EFFECT OF PLANT EXTRACT ON SEED HEALTH STATUS OF THREE SELECTED MAIZE VARIETIES

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CERTIFICATE

This is to certify that the thesis entitled, **"EFFECT OF PLANT EXTRACT ON SEED HEALTH STATUS OF THREE SELECTED MAIZE VARIETIES"** submitted to the Department of Plant Pathology, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in the partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (M. S.) IN PLANT PATHOLOGY,** embodies the result of a piece of bona fide research work carried out by **MD. AL-MAMUN, REGISTRATION NO. 09-03701** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

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: 26.05.2016
Place: SAU, Dhaka-1207

(Dr. Nazneen Sultana)
Professor
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Dedicated
To
My Beloved
Parents

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The Author

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ABSTRACT

Experiment was conducted to know the effect of plant extract on seed health status of three selected maize variesties BHM-3, BHM-7, BHM-9 during the period January 2015 to February 2016 in the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh. Four plant extracts viz. neem leaf, garlic bulb, mustard seed, alamonda leaf were used. In the experiment seed germination was the highest (97.22%) in seed treated with neem leaf extract. Three fungi *Aspergillus flavus*, *Aspergillus niger* and *Fusarium moniliforme* were identified. The lowest incidence (15.22%) of *Aspergillus flavus* and *Fusarium moniliforme* (4.77%) were observed in seed treated with neem leaf extract. The lowest incidence (13.78%) of *Aspergillus niger* was observed in seed treated with alamonda leaf extract. Seedling symptom test yielded the highest seed germination, normal seedling and the lowest disease incidence in maize variety BHM-9. Seedling vigor index was found promising in neem leaf extract. Maximum germination (95.56%), maximum number of healthy seedling (84.67%), minimum number of dead seedling (3.88%), minimum number of diseased seedlings (7.11%), abnormal seedling (3.22%) and the highest vigor index (2198) were observed in seed treated with neem leaf extract. Farmers are therefore, advised to use neem leaf extract for treating maize seed before sowing.

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LIST OF ABBREVIATED TERMS

ABBREVIATION	FULL WORD
et al.	And others
BARI	Bangladesh Agricultural Research Institute
Cm^3	Centimeter cube
^0C	Degree centigrade
Etc.	Etcetera
Ed.	Edited
Eds.	Edition
G	Gram
J.	Journal
No.	Number
PDA	Potato Dextrose Agar
LSD	Least Significant Difference
%	Percent
CRD	Completely Randomised Design
Res.	Research
SAU	Sher-e-Bangla Agricultural University
Viz.	Namely
Var.	Variety

CHAPTER I

INTRODUCTION

INTRODUCTION

Maize (*Zea mays* L.) belongs to the family Gramineae is one of the most leading cereal in the world next to rice and wheat (Aldrich *et al.*, 1975). Central America or Mexico is the most likely center of origin of this crop and South America is the possible secondary origin (Martin and Leonard, 1975). Africa, Asia and some Central and South American countries use maize as an important staple food but it is mostly used as animal food. Bangladesh has good potentiality to adopt it as a cereal crop due to its low cost of production, wide adaptability and diversified uses. Maize is now a popular crop because of its high yield potential. Kharif is main season, although it can be cultivated in both Rabi and Kharif season. Maize kernels have high nutritive value contains 66.2% starch, 11.1% protein, 7.1% oil and 1.5% minerals (Hulse *et al.*, 1980). It also contains 90 mg carotene, 1.8 mg niacin, 0.8 mg thiamin and 0.1 mg riboflavin per 100 g grains (Chowdhury and Islam, 1993). Better yield, improved color, greater uniformity, disease resistance which are the main point to choose hybrid variety. Maize acreage and production have an increasing tendency. After Introduction of hybrid since 1993 area, production and yield of maize have increased by 17%, 33%, and 16%, respectively which reflect the effect of adopting improved technology (Mohiuddin, 2003). The population growth in Bangladesh is high which puts great pressure on the country's food production. Cereal is still staple one for Bangladeshi people. Now maize is the third position next to rice and wheat in the country in terms of human consumption. Average yield of maize in Bangladesh is considerably low. The national average yield is only 11.24 t/ha (BBS, 2010), whereas the newly released hybrid varieties have the potential to produce more than 8.0 t/ha.

Seed-borne diseases is one of the main factor for lower average yield of maize in Bangladesh compared to other countries. Though hybrid varieties were treated, some fungi may be found associated with the seeds.

Infected seed failed to germinate and may transmit pathogens to seedling and growing plant in field that interfere with production. Seed is the vital carrier of plant pathogens. Thus seed-borne fungi create a great threat to the production of crop in Bangladesh. Seed is the basic material and vital input of agriculture. Healthy seeds which are free from seed borne pathogens are prerequisite for successful crop production. Maize seeds are infected by three major categories of pathogens namely fungi, bacteria and viruses that affect seed health and quality (Avinder and Rai, 1991). Diseases play a significant role among the various factors responsible for low yield of maize. As many as 112 diseases are known to occur in maize crops (USDA, 1960) where more than 70 diseases are seed-borne. Fungi, bacteria and viruses all the three major groups of pathogens can be seed-borne and affect the seed health quality of maize. Again of all the seed-borne pathogens, fungi are predominant and about 60 fungi are known to be seed-borne or seed transmitted in maize. Important or devastating diseases of the crop are coincidentally seed-borne and caused by fungi. Seed-borne fungal diseases of international importance occurring on maize are leaf spot/leaf blight (*Bipolaris turcicum*) cob rot and seed rot (*Aspergillus* sp., *Fusarium* sp., *Penicillium* sp.), kernel rot *(Acremonium strictum*, *Botryodiplodia* sp., *Cladosporium* sp.), scutellum rot (*Rhizopuus* sp.), seedling blight (*Gibberella zeae*), anthracnose (*Colletotrichum graminicola*) and head smut caused by (*Sphacelotheca reiliana*) (Richardson, 1990).

Seed health status is an important factor for the management of crop diseases. The present study was carried out to assess seed health status of the mostly cultivated maize varieties (three hybrid varieties) to determine the whether those were pathogen free or contaminated. Seed infection or contamination could be reduced by seed treatments by using chemicals but chemical control have some hazards. Use of plant extracts against plant disease is however, a recent approach to manage plant diseases. It helps to avoid environmental pollution by chemicals.

Though plant extracts have been used in controlling seed-borne infection in certain crops, their efficiency in controlling seed-borne infections of fungal pathogens in maize seeds has not yet been critically evaluated. Thus this study was under taken with the following objectives.

OBJECTIVES:

1. To know the seed health status of three selected hybrid maize varieties
2. To determine the effect of some plant extracts on seed germination and seedling vigor of three selected hybrid maize varieties

CHAPTER II

REVIEW OF LITERATURE

REVIEW OF LITERATURE

2.1. Origin and botany of maize

Maize (*Zea mays* L.), also known in some countries as corn, is a cereal grain domesticated in Mesoamerica and subsequently spread throughout the American continents (Sauer, 1993).

Maize was domesticated in Mexico about 7000 years ago and by the time Columbus arrived in the New World in 1492 AD, there were already many varieties. It was introduced to Africa in the 16th century and over time came to replace sorghum as the staple food in all but the drier areas (Biodiversity, 2010).

Maize is a direct domesticate of the teosinte, *Zea mays* sp. *parviglumis*, native to the Balsas River Valley of southern Mexico, with up to 12% of its genetic material obtained from *Zea mays* sp. *Mexicana* through introgression (Eurekalert, 2009).

2.2. Importance of maize

According to the International Institute for Tropical Agriculture (IITA) maize serves as staple food for more than 300 million of Africa's most vulnerable inhabitants and the most important staple food on the continent (Essiet, 2010).

Maize as cornmeal (maize flour) constitutes a staple food in many regions of the world. Maize can be harvested and consumed in the unripe state, when the kernels are fully grown but still soft. The cooked unripe kernels may also be shaved off the cob and served as a vegetable in side dishes, salads, garnishes, etc (Wikipedia, 2009).

The primary uses for maize in North America are the production of corn sweeteners like corn syrup, as a feed for livestock, and the production of ethanol. Ethanol, a type of alcohol, is mostly used as an additive in gasoline to increase the octane rating. Maize cobs are used as a biomass fuel source. Maize is relatively cheap and a home heating furnace has been developed which uses maize kernels as a fuel (Bambooweb, 2009).

Starch from maize can also be used in the manufacturing of plastics, fabrics, adhesives and many other chemical products. The corn steep liquor, a plentiful watery by-product of maize wet milling process, is widely used in the biochemical industry and research as a culture medium to grow many kinds of microorganisms (Wikipedia, 2010a).

An analysis based on 1987 data showed that maize and maize-based foods accounted for 10.8% of household food expenditures by the poor and 10.3% of food expenditures by all income groups (Boateng *et al*., 1990)

2.3. Maize production trends

The average yield of maize in Bangladesh is considerably low. The national average yield is only 11.24 t/ha, whereas the newly released hybrid varieties have the potential to produce more than 8.0 t/ha. Both of the two key determinants of production (area planted and yield) have increased over the longer term, although the upward trends have been characterized by high year-to-year variability typical of rainfed crops (BBS, 2010).

The rising importance of the transition zone as a source of maize supply can be attributed to a combination of factors, including the presence of favorable agro-

ecological conditions (such as annual rainfall, type of soil, soil fertility, and temperature), availability of improved production technology, a relative abundance of underutilized land, and a well-developed road transport system (Morris *et al*., 1999)

2.4. Diseases of maize

There are many causes of low maize yield of which diseases play a significant role. Moreover, seed-borne diseases cause enormous losses both in storage as well as in the field. A total of 112 diseases are known to occur in maize crops (USDA, 1996) and among them, more than 70 are seed-borne. Important seed-borne diseases of maize are leaf spot, leaf blight, Collar rot, kernel rot, scutellum rot, seedling blight, anthracnose and head smut (Richardson, 1990).

2.4.1. Major fungal diseases of maize

A field survey was in Bangladesh for pathogen risk analysis of maize and detected *Aspergillus flavus, A. niger, Penicillium* sp., *Rhizopus* sp., *Fusarium moniliforme* and *Xanthomonas* sp. through seed health testing (Faruq *et al.*, 2014).

Alves and Pozza evaluated scanning electron microscopy detection of seed-borne fungi in blotter test and standard SEM method. They identified *Fusarium* sp. on maize, *Colletotrichum gossypii* and *Fusarium oxysporum* on cotton, *A. flavus*, *Rhizopus* sp. and *Mucor* sp. on common bean in a blotter test (Alves and Pozza, 2012).

A study was conducted to find out seed borne-fungi associated with seeds of different variety of maize viz. Badsha-1, Khai Bhutta, Bornali, Mohor, BARI Bhutta-5 and BARI Bhutta-6 by blotter method and identified *A. niger, A. flavus, Fusarium* sp., *P. oxalicum, Curvularia lunata* and *R. stolonifer* from seeds of these maize varieties (Debnath *et al.*, 2012).

The pathogenicity of two most predominant fungal species *F. moniliforme* and *A. niger* on maize those collected from different localities of Azad Jammu and Kashmir (AJK), Pakistan and found *F. moniliforme* had 50.2% pathogenicity on seeds and 6.55% on seedlings, while *Aspergillus niger* had 62.87% on seeds and 11.24%on seedlings. These depicts that mycoflora had significant detrimental impacts on seeds and seedling's life of maize (Hussain *et al.,* 2012).

Eleven seed-borne fungal species were identified from 18 maize seed samples collected from 6 maize growing districts of Punjab and Khyber-Pakhtoonkhwa, Pakistan. *A. flavus*, *A. niger* and *F. moniliforme* were the most prevalent species with the incidence range up to 94, 62 & 43%, respectively in maize seed (Saleem *et al.,* 2012).

Seed mycoflora of four maize varieties (SUWAN IESR, DMR ESR, ACR 97 STR, ACR 97 TZLC) and the pathogenicity of fungal isolates were determined *Aspergillus* sp. were isolated from seeds of the four maize varieties while *Penicillinm* sp., *Cladosporium* sp., *Fusarium* sp. were encountered in three of the maize varieties (Fawole *et al.*, 2010).

An experiment was conducted on screening of seven medicinal plants for antifungal activity against seed borne fungi of maize seeds. They identified *A. flavus, A. niger*, *F. moniliforme*, *F. graminearum*, *P. chrysogenum* and *P. notatum* from seed of maize (Kiran *et al.*, 2010).

Twelve yellow and ten white seed maize hybrids seeds were sown randomly. Fungi like *Fusarium* sp., *Penicillium* sp., and Aspergillus sp. were identified. White hybrids had 34, 52 and 22% less infection by *F. verticillioides*, *A. flavus*, and *A. niger* then yellow hybrids, respectively, and almost the double infection

with *Penicillium* sp. White hybrids presented 51% more healthy grains than the yellow hybrids (Montes *et al.*, 2009).

