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Effect of aqueous extract of some plants on some stored and field fungi

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Abstract: Aqueous extract of leaf and seed of *Azadirachta indica, Calotropis procera, Croton spasriflorous, Luffa cylindrica, Putranjiva roxburghii, Salvadora persica, Senna alata, , Trema orientalis*, and *Trichosanthes dioica* were used to treat brinjal seeds to control seed borne fungi. The result revealed that, among these botanical extract, leaf extract of *Azadirachta indica* and leaf extract of *Putranjiva roxburghii* recorded only 4% seed infection followed by leaf extract of *Salvadora persica* and *Calotropis procera* (5.33%), leaf extract of *Trema orientalis* (8%), fruit extract of *Luffa cylindrica* (8%), leaf extract of *Senna alata* (8%), leaf extract of *Croton spasriflorous* and fruit extract of *Putranjiva roxburghii* (9.33%); while control treatment showed 66% seed infection . These crude plant extracts have good effect on germination of seeds. The extracts are successfully checked the mycelial growth (above 95%) of all the test fungi.
Key words: Botanical extract, Antifungal potentiality, seed borne diseases.

Introduction

Different fungi are significant destroyers of seeds during storage and crops at seedling stage. They are also remain dormant in seed and transmit to seedlings and mature plant and showing different symptoms (Zeringue and Bhatnager, 1990). Fungi caused germination failure and seedling death (Goldblatt, 1971). So the growers need to pay more attention to the good health of seeds and the seedlingsby using chemical pesticides which causing health hazard also.The main toxic effects are carcinogenicity, genotoxicity, terrotogenicity, hepetotoxicity, reproductive disorder and immuno suppression (Lacey,1988). The poor people of third world countries like Bangladesh and many countries of Africa are the victim of this mycotoxin produced by fungus, poisonous heavy metals and chemicals due to indiscriminate use of pesticides. It imbalances our ecology, interferes our food chain, causes many abnormalities to the environment So it is time to search better alternatives of fungicides & pesticides. Plant extracts of many higher plants have been reported to exhibit antibacterial, antifungal and insecticidal properties under laboratories trials (Bhatnagar *et al. 1990;* Mohana and Raveesha, 200*6*). Plant metabolities and plant based pesticides appear to be one of the better alternatives as they known to have minimal environmental impact and danger to consumers in contrast to the synthetic pesticides (Verma and Dubey, 1999). Therefore the present study was designed to screen *in vitro* a number of plants for antifungal potential against important seed borne fungal species like *Phomopsis vexans,Fusarium oxysporum, Aspergillus flavus, ,Curvularia lunata* with the ultimate aim of developing plant based formulations for plant disease management, the quality seeds for sowing and storage grains.

Materials and Methods

Plant material: Fresh diseased free leaves and fruits of Neem (*Azadirachta indica*), Akanda (*Calotropis procera*), Bonmirca (*Croton spasriflorous*), Dhundal (*Luffa cylindrica*), Paten java (*Putranjiva roxburghii*), Meswak (*Salvadora persica*) Dadmordan (*Senna alata*), Givon (*Trema orientalis*), Patol (*Trichosanthes dioicahae*) species were collected from Kaligonj thana under Jhenidah district of Bangladesh and Botanical Garden of Bangladesh Agricultural University, Mymensingh, Bangladesh .

Preparation of extract

Aqueous extract: Green leaf and fruit samples (100g) of selected plants were collected ground with conventional grounder called "HAMAN DISTA" (Mortar and pastels). Then the ground materials are dipped into 100 ml distilled water for 48 hours for complete extraction of the active ingredient from the extracted samples. After that, the water and ground materials were filtered with the help of a very fine and clean piece of cloth separately for every plant species. Then the crude extracts were preserved in glass bottle in a refrigerator at 5 ± 2 ºC for further use.

Seed treatment: Previously collected brinjal seeds were used for this study required amount of seeds are treated in the aqueous botanical extracts for 30 minutes. The concentration of the solution was 100% (v/v). For comparison Vitavax 200 was used to treat the seeds for 30 minutes with recommended dose (25% of seed wt.). Seeds are dipped only in distilled water for 30 minutes was considered as control.

