IN VITRO EVALUATION OF SELECTED PLANT EXTRACTS FOR SEED TREATMENT OF HYBRID RICE

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DHAKA -1207

DECEMBER, 2013

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REGISTRATION NO. 07-02250

A Thesis

Submitted to the Faculty of Agriculture,
Sher-e-Bangla Agricultural University,
Dhaka, in partial fulfillment
of the requirements
for the degree
of

MASTER OF SCIENCE
IN
PLANT PATHOLOGY

SEMESTER: JULY- DECEMBER, 2013

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CERTIFICATE

*This is to certify that thesis entitled, "**IN VITRO EVALUATION OF SELECTED PLANT EXTRACTS FOR SEED TREATMENT OF HYBRID RICE.**" submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE IN PLANT PATHOLOGY,** embodies the result of a piece of bona fide research work carried out by, **Registration No. 07-02250** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma in any institute.*

I further certify that such help or source of information, as has been availed of during the course of this investigation has duly been acknowledged.

Dated: 20 November, 2014
Place: Dhaka, Bangladesh

……..
(Abu Noman Faruq Ahmmed)

Supervisor

DEDICATED TO

MY

BELOVED PARENTS

ACKNOWLEDGEMENTS

All praises are due to the "Almighty Allah" who enabled the author to pursue higher education in Plant Pathology and to submit the thesis for the degree of Master of Science (M.S.) in Plant Pathology.

The author wishes to express his profound sense of appreciation and heartiest gratitude to his Supervisor, **Abu Noman Faruq Ahmmed,** *Assistant Professor, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka for his help, scholastic supervision, continuous encouragement and constructive suggestion throughout the period of research and for taking immense care in preparing this manuscript.*

The author expresses his immense gratitude to his Co-supervisor, ***Dr. F.M. Aminuzzaman,*** *Professor and Chairman, Department of* ***Plant Pathology****, Sher-e-Bangla Agricultural University, Dhaka for his valuable advice and constructive criticism during the critic period of research work.*

The author is grateful to ***Nazneen Sultana,*** *Professor, Department of Plant Pathology, Sher-e-Bangla Agricultural University for giving necessary suggestions.*

The author would like to extend his appreciation to all teachers and staff of the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka for their co-operation and encouragement during the study period.

The author feels proud of expressing his sincere appreciation and gratitude to Ministry of Science and Technology for selecting him NST fellow and funding.

The author is also thankful to Sher-e-Bangla Agricultural University Research System (SAURES) for providing research support.

The author can never repay the debt of his beloved parents, uncle, aunty, sisters, brothers and well wishers for their inspiration, constant encouragement and sacrifice for his higher education.

The author expresses his immense gratefulness to all of them who assisted and inspired him to achieve higher education and regret for his inability for not to mention every one by name.

The Author

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ABSTRACT

An investigation was carried out to evaluate the efficacy of some selected plant extract for seed treatment of hybrid rice in the seed health laboratory of Department of Plant Pathology of Sher-e-Bangla Agricultural University during the period from January 2012 to June 2013. Seed health test as well as seed treatment where nine plant extracts viz. *Allium cepa* bulb extract, *Nigella sativa* seed extract, *Allamanda cathartica* leaf extract, *Allium sativum* clove extract, *Azadirachta indica* leaf extract, *Datura metel* leaf extract, *Curcuma longa* rhizome extract, *Polygonum hydropiper* leaf extract and *Salmalia malabarica* leaf extract were evaluated against seed borne pathogens of hybrid rice. All of the botanical were used as per 1:1 (w/v) ratio. Seven seed borne fungi were identified which were two strains of *Xanthomonas oryzae* pv. *oryzae*, *Rhizopus stolonifer*, *Aspergillus flavus*, *Aspergillus niger*, *Fusarium moniliforme*, *Bipolaris oryzae*, *Curvularia lunata* and *Chaetomium globosum*. Out of these pathogens, *Xanthomonas* spp., *Rhizopus stolonifer*, *Aspergillus* spp., *Fusarium moniliforme* were predominant. Pathogenicity of *Xanthomonas oryzae* pv. *oryzae* was studied. *Xanthomonas oryzae* pv. *oryzae* was found to be associated with all tested varieties of hybrid rice. Hira 2, BRRI hybrid dhan2 and SL 8 were more susceptible to the strain 1. All of the plant extracts significantly control seed borne fungi. Among the plant extracts, *Datura metel* leaf extract, *Curcuma longa* rhizome extract; *Allamanda cathartica* leaf extract and *Allium sativum* clove extract showed best performance against the seed borne pathogen. *Nigella sativa* seed extract, *Polygonum hydropiper* and *Salmalia malabarica* leaf extract also showed promising effect against seed borne fungi only. *Datura metel* leaf extract and *Curcuma longa* rhizome extract resulted highest anti bactericidal and anti fungal effect out of the plant extracts used. From the findings of the study, it is very clear that the seed health status of hybrid rice seed was not at satisfactory level. Considering the overall performance of plant extracts, *Datura metel* leaf extract (1:1 w/v) and *Curcuma longa* rhizome extract (1:1 w/v) could be used for seed treatment of hybrid rice as an eco-friendly approach and may be advised to the farmer for profitable hybrid rice production but more research need to be carried out in this respect.

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CHAPTER 1
INTRODUCTION

Rice (*Oryza sativa*) is the staple food of Bangladesh and it constituted about 90% of the total food grain production (Huda, 2001). It covers about 75% of the total cultivable land in Bangladesh (BBS, 2008). The average world yield of rice is 4.5 tons/ha in 2012 but the average yield of rice in Bangladesh is 2.96 ton/ha (BBS, 2013). In Bangladesh, hybrid rice has taken up significant land coverage due to active government attention, promotion of private seed companies and publication of mass media. In the present time, several seed companies have imported different hybrid rice varieties to increase rice production. The Bangladesh Government has also heavily promoted hybrid rice cultivation aims to boost rice production. Different seed companies are importing hybrid rice seed from China, India and Philippines. First, it was considered as an alternative technology for breaking the present yield ceiling of modern varieties. However, sometimes the yield of those varieties is hampered by different factors. Major constrains in hybrid rice adoption were identified; these were high cost of seed, requirement of more crop care and management time, high pest and disease attack, low profits and lack of suitability for home consumption (AAS, 1999). In last few years Bacterial Leaf Blight, Bacterial Leaf Streak and Blast disease appear seriously in the hybrid rice in different areas of the country. Objection was made from some corner that those seed borne diseases extremely appear in the hybrid rice varieties.

Seed is the most important input for crop production. Pathogen free healthy seed is essential for desired plant population and good harvest. Many plant pathogens are seed-borne, which can cause enormous crop losses. In Bangladesh, 16% annual crop losses occur due to plant diseases, at least 10% loss is incurred due to seed-borne diseases (Fakir, 1983). Coincidentally important or devastating crop diseases are seed-borne and caused by mostly fungi. Seed is a common carrier of plant pathogens. It acts as the primary source of many diseases. Most of the major diseases of rice are seed-borne

(Fakir, 2002). In Bangladesh, approximately 2.5 million tons of rice worth more than Tk. 12000 million is lost annually due to diseases caused by seed-borne pathogens (Fakir *et al*., 2003). Rice suffers from more than 60 different diseases. In Bangladesh, 43 diseases are known to occur on the rice crop. Among these diseases 27 are seed-borne of which 14 are of major importance. Fungi are the principal organisms associated with seed in storage. Of all the seed-borne diseases of rice, 22 are caused by fungi (Fakir, 2000).

The common seed borne pathogen of rice are *Drechslera oryzae* (Brown spot), *Pyricularia oryzae* (Blast), *Fusarium moniliforme* (Foot rot), *Garlecia oryzae* (Leaf scald) and *Sarocladium oryzae* (Narrow brown leaf spot) as reported by Ashrafuzzaman, (1991). Fakir (1988) reported 37 seed borne pathogens of rice in Bangladesh. Out of them 32 are fungi, 3 are bacteria, each of one are virus and nematode. Brown spot (*Bipolaris oryzae*), Bakanae (*Fusarium moniliforme*), Blast (*Pyricularia oryzae*), Sheath blight (*Rhizoctonia solani*), Sheath rot (*Sarocladium oryzae*), Stem rot (*Sclerotium oryzae*), Bacterial leaf blight (*Xanthomonas campestris* pv. *oryzae*), Bacterial leaf streak (*Xanthomonas campestris* pv. *oryzicola*) and Tungro (Rice Tungro virus) are the most important seed borne diseases of rice in Bangladesh (Fakir, 1988).

Various methods have been practiced to control these pathogens. The acceptable method for controlling of these seed borne diseases is sowing of pathogen free seeds. Use of chemical can give quick result. Farmers are therefore, interested to use chemical for controlling plant diseases. Treatment of seed with seed-dressing fungicides was found to improve germination and decrease infection of seed borne pathogen from different crop. Use of chemical to control a disease is the most favorable mean to our farmers till now. An appreciable amount of work has been done on the control of seed-borne pathogens of rice by fungicidal seed treatment at home and abroad (Misra and Vir, 1990; Suratuzzaman *et al*. 1994; Parisi *et al*. 2001). However, most of the chemicals are costly, so the farmers have to spend a large amount of money to buy these chemicals. Use of precise dose of the chemical for its application to

the field is a difficult job for them. So, indiscriminate and long time use of chemicals affect the soil health and also increase pathogen resistance. Harmful chemical substances also enter into the food chain that ultimately causes serious human diseases. Now-a-days use of chemical for management of crop disease is being discouraged due to health hazards and environmental pollution. Use of alternate methods instead of seed treating chemicals is of great concern now a day to save our environment.

Therefore, it is judicious to explore less expensive, less risky non-chemical components to treat seeds for freedom from the seed-borne pathogens. In this respect, seed treatment with different plant extracts has been shown effective to obtain apparently healthy seed in controlling seed borne fungal pathogen. Seed treatment with botanicals is an effective way to control seed borne diseases. Islam (2006) reported that seed treatment with garlic clove extract and bishkatali extract was found effective in reducing the incidence of *Bipolaris sorokiniana* as well as leaf blight severity in the field. Rahman (2007) also reported that seed treatment with garlic clove extract and turmeric rhizome extract resulted remarkable reduction of leaf blight severity of wheat. Botanical extracts are biodegradable and their use in crop protection is a practical sustainable alternative. It reduces environmental contamination and health hazards (Devlin and Zettel, 1999; Grange and Ahmed, 1988). Research on the active ingredients, fungicide preparation, application rates and environmental impact of botanical fungicides is a prerequisite for sustainable agriculture (Buss and Park, 2002). Botanical fungicides are unique because they can be produced easily by the farmers and small industries (Roy *et al.,* 2005). Few works have been done by using tobacco, neem, garlic and some other plant extracts to control some other fungi. Antifungal activities of garlic, neem, allamanda have been reported by many researchers (Islam, 2005; Rahman *et al.,* 1999; Arun *et al.,* 1995; Mohanty *et al.,* 1995).

The cultivable land area of our country is decreasing gradually but population is increasing. Due to fulfill the demand of increasing population, the cultivation

of hybrid rice is increasing day by day. Almost all of the hybrid rice varieties are imported from abroad. Rice seed play an important role to carry pathogen in quarantine aspect. Farmer's usually used different hybrid rice varieties and face the difficulties of many diseases. In the last few years the cultivation of imported hybrid rice in Bangladesh increases rapidly. Last few years Bacterial Leaf Blight, Bacterial Leaf Streak and blast diseases appear seriously in most of the hybrid rice cultivated area. Objection was made from some corner that those diseases extremely appear in the hybrid rice seeds. It is thus dire necessity to work extensively to examine the effect of different plant extracts to control the seed borne diseases of hybrid rice. These botanical pesticides are affordable by low income farmers. They have the potentiality to use botanicals in agriculture, especially with the dramatic increase towards the consumption of organically produced plants and ensure the sound ecology and friendly environment without any pollution. Considering the above facts, the study was undertaken:

1. To evaluate the seed health status of three selected hybrid rice varieties;
2. To evaluate the efficacy of different plant extracts as seed treating agent against different seed borne pathogens of hybrid rice; and
3. For *in vitro* screening of datura leaf extract and turmeric rhizome extract against seed borne *Xanthomonas* bacteria of hybrid rice.

CHAPTER 2

REVIEW OF LITERATURE

Use of plant extracts instead of chemical fungicides in controlling disease is one of the recent approaches for plant disease control. No research works directly have been carried out in Bangladesh to control seed borne pathogens of hybrid rice by botanicals. However, some research works have been carried out on controlling seed borne diseases of different crops (Hossain *et al.*, 2005; Hossain *et al.*, 1997; Ganguli, 1994; Ahmed and Sultana, 1984; Alice, and Rao, 1987).

2.1. Review of literature on garlic clove extract

Ahmed and Sultana (1984) observed that the bulb extract of garlic was most effective against major seed borne pathogens of jute. Seed treatment with botanicals is an effective way to control leaf blight of wheat.

Islam *et al.* (2006) reported that seed treatment with garlic clove extract and bishkatali extract was found effective in reducing the incidence of *Bipolaris sorokiniana* as well as leaf blight severity in the field. Control of plant disease by biological means instead of chemicals has drawn special attention all over the world. Some researchers have already been successfully used plant extracts in controlling leaf blight of wheat (Hossain and Schlosser, 1993; Ashrafuzzaman and Hossain, 1992).

Alice and Rao (1987) evaluated 31 plant extracts *in vitro* against *Dreschlera oryzae* in rice using inhibition zone technique and found that maximum inhibition was obtained with *Mentha piperita* followed by *Piper nigrum* seed extracts and *Allium sativum* extract.

Shetty *et al*. (1989) found that rice seeds soaked in 10, 20, and 30% extracts (w/v) of garlic bulb and rhizome of ginger significantly reduced seed-borne infection of *Trichoconiella* (*Alternaria*) *padwickii*.

Miah *et al.* (1990) examined the efficacy of extract of eight different plant species against seed-borne fungi of rice through eight hrs seed soaking. Out of the plant species tested, extracts of *Allium sativum* and *Curcuma longa* reported to be promising.

Tariq and Magee (1990) observed the effect of volatile component of crude aqueous extracts of garlic bulb on the germination of micro conidia and hyphal extension in *Fusarium oxysporum* f. sp. *lycopersici* in axenic culture. The inhibitory effects were reversible except when micro conidia were exposed to volatile from extracts containing a high conc. of garlic (500 mg /ml) while those extracts containing only 10 ml garlic promoted formation of the latter spore type.

Fakir and Khan (1992) reported that garlic bulb extract was effective in controlling seed-borne fungal pathogen of jute such as *Macrophomina phaseolina* and *Fusarium* sp. by seed treatment.

Suratuzzaman *et al.* (1994) found the garlic extract effective in controlling *Pyricularia oryzae* and *Curvularia lunata*.

Arun *et al.* (1995) observed that extract of garlic, marsh peppers, mart weed and vitavex 200 effectively suppressed seed-borne fungal pathogen of rice seeds. They found highest reduction of fungal population with vitavex 200 followed by garlic extract.

Bisht and Khulbe (1995) studied the efficacy leaf of extract of *Allium sativum* in controlling the growth of *Drechslera oryzae*. The fungitoxic properties of *Allium sativum* have been observed and significant reduction of the mycelial growth compared with the control was obtained.

Khan and Fakir (1995) observed that seed treatment with garlic extract at different conc. significantly reduced seed-borne infection of *Colletotrichum corchori*, *Fusarium* spp. and *Macrophomina phaseolina* in jute. They also obtained good germination in garlic extract treated seed.

Hossain *et al*. (1997) reported that the extract of *Allium sativum* and *Lawsonia alba* showed marked effect in controlling the spore germination and mycelial growth of *Bipolaris sorokiniana* and pathogenecity to wheat leaves and *Nigella sativa* showed positive antifugal activity in reducing the pathogenicity of *Bipolaris sorokiniana* of wheat leaves.