Ten pathogenic fungi on maize seeds in a moist blotter test consisted of *Acremonium strictum* (infection ranging from 2 to 96%), *Bipolaris maydis* (1 to 30%), *B. theobromae* (1 to 17%), *C. graminicola* (2 to 8%), *Curvularia* sp. (1 to 39%), *Exserohilumro stratum* (1 to 13%), *F. moniliforme* (38 to 99%), *F. equiseti* (1 to 15%), *F. pallidoroseum* (1 to 23%) and *Phoma* sp. (2 to 50%). *A. flavus* (1 to 99%), *A. niger* (1 to 99%), *Cladosporium* sp. (1 to 93%), *Penicillium* sp. (12 to 100%) and *Rhizopus* sp. (1 to 51 %) (Somda *et al.*, 2008).

Ten seed-borne fungi viz. *Alternaria tenuis*, *A. niger*, *B. sorghicola*, *Botrytis cinerea*, *Colletotrichum graminicola*, *Curvularia lunata*, *C. trifolii*, *F. moniliforme*, *P. oxalicum* and *Phoma sorghina* in sorghum seeds was recorded by Karim (2005).

A. flavus, *Stenocarpella maydis*, *Fusarium graminearum*, *F. verticillioides*, and *Trichoderma viride* isolated from infected severely discolored grain and protective endophytes, including myco parasites that live asymptomatically in maize, are not readily distinguished from uninfected grains and represent confounding variables in maize variety trials for fungus mycotoxin resistance (Wicklow and Pearson, 2005).

BARI and Alam described 28 diseases of maize. They mentioned seed-borne diseases like leaf blight (*Bipolaris turcicum*, *B. maydis*), leaf spot (*C. lunata*), banded leaf and sheath spot (*Rhizotonia solani*), cob rot (*Aspergillus* sp.), damping off (*F. moniliforme*) and anthracnose (*Colletotrichum* sp.). However there was no proof about the association of these fungal pathogens with the seeds of maize in Bangladesh (Bari and Alam, 2004).

Six fungi was found namely *Alternaria alternate*, *A. niger*, *F. moniliforme*, *Fusarium* sp., *Penicillium* sp. and *Ustilago zeae* associated with maize seed and among the fungi identified fugi the highest incidence was found with *F. moniliforme* and the lowest in *Penicillium* sp. (Basak and Lee,2002).

Eleven seed-borne diseases was found which occurring on maize in Bangladesh namely, kernel mould (*A. flavus*, *Penicillium* sp.), cob rot (*Aspergillus* sp., *Gibberella zeae*), kernel rot (*B. theobromae*), leaf blight (*B. turcica*), seed rot (*F. moniliforme*, *F. oxysporum*, *Penicillium* sp.), germination failure (*Aspergillus* sp.), seedling blight (*Gibberella zeae*), blue eye (*Penicillium* sp.), brown spot (*Physodermazeae maydis*), scutellum rot (*Rhizopus* sp.) and smut (*Ustilago zeae*). However, no attempt was made to detect these fungal organisms in seeds of the crop (Fakir, 2001).

An experiment was conducted to determine seed-borne fungal diseases on maize plants and observed seed rot, seedling blight and damping-off (*F. moniliforme*, *Penicillium* sp., *Aspergillus* sp., *Bipolaris* sp., *Rhizoctonia* sp. and *Alternaria* sp.) and stalk rots, ear rots and kernel rots (*Gibberella* sp. *Diplodia* sp., *Fusarium* sp., *Pylhium* sp., *Penicillium* sp. and *Aspergillus* sp. All the fungi were found associated with the recorded diseases were seed-borne (White, 1999).

An examined 69 commercially treated maize seed samples and 59 untreated maize seed samples for infection with fungi. *F. moniliforme*, *Penicillium* sp. and *Cephalosporium* sp. were observed at higher incidences. The highest incidence of *D. maydis* and *F. graminearum* was observed in the Southern region and *Fusarium* sp. and *Cephalosporium* sp. in the Southeast region of Brazil (Casa *et al.*, 1998).

An isolation was conducted with 63 species of fungi belonging to 21 genera from maize grains in Egypt. *Aspergillus* (15 species), *Penicillium* (17 species) and

Fusarium (4 species) were the dominant genera isolated from the 3 types of maize. Of the four species of Fusaria, *F. moniliforme* was the dominant species. *F. oxysporum* and *F. subglutinans* were isolated exclusively from yellow corn (Maize) (El-Maghraby *et al.* (1995).

Sixty fungal pathogens was listed on maize seeds and important seed-borne diseases were recorded on the crop caused by the fungal pathogens were leaf spot/leaf blight (*Cochliobolus heterostrophus*, *Epicoccum* sp.), cob rot and seed rot (*Aspergillus* sp., *Fusarium* sp., *Penicillium* sp.), kernel rot (*Acremonium strictum*, *Bolryodiplodia* sp., *Cladosporium* sp.), scutellum rot (*Rhizopus* sp.), seedling blight (*Gibberella zeae*), anthracnose (*Colletotrichum graminicola*) and head smut (*Sphacelothe careiliana*). (Richardson, 1990). Maize kernels infected by *F. moniliforme*. *A. flavus* were frequently observed in the apical section of the cobs (Zummo and Scott, 1990).

2.5. Effect of seed-borne fungal diseases on man and animal

For the safety of human food, food-borne bacteria constitute the greatest hazard, followed by mycotoxins. Conversely, in terms of livestock feeds, mycotoxins pose the greatest threat. Mycotoxins have attracted worldwide attention due to the significant losses associated with their impact on human and animal health, and consequent national economic implications (Bhat and Vashanti, 1999).

Mycotoxicosis is the consequence or effect (disease or pathological abnormalities) of ingesting toxin-contaminated foods by man and animals. It may also result indirectly from consumption of animal products such as milk from livestock exposed to contaminated feed. Over 300 'mycotoxins' have been reported (Coker, 1979). However, based on extensive analytical studies (IARC, 1993) and detailed study of the distribution of fungi in nature, the five agriculturally important toxins

from fungi are aflatoxins, fumonisins, ochratoxin A, zearalenone and deoxynivanelol. Fungal toxins can cause acute or chronic intoxications, depending on the animal, sex, breed and dosage (Coker, 1979).

The only toxin that has gained prominence in scientific literature in food products from the West African sub-region is aflatoxin, while there are few studies conducted on fumonisin and ochratoxin A (Bankole and Adebanjo, 2003).

Fusarium sp. can produce a number of toxic compounds including fusaric acid, fusarins, gibberellins, moniliformin and fumonisins (Marasas *et al.*, 1981; Nelson, 1992; Nelson *et al.*, 1993). Recently, fumonisins have received the most attention, because fumonisin B1 is the most common toxin found in maize infected by *F. moniliforme* (Leslie *et al.*, 1992; Nelson *et al.*, 1993). Fumonisin B1 has been shown to cause a number of health problems in livestock and laboratory animals (Nelson *et al.*, 1993, Osweiler *et al.*, 1992; Riley *et al.*, 1993) and has been associated with 1increased incidence of human oesophageal cancer in some parts of the world (Nelson *et al.*, 1993). Fumonisins can be found in moldy and symptomless infected kernels of maize (Bullerman and Tsai, 1994; Munkvold and Stahr, 1994; Nelson, 1992; Nelson *et al.*, 1993).

2.6. Control of seed-borne diseases

Seed borne diseases are controlled by seed treatment practices. Seed treatment is the oldest practice in plant protection. Its origins can be traced to the 18th century with use of brine for the control of cereal smuts (Neergaard, 1988). The modern era of seed treatments began with the introduction of organo-mercury fungicides in 1912 which were widely used for several decades (McGee, 1995).

2.6.1. Types of seed treatment

Seed treatments can be classified as physical, biological, chemical and botanical. Regardless of type, successful seed treatment practices must satisfy the following biological requirements (McGee, 1995).

- Consistantly effective.
- Safe to operators during handling and planting.
- Safe to wildlife.
- Compatible with other materials used on seeds.
- Should not produce harmful residues on plant or soil.
- Chemical or biological methods should have desirable qualities with respect to application and retention on the seeds.

2.6.2. Plant or botanical extracts with fungicidal properties

The effect of garlic extract (1:2, 1:3), neem extract (1:2, 1:3) and BAU-Biofungicide @ 2.5% on vigour index of maize cv. BARI Bhutta-6 were evaluated Fig.1. The vigour index of seedling varied from 2856 to 1885 where the significantly lowest (1885) vigour index was recorded in control and the highest (2856) vigour index was recorded from the seeds treated with BAU-Biofungicide which was 51.51% higher over control. The least but similar vigour index was recorded in rest of the tested varieties (Sultana *et. al*., 2012).

Five different plant extracts were used in controlling seed-borne fungi of rice. In case of variety BR6 the highest germination (91.67%) was found when the seeds were treated with garlic extract (*Allium sativum*) @ 1:1 dilution and it increased germination by 67.68% over control (Table 1).

Among the other extracts neem (1:1) and chirata (1:1) also increased germination by 59.74% and 46.94%, respectively over control (Plate 2) (Ahmed *et al*., 2013).

An experiment of seed treatment with the garlic extract, neem, gagra, vatpata, Bishkatali leaf extracts reduced seed-borne prevalence and increased germination percentage of wheat seeds. Among them garlic and neem bark gave better results. Khan and Kumar observed that bishkatali, garlic, ginger and neem extract were effective against seed-borne *Curvularia lunata, Fusarium* sp. of wheat. Ahmed observed that neem and garlic extracts at 1:1 dilution were effective against *Bipolaris oryzae* (Mondall *et. al,* 2013).

Percent seedling germination for wheat cultivars tested was > 85% and was greatly increased by either fungicide or garlic juice treatments depending on the wheat cultivar tested non-treated seed (Perello *et al.,* 2013).

Aspergillus flavus, Rhizopus stolonifer, Fusarium moniliforme, Colletotrichum capsici and *Aspergillus niger* were the predominant seed borne fungi in the chili seeds. All the seed treating agents completely eliminated the fungi *Colletotrichum capsici* and *Fusarium moniliforme*. Among the fungi, *Curvularia lunata* was the most prevalent ones followed by *Rhizopus stolonifer*, *Colletotrichum capsici, Fusarium moniliforme* and *Aspergillus flavus*. The highest (14.67%) seed borne *Curvularia lunata* was found in case of untreated seeds, whereas the lowest (2.00%) seed borne *Curvularia lunata* was found when the seeds treated with BAU Bio-fungicide + Ginger rhizome extracts. The highest (4.67%) seed borne *Aspergillus flavus* was found in case of untreated seeds whereas the lowest (2.00%) seed borne *Aspergillus flavus* was found when the seeds treated with alamanda leaf extracts. The highest (10.33%) seed borne *Rhizopus stolonifer* was found in case of untreated seeds, whereas no seed borne *Rhizopus stolonifer* was found when the seeds treated with BAU Bio-fungicide, thiovit and allamonda leaf extracts (Asalmol *et al*., 2001).

An experiment was applied with BARI Morich-1, to determine percent seed germination, percent healthy seedling and seedling height significantly differed in response to different seed treating agents (physical, chemicals, biological and botanicals) compared to control. The lowest seed germination (61.33%) was obtained from control. The highest seed germination (93.33%) was obtained when the seed was treated with BAU Bio-fungicide followed by seed treatment with neem leaf extract (93.00%). The highest percent healthy seedling (95.33%) was obtained when the seed was treated with allamonda leaf extract and seed treatment with BAU Bio-fungicide. Percent healthy seedling was increased by 61.58% under allamonda leaf extract and BAU Bio-fungicide over control. The lowest vigour index (214.66) was recorded in case of untreated seeds, whereas the highest vigour index (681.69) was recorded when the seeds were treated with neem leaf extracts which was closely followed by seed treatment with BAU Biofungicide and allamonda leaf extracts (Xiao-Fang *et al*., 2012).

Hasan *et al*. (2005) found that alcoholic extracts of neem and garlic completely inhibit the presence of *Fusarium* sp. *Aspergilus* sp. *Rhizopus* sp. On treated wheat seed whearer the highest percentage of *Fusarium* sp. (24.33%) *Aspergilus* sp. (17.07%) *Rhizopus* sp. (17.67%) (Hasan *et al*., 2005).

Seeds of the Neem tree (*Azadirachta indica)* contain Azadirachtin which is believed to have antifungal properties. Aqueous extracts of *Azadirachta indica* seeds, garlic bulbs, ginger rhizomes and basil leaves were used to control *Alternaria padwickii* in rice seeds (Shetty *et al*., 1989), while extracts from peppermint and garlic reduced rice seed infection by *Cochliobolus miyabeanus* (Alice and Rao, 1986).

Garlic bulb extract inhibited the spore germination and mycelial growth of seed borne fungal pathogens of jute, including *Macrophomina phaseolina, Botryodiplodi atheobromae* and *Colletotrichum corchori* (Ahmed and Sultana, 1984). Neem contains azadirachtin which has demonstrated antifungal activity (Natarajan *et al*., 2003).

CHAPTER III

MATERIALS AND METHODS

MATERIALS AND METHODS

3.1. Experimental site

The experiment was conducted in Seed Health Laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University (SAU), Sher-e-Bangla Nagar, Dhaka-1207.

