Journal of Agricultural Technology 2011 Vol. 7(4): 1139-1153
Available online http://www.ijat-aatsea.com
ISSN 1686-9141

Antifungal potentiality of some botanical extracts against important seedborne fungal pathogen associated with brinjal seeds, *Solanum melongena* L.

Kuri S. K*., Islam, M.R. and U. Mondal

Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.

Kuri S. K., R.M.Islam and U. Mondal. (2011). Antifungal potentiality of some botanical extracts against important seedbornd fungal pathogen associated with brinjal seeds , *Solanum melongena* L. Journal of Agricultural Technology 7(4):1139-1153.

Aqueous leaf extracts of *Azadirachta indica, Calotropic procera, Clerodendron spp.,Croton spasriflorous,* leaf and seed of *Lantana camara,* leaf of *Luffa cylidrica , Moringa oleifera,* leaf and seed of *Putranjiva roxburghii,* leaves of *Salvadora persica, Senna alata , Trema orientales* and *Trichosanthes dioica* showed some potentiality to inhibit the growth of some seed borne fungi, *Phomopsis vexans, Fusarium oxysporum, Aspergillus flavus, Aspergillus nigar,Curvularia lunata, Penicillium* spp., and enhanced the seed germination of brinjal (*Solanum melongena*). Leaf extract of *M. oleifera* increased seed germination of 92% as compared with control treatment. Leaf extracts of *A. indica* and *P. roxburghii* gave the best potential against all tested pathogen with 4% seed infection, followed by leaf extract of *S. persica* and *C. procera* were shown activities of 5.33% seed infection against tested pathogens. Extracts of *A. indica* and *P. roxburghii* were significantly highest inhibited the tested fungi of 4 % seed infection. Leaf extract of *S. persica* and *C. procera* showed seed infection of 5.33%. Extracts of *L. camara* and *Clerodendron* spp significantly inbibited as seed infection was 6.67 %. The leaf extracts of *S. alata*, *T. orientales* and *L. cylidrica* showed seed infection of 8 % and leaf extracts of *M. oleifera*, *C. spasriflorous*, *P. roxburghii*, *T. dioicahae* were also shown 9.33 % seed infection with non- significantly differ from Vitavax 200 treatment, but significantly different from the control which was 66 % seed infection. All plant extracts were mostly significantly inhibited the tested fungi associated with brinjal seeds (*S. melongena* L).

Key words: Botanical extract, Antifungal potentiality, seed borne fungi

Introduction

Many third world countries like Bangladesh peasants are not too worry about their crop health, own health, as well as environment. They are indiscriminately using different types of dangerous and poisonous chemicals

* Corresponding auther: Kuri S. K; e-mail: subratobau@gmail.com

in their fields for controlling a variety of field diseases. Among these diseases, fungi are responsible for a significant disease production. So, the farmers have been used a large quantity of fungicides in their fields for controlling fungal diseases. The main sources of fungal diseases are seeds, crop refuses, soil and other sources. This fungi are attacked or harbor in the seeds during fruiting stage, storing, even after sowing of seeds. Different fungi are significant destroyers of seeds during storage and crops at seedling stage. They are also dormant in seed and transmit to seedlings and mature plant and showing different symptoms (Zeringue, *et.al* D1990). Fungi are caused a hand-some amount of germination failure, seedling death. (Goldblatt, L.A., 1971). So, the growers need to pay more attention for good health of seeds and the seedlings. It is resulted in extra addition of cost, time, labors, poisonous chemicals, as well as extra headache of the farmers. Rather more than 25% of the world cereals are contaminated with known mycotoxin and more than 300 fungal metabolities are reported to be toxic to man and animals (Galvano, *et al.,* 2001). The main toxic effects are carcinogenicity, genotoxicity, terrotogenicity, hepetotoxicity, reproductive disorder and immunosuppression (Lacey, 1988; Desjardins, *et al.,* 2000). The poor people of third world countries like Bangladesh and many countries of Africa are the victim of this mycotoxin produced by fungi, poisonous heavy metals and chemicals due to indiscriminate use of pesticides. It is a threat for us and our future generation. These seed treating chemical originated fungicides are able to kill the fungi, rather they also caused damage to the seedlings due to phytotoxicity by excess application (ignorance of farmers), causing death to soil beneficial flora & fauna, wash out by rain or irrigation or flood to the water source and caused serious health problem of the human, animals, fishes, birds, snakes, frogs etc. This phenomenon is not safe for our normal ecology. It imbalances our ecology, interferes our food chain, causes many abnormalities to the environment So it is time to search better alternatives of botanical fungicides. Plant extracts of many higher plants have been reported to exhibit antibacterial, antifungal and insecticidal properties under laboratories trials (Satish, *et al.,* 1999*;* Okigbo and Ogbonnaya,2006;Shariff*, et al.,2006;*Bouamama, *et al.,*2006; Ergene, *et al*., 2006; Kiran and Raveesha, 2006; Mohana and Raveesha, 2006). Plant metabolities and plant 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). This inspired the authors to screen *in vitro* a large number of plants for antifungal potential against important seed borne fungal species like *Phomopsis vexans,Fusarium oxysporum, Aspergillus*