Hossain *et al.* (1993) reported that extracts of *Lawsonia alba*, *Ipomoea fistulosa*, *Allium sativum* and *Leucas aspera* against *Rhizoctonia solani* and *Bipolaris sorokiniana*. Among the test extracts, *A. sativum* completely inhibited the mycelial growth of the fungi at dilution ratio of 1:4 (w/v).

Mohanty *et al.* (1995) reported that garlic bulb extract (1:1) sprays in the field reduced phomopsis blight and fruit rot by 66%.

Bisht and Khulbe (1995) found promising effect of leaf extract of *Allium sativum* in controlling the mycelial growth of *Drechslera oryzae*.

Rahman *et al.* (1999) found that bishkatali (*Polygonum hydropiper*), garlic (*Allium sativum*), ginger (*Zingiber officinale*) and neem (*Azadirachta indica*) extracts were effective against seed borne infections by *Alternaria tenuis, Bipolaris sorokiniana, Curvularia lunata, Fusarium* spp. of wheat. However, garlic was found superior to ginger and neem.

Dubey and Dwivedi (1991) found that fungitatic properties of extracts of leaves, bulb of onion and garlic and fruit, bark of *Allium cepa* against vegetative growth, *Sclerotial* viability of *Macrophomina phaseolina*. They observed that all the extracts inhibited growth but garlic bulb extract was more effective than other extracts employed in the tests.

Khaleduzzaman (1996) evaluated the effect of plant extracts viz bishkatali (*Polygonum hydropiper*), garlic (*Allium sativum*), ginger (*Zingiber officinale*), neem (*Azadirachta indica*) and a seed dressing chemical Vitavax 200 (Carboxin) on incidence of seed-borne fungi of wheat following blotter method of seed health testing. Vitavax 200 was found best in reducing seed–borne infection and increasing germination of seeds. All the four plant extracts were

found effective against seed-borne fungi of wheat resulting statistically similar effect like Vitavax 200. However, garlic was turned up as superior among the extract followed by ginger and neem.

2.2. Review of literature on neem leaf extract

Dharam and Sharma (1985) reported that neem oil inhibited the growth of *Alternaria alternata* by 61% and 100% at 1% and 10% concentration, respectively.

Khan and Kumar (1992) observed the antifungal activity of leaves extract of neem (*Azadirachta indica*) with different dilutions of wheat seeds mycoflora. They recorded a marked reduction in seed mycoflora and enhance seed germination of wheat seeds.

Hossain and Schlosser (1993) observed that the possibility of using neem plant **(***Azadirachta indica*) extract as a means of controlling seedborne pathogen of wheat of wheat. Neem seed extracts or cake was found effective against *Bipolaris sorokiniana.* The extract inhibited the growth of the fungus and also reduced its pathogenecity on wheat leaves. Germination rate of wheat seeds increased after treatment with extracts of neem seed and cake.

Govindachari *et al*. (1998) observed the potentiality of neem oil (*Azadirachta indica*) against *Drechslera oryzae* (*Cochliobolus miyabeanus*), *Fusarium oxysporum* and *Alternaria tenuis* (*A. alternata*). They observed that the active fractions of those plant extracts contained major compound such as 6-deacetylnimbin, azadiraduione, nimbin, salannin and epoxyazadiradione. Pure azadradione, nimbin, salannin and epoxyazadiradione did not show antifungal activity. However, when terpenoids (Methanol extraction) were mixed and bioassayed, they showed antifungal activity, suggesting possible additives/ synergistic effects.

2.3. Review of literature on datura leaf extract

Thakhur *et al*. (1991) evaluated the extracts of medicinal plants against cotton pathogens *Myrothecium roridum*, *Alternaria tenuis* and *Xanthomonus*

campestris pv. *malvacearum* showed that among the nine extract tested, *Punica granatum* and *Dutra metel* had the best antifungal and antibacterial activity against cotton pathogens.

2.4. Review of literature on allamanda leaf extract

Mohanty *et al.* (1995) reported that allamanda leaf extract sprays in the field reduced phomopsis blight and fruit rot by 75%.

Mostafa (2004) studied effective of some plant extracts (Rhizome of ginger, Turmeric and bon ada, leaf of neem, Allamanda pitraj, Marigold, plant of Biskatali and fruits of pepper) on the incidence and severity of the viral diseases of tomato through a field experiment. Among the plant extracts Allamanda was most effective. The lowest percent plant infection (3.75) observed in Allamanda extract treated plots.

Howlader (2003) observed that seed treatment with *Allamanda* leaf extract (1:1) effectively increased germination of egg plant seeds and tremendously decreased nursery diseases.

Khan (1999) studied the effect of plant extracts (allamanda, bel and neem) for the management of phomopsis blight/fruit rot of eggplant in field condition by spraying and observed that among the 3 plant extracts, allamanda was most effective than bel and neem extract.

2.5. Review of literature on biskatali leaf extract

Ashrafuzzaman and Khan (1992) found that bishkatali (*Polygonum hydropiper*) extracts inhibited the mycelial growth and spore germination of *Rhizoctonia solani* effectively.

Islam *et al*. (2006) evaluated eight plant extracts including Vitavax 200 against leaf spot (*Bipolaris sorokiniana*) of wheat. Among eight plant extracts, onion, garlic, kalijira, ginger, bishkatali and neem extract showed statistically similar grain yield as of seed treatment with Vitavax 200. Seed treatment with bishkatali extract increased 29.74% grain yield over untreated control.

2.6. Review of literature on turmeric rhizome extract

Rahman (2007) evaluated 13 plant extracts in controlling leaf blight of wheat in the field. He found turmeric extract very promising against leaf blight of wheat with increasing grain yield.

2.7. Review of literature on onion bulb extract

Rahman *et al*. (1999) evaluated extracts of 33 plant species in *vitro* against *Bipolaris sorokiniana*. The extracts of kalijira, turmeric, ginger, garlic, onion, neem, allamanda, nayantara, mandar, naglingam and duranta showed good effect in controlling the mycelial growth of *Bipolaris sorokiniana*.

2.8. Review of literature on kalijira seed extract

Alam *et. al*. (2010) reported that chloroform and ethanol extracts of black cumin seeds (*Nigella sativa*) were analyzed for antibacterial activity against five food and water borne pathogens, namely, *Staphylococcus aureus*, *Bacillus cereus*, *Vibrio cholerae*, *Klebsiella pneumoniae*, *Escherichia coli*, and one food spoilage bacteria *Bacillus subtilis*, all of which were previously found to be resistant to different antibiotics. The antibacterial activities of the extracts were determined by disc diffusion and tube dilution methods. All the bacterial strains except *E. coli and K. pneumoniae*, showed sensitivity to the chloroform extract as well as the bacterial strains except the *K. pneumoniae* showed sensitivities to ethanol extract. The minimum inhibitory concentration (MIC) and minimum

lethal concentration (MLC) of both extracts were also evaluated. Ethanol extract of black cumin was as found to be highly effective for *B. subtilis* (MIC value 375 µg/ml), followed by *S. aureus* (MIC value 1125 µg/ml).

2.9. Review of literature on over all plant extract

Khan and Hossain (1993) observed that extracts of *Allium cepa*, *A. sativum*, *Datura stramonium*, *D. plumeiri*, *Lawsonia alba*, *Ricinus communis*, *Leomurus sibiricus* and *Metha viridis* completely inhibited spore germination of *B. sorokiniana* at 1:3 (w/v) dilution ratio.

Ganguly (1994) reported that leaf extracts of *Vinca rosea, Lantana camada, Ocimum tenuiflorum, Solanum melongena, Azadirachta indica, Polyanthia longifolia, Aegle marmelos* and *Datura metel* showed antifungal activity against *Pyricularia oryzae* and *H. oryzae in vitro*. Extracts of *V. rosea* showed inhibition of mycelial growth and spore germination.

Ahmed (2000) found twelve rice seed samples infected by *B. oryzae* the cause of brown spot disease. Four fungicides viz. Bavistin, Homai, Tilt 250 EC and Dithane M-45 and four plant extracts viz. Biskatali, Onion, Garlic and Neem were evaluated against *B. oryzae*. Dithane M-45 was the best with 100% inhibition of the myclial growth at 0.3%. Neem and Garlic were effective against *B. oryzae* at 1:1 dilution. All test fungicides and plant extracts were effective against *B. oryzae* at higher concentration.

Hossain *et al.* (2005) tested different botanicals viz. bishkatali, vatpata, garlic, gagra, bitter guard and neem against seed borne fungi of wheat. Seed treatment of wheat by crude extract and alcoholic extract both in undiluted and diluted form for 24 hours reduced the incidence of *Bipolaris sorokiniana*, *Alternaria tenuis*, *Curvularia lunata*, *Fusarium* spp. and *Aspergillus* spp. and increased seed germination.

Ahmed *et al.* (2013) collected six rice varieties from Parshuram upazila, Feni district of Bangladesh and nine seed-borne fungi were detected from these seed samples. The identified fungi were *Fusarium oxysporum*, *F. moniliforme*,

Bipolaris oryzae, Alternaria padwickii, Curvularia lunata, Aspergillus flavus, Aspergillus niger, Penicillium sp. and *Nigrospora oryzae*. Five different plants extracts viz. garlic, allamanda, neem, chirata and bishkatali with two dilutions (1:1 & 1:2) were tested for seed treatment. Garlic extract (1:1) dilution found best for three varieties which successfully reduced seed-borne infection and also increased seed germination up to 68.39% over control. Neem (1:1) and Chirata (1:1) extracts also increased seed germination up to 66.09% and 67.81%, respectively.

Yeasmin *et. al.* (2012) treated rice seed with Garlic (*Allium sativum*) clove extract @ 1:0, 1:1, 1:2 dilutions in water, allamanda (*Allamanda cathartica)* leaf extract @ 1:1, 1:2, 1:3 dilutions in water and Provax-200 @ 0.3% for controlling seed borne fungi, where the seed samples of three rice varieties viz. Katharee, Gutee Aus and Kalijira were collected from farmer's storages of Bangladesh. The seed germination under control ranged from 64 to 77%, where treatments resulted up to 100% germination. The identified seed borne fungi of rice were *Bipolaris oryzae, Curvularia oryzae, Fusarium oxysporum, F. moniliforme, Nigrospora oryzae, Aspergillus flavus, Aspergillus niger* and *Penicillium* sp., where prevalence of *Bipolaris oryzae* (7.5%) and *Fusarium moniliforme* (8.3%) were the maximum. All the treatments significantly reduced the seed borne fungi up to 100% over the control, where Provax was found best and was statistically similar to garlic (1:1) extract against seed borne pathogen of rice.

Rahman *et. al.* (2006) evaluated the efficacy of some indigenous plant extracts in controlling leaf blight (*Bipolaris sorokiniana*) of wheat. Primarily 33 plant species were evaluated for their antifungal activity against *Bipolaris sorokiniana* by an in vitro test. Among them 13 species were found promising and selected for pot and field evaluation against leaf blight disease. Significantly higher grain yield/pot was obtained by treating seeds with allamanda leaf extract.

CHAPTER 3

MATERIALS AND METHODS

3.1. Experimental sites

The research activities were carried out in the Seed Pathology Laboratory (SPL) and Plant Disease Diagnostic Laboratory (PDDL) of the Department of Plant Pathology, Sher-e-Bangla Agricultural University (SAU), Dhaka.

3.2. Collection of seed samples

Altogether nine seed samples of three hybrid rice varieties were collected from local seed importer, research institute and local market of Bangladesh. Three seed samples were collected for each variety. BRRI hybrid dhan2 collected from Bangladesh Rice Research Institute, SL 8 collected from Bangladesh Agricultural Development Corporation (BADC) and Hira 2 collected from Supreme Seed Co. Ltd.

3.3. Test material (Hybrid rice varieties)

- Hira-2
- BRRI hybrid dhan2
- SL-8

3.4. Collection of botanicals

Botanicals were collected from different places (Table 1 and Plate 1). Garlic, kalizira, turmeric and onion were collected from the Agargoan market, Tejgaon, Dhaka. Leaves of neem, allamanda, bishkatali, datura, shimul were collected from Sher-e-Bangla Agricultural University campus.

Table 1. The particulars of botanicals used in this study

Common name	**English name**	**Scientific name**	**Plant parts used**	**Dilution (w/v)**
Neem	Margosa tree	*Azadirachta indica*	Leaf	1:1
Allamanda	Allamanda	*Allamanda cathartica*	Leaf	1:1
Garlic	Garlic	*Allium sativum*	Clove	1:1
Onion	Onion	*Allium cepa*	Bulb	1:1
Turmeric	Turmeric	*Curcuma longa*	Rhizome	1:1
Kalijira	Cumin black	*Nigella sativa*	Seed	1:1
Bishkatali	Smart weed	*Polygonum hydropiper*	Leaf	1:1
Datura	Datura	*Datura metel*	Leaf	1:1
Shimul	Silk cotton tree	*Salmalia malabarica*	Leaf	1:1

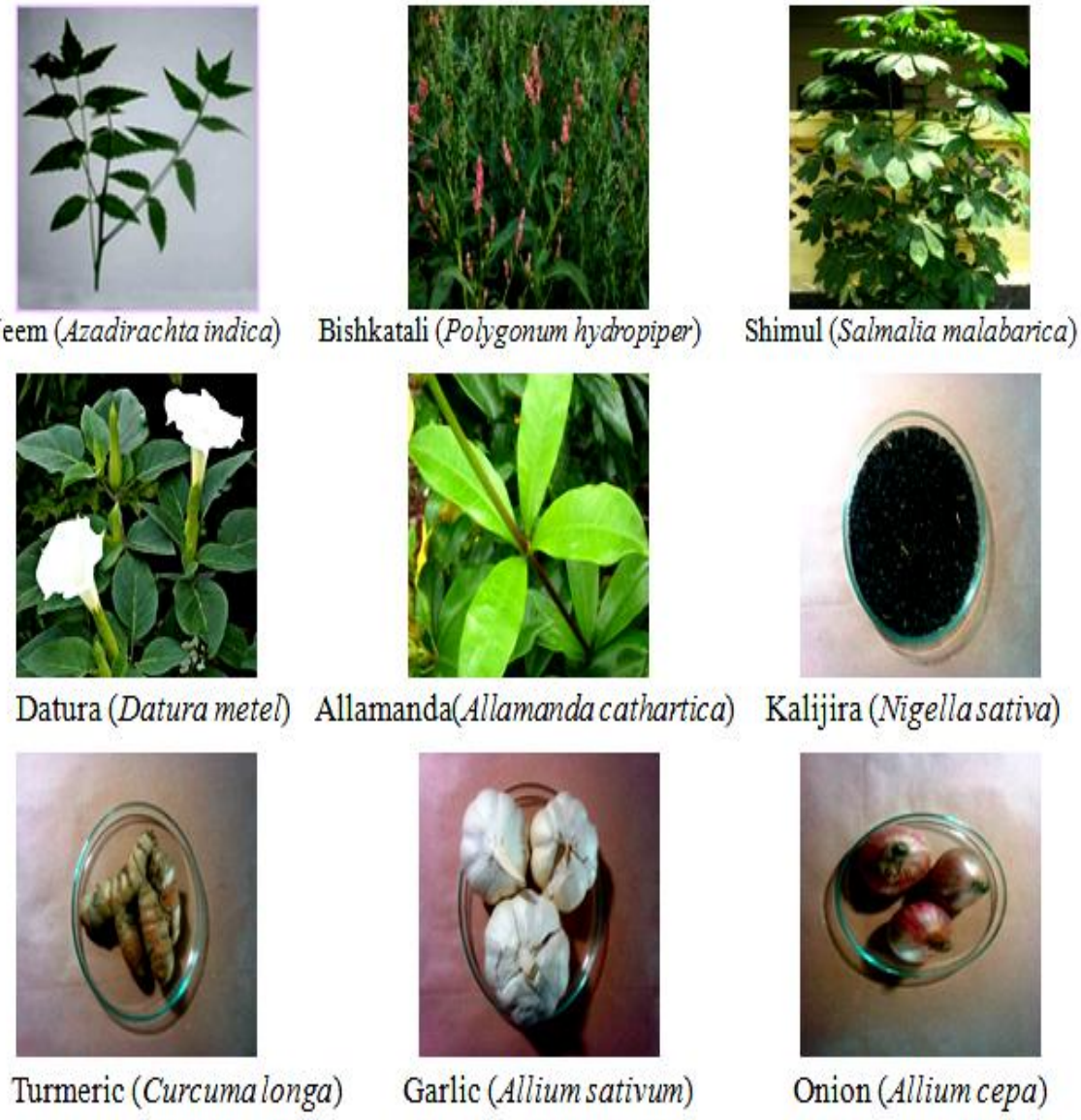


Plate 1: Botanicals used for seed treatment to test antifungal and antibacterial activities in hybrid rice seed

3.5. Treatments used in the experiment

T_1=Control

T_2= Seed treated with onion bulb extract (1:1 w/v)

T_3= Seed treated with kalijira seed extract (1:1 w/v)

T_4= Seed treated with allamonda leaf extract (1:1 w/v)

T_5= Seed treated with garlic clove extract (1:1 w/v)

T_6= Seed treated with neem leaf extract (1:1 w/v)

T_7= Seed treated with datura leaf extract (1:1 w/v)

T_8= Seed treated with turmeric rhizome extract (1:1 w/v)

T_9= Seed treated with biskatali leaf extract (1:1 w/v)

T_{10}= Seed treated with shimul leaf extract (1:1 w/v)

3.6. Laboratory experiment

Assaying of different seed treating techniques for reducing pathogen contamination and infection of imported hybrid rice seeds was done by Blotter method.