3.2. Experimental period

The experiment was conducted during the period of January-2015 to February-2016.

3.3. Collection of seeds

The mostly cultivated three hybrid varieties of maize namely BARI HYBRID MAIZE VARIETY-3, BARI HYBRID MAIZE VARIETY-7, BARI HYBRID MAIZE VARIETY-9 were collected from Bangladesh Agriculture Research Institute, Gazipur.

3.4. Treatments

A total of five treatments (with one control) were used in the experiment are as follows:

T_1 = Control

T_2 = Neem leaf extract (1:2)

T_3 = Garlic extract (1:2)

T_4 = Mustard seed extract (1:2)

T_5 = Alamanda leaf extract (1:2)

3.5. Prevalence of fungi associated with three selected hybrid maize varieties

The collected seed samples of maize were analyzed for the presence of major seed borne fungal pathogens by blotter paper method following the International rules for Seed Health Testing. Four hundred seeds were tested for each variety randomly selected from the collected seed sample. Nine seeds were placed on three layers of moist blotting paper (Whatman No.1) in each glass petridish (Fig. 1). The petridishes containing seeds were incubated at 20 ± 10^{0}C under 12 hours photoperiod for 7 days. After incubation seeds were observed under stereomicroscope in order to record the presence of fungi on seed and based on growth habit. In doubtful cases temporary slides were prepared and observed under the compound microscope. Appropriate keys were followed consulted for identification of the fungi. The presence of fungi was recorded as percentage. The results were presented as percent incidence. Germination of the seeds was also recorded.

$$\% \text{ disease incidence} = \frac{\text{Number of infected seeds/seedlings}}{\text{Total number of inspected seed / seedlings}} \text{ X } 100$$

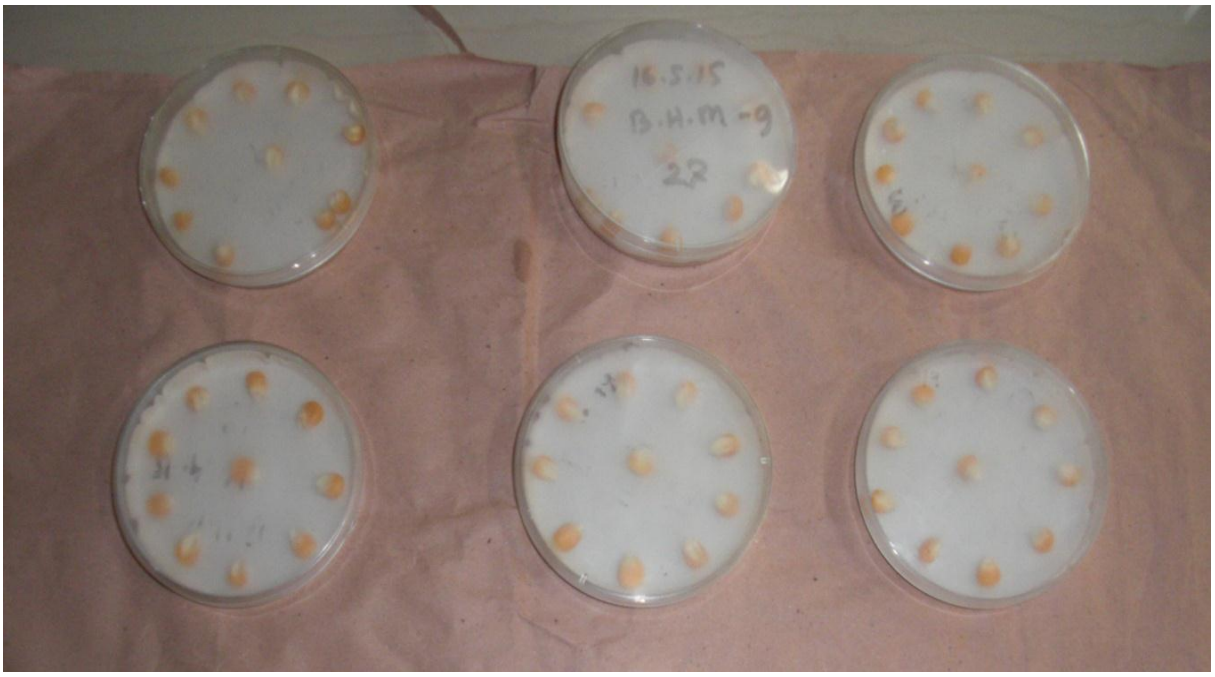

Fig .1. Maize seeds plated on blotter paper (blotter method)

3.6. Isolation and purification of fungi from incubated seeds

The fungi growing on the incubated seeds were transferred aseptically to PDA media containing petri-plates. Pure culture of the each of the test fungus was made following Riker and Riker (1921). The transferred single hyphal tip of the each test fungus was allowed to grown on PDA media at 22 ± 2^0C for 7 days.

3.6.1. Preparation of potato dextrose agar media (PDA media)

PDA was prepared as described by Islam (2009). Two hundred gram peeled and sliced potato was boiled in 500 ml water for about half an hour. Then the potato extract was filtered through cheese cloth. The two ingredients viz. 20g dextrose and 17 g agar were added in the extract followed by added water volume up to 1 L. The prepared standard PDA was poured in 1000 ml conical flask and sterilized in an autoclave at 121^0C under 15 psi for 30 min.

3.6.2. Identification of fungi

Existing seed borne-fungi with maize seeds were identified by observing their key characters on the incubated seeds. The fungi were identified to species level, wherever possible, following the keys of Malone and Musket (1964), Raper and Funnel (1965), Ramnath *et al.* (1970), Booth (1971), Ellis (1971), Mathur and Kongsdal (2003).

3.6.3. Preservation of fungal culture

To obtain pure culture of the pathogen, the hyphal tips were transferred aseptically onto PDA plate by using the flame sterilized tip of an inoculation needle. The plate was incubated at room temperature for 7 days. Advanced hyphae were collected and transferred into the test tube slants containing PDA media and incubated at room temperature for 7 days. After incubation, the slants were carefully checked for contamination and then preserved in a refrigerator at 40^0C for further use.

3.7. Seedling symptom test through water agar test tube method

The test tube seedling symptom test developed by Khare *et al.* (1977) was used for this study. Test tube slants were prepared by pouring 6 ml of 2.0% water agar and sterilized in autoclaved at 121^0C for 10 minutes under 15 lb pressure. Two hundred seeds for each sample were used at the rate of one seed per test tube. Then the test tubes with the seeds were then incubated in the laboratory desk at room temperature. The test tubes were properly plugged with cotton followed by placed on the wooden test incubation (Fig.2). The germinating seeds and seedlings in the test tube were examined for the presence of visible symptoms (seed rot, germination failure and infection or death of emerged seedlings) caused by the pathogens present in the seed. The symptoms produced on the germinating seeds and seedlings by the associated pathogen were confirmed by examining the seeds under stereo- binocular microscope. Data on germination, number of normal seedlings, abnormal seedlings, diseased seedlings and dead seeds were recorded.

Fig .2. Seedling symptom test by water agar test tube method

3.8. Determination of vigor index of three selected hybrid maize varieties (rolled paper towel method)

Seedling infection and seedling vigor test was done in rolled paper towel method (Warham, 1990). In this method, 200 seeds were randomly selected from each treatment and placed uniformly between a pair of moist paper towels. The towels were rolled and the two ends also closed with rubber band so that moist could not remove easily (Fig. 3). Then the rolled papers containing seeds were placed in an upright position for 7 days at room temperature under 12 hours photoperiod for 7 days. Length of shoot was measured from the base of the stem up to the growing point of the youngest leaf. Similarly, length of root was measured from starting point of the root to the largest available lateral root apex. Vigor of the seedling was determined by the following formula (Baki and Anderson, 1972).

Vigor index = (Mean of root length + Mean of shoot length) X % of seed germination.

Fig .3. Seeds (controls) placed on paper towel

Fig .4. Treated seeds (neem leaf extract) placed on paper towel

Fig .5. Treated seeds (garlic bulb extract) placed on paper towel

Fig .6. Treated seeds (mustard seed extract) placed on paper towel

Fig .7. Treated seeds (almanda leaf extract) placed on paper towel

Fig .8. Seedling vigor test in rolled paper towel method

3.9. Effect of plant extract on prevalence of fungi seedling vigor of three selected maize varieties

3.9.1. Treatments

3.9.2. Preparation of plant extracts

The collected plant parts were chopped after cleaning in running tap water. The extracts were prepared by crushing the plant parts or clove in a blender with distilled water in 1:2 ratio (eg. 1:2 = 100 gm plant material crushed in 200 ml water). The extracts were filtered through cheese cloth. The extracts thus obtained were kept in a refrigerator at $4°\pm1^0$c until use.

3.9.3. Seed treatment with plant extracts

Selected seed samples of maize were treated following dipping method. The seeds were dipped in 1:2 dilutions for 1 hour in previously prepared garlic clove, mastard, alamanda, neem, leaf extracts. After 1 hour, plant extracts were drained out from the petridishes. The treated seeds were allowed to be dried up on filter paper for some time and were tested following the standard blotter method to observe the growth of different fungal colonies on the seeds. For each treatment 400 seeds were placed on 40 petridishes. Then water agar method and paper towel method were applied.

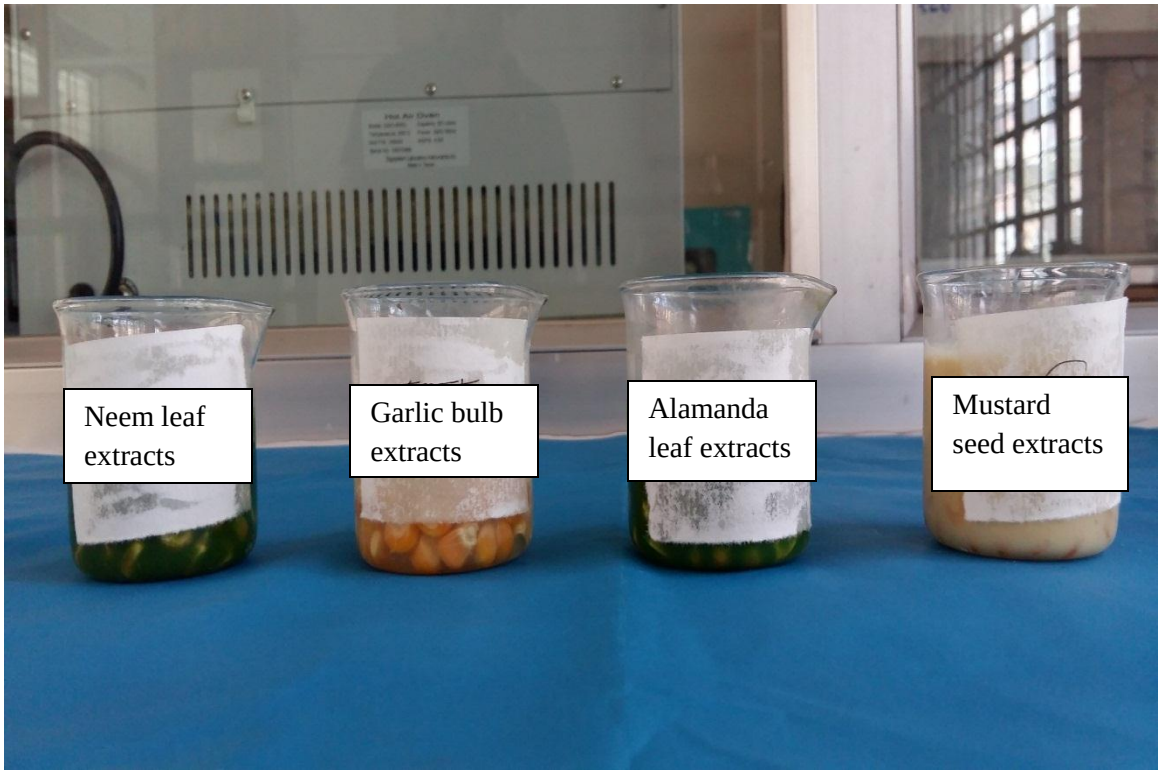

Fig .9. Seed treated with different plant extracts

3.9.4. Effect of plant extracts on prevalence of seed borne fungi (blotter paper method)

Treated seeds were tested following the procedure described in 3.5

3.9.5. Interaction effect of plant extract with variety on seedling vigor of three selected maize varieties

Treated seeds were tested in rolled paper towel method following 3.8

3.10. Statistical analysis

The recorded data were analyzed using MSTAT-C computer package programme following the statistical procedures of Gomez and Gomez (1984) for Lab experiment were designed in CRD double factor for blotter paper method and roll paper towel method and single factor for water augar method. The mean differences were judged by least significantly difference (LSD) or the 5% level of significance.