Journal of Agricultural Technology 2011 Vol. 7(4): 1139-1153
Available online http://www.ijat-aatsea.com
ISSN 1686-9141

flavus, Aspergillus niger, Curvularia lunata, Penicillium spp. with the ultimate aim of developing plant based formulations for plant disease management and the quality seeds for sowing and storage grains.

Materials and methods

Plant materials

Fresh diseased free leaves and fruits of twelve plant species were collected from Kaligonj Thana under Jhenidah district of Bangladesh and Botanical Garden of Bangladesh Agricultural University, Mymensingh, Bangladesh. A voucher specimen of plant images has been deposited in the author scientific album and all extracts are also deposited in the refrigerator of M.S Laboratory, Department of Plant Pathology Bangladesh Agricultural University, Mymensingh, Bangladesh.

Table 1. List of plant species tested for antifungal activity

Sl. no	Local Name	English name	Scientific name	Family name	Plant parts used
1	Neem	Indian Lilac	*Azadirachta indica*	Meliaceae	Leaf
2	Akanda	Apple of sodom	*Calotropic procera*	Asclepiadace	Leaf
3	Vait	Glory bower	*Clerodendron spp*	Verbenaceae	Leaf
4	Ban mirca	Croton plant	*Croton spasriflorous*	Euphorbiaceae	Leaf
5	Lantana	Lantana	*Lantana camara*	Verbenaceae	Leaf &seed
6	Dhundal	Spongegourd	*Luffa cylidrica*	Cucurbitaceae	Fruit
7	Sajina	Drum stick	*Moringa oleifera*	Moringaceae	Leaf
8	Paten java	Paten java	*Putranjiva roxburghii*	Euphorbiaceae	Leaf& seed
9	Meswak	Siwak	*Salvadora persica*	Salvadoraceae	Leaf
10	Dadmordan	Candle Bush	*Senna alata*	Leguminosae	Leaf
11	Givon	Givon	*Trema orientales*	Ulmaceae	Leaf
12	Patol	Pointed gourd	*Trichosanthes dioicahae*	Cucurbitaceae	Leaf

Preparation of extracts

Aqueous extracts

Green leaf and fruit samples (100g) of different plants were collected and washed very carefully with distilled water. Then the plant parts are ground with conventional grounder called "HAMAN DISTA" (mortar and pastels) which is available and popular in Bangladeshi farmers' family. Then the grounded materials were dipped into 100ml 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. In every time after separation, the cloth is washed with antibacterial soap carefully

followed by washing with distilled water. Then, the crude extracts were preserved in glass bottles in a refrigerator at 5 ± 2 ºC for further use. This indigenous methodology was practiced to justify the effectiveness of this method, so that the poor farmers of our country would practice this method in their house without facing any technological troubles for preparing a plant extracts for using in their own field crops. The authors tried to make this methodology which too easy and practice without any kind of scientific gorgon to the illiterate person in the country.

Test fungi

Farmers' stored Brinjal (*Solanum melongena*) seeds were collected from the farmers' house. The tested seeds were not treated with any seed treating chemicals. Then seeds were placed in blotter method and placed in incubation at 22 ± 2 ºC for growth of fungi. After 10 days pletting of seeds the growth of seed borne fungi on the seeds were recorded. Then the infected seeds are marked and placing in the Potato Dextrose Agar (PDA) medium. After that, the inocula of fungi are growing in the PDA medium. Lastly, the individual inoculum was isolated and set in PDA medium for pure culture. After 5 days of inoculation, the individual pathogenic fungi were identified by Stereomicroscope with the specific characters of mycelia and conidia. Then, permanent slides were made for each fungi and storing in the Mycology Laboratory of Seed Pathology Centre, Bangladesh Agricultural University, Mymensingh, Bangladesh. Finally, it was confirmed that the the experimented brinjal seeds contained *Phomopsis vexans, Fusarium oxysporum, Aspergillus flavus, Aspergillus niger, Curvularia lunata, Penicillium* spp.