3.6.1. Blotter method

Seed samples treated with different seed treating agent was subjected to seed health analysis for collecting data on suppressing seed borne pathogen. Blotter method was done following International Rules for Seed Health Testing (ISTA, 1996). In this method 9 cm diameter glass petridish (pyrex, USA) and Whatman no.1 filter paper was used. Four hundred seeds from each sample was taken randomly and placed on the moist filter paper in eight replicate petridishes. The petridishes with treated seeds were incubated at 22 ± 2 °C for seven days in the laboratory under the alternate cycle of 12 hours NUV light and under 12 hours darkness for 7 days. After incubation the seeds were examined under stereomicroscope and the pathogens were identified following the key of Mathur and Kongsdal (2003). Appropriate keys (Booth, 1971; Misra *et al*., 1994 and Malone and Muskette, 1964.) were consulted for identification of the fungi and bacteria. The results were presented as percent incidence for individual pathogen. Germination of the seeds was also recorded.

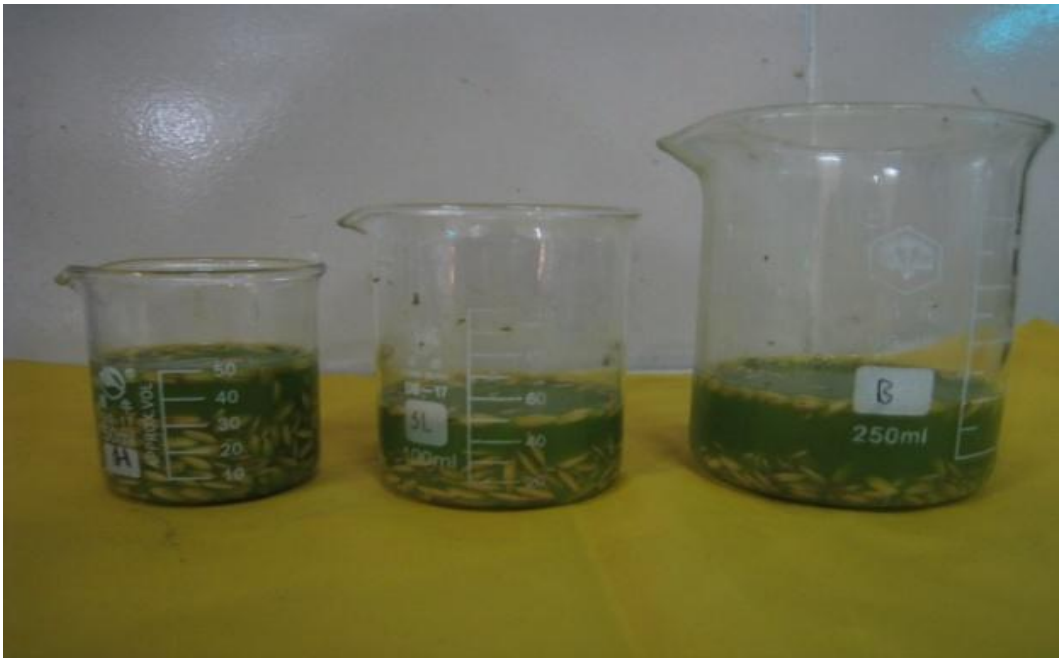
Each individual incubated seed was observed under stereomicroscope at 16x and 25x magnification in order to record the incidence of seed borne fungi. Most of the associated pathogens were detected by observing their growth characters on the incubated seeds on blotter paper following the keys outlined by Mathur and Kongsdal (2003). For proper identification of fungi temporary slides were prepared from the fungal colony and observed under compound microscope at 100x and 400x, and identified with the help of Keys suggested by Malone and Muskette (1964), Booth (1971), Ellis (1971), Chidambaram and Mathur (1975).

The fungi from the incubated seeds were also transferred to PDA when needed. The culture was incubated at 25±10°c for 3-7 days. Temporary slides prepared from the fungal colony and observed under compound microscope. The fungi were identified with the help of different books, manuals and publications (Ellis, 1971; Agarwal and Sing, 1974; Agarwal *et al.*, 1990). The results were presented as percent incidence for individual pathogen.

3.7. Preparation of plant extract

The extracts were prepared (Photograph 1 to 9) by using the method of Ashrafuzzaman and Hossain, (1992). For preparation of extracts, collected leaves were weighted in an electric balance and then washed in the water. After washing the big leaves were cut into small pieces. For getting extract, weighted plant parts were blended in an electric blender and then distilled water was added into the jug of the blender. The pulverized mass was squeezed through 3 folds of fine cotton cloth. For getting 1:1 (w/v) ratio 100 ml of distilled water was added with 100 g plant parts.

Photograph 1: Seed treatment with alamonda leaf extract



Photograph 2: Seed treatment with neem leaf extract

Photograph 3: **Seed treatment with garlic clove extract**

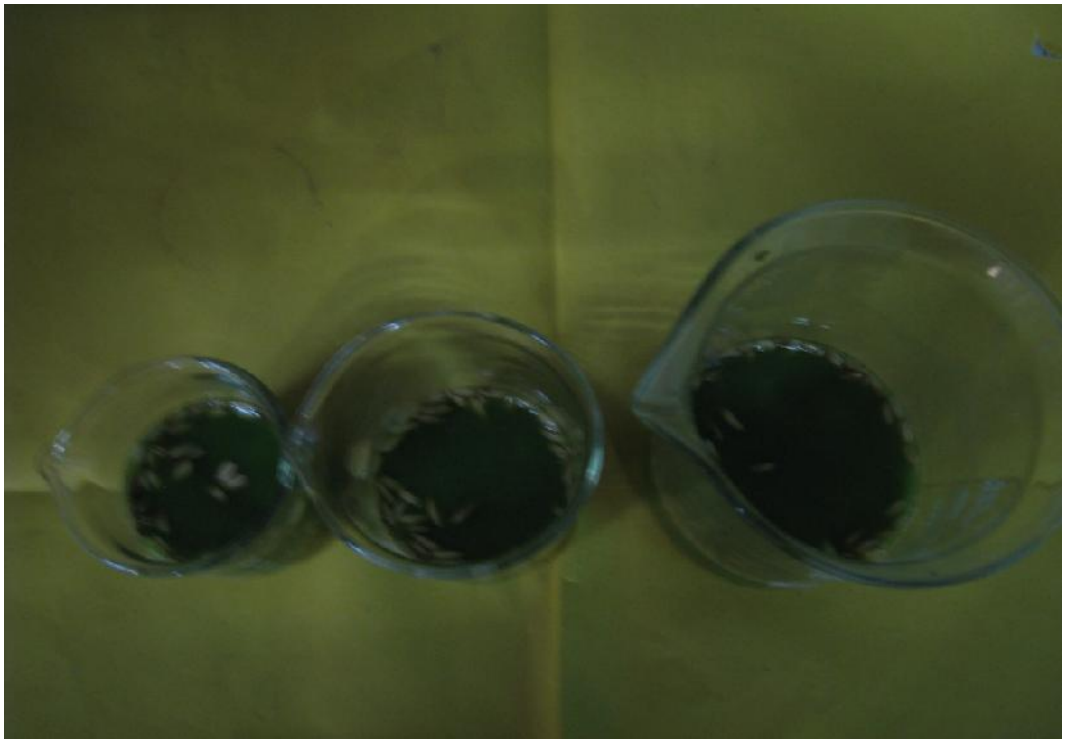
Photograph 4: **Seed treatment with onion bulb extract**

Photograph 5: **Seed treatment with datura leaf extract**

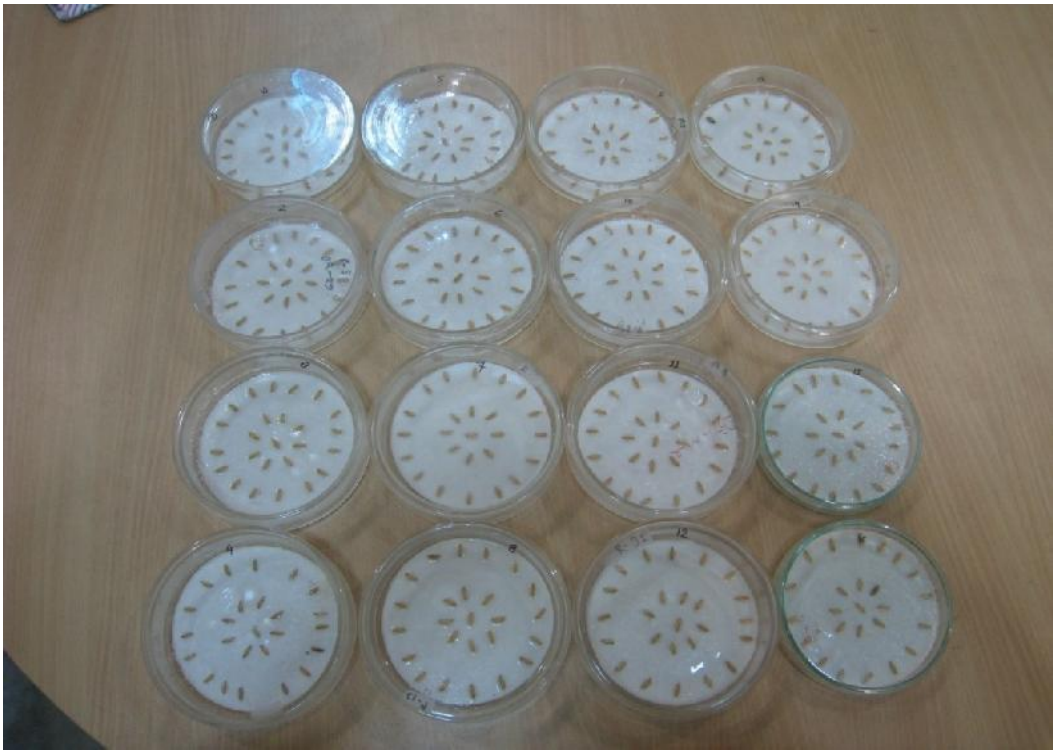
Photograph 6: **Seed treatment with shimul leaf extract**

Photograph 7: **Seed treatment with turmaric rhizom extract**

Photograph 8: **Seed treatment with biskatali leaf extract**



Photograph 9: **Seed treatment with kalijira seed extract**



Photograph 10: **Seed health study by blotter Method**

3.8. Preparation of culture media

3.8.1 Nutrient agar medium (NA)

Nutrient agar (15g) was taken in the Erlenmeyer flask containing 1000 ml distilled water. Peptone (5g) and beef extract (3g) were added to flask. For mixing properly the nutrient agar was shaked thoroughly for few minutes. Flask was then plugged with cotton and wrapped with a piece of brown paper and tied with thread. It was then autoclaved at 121^{o}C under 15 lbs pressure for 15 minutes. After autoclaving, the liquid medium was poured in the petridishes and solidified.

3.8.2. Isolation and identification of bacteria

Bacterial strains were cultured (Photograph 11 to 12) in NA media following streak plate method. After incubation at 30±1°C for 48 hrs individual colony was formed. Thus pure cultures of strains were prepared.

Photograph 11: **Pure culture of *Xanthomonas oryzae* pv. *oryzae* (Strain 1)**

Photograph 12: Pure culture of *Xanthomonas oryzae* pv. *oryzae* (Strain 2)

3.9. Pathological Study

3.9.1. Pathogenicity test

Cultured strains of bacteria (Photograph 13 to 14) were taken in plates and 5 ml sterile water was poured on the bacterial colonies grown in each plate. The suspension was poured in 250 ml Erlenmeyer flask. Bacterial suspensions of two strains were taken in the test tubes separately and rice plants (30 days after transplanting in pots) were inoculated by cutting leaf tip with scissors dipped in test tubes following the method of Kauffman *et al.* (1973). For control leaves were dipped in water. After inoculation, reaction was assessed by the following standard evaluation system.

Photograph 13: *Xanthomonas oryzae* pv. *oryzae* (Strain 1)

Photograph 14: *Xanthomonas oryzae* pv. *oryzae* (Strain 2)

Photograph 15: 30 days old seedling of (i) Hira 2, (ii) BRRI hybrid dhan2 and (iii) SL-8 respectively

Photograph 16: Inoculation with *Xanthomonas oryzae* pv. *oryzae* (Strain 1) to BRRI hybrid dhan2

Photograph 17: Inoculation with *Xanthomonas oryzae* pv. *oryzae* (Strain 2) to BRRI hybrid dhan2

Photograph 18: Inoculation with *Xanthomonas oryzae* pv. *oryzae* (Strain 1) to Hira 2

Photograph 19: Inoculation with *Xanthomonas oryzae* pv. *oryzae* (Strain 2) to Hira 2

Photograph 20: Inoculation with *Xanthomonas oryzae* pv. *oryzae* (Strain 1) to SL-8

Photograph 21: Inoculation with *Xanthomonas oryzae* pv. *oryzae* (Strain 2) to SL-8

Photograph 22: BLB symptom developed by *Xanthomonas oryzae* pv. *oryzae* (strain 1) on Hira 2

Photograph 23: BLB symptom developed by *Xanthomonas oryzae* pv. *oryzae* (strain 2) on Hira 2

Photograph 24: BLB symptom developed by *Xanthomonas oryzae* pv. *oryzae* (strain 1) on SL 8

Photograph 25: BLB symptom developed by *Xanthomonas oryzae* pv. *oryzae* strain 2 on SL 8

Photograph 26: BLB symptom developed by *Xanthomonas oryzae* pv. *oryzae* (strain 1) on BRRI hybrid dhan2

Photograph 27: BLB symptom developed by *Xanthomonas oryzae* pv. *Oryzae* (strain 2) on BRRI hybrid dhan2

3.9.2. Identification of bacterial strain

Pathogenicity test is the most reliable to identify *Xanthomonas* bacteria. The symptom develops in pathogenicity test is characteristic to the symptom of *Xanthomonas* so bacterial strain is identified as *Xanthomonas.* (Mew and Mishra,1994)

3.9.3. Measurement of percent leaf area diseased

To measure leaf area disease, a scale developed by Hossain and Azad in 1992 was used. Some modification of the scale was done for convenience.