CHAPTER IV

RESULTS

RESULTS

4.1. Germination and prevalence of fungi associated with three hybrid maize varieties

Significant variations were observed among the varieties in respect of percent seed germination and incidence of fungi associated with maize seeds (Table 1). The highest (95.27%) seed germination was recorded in BARI hybrid maize variety-3 (V_1) and the lowest (92.90%) seed germination was recorded in BARI hybrid maize variety-9 (V_3). Three fungal species viz. *Aspergillus flavus*, *Aspergillus niger* and *Fusarium moniliforme* were detected from maize seeds. The incidence of *A. flavus* ranged from 10.67- 11.71%. The highest incidence was recorded in BARI hybrid maize variety-3 (V_1) which was statistically identical with BARI hybrid maize variety-79 (V_3) and the lowest incidence (10.67%) was recorded in BARI hybrid maize variety-7 (V_2). The highest incidence (11.20%) of *A. niger* was found in BARI hybrid maize variety-9 (V_3) where the lowest incidence (10.07%) was recorded in BARI hybrid maize variety-3 (V_1) which was statistically identical with BARI hybrid maize variety-7 (V_2). The incidence of *F. moniliforme* was varied from 8.33% to 12.13% where the highest incidence (12.13%) was recorded in BARI hybrid maize variety-3 (V_1) and the lowest incidence (8.33%) was found in BARI hybrid maize variety-7 (V_2) (Table 1).

Fig .10. *Aspergillus flavus* on maize seed

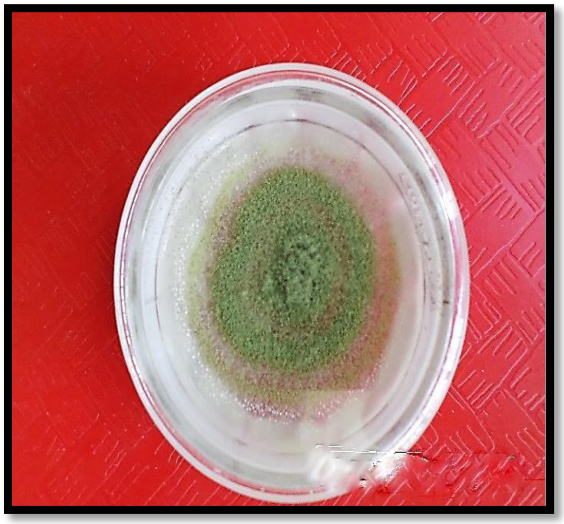

Fig .11. Pure culture of *Aspergillus flavus* on PDA medium

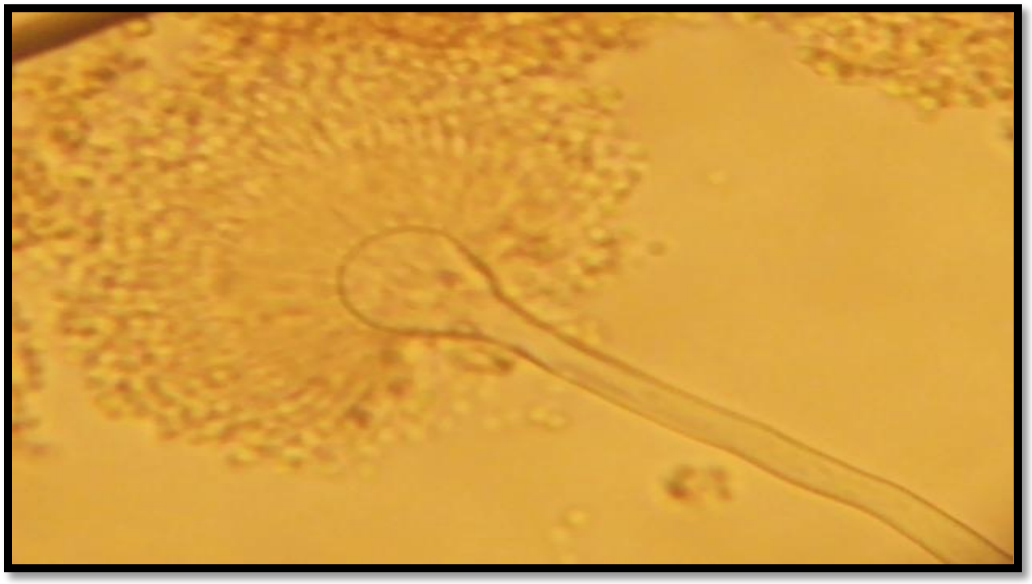

Fig .12. Conidia and conidiofore of *Aspergillus flavus* (under compound microscope at 40X)

Fig .13. *Aspergillus niger* on maize seed

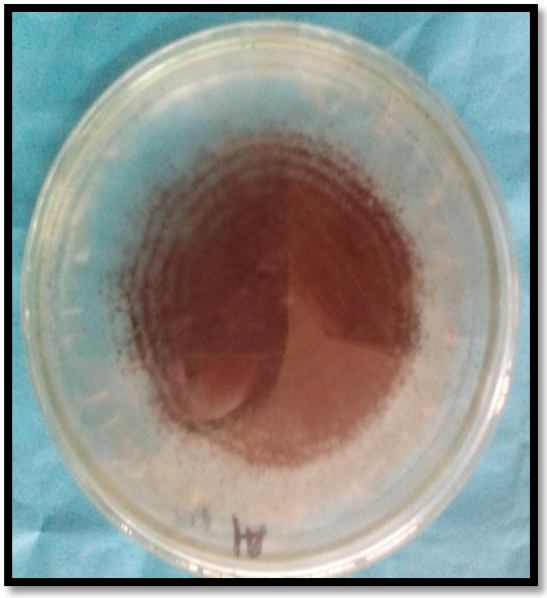

Fig .14. Pure culture of *Aspergillus niger* on PDA medium

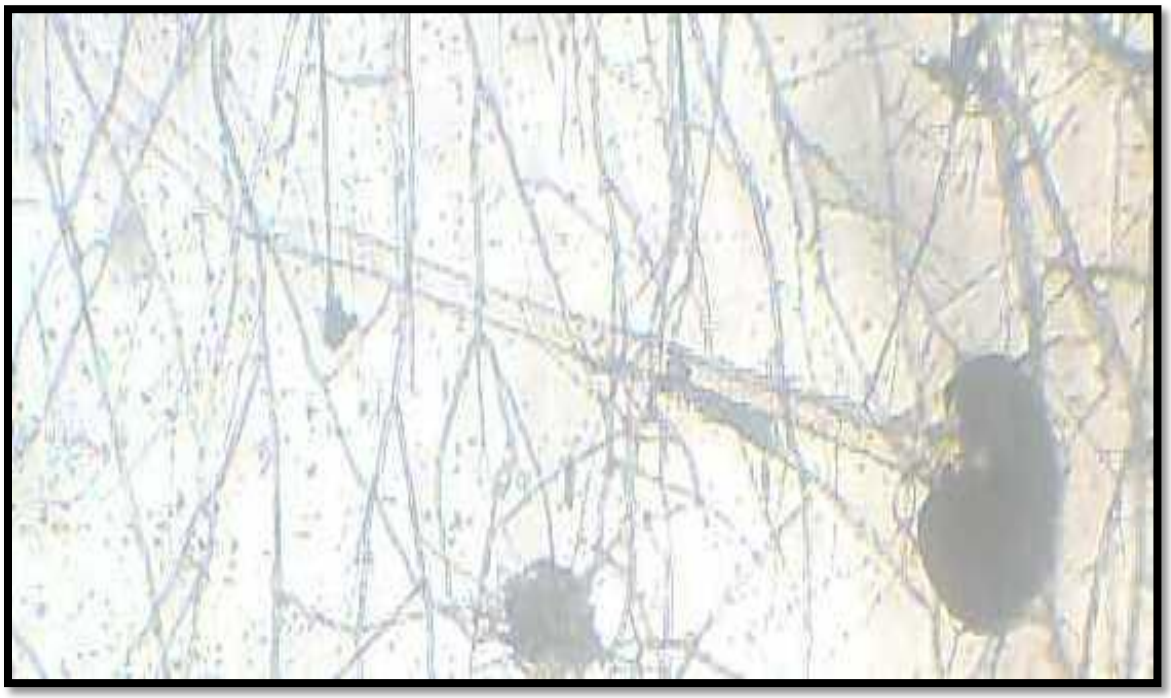

Fig .15. Conidia and conidiofore of *Aspergillus niger* (under compound microscope at 10X)

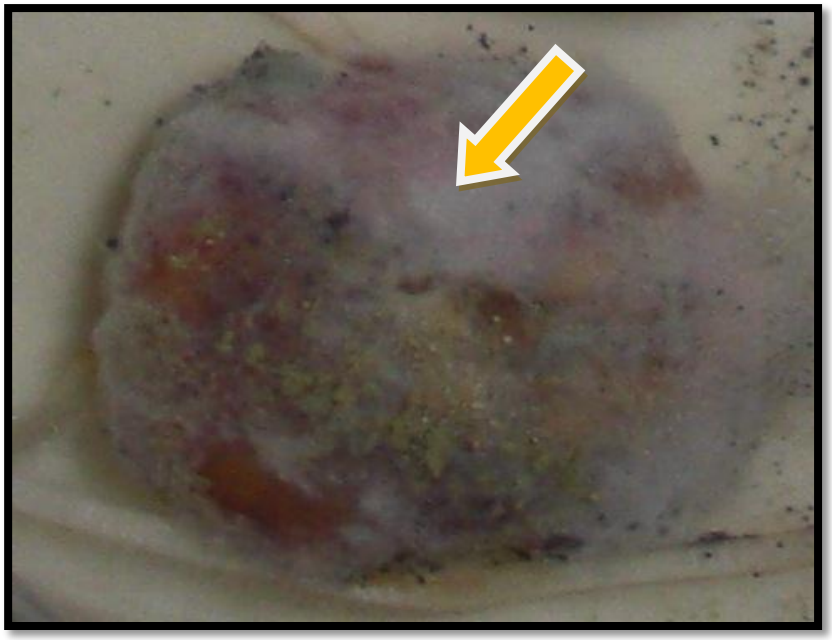

Fig .16. *Fusarium moniliforme* on maize seed

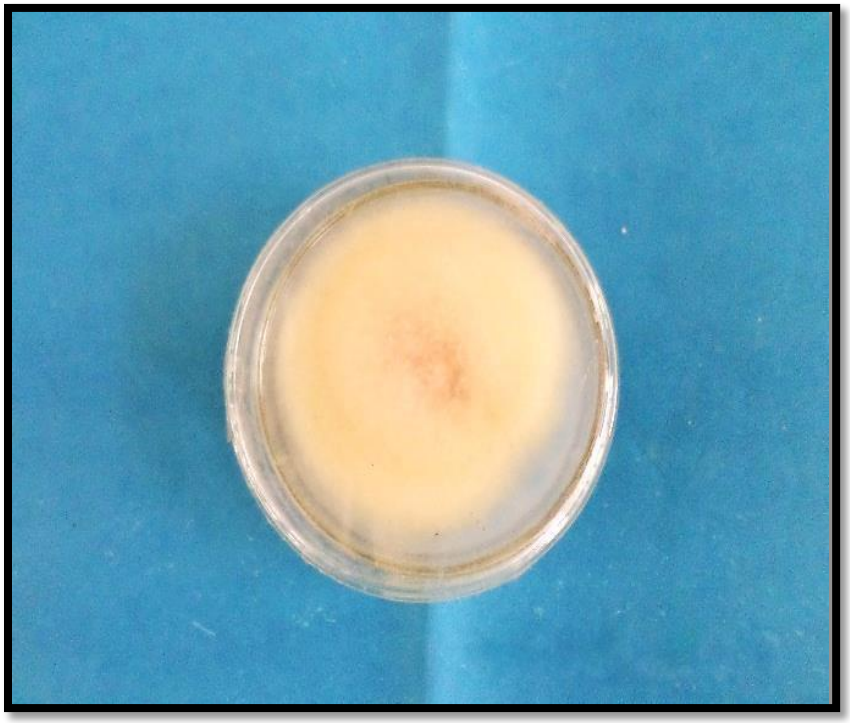

Fig .17. Pure culture of *Fusarium moniliforme* on PDA medium

4.1.2. Effect of plant extract on germination and prevalence of seed borne fungi of selected three hybrid maize varieties (blotter paper method)

Effect of plant extract on seed borne fungi on seedlings of selected hybrid maize varieties were determined and significant variation was observed among the varieties regarding germination and incidence of seed borne pathogens (table 1). The highest germination (97.22%) was observed in seeds treated with neem leaf extract (T_2) which is statistically similar with control (T_1). Incidence of *Aspergilus flavus* varied 7.78-22.47% where the lowest was observed in garlic bulb extract (T_3) treated seeds which was statistically same with alamanda (T_5) and the highest was in control (T_1). Incidence of *Aspergilus niger* was lowest (8.12%) in seed treated with T_5 (8.12%) which is statistically same with neem leaf extract (T_3) and highest (21.22%) in seed treated with control (T_1). Incidence of *Fusarium moniliforme* varied from 7.11-19.89%. The lowest value (7.11%) was observed in alamanda leaf extract which is statistically same with neem leaf extract and the highest value (19.89%) was observed in control (T_1).