After treating, seeds are placed in petridish. Before seed pletting petridishes were sterilized and 4 layers of blotting paper were soaked into distilled water and placed into the Petridishes. Each dish containing 25 seeds and 3 dishes were selected as replicates are for each extract. Then the petridishes were kept in the incubation chamber at 22 ± 2 ºC and data were recorded after 7, 10, 14 days after sowing (DAS).Then the total germination and percent seed infection of seeds are counted manually. But the seed infection by specific fungi are identified and counted by observing the petridishes under simple stereo binocular microscope.

Poisoned Food Technique: PDA medium with 25% concentration of the aqueous extracts of the test plants were prepared. About 20 ml of the medium was poured into each petridish and allowed to solidify. Five mm disc of 7-day-old culture of the test fungi were placed at the center of the petridish anal incubated at 25 ± 2 °C for seven days. After incubation the colony diameter was measured in millimeter. For each treatment four replicates were maintained. PDA medium without the aqueous extract served as control. The fungitoxicity on the extracts in terms of percentage inhibition of mycelia growth was calculated by using the formula: % inhibition =dc – dt/dc.100; Where, dc = average increase in mycelial growth in control, dt = Average increase

in mycelial growth in treatment (Singh and Tripathi, 1999).

Synthetic seed treating chemical i.e. Vitavax200, were also tested at their recommended dosage (2gm/L) for antifungal activity by poisoned food technique.

Results and Discussion

Effects of botanical extracts on percent germination of brinjal seeds: Among all the plant extract, brinjal seeds treated with leaf extract of *Calotropis procera*, *Senna alata*, *Trema orientalis*, *Croton spasriflorous*, seed extract of *Putranjiva roxburghii* and Vitavax 200 treated seeds gave 86.67%, 82.67%, 80.33%, 80.67%, 81.33%, 82.67%, 82.67% germination, respectively. The leaf extract of *Azadirachta indica*, *Putranjiva roxburghii*, *Salvadora persica* and *Luffa cylindrica* also gave higher germination compare to that or control (Table 1). This result indicates that the botanical extracts have a very good effect on germination. It also indicates that the botanical extracts helps to increase germination rather inhibit the germination of seeds.

Table 1. Effect of different botanical extracts on germination (%) and seed borne infection (%)

Treatments	Germination (%)	Seed nfection (%)	% Reduction of seed borne infection
T_1-(*Azadirachta indica*)	78.67cd	4.00f	93.3
T_2-(*Putranjiva roxburghii*)	74.67de	4.00f	93.3
T_3-(*Salvadora persica*)	77.33cd	5.33ef	91.9
T_4-(*Calotropic procera*)	86.67ab	5.33ef	91.9
T_5-(*Senna alata*)	82.67bc	8.0cd	87.8
T_6-(*Trema orientales*)	0.33bcd	8.00cd	87.8
T_7-(*Luffa cylidrica*)	70.67e	8.00cd	87.8
T_8- (*Croton spasriflorous*)	0.67bcd	9.33bc	85.8
T_9-(*Putranjiva roxburghii*)	81.33bc	9.33bc	85.8
T_{10}-(*Trichosanthes dioicahae*)	78.67cd	9.33bc	85.8
T_{11}-Vitavax 200	82.667bc	9.33bc	85.8
T_{12}-Control	50.667f	66.00a	

Means in a column followed by same letter are not significantly different (p=0.01) according to DMRT.