Anti fungal activity assay

Seed treatments

Required amount of seeds were treated in the aqueous botanical extracts for 30 minutes. The concentration of the solution was 100% (v/v). For all twelve individual plant species were separately done. Then, to observe the comparison between botanical and chemical seed treating material, Vitavax 200 was used to treat the seeds for 30 minutes with recommended dose (25% of seed wt.), and in another plate seeds are dipped in distilled water for 30 minutes as control treatment.

Testing potentiality

After treating, seeds were placed in sterilized Petri dishes and four layers of blotting paper were soaked into distilled water and placed into the Petri

Journal of Agricultural Technology 2011 Vol. 7(4): 1139-1153
Available online http://www.ijat-aatsea.com
ISSN 1686-9141

dishes. Each dish containing 25 seeds and 3 dishes were selected as replicates for each extract. Then, the Petri dishes were kept in the incubation chamber at 22 ± 2 ºC at light cycle 12/12 hours and data were recorded after 7, 10, 14 days after sowing (DAS). The total seed germination and percent seed infection are counted manually. But, the seeds were infected by specific fungi that identified and counted by observing the Petri dishes under Stereo Binocular Microscope and compared with the catalogue of Seed Mycology (Mathur *et al*., 1994).

Experimental design and statistical analyses

The experiment was done using Compleltly Randomized Design with four replications and treatments were designed as follows:- T1=Leaf extract of *A. indica,* T2=leaf extract of *P. roxburghii,* T3=leaf extract of *S. persica*, T4=leaf extract of *C. procera*, T5=seed extract of *L. camara,* T6=leaf extract of *Clerodendron* spp, T7=leaf extract of *S. alata*, T8=leaf extract of *T. orientales*, T9 =leaf extract of *L. cylidrica*, T10=leaf extract of *M. oleifera*, T11=leaf extract of *C. spasriflorous*, T12=seed extract of *P. roxburghii*, T13=leaf extract of *T. dioicahae*, T14=leaf extract of *L. camara(leaf)*, T15=Vitavax 200 and T16=Control (no chemicals). Data were statistical analysed analysis of variance (ANOVA) and treatment means were compared using Duncan's Muly=tiple Range Test (DMRT) at P=0.01.

Results and discussion

Effects of botanical extracts on germination of brinjal seeds

All the plant extract brinjal seeds treated with leaf extract of *M. oleifera* showed high percentage of seed germination (92%). The treated seeds with leaf extract of *C. procera, Clerodendron* spp*, S. alata,T. orientales, C. spasriflorous*, seed extract of *P. roxburghii,* leaf extract of *L. camara* and Vitavax 200 showed over 80% seed germination of 86.67%, 82.67%, 82.67%, 80.33%, 80.67%, 81.33%, 82.67%, 82.67% ,respectively. The seed treated with the leaf extract of *A. indica,* leaf extract of *Putranjiva, Roxburghii,* leaf extract of *S. persica,* seed extract of *L. camara,* leaf extract of *L. cylidrica* and *T. dioicahae* showed seed germination of 78.67%, 74.67%, 77.33%, 70.67%, 70.67%, 70.67% and 78.67%, respectively while the untreated control seeds was 50.67% seed germination.

Result indicated that the treted seeds with botanical extracts gave a very good effect on germination. It also indicated that the botanical extracts that increased in seed germination rather inhibited seed germination. The result was

some contradicted and agreed with the findings of Edwin, *et al,* (1968); Jeffersonand and Pennacchio (2003**).** They found that botanical leaf and fruit extracts of some plants showed allelopathic effect and inhibited seed germination. Extracts from potato, sugarbeet, sagebrush, green soybean, alfalfa, pea, and bean were extremely toxic for seed germination, while the extracts from Douglas-fir, peat moss and sphagnum moss stimulated seed germination. The degree of toxicity was dependent upon the maturity of the plant residues, the extract concentration and plant species.