Table 1. Germination and prevalence of fungi associated with three selected hybrid maize varieties

Variety	Germination (%)	*Aspergilus flavus (%)*	*Aspergilus niger (%)*	*Fusarium moniliforme (%)*
V_1	95.27 a	11.40 a	10.07 b	12.13 a
V_2	94.20 b	10.67 b	10.27 b	8.333 b
V_3	92.90 c	11.71 a	11.20 a	8.800 b
CV%	5.74	4.10	4.52	6.89
LSD(0.05)	1.25	0.54	0.76	2.40

V_1 = BARI hybrid maize variety-3

V_2 = BARI hybrid maize variety-7

V_3 = BARI hybrid maize variety-9

In a column means having similar letter (s) are statistically identical and those having dissimilar letter(s) differ significantly as per 0.05 level of probability

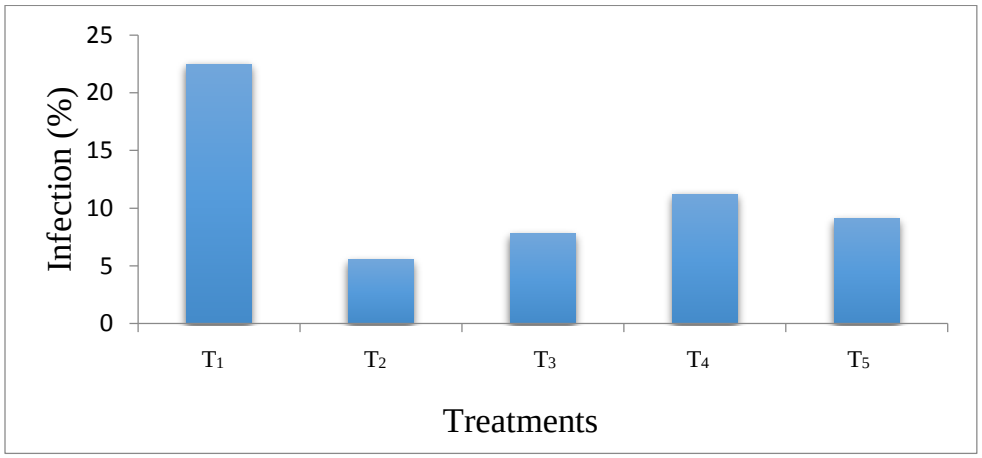

Fig .18. Interaction between seed borne infection by *Aspergillus flavus* and different plant extracts T_1 = Control, T_2 = Neem leaf extract, T_3 = Garlic bulb extract, T_4 = Mustard seed extract, T_5 = Alamanda leaf extract

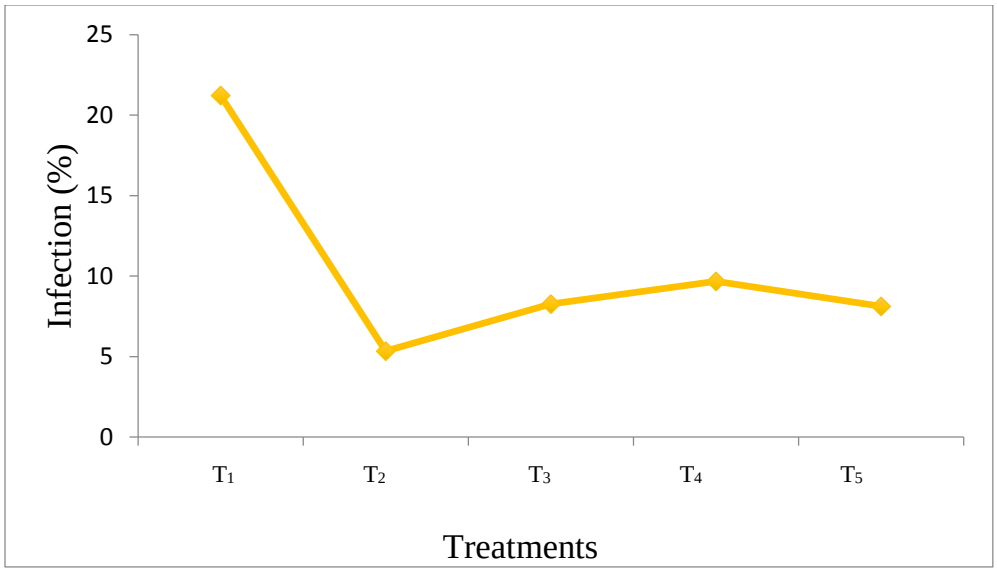

Fig .19. Interaction between infection of *Aspergillus niger* and different plant extracts .T_1 = Control, T_2 = Neem leaf extract, T_3 = Garlic bulb extract, T_4 = Mustard seed extract, T_5 = Alamanda leaf extract

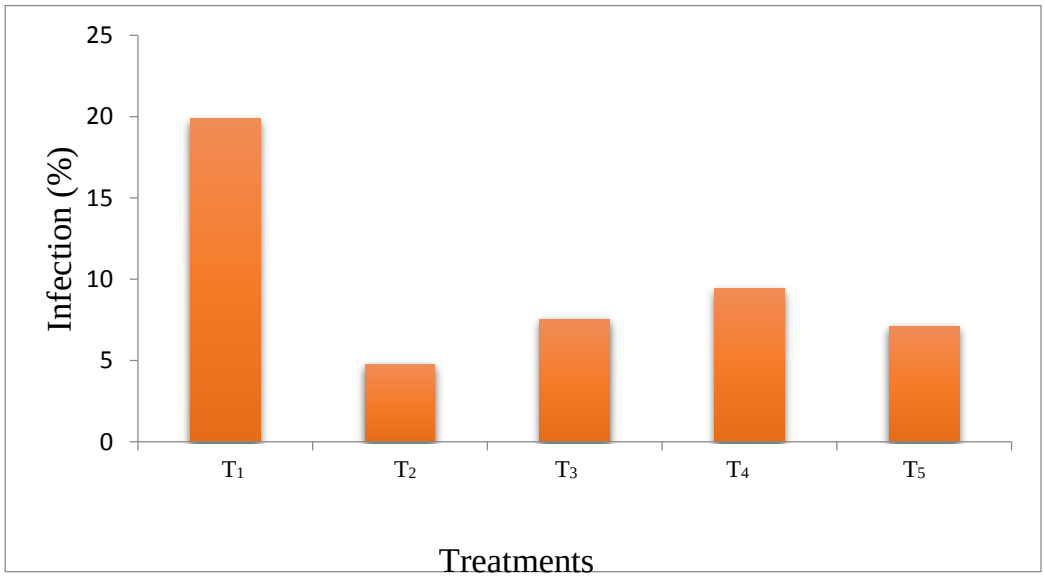

Fig .20. Interaction between infection of *Fusarium moniliforme* and different plant extracts. T_1 = Control, T_2 = Neem leaf extract, T_3 = Garlic bulb extract, T_4 = Mustard seed extract, T_5 = Alamanda leaf extract

4.1.3. Interaction effect of plant extracts with variety on germination and prevalence of seed borne fungi

Significant interaction effect of plant extract with variety on germination and prevalence of pathogen were observed (Table 2). The highest seed germination (97.33%) was recorded in V_2T_2 where BARI hybrid maize variety-7 treated with neem leaf extract which is statistically identical with V_3T_2 when BARI hybrid maize variety-9 treated with neem leaf extract and the lowest (83.33%) in V_3T_1 when BARI hybrid maize variety-9 untreated control.

Significant interaction effect of plant extract with variety on disease incidence was observed. The highest *Aspergilus flavus* (23.33%) was recorded in V_2T_1 when BARI hybrid maize variety-7 used with control treatment and the lowest (4.65%) was recorded in V_2T_2 when Bari hybrid maize variety-7 treated with neem leaf extract. The highest *Aspergilus niger* (21.67%) was recorded in V_3T_1 when BARI hybrid maize variety-9 untreated with control treatment which is statistically identical with V_2T_1 and V_1T_1 and the lowest (4.31%) was recorded in V_2T_2 when BARI hybrid maize variety-7 treated with neem leaf extract. The highest *Fusarium moniliforme* (23.34%) was recorded in V_1T_1 when BARI hybrid maize variety-3 untreated with control treatment and the lowest (3.41%) was recorded in V_2T_2 when BARI hybrid maize variety-7 was treated with neem leaf extract.

Table 2. Effect of plant extract on germination and prevalence of seed borne fungi of selected three hybrid maize varieties (blotter paper method)

Treatments	Germination (%)	*Aspergilus flavus*(%)	*Aspergilus niger*(%)	*Fusarium moniliforme*(%)
V_1T_1	91.67 e	21.67 b	20.33 a	23.34 a
V_1T_2	97.00 ab	5.68 gh	5.29 gh	6.66 g
V_1T_3	95.00 cd	7.66 e	9.35 cd	9.38 de
V_1T_4	96.67 abc	11.36 c	6.67 efg	11.28 c
V_1T_5	96.00 abc	10.62 c	8.58 cde	10.00 d
V_2T_1	86.67 f	23.33 a	21.67 a	18.33 b
V_2T_2	97.33 a	4.65 h	4.31 h	3.41 i
V_2T_3	96.67 abc	7.34 ef	7.62 def	6.68 g
V_2T_4	95.00 cd	10.63 c	10.67 bc	8.58 ef
V_2T_5	95.33 bcd	7.41 ef	7.00 efg	4.62 h
V_3T_1	83.33 g	22.58 ab	21.66 a	18.00 b
V_3T_2	97.33 a	6.33 fg	6.36 fgh	4.33 h
V_3T_3	96.33 abc	8.29 de	7.66 def	6.71 g
V_3T_4	94.00 d	11.60 c	11.67 b	8.37 f
V_3T_5	96.00 abc	9.31 d	8.62 cde	6.68 g
CV%	5.74	4.10	4.52	6.89
LSD(0.05)	1.93	1.06	2.30	0.92

T_1 = Control, T_2 = Neem leaf extract, T_3 = Garlic bulb extract, T_4 = Mustard seed extract, T_5 = Alamanda leaf extract and V_1 = BARI hybrid maize variety-3, V_2 = BARI hybrid maize variety-7, V_3 = BARI hybrid maize variety-9

4.2.1. Effect of fungi associated with three selected maize varieties on seedlings vigor (rolled paper towel method)

Effect of seed borne fungi on seedling vigor of selected hybrid maize varieties were determined and significant results were found regarding seed germination, normal seedlings, diseased seedlings, dead seed and vigor index (Table 3). The highest seed germination (92.47 %) was recorded in BARI hybrid maize variety-3 (V_1) which was statistically identical with BARI hybrid maize variety-9 and the lowest germination (90.73%) was found in BARI hybrid maize variety-7. Percent of healthy seedlings varied from 75.81% - 83.80% where the highest value was counted in BARI hybrid maize variety-7 (V_2) and the lowest value was found in BARI hybrid maize variety-9 (V_3). Dead seed percent was ranged from 5.13-7.93% where the highest value counted in BARI hybrid maize variety-7 (V_2) and the lowest percent was recorded in BARI hybrid maize variety-9 (V_3) which was statistically similar with BARI hybrid maize variety-3 (V_1). In case of abnormal seedlings the highest percent (5.06%) was recorded from BARI hybrid maize variety-3 (V_1) which was statistically identical with BARI hybrid maize variety-9 (V_3) and the lowest percent (3.13%) was found in BARI hybrid maize variety-7 (V_2). Disease Incidence was the highest value (14.13%) was counted in BARI hybrid maize variety-9 (V_3) and lowest (5.13%) was counted in BARI hybrid maize variety-7 (V_2). In case of vigor index, the maximum vigor index (2087) was recorded in BARI hybrid maize variety-7 (V_2) which was statistically similar with BARI hybrid maize variety-3 (V_1) and the minimum vigor index (1664) was observed in BARI hybrid maize variety-9 (V_3).

Table 3. Effect of seed borne fungi on seedlings vigor (rolled paper towel method)

Variety	Germination (%)	Healthy seedling (%)	Dead seedling (%)	Abnormal seedling (%)	Disease seedling (%)	Vigor Index (%)
V_1	92.47 a	81.27 b	5.86 b	5.06 a	7.20 b	2031 a
V_2	90.73 b	83.80 a	7.93 a	3.13 b	5.13 c	2087 a
V_3	91.87 b	75.80 c	5.13 b	4.93 a	14.13 a	1664 b
CV%	5.05	3.06	6.62	5.97	4.64	6.14
LSD(0.05)	1.25	2.34	2.66	1.45	2.04	395

V_1 = BARI hybrid maize variety-3

V_2 = BARI hybrid maize variety-7

V_3 = BARI hybrid maize variety-9

In a column means having similar letter (s) are statistically identical and those having dissimilar letter(s) differ significantly as per 0.05 level of probability

4.2.2. Effect of plant extract on fungi associated with three selected maize varieties on seedlings vigor (rolled paper towel method)

Effect of plant extract on seed borne fungi on seedling vigor of selected hybrid maize varieties were determined and significant results found regarding germination, normal seedlings, diseased seedlings, dead seed and vigor index (Table 4). The highest seed germination (95.56%) was recorded in T_2 where seeds were treated with neem leaf extract and the lowest germination (87.67%) was found in T_1. Percent of normal seedlings varied from 74.22% - 84.67 % where the highest value was counted in T_2 where seeds were treated with neem leaf extract and the lowest value was found in T_1 (control treatment). Dead seed percent was ranged from 3.88-9.44% where the highest value counted in T_1 untreated control treatment and the lowest percent was recorded in T_2 where seeds were treated with neem leaf extract. In case of abnormal seedlings the highest percent (5.33%) was recorded from (T_5) and the lowest percent (3.22%) was found in T_2 where seeds were treated with neem leaf extract. Disease Incidence was the highest in T_3 (10.89%) and lowest was counted in T_2 (7.11%) where seeds were treated with neem leaf extract. In case of vigor index, the maximum vigor index was recorded in T_2 (2198) where seeds were treated with neem leaf extract and the minimum vigor index 1588 was observed untreated control T_1.