Table 2. Efficacy of different botanical extracts on the percent (%) seed infection with different fungi species

Treatments	Seed borne infection (%)			
	Aspergillus flavus	*Fusarium oxysporum*	*Curvularia lunata*	*Phomopsis vexns*
T_1-(*Azadirachta indica*)	1.33	1.33	1.33	0.00
T_2-(*Putranjiva roxburghii*)	0.00	0.00	0.00	0.00
T_3-(*Salvadora persica*)	0.00	0.00	0.00	0.00
T_4-(*Calotropic procera*)	2.66	1.33	1.33	0.00
T_5-(*Senna alata*)	0.00	2.66	2.66	2.66
T_6-(*Trema orientales*)	0.00	0.00	0.00	0.00
T_7-(*Luffa cylidrica*)	0.00	1.33	1.33	0.00
T_8- (*Croton spasriflorous*)	0.00	0.00	0.00	8.00
T_9-(*Putranjiva roxburghii*)	0.00	0.00	0.00	4.00
T_{10}-(*Trichosanthes dioicahae*)	1.33	1.33	1.33	0.00
T_{11}-Vitavax 200	0.00	0.00	0.00	0.00
T_{12}-Control	9.33	10.66	10.66	21.33

*Data given are mean of four replicates.

Effects of botanical extracts on percentage of seed infection: Aqueous extract of leaf of *Azadirachta indica* and *Putranjiva Roxburghii* have recorded significant antifungal activity against all test fungi. 93.3% of seed infection was inhibited against these crude extract. Then, leaf extract of *Salvadora persica and Calotropis procera* showed good result against all tested fungi which showed only 91.3% seed infection is suppressed in comparison to control. Leaf extract of *Luffa cylindrica*, *Trema orientalis*, *Senna alata* inhibited 87.8% seed infection. Seed treated with leaf extract of *Trichosanthes dioicahae*, seed extract of *Putranjiva roxburghii*, leaf extract of *Croton spasriflorous* suppressed 85.8% seed infection. Vitavax 200 was inhihit 85.8% seed infection against all pathogens whereas control treatment without any botanical extract or chemical treatment is shown highly susceptible against all fungi at 66% seed infection.

The results from this study strongly suggest that seed borne pathogens are fairly managed by using this botanical extracts. This botanical extracts have a very good potentiality against seed borne pathogen of brinjal. The result has an agreement with the findings of (Alberts *et al*. 2006**).**They said that pre- and post harvest bio-deterioration and spoilage of seeds, grains, vegetables,

fruits and agricultural produce due to infestation by insects and micro organisms may cause losses up to 100%. Association of variety of fungi including *Phomopsis vexans, Fusarium oxysporum, Curvularia lunata, Aspergillus flavus* causing significant loss in seed quality of the seeds and grains have been reported.

Effects of botanical extracts against individual seed borne fungal species: In case of *Aspergillus flavus*- leaf extract of *Putranjiva roxburghii,* leaf extract of *Salvadora persica,* leaf extract of *Senna alata,* leaf extract of *Trema orientalis,* leaf extract of *Luffa cylindrica,* leaf extract of *Croton spasriflorous,* seed extract of *Putranjiva roxburghii* & Vitavax 200 were showing best performance against *Aspergillus flavus,* these botanical plant extracts were completely inhibited all seed borne *Aspergillus flavus* species, so, there is zero percent (0%) seed infection. Treated seeds with leaf extract of *Azadirachta indica,* leaf extract of *Calotropis procera* treated seeds showed 2.66 % seed infection where as the control treatment was shown 9.33% seed infection by the infection of *Aspergillus flavus.*

Table 3. Antifungal activity of different plant extracts at 25% (v/v) or (10ml in1000ml) against tested fungal species

Plant Species	% mycelial growth inhibition(Piosion food technique)			
	Aspegillus flavus	*Fusarium oxysporum*	*Curvularia Lunata*	*Phomopsis vexans*
Luffa cylidrica	98.89	98.92	99.22	99.0
Salvadora persica	98.89	98.92	99.22	98.8
Calotropic procera	98.89	99.23	99.33	99.0
Azadirachta indica	99.22	98.92	99.44	99.0
Croton spasriflorous	99.44	98.77	99.44	98.8
Putranjiva roxburghii	99.22	99.23	99.44	99.0
Vitavax200	95.00	96.33	99.87	95.0

