

**EFFICACY OF SEED TREATMENT WITH PLANT EXTRACTS  
ON LEAF BLIGHT (*Bipolaris sorokiniana*) DEVELOPMENT  
AND YIELD OF WHEAT**

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**EFFICACY OF SEED TREATMENT WITH PLANT EXTRACTS  
ON LEAF BLIGHT (*Bipolaris sorokiniana*) DEVELOPMENT  
AND YIELD OF WHEAT**

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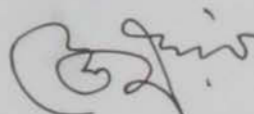
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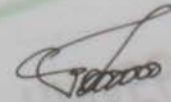
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## CERTIFICATE

This is to certify that the thesis entitled, "*EFFICACY OF SEED TREATMENT WITH PLANT EXTRACTS ON LEAF BLIGHT (*Bipolaris sorokiniana*) DEVELOPMENT AND YIELD OF WHEAT*" 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 *Md. Mosiur Rahman*, Registration No. 27559/00722, under my supervision and guidance. No part of this thesis has been submitted for any other degree or diploma.

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

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*Dedicated to  
My  
Beloved Parents*

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**The Author**

# EFFICACY OF SEED TREATMENT WITH PLANT EXTRACTS ON LEAF BLIGHT (*Bipolaris sorokiniana*) DEVELOPMENT AND YIELD OF WHEAT

BY

MD. MOSIUR RAHMAN

## ABSTRACT

Wheat (*Triticum aestivum* L.), an important cereal crop, is produced all over the country. Leaf blight caused by *Bipolaris sorokiniana* is a common constraint of limiting wheat production in Bangladesh. Use of plant extracts as a biocontrol agent against plant diseases is a relatively recent approach. Efficacy of plant extracts in controlling leaf blight (*Bipolaris sorokiniana*) of wheat was studied during the cropping season 2006-2007. The experiments were conducted in the Seed Health Laboratory, Department of Plant Pathology and in the farm of the Sher-e-Bangla Agricultural University, Dhaka. Initially 33 plant species were evaluated for their efficacy against *Bipolaris sorokiniana* by an *in vitro* test. Among them 13 species were found promising and selected for field evaluation against leaf blight disease. A remarkable reduction of the severity of leaf blight was achieved by treating seeds with botanicals. The treatments showed significant effect in respect of leaf blight severity (0-5 scale) for flag leaf and penultimate leaf at flowering, milking and hard dough stages. The lowest severity of flag leaf and penultimate leaf in every stage was found in the treatment T<sub>12</sub> (Seed treatment with turmeric rhizome extract) followed by treatment T<sub>2</sub> (Seed treatment with garlic clove extract) whereas the highest disease severity was recorded under the treatment T<sub>1</sub> (untreated control). Significantly the highest plant height (93.09 cm) was found in the treatment T<sub>2</sub>, ear length (15.58 cm) in treatment T<sub>8</sub> (Seed treatment with kalijira seed extract) and the number of spikelets /ear (19.11) in treatment T<sub>5</sub> (allamanda leaf extract). The number of grains/ear, number of healthy grains/ear, weight of grains/ear and weight of healthy grains/ear were increased by using seeds treated with extracts of kalijira (T<sub>8</sub>), garlic (T<sub>2</sub>), allamanda(T<sub>5</sub>) and turmeric (T<sub>12</sub>). Seed treatment with plant extracts significantly increased grain yield over untreated control. Among the plant extracts, turmeric rhizome extract treated seeds resulted the highest grain yield (3.60 t/ha) which was 39.44 % higher over untreated control. Thus plant extracts have definite antifungal effect on *Bipolaris sorokiniana* which could be employed as an ecofriendly approach in controlling leaf blight of wheat.



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## ABBREVIATIONS USED

AEZ	=	Agro-Ecological Zone
@	=	At the rate
Anon.	=	Anonymous
AUDPC	=	Area under disease progress curve
BARI	=	Bangladesh Agricultural Research Institute
BAU	=	Bangladesh Agricultural University
CIMMYT	=	International Maize and Wheat Improvement Center
cm	=	Centimeter
CT	=	Conventional tillage
cv.	=	Cultivar (s)
DAS	=	Days after sowing
DMRT	=	Duncan's Multiple Range Test
e.g.	=	Example
g	=	Gram
FAO	=	Food and Agriculture Organization
GY	=	Grain yield
ha	=	Hectare
HLB	=	<i>Helminthosporium</i> leaf blight
hr	=	Hour
i.e.	=	That is
K	=	Potassium
Kg	=	Kilogram
lb	=	Pound
LSD	=	Least significant difference
m	=	Meter
mm	=	Millimeter
MP	=	Muriate of potash

N	=	Nitrogen
NT	=	Not conventional tillage
P	=	Phosphorus
PDA	=	Potato Dextrose Agar
RCBD	=	Randomized Complete Block Design
S	=	Sulphur
SAU	=	Sher-e-Bangla Agricultural University
T	=	Treatment
t / ha	=	Ton per hectare
TSP	=	Triple Super Phosphate
UNDP	=	United Nation Development Program
wt.	=	Weight
w/v	=	weight/ volume
Zn	=	Zinc
<sup>0</sup> C	=	Degree Centigrade
%	=	Percent





# Chapter 1

## Introduction



## 1. INTRODUCTION

Wheat (*Triticum aestivum* L.), an important cereal crop is produced widely and extensively all over the world. About two third of the world population use wheat as staple food (Majumder, 1991). More land is already devoted worldwide to the production of wheat than any other commercial crop covering about 215.89 million ha (FAO, 2005). Wheat is the second most important cereal crop next to rice in Bangladesh. It has gained much popularity among the farmers of Bangladesh due to its higher nutritive value and lower cost of production than that of rice. Bangladesh has moved from the ranges of non traditional wheat growing countries into traditional wheat growing countries (Klatt, 1988). In Bangladesh, about 0.56 million hectares of land was under wheat cultivation and total production was about 0.98 million metric tons (FAO, 2005). According to FAO (2005), the average yield of wheat was 1.7 t ha<sup>-1</sup> in our country which was lower in comparison to the developed countries of the world like Japan, France, Germany and UK producing 3.76, 7.12, 7.28 and 8.00 t ha<sup>-1</sup> respectively.

There are various factors which are responsible for lower yield of wheat. Among them, disease plays a vital role. The production of wheat has undergone a historic revolution in many developing nations of Asia and Africa where the crop is grown under different environmental conditions ranging from humid to arid, subtropical to temperate and sea level to an

altitude of 3000 meters (Sarri and Wilcoxon, 1974). Wheat suffers from as many as 26 seed borne pathogens causing 14 seed borne diseases (Fakir, 1999). Among the diseases, leaf blight is the major constraint that limiting wheat production in the tropical and sub tropical regions of the world (Duveiller and Gilchrist, 1994; Mehta, 1997). Leaf blight caused by *Bipolaris sorokiniana* (Sacc. in Sork.) Shoemaker, teleomorph *Cochliobolus sativus* (Ito & Kuribayashi) Dreshs. Ex Dastur, (Syn. *Helminthosporium sativum* Pamm. King and Blakke) is now a common disease of wheat in Bangladesh (Hossain *et al.*, 1993; Alam and Shah, 1991 and Alam *et al.*, 1995). There is no cultivar found to resistant against leaf blight of wheat (Hossain and Azad, 1992).

Leaf blight reduces the yield of wheat by producing thinner stand with lower number, size and kernel weight. In Bangladesh yield loss of wheat due to leaf blight has been reported to be 20% in var. Sonalika, whereas 14% and 18% in Akbar and Kanchan, respectively (Razzaque and Hossain, 1991). In farmers' field, the yield loss was estimated to be 14.97%, whereas 29% yield reduction was recorded during 1991-92 in Kanchan (Alam *et al.* 1995). 100% yield loss of wheat may be occurred if the plants attacked severely (Hossain and Azad, 1994). Ahmed and Hossain (2005) found 43.75% yield loss in an inoculated wheat field.

Use of chemical to control a disease is the most favorable mean to our farmers till now. But 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 chemical affect the soil health. Harmful chemical substances enter into the food chain that ultimately causes serious human diseases. Eco-friendly management of plant diseases such as use of plant extracts has a great chance to save the beneficial soil micro-organisms.

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). Seed treatment with botanicals is an effective way to control leaf blight of wheat. 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.

In the view of above circumstances the present study was designed with the following objectives:-

1. To determine the efficacy of plant extracts in controlling growth of *Bipolaris sorokiniana* in the laboratory.
2. To evaluate the efficacy of selected plant extracts in controlling leaf blight of wheat under field condition.
3. To determine the efficacy of seed treatment with plant extracts on yield and yield contributing characters of wheat.



## Chapter 2

# Review of literature

## 2. REVIEW OF LITERATURE

Leaf blight of wheat caused by *Bipolaris sorokiniana* is a worldwide important disease. This disease is considered as the most devastating one and has profound effect on wheat production in Bangladesh. The concept of management of diseases employing eco-friendly materials gained momentum as mankind became more and more environment conscious. Use of botanicals instead of chemical fungicides is one of the recent approaches for plant disease control. Reviews related to control of leaf blight of wheat with plant extracts are presented in this chapter.

Lapis and Dumancas (1978) screened crude extracts of 93 plants against *Helminthosporium oryzae* and found that 42 inhibited its growth. The extracts were active at 1:10 aq. solutions except *Euphorbia pulcherrima* which was only active in its natural concentration form. The precipitate was generally more active than the supernatant and activity decreased with time. *Impatiens balsamina*, *Pseudocalyma alliaceum*, garlic and *Tagetes erecta* were further evaluated in vitro. *Impatiens balsamina* extracts were active as a therapeutant and protectant against the pathogen on rice while other 3 were effective as therapeutants only.

Singh and Sharma (1978) compared the antifungal activity of 34 crude extracts of indian flowering plants which strongly inhibited *Helminthosporium sativum*, *Colletotrichum falcatum* and *Fusarium oxysporum*.

Tripathi *et al.* (1978) reported the fungitoxic effect of the extract of higher plants *Lawsonia inermis*. They found that the extract of *Lawsonia* leaves exhibited strong fungitoxicity. The antifungal component was 2-hydroxy 1,4-naphthoquinone (Lawnone) which was effective against the test fungus *Helminthosporium oryzae* (*Cochliobolus miyabeanus*) at 1000 ppm. Lawnone is fungicidal, with a wide fungitoxic spectrum and is nonphytotoxic.

Ahmed and Sultana (1984) observed that bulb extract of garlic was most effective against the major seed borne pathogen of jute viz *Macrophomina phaseolina*, *Botryodiplodia theobromae* and *Colletotrichum corchori*. They reported that jute seeds treated with garlic paste increased seed germination and decreased the rate of post emergence seedling mortality over untreated control. The antifungal property of garlic juice was also demonstrated against *Fusarium* wilt of watermelon caused by *Fusarium oysporum* f. sp. *niveum* (El-Shami *et al.* 1986). They observed that garlic extract successfully inhibited spore germination and mycelial growth of fungus.