4.2.3. Interaction of plant extract with variety on fungi associated with three selected maize varieties on seedlings vigor (rolled paper towel method)

Effect of plant extract on seed borne fungi on seedling vigor of selected hybrid maize varieties were determined and significant results were found regarding germination, normal seedlings, diseased seedlings, dead seed and vigor index (Table 4). The highest seed germination (92.47 %) was recorded in V_2T_2 when BARI hybrid maize variety-7 treated with neem leaf extract and the lowest seed germination (87.33%) was found in V_3T_1 when BARI hybrid maize variety-7 used with control treatment. Percent of normal seedlings varied from 64.00% - 89.33% where the highest value was counted in V_2T_2 when BARI hybrid maize variety-7 treated with neem leaf extract and the lowest value was found in V_3T_1 when BARI hybrid maize variety-9 used with control treatment. Dead seed percent was ranged from 2.66-11.33% where the highest value was counted in V_3T_1 and the lowest percent was recorded in V_3T_2. In case of abnormal seedlings the highest percent (6.33%) was recorded from V_1T_5 where BARI hybrid maize variety-3 treated with alamanda leaf extract and V_3T_1 where BARI hybrid maize variety-9 untreated with control treatment and the lowest percent (2.66%) was found in V_1T_2 when BARI hybrid maize variety-3 treated with neem leaf extract and V_2T_3 when BARI hybrid maize variety-7 treated with garlic bulb extract. Disease Incidence was the highest value counted in V_3T_1 (18.33%) and lowest was counted in V_1T_2 (7.66%) when BARI hybrid maize variety-3 treated with neem leaf extract which is statistically similar with V_1T_1 when BARI hybrid maize variety-3 untreated with control treatment and V_1T_3. In case of vigor index, the maximum vigor index was recorded in V_2T_2 (2571) when BARI hybrid maize variety-7 treated with neem leaf extract and the minimum vigor index was observed in V_3T_1 (1310) when BARI hybrid maize variety-9 with control treatment.

Table 4. Effect of plant extract on seed- borne fungi on seedlings vigor (rolled paper towel method)

Treatment	Germination	Healthy seedling (%)	Dead seedling (%)	Abnormal seedling (%)	Disease seedling (%)	Vigor Index
T_1	87.67 c	74.22 d	9.44 a	4.44 b	10.89 a	1588 d
T_2	95.56 a	84.67 a	3.88 c	3.22 c	7.11 d	2198 a
T_3	91.67 b	81.67 b	5.44 b	4.33 b	9.33 b	1902 c
T_4	92.11 b	81.44 b	6.22 b	4.33 b	8.11 cd	1930 c
T_5	91.44 b	79.44 c	6.55 b	5.33 a	8.66 bc	2018 b
CV%	5.05	3.06	6.62	5.97 a	4.64	6.14
LSD(0.05)	2.65	1.59	1.19	1.08	1.07	110

T_1 = Control,

T_2 = Neem leaf extract,

T_3 = Garlic bulb extract,

T_4 = Mustard seed extract,

T_5 = Alamanda leaf extract

In a column means having similar letter (s) are statistically identical and those having dissimilar letter(s) differ significantly as per 0.05 level of probability

Table 5. Effect of interaction of plant extract with variety on seed- borne fungi on seedlings vigor (rolled paper towel method)

Interaction	Germination	Healthy seedling (%)	Dead seedling (%)	Abnormal seedling (%)	Disease seedling (%)	Vigor Index
V_1T_1	88.00 fg	79.00 fg	7.33 cd	2.66 e	8.00 e	1671 g
V_1T_2	95.67 ab	83.33 bc	3.66 ef	5.33 abc	7.66 e	2200 b
V_1T_3	93.67 bc	81.00 cdef	5.33 de	5.66 ab	8.00 e	2029 de
V_1T_4	94.00 abc	82.67 cd	5.33 de	5.33 abc	6.66 ef	2163 bc
V_1T_5	91.00 de	80.33 defg	7.66 bc	6.33 a	5.66 fg	2092 bcd
V_2T_1	87.67 g	79.67 efg	9.66 ab	4.33 cd	6.33 ef	1783 fg
V_2T_2	96.33 a	89.33 a	4.33 ef	1.33 f	3.66 h	2571 a
V_2T_3	90.33 def	86.00 b	8.33 bc	2.66 e	4.33 gh	2017 de
V_2T_4	88.67 efg	81.67 cdef	9.66 ab	3.00 e	5.66 fg	2159 bc
V_2T_5	90.67 de	82.33 cde	7.66 bc	4.33 cd	5.66 fg	1905 ef
V_3T_1	87.33 g	64.00 i	11.33 a	6.33 a	18.33a	1310 i
V_3T_2	94.67 abc	81.33 cdef	2.66 f	5.00 bc	10.00 d	1825 f
V_3T_3	91.00 de	78.00 gh	3.66 ef	3.66 de	15.67 b	1660 g
V_3T_4	93.67 bc	80.00 defg	3.66 ef	4.33 cd	12.00 c	1468 h
V_3T_5	92.67 cd	75.67h	4.33 ef	5.33 abc	14.67 b	2056 cd
CV%	5.05	3.06	6.62	5.97	4.64	6.14
LSD(0.05)	2.26	2.75	2.06	1.16	1.86	130

T_1 = Control, T_2 = Neem leaf extract, T_3 = Garlic bulb extract, T_4 = Mustard seed extract, T_5 = Alamanda leaf extract and V_1 = BARI hybrid maize variety-3, V_2 = BARI hybrid maize variety-7, V_3 = BARI hybrid maize variety-9

Fig .21. Normal seedling of maize

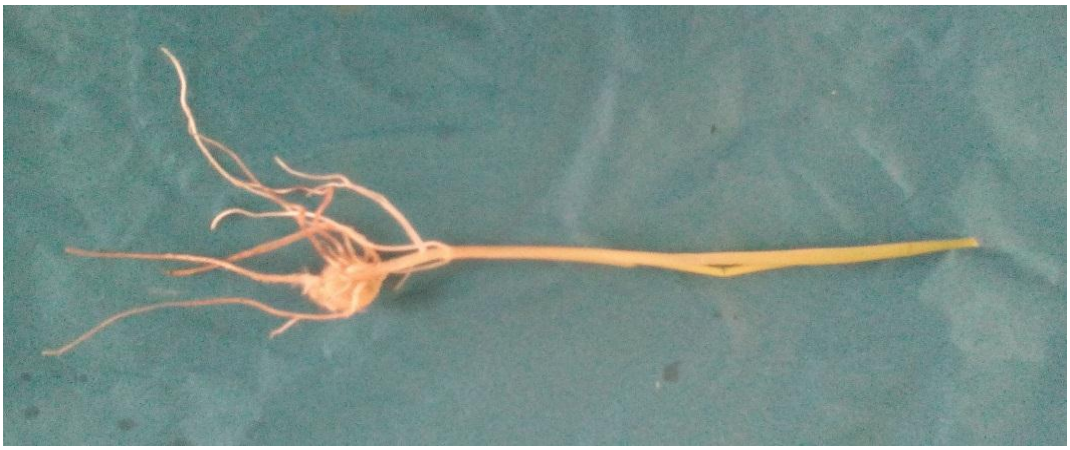

Fig .22. Diseased seedling of maize

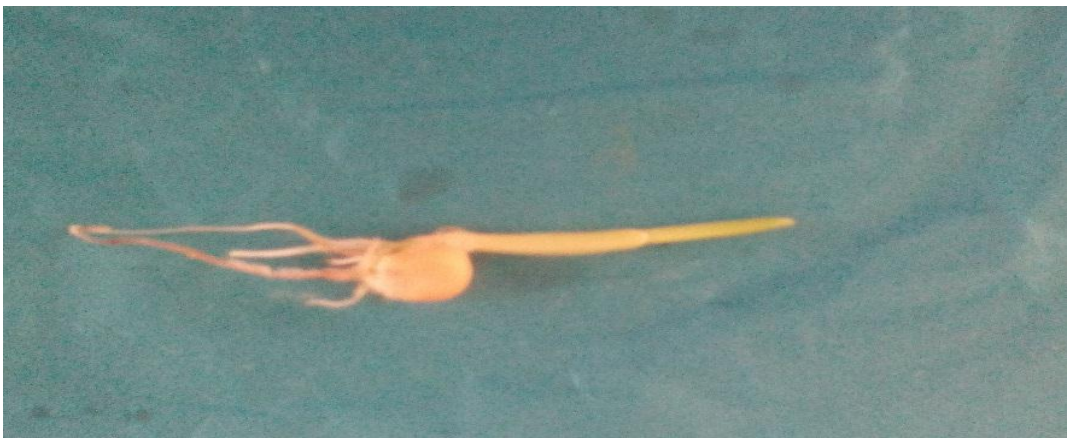

Fig .23. Abnormal seedling of maize

4.3. Effect of fungi associated with three selected maize varieties on seedlings (water agar test tube method)

Effect of seed-borne fungi on seedlings of selected hybrid maize varieties were determined and significant variation was observed among the varieties regarding germination, dead seed healthy seedlings, abnormal seedlings and diseased seedlings. In water agar test tube method the highest percent of germination (94.36%) was recorded in BARI hybrid maize variety-9 (V_3) and the lowest germination (89.78%) was recorded in BARI hybrid maize variety-3 (V_1). Number of healthy seedlings varied from 79.66-84.33% where the highest 84.33% number recorded from BARI hybrid maize variety-9 (V_3) which is statistically similar with BARI hybrid maize variety-7 (V_2) (83.66%) and the lowest numbers of healthy seedlings were (79.66%) found in BARI hybrid maize variety-3 (V_1). Dead seed percent was ranged from 6.12-9.68% where the highest value counted in BARI hybrid maize variety-3 (V_1) and the lowest percent was recorded in BARI hybrid maize variety-9 (V_3). In case of abnormal seedlings significant variation was not observed. The highest percent (2.66%) was recorded from BARI hybrid maize variety-7 (V_2) and the lowest percent (2.00 %) was found in BARI hybrid maize variety-3 (V_1). Disease Incidence was highest value was (8.66%) counted in BARI hybrid maize variety-3 (V_1) and the lowest was (6.02%) counted in BARI hybrid maize variety-7 (V_2).

Table 6. Effect of fungi associated with three selected maize varieties on seedlings (water agar test tube method)

Variety	Germination (%)	Healthy seedling (%)	Dead seedling (%)	Abnormal seedling (%)	Disease seedling (%)
V_1	89.78 c	79.66 b	9.68 a	2.00	8.66 a
V_2	92.24 b	83.66 a	7.66 b	2.66	6.02 c
V_3	94.36 a	84.33 a	6.12 c	2.43	7.00 b
CV%	6.32	5.47	4.07	3.52	5.12
LSD(0.05)	1.96	4.35	1.58	----	1.00

V_1 = BARI hybrid maize variety-3

V_2 = BARI hybrid maize variety-7

V_3 = BARI hybrid maize variety-9

CHAPTER V

DISCUSSION

DISCUSSION

The experiment was conducted in Seed Health Laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University (SAU), Dhaka to determine the prevalence of fungi associated with three hybrid maize varieties namely BARI hybrid maize variety-3, BARI hybrid maize variety-7, BARI hybrid maize variety-9 and the effect of plant extracts on the seed germination and seedling vigor. Three fungal species were found associated with the seeds viz *Aspegillus flavus, A. niger* and *Fusarium moniliforme.* A large number of seed-borne pathogens of maize namely *A. flavus*, *A. niger*, *Penicillium* sp., *Rhizopus* sp., *F. moniliforme* and *Xanthomonas* sp. were detected from maize seed (Faruq *et al.*, 2014). Debnath *et al.* (2012) tested six maize varieties by blotter method and identified *A. niger, A. flavus, Fusarium* sp., *P. oxalicum, C. lunata* and *R. stolonifer*. Six fungi namely *Alternaria alternata*, *A. niger*, *F. moniliforme*, *Fusarium* sp., *Penicillium* sp. and *Ustilago zeae* were found associated with maize seeds. Among the fungi identified the highest incidence was found with *F. moniliforme*. In agreement with this result Fakir (2001) and Alam (2004) reported the more or less the same result. The present results were partialy supported by Basak and Lee (2002). Hussain *et al.* (2012) evaluated the pathogenicity of two mostly prevailing fungal species *F. moniliforme* and *A. niger* on maize and found *F. moniliforme* had 50.2% pathogenicity on seeds and 6.55% on seedlings, while *A. Niger* had 62.87% on seeds and 11.24% on seedlings. These findings proves that mycoflora had significant detrimental impacts on seeds and seedling's life of maize. Ahmed *et al.* (2013) worked with ten plant extract on rice seedling and found that promising performance of the basis of germination and disease is garlic, charita and neem extraction seed and seedling health. Joseph Adjei (2011) described that Aqueous extracts of neem (*Azadirachta indica*) seed, lemongrass (*Cymbopogon citratus*) leaves and *Chromolaena odorata* leaves were tested for their efficacy in controlling seed borne fungal pathogens and reported that aqueous extract of neem

seed treatment for 24 hours was active in reducing infection by *Fusarium moniliforme* in maize seed by reducing it from 68% to 18%. Similar results were obtained by Masum *et al.* (2009) in which neem seed extract reduced incidence of *F. moniliforme* and other seed borne fungal infection in sorghum.