3.10. *In vitro* screening of botanicals against *Xanthomonas oryzae* pv. *Oryzae*

3.10.1. Sterilization of plant extract

Plant extracts were prepared (Photograph 1 to 9) as previously mentioned method. For sterilization milipore filter was used. (Photograph 28)

Photograph 28: Plant extract sterilization by milipore filter

3.10.2. Bioassay

A supply of cotton swabs on wooden applicator sticks was prepared following the method described by Vandipitte *et al*. (1991). Two selected botanicals viz. Turmeric rhizome extract and Datura leaf extract against the bacterium by well diffusion method measuring the inhibition zone (Anon, 1996). Four wells of 5 mm in diameter were made in the same NA plate maintaining equal distance and the broth culture of *Xanthomonas oryzae* pv. *oryzae.* was spreaded uniformly on it with sterile cotton swabs. Plant extracts at definite concentration added into the well each at three replications. In case of control, only sterile water was used instead of chemical. The plates were incubated at 30±1ºC. Zone of inhibition around the wells were measured and recorded after every 24 hours for 5 days.

Here treatments were four different concentrations of turmeric rhizome extract and datura leaf extract with water. These are given below:

T_1 = Control

T_2= 1:1 (w/v)

T_3= 1:2 (w/v)

T_4= 1:4 (w/v)

3.11. Design of experiment: The laboratory experiment was conducted following Completely Randomized Design (CRD) with four replications according to ISTA rule.

3.12. Analysis of data: Data were analyzed by MSTAT-C computer package program and treatment means were compared by DMRT. Data were transformed by square root transformation method to reduce co-efficient of variation.

CHAPTER 4

RESULTS

4.1. Pathological properties of two bacterial strains obtained from hybrid rice seed

4.1.1. Pahtogenicity test

Both strains showed pathogenic reaction in pathogeniciy test in all of the varieties of hybrid rice. Leaf area diseased (% LAD) was recorded at 5, 10, 15, 20 and 30 days after inoculation on the variety of Hira-2, BRRI hybrid dhan2 and SL-8 (Table 2). The maximum leaf area diseased (more than 50%) was observed in SL 8 (Strain 2) and BRRI hybrid dhan2 (Strain 1) at 30 days after inoculation.

Table 2. Effect of two strains of *Xanthomonas oryzae* pv. *oryzae* on the development of BLB disease in hybrid rice seedlings

Variety	**Strains**	**Severity (%)**				
		5 DAI	**10 DAI**	**15 DAI**	**20 DAI**	**30 DAI**
SL-8	Strain 1	-	+	++	+++	++++
	Strain 2	+	++	+++	++++	+++++
BRRI hybrid dhan2	Strain 1	+	++	+++	++++	+++++
	Strain 2	-	-	+	++	+++
Hira-2	Strain 1	+	++	++	+++	++++
	Strain 2	+	+	++	++	++

Indication

- = No symptom

+ = Less than 5% leaf area damaged

++ = 5-10% leaf area damaged

+++ = 11-25% leaf area damaged

++++ = 26-50% leaf area damaged

+++++ = More than 50% leaf area damaged

DAI = Days after inoculation

4.2. Determination of seed health status of hybrid rice by blotter method

4.2.1. Identified Pathogens

The identified pathogens were *Xanthomonas oryzae* pv. *oryzae* (two strains), *Rhizopus stolonifer*, *Aspergillus flavus*, *Aspergillus niger*, *Fusarium moniliforme*, *Bipolaris oryzae*, *Curvularia lunata*, *Chaetomium globosum* and one unknown fungus (Table 3).

4.2.2. Incidence of seed borne pathogens

Results regarding the incidence of different seed borne fungi and bacteria in hybrid rice seed are presented in Table 3 and Photograph 29 to 44. In blotter method, seven fungi, two strains of bacteria and one unknown fungus were detected. It was observed that, incidence of *Xanthomonas oryzae* pv. *oryzae* (strain 1) ranged from 7.35 to 9.54 %. The highest incidence was observed in BRRI hybriddhan2 (9.54 %) and the lowest on Hira 2 (7.35%). Incidence of *Xanthomonas oryzae* pv. *oryzae* (strain 2) ranged from 2.50 to 4.55 % where the highest incidence was recorded on SL 8 (4.55 %) and the lowest in Hira 2 (2.50%). The incidence of *Aspergilllus flavus* and *Aspergilllus niger* ranged from 2.33 to 2.63 % and 0.88 to 1.57 %, respectively. The highest incidence of

Aspergilllus flavus was observed on BRRI hybrid dhan2 (2.63%) and lowest in SL 8 (0.88%) and highest incidence of *Aspergilllus niger* was observed in BRRI hybrid dhan2 (1.57%) and Hira 2 (0.88%) . The incidence of *Rhizophus stolonifer* ranged from 1.94 to 4.26 %. The highest incidence was recorded in SL 8 (4.26%). The incidence of *Fusarium moniliforme* ranged from 4.16 to 5.56%. The highest incidence was observed in Hira 2 (5.56%) and lowest in SL 8 (4.16%). The incidence of *Chaetomium globosum* ranged from 1.46 to 0.71 %.The highest incidence was observed in BRRI hybrid dhan2 (1.46 %) and lowest in Hira 2 (0.71%). The incidence of *Bipolaris oryzae* was recorded from 0.95 to 0.71%. The highest incidence was observed in Hira 2 (0.95 %) and the lowest in SL 8 (0.71%) and BRRI hybrid dhan2 (0.71%). The incidence of *Curvularia lunata* ranged from 1.97 to 1.06%. The highest was found in BRRI hybrid dhan2 (1.97%) and the lowest was observed in SL 8 (1.06%). One unknown fungus was also found in all tested varieties ranged between 2.88 to 1.96%, the highest incidence was found on Hira 2 (2.88%) and the lowest was observed in SL 8 (1.96%).

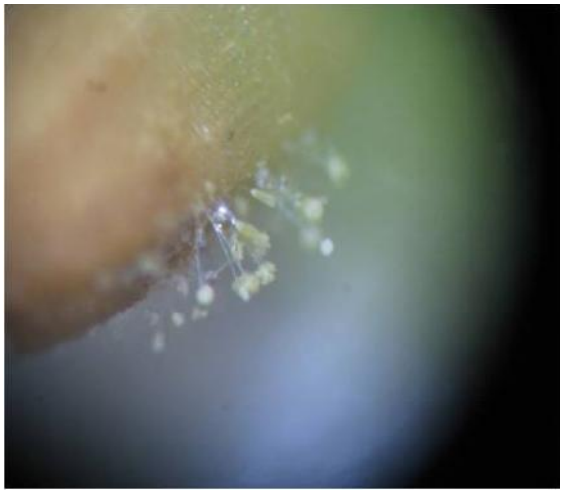
Table 3: Percent incidence of different seed borne pathogens on hybrid rice varieties by blotter method

Hybrid Rice Varieties	% Incidence									
	***Xanthomonas oryzae* pv. *oryzae* (strain 1)**	***Xanthomonas Oryzae* pv. *oryzae* (strain 2)**	***Aspergillus flavus***	***Aspergillus niger***	***Rhizopus stolonifer***	***Fusarium moniliforme***	***Chaetomium globosum***	***Bipolaris oryzae***	***Curvularia lunata***	**Unknown fungus**
Hira 2	7.350 b	2.500 b	2.385 a	0.8850 a	2.525 b	5.565 a	0.7100 a	0.9575 a	1.790 ab	2.880 a
SL 8	9.205 a	4.557 a	2.335 a	0.9575 a	4.265 a	4.168 b	1.030 a	0.7100 a	1.060 b	1.965 a
BRRI hybrid dhan2	9.540 a	2.588 b	2.638 a	1.575 a	1.947 b	4.262 b	1.465 a	0.7100 a	1.977 a	2.013 a
$LSD_{0.05}$	1.178	1.327	0.6972	0.8014	0.8569	1.298	1.019	0.3077	0.8479	1.086
CV%	8.46	25.80	17.79	44.01	18.38	17.39	59.65	24.13	32.93	29.69

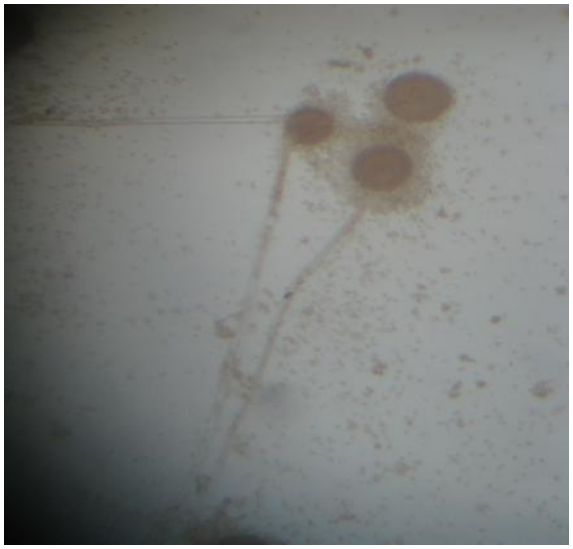
Values with different letters with in a column differ significantly at 5% level of significance as per DMRT Data are transformed by square root method

4.2.2.1. Characteristics of *Aspergillus flavus*

The growth of the fungus on seed was characterized by immature, white head and mature heads in shades ranging from yellow cream to green (Photograph 29 and 30). Conidiophores bearing the head were clearly seen when the growth was light, long and hyaline terminating in bulbous heads. Conidia globose to subglobose, usually were rough, yellowish green.



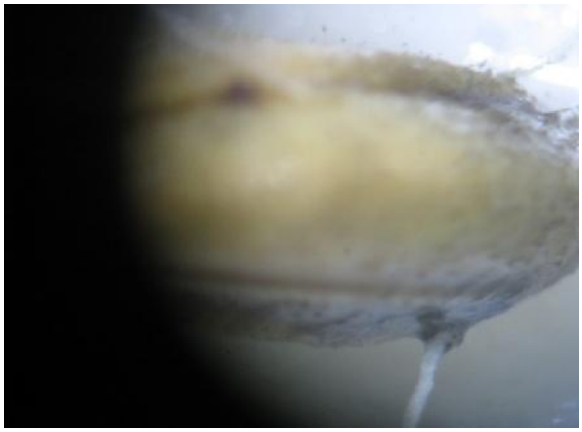
Photograph 29: *Aspergillus flavus* on rice seed under steriomicroscope (60x)



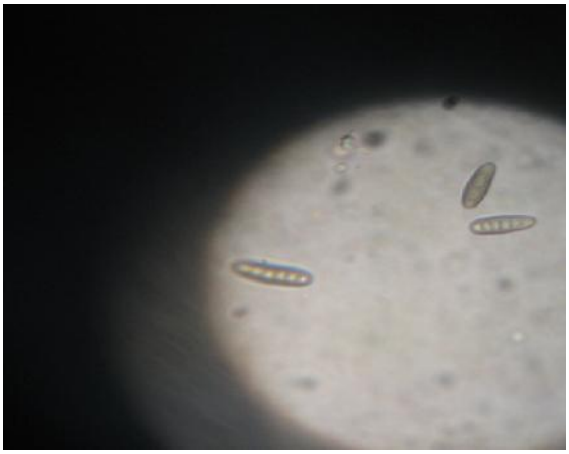
Photograph 30: *Aspergillus flavus* on rice seed under compound microscope (100x)

4.2.2.2. Characteristics of *Bipolaris oryzae*

Mycelium scanty, conidiophores straight or flexuous, brown, bring dark brown conidia arranged acropleurogenously. Conidia not curved, tapering towards the end, olivacious to dark brown. Conidia cylindrical, pale to mid golden brown, smooth, widest around the middle, tapering to rounded ends, base more rounded, hilum in most cases inconspicuous , within the contour of the basal cell seen as a papilla like structure, but in few cases a clear scar is visible (Photograph 31 and 32).



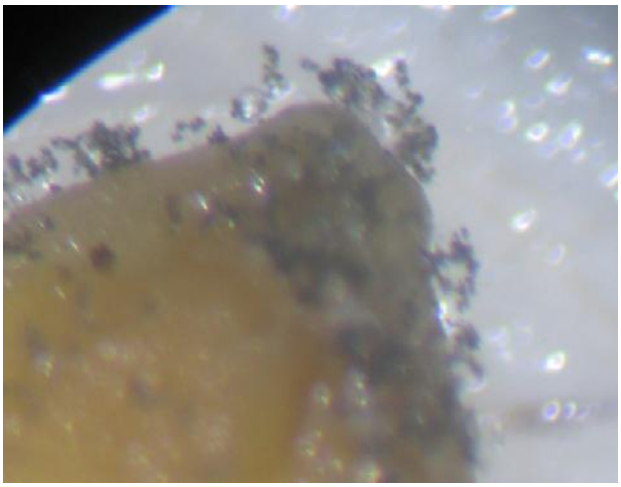
Photograph 31: *Bipolaris oryzae* on rice seed under stereomicroscope (60x)



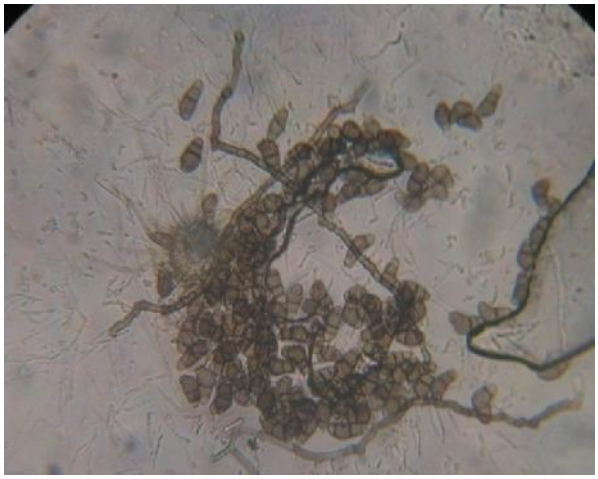
Photograph 32: *Bipolaris oryzae* on rice seed under compound microscope (400x)

4.2.2.3. Characteristics of *Curvularia lunata*

Aerial mycelia were light brown to brown with abundant branching. Conidiophores were solitary or in groups; dark brown; straight, sometime bent; simple; arising directly from the seed surface. Conidia were borne more or less at the tip in a whorl or in thick panicles (Photograph 33 and 34).



Photograph 33: *Curvularia lunata* on rice seed under stereomicroscope (60x)

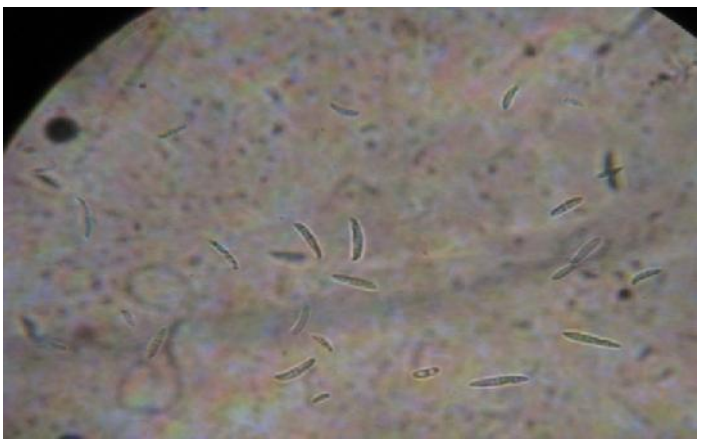


Photograph 34: Conidia of *Curvularia lunata* under compound microscope (400x)

4.2.2.4. Characteristics of *Fusarium monilimorme*

Aerial mycelia were scanty, creeping close to the seed surface and loosely branched. Aerial mycelia appear white with small, dirty to creamy white false heads borne on long, simple, upright to slightly bent conidiophores. Micro and macro conidia were present. Micro conidia were two celled and macroconidia were three to five celled (Photograph 35 and 36).