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

El-Shami *et al.* (1986) compared antifungal property of garlic clove juice with recommended dose of fungicidal treatments against *Fusarium* wilt

of watermelon. The garlic extract inhibited spore germination and reduce mycelial growth of *Fusarium oxysporum* f. sp. *nivium* in extent similarly to five different fungicides *in vitro*. But *in vivo* experiment, soaking water melon seeds in the extract gave better control of seedling infection than that of seed treatment with Benlate, Vitavax, Carboxin (captan + carboxin) or Thiram.

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

Assadi and Behroozin (1987) conducted an experiment to evaluate the efficacy of bulb extracts of onion and clove extracts of garlic against mycelial growth of *Fusarium spp.* and *Sclerotium cepivorum*. Garlic extract was found more active than that of onion in inhibiting growth of *Fusarium solani*, *Fusarium oxysporum* and *Fusarium acuminatum*.

Chalfo and Carvalho (1987) compared the garlic extract and chemical fungicide Captafol in controlling mycelial growth of *Gibberella zeae*. All treatments inhibited mycelial growth of *Gibberella zeae* where most effective concentration being 8000 ppm for garlic extract and 10000 ppm for Captafol.

Singh and Dwivedi (1987) estimated that hyphal dry weight and sclerotia production of *Sclerotium rolfsii* Sacc. were significantly reduced by bark extracts of *Acacia arabia*. They evaluated bulb and leaf extracts of garlic and onion, leaf extracts of *Rauwolfia serpentina*, *Lawsonia alba*, *Datura stramonium*, *Solanum xarhocarpum*, *Calotropis procera*, *Eucalyptus globus*, *Emblica officinalis*, fruit extract of *Azadirachta indica* and rhizome extracts of turmeric and ginger against *Sclerotium rolfsii* and found that those extracts more or less effective in inhibiting the growth of the fungus.

Naidy (1988) reported that extract of deshi patabahar (*Codiaeum variegatum*) possessed antifungal activity and found effective against *Alternaria alternata* and *Fusarium oxysporum* in vitro.

Meah and Hossain (1989) used extracts of mustard var. Sambal leaves to control leaf blight of mustard. They recorded the reduced number of diseased leaves per plant, percent leaf area diseased and number of spots per pod in plots of mustard var. Sonali, Sampad and Sambal that received spray of the botanicals. They recorded 34.0 and 68.2% reduction of leaf area diseased and percent pod infection respectively over control.

Mishra and Dixit (1989) investigated the fungitoxic effect of lemon (*Citrus medica*) extract against *Aspergillus flavus* and found that the extract inhibited the fungus considerably.



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*.

Extract of pan (*Piper betel*) found to be effective against collar rot pathogen, *Thanatephorus cucumeris* (Lakshmonan *et al.* 1990).

Lakshmonan *et al.* (1990) found that garlic clove extract was most effective in inhibiting mycelial growth and spore germination of *Corynespora cassiicola*.

Miah *et al.* (1990) reported that gada (*Tagetes erecta*) was effective against *Monographella alboscens*, *Pyricularia oryzae* and *Rhizoctonia solani*.

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 *F. 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.

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.

Tewari and Mandakini (1991) reported that extract of *Piper betle*, *Ocimum sanctum*, *Nyctanthes arbortristis* and *Citrus limon* were effective in reducing the radial growth of *Pyricularia oryzae*, *C. miyabeanus* and *Rhizoctonia solani* in vitro, with extracts of *P. betle*, followed by *O. sanctum* were the most effective.

Thakhur *et al.* (1991) studied on extracts of medicinal plants against cotton pathogens *Myrothecium roridum*, *Alternaria tenuis* and *Xanthomonas 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.

Achimu and Schlosser (1992) studied the effect of neem seed extracts against downy mildew (*Plasmopara viticola*) of grapevine. They found that raw neem seed extract and commercial neem products (margo-san-o,

neem oil and neem-Azal-S) had high (80-90%) antifungal properties against *Plasmopara viticola* in field trial.

Ashrafuzzaman and Hossain (1992) evaluated pudina (*Mentha viridis*) extract against *Bipolaris sorokiniana* and observed that the extract inhibited mycelial growth and spore germination. In the same work they found that extract of castor (*Ricinus communis*) and dantha kalash (*Leucas aspera*) were inhibitory against mycelial growth and spore germination of *Bipolaris sorokiniana*.

Ashrafuzzaman and Khan (1992) evaluated thankuni (*Hydrocotyl asiatica*), mehedi (*Lawsonia alba*) and duranta (*Duranta plumeiri*) against *Rhizoctonia solani* and found all the extracts effective in reducing mycelial growth and sclerotia formation effectively.

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

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 spp.* by seed treatment.

Hashim *et al.* (1992) studied on seed mycoflora of lentil and isolated 21 fungal species from 4 lentil cultivars of which *Fusarium moniliforme*

(*Gibberella zeae*), *F. oxysporum* and *F. semitectum* (*F. pallidoroseum*) were prevalent. They treated seeds with 80 ppm neem (*Azadirachta indica*) extract controlled the seed-borne mycoflora of lentil.

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) found neem (*Azadirachta indica*) seed extracts/cake effective against *Bipolaris sorokiniana*. The extract inhibited the growth of the fungus and also reduced its pathogenicity on wheat leaves. Germination rate of wheat seeds increased after seed treatment with extracts of neem seed and cake.

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

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 metal* showed antifungal activity against *Pyricularia oryzae* and *H. oryzae in vitro*. Extracts of *V. rosea* showed inhibition of mycelial growth and spore germination.

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

Arun *et al.* (1995) found that the extract of garlic bulb was effective in suppressing radial growth of *Fusarium sp* and *Sclerotium sp*.

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.

Mohanty (1995) demonstrated that leaf extract of neem (*Azadirachta indica*) was significantly effective causing 52.23% growth inhibition of *Phomopsis vexans*, the causal agent of phomopsis blight and fruit rot of brinjal.

Mohanty *et al.* (1995) reported that garlic bulb extract (1:1) and allamanda leaf extract sprays in the field reduced phomopsis blight and fruit rot by 66% and 75% respectively.

Suratuzzaman (1995) performed an experiment with plant extracts to control seed-borne *Colletotrichum dematium* var. *truncatum*, *Macrophomina phaseolina* and *Cercospora kikuchi* of soyaben seed. Seed treatment with garlic and ginger extracts gave excellent control of pathogens.

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.

Panda *et al.* (1996) tested the efficacy of leaf extracts from *Polyalthia longifolia*, *Aegle marmelos*, *Ocimum sanctum*, *Azadirachta indica*, *Carthamus roseus* and *Allamanda cathartica* for the control of phomopsis blight (caused by *Phomopsis vexans*). Leaf extract of *Allamanda cathartica* had excellent potential as fungicide.

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

Kurucheve and Padmavathi (1997) evaluated five selected plant products against *Pythium aphanidermatum*, the causal organism of damping off of chilli. Among them *Allium sativum* (garlic) bulb recorded the minimum mycelial growth (176 mg) followed by *Lawsonia inermis* leaf extract. Maximum percentage of seed germination, growth and vigour of chilli seedlings were observed with extract of garlic bulbs.

Mahfuzul (1997) evaluated some plant extract viz. garlic (*Allium sativum*), ginger (*Zingiber officinale*), nisinda (*Vitex negundo*), dolkalmi (*Ipomoea fistulosa*) and marigold (*Tagetes erecta*) against major seed-borne fungal pathogens of chilli. Among the plant extracts garlic was found to be most effective followed by neem leaf. The garlic and neem leaf extracts at the dilution ratio of 1:1 were almost equally effective.

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, azadiradione, nimbin, salannin and epoxyazadiradione. Pure azadiradione, 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.

Parveen (1998) studied the effect of lemon grass oil for controlling sheath blight of rice in the net house and in the field. She assessed the effect of lemon grass oil at (1:80) dilution in controlling sheath blight of rice and found that lemon grass oil was very effective in controlling sheath blight disease of rice in Binasail and TN1 variety.

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.

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.

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

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<sup>0</sup>c, 52<sup>0</sup>c, 54<sup>0</sup>c, 56<sup>0</sup>c and 58<sup>0</sup>c 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.

Hossain *et al.* (2005) reported that extract of different plant; viz. bishkatali, vatpata, garlic, gagra, bitter guard and neem were effective against fungi associated with wheat seed. Out of six plant species, neem extract was turned up as superior among the selected extracts followed by garlic, bishkatali and vatapta.

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.





## Chapter 3

# Materials and Methods

### **3. MATERIALS AND METHODS**

#### **3.1. Laboratory experiment**

The experiment was conducted at the Seed Pathology Laboratory of the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh.

##### **3.1.1. Collection of seeds**

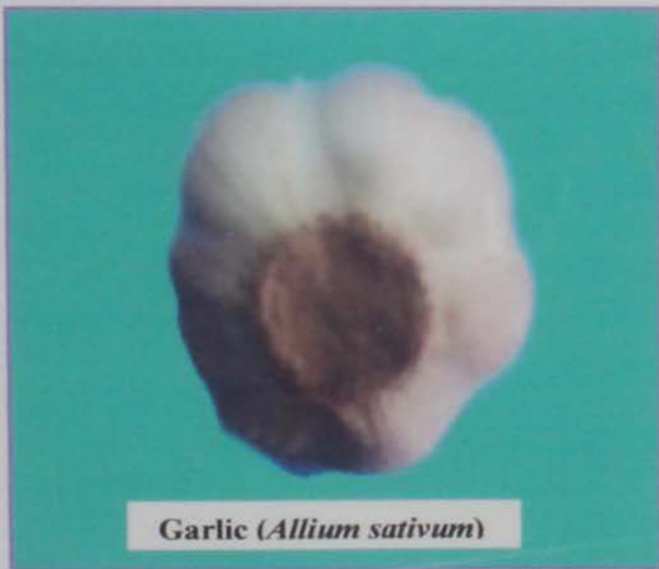
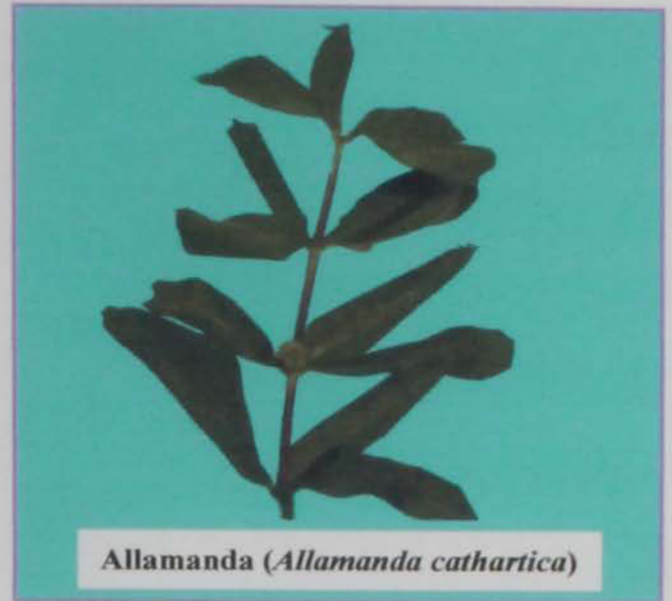
About 10 kg seed sample of wheat cv. Kanchan was collected from a farmer of Village- Kamalakantopur, Upazilla- Shibganj, District- Chapai Nawabganj. After collection, the seeds were kept in a plastic container with air tight lid and the container was stored in normal room temperature in MS laboratory of the Department of Plant Pathology, Sher-e-Bangla Agricultural University.