Table2. Effect of different botanical extracts on germination and seed infection

	Treatments	Germination (%)	Seed infection (%)
T1	*Azadirachta indica*	78.67 cd	4.00 f
T2	*Putranjiva roxburghii(Leaf)*	74.67 de	4.00 f
T3	*Salvadora persica*	77.33 cd	5.33 ef
T4	*Calotropic procera*	86.67 ab	5.33 ef
T5	*Lantana camara(fruit)*	70.67 e	6.67 de
T6	*Clerodendron spp*	82.67 bc	6.67 de
T7	*Senna alata*	82.67 bc	8.00 cd
T8	*Trema orientales*	80.33 bcd	8.00 cd
T9	*Luffa cylidrica*	70.67 e	8.00 cd
T10	*Moringa oleifera*	92.00 a	9.33 bc
T11	*Croton spasriflorous*	80.67 bcd	9.33 bc
T12	*Putranjiva roxburghii(seed)*	81.33 bc	9.33 bc
T13	*Trichosanthes dioicahae*	78.67 cd	9.33 bc
T14	*Lantana camara(leaf)*	82.667 bc	10.67 b
T15	Vitavax 200	82.667 bc	9.33 bc
T16	Control (no chemicals)	50.667 f	66.00 a
	LSD (P = 0.01)	5.618	2.11

Means in a column followed by same letter are not significantly different (P=.01) according to DMRT.
*Data given are means of four replicates.

Effects of botanical extracts on seed infection of brinjal seeds

Aqueous leaf extracts of *A. indica, leafextract, Putranjiva, Roxburghii* were recorded significantly inhibited as antifungal activity against all tested fungi. These aqueous extracts were stromglyinhibited seed infection. Seed infection was recorded against these crude extracts averaged 4 %. Then, leaf extract of *S. persica* and *C. procera* showed good result against all tested fungi which was only 5% seed infection. The treated seeds with the extracts of *L. camara* and *Clerodendron* spp were infected by tested fungi of 6.66%. All tested fungi were infected the treated seeds with leaf extract of *L. cylidrica*, *T. orientales*, *S. alata* of 8 %. Seed treated with extracts of *T. dioicahae, P.*

roxburghii, C. spasriflorous, *M. oleifera* protected infection as seed infection was 9.33%. Leaf extract of *L. camara*e showed the highest seed infection against all tested fungi which was 10.66 %. Vitavax 200 treatment showed seed infection of 9.33%. Whereas, control treatment without any botanical extracts or chemical treatment showed highly seed infection against all tested of 66%.

The results are strongly suggested that seedborne fungi that would possible managed by using these botanical extracts. These botanical extracts gave a very good potentiality against seedborne pathogen of brinjal. The result was an agreement with the findings of Koirala *et al*.(2005); Usha, *et al*.(1993);Alberts,*et al*.(2006**).** They reported that pre and post harvest bio-deterioration and spoilage of seeds, grains, vegetables, fruits and agricultural pruduce due to infestation by insects and microorganisms may cause losses up to 100%. Association of fungal variety including *P. vexans, F. oxysporum, C. lunata, A. flavus, A. niger* and *Penicillium* spp. causing significant loss in seed quality were reported. Satish, *et al.* (2007) found that biologically active plant derived pesticides are expected to play an increasingly significant role in crop protection strategies. There was a comparative study tin bar diagram (Fig. 1) indicated an increased in per cent germination of all individual treatment as compared with control. Results showed that extracts of *A. indica* and *P. roxburghii* were significantly highest inhibited the tested fungi of 4 % seed infection. Leaf extract of *S. persica* and *C. procera* showed seed infection of 5.33%. Extracts of *L. camara* and *Clerodendron* spp inbibited seed infection of 6.67 %. Leaf extracts of *S. alata*, *T. orientales* and *L. cylidrica* were shown seed infection of 8 %. Leaf extracts of *M. oleifera*, *C. spasriflorous*, *P. roxburghii*, *T. dioicahae* were also shown seed infection of 9.33 % with non-significantly differ from Vitavax 200 treatment, but significantly different from the control which was 66 % seed infection. All plant extracts were significantly decreased in seed infection against all seedbornefungi as comparison with control.