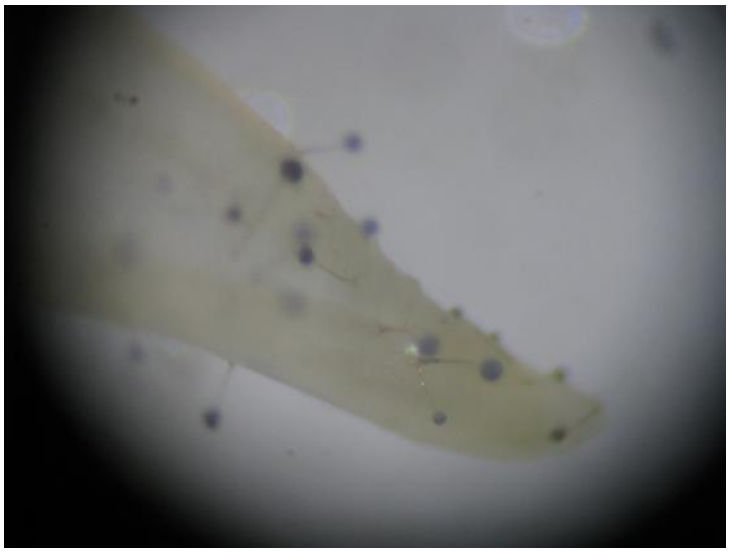
Photograph 35: *Fusarium moniliforme* on rice seed under stereo microscope (60x)



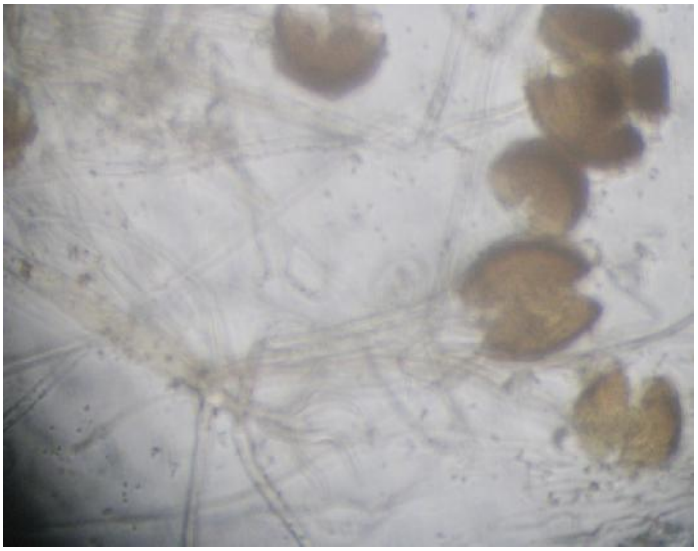
Photograph 36: Conidia of *Fusarium moniliforme* under compound microscope (400x)

4.2.2.5. Characteristics of *Rhizopus stolonifer*

Gray sporangia were present on whole seed. Rhizoids, sporangiophores, sporangium and sporangiospores were clearly visible (Photograph 37 and 38).



Photograph 37: ***Rhizopus stolonifer*** **on rice seed under stereomicroscope (60x)**

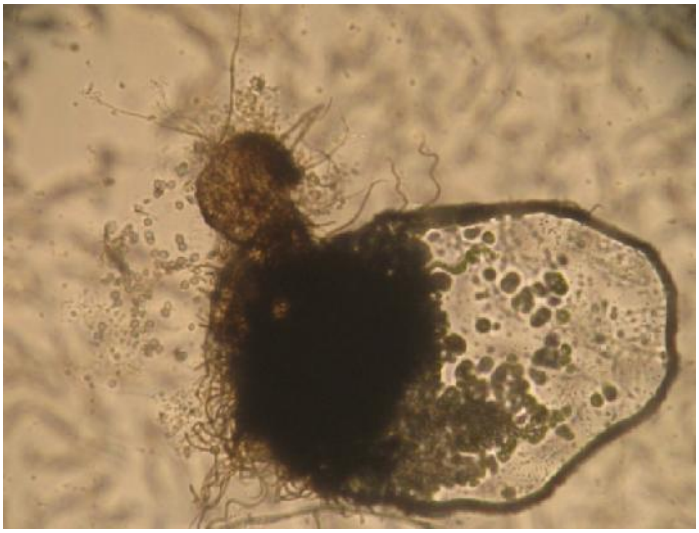


Photograph 38: mycelia and sporangia of *Rhizopus stolonifer* under compound microscope (400x)

4.2.2.6. Characteristics of *Chaetomium globosum*

Perithecium was dark brown, ostiolate, sub-globose with brown flexuous hairs. Awn and lemma were present (Photograph 39).

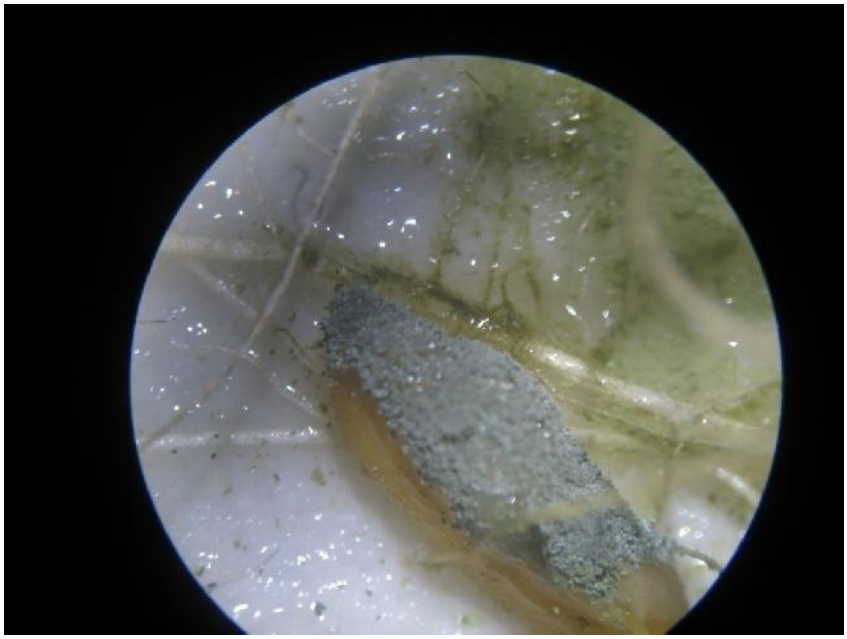
Photograph 39*:* ***Chaetomium globosum*** **on Rice seed under stereo microscope (60x)**



Photograph 40: **Spore of *Chaetomium globosum* under compound microscope (400x)**

Photograph 41: **Bacterial ooze of *Xanthomonas oryzae* pv. *oryzae* strain 1 under stereomicroscope (60x)**

Photograph 42: **Bacterial ooze of *Xanthomonas oryzae* pv. *oryzae* strain 2 under stereomicroscope (60x)**



Photograph 43: **An unknown fungus under stereomicroscope**

4.3. Evaluation of some selected plant extracts against seed borne pathogens of Hira 2 hybrid rice

The effect of different plant extracts on pathogen incidence of hybrid rice (Hira 2) is shown in Table 4. The incidence of seed borne pathogens at different treatments was significantly differing from one to another. The highest incidence of *Xanthomonas oryzae* pv. *oryzae* strain 1 was recorded in T_1 (7.35%) followed by T_2 (7.06%), T_3 (5.11%), T_4 (3.96%), T_9 (3.45%) and the lowest incidence was recorded in T_7 (1.82%) followed by T_8 (2.11%), T_6 (2.38%), T_5 (2.69%), T_{10} (3.34%). No significant difference was observed among (T_1 and T_2); (T_4, T_9 and T_{10}); (T_5 and T_6) and (T_7 and T_8). The highest incidence of *Xanthomonas oryzae* pv. *oryzae* strain 2 was recorded in T_1(2.5%) followed by T_2 (1.23%), T_{10} (1.03%), T_9 (0.88%), T_7 (0.88%) and the lowest incidence was recorded in T_8 (0.71%) and rest of the treatments showed same percentage of incidence. No significant difference was observed among (T_2, T_3, T_4, T_5, T_6, T_7, T_8, T_9 and T_{10}. The highest incidence of *Aspergillus flavus* was recorded in T_1 (2.38%) followed by T_9 1.76%), T_6 (1.38%), T_{10} (1.38%), T_7 (1.14%) and the lowest incidence was recorded in T_8 (0.71%) followed by T_5 (0.88%) and incidence among T_2, T_3, T_4 was 0.710%.Treatments T_2, T_3, T_4, T_5, T_7 and T_8 were statistically similar. The highest incidence of *Aspergillus niger* was recorded in T_1 (0.88%) and the rest of the treatments showed 0.710% of incidence. The highest incidence of *Rhizopus stolonifer* was recorded in T_1 (2.20%) followed by T_{10} (1.03%) and rest of the treatments showed 0.71% incidence. The highest incidence of *Fusarium moniliforme* was recorded in T_1 (5.56%) followed by T_2 (4.05%), T_6 (1.46%), T_{10} (1.41%), T_3 (1.41%) and the lowest incidence was 0.710% recorded in T_8,T_9 and T_5 followed by T_7 (1.06%)and T_4 (1.06%). No significant difference was observed among T_3, T_4, T_5, T_6, T_7, T_8, T_9 and T_{10} treatments. The highest incidence of *Bipolaris oryzae* and *Curvularia lunata* were observed in T_1 (0.95% and 1.79% respecively) and 0.71% incidence was recorded in rest of the treatments. The highest incidence of an unknown fungi was recorded in T_1 (2.880%) followed by T_9 (1.53%), T_6

(1.35%) while lowest incidence was observed in T_8 (0.71%) and rest of the treatments resulted 0.71% incidence as well.

Table 4: Evaluation of some selected plant extract against seed borne pathogen of Hira 2 hybrid rice

Treatments	% Incidence								
	***Xanthomonas oryzae* pv. *oryzae* (strain1)**	***Xanthomonas oryzae* pv. *oryzae* (strain 2)**	***Aspergillus flavus***	***Aspergillus niger***	***Rhizopus stolonifer***	***Fusarium moniliforme***	***Bipolaris oryzae***	***Curvularia lunata***	**Unknown fungus**
T_1 (Control)	7.350 a	2.500 a	2.385 a	0.885 a	2.203 a	5.565 a	0.957 a	1.790 a	2.880 a
T_2 (Onion)	7.065 a	1.235 b	0.710 d	0.710 b	0.710 b	4.055 b	0.710 b	0.710 b	0.710 c
T_3 (Kalijira)	5.110 b	0.710 b	0.710 d	0.710 b	0.710 b	1.415 c	0.710 b	0.710 b	0.710 c
T_4 (Allamonda)	3.960 c	0.710 b	0.710 d	0.710 b	0.710 b	1.060 c	0.710 b	0.710 b	0.710 c
T_5 (Garlic)	2.693 def	0.710 b	0.885 cd	0.710 ab	0.710 b	0.710 c	0.710 b	0.710 b	0.710 c
T_6 (Neem)	2.382 ef	0.710 b	1.382 bc	0.710 ab	0.710 b	1.467 c	0.710 b	0.710 b	1.352 b
T_7 (Datura)	1.852 f	0.885 b	1.140 cd	0.710 ab	0.710 b	1.060 c	0.710 b	0.710 b	0.710 c
T_8 (Turmeric)	2.112 f	0.710 b	0.710 d	0.710 ab	0.710 b	0.710 c	0.710 b	0.710 b	0.710 c
T_9 (Bishkatali)	3.455 cd	0.885 b	1.765 b	0.710 ab	0.710 b	0.710 c	0.710 b	0.710 b	1.530 b
T_{10} (Shimul)	3.340 cde	1.033 b	1.382 bc	0.710 ab	1.033 b	1.410 c	0.710 b	0.710 b	0.710 c
$LSD_{0.05}$	1.015	0.6093	0.5667	0.1582	0.5518	0.9568	0.1515	0.3417	0.4235
CV (%)	17.88	41.87	33.28	15.21	42.83	36.49	14.25	29.02	27.35

Values with different letters with in a column differ significantly at 5% level of significance as per DMRT Data are transformed by square root method

4.4. Evaluation of some selected plant extracts against seed borne pathogens of SL 8 hybrid rice

The effect of different plant extracts on pathogen incidence of hybrid rice (SL 8) is shown in Table 5. The incidence of seed borne pathogens at different treatments was significantly differing from one to another. The highest incidence of *Xanthomonas oryzae* pv. *oryzae* strain 1 was recorded in T_1 (9.20%) followed by T_4 (7.53%), T_2 (6.91%), T_5 (6.53%), T_6 (6.10%) and the lowest incidence was recorded in T_8 (3.44%) followed by T_9 (3.56%), T_7 (3.66%), T_{10} (4.52%), T_3 (4.63%). No significant difference was observed between (T_2 and T_5) and (T_7, T_8 and T_9). The highest incidence of *Xanthomonas oryzae* pv. *oryzae* strain 2 was recorded in T_1 (4.55%) followed by T_3 (2.28%), T_7 (1.81%), T_6 (1.64%), T_8 (1.64%) and the lowest incidence was recorded in T_{10} (0.71%) followed by T_2 (1.06%), T_4 (1.06%), T_5 (1.38%) and T_9 (1.46%). No significant difference was observed among (T_2, T_4, T_5, T_6, T_7, T_8, T_9 and T_{10}). The highest incidence of *Aspergillus flavus* was recorded in T_1 (2.33%) followed by T_6 (1.23%), T_9 (1.06%), T_{10} (0.88%), and the lowest incidence was recorded in T_8 (0.71%) and incidence among T_2, T_3, T_4, T_5 and T_7 was 0.71%.Treatments among T_2, T_3, T_4, T_5, T_7,T_8 and T_{10} were statistically similar. No significant difference was observed between treatment T_6 and T_9. The highest incidence of *Aspergillus niger* was recorded in T_6 (1.81%) followed by T_1 (0.95%), T_2 (0.88% and rest of the treatments showed 0.71% of incidence. The highest incidence of *Rhizopus stolonifer* was recorded in T_1 (4.26%) and rest of the treatments showed 0.71% incidence. The highest incidence of *Fusarium moniliforme* was recorded in T_1 (4.16%) followed by T_6 (2.41%), T_2 (1.64%), T_7 (1.06%), T_5 (1.03%) and the lowest incidence was 0.71% recorded in T_3,T_4, T_9 and T_{10} followed by T_8 (0.88%). No significant difference was observed among T_3, T_4, T_5, T_7, T_8, T_9 and T_{10} treatments. The highest incidence of *Chaetomium globosum* was in T_1 (1.03%) followed by T_9 (0.95%) and rest of the treatments showed 0.71% incidence. *Curvularia lunata* was observed maximum in T_1 (1.06%) and 0.71% incidence rest of the treatments showed. The highest incidence of an unknown fungi was recorded in T_1 (1.96%)

followed by T_3 (1.23%),T_6 (1.06%), T_9 (0.88%), T_2 (0.53%) while lowest incidence was observed in T_8 (0.71%) and rest of the treatments showed 0.71% incidence as well.