##### **3.1.2. Collection of botanicals**

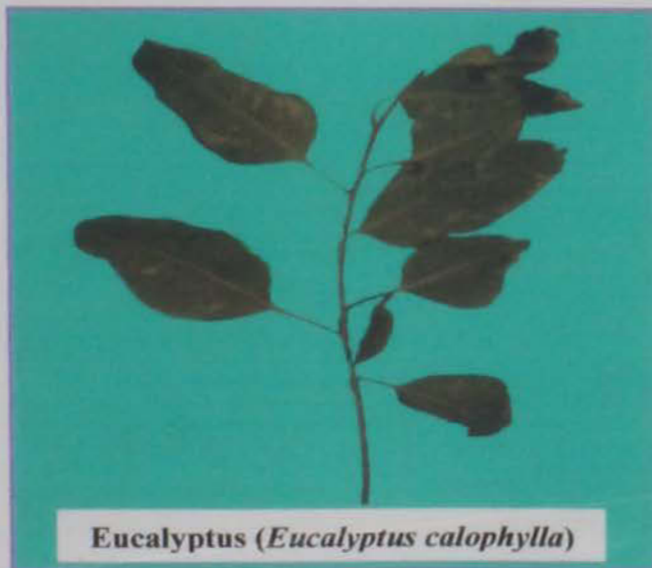
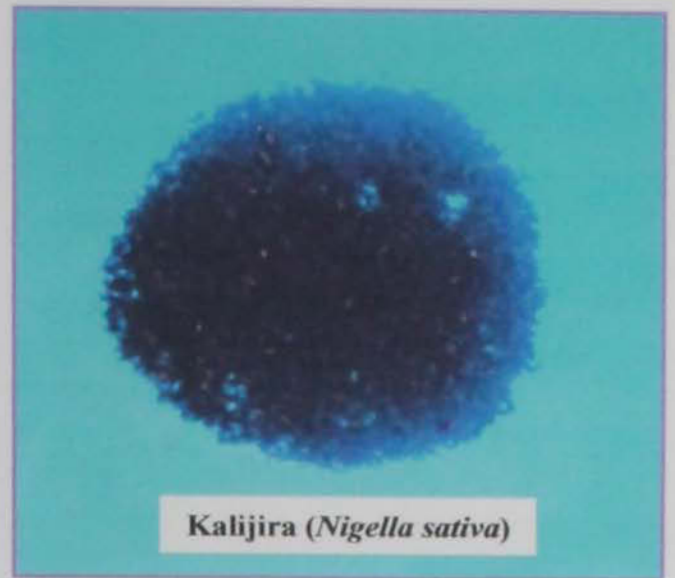
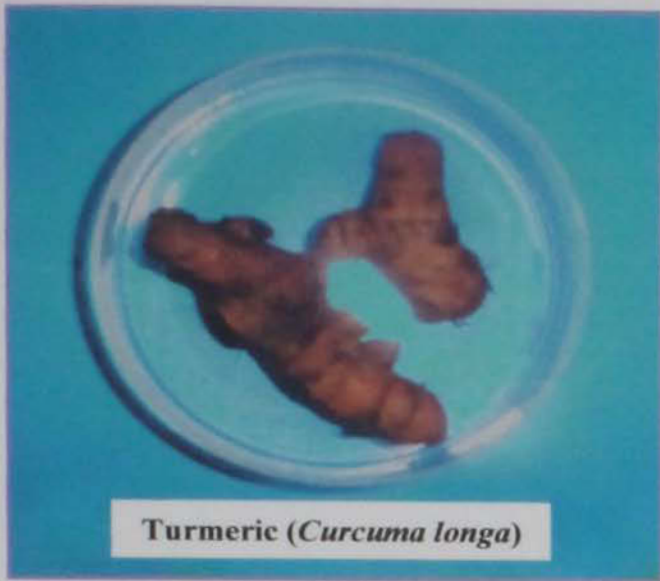
Botanicals were collected from different places (Plate 1-9). Garlic, ginger, kalizira, turmeric and onion were collected from the agargoan market, Tejgaon Dhaka. Leaves of neem, allamanda, katamehedi, tulsi, naglingam, mander, eucalyptus, chita, noyantara, durba, bel, guava, gada, babla, jaam, arjun, debderu, gandhaviduli, lemon, sajna, vat, justicia, mechania lata, roots with stems of muthaghass, leaves with stems of duranta, dhonia and root of kachuripana were collected from Sher-e-Bangla Agricultural University campus.

**Table 1. The particulars of plant species used in this study**

<b>Common name</b>	<b>English name</b>	<b>Scientific name</b>	<b>Plant parts used</b>
Neem	Margosa tree	<i>Azadirachta indica</i>	Leaf
Allamanda	Allamanda	<i>Allamanda cathartica</i>	Leaf
Garlic	Garlic	<i>Allium sativum</i>	Clove
Onion	Onion	<i>Allium cepa</i>	Bulb
Turmeric	Turmeric	<i>Curcuma longa</i>	Rhizome
Kalijira	Cumin black	<i>Nigella sativa</i>	Seed
Katamehedi	Henna	<i>Lawsonia alba</i>	Leaf
Ginger	Ginger	<i>Zingiber officinale</i>	Rhizome
Tulsi	Basil	<i>Ocimum basilicum</i>	Leaf
Naglingam	Cannon ball tree	<i>Couroupita guianensis</i>	Leaf
Mandar	Cortal tree	<i>Erythrina indica</i>	Leaf
Eucalyptus	Eucalyptus	<i>Eucalyptus calophylla</i>	Leaf
Chita	Jew Bush	<i>Pedilanthus tithymaloides</i>	Leaf
Noyantara	Periwinkle	<i>Vinca rosea</i>	Leaf
Durba	Bermuda grass	<i>Cynodon dactylon</i>	Leaf
Bel	Bel	<i>Aegle marmelos</i>	Leaf
Muthaghass	Mutha	<i>Cyperus sp</i>	Leaf and root
Kachuripana	Water hyacinth	<i>Eichhornia crassipes</i>	Root
Guava	Guava	<i>Psidium guajava</i>	Leaf
Gada	Marigold	<i>Tagetes erecta</i>	Leaf
Babla	Indian gum tree	<i>Acacia arabica</i>	Leaf
Jaam	Jamun	<i>Syzygium cumini</i>	Leaf
Duranta	Lantana	<i>Lantana camora</i>	Leaf and stem
Arjun	Arjun	<i>Terminalia arjuna</i>	Leaf
Debdaru	Mast tree	<i>Polyalthia longifolia</i>	Leaf
Gandhaviduli	Goat weed	<i>Ageratum conyzoides</i>	Leaf
Lemon	Lemon	<i>Citrus limon</i>	Leaf
Sajna	Drum stick	<i>Moringa oleifera</i>	Leaf
Dhonia	Coriander	<i>Coriandrum sativum</i>	Leaf and stem
Vat	Vat	<i>Glycosmis arborea</i>	Leaf
Justicia	Justicia	<i>Justicia aurea</i>	Leaf
Mechania lata	Mikania	<i>Mikania seandens</i>	Leaf
Ipil-ipil	Ipil-ipil	<i>Leucaena leucocephala</i>	Leaf



**Plate 1: Botanicals used to test antifungal activity against *Bipolaris sorokiniana***



**Plate 2: Botanicals used to test antifungal activity against *Bipolaris sorokiniana***



**Plate 3: Botanicals used to test antifungal activity against *Bipolaris sorokiniana***





**Plate 4: Botanicals used to test antifungal activity against *Bipolaris sorokiniana***



**Plate 5: Botanicals used to test antifungal activity against *Bipolaris sorokiniana***

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Duranta (*Lantana camara*)



Arjun (*Terminalia arjuna*)

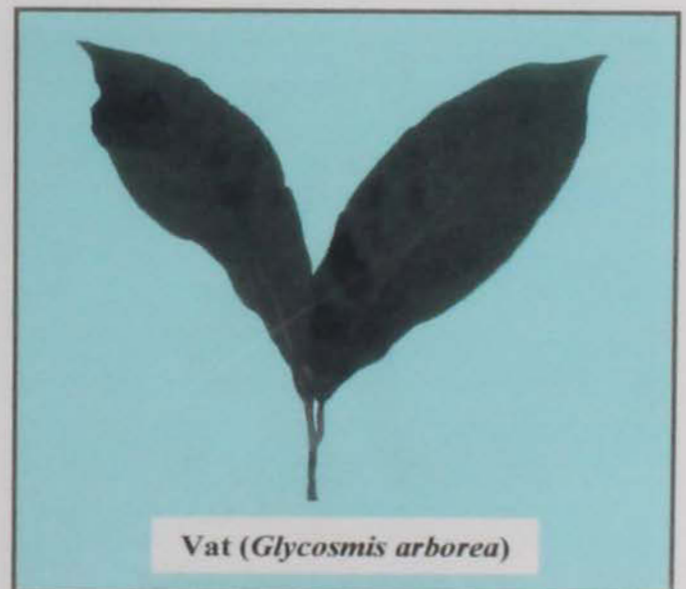
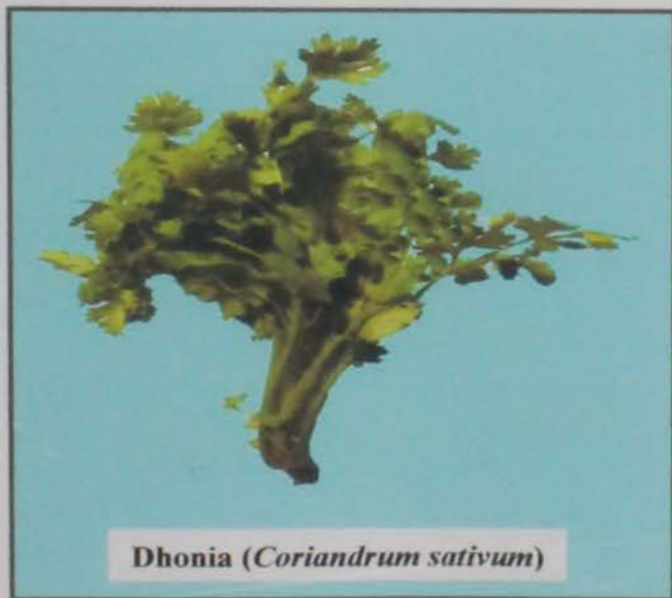


Debbaru (*Polyalthia longifolia*)



Gandhaviduli (*Ageratum conyzoides*)

**Plate 6: Botanicals used to test antifungal activity against *Bipolaris sorokiniana***



**Plate 7: Botanicals used to test antifungal activity against *Bipolaris sorokiniana***



**Plate 8: Botanicals used to test antifungal activity against *Bipolaris sorokiniana***



**Kachuripana (*Eichhornia crassipes*)**

**Plate 9: Botanicals used to test antifungal activity against *Bipolaris sorokiniana***

The extracts were prepared 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 (Plate 10). The pulverized mass was squeezed through 3 folds of fine cotton cloth. For getting 1:2 (w/v) ratio 200 ml of distilled water was added with 100 g plant parts. The particulars of the botanicals used for the experiment are listed in Table 1.