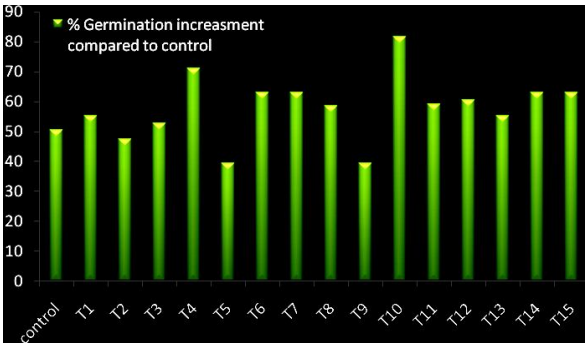

Fig 1. Comparative performance of all the botanical extracts and chemical fungicides as compared to control treatment respect of percent germination increasing.
*Data are means of four replicates.

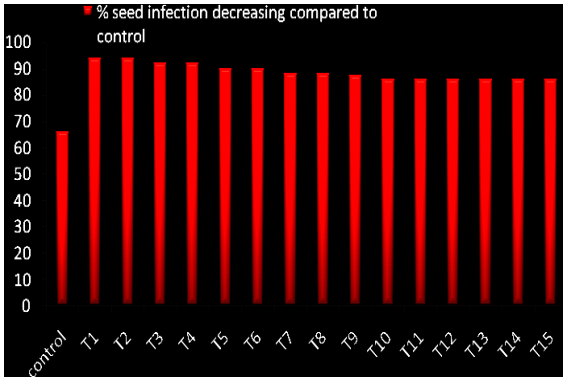

Fig 2: Comparative performance of all the botanical extracts and chemical fungicides as compared to control treatment respect of percent seed infection decreasing.
*Data are means of four replicates

Effects of botanical extracts against individual seed borne fungi

Extracts of *A. indica*, *P. roxburghii*, *S. persica, C. procera, Clerodendron* spp, *L. camara, T. orientales, L. cylidrica, T. dioicahae* and Vitavax 200 showed the highest inhibition of *P. vexans*. These extracts were completely inhibited of *P. vexans* on seed treatments with extracts of *M. oleifera, S. alata, C. spasriflorou, L. camara, P. roxburghii* which showed seed infection at 1.33%, 2.66%, 2.66%, 2.66% and 4% respectively, whereas the control treatment was 21.33% seed infection. Leaf extract of *A. indica* treated seeds was the lowest infection of *F. oxysporum*, which completely eliminated all inocula of *F. oxysporum*. Seeds treated with leaf extracts of *C. procera, L.*

Journal of Agricultural Technology 2011 Vol. 7(4): 1139-1153
Available online http://www.ijat-aatsea.com
ISSN 1686-9141

cylidric, P. roxburghii, seed extract of *L. camara,* leaf extract of *Clerodendron* spp, leaf extract of *S. alata* and Vitavax 200 showed seed infection of 2.66 %, respectively, except for leaf extracts of *C. procera*which was 1.33 % seed infection. Leaf extract of *S. persica,* seed extract of *P. roxburghii,* treated seeds was shown 4 % seed infection. Leaf extracts of *T. dioicahae, L. camara* treated seeds showed 5.33 % and 6.66% seed infection, respectively, while the control treatment was 16 % seed infection of *F. oxysporum.* Leaf extract of *P. roxburghii, S. persica, T. orientales, M. oleifera*, *C. spasriflorous* and seed extract of *P. roxburghii,* leaf extract of *L. camara* and Vitavax 200 treated seeds were the lowest infection of *C. lunata* as observation was not seen any seed infection. Leaf extracts of *A.indica*, *C. procera, L. cylidrica, T. dioicahae* showed 1.33% seed infection. Seeds treated with seed extract of *L. camara,* leaf extract of *Clerodendron* spp, leaf extract of *S. alata* showed 2.66 % seed infection of *C. lunata* whereas the control treatment was 10.66 % seed infection. Leaf extracts of *P. roxburghii*, *S. persica, Clerodendron* spp, *S. alata, T. orientales*, *L. cylidrica*, *M. oleifera*, *C. spasriflorous,* seed extract of *P. roxburghii* and Vitavax 200 showed best performance against *A. flavus.* These botanical plant extracts were completely inhibited *A. flavus* as shown zero percent (0%) seed infection. Treated seeds with leaf extract of *A. indica,* seed extract of *L. camara,* leaf extract of *L. camara* showed 1.33% seed infection. Leaf extract of *C. procera* treated seeds showed 2.66% seed infection where the control treatment was 9.33% seed infection of *A. flavus.* Leaf extracts of *S. persica , C. procera*, seed extract of *L. camara,* leaf extract of *S. alata*, seed extract of *P. roxburghii,* leaf extract of *L. camara* treated seeds showed the lowest infection of *A. niger* as 0 % seed infection. Seeds treated with leaf extracts of *A. indica*, *P. roxburghii, Clerodendron* spp showed 1.33 % seed infection. Leaf extracts of *T. orientales*, *M. oleifera, T. dioicahae* showed 2.66% seed infection. Leaf extract of *Luffa cylidrica* and Vitavax 200 treated seeds were 5.33 % seed infection where as control treatment was shown 9.33% seed infection of *A. niger.* Seeds treated with leaf extracts of *A. indica*, *P. roxburghii*, seed extract of *L. camara*, leaf extracts of *C. procera*, *S. persica, Clerodendron* spp, *S. alata*, *T. orientales*, *L. cylidrica*, *M. oleifera*, *C. spasriflorous*, *L. camara*, *T. dioicahae* showed best potentiality to inhibit against *Penicillium* spp., as there were no recorded seed infection. Leaf extract of *S. persica*, seed extract of *P. roxburghii* and Vitavax 200 showed 1.33% seed infection while the control treatment showed 6.66% seed infection of *Penicillium* spp.