In paper towel method fungal incidence was recorded in BARI hybrid maize variety-3, BARI hybrid maize variety-7, BARI hybrid maize variety-9. The highest germination was obtained from BARI hybrid maize variety-7 when treated with neem leaf extract and the lowest in BARI hybrid maize variety-9 in control treatment which is statistically same with BARI hybrid maize variety-7 when seeds were untreated with control treatment. Highest vigour index was recorded in BARI hybrid maize variety-7 which is treated with neem leaf extract where significantly the lowest vigour index was recorded in control. Debnath *et al.*, (2012) reported the seed treated with neem, garlic extract, BAU fungicide on BARI Bhutta- 6 and found that the vigour index of seedling varied from 2856 to 1885 where significantly the lowest (1885) vigour index was recorded in control and the highest (2856). The present findings were also supported by Islam and Borthakur (2012) and Utobo *et al.* (2011). Islam and Borthakur (2012) reported that *F. moniliforme*, *R. nigricans* and *P. oxalicum*ca used marked reduction in shoot length, whereas *Chaetomium herbasum* and *F. moniliforme* caused marked reduction in root length. *F. moniliforme*, *F. chlamydosporum* and *A. niger* caused reduction in vigor index.

In water agar test tube method the highest germination, minimum number of abnormal seedling, minimum number of diseased seedlings and minimum number of dead seeds were recorded in BARI hybrid maize variety-9 (V_3). Minimum number of diseased seedlings found in BARI hybrid maize variety-9 (V_2).

Regarding seeds and seedlings health BARI hybrid maize variety-3 (V_1) showed comparatively poor performance. This findings keep with the findings of Guerrero *et al.* (1972) and Islam *et al.* (2000). Guerrero *et al.* (1972) reported that most of seed-borne fungi caused abnormal seedlings and Islam *et al.* (2000) observed that the highest lethal seed infection caused by seed-borne fungi. Basak and Lee (2002) evaluated transmission of all seed-borne pathogens from seeds to seedlings detected by test tube seedling symptom test. Among the seed-borne fungi, *A. alternata*, *F. moniliforme* and *Fusarium* sp. caused distinct seed rot and seedling infection. All the transmitted seed-borne fungi might be primary sources of infection to the maize crop. The present findings were in agreement with the information of seed borne nature of the pathogen reported by Marley and Gbenga (2004). The pathogenicity test with two most frequently isolated fungi *F. moniliforme* and *A. niger* was carried out and showed pathogenic effects on seeds germination. *Fusarium moniliforme and A. flavus*, *A. niger* highly pathogenic seeds borne fungi that were frequently recorded almost with all samples from all localities and were also reported pathogenic by several other studies (Richardson, 1979; Iftikhar, 1991; Fakhrunnisa and Hashmi, 1992, Ahmad *et al.*, 1993). *F. moniliforme* was found to be highly infective by producing mycotoxins that are involved in retarding seed germination and seedlings growth as also reported by Yates *et al.* (1997).

From the present study it is revealed that hybrid maize seeds were found associated with some fungi harmful for seeds and seedling of maize. The findings of the present study need to be explored under field condition for its feasibility in the farmer's field. Therefore, further research works taking more options in the area is advocated.

CHAPTER VI

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

The present study was conducted to know the effect of plant extract on seed germination and seedling health of three selected hybrid maize varieties. The research work was carried out in the Seed Pathology Laboratory**,** Department of Plant Pathology, Sher-e-Bangla Agricultural University (SAU), Dhaka-1207 during the period January-2015 to February- 2016. Three selected hybrid maize varieties namely BARI hybrid maize variety-3, BARI hybrid maize variety-7, BARI hybrid maize variety-9 were used. In blotter method, three fungal species were identified associated with the seeds viz. *Aspegillus flavus*, *A. niger* and *Fusarium moniliforme.*

In water agar test tube method the highest seed germination percentage (94.36%) was found in BARI hybrid maize variety-9. The maximum number of abnormal seedlings (2.66%) were found in BARI hybrid maize variety-7. The highest diseased seedlings (8.66%) were recorded from BARI hybrid maize variety-3. The highest percentage of dead seed were (2.43%) recorded from BARI hybrid maize variety-3.

In rolled paper towel method the best performance showed by seeds treatment with neem leaf extract regarding maximum number of seed germination, highest vigority index, the maximum number of healthy seedling, minimum number of disease, minimum number of dead seedling was observed.

In this experiment total five treatments were applied on three hybrid maize varieties. The treatments were neem leaf extract, garlic bulb extract, mustard seed extract, alamanda leaf extract and control. Effect of plant extracts on germination and prevalence of seed borne fungi were identified. Above this treatment neem leaf extract performance was found best.

Seed is a common carrier of plant pathogens. Pathogen free seed is the important input material in agriculture and high quality seed is not only important for increasing crop production but proper management in storage is also an important factor for seed health. The present experiment showed few number of seed-borne fungi were found associated with hybrid maize seeds though the hybrid maize seeds were treated with fungicides. Seed-borne fungi appeared may be due to improper management in storage.

Considering of over-all findings it can be said that plant extract can increase seed germination and decrease disease incidence. Farmers are therefore advised to treatment maize seeds before sowing with plant extracts.

REFERENCES

Agarwal, P., Mortensen C. and Mathur, S. B. (1989). Seed-borne diseases and seed health testing of rice. Danish Govt. Inst. Seed Path., Copenhagen, Denmark. p. 14.

Ahmad, D., Iftikhar, S. and Bhutta A. R. (1993). Seed-borne microorganisms in Pakistan Checklist 1991. PARC, Islamabad, Pakistan. p. 32.

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

Aldrich, S. R., Scott, W. O. and Leng, E. R. (1975). Modern corn production. 2nd edition. United States of America. pp. 1-5.

Alice, D. and Rao, A. V., (1986). Management of seed borne *Drechslera oryzae* of rice with plant extracts. *International Rice Research Newsletter*. **11**:19.

Alves M. C. and Pozza E. M. (2012). Scanning electron microscopy detection of seed-borne fungi in blotter test. Current Microscopy Contributions to Advances in Science and Technology (A. Méndez-Vilas, Ed.)

Anonymous (2014). Improving Corn; based on "Hybrid Corn", published in the Yearbook of Agriculture, 1962". United States Department of Agriculture. Retrieved 14 December 2014.

Asalmol, M.N. Kale, V.P. and Ingle, S.T. 2001. Seed Borne Fungi of Chilli, Incidence and Effect on Seed Germination. *Seed Res.*, **29**(1): 76-79.

Avinder, R. and Rai, B. K. (1991). Seed mycoflora of *Zea mays* in tribal area. *Indian Phytopathol*. **44**(4): 526.

Baki, A. A. and Anderson, J. D. (1973). Vigor determination in soybean seed by multiple criteria. *Crop Sci*. **13**: 630-633.

Bangladesh Bureau of Statistics (BBS). (2010). Statistical Year Book of Bangladesh, Statistics Division, Ministry of Planning: Govt. of the People's Republic of Bangladesh, Dhaka.p. 36.

Bankole, S. A. and Adebanjo, A. (1995). Inhibition of growth of some plant pathogenic fungi using extracts from some Nigerian plants. *Intl. J. Trop. Plant Dis*., **13**: 91-95.

Bankole, S. A. and Adebanjo, A. (2003). Mycotoxins in food in West Africa: current situation and possibilities of controlling it. *African Journal of Biotechnology* **2** (9): 254-263.

Bari, M. A. and Alam, M. S. (2004). Major diseases of wheat and maize and their control. A Bengali Booklet published from Dept. of Plant Pathology, BARI, Joydebpur, Gazipur. **2**:12-16.

Basak, A. B. and Lee, M. W. (2002). Prevalence and transmission of seed-borne fungi of maize grown in a farm of Korea. *The Korean Society of Mycology*. **30**(1): 47-50.

Bhat, R. V., Vashanti, S. (1999). Occurrence of aflatoxins and its economic impact on human nutrition and animal feed. *The New Regulation*. Agric. Develop. No **23**: 50-56.

Boateng, E.O., Ewusi, K., Kanbur, R. and McKay. A. (1990). *A Poverty Profile for Ghana, 1987–88*. Social Dimensions of Adjustment in Sub-Saharan Africa Working Paper No. 5. Washington, D.C.: The World Bank.

Booth, C. (1971). The Genus Fusarium. *Commonwealth Mycol. Inst. Kew, Surrey, England*. p. 236.

Brekalo, J., Palaversic, J. B. and Rojic, M. (1991). Monitoring the occurrence and severity of maize disease in Croatia from 1985 to 1989. *Zastita bilja*. **42**(1): 51-60.

Casa, R. T., Reis E. M. and Zambolim, L. (1998). Fungi associated with maize seeds from Southern and Southeast regions of Brazil. Departmento de Fitopatologia, Universedadc Federal de Vicosa, Brazil. *Fitopatologia-Brasileira*. **23**(3): 370-373.

Chidambaram, P. Mathur, S. B. and Neergaard, P. (1973). Identification of seed-borne Drechslcra species. The Danish Government Institute of seed Pathology for developing countries. Copenhagen, Denmark. PRIESIA. **10**:165-207.

Chowdhury, M. K. and Islam, M. A. (1993). Production uses of maize (in Bengali) Published by On-Farm Research Division of Bangladesh Agricultural Research Institute, Joydebpur, Gazipur. pp. 1-189. 37

Cook, R. J. (1993). Making greater use of introduced micro-organisms for biological control of plant pathogens. *Annual Review of Phytopathology* **31**:53-151.

Debnath, M., Sultana, A. and Rashid, A. Q. M. B. (2012). Effect of seed-borne fungi on the germinating seeds and their bio-control in maize. *J. Environ. Sci. & Natural Resources*. **5**(1): 117 – 120.

Deepavali, S. D. and NIlima, W. K. (2013). Incidence of seed borne mycoflora on maize and its effect on seed germination. *International Journal of Current Research*. **12**(5): 4151-4155.

Ellis, M. B. (1971). Dcmatiaceous hypomycetes. *Commonwealth Mycol. Inst. Kew, Surrey, England*. p. 608.

El-Maghraby, O. M. O., El-Kady, A. and Soliman, S. (1995). Mycoflora and *Fusarium* toxins of three types of corn grains in Egypt with special reference to production of trichothcccne toxins. Department of Botany, Faculty of Science, Assiut University, Sohag, Egypt. *Microbiol. Res.* **150**(3): 225-232.

Essiet, D. (2010). Maize is staple food to 300 million Africans. Africa News Network. http://www.africanagricultureblog.com/2010/05/maize-is-staple-food-to-300-million.html [Accessed on: 20th June 2010]

Eurekalert, (2009). "Wild grass became maize crop more than 8,700 years ago", National Science Foundation, News Release at *Eurekalert* March 24, 2009. *http://www.eurekalert.org/pub_releases/2009-03/nsf-wgb032309.php* [Accessed on: 25th October 2009]

Fakhrunnisa H, Hashmi M (1992). Seed-borne mycoflora of corn, millet and paddy. In: Status of Plant Pathology in Pakistan. (Eds.): Ghaffar A. and Shahzad S. Dept. Bot., Univ. Karachi, Karachi-75270, Pakistan. pp. 125-129.

Fakir, G. A. (2000). An annonated list of seed borne disease in Bangladesh. Seed Pathology Center, Dept. of Plant Pathology, Bangladesh Agricultural University, Mymensingh.

Fakir, G. A. (2001). List of seed borne diseases of important crops occurring in Bangladesh. Seed Pathology Lab., Dept. of Plant Pathology, Bangladesh Agricultural University, Mymensingh. p.38.

Fakir, G.A. (2001). List of seed-borne diseases of important crops occurring in Bangladesh. Seed Pathology Laboratory, Dept. of Plant Pathology, BAU, Mymensingh. p. 9.