#### **3.1.4. Isolation of seed-borne *Bipolaris sorokiniana***

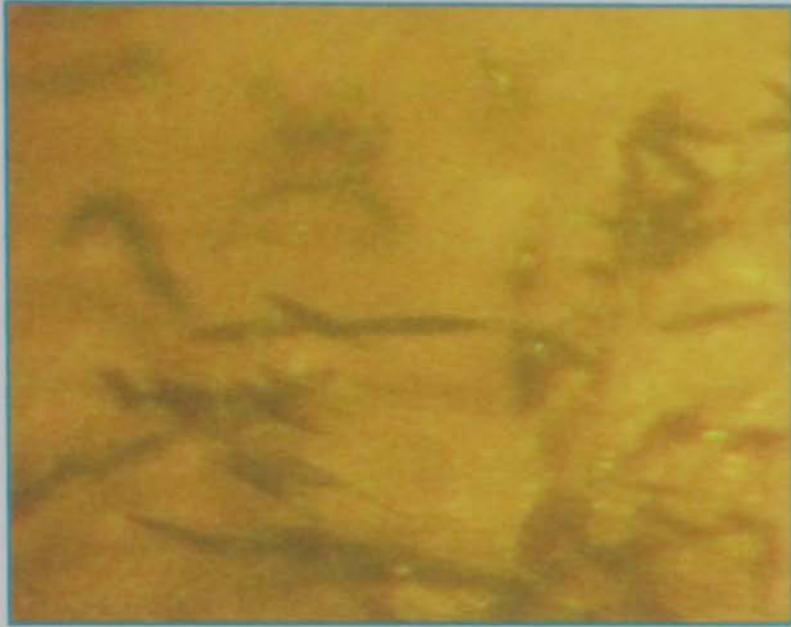
Seed-borne *Bipolaris sorokiniana* was isolated by using the blotter method of ISTA, 1996. In this method 3 layers of blotter was soaked in sterilized water and placed at the bottom of the sterilized plastic petridish. Then 25 seeds were plated on the blotting paper (Whatman) in a petridish maintaining equal distance and covered with the lid. The petridish were incubated in an air cooled room at about 20<sup>0</sup> c temperature for 7 days maintaining 12hr/12hr alternative cycle of NUV light and darkness in the laboratory. Time to time watering was done to keep the filter paper moist. After 7days of incubation the seed were



**Plate 10. Extract preparing blender**



the stereoscopic binocular microscope was confirmed by preparing temporary slides and examined under the compound microscope with the help of relevant taxonomic books (Booth, 1971 and Ellis1971). Then the conidia of *Bipolaris sorokiniana* were carefully picked up and transferred on to fresh PDA plate and incubated at 20<sup>0</sup> c for 7 days. The axenic culture of *Bipolaris sorokiniana* was preserved in refrigerator at 4<sup>0</sup> c for future use.

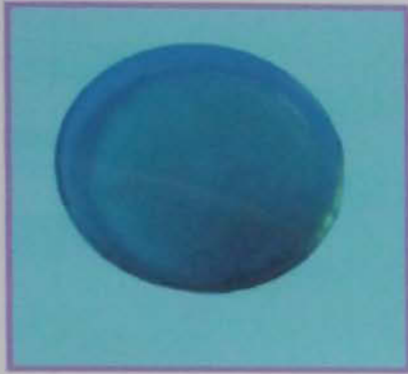


**Plate 11. Habit characteristic of *Bipolaris sorokiniana* on incubated wheat seed under stereomicroscope (X 45)**

### 3.1.5. In vitro test of effectiveness of plant extracts against *Bipolaris sorokiniana*

The effectiveness of the extracts was evaluated by two methods:

- i.) Cup method: In this method, petriplates of 15 ml acidified PDA media were prepared. After solidification, three 5mm disc of the medium were scooped from three places by a disc cutter. Three drops of extract were put into each hole and the plates were stored overnight at room temperature for allowing diffusion of the extract in the medium around the hole. Next day, a 5mm culture block of *Bipolaris sorokiniana* was cut and placed at the middle of the PDA plate. Each treatment was replicated thrice. For control treatment, only sterile water was used instead of plant extracts. The plates were then placed at  $25\pm 1^{\circ}\text{C}$  for 7 days and the radial mycelial growth of the fungus was recorded (Plate 12).
- ii.) Disc method: In this method, 1 ml of spore suspension (60,000 spores/ml) was added and spreaded on the PDA plates. Filter paper disc (7 mm) that previously soaked in plant extracts was placed at the centre of the PDA plate. Then the plates were incubated for 7 days at  $25\pm 1^{\circ}\text{C}$ . For control treatment, only sterile water was used instead of plant extracts. Each treatment was replicated thrice. After the incubation period, the inhibition zones between plant extracts soaked paper disc and the germinated spores were recorded (Plate 13).



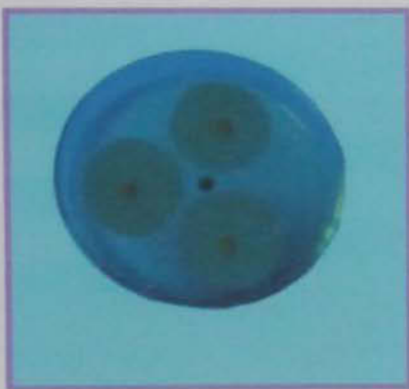
**PDA plate**



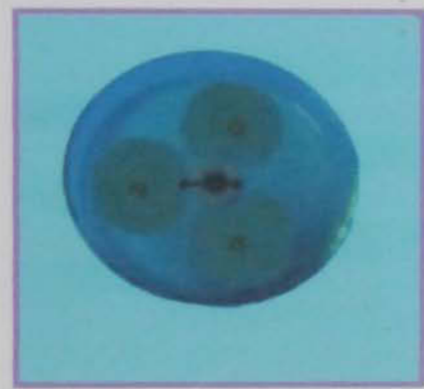
**Cup/Grove**



**Extracts within the cup**

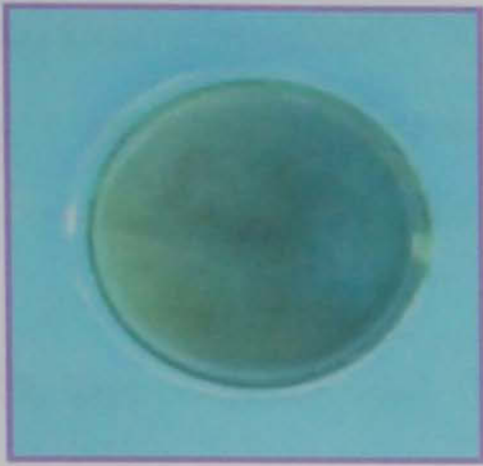


**Inoculation of fungus**

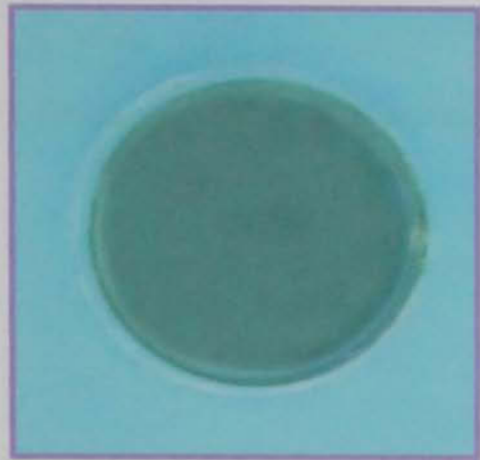


**Radial growth**

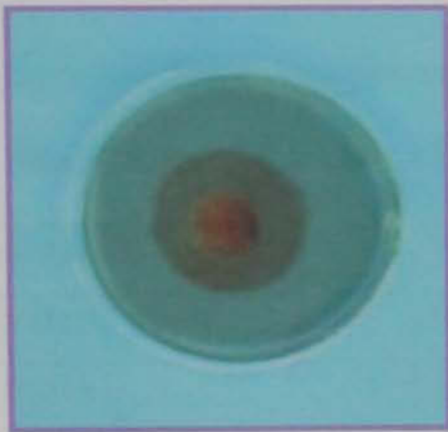
**Plate 12. Test of effectiveness of plant extracts against *Bipolaris sorokiniana* through Cup method**



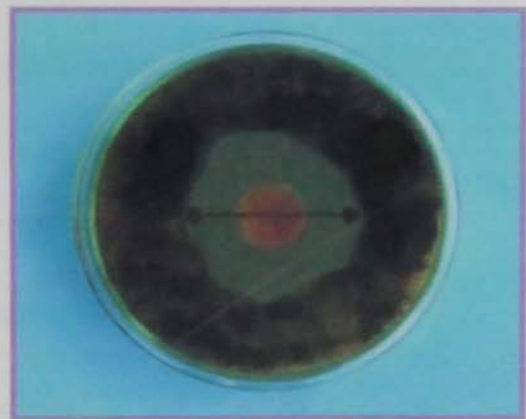
**PDA plate**



**Spore suspension spread over the PDA**



**Extract soaked filter paper disk on the centre of the plate**



**Growth inhibition zone**

**Plate 13. Test of effectiveness of plant extracts against *Bipolaris sorokiniana* through Disc method**



### 3.2. Field Experiment

#### 3.2.1. Experimental site

The experiment was conducted in the farm of Sher-e-Bangla Agricultural University, Dhaka under natural condition.

#### 3.2.2. Experimental period

The experiment was carried out during the period from November, 2006 to March, 2007.

#### 3.2.3. Soil type

The soil of the experimental plots was a medium high land with silty loam in texture belonging to Modhupur tract under the Agro-Ecological Zone (AEZ) 28.

The information about AEZ 28 is given below:

Land Type	Medium high land
General soil type	Non-Calcareous Darkgray floodplain soil
Soil series	Tejgaon
Topography	Upland
Elevation	8.45
Location	SAU Farm, Dhaka.
Field level	Above flood level
Drainage	Fairly good
Firmness (consistency)	Compact to friable when dry

### 3.2.4. Seed samples used

Wheat (*Triticum aestivum* L.) seed sample of variety Kanchan was collected from local farmer of Village: Kamalakantopur, Upazilla: Shibganj, District: Chapai Nawabganj.

### 3.2.5. Design and layout of the experiment

The experiment was carried out in Randomized Complete Block Design (RCBD) with three replications. The field was divided into three blocks with 14 unit plots in each. Each unit plot size was 2m × 1m. Block to block and plot to plot distance was 1m and 1m respectively (Appendix I).