Table 5: Evaluation of some selected plant extract against seed borne pathogen of SL 8 hybrid rice

Treatments	% Incidence								
	***Xanthomonas oryzae* pv. *oryzae* (strain 1)**	***Xanthomonas oryzae pv. oryzae* (strain 2)**	***Aspergillus flavus***	***Aspergillus niger***	***Rhizopus stolonifer***	***Fusarium moniliforme***	***Chaetomium globosum***	***Curvularia lunata***	**Unknown fungus**
T_1 (Control)	9.205 a	4.557 a	2.335 a	0.957 b	4.265 a	4.168 a	1.030 a	1.060 a	1.965 a
T_2 (Onion)	6.912 bc	1.060 cd	0.710 c	0.885 b	0.710 b	1.643 c	0.710 b	0.710 b	0.532 d
T_3 (Kalijira)	4.637 e	2.287 b	0.710 c	0.710 b	0.710 b	0.710 d	0.710 b	0.710 b	1.235 b
T_4 (Allamonda)	7.533 b	1.060 cd	0.710 c	0.710 b	0.710 b	0.710 d	0.710 b	0.710 b	0.710 cd
T_5 (Garlic)	6.537 cd	1.382 bcd	0.710 c	0.710 b	0.710 b	1.033 cd	0.710 b	0.710 b	0.710 cd
T_6 (Neem)	6.105 d	1.643 bcd	1.235 b	1.817 a	0.710 b	2.415 b	0.710 b	0.710 b	1.060 bc
T_7 (Datura)	3.660 f	1.817 bc	0.710 c	0.710 b	0.710 b	1.060 cd	0.710 b	0.710 b	0.710 cd
T_8 (Turmeric)	3.445 f	1.643 bcd	0.710 c	0.710 b	0.710 b	0.885 d	0.710 b	0.710 b	0.710 cd
T_9 (Bishkatali)	3.563 f	1.467 bcd	1.060 bc	0.710 b	0.710 b	0.710 d	0.957 a	0.710 b	0.885 bcd
T_{10} (Shimul)	4.523 e	0.710 d	0.885 c	0.710 b	0.710 b	0.710 d	0.710 b	0.710 b	0.710 cd
$LSD_{0.05}$	0.7292	0.9481	0.3197	0.3197	0.2924	0.5884	0.1991	0.1827	0.3875
CV (%)	8.99	37.25	22.56	25.58	18.96	29.03	18.10	17.15	29.00

Values with different letters with in a column differ significantly at 5% level of significance as per DMRT Data are transformed by square root method

4.5. Evaluation of some selected plant extracts against seed borne pathogens of BRRI hybrid dhan2 hybrid rice

The effect of different plant extracts on pathogen incidence of hybrid rice (BRRI hibriddhan 2) is shown in Table 6. The incidence of seed borne pathogens at different treatments was significantly differing from one to another. The highest incidence of *Xanthomonas oryzae* pv. *oryzae* strain 1 was recorded in T_1 (9.54%) followed by T_4 (8.42%), T_3 (6.85%), T_2 (6.59%), T_5 (5.60%) and the lowest incidence was recorded in T_8 (3.29%) followed by T_7 (3.78%), T_9 (4.63%), T_{10} (5.34%), T_5 (5.60%). No significant difference was observed among T_2 and T_3; T_5,T_6,T_9 and T_{10}; and T_7 and T_8. The highest incidence of *Xanthomonas oryzae* pv. *oryzae* strain 2 was recorded in T_6 (2.59%) followed by T_1 (2.58%), T_9 (1.81%), T_5 (1.64%), T_7 (1.53%) and the lowest incidence was recorded in T_4 (0.53%) followed by T_{10} (0.88%), T_3 (1.03%), T_8 (1.05%) and T_2 (1.46%). No significant difference was observed among T_2, T_3, T_5, T_6, T_7, T_8, and T_9. The highest incidence of *Aspergillus flavus* was recorded in T_1 (2.63%) followed by T_9 (1.56%), T_2 (1.53%), T_{10} (1.38%), T_7 (0.88%) and the lowest incidence was recorded in T_8 (0.71%) and incidence among T_3, T_4, T_5, T_6, T_{10} was 0.71%. Treatments T_3, T_4, T_5, T_6, T_{10} were statistically similar. The highest incidence of *Aspergillus niger* was recorded in T_1 (1.57%) followed by T_6 (0.88%) and lowest incidence was observed in T_8 and the rest of the treatments showed 0.71% of incidence. The highest incidence of *Rhizopus stolonifer* was recorded in T_1 (1.94%) and rest of the treatments showed 0.71% incidence. The highest incidence of *Fusarium moniliforme* was recorded in T_1 (4.26%) followed by T_2 (3.48%), T_6 (2.94%), T_4 (1.06%), T_9 (0.88%), T_7 (0.88%) and the lowest incidence was 0.71% recorded in T_8,T_3 followed by T_5 (0.88%). No significant difference was observed among (T_2 and T_6); T_3, T_4, T_5, T_7, T_8, T_9 and T_{10} treatments. The highest incidence of *Chaetomium globosum* and *Curvularia lunata* were observed in T_1 (1.32% and 1.54% respecively) and 0.71% incidence was recorded in rest of the treatments. The highest incidence of an unknown fungi was recorded in T_1 (2.01%) followed by T_6 (1.06%), T_3 (0.96%), T_9 (0.88%)

while lowest incidence was observed in T_8 (0.71%) and rest treatments resulted 0.71% incidence as well.

Table 6: Evaluation of some selected plant extract against seed borne pathogen BRRI hybrid dhan2 of hybrid rice

Treatments	% Incidence								
	***Xanthomonas oryzae* pv. *oryzae* (strain 1)**	***Xanthomonas oryzae* pv. *oryzae* (strain 2)**	***Aspergillus flavus***	***Aspergillus niger***	***Rhizopus stolonifer***	***Fusarium moniliforme***	***Chaetomium globosum***	***Curvularia lunata***	**Unknown fungus**
T_1 (Control)	9.540 a	2.588 a	2.638 a	1.575 a	1.947 a	4.262 a	1.320 a	1.543 a	2.013 a
T_2 (Onion)	6.590 c	1.465 abc	1.530 b	0.710 b	0.710 b	3.480 b	0.710 b	0.710 b	0.710 b
T_3 (Kalijira)	6.858 c	1.030 bc	0.710 c	0.710 b	0.710 b	0.710 c	0.710 b	0.710 b	0.965 b
T_4 (Allamonda)	8.427 b	0.530 c	0.710 c	0.710 b	0.710 b	1.060 c	0.710 b	0.710 b	0.710 b
T_5 (Garlic)	5.608 d	1.643 abc	0.710 c	0.710 b	0.710 b	0.885 c	0.710 b	0.710 b	0.710 b
T_6 (Neem)	5.030 de	2.598 a	0.710 c	0.885 b	0.710 b	2.945 b	0.710 b	0.710 b	1.060 b
T_7 (Datura)	3.785 f	1.530 abc	0.885 c	0.710 b	0.710 b	0.885 c	0.710 b	0.710 b	0.710 b
T_8 (Turmeric)	3.298 f	1.058 bc	0.710 c	0.710 b	0.710 b	0.710 c	0.710 b	0.710 b	0.710 b
T_9 (Bishkatali)	4.635 e	1.817 ab	1.563 b	0.710 b	0.710 b	0.885 c	0.710 b	0.710 b	0.885 b
T_{10} (Shimul)	5.345 de	0.885 bc	0.710 c	0.710 b	0.710 b	0.885 c	0.710 b	0.710 b	0.710 b
$LSD_{0.05}$	0.6986	1.078	0.5884	0.3653	0.2416	0.7490	0.3766	0.2583	0.5556
CV(%)	8.18	49.29	37.46	31.18	19.98	31.02	33.73	22.70	41.95

Values with different letters with in a column differ significantly at 5% level of significance as per DMRT Data are transformed by square root method

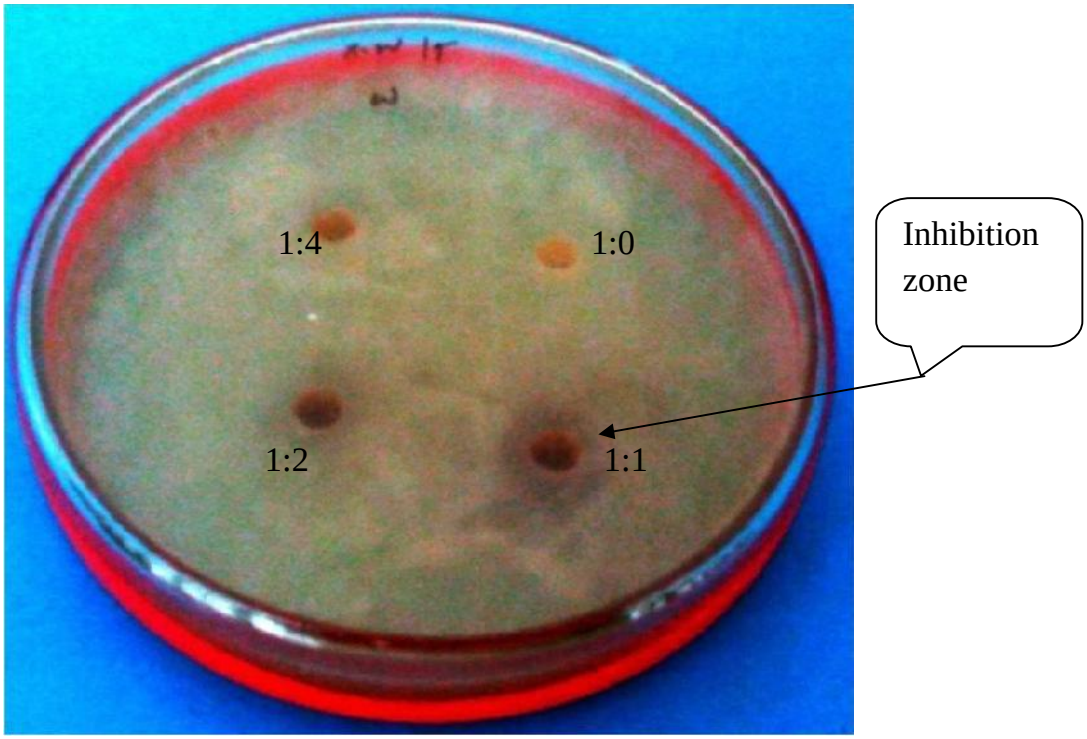
4.6. Efficacy of turmeric rhizome extract against *Xanthomonas oryzae* pv. *oryzae* strain 1

The effect of different concentrations of turmeric rhizome extract were tested against *Xanthomonas oryzae* pv. *oryzae* strain 1 and only T_2 showed inhibition zone (Table 7 and Photograph 44). In T_2 after 24 hour no inhibition zone was observed. After 48 hour it was 7.33 mm, followed by 9.33 mm after 72 hours,12.67 mm after 96 hours and 15.67 mm after 120 hours. Rest of the treatments did not result any inhibition zone.

Table 7: **Comparative efficacy of turmeric rhizome extract against *Xanthomonas oryzae* pv. *oryzae* strain 1**

Treatment	Inhibition zone (mm) after				
	24hr	**48hr**	**72hr**	**96hr**	**120hr**
T_1 (Control)	0	0 b	0 b	0 b	0 b
T_2 (1:1 w/v)	0	7.33 a	9.33 a	12.67 a	15.67 a
T_3 (1:2 w/v)	0	0 b	0 b	0 b	0 b
T_4 (1:4 w/v)	0	0 b	0 b	0 b	0 b
$LSD_{0.05}$	-	0.5065	0.5065	0.5065	0.5065
CV%	-	6.82	5.36	3.95	3.19

Values with different letters with in a column differ significantly at 5% level of significance as per DMRT



Photograph 44: **Inhibition zone occurred by turmeric rhizome extract against *Xanthomonas oryzae* pv. *oryzae* strain 1**

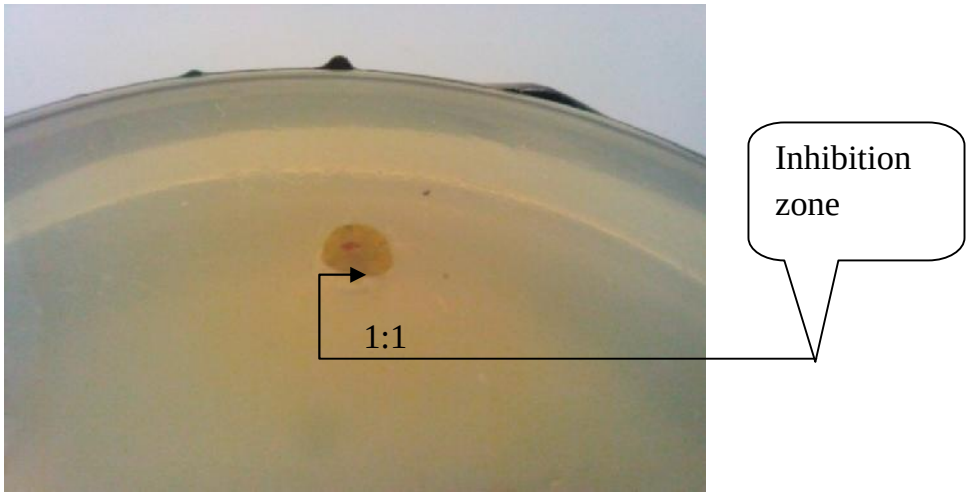
4.7. Comparative efficacy of datura leaf extract against *Xanthomonas oryzae* pv. *oryzae* strain 1

The effect of different concentrations of datura leaf extract were tested against *Xanthomonas oryzae* pv. *oryzae* strain 1 and only T_2 showed inhibition zone in (Table 8 and Photograph 45). In T_2 after 72 hour no inhibition zone was observed. After 96 hour it was 6.33 mm, followed by 8.33 mm after 120 hours. Rest of the treatments did not result inhibition zone.

Table 8: **Comparative efficacy of datura leaf extract against *Xanthomonas oryzae* pv. *oryzae* strain 1**

Treatment	Inhibition zone (mm) after		
	72hr	96hr	120hr
T_1 (Control)	0	0 b	0 b
T_2 (1:1 w/v)	0	6.33 a	8.33 a
T_3 (1:2 w/v)	0	0 b	0 b
T_4 (1:4 w/v)	0	0 b	0 b
$LSD_{0.05}$	-	0.5065	0.5065
CV%	-	7.89	6.00

Values with different letters with in a column differ significantly at 5% level of significance as per DMRT



Photograph 45: **Inhibition zone occurred by datura leaf extract against *Xanthomonas oryzae* pv. *oryzae* strain 1**

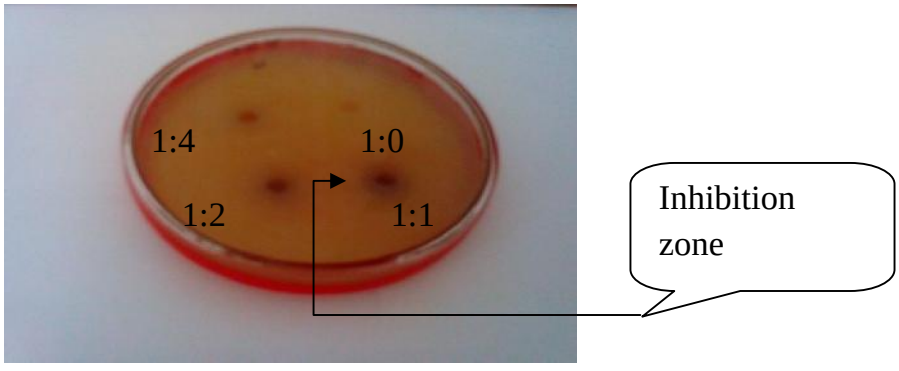
4.8. Comparative efficacy of turmeric rhizome extract against *Xanthomonas oryzae* pv. *oryzae* strain 2

The effects of different concentrations of turmeric rhizome extract were tested against *Xanthomonas oryzae* pv. *oryzae* strain 2 and only T_2 resulted inhibition zone in (Table 9 and Photograph 46. In T_2 after 24 hour no inhibition zone was observed. After 48 hour it was 7.33 mm, followed by 9.33 mm after 72 hours, 13.33 mm after 96 hours and 15.33 mm after 120 hours. Rest of the treatments did not resaulted inhibition zone.

