro* evaluation of plant extracts against *Alternaria alternata* by Poison food technique.

Isolated Fungi	Control		*E. globulus* 20% leaf extract		*C. procera* 20% leaf extract		*M. azedarach* 20% leaf extract		*D.stramonium* 20%leaf extract		*A. indica* 20% leaf extract	
	SBM	APM	SBM	APM	SBM	APM	SBM	APM	SBM	APM	SBM	APM
	RF	RF	RF	RF	RF	RF	RF	RF	RF	RF	RF	RF
Alternaria alternata	65.05	40.5	26.5	24.3	30.6	28.5	34.2	32.6	37.5	35.6	41.5	39.5
Rhizopus spp.	52.12	24.2	0	0	0	0	4.5	3.5	6.2	5.3	9.1	8.3
Mucor spp.	48.5	19.6	0	0	0	0	3.1	2.3	5.4	4.5	7.6	6.2
Fusarium moniliforme	41.4	31.4	13.6	12.5	15.3	13.6	18.6	16.4	21.3	19.6	25.2	23.6
Aspergillus flavus	35.2	17.3	18.3	16.2	21.5	19.3	24.2	22.5	28.6	26.5	32	30.5
Aspergillus niger	32.6	35.2	11	10.6	14.2	12.4	15	13.6	19.4	18.2	24.5	22.4
Penicillium spp.	28.05	12.8	9.2	8.3	10.4	9.2	12.5	11.3	15.5	14.6	18.4	16.5
Drechslera australiensis	19.5	28.4	4.1	3.2	5.3	4.6	7.4	6.5	9.6	8.1	12.6	11.3
Curvularia lunata	16.1	25.6	0	0	0	0	2.3	1.3	5.1	4.2	9.3	6.5
Cladosporium spp.	10.5	0	0	0	0	0	0	0	0	0	0	0
Stemphylium spp.	4.8	0	0	0	0	0	0	0	0	0	0	0
Ulocladium spp.	2.3	0	0	0	0	0	0	0	0	0	0	0
LSD at 0.05	**2.07**	**1.39**	**0.63**	**0.59**	**0.75**	**0.68**	**0.86**	**0.77**	**0.99**	**0.92**	**1.17**	**1.08**
% germination	42	46	60	64	56	60	52	56	50	54	46	50

SBM = Standard Blotter Method, APM = Agar Plate Method, RF = Relative Frequency

Table 2: Effect of leaf extracts as seed treatment against seed-borne mycoflora of barley.

(14.2%), *Penicillium* spp., (10.4%) and *D. australiensis* (5.3%) while in agar plate method highest frequency was observed in *A. alternata* (28.5%) followed by *A. flavus* (19.3%), *F. moniliforme* (13.6%), *A. niger* (12.4%), *Penicillium* spp., (9.2%) and *D. australiensis* (4.6%) as compared to control. The germination percentage of seeds treated with *C. procera* leaf extract was 56% in blotter method and 60% in agar plate method. These leaf extracts eliminate majority of fungi such as *Rhizopus* spp., *Mucor* spp., *C. lunata, Cladosporium* spp., *Stemphylium* spp., and *Ulocladium* spp. The germination of seed has also been shown improvement as compared to control.