### 3.2.6. Treatments

There were fourteen different treatments which were as follows:

- T<sub>1</sub> = Neem leaf extract (1: 2 w/ v)
- T<sub>2</sub> = Garlic clove extract (1: 2 w/ v)
- T<sub>3</sub> = Onion bulb extract (1: 2 w/ v)
- T<sub>4</sub> = Durba leaf extract (1:2 w/ v)
- T<sub>5</sub> = Allamanda leaf extract (1: 2 w/ v)
- T<sub>6</sub> = Naglingam leaf extract (1: 2 w/ v)
- T<sub>7</sub> = Noyantara leaf extract (1: 2 w/ v)
- T<sub>8</sub> = Kalijira seed extract (1: 2 w/ v)
- T<sub>9</sub> = Ginger rhizome extract (1: 2 w/ v)
- T<sub>10</sub> = Mutha leaf and root extract (1: 2 w/ v)
- T<sub>11</sub> = Bel leaf extract (1:2 w/ v)
- T<sub>12</sub> = Turmeric rhizome extract (1:2 w/ v)
- T<sub>13</sub> = Eucalyptus leaf extract (1: 2 w/ v)
- T<sub>14</sub> = Control

### 3.2.7. Land preparation

The land was thoroughly prepared by ploughing and cross ploughing with a power tiller followed by laddering. The clods were broken and the soil was leveled until the desired tilth was obtained for sowing the wheat seeds.

### 3.2.8. Application of fertilizer and manures

Fertilizers were applied as per recommendation of Bangladesh Agricultural Research Institute (BARI), Krishi Projucti Handbook, 2000. The following dose of fertilizers and manures were applied to the plot for wheat cultivation.

Fertilizers/ Manures	Dose/ha
Urea	220 kg
TSP	180 kg
MP	50 kg
Gypsum	120 kg
Cow dung	10 tons

One third of Urea, total amount of TSP, MP and Gypsum were applied at the time of final land preparation. Cow dung was applied two weeks before sowing



during the land preparation. Remaining two-third of Urea was applied as splits at the growth stages after 3 and 7 weeks of sowing.

### **3.2.9. Collection of botanicals and preparation of extracts**

The plant extracts were prepared using the method of Ashrafuzzaman and Hossain (1992) as described in laboratory experiment (3.1.2.).

### **3.2.10. Seed treatment procedure**

Seeds were treated by dipping separately in different extracts. For this, different plant extracts were poured in different sterilized conical flask. Then four hundred seeds per treatment were dipped in the solution for 30 minutes. Then the excess extract was drained off and treated seeds were kept in blotting paper to remove excess moisture from seed surface.

### **3.2.11. Sowing of Seeds**

The seeds were sown in the field on 15<sup>th</sup> November, 2006 at the rate of 130 kg / ha. Seeds were placed continuously in lines at the depth of 5 cm and covered by soil with the help of hand.

### **3.2.12. Irrigation**

The field plots were irrigated twice; first irrigation was done at 21 days after sowing (DAS). Second irrigation was done at 50 DAS.

### **3.2.13. Weeding**

Weeding was done thrice in the whole experimental period at 30 days after sowing, 45 days and 55 days after sowing.

### **3.2.14. Isolation and identification of *Bipolaris sorokiniana***

The diseased leaves were collected from the wheat field and taken to the Seed Health Laboratory of the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka. The diseased leaves were cut into small pieces (0.5 cm) and these were surface sterilized with Clorox (1:1000) for one minute. The cut pieces were then washed into sterilized water thrice and placed on a PDA media. The PDA plates containing pieces (three pieces per plate) were incubated at  $25 \pm 1^{\circ}\text{C}$  for seven days. Then the organism grew freshly on to the culture and isolated by means of hyphal tip culture method aseptically and cultured again on another PDA plate to have pure culture. The pathogen was identified as *Bipolaris sorokiniana* (Plate 14 and Plate 15) following the key of Booth, 1971 and then preserved at  $4^{\circ}\text{C}$  in the refrigerator for future use.



**Plate 14. Pure culture of *Bipolaris sorokiniana* on PDA media**



**Plate 15. Mycelia and Conidia of *Bipolaris sorokiniana* under compound microscope (X 250)**

### 3.2.15. Recording of disease severity

Leaf spot severity was recorded from fifteen selected (tagged) plants per plot (three plants per line) on flag leaf and penultimate leaves (2<sup>nd</sup> leaf from top).

The data was recorded in flowering stage, milking stage and hard dough stage.

The disease severity was recorded following 0-5 rating scale (Plate 16) as used by Hossain and Azad (1992) and the rating scale is given below:

**0** = No infection

**1** = Few minute lesions on leaves

**2** = Black lesions with no distinct chlorotic halos covering  $\leq 10\%$  of the leaf area

**3** = Typical lesions surrounded by distinct chlorotic halos covering 10-50% of the leaf area

**4** = Severe lesions on leaves with ample necrotic zones, drying over a part of the leaf, covering  $\geq 50\%$  of the leaf area and

**5** = Severe infection, drying of the leaf, spike infected to some extent



**Plate 16. Leaf spot severity of wheat showing 0-5 rating scale  
(Hossain and Azad, 1992)**

### 3.2.16. Harvesting and recording data on plant growth and yield attributes

The crop was harvested on 11th march, 2006 at full ripening stage. The data on the following parameters were recorded from the randomly selected 15 tagged plant of each unit plot:

- I. Plant height (cm)
- II. Ear length (cm)
- III. Length between the point flag leaf initiation and base of ear (cm)
- IV. Number of spikelets/ear
- V. Number of grains/ear
- VI. Number of healthy grains/ear
- VII. Number of diseased grains/ ear
- VIII. Weight of grains /ear(g)
- IX. Weight of healthy grains /ear (g)
- X. Weight of diseased grains/ear (g)
- XI. 1000 grains weight (g)
- XII. Weight of straw (kg/plot )
- XIII. Straw yield (t/ha)
- XIV. Weight of grain (kg/plot)
- XV. Grain yield (t/ha)

### **3.2.17. Grading of seeds**

The grading of seeds was done using 0-5 rating scale (Plate17) of CIMMYT (Gilchrist 1985) as follows:

0 = Free from infection

1 = Only embryo blackish

2 = Embryo and its adjacent area slightly infected

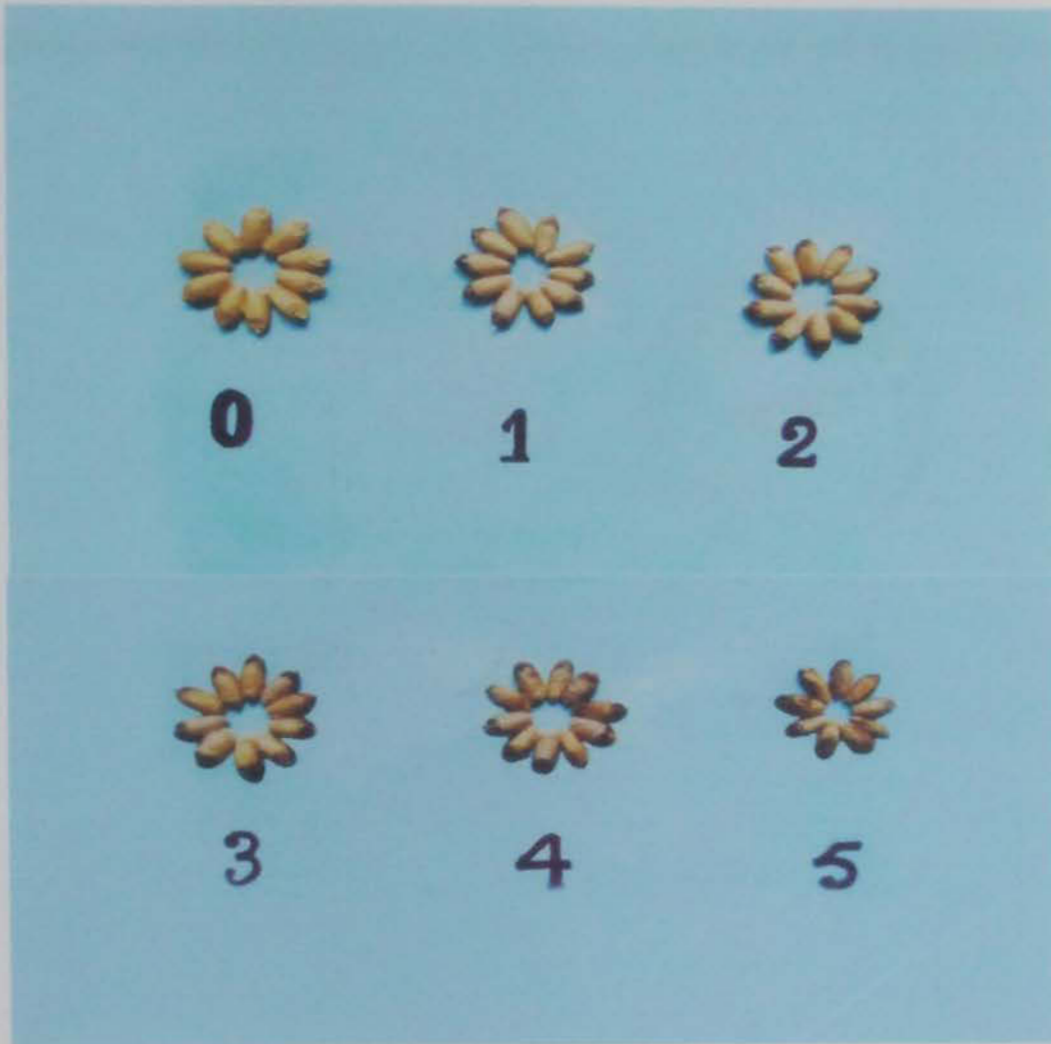
3 = Embryo and less than  $\frac{1}{4}$  of grain are discolored

4 = Embryo and  $\frac{1}{2}$  of grain are infected

5 = Grains are shriveled, almost completely discolored or more than  $\frac{1}{2}$  of grains are discolored.

### **3.2.18. Statistical analysis**

The collected data on different parameters were analyzed statistically by using MSTAT-C package program. The means for all the treatments were compared by DMRT (Duncan Multiple Range Test). The significance of the difference among the means was calculated by LSD test (Least Significance Difference).



**Plate 17. Grading of wheat seeds (0-5 scale)**



### 3.2.19. Weather report

The monthly average data on temperature, rainfall and humidity during experimental period were collected from the authority of Bangladesh Meteorological Department, Agargoan, Dhaka, and presented in Appendix II.





## Chapter 4

# Results

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## 4. RESULTS

### 4.1. Laboratory experiment

#### 4.1.1. Efficacy of botanicals on radial mycelia growth of *Bipolaris sorokiniana* in vitro (Poisoned food technique)

##### 4.1.1.1. Cup method

Efficacy of botanicals on mycelial growth of *Bipolaris sorokiniana* is shown in Table 2. Plant extracts have profound effect on mycelial growth of the fungus. All the tested botanicals significantly reduced radial mycelial growth of the fungus. Radial mycelial growth for all the tested botanicals ranged from 6.50 mm to 25.21 mm. Significantly lowest radial growth (6.50 mm) of *Bipolaris sorokiniana* was recorded in allamanda leaf extract and highest growth (25.21 mm) was recorded in untreated control. Garlic clove extract, kalijira seed extract, neem leaf extract, onion bulb extract, ginger rhizome extract, turmeric rhizome extract, mutha leaf and root extract, periwinkle leaf extract, bel leaf extract, eucalyptus leaf extract, naglingam leaf extract and durba leaf extracts were also found promising in reducing the growth of the fungus in the laboratory.