Table 9: Comparative efficacy of turmeric rhizome extract against *Xanthomonas oryzae* pv. *oryzae* strain 2

Treatment	Inhibition zone (mm) after				
	24hr	48hr	72hr	96hr	120hr
T_1 (Control)	0	0 b	0 b	0 b	0.00 b
T_2 (1:1 w/v)	0	7.33 a	9.33 a	13.33 a	15.33 a
T_3 (1:2 w/v)	0	0 b	0 b	0 b	0 b
T_4 (1:4 w/v)	0	0 b	0 b	0 b	0 b
$LSD_{0.05}$	-	0.5065	0.5065	0.5065	0.5065
CV%	-	6.82	5.36	3.75	3.26

Values with different letters with in a column differ significantly at 5% level of significance as per DMRT



Photograph 46: **Inhibition zone occurred by turmeric rhizome extract against *Xanthomonas oryzae* pv. *oryzae* strain 2**

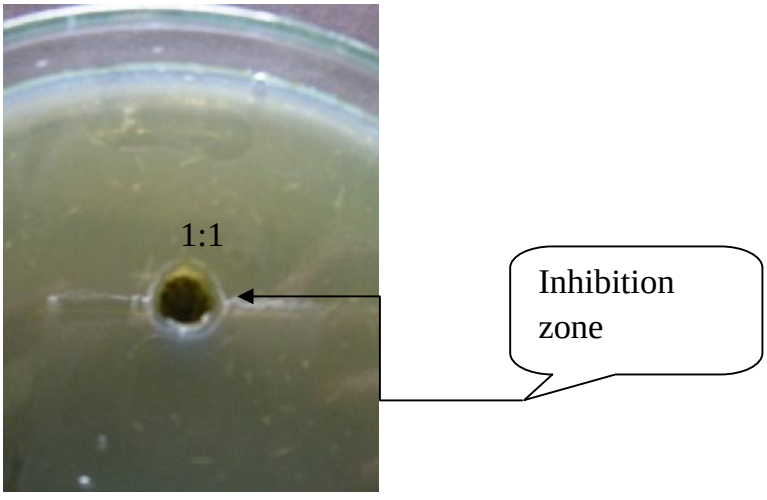
4.9. Comparative efficacy of datura against *Xanthomonas oryzae* pv. *oryzae* strain 2

The effect of different concentrations of datura leaf extract were tested against *Xanthomonas oryzae* pv. *oryzae* strain 2 and only T_2 resulted inhibition zone (Table 10 and Photograph 47). In T_2 after 72 hour no inhibition zone was observed. After 96 hour it was 6.33 mm, followed by 8.33 mm after 120 hours. Rest treatments did not resulted inhibition zone.

Table 10: Comparative efficacy of datura leaf extract against *Xanthomonas oryzae* pv. *oryzae* strain 2

Treatment	Inhibition zone (mm) after		
	72hr	96hr	120hr
T_1 (Control)	0	0 b	0 b
T_2 (1:1 w/v)	0	6.33 a	8.33 a
T_3 (1:2 w/v)	0	0 b	0 b
T_4 (1:4 w/v)	0	0 b	0 b
$LSD_{0.05}$	-	0.5065	0.5065
CV%	-	7.89	6.00

Values with different letters with in a column differ significantly at 5% level of significance as per DMRT



Photograph 47: **Inhibition zone occurred by datura leaf extract against *Xanthomonas oryzae* pv. *oryzae* strain 2**

CHAPTER 5

DISCUSSION

Nine plant extracts (onion bulb extract, kalijira seed extract, allamonda leaf extract, garlic clove extract, neem leaf extract, datura leaf extract, turmeric rhizome extract, turmeric rhizome extract, shimul leaf extract) were evaluated against three hybrid varieties of rice to determine efficacy against seed borne pathogen. *In vitro* evaluation of datura leaf extract and turmeric rhizome extract was also conducted against *Xanthomonas oryzae* pv. o*ryzae*. A considerable amount of seed borne pathogenic fungi and bacteria were observed by using blotter method.

In total nine pathogens were associated with the collected seed samples as detected by blotter method. The incidences of different pathogens were found to vary individually and independently among the hybrid varieties of rice seed.

In blotter method, nine seed borne pathogens were identified. These are *Xanthomonas oryzae* pv. *oryzae* (two strains), *Rhizopus stolonifer, Aspergillus flavus, Aspergillus niger*, *Fusarium moniliforme, Bipolaris oryzae, Curvularia lunata*, *Chaetomium globosum* and one unknown fungus. The incidence of *Xanthomonas oryzae* pv. *oryzae* (strain 1) ranged from 7.35 to 9.54% and *Xanthomonas oryzae* pv. *oryzae* (strain 2) ranged from 2.50 to 2.58%. The highest incidence was observed on BRRI hybrid dhan2 (9.54%). Bhutta and Ahmed (1994) reported that maximum seed infection due to *Xanthomonas oryzae* pv. *oryzae* was 11 to 12% in variety IRRI-6 at Lahore and Hydrabad, respectively. The incidence of *Rhizopus stolonifer* ranged from 1.94 to 4.26%. The highest incidence was observed on SL 8 (4.26%). The incidence of *Aspergillus flavus* ranged from 2.33 to 2.63%. The highest incidence was observed on BRRI hybrid dhan2 (2.63%). The incidence of *Aspergillus niger* ranged from 0.88 to 1.57%. The highest incidence was observed on BRRI hybrid dhan2 (1.57%). The incidence of *Fusarium moniliforme* ranged from 4.16 to 5.56%. The highest incidence was observed on Hira 2 (5.56%). The

incidence of *Bipolaris oryzae* ranged from 0.71 to 0.95%. The highest incidence was observed on Hira 2 (0.95%). The incidence of *Curvularia lunata* ranged from 1.06 to 1.97%. The highest incidence was observed on BRRI hybrid dhan2 (1.97%). The incidence of *Chaetomium globosum* ranged from 0.71 to 1.46%. The highest incidence was observed on BRRI hybrid dhan2 (1.46%). The incidence of an unknown fungus ranged from 1.96 to 2.88%. The highest incidence was observed on Hira 2 (2.88%).

The present findings were supported previous research reports (Fakir and Ahmed, 1974; Hossain and Fakir, 1974 and Sharma *et al.,* 1992) detected 10 fungal species of fungi from the rice seeds where *Fusarium moniliforme (Gibberella fujikuroi), Curvularia lunata (Cochliobolus lunata), Aspergillus flavus and Rhizopus stolonifer* were the most common. Of all the pathogens, *Xanthomonas* spp., *Rhizopus stolonifer, Aspergillus* spp., *Fusarium moniliforme* were predominant. These pathogen were designated as predominant, because each of them constituted at least 5.0% of the total seed borne pathogens infection. Mian and Fakir (1989) reported that the most predominant fungi in order of prevalence were *Helminthosporium oryzae, Curvularia lunata, Cladosporium cladosporioides, Aspergillus* spp. and *Trichoconis padwickii.*

Seeds of Hira 2 are treated by nine plant extracts where the incidence of *Xanthomonas oryzae* pv. *oryzae* (strain 1) ranged from 1.85 to 7.35%. The lowest incidence was observed in T_7 (1.85%). The incidence of *Xanthomonas oryzae* pv. *oryzae* (strain 2) ranged from 0.71 to 2.5%. The lowest incidence was observed in T_8 and (0.71%). The incidence of *Aspergillus flavus* ranged from 0.71 to 2.38%. The lowest incidence was observed in T_8 (0.71%). The incidence of *Aspergillus niger* ranged from 0.71 to 0.88%. The lowest incidence was observed in T_8 and T_7 (0.71%). The incidence of *Rhizopus stolonifer* ranged from 0.71 to 2.20%. The lowest incidence was observed in T_8 and T_7 (0.71%). The incidence of *Fusarium moniliforme* ranged from 0.71 to 5.56%. The lowest incidence was observed in T_8 (0.71%). The incidence of

Bipolaris oryzae ranged from 0.71 to 0.95%. The lowest incidence was observed in T_8 and T_7 (0.71%). The incidence of *Curvularia lunata* ranged from 0.71 to 1.79%. The lowest incidence was observed in T_8 and T_7 (0.71%). The incidence of an unknown fungus ranged from 0.71 to 2.88%. The lowest incidence was observed in T_8 and T_7 (0.71%).

Seeds of SL 8 are tested by nine plant extracts the incidence of *Xanthomonas oryzae* pv. *oryzae* (strain 1) ranged from 3.44 to 9.20%. The lowest incidence was observed in T_8 (3.44%). The incidence of *Xanthomonas oryzae* pv. *oryzae* (strain 2) ranged from 0.71 to 4.55%. The lowest incidence was observed in T_{10} (0.71%). The incidence of *Aspergillus flavus* ranged from 0.71 to 2.33%. The lowest incidence was observed in T_7 (0.71%). The incidence of *Aspergillus niger* ranged from 0.71 to 1.81%. The lowest incidence was observed in T_8 and T_7 (0.71%). The incidence of *Rhizopus stolonifer* ranged from 0.71 to 4.26%. The lowest incidence was observed in T_8 and T_7 (0.71%). The incidence of *Fusarium moniliforme* ranged from 0.71 to 4.16%. The lowest incidence was observed in T_3, T_4, T_9 and T_{10} (0.71%). The incidence of *Chaetomium globosum* ranged from 0.71 to 1.03%. The lowest incidence was observed in T_8 and T_7 (0.71%). The incidence of *Curvularia lunata* ranged from 0.71 to 1.06%. The lowest incidence was observed in T_8 and T_7 (0.71%). The incidence of an unknown fungus ranged from 0.71 to 1.96%. The lowest incidence was observed in T_8 and T_7 (0.71%).

Seeds of BRRI hybrid dhan2 are tested by nine plant extracts the incidence of *Xanthomonas oryzae* pv. *oryzae* (strain 1) ranged from 3.29 to 9.54%. The lowest incidence was observed in T_8 (3.29%). The incidence of *Xanthomonas oryzae* pv. *oryzae* (strain 2) ranged from 1.05 to 2.58%. The lowest incidence was observed in T_8 (1.05%). The incidence of *Aspergillus flavus* ranged from 0.71 to 2.63%. The lowest incidence was observed in T_8 (0.71%). The incidence of *Aspergillus niger* ranged from 0.71 to 1.57%. The lowest incidence was observed in T_8 and T_7 (0.71%). The incidence of *Rhizopus stolonifer* ranged from 0.71 to 1.94%. The lowest incidence was observed in T_8

and T_7 (0.71%). The incidence of *Fusarium moniliforme* ranged from 0.71 to 4.26%. The lowest incidence was observed in T_3 and T_8 (0.71%). The incidence of *Chaetomium globosum* ranged from 0.71 to 1.32%. The lowest incidence was observed in T_8 and T_7 (0.71%). The incidence of *Curvularia lunata* ranged from 0.71 to 1.54%. The lowest incidence was observed in T_8 and T_7 (0.71%). The incidence of an unknown fungus ranged from 0.71 to 2.01%. The lowest incidence was observed in T_8 and T_7 (0.71%).

Ganguly (1994) reported that leaf extracts of *Vinca rosea, Lantana camada, Ocimum tenuiflorum, Solanum melongena, Azadirachta indica, Polyanthia longifolia, Aegle marmelos* and *Datura metal* showed antifungal activity against *Pyricularia oryzae* and *H. oryzae in vitro*. Extracts of *V. rosea* showed inhibition of mycelial growth and spore germination.

Chowdhury (2005) observed that highly infected/ contaminated seed samples with seed borne fungi of rice, wheat, cosmos, zinnia, sunflower and radish were subjected to seed treatment with 1:0,1:1, 1:5,1:10 and 1:20 dilution of crude/ nascent extract of garlic, datura and turmeric; 1:1, 1:5, 1:10 and 1:20 dilution of commercially available oil extracts of neem, mahogany and koromcha; hot water treatment for 15 minutes at 50^0c, 52^0c, 54^0c, 56^0c and 58^0c temperatures and chemical seed treatment with Vitavax-200 @ 0.1%, 0.2% and 0.3% of the seed weight. Botanicals at all concentrations reduced the occurrence of mycoflora on the seed significantly and thereby increased seed germination. Some fungi were totally removed at 1:10 dilution of commercially available plant oil extract.

Rahman *et. al.* (2007) showed that seed treatment with *Curcuma longa* rhizome extract and *Allium sativum* caused remarkable reduction of leaf blight severity of wheat.

CHAPTER 6

SUMMARY AND CONCLUSION

The present study was conducted to find out the seed health condition of some hybrid rice varieties, to detect and identify seed borne pathogens and to evaluate plant extract for seed treatment of hybrid rice. The experiment was carried out in the Seed Health Laboratory (SHL) of Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh during the period from January 2012 to June 2013. The experiment was carried out following Completely Randomized Design with four replications. Data were analyzed by MSTAT-C computer package program and treatment means were compared by DMRT.

In this experiment, nine seed borne pathogens were found and eight pathogens were identified by blotter method. Two strain of one seed borne bacteria and six fungi were identified. One unknown fungus was also found on the seed. The identified pathogens were *Xanthomonas oryzae* pv. *oryzae* (two strains), *Rhizopus stolonifer*, *Aspergillus flavus*, *Aspergillus niger*, *Fusarium moniliforme, Bipolaris oryzae, Curvularia lunata* and *Chaetomium globosum*. Of all the pathogens, *Xanthomonas* spp., *Rhizopus stolonifer, Aspergillus* spp., *Fusarium moniliforme* were predominant.

Xanthomonas oryzae pv. *oryzae* was identified by the pathological study. Bacteria of 48 hours old culture were observed. Single colony was seen convex and circular form. The colony colors of the strains were yellowish and whitish. The both strains showed pathogenic reaction in pathogeniciy test. Leaf area diseased (% LAD) was recorded at 5, 10, 15, 20 and 30 days after inoculation on the variety of Hira-2, BRRI hybrid dhan2 and SL-8. The maximum leaf area diseased (more than 50%) was observed in SL 8 (Strain 2) and BRRI hybrid dhan2 (Strain 1) at 30 days after inoculation. The both strains showed positive reaction in all of the varieties of hybrid rice. From the result it was observed that, all of the varieties are highly susceptible to the bacterium named

Xanthomonas oryzae pv. *oryzae.* Hira 2 and BRRI hybrid dhan2 are more susceptible to the strain 1 where as SL 8 is more susceptible to the strain 2.

Nine plant extracts viz. onion bulb extract, kalijira seed extract, allamonda leaf extract, garlic clove extract, neem leaf extract, datura leaf extract, turmeric rhizome extract, biskatali leaf extract and shimul leaf extract were evaluated against seed borne pathogens of hybrid rice. All of the botanical were used as per 1:1 (w/v) ratio. From the findings of this experiment, it was reveal that all of the plant extracts significantly control seed borne fungi. Four plants extract named, datura leaf extract, turmeric rhizome extract; allamonda leaf extract and garlic clove extract showed best performance against seed borne pathogens of hybrid rice. Kalijira seed extract, bishkatali and shimul leaf extract also showed promising effect against seed borne fungi only. Onion bulb extract did not show efficiency against *Fusarium moniliforme* and *Curvularia lunata*. Neem leaf extract did not show significant effect against *Fusarium moniliforme* and *Aspergillus flavus.* Datura leaf extract and turmeric rhizome extract have the highest bactericidal and ant ifungal efficacy among the plant extracts used in the experiment. Onion, kalijira, allamonda, bishkatali and shimul leaf extract did not showed any significant effect against seed borne bacteria of hybrid rice.

From the findings of the study, it is very clear that the seed health status of hybrid rice seed was not at satisfactory level. Considering the overall performance of plant extracts as a seed treating agent for hybrid rice, datura leaf extract (1:1 w/v) and turmeric rhizome extract (1:1 w/v) could be used as eco friendly approach and may be advised to the farmer for profitable hybrid rice production. However, further intensive study need to be carried out to evaluate the performance of plant extracts at different dilution with different types of organic solvent viz. acetone, ethanol, mineral oil and benzene.

REFERENCES

AAS. (1999). Performance of Rice Hybrids under Bangladesh Conditions: 1998-99 Boro Season, Agricultural Advisory Society, Dhaka.