The result was an agreement with the findings of Reddy, *et al.* (2008**).** They repoatred that the plant extracts completely inhibited *A. flavus* as also reported by Howard, *et al* (2002) who stated that botanical extracts of some higher plants can inhibit the growth of *P. vexans, F. oxysporum.* Antifungal effect of leaf extract of some medicinal plants against *F. oxysporum* causing wilt disease of *Solanum melogena L.* Siva1, *et al.*(1992) and Majid Avijgan *et al.*(2005) found that some antifungal effect of botanical plant extracts against *C. lunata*, *A. niger*, *Penicillium* spp. Some important seed borne pathogen like *F. oxysporum, A.niger, Penicillium* spp. *P. vexans, A. flavus* are managed by using some botanical plant extracts (Devi, *et al*. 1999). Exploitation of naturally available chemicals from plantprotection would be a prominent role in development of future commercial pesticides for crop protection strategies, with special reference to manage plant diseases (Varma and Dubey,1999; Gottileb, *et al*., 2002).

Table 3. Efficacy of different botanical extracts on seed infection with different fungal species.

N o.	Treatmenta	Seed infection (%)					
		Phomopsis vexns	***Fusarium oxysporum***	***Curvularia lunata***	***Aspergillus flavus***	***Aspergillus niger***	***Penicilium Spp.***
T1	*Azadirachta indica*	0	1.33	1.33	1.33	1.33	0
T2	*Putranjiva roxburghii*	0	0	0	0	1.33	0
T3	*Salvadora persica*	0	0	0	0	0	1.33
T4	*Calotropic procera*	0	1.33	1.33	2.66	0	0
T5	*Lantana camara*	0	2.66	2.66	1.33	0	0
T6	*Clerodendron spp*	0	2.66	2.66	0	1.33	0
T7	*Senna alata*	2.66	2.66	2.66	0	0	0
T8	*Trema orientales*	0	0	0	0	2.66	0
T9	*Luffa cylidrica*	0	1.33	1.33	0	16	0
T10	*Moringa oleifera*	1.33	0	0	0	1.33	0
T11	*Croton spasriflorous*	8	0	0	0	0	0
T12	*Putranjiva roxburghii*	4	0	0	0	0	1.33
T13	*Trichosanthes dioica*	0	1.33	1.33	1.33	1.33	0
T14	*Lantana camara*	2.66	0	0	1.33	0	0
T15	Vitavax 200	0	0	0	0	5.33	1.33
T16	Control	21.33	10.66	10.66	9.33	9.33	6.66

*Data are means of four replicates.

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.

Acknowledgements

The authors are grateful to Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh, Mycology Laboratory, Dnida Seed Pathology

Centre, Bangladesh Agricultural University, Mymensingh for providing technical facilities. The authors are also grateful to Suchitra Rani Kuri for her cordial helps to collect the plants.

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(Received 8 March 2010; accepted 30 May 2011)