#### 4.1.1.2. Disc method

From the present study it has been found that all the botanicals have strong effect to produce inhibition zone against *Bipolaris sorokiniana* in culture media. Among them onion bulb extract produced the largest inhibition zone (63.91 mm) followed by garlic clove extract (63.67 mm), allamanda leaf extract (63.00 mm), periwinkle leaf extract (62.83 mm), chita leaf extract (62.50 mm), ginger rhizome extract (62.33 mm), bel leaf extract (62.33 mm), kalijira seed extract (62.17 mm), mandar leaf extract (62.17 mm) and turmeric rhizome extract (61.50 mm). No inhibition zone found in case of untreated control treatment.

**Table 2. Efficacy of botanicals on radial growth of *Bipolaris sorokiniana* in vitro (Poisoned food technique)**

Treatments	Radial mycelial growth (mm) (Cup method)	Diameter (mm) of growth inhibition zone (Disc method)
T <sub>1</sub> = Neem leaf extract	7.41j-l	60.17a-e
T <sub>2</sub> = Allamanda leaf extract	6.50l	63.00a-c
T <sub>3</sub> = Garlic clove extract	6.66kl	63.67ab
T <sub>4</sub> = Onion bulb extract	8.50i-l	63.91a
T <sub>5</sub> = Turmeric rhizome extract	9.33h-l	61.50a-d
T <sub>6</sub> = Kalijira seed extract	7.33j-l	62.17a-d
T <sub>7</sub> = Katamehedi leaf extract	18.67bc	47.50h
T <sub>8</sub> = Ginger rhizome extract	8.91h-l	62.33a-d
T <sub>9</sub> = Tulsi leaf extract	15.33c-f	44.67hi
T <sub>10</sub> = Debdaru leaf extract	20.00b	48.33gh
T <sub>11</sub> = Mandar leaf extract	10.83g-k	62.17a-d
T <sub>12</sub> = Kachuripana root extract	17.17b-e	52.17c-h
T <sub>13</sub> = Periwinkle leaf extract	10.50g-l	62.83a-c
T <sub>14</sub> = Chita leaf extract	15.17c-f	62.50a-d
T <sub>15</sub> = Durba leaf extract	11.33f-j	60.50a-e
T <sub>16</sub> = Bel leaf extract	10.58g-l	62.33a-d
T <sub>17</sub> = Mutha leaf and root extract	10.50g-l	59.50a-f
T <sub>18</sub> = Eucalyptus leaf extract	11.33f-j	59.17a-g
T <sub>19</sub> = Guava leaf extract	13.00e-h	43.83hi
T <sub>20</sub> = Gada leaf extract	15.33c-f	47.50h
T <sub>21</sub> = Babla leaf extract	13.83e-g	44.00hi
T <sub>22</sub> = Jaam leaf extract	13.83e-g	49.67e-h
T <sub>23</sub> = Duranta leaf and stem extract	13.00e-h	53.33a-h
T <sub>24</sub> = Arjun leaf extract	14.50c-g	49.00f-h
T <sub>25</sub> = Naglingam leaf extract	11.33f-j	46.83hi
T <sub>26</sub> = Gandhaviduli leaf extract	18.50b-d	52.67b-h
T <sub>27</sub> = Lemon leaf extract	15.50c-f	48.83f-h
T <sub>28</sub> = Sajna leaf extract	15.50c-f	43.17hi
T <sub>29</sub> = Dhonia leaf and stem extract	12.50f-i	36.17i
T <sub>30</sub> = Vat leaf extract	14.67c-g	44.83hi
T <sub>31</sub> = Justicia leaf extract	17.17b-e	50.33e-h
T <sub>32</sub> = Mechania lata leaf extract	12.67f-i	42.67hi
T <sub>33</sub> = Ipil-ipil leaf extract	14.33d-g	51.50d-h
T <sub>34</sub> = Control	25.21a	0.00j
<b>LSD(P=0.05)</b>	<b>3.535</b>	<b>9.236</b>

## 4.2. Field experiment

### 4.2.1. Effect of seed treatment with plant extracts (1:2 w/v) on leaf spot

#### *(Bipolaris sorokiniana)* severity of wheat at flowering stage

The effect of seed treatment with plant extracts on disease severity of leaf spot at flowering stage of wheat plants was determined and presented in Table 3. The disease severity of flag leaf as well as penultimate leaf under different treatments was found to differ significantly from one to another. The lowest disease severity of flag leaf was found with the treatment T<sub>12</sub> (turmeric rhizome extract) and the highest in the treatment T<sub>1</sub> (Control). It has been recorded that the other treatments T<sub>2</sub> (garlic clove extract), T<sub>3</sub> (onion bulb extract), T<sub>4</sub> (durba leaf extract), T<sub>5</sub> (allamanda leaf extract), T<sub>6</sub> (naglingam leaf extract), T<sub>7</sub> (periwinkle leaf extract), T<sub>8</sub> (kalijira seed extract), T<sub>9</sub> (ginger rhizome extract), T<sub>11</sub> (bel leaf extract), T<sub>13</sub> (eucalyptus leaf extract) and T<sub>14</sub> (neem leaf extract) did not show significant variation in respect of disease severity.

In the case of penultimate leaf, the lowest disease severity (0.24) was also found with the treatment T<sub>12</sub> (turmeric rhizome extract) which was nearly similar (0.28) with the treatment T<sub>2</sub> (garlic clove extract) whereas the highest severity (1.03) was found in the treatment T<sub>1</sub> (Control).

The average leaf spot severity of flag leaf and penultimate leaf ranged from 0.15 to 0.97, where the lowest severity was found with the treatment T<sub>12</sub> (turmeric rhizome extract) and the highest in the treatment T<sub>1</sub> (Control).

**Table 3. Effect of seed treatment with plant extracts (1:2 w/v) on leaf spot (*Bipolaris sorokiniana*) severity of wheat at flowering stage**

Treatments	Disease severity (0-5 scale)		
	Flag leaf	Penultimate leaf	Average
T <sub>1</sub> = Control	0.91a	1.03a	0.97a
T <sub>2</sub> = Garlic clove extract	0.11bc	0.28de	0.19de
T <sub>3</sub> = Onion bulb extract	0.17bc	0.53bc	0.35b-d
T <sub>4</sub> = Durba leaf extract	0.19bc	0.63b	0.41bc
T <sub>5</sub> = Allamanda leaf extract	0.19bc	0.49b-d	0.34b-d
T <sub>6</sub> = Naglingam leaf extract	0.24bc	0.51bc	0.45bc
T <sub>7</sub> = Periwinkle leaf extract	0.20bc	0.62b	0.41bc
T <sub>8</sub> = Kalijira seed extract	0.19bc	0.36c-e	0.28c-e
T <sub>9</sub> = Ginger rhizome extract	0.28bc	0.71b	0.49b
T <sub>10</sub> = Mutha leaf and root extract	0.36b	0.68b	0.52b
T <sub>11</sub> = Bel leaf extract	0.17bc	0.63b	0.40bc
T <sub>12</sub> = Turmeric rhizome extract	0.07c	0.24e	0.15e
T <sub>13</sub> = Eucalyptus leaf extract	0.28bc	0.67b	0.37b-d
T <sub>14</sub> = Neem leaf extract	0.20bc	0.34c-e	0.27c-e
<b>LSD ( P = 0.05)</b>	<b>0.2313</b>	<b>0.1986</b>	<b>0.1592</b>

#### **4.2.2. Effect of seed treatment with plant extracts (1:2 w/v) on leaf spot (*Bipolaris sorokiniana*) severity of wheat at milking stage**

Highly significant variations were observed among the different treatments in respect of disease severity grade for the flag leaf and penultimate leaf at milking stage. In case of flag leaf, the lowest disease severity (0.13) was found in the treatment T<sub>12</sub> (turmeric rhizome extract) that was nearly similar in the treatment T<sub>2</sub> (garlic clove extract). On the other hand, the highest disease severity (1.15) was found in the treatment T<sub>1</sub> (Control).

Similar result was found in the penultimate leaf at milking stage where the lowest disease severity (0.29) was observed in the treatment T<sub>12</sub> (turmeric rhizome extract) and the highest disease severity (1.35) was recorded in control treatment (T<sub>1</sub>).

The average leaf spot severity grade for flag leaf and penultimate leaf varied from 0.21 to 1.25. The lowest disease severity was obtained in the treatment T<sub>12</sub> (turmeric rhizome extract) and the highest in the treatment T<sub>1</sub> (Control) whereas T<sub>2</sub> (garlic clove extract), T<sub>8</sub> (kalijira seed extract) and T<sub>14</sub> (neem leaf extract) showed moderate disease severity.



**Table 4. Effect of seed treatment with plant extracts (1:2 w/v) on leaf spot (*Bipolaris sorokiniana*) severity of wheat at milking stage**

Treatments	Disease severity (0-5 scale)		
	Flag leaf	Penultimate leaf	Average
T <sub>1</sub> = Control	1.15a	1.35a	1.25a
T <sub>2</sub> = Garlic clove extract	0.17cd	0.34d	0.35cd
T <sub>3</sub> = Onion bulb extract	0.26b-d	0.64bc	0.45bc
T <sub>4</sub> = Durba leaf extract	0.38b-d	0.75b	0.57bc
T <sub>5</sub> = Allamanda leaf extract	0.29b-d	0.61bc	0.45bc
T <sub>6</sub> = Naglingam leaf extract	0.36b-d	0.65bc	0.50bc
T <sub>7</sub> = Periwinkle leaf extract	0.34b-d	0.71b	0.52bc
T <sub>8</sub> = Kalijira seed extract	0.26b-d	0.44cd	0.35cd
T <sub>9</sub> = Ginger rhizome extract	0.46bc	0.80b	0.63b
T <sub>10</sub> = Mutha leaf and root extract	0.45bc	0.76b	0.61b
T <sub>11</sub> = Bel leaf extract	0.48b	0.78b	0.63b
T <sub>12</sub> = Turmeric rhizome extract	0.13d	0.29d	0.21d
T <sub>13</sub> = Eucalyptus leaf extract	0.51b	0.81b	0.66b
T <sub>14</sub> = Neem leaf extract	0.28b-d	0.43cd	0.36cd
<b>LSD ( P = 0.05)</b>	<b>0.26</b>	<b>0.2123</b>	<b>0.2123</b>

#### **4.2.3. Effect of seed treatment with plant extracts (1:2 w/v) on leaf spot (*Bipolaris sorokiniana*) severity of wheat at hard dough stage**

Effect of seed treatment with plant extract on leaf spot severity of wheat at hard dough stage is shown in Table 5. Wheat plant showed significant differences in the disease severity at hard dough stage when different treatments (plant extracts) were applied. The lowest disease severity was found in the treatment T<sub>12</sub> (turmeric rhizome extract) that was statistically similar with the treatment T<sub>2</sub> (garlic clove extract) for every cases of flag leaf, penultimate leaf and their average disease grade. On the other hand, the highest disease severity was found with the treatment T<sub>1</sub> (Control) in every case. It was observed that the treatment T<sub>3</sub> (onion bulb extract), T<sub>5</sub> (allamanda leaf extract) and T<sub>14</sub> (neem leaf extract) showed moderate disease severity.