However, in case of *M. azedarach, D. stramonium* and *A. indica* leaf extracts was also found to be effective to eliminate seed-borne fungi such as *Cladosporium* spp., *Stemphylium* spp, and *Ulocladium* spp. In this treatment highest frequency was observed in *A. alternata* (34.2%) followed by *A. flavus* (24.2%), *F. moniliforme* (18.6%), *A. niger* (15%), *Penicillium* spp., (12.5%), *D. australiensis* (7.4%), *Rhizopus* spp. (4.5%) *Mucor* spp. (3.1%) and *C. lunata* (2.3%), while in agar plate method highest frequency was observed in *A. alternata* (32.6%) followed by *A. flavus* (22.5%), *F. moniliforme* (16.4%), *A. niger* (13.6%), *Penicillium* spp., (11.3%), *D. australiensis* (6.5%), *Rhizopus* spp. (3.5%) *Mucor* spp. (2.3%) and *C. lunata* (1.3%) in comparison to control. The germination percentage of seeds treated with *M. azedarach* leaf extract was 52% in standard blotter method and 56% in agar plate method. Similarly in *D. stramonium* leaf extracts highest frequency was observed in *A. alternata* (37.5%) followed by *A. flavus* (28.6%), *F. moniliforme* (21.3%), *A. niger* (19.4%), *Penicillium* spp., (15.5%), *D. australiensis* (9.6%), *Rhizopus* spp. (6.2%) *Mucor* spp. (5.4%) and *C. lunata* (5.1%), while in agar plate method highest frequency was observed in *A. alternata* (35.6%) followed by *A. flavus* (26.5%), *F. moniliforme* (19.6%), *A. niger* (18.2%), *Penicillium* spp., (14.6%), *D. australiensis* (8.1%), *Rhizopus* spp. (5.3%), *Mucor* spp. (4.5%) and *C. lunata* (4.2%) as compared to control. The germination percentage of seeds treated with *D. stramonium* leaf extract was 50% in standard blotter method and 54% in agar plate method. In *A. indica* leaf extracts highest frequency was observed in *A. alternata* (41.5%) followed by *A. flavus* (32%), *F. moniliforme* (25.2%), *A. niger* (24.5%), *Penicillium* spp., (18.4%), *D. australiensis* (12.6%), *C. lunata* (9.3%), *Rhizopus* spp. (9.1%) and *Mucor* spp. (7.6%), while in agar plate method highest frequency was observed in. *A. alternata* (39.5%) followed by *A. flavus* (30.5%), *F. moniliforme* (23.6%), *A. niger* (22.4%), *Penicillium* spp., (16.5%), *D. australiensis* (11.3%), *Rhizopus* spp. (8.3%), *C. lunata* (6.5%) and *Mucor* spp. (6.2%) as compared to untreated control. The germination percentage of seeds treated with *A. indica* leaf extract was 46% in standard blotter method and 50% in agar plate method.

Discussion

Seed is the most important unit of crop production and its health plays important role in agriculture, which determines the plant population and final yield. One of the major constraints that deteriorate the seed quality is the seed-borne fungi present inside or on the surface of seeds [7]. Leaf extracts of many higher plants have been reported to possess antifungal activity under laboratory trails [8]. In the present investigation antifungal activity of five plants extracts viz., *E. globulus*, *C. procera*, *M. azedarach*, *D. stramonium* and *A. indica* was assesed

at the rate of 5%, 10% and 20% concentration against the mycelial growth and sporulation of *A. alternata* by poison food technique. Similar investigation on antifungal activity of plant extracts against seed-borne mycoflora was reported by many workers [9-11]. Among these *E. globulus* leaf extract was found more effective in inhibition of mycelial growth against *A. alternata* than other plants. Antifungal activity of *E. globulus* leaf extract at different concentrations against seed-borne fungi was also checked and found to be effective [12,13] because of essential oil a mixture of terpenoids, aromatic phenols and many other compounds such as 1, 8-Cineole proved to have pesticidal properties [14].

Further seed treatment was done at 20% concentration as found effective in poison food technique. Seed treatment is the safest and the cheapest way to control the seed-borne fungal diseases and is used to prevent biodeterioration of grains [15]. Seed treatment with *E. globulus* leaf extract was found most effective in reduction of seed-borne incidence and improvement of germination in both standard blotter and agar plate methods. It is known that plants synthesize a variety of bioactive compounds in plant tissues like alkaloids, flavonoids, tannins, terpenoids, saponins, and other compounds, reported to have *in vitro* antifungal properties [16]. These antifungal compounds stop or inhibit the development of mycelia growth, inhibition of germination or reduce sporulation of fungal pathogens, it is considered that these compounds obtained from plants are biodegradable and safe for use as a substitute for disease control in a traditional production system [17,18]. These are also evidences from the earlier workers, that plants possess the antifungal activity that can play a pivotal role in the management of the plant disease which are cheap, locally available, and biodegradable and environment friendly.

Conclusion

From both the standard blotter method and agar plate method, *A. alternata* was found most frequently isolated fungi. Among the various plant extracts tested in poison food technique, *E. globulus* leaf extract at 20% concentration was found most effective in inhibition of mycelial growth of *A. alternata*.. Seed treatment with all the plant extracts at 20% concentration which were found effective in poison food technique was also found to be effective in both standard blotter methods and agar plate methods. Among all the tested plants *E. globulus* leaf extract at 20% concentration was found more effective in reducing seed-borne incidence and improved germination in both methods. Further studies should be taken to find the exact mechanism of action by which extracts exert their antifungal effect and to find the active compounds responsible for plant biological activity.

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