Agarwal, P.C., Mortensen, C.N. and Mathur, S.B. (1990). Seed borne disease and seed health testing of rice. *Phytopathological Papers*. **30**: 106 p.

Agarwal, V. K. and Sing, O.V. (1974). Relative percentage incidence of seed borne fungi associated with different varieties of rice seeds. *Review of Plant Pathology*. **55(1):** 38.

Ahmed, M. F. (2000). Efficacy of some fungicide and plant extracts against *Bipolaris oryzae.* M.S. Thesis, Department of Plant Pathology, BAU, Mymensingh.

Ahmed, M., Hossain, M., Hassan, K. and Dash, C. K. (2013). Efficacy of Different Plant Extract on Reducing Seed Borne Infection and Increasing Germination of Collected Rice Seed Sample. *Universal Journal of Plant Science.* **1(3):** 66-73.

Ahmed, N. and Sultana, K. (1984). Fungitoxic effect of garlic on treatment of jute seed. *Bangladesh J. Bot*. **13(2):** 130-136.

Alam, M. M., Yasmin, M., Nessa, J. and Ahsan, C. R. (2010). Antibacterial activity of chloroform and ethanol extracts of black cumin seeds (*Nigella sativa*) against multi-drug resistant human pathogens under laboratory conditions. *J. of Medicinal Plants Research* **4(18):** 155-161.

Alice, D. and Rao, A. V. (1987). Antifungal effect of plant extracts on *Drechslera oryzae* in rice. *Res. News Letter*. **12(2):** 28.

Anon. (1996). The Indian Pharmacopoeia. 3rd ed. Ministry of Health and family welfare. Government of India, New Delhi, India, 48 p.

Arun, A., Rekha, C. and Chitra, A. (1995). Effect of Alicia and extracts of Garlic and Bigononia on two fungi. *Indian J. Mycol. and Plant Path*. **25(3):** 316-318.

Ashrafuzzaman, M. H. and Hossain, I. (1992). Antifungal activity of crude extracts of plants against *Rhizoctonia solani* and *Bipolaris sorokiniana*. *Proc, BAU. Res. Prog*. **6:** 188-192.

Ashrafuzzaman, M. H. and Khan, A. R. (1992). Antifungal activity in vitro of some plant extracts on *Rhizoctonia solani*. *Bangladesh J. Sci. Res*. **10(2):** 243-244.

Ashrafuzzaman, M. H. (1991). A Text Book of Plant Pathology. Bangladesh Agril. Res. Council. 526-527 pp.

BBS. (2008). Bangladesh Bureau of Statistics. Year Book of Agricultural Statistics of Bangladesh. Stat. Div. Min. Planning, Govt. of the Peoples Republic of Bangladesh, Dhaka. Bangladesh. 136-140 pp.

BBS. (2013). Bangladesh Bureau of Statistics. Statistical Pocketbook Bangladesh. Bur. Stat. Div. Min. Planning, Dhaka. Bangladesh. 203 p.

Bhutta, A and Ahmed. S. I. (1994). Detection of bacterial pathogens in paddy seed lots in Pakistan. *Pakistan J. Scientific. Ind. Res*. **37(9):** 382.

Bisht, G. S. and Khulbe, R. D. (1995). Efficacy of leaf extracts of certain indigenous plant against brown leaf spot pathogen of rice. *Indian Phytopath*. **48(4):** 480-482.

Booth, C. (1971).The genus *Fusarium.* CMI, Kew, Surrey, England. 238 p.

Buss, E. A. and Park, S. G. (2002). Natural Products for Insect Pest Management. ENY-350 (IN197), one of a series of the Department of Entomology and Nematology, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. **10(4):** 311-318.

Chidambaram, P. S. and Mathur, S. B. (1975). Deterioration of stored grains by fungi. *Ann. Rev. Phytopathology*. **3**: 69-89.

Chowdhury, S. D. (2005). Comparative effect of plant extracts, physical and chemical agents in seed treatment. MS thesis, Dept. of Plant Pathol. Bangladesh Agricultural University, Mymensingh, Bangladesh.

Delvin, J. F. and Zettel, T. (1999). Eco-agriculture: Initiatives in Eastern and Southern Africa**.** Environmental Round Table Series – University of Guelph (Canada). **6(2):** 150-152.

Dharam and Sharma, R. K. (1985). Effecacy of fungicides studies on the fugicidal properties of Neem oil. *Indian J. Plant Path*. **3(2):** 241-242.

Duan, Y.J., Shang, S.G., Lu, X.D. and J.Y. Tang. (1983). Studies on the strains of *Xanthomonas oryzae* pv. *oryzae*. *Xunnan Agric. Sci. and Technology*. No. **1**, 1-6 pp.

Dubey, R.C. and Dwivedi, R. S. (1991). Fungitoxic properties of some plant extracts against vegetables growth and Sclerotial viability of *Macrophomina phaseolina* . *Indian Phytopath*. **44 (3):** 411-413.

Ellis, M. B. (1971). Dematiaceous Hyphomycetes. Commnwealth Mycol, Inst. Kew, Surrey, England. 608 pp.

Fakir, G. A. (1983). Teaching, Research and Training Activities on Seed Pathology in Bangladesh. *Seed Sci. & Tech*. **11:** 1345-1352.

Fakir, G. A. (2000). An annotated list of seed-borne disease in Bangladesh. Seed Pathology Laboratory. Dept. of Plant Pathology, Bangladesh Agricultural University, Mymensingh. 41 p.

Fakir, G. A. (2002). An annotated list of seed-borne disease in Bangladesh. Seed Pathology Centre. Dept. of Plant Pathology, Bangladesh Agricultural University, Mymensingh.

Fakir, G. A. and Khan, A. A. (1992). Control of some selected seed-borne fungal pathogen of jute by seed treatment with garlic extract. *Proc. Bangladesh Agricultural University, Research Prog*. **6:**176-180

Fakir, G. A. and Ahmed. M. U. (1974). Microflora of freshly harvested rough rice grains of Tepi boro. *Bangladesh Agril. Sci. Abst*. **2:** 160.

Fakir, G. A., Hossain, I., Ahmed, M. U., Anam, M. K., Alam, M. N. and Rahman. M. (2003). Effect of ash, chalk powder and neem leaf on the

quality of boro rice seed stored in gunny bag, motka, plastic drum and tin. Proceeding of review and planning meeting of the Rice Seed Improvement Sub-project held ay BRII, Gazipur, Bangladesh during 21-22 April, 2003.1-37 pp.

Fakir, G.A. (1988). An annoted list of seed borne diseases of important crop in Bangladesh. SPC, Bangladesh Agricultural University, Mymensingh.

Ganguli, L. K. (1994). Fungitoxic effect of certain plant extracts against rice blast and brown spot pathogen. *Environment and Ecology*. **12(3):** 731-733.

Govindachari, T. R., Suresh, Gopalakrishnan, G., Banumathy, B. and Masilamani, S. (1998). Identification of antifungal compound from the seed oil of *Azadirachta indica*. *Phytoparasitica*. **26(2):** 109-116.

Grange, N. and Ahmed, S. (1988). Handbook of Plants with Pest Control Properties. First edition. Willey-Interscience, Hoboken, NJ, United States of America. **7(5):** 75-78.

Hossain, I. and Schlosser, E. (1993). Control of *Bipolaris sorokiniana* in wheat with Neem extracts. *Bangladesh J. Mycrobial*. **10(1):** 39-42.

Hossain, I., Ashrafuzzaman, H. and Khan, M. H. H. (1993). Biocontrol of *Rhizoctonia solani. BAU Res. Prog*. **7:**264-269.

Hossain, I., Mahamud, H. and Ashrafuzzaman, H. (1997). Effect of plant extracts on fungi (*Bipolaris sorokiniana* and *Bipolaris solani*) and okra mosaic disease. *Ecoprint* **4(1):** 35-42.

Hossain, N and Fakir, G. A. (1974). Mycoflora of rice grains in Bangladesh. III- Prevalence of mcroflora associated with the freshly harvested rough grains of three Aus Varieties of rice. *Bangladesh Agril. Sci. Abs*. **2:** 161.

Hossain. M. M., Khalequzzaman, K. M., Aminuzzaman, F. M., Mollah, M. R. A. and Rahman, G. M. M. (2005). Effect of plant extract on the incidence of seed-borne fungi of wheat. *J. of Agric. and Rural Dev*. **3(1 and 2).** 39-43.

Howlader, A. N. (2003). Effect of seed selection and seed treatment on the development of phomopsis blight and fruit rot of egg plant. M.S. Thesis. Dept. of Plant Pathology, BAU, Mymensingh, Bangladesh.

Huda, M. Z. (2001). Regional development of irrigation technologies and its impact on food grain production in Bangladesh. M.S. Thesis. Department of Agril. Economics. Bangladesh Agricultural University, Mymensingh. 406-68 pp.

Islam, M. A. (2005). Country news, Holiday Publication Limited, **8:** 3-4.

Islam, M. A. (2006). Efficacy of selected plant extracts on leaf spot (*Bipolaris sorokiniana*) and grain yield of wheat. MS thesis. Dept. of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, 72 pp.

Islam, M. A., Aminuzzaman, F. M., Islam, M. R. and Zamal, M. S. (2006). Seed treatment with plant extract and Vitavax-200 in controlling leaf spot (*Bipolaris sorokiniana*) with increasing grain yield of wheat. *Int. J. of Sustain. Agril. Tech*. **2(8):** 15-20.

ISTA. (1996). International Rules for Seed Testing. International Seed Testing Association. *Seed Science and Technology*.**24:** 39-42.

Kauffman, H.E., Reddy, A.P.K., Hzieh, S.P.Y. and Merca. S. D. (1973). An improved technique for evaluation resistance of rice varieties to *Xanthomonas oryzae*. *Plant Dis. Reptr*. **57:** 537-541.

Khaleduzzaman, S. M. (1996). Control of seed borne prevalence by seed treatment in wheat (*Triticum aestivum* L.). M.S. Thesis. Dept. of Pl. Pathol. BAU, Mymensingh, Bangladesh. 1 p.

Khan, A. A. and Fakir, G. A. (1995). Seed treatment with garlic extract to control seed-borne pathogen of jute. *Bangladesh J. Pl. Pathol*. **11(1-2).**

Khan, M. H. H. and Hossain, I. (1993). Antifungal activity of crude plant extracts against *Bipolaris sorokiniana*. Paper presented 5th Biennial conference. Bangladesh phytopathological society, 27-28 June-1993. Bangladesh Agricultural University, Mymensingh. 10 p.

Khan, M. I. and Kumar, R. (1992). Antifungal activity of leaf extracts neem on seed mycoflora of wheat. *Indian J. Seed Abs*. **15(7):** 299.

Khan, N. U. (1999). Studies on epidemiology, seed borne nature and management of *Phomopsis* fruit rot of brinjal. M.S. Thesis. Dept. of Pl. Pathol. BAU, Mymensingh, Bangladesh.

Malone, G.P. and Muskette, A. E. (1964). Seed borne fungi. Description of 77 fungal species. *Proc. Int. Seed Test. Ass*. **29(2):** 180-183.

Mathur,S.B. and Kongsdal, O. (2003). Common Laboratory Seed Health Testing Method for Detecting Fungi. First edition. International Seed Testing Association, Bassersdorf, Switzerland. 425pp.

Mew, T. W. and Misra, J. K. (1994). A Manual of Rice Seed Health Testing. 41 pp.

Miah, A., Ahmed, M. U., Sharma, N. R., Ali and Miah, S. A. (1990). Antifungal activity of some plant extracts. *Bangladesh J. Botany*. **19(1):** 5-20.

Mian, I. H. and Fakir, G. A. (1989). Fungi, moisture content and germinability of rough rice grains during storage. *Seed Research*. **17:** 169-173.

Misra, A. K. and Vir. D. (1990). Efficacy of fungicides-XLV1: effect of fungicidal seed treatment against heavy inoculums pressure of certain fungi causing discoloration of paddy seeds. *Indian Phytopathology*. **43 (2):** 175-178.

Misra, J. K. and Gergon and Mew. T. W. (1994). Occurance, distribution and phenology of seed borne fungi of rice in certain proviences of Philippine. *Plant Path*. Bulletin **3(4)**:229-239.

Mohanty, A. K., Kar, A. K. and Setti, P. N. (1995). Efficacy of crude plant extracts of some selected plants in controlling brinjal blight and fruit rot pathogen, *Phomopsis vexans*. *Crop Research Hisar*. **9(3):** 447-448.

Mostafa, M. (2004). Effect of some plant extracts on viral diseases of tomato. An M.S. thesis submitted to the Department of Plant Pathology, Bangladesh Agriculture University, Mymensingh. 1-78 pp.

Ou, S.H. (1985). Rice Diseases. Second Edition, CMI, Kew, Surrey, England. P. 38-41.

Parisi, J. J. D.; Malavolta. V.M.A. and Leonel Junior. F. L. (2001). Chemical control of seed-borne fungi in rice seeds (*Oryza sativa* L.). *Summa Phytopathologica*. **27 (4):** 403-409.

Rahman, G. M. M., Islam, M. R. and Wadud, M. A. (1999). Seed treatment with plant extracts and hot water: a potential biological method of controlling seed borne infection of wheat. *Bangladesh Journal of Training and Development*. **12(1-2):** 185-190.

Rahman. M. M., Islam. M. A., Aminuzzaman. F. M. and Islam. M. R. (2006). Antifungal activity of indigenous plant extracts against *Bipolaris sorokiniana*. *J. agric. Technol*. **9(1&2):** 101-106.

Rahman. M. M., Aminuzzaman. F. M. and Islam, M. R. (2007). Efficacy of plant extract in controlling leaf blight (*Bipolaris sorokiniana*) with increase yield of wheat. *Int. J. Sustain. Agril. Tech.* **3(6):** 22-27.

Rahman. M. M. (2007). Efficacy of seed treatment with plant extracts on leaf blight (*Bipolaris sorokiniana*) development and yield of wheat. MS thesis. Dept. of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, p iii.

Roy, B., Amin, R., Uddin, M. N., Islam A. T. M. S., Islam, M. J. and Halder, B. C. (2005). Leaf extracts of Shiyalmutra (*Blumea lacera*) as botanical pesticides against lesser grain borer and rice weevile. *Journal of Biological Sciences*, **5(2):** 201-204.

Sharma, H.L., Randhawa, H.S., Kapur, A. and Singh. S. (1992). Seed discoloration in rice. *Seed Res. and Prodn. Unit*. **24(1):** 37-41.

Shetty, S. A., Prakash, H. S. and Shetty, H. S. (1989). Efficacy of certain plant extracts against seed-borne prevalence of *Trichoconiella padwickii* in paddy (*Oryza sativa*). *Canadian J. Bot.* **67(7):** 1956-1958.

Suratuzzaman, M. Hossain, I. and Fakir, G. A. (1994). Control of seed borne fungi of two rice varieties with some plant extracts. *Progress. Agric*. **5(1):** 11-15.

Tariq, V. N. and Magee, A. C. (1990). Effect of volatiles from garlic bulb extracts on *Fusarium oxysporum f.sp. lycopersici*. *Mycological Res*. **94(5):** 617-620.

Thakhur, K. D. Khune, N. N. and Sable, J. E. (1991). Efficacy of some plant extracts on inhibition of cotton pathogens. *Orissa J. Agril. Res*. **4 (1-2):** 90-94.

Vandepitte, J., Engbreak, K., Piot, P. and Heuck, C. C. (1991). Basic Laboratory Procedure in Clinical Bacteriology. World Health Organization, Geneva.

Yeasmin, F., Ashrafuzzaman, M. and Hossain I. (2012). Effects of garlic extract, allamanda leaf extract and Provax -200 on seed borne fungi of rice. *The Agriculturists*. **10 (1):** 46-50.

APPENDICES

Appendix I. Composition of Nutrient ager medium in 1000 ml water

Nutrient ager	15g
Peptone	5g
Beef extract	5g