**Table 5. Effect of seed treatment with plant extracts (1:2 w/v) on leaf spot (*Bipolaris sorokiniana*) severity of wheat at hard dough stage**

Treatments	Disease severity (0-5 scale)		
	Flag leaf	Penultimate leaf	Average
T <sub>1</sub> = Control	1.95a	2.12a	2.04a
T <sub>2</sub> = Garlic clove extract	0.42e	0.48e	0.45e
T <sub>3</sub> = Onion bulb extract	0.71cd	0.82cd	0.77cd
T <sub>4</sub> = Durba leaf extract	0.95bc	1.13b	1.04b
T <sub>5</sub> = Allamanda leaf extract	0.74cd	0.84cd	0.79cd
T <sub>6</sub> = Naglingam leaf extract	1.00b	1.13b	1.07b
T <sub>7</sub> = Periwinkle leaf extract	0.93bc	1.01bc	0.97bc
T <sub>8</sub> = Kalijira seed extract	0.65d	0.72d	0.69d
T <sub>9</sub> = Ginger rhizome extract	1.10b	1.22b	1.16b
T <sub>10</sub> = Mutha leaf and root extract	1.05b	1.16b	1.11b
T <sub>11</sub> = Bel leaf extract	1.01b	1.18b	1.09b
T <sub>12</sub> = Turmeric rhizome extract	0.41e	0.46e	0.44e
T <sub>13</sub> = Eucalyptus leaf extract	1.15b	1.28b	1.22b
T <sub>14</sub> = Neem leaf extract	0.70cd	0.87cd	0.78cd
<b>LSD ( P = 0.05)</b>	<b>0.2252</b>	<b>0.2374</b>	<b>0.2252</b>

#### **4.2.4. Efficacy of seed treatment with plant extracts on plant height, ear length, length between the point of flag leaf initiation and base of ear and number of spikelets /ear**

Seed treatments with plant extracts were found to differ significantly in respect of plant height, ear length, length between the point of flag leaf initiation and base of ear and the number of spikelets /ear (Table 6).

The highest plant height was observed in the treatment T<sub>2</sub> (93.09 cm) where as the lowest plant height (79.12 cm) was recorded in control (T<sub>1</sub>). Treatment T<sub>12</sub> showed the second highest plant height followed by the treatments T<sub>3</sub>, T<sub>4</sub>, T<sub>10</sub>, T<sub>6</sub>, T<sub>7</sub>, and T<sub>5</sub>.

The length of ear ranged from 12.60 cm to 15.58 cm where the highest ear length was found in treatment T<sub>8</sub> and the lowest ear length was found in control (T<sub>1</sub>) treatment. The treatments T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub>, T<sub>5</sub>, T<sub>6</sub>, T<sub>9</sub>, T<sub>10</sub>, T<sub>11</sub>, T<sub>12</sub>, T<sub>13</sub> and T<sub>14</sub> showed statistically similar ear length and differed significantly from untreated control (T<sub>1</sub>).

The length between the point of the flag leaf initiation and the base of ear ranged from 11.11 cm to 16.45 cm where the highest and the lowest lengths were observed under the treatments T<sub>2</sub> and T<sub>1</sub>, respectively. The highest number of spikelets/ear was found in the treatment T<sub>5</sub> (19.11) and the lowest number of spikelets/ear was observed in the T<sub>1</sub> treatment (14.39).

**Table 6. Efficacy of seed treatment with plant extracts on number of plant height, ear length, length between the point of flag leaf initiation and base of ear and number of spikelets /ear**

Treatments	Plant height (cm)	Ear length (cm)	Length between the point of flag leaf initiation and base of ear (cm)	Number of spikelets /ear
T <sub>1</sub> = Control	79.12d	12.60c	11.11c	14.39c
T <sub>2</sub> = Garlic clove extract	93.09a	15.14ab	16.45a	17.76ab
T <sub>3</sub> = Onion bulb extract	88.93a-c	14.40ab	15.39ab	16.69b
T <sub>4</sub> = Durba leaf extract	88.68a-c	14.36ab	15.20ab	18.07ab
T <sub>5</sub> = Allamanda leaf extract	87.30a-c	14.62ab	14.48ab	19.11a
T <sub>6</sub> = Naglingam leaf extract	87.46a-c	14.85ab	14.05a-c	18.02ab
T <sub>7</sub> = Periwinkle leaf extract	87.34a-c	14.23b	14.96ab	16.96b
T <sub>8</sub> = Kalijira seed extract	83.24cd	15.58a	12.78bc	17.49ab
T <sub>9</sub> = Ginger rhizome extract	84.04b-d	14.50ab	13.19a-c	17.52ab
T <sub>10</sub> = Mutha leaf and root extract	88.63a-c	14.69ab	15.23ab	17.31b
T <sub>11</sub> = Bel leaf extract	83.51cd	15.43ab	12.60bc	17.58ab
T <sub>12</sub> = Turmeric rhizome extract	89.95ab	14.66ab	15.74ab	17.55ab
T <sub>13</sub> = Eucalyptus leaf extract	87.10a-c	14.82ab	14.51ab	17.62ab
T <sub>14</sub> = Neem leaf extract	85.39bc	14.61ab	13.25a-c	17.69ab
<b>LSD (P = 0.05)</b>	<b>5.407</b>	<b>1.094</b>	<b>2.796</b>	<b>1.518</b>

#### **4.2.5. Efficacy of seed treatment with plant extracts on grain formation and grain weight/ear of wheat cv. Kanchan**

It has been observed that the treatments differed significantly from one to another in respect of grain formation as well as grain weight of wheat cv. Kanchan (Table 7).

The number of grains/ear ranged from 36.68 to 43.83 where the lowest and the highest counts were made under the treatments T<sub>1</sub> and T<sub>8</sub>, respectively. The treatments T<sub>2</sub>, T<sub>5</sub> and T<sub>8</sub> resulted statistically similar effect regarding number of grains/ear.

The number of healthy grains/ear ranged from 33.67 to 42.44 where the lowest and the highest healthy grains were counted under the treatments T<sub>1</sub> and T<sub>8</sub>, respectively. On the other hand, the highest number of diseased grains/ear (3.01) was recorded under the treatment T<sub>1</sub>. Seed treatment with botanicals resulted significantly lower number of diseased grains/ear. The lowest number of diseased grains/ear (1.04) was observed in treatment T<sub>14</sub>.

In case of weight of grains/ear, it has been found that the highest (1.75g) and the lowest (1.33g) weight of grains/ear were obtained under the treatments T<sub>8</sub> and T<sub>1</sub> respectively. The treatments T<sub>2</sub>, T<sub>3</sub>, T<sub>7</sub>, T<sub>8</sub>, T<sub>12</sub> and T<sub>14</sub> resulted statistically similar effect regarding weight of grains/ear.

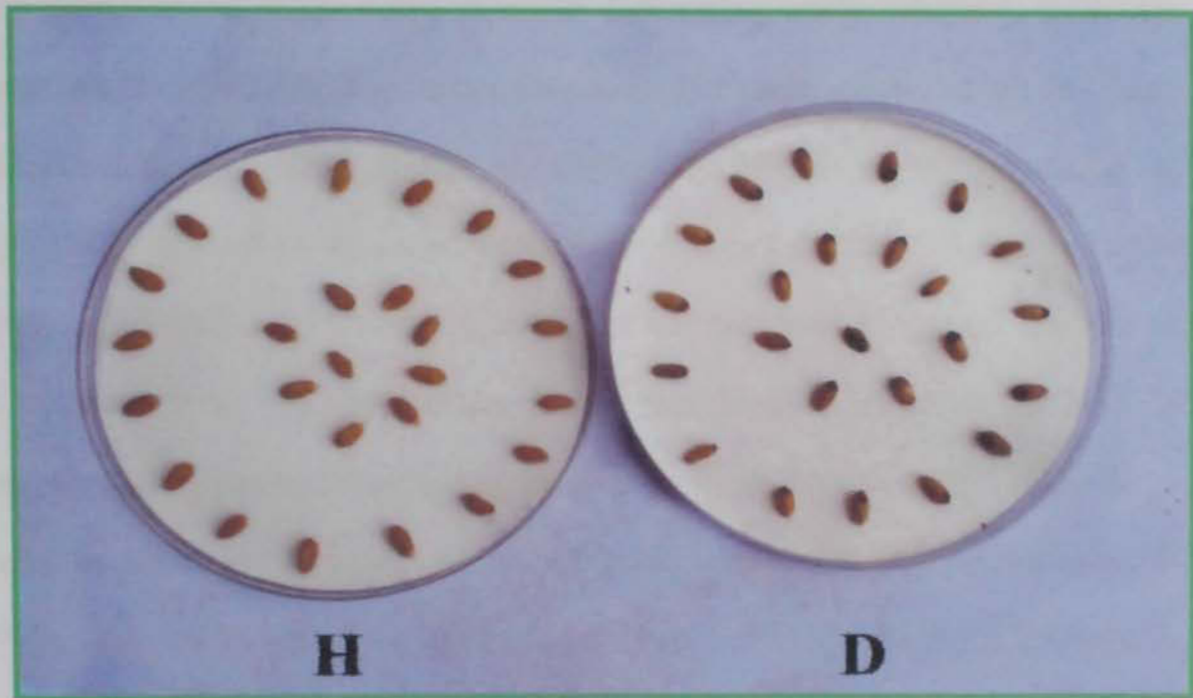
The weight of healthy grains/ ear ranged from 1.07g to 1.71g where the lowest and highest weight was recorded under the treatments T<sub>9</sub> and T<sub>8</sub>, respectively. The treatment T<sub>12</sub> has been resulted statistically similar effect with T<sub>8</sub> regarding weight of healthy grains/ear. The highest weight of diseased grains/ear (0.17g) was recorded under the treatment T<sub>6</sub> and the lowest (0.03) was found under the treatments T<sub>8</sub> and T<sub>12</sub>.