Faruq, A. N., Alam, M. M., Chowdhury, M. S. M., Khaiyam, M. O., Rahman, M. A. and Haque, S. (2014). Pathogen risk analysis of maize in Bangladesh. *App. Sci. Report*. **8**(2): 75-82.

Fatima, R., Mathur, S. B. and Neergaard, P. (1974). Importance of *Drechsleria maydis* on seed of crops other than maize. *Seed Sci. Technol*. **2**:371-383.

Futrell, M. C. and Kilgore, M. (1969). Poor stands of corn and reduction of root growth caused by *Fusarium moniliforme. Plant Dis. Rep*. **53**: 213-215.

Gomez, K. A. and Gomez, A. A. (1984). Statistical procedures for Agricultural Research. 2nd edition. John Wiley and Sons, New York. pp. 207-215.

Guerrero, F. C., Mathur, S. B. and Neergaard, P. (1992). Seed health testing of rice. Seed borne fungi associated with abnormal seedlings of rice. *Proc. Int. Seed Test. Assoc*. **37**: 985-997.

Habib, A., Sahi, S.T., Javed, N. and Ahmad, S. (2011). Prevalence of seed borne fungi on wheat during storage and its impact on seed germination. *Pakistan J. Phytopathol*. **23**(1): 42-47.

Hall, J. S, and Harman, G. E. (1991). Efficacy of oil treatment of legume seeds for control of *Aspergillus* and *Zabrotes*. *Crop Prot*. **10**:315-9.

Haque, A. H. M. M., Akon, M. A. H., Islam, M. A., Khalequzzaman, K. M. and Ali, M. A. (2007). Study of seed health, germination and seedling p.39.

HGCA. (2010). USDA sees world maize production up at 804Mt. *http://www.hgca.com/content.output/4477/4477/Markets/Market%20News/USDA%20sees%20world%20maize%20production%20up%20at%20804Mt.mspx* [Accessed on: 20th June 2010].

Howlader, A. N. 2003. Effect of seed selection and seed treatment on the development of phomopsis blight and fruit rot of egg-plant. An M.S. Thesis submitted to the Dept. of Plant Pathology, BAU, Mymensingh. pp. 40-68.

Hulse, J. H., Laing, E. M. and Pearson, O. E. (1980). Composition and nutritive value of corn and millets. 3rd edition. *Academic Press*, New York. pp. 2-6.

Hussain, N., Hussain, A., Ishtiaq, M., Azam, S. and Hussain, T. (2013). Pathogenicity of two seed-borne fungi commonly involved in maize seeds of eight districts of Azad Jammu and Kashmir, Pakistan. *African J. Biotechnol.* **12**(12): 1363-1370.

International Seed Testing Association [ISTA] (2007). International Rules for Seed Testing. *Proceedings of International Seed Testing Association*. ISTA, 8303 Bassersdorf, Switzerland.

Islam, M. S., Jahan, Q. S. A., Bunnarith, K., Viangkum, S. and Merca, S. D. (2000). Evaluation of seed health of some rice varieties under different conditios. *Bot. Bull. Acad. Sci.* **41**: 293-297.

Islam, T. (2009). Population dynamics of *Phomopsis vexan*, *Sclerotium rolfsii*, *Fusariam oxsysporum* pv. *Lycopersici* and *Trichoderma* in the soil of eggplant field. An M.S. thesis submitted to the Dept. of plant Pathology, Bangladesh Agricultural University, Mymensingh. pp. **48**-57.

ISTA. (1976). International rules for seed testing. Seed Sci. and Technol. **4**: 3-49.

ISTA. 1976. International Rules for Seed Testing Association. *Int. Seed test. Assoc.* **31**: 107-115.

Jacqua, G. (1989). Contribution to the study of the principle foliar diseases of maize in Guatemala. The possibilities of coexistence of *Bipolaris maydis* and *B*. turcicum. Agronomic Tropicale. **44**(2): 111-114.

Joshi, R. N. (2006). Neem: A Tree for Solving Global Problems**.** Latest Reviews 4 (1):2006. In: *Pharmainfo. net http://www.pharmainfo.net/reviews/neem-tree-solving-global-problems.*

Karim, M. (2005). Prevalence of fungi associated with seeds of some minor cereals. An M.S. Thesis. Department of Plant Pathology. Bangladesh Agricultural University, Mymensingh. p. 97.

Khare, M. N., Mathur, S. B. and Neergaard, P. (1977). A seedling symptoms for detection of *Septorianodorum* in wheat. *Seed Sci. and Tech*. **5**: 613-617.

Kiran B., Lalitha, V. and Raveesha, K. A. (2010). Screening of seven medicinal plants for antifungal activity against seed borne fungi of maize seeds. *African J. Basic and Appl. Sci.* **2**(3-4):99-103.

Malone, G. P. and Musket, A. E. (1964). Seed-borne fungi. Description of 77 fungus species. *Proc. Intl. Seed Test*. **29**(2): 180-183.

Marasas, W.F.O., Wehner, F.C., van Rensburg, S.J. and Van Schalkwyk, D.J., (1981). *Mycoflora* of corn produced in human oesophageal cancer areas in Transkei, Southern Africa. *Phytopathol*. **71**: 792-6.

Marley, P. S. and Gbenga, O. (2004. Fungicide control of *Stenocarpella maydi* in the Nigerian Savanna. *Arch. Phytopathol.* and *Plant Prot*. **3**(1): 19-28.

Martin, J. H. and Leonard, W. H. (1975). Principles of Field Crops Production. The Macmillan Company, Collier-Macmillan Limited. pp. 291-295.

Martinez, Maria, B., Schieber, R., Gomej, B. and Bressani, R. (1970). Prevalence of fungi in maize grains of Guatemala. Turrialba. **20**(3): 311-319.

Mathur, S. B. and Kongsdal O. (2003). *Common Laboratory Seed Health Testing* Methods for Detecting Fungi. Pub. International Seed Testing Association, 8303 Bassersdorf, CH-Switzerland.

Mathur, S. B. and Kongsdal, O. (2003). Common Laboratory Seed Health Testing Methods for Detecting Fungi. Danish Govt. Institute of Seed Pathology for

Developing Countries, Copenhagen, Denmark. Published by ISTA, Switzerland. p. 425.

McGee, D. C. (1995). Advances in seed treatment technology. Paper presented at ASIAN SEED ’95. New Delhi, India. McGee, D. C., Iles, A. and Misra, M. K., (1989). Suppression of storage fungi in grain with soybean oil. *Phytopathology* **79**:1140 (Abstr.)

McGee, D.C. (1981). Seed pathology: its place in modern seed production. *Plant Dis*. **65**:638-42.

Mian, M. A. W., Moniruzzaman, F. M., Sattar, M. A. Miah, M. A. Pal S. K. and Reazul (2001). Agricultural Research in Bangladesh in the 20th Century. BARC/BAAG. Dhaka, Bangladesh. p. 528.

Mohiuddin, M. (2003). Efficiency and sustainability of maize cultivation in an area of Bangladesh, Published Master’s Degree Thesis, BAU, Mymensingh.

Mondall, N. K., A. S. k. Mojumdar, A. Chatterje, J. K. Banerjee and S. Gupta.2009. Antifungal activities and chemical characterization of neem leaf extracts on the growth of some selected fungal species in vitro culture medium. Journal of Applied Sciences and Environmental Management **13**(1):49-53.

Montes, G. N., Reyes, M. C. A., Montes, R. N. and Cantu, A. M. A. (2009). Incidence of potentially toxigenic fungi in maize (*Zea mays* L.) grain used as food and animal feed. *J. Food*. **7**(2):119-125.

Natarajan, V., Venugopal, P. V. and Menon T. (2003). Effect of azadirachta indica (neem) on the growth pattern of dermatophytes. *Indian Journal of Med. Microbiol*. **21**(2):98-101.

National Research Council (NRC), 1992. *Neem: A Tree for Solving Global Problems*. National Academy Press, Washington D.C

Naznin, H. A. and Hossain, I. 2004. Effect of BAU-Biofungicide on germination and seedling vigour of some summer vegetables. *Bangladesh J. Seed Sci. & Tech.*, **8** (l&2): 85-90.

Rake, K., Khanna, K.K., Sudhir C., Khanna, R. and Chandra, S. (1989). Effect of homoeopathic drugs on seed mycoflora of wheat. *Nat Acad. Sci. Let*. **12**:39-41.

Ramnath, P., Neergaard. andMathur, S. B. (1970). Identification of *Fusarium* species on seeds as they occur in blotter test. *Proc. of the Intl. Seed Test. Assoc*. **35**: 121-144. 42

Raper, K. B. and D. I. Fennel. (1965). The genus *Aspergillus.* The Williams and Wilkins Co., Baltimore. p. 686.

Richardson M. J. (1979). An annotated list of seed-borne diseases (*Int. Seeds Test Assoc.* Zurich, Switzerland), 3rd Ed. p. 320.

Richardson, M. J, (1986). An assessment of the need for routine use of organo-mercurial cereal seed treatment. *Field Crop Res*. **13**:3-24.

Riker, J. J. and Riker C. J. (1921). Introduction to phytopathological methods. Cornell University. Ithaca, New York. p. 131.

Saleem M. J., Bajwa R., Hannan A. and Qaiser A. (2012). Maize seed storage mycoflora in Pakistan and its chemical control. *Pakistan. J. Bot*. **44**(2): 807-812.

Somda I., Sanou J. and Sanon P. (2008). Seed borne infection of farmer-saved maize seeds by pathogenic fungi and their transmission to seedlings. *Plant Pathol. J*. **7**(1): 98-103.

Somda, I., Leth, V. and Sereme, P., (2007). Evaluation of Lemongrass, Eucalyptus and Neem Aqueous Extracts for Controlling Seed-Borne Fungi of Sorghum Grown in Burkina Faso. *World journal of agricultural sciences*, **3**(2): 218-223, 2008.

USDA, (1996). Index of Plant Disease in the United States. *Agricultural Hand Book*. No. 165: 531 pp. In: Rahman, M.M.E., Ali, M.E., Ali, M.S., Rahman, M.M. and Islam, M. N. (2008). Hot Water Thermal Treatment for Controlling Seed-Borne Mycoflora of Maize. *International Journal on Sustainable Crop Production.* **3**(5): 5-9.

USDA. (1960). Index of plant Disease in the United States. Agricultural hand book. No. pp. 165:531.

Utobo, E. B., Ogbodo, E. N. and Nwogbaga, A. C. (2011). Seed-borne mycoflora associated with rice and their influence on growth at Abakaliki, Southest Agro-Ecology, Nigeria. *Libyan Agric. Res. Cen. J. Intl*. **2** (2): 79-84.

Vaidehi, B. K. and Ramarao, P. (1978). Fungi from maize seed. *Nat. Acad. Sci. Lel*. **1**(8): 283-284.

Warham, E. J. (1990). Effect of *Tilletia indica* infection on viability, germination and vigor of wheat seed. *Plant Dis.* **74**:130-135.

Warren, H. L. and Kommedahl, T. (1973). Prevalence and Pathogenicity to corn of *Fusarium* species from corn roots, rhizospheres, residues and soil. *Phytopathology*. **63**:1288-1290.

White, D. G. (I999). Compendium of Corn Diseases. APS Press. *The American Phytopatho. Soci*. pp. 10-46.

Wicklow D. T., Weaver D. K. and Throne J. E. (1998). Fungal colonists of maize grain conditioned at constant temperatures and humidities. *J. Stored Product Res*. **34**: 355-361.

APPENDICX

APPENDICES

APPENDIX

ANALYSIS OF VARIANCE

1. Analysis of variance of the data on prevalence of fungi associated with three selected hybrid maize varieties (blotter method)

Source of variation	**Degrees of freedom (df)**	**Germination (%)**	***Aspergilus flavus* (%)**	***Aspergils niger* (%)**	***Fusarium moniliforme* (%)**
Factor A (variety)	2	50.156*	87.756*	26.356**	35.422*
Factor B (treatment)	4	145.256**	628.367**	570.944**	194.700**
A×B	8	42.435*	78.950*	23.658*	32.442*
Error	30	11.989	18.667	5.328	8.033

* Significant at 0.05 level of probability; ** Significant at 0.01 level of probability and NS Non-significant

2. Analysis of variance of the data on prevalence of fungi associated with three selected hybrid maize varieties (Roll paper towel method)

Source of variation	Degrees of freedom (df)	Germination (%)	Healthy seedling (%)	Dead seedling (%)	Abnoral seedling (%)	Disease seedling (%)	Vigor Index
Factor A (variety)	2	24.622*	250.756**	31.622**	17.489**	333.356**	93288.576**
Factor B (treatment)	4	70.578**	134.811**	37.133**	10.644**	17.978**	43972.773**
A×B	8	21.594*	31.561**	11.233**	5.044*	10.078**	20402.538*
Error	30	5.844	2.733	1.533	1.489	1.244	6076.422

* Significant at 0.05 level of probability; ** Significant at 0.01 level of probability and NS Non-significant