In case of *Curvularia lunata*- leaf extract of *Putranjiva roxburghii,* leaf extract of *Salvadora persica,* leaf extract of *Trema orientalis,* leaf extract of *Croton spasriflorous,* seed extract of *Putranjiva roxburghii* and Vitavax 200 treated seeds were shown lowest susceptibility against *Curvularia lunata,* here no seed infection was occurred due to infection of *Curvularia lunata* (0%). Leaf extract of *Azadirachta indica,* leaf extract of *Calotropic procera,* leaf extract of *Luffa cylindrica &* leaf extract of *Trichosanthes dioicahae* showed 1.33% seed infection. Seeds treated with leaf extract of *Senna alata* showed 2.66 % susceptible to *Curvularia lunata* whereas the control treatment was shown 10.66 % seed infection causing *Curvularia lunata.*

In case of *Fusarium oxysporum*- leaf extract of *Azadirachta indica* treated seeds were shown lowest susceptibility against *Fusarium oxysporum,* this extract was completely eliminated all inocula of *Fusarium oxysporum* presented in seeds. Seeds treated with leaf extract of *Calotropis procera,* are leaf extract of *Putranjiva roxburghii,* leaf extract of *Senna alata &* Vitavax 200 showed 1.33%, 2.66%, 2.66%, 2.66%, 2.66%, 2.66%, 2.66% seed infection respectively. Leaf extract of *Salvadora persica,* seed extract of *Putranjiva roxburghii,* treated seeds are shown 4 % seed infection. Leaf extract of *Trichosanthes dioicahae* treated seeds showed 5.33 % and 6.66% seed infection while the control treatment shown 16 % seed infection by the infection of *Fusarium oxysporum*

In case of *Phomopsis vexans*- leaf extract of *Azadirachta indica,* leaf extract of *Putranjiva roxburghii,* leaf extract of *Salvadora persica,* leaf extract of *Calotropis procera,* leaf extract of *Trema orientalis,* leaf extract of *Luffa cylidrica,* leaf extract of *Trichosanthes dioicahae* and Vitavax 200 were showing best performance. They were completely eliminate all *Phomopsis vexans* prevailing in seeds Seeds treated with leaf extract of *Senna alata,* leaf extract of *Croton spasriflorou &* seed extract of *Putranjiva roxburghii* were showed percent seed infection at 1.33%, 2.66%, 2.66%, 2.66%,4% respectively, whereas the control treatment showed 21.33% seed infection by the infection of *Phomopsis vexans.*

In Table 3, it is found that aqueous extract of all the plant control the mycelial growth of all the tested fungal species i.e. *Aspegillus flavus, Fusarium oxysporum, Curvularia lunata, Phomopsis vexans* above 95%. It was observed that mycelial growth of *Aspergillus flavus* by 98.88%, 98.88%, 98.88%, 99.22%, 99.44%, *Fusarium oxysporium* (98.92%, 98.92%, 99.29%, 99.29%, 98.76%, 99.23%), *Curvularia lunata* (99.22%, 99.22%, 99.33%, 99.44%, 99.44%, 99.44%), *Phomopsis vexans* (99%, 98.8%, 99%, 99%, 98.8%, 99%) (Table 3). All the botanical extracts were successfully control the mycelial growth of the fungi. The results from this study strongly suggest that the tested seed borne pathogens are fairly managed by using this botanical extracts. The result has an agreement with the findings of (Reddy *et al.*, 2005**).** They found that the plant extracts showed complete inhibition of *Aspergillus flavus* growth. In poisoned feed technique all plant extract showing above 90% mycelia growth. Some important seed borne pathogen like *Fusarium oxysporum, Phomopsis vexans, Aspergillus flavus* are managed through with the help of some botanical plant extracts (Devi *et al*. 2001). So Exploitation of naturally available chemicals from plant protection and will have a prominent Role in development of future commercial pesticides for crop protection strategies, with special reference to the management of plant diseases (Verma and Dubey, 1999).

The present investigation is an important step in developing plant based pesticides and seed testing chemicals which are ecofriendly for the management of the important seed borne fungi and development of commercial formulation based field trial and toxicological experiment.

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