**Table 7. Efficacy of seed treatment with plant extracts (w/v) on grain formation and grain weight/ ear of wheat cv. Kanchan**

Treatments	Number of grains /ear	Number of healthy grains /ear	Number of diseased grains /ear	Weight of grains/ ear (g)	Weight of healthy grains/ ear (g)	Weight of diseased grains/ ear (g)
T <sub>1</sub> = Control	36.68i	33.67e	3.01a	1.33g	1.18d-f	0.14b
T <sub>2</sub> = Garlic clove extract	43.43ab	41.92ab	1.51b-d	1.74a	1.67ab	0.06gh
T <sub>3</sub> = Onion bulb extract	41.42de	39.70c	1.72bc	1.65ab	1.57a-c	0.08c-f
T <sub>4</sub> = Durba leaf extract	41.33d-f	39.44c	1.89b	1.52cd	1.43a-e	0.09c-e
T <sub>5</sub> = Allamanda leaf extract	43.15ab	41.49ab	1.66bc	1.61bc	1.54a-d	0.07fg
T <sub>6</sub> = Naglingam leaf extract	39.29h	37.53d	1.76bc	1.42e-g	1.24c-f	0.17a
T <sub>7</sub> = Periwinkle leaf extract	42.01cd	40.87b	1.14cd	1.67ab	1.59a-c	0.07e-g
T <sub>8</sub> = Kalijira seed extract	43.83a	42.44a	1.39b-d	1.75a	1.71a	0.03i
T <sub>9</sub> = Ginger rhizome extract	40.61e-g	39.09c	1.52b-d	1.44d-f	1.07f	0.09cd
T <sub>10</sub> = Mutha leaf and root extract	39.05h	37.25d	1.80b	1.54cd	1.46a-e	0.08d-f
T <sub>11</sub> = Bel leaf extract	40.46fg	39.04c	1.42b-d	1.41fg	1.30b-f	0.10c
T <sub>12</sub> = Turmeric rhizome extract	42.75bc	41.28ab	1.47b-d	1.71ab	1.67a	0.03i
T <sub>13</sub> = Eucalyptus leaf extract	40.22g	39.06c	1.16cd	1.51de	1.14ef	0.09cd
T <sub>14</sub> = Neem leaf extract	42.16cd	41.12b	1.04d	1.68ab	1.63ab	0.04hi
<b>LSD (P = 0.05)</b>	<b>0.8640</b>	<b>1.102</b>	<b>0.5334</b>	<b>0.09193</b>	<b>0.3184</b>	<b>0.01678</b>





**Plate 18. Healthy (H) and diseased seeds (D) of wheat**

#### **4.2.6. Effect of seed treatment with plant extracts on the formation of healthy grains and disease grains of different grades (0-5 scale) of wheat cv. Kanchan**

Grading of diseased seeds were done in 1-5 rating scale where 1 indicates minimum and 5 indicates maximum diseased symptom showing seeds (Plate 18). The treatments were found to differ significantly on formation of healthy grains as well as diseased grains of different grades. Highest percent (69.76%) of healthy seeds were observed in the treatment T<sub>12</sub> and the lowest in the treatment T<sub>1</sub>. On the other hand, the highest percent (34.48) of diseased grains were observed in the treatment T<sub>1</sub> and lowest (20.21) in T<sub>12</sub>. In case of grade-1, the highest percent of grain was found in the treatment T<sub>1</sub> (17.17%) which was followed by T<sub>2</sub> (14.00%), T<sub>3</sub> (13.75%), T<sub>4</sub> (13.55%), T<sub>5</sub> (13.28%), T<sub>6</sub> (13.27%), T<sub>7</sub> (12.77%), T<sub>8</sub> (12.75%), T<sub>9</sub> (11.18%), T<sub>10</sub> (9.34%), T<sub>11</sub> (8.89%) and T<sub>12</sub> (8.87%). The lowest percent of grade-1 grain was observed in the treatment T<sub>14</sub> (7.45%) which is statistically similar with the treatment T<sub>13</sub> (7.93%).

Considering grade-2, the highest percent of diseased grains was observed in the treatment T<sub>1</sub> (7.00%) and the lowest diseased grains was found in the treatment T<sub>14</sub> (2.67%) which is statistically similar with T<sub>13</sub> (2.97%).

Maximum grade-3 seeds were found in  $T_1$  (3.94%) and minimum in  $T_{14}$  (0.87%). Considering grade-4, the treatment  $T_1$  (4.57%) showed the highest percent of diseased grains whereas the lowest counts were made in  $T_{14}$  (0.20%).

In case of grade-5, very negligible percent of diseased grains (0.50%) were counted with the treatments  $T_{11}$ ,  $T_{12}$ ,  $T_{13}$  and  $T_{14}$  where the treatment  $T_1$  (4.10%) showed the highest percent of diseased grains of grade-5.

**Table 8. Effect of seed treatment with plant extracts on the formation of healthy grains and diseased grains of different grades (0-5 scale) of wheat cv. Kanchan**

Treatments	%Healthy grains	%Diseased grains	%Diseased grain under different grades				
			1	2	3	4	5
T <sub>1</sub> = Control	55.48d	34.48a	17.17a	7.00a	3.94a	4.57a	4.10a
T <sub>2</sub> = Garlic clove extract	67.24ab	22.73cd	14.00ab	6.24b	3.60ab	3.72b	3.72b
T <sub>3</sub> = Onion bulb extract	61.84c	28.19b	13.75a-c	6.09b	3.31b	3.56b	3.16c
T <sub>4</sub> = Durba leaf extract	58.26d	31.70a	13.55a-c	5.97b	3.25b	2.56c	2.89cd
T <sub>5</sub> = Allamanda leaf extract	57.62d	32.34a	13.28a-c	5.40c	2.55c	2.41c	2.87cd
T <sub>6</sub> = Naglingam leaf extract	56.61d	33.35a	13.27a-c	5.40c	2.53c	2.34c	2.86cd
T <sub>7</sub> = Periwinkle leaf extract	59.16cd	30.80ab	12.77a-c	5.17cd	2.52c	2.27c	2.75d
T <sub>8</sub> = Kalijira seed extract	67.47ab	22.50cd	12.75a-c	5.06cd	2.30c	1.67d	2.07e
T <sub>9</sub> = Ginger rhizome extract	57.58d	32.38a	11.18b-d	4.77d	1.69d	1.60d	1.80e
T <sub>10</sub> = Mutha leaf and root extract	58.81cd	31.16ab	9.340b-d	4.67d	1.65d	1.57d	1.17f
T <sub>11</sub> = Bel leaf extract	57.35d	32.62a	8.890cd	4.01e	1.41de	1.03e	0.50g
T <sub>12</sub> = Turmeric rhizome extract	69.76a	20.21d	8.875cd	3.47f	1.28d-f	0.89ef	0.50g
T <sub>13</sub> = Eucalyptus leaf extract	57.30d	32.72a	7.930d	2.97g	1.21ef	0.63f	0.50g
T <sub>14</sub> = Neem leaf extract	65.66b	24.30c	7.456d	2.67g	0.87f	0.20g	0.50g
<b>LSD (P = 0.05)</b>	<b>3.196</b>	<b>3.194</b>	<b>4.306</b>	<b>0.4831</b>	<b>0.3984</b>	<b>0.3204</b>	<b>0.3347</b>

0 = Free from infection

1 = Only embryo blackish

2 = Embryo and its adjacent area slightly infected

3 = Embryo and less than ¼ of grains are discolored

4 = Embryo and ½ of grains are infected and

5 = Grains are shriveled, almost completely discolored or more than ½ of grains are discolored

#### **4.2.7. Efficacy of seed treatment with plant extracts on 1000-seed weight and yield of wheat cv. Kanchan**

The treatments were found to differ significantly in respect of 1000 grain weight, grain yield and straw yield (Table 9). The 1000 grain weight (g) of wheat ranged from 35.61 to 41.50 g where the highest weight was recorded in the treatment  $T_2$  which was statistically similar with the treatment  $T_{12}$  (41.46g). The lowest weight of 1000 grains was recorded in the control treatment  $T_1$  (35.61g).

Considering straw yield (Kg/plot) it was observed that the highest yield was found in the treatment  $T_{12}$  (1.43 Kg) where seeds were treated with turmeric rhizome extract and untreated control treatment ( $T_1$ ) showed the lowest straw yield (0.61 Kg/plot).

Yield of wheat (g/plot) profoundly varied from one treatment to another, ranging from 436.8 to 721.0 g/plot. Highest yield was recorded in plots under treatment  $T_{12}$  (seed treated with turmeric rhizome extract) where the lowest was found under control treatment. In case of grain yield of wheat (t/ha), treatment  $T_{12}$  resulted the highest yield (3.60 t/ha) and treatment  $T_1$  showed the lowest yield (2.18 t/ha) which was statistically similar with treatment  $T_{11}$  (seed treated with bel leaf extract).

**Table 9. Efficacy of seed treatment with plant extracts on 1000-seed weight and yield of wheat cv. Kanchan**

Treatments	1000 grain weight (g)	Straw yield (kg/plot)	Straw yield (t/ha)	Grain yield (g/plot)	Grain yield (t/ha)
T <sub>1</sub> = Control	35.61f	0.61d	3.05d	436.8d	2.18e
T <sub>2</sub> = Garlic clove extract	41.50a	1.17a-c	5.86a-c	623.9a-c	3.11a-c
T <sub>3</sub> = Onion bulb extract	36.56ef	1.19ab	5.98ab	503.2b-d	2.51b-e
T <sub>4</sub> = Durba leaf extract	38.94b-d	0.90b-d	4.51b-d	590.3a-c	2.95a-d
T <sub>5</sub> = Allamanda leaf extract	38.23b-d	1.02bc	5.10bc	572.2b-d	2.85b-e
T <sub>6</sub> = Naglingam leaf extract	38.02c-e	0.81cd	4.05cd	480.5cd	2.40de
T <sub>7</sub> = Periwinkle leaf extract	37.53de	0.94b-d	4.70b-d	484.8cd	2.42c-e
T <sub>8</sub> = Kalijira seed extract	39.70b	1.14a-c	5.71a-c	634.8ab	3.16ab
T <sub>9</sub> = Ginger rhizome extract	37.69de	0.86b-d	4.33b-d	620.4a-c	3.10a-d
T <sub>10</sub> = Mutha leaf and root extract	39.76b	0.98bc	4.91bc	570.3b-d	2.84b-e
T <sub>11</sub> = Bel leaf extract	38.36b-d	1.14a-c	5.70a-c	440.1d	2.19e
T <sub>12</sub> = Turmeric rhizome extract	41.46a	1.43a	7.15a	721.0a	3.60a
T <sub>13</sub> = Eucalyptus leaf extract	39.36bc	0.92b-d	4.60b-d	541.8b-d	2.70b-e
T <sub>14</sub> = Neem leaf extract	39.36bc	0.98bc	4.93bc	640.4ab	3.20ab
<b>LSD (P = 0.05)</b>	<b>1.353</b>	<b>0.3228</b>	<b>1.619</b>	<b>124.0</b>	<b>0.6189</b>



Chapter 5

Discussion

## 5. DISCUSSION

### 5.1. Laboratory experiment

In the present study among 33 species, all the tested botanicals significantly reduced radial mycelial growth of the fungus. Significantly lowest radial growth of *Bipolaris sorokiniana* was recorded in allamanda leaf extract and highest growth was recorded in untreated control. It has been also found that all the botanicals have strong effect to produce inhibition zone against *Bipolaris sorokiniana* in culture media. Among them onion bulb extract produced the largest inhibition zone where as no inhibition zone found in untreated control. The present findings were well supported by the reports of Ashrafuzzaman and Hossain (1992) and Hossain *et al.* (1997).

Ashrafuzzaman and Hossain (1992) reported that bishkatali (*Polygonum hydropiper*) extract inhibited the mycelial growth and spore germination of *Bipolaris sorokiniana* effectively. They also reported that neem seed extract was found effective against *Bipolaris sorokiniana* and the extract inhibited the growth of the fungus in wheat seeds.



In the present study, garlic clove extract showed an excellent result to inhibit the mycelial growth of *Bipolaris sorokiniana* in vitro. Hossain *et al.* (1997) also observed good antifungal activity of garlic extract against *Bipolaris sorokiniana* of wheat. They reported that garlic extract completely inhibited the mycelial growth of *Bipolaris sorokiniana* at 1:4 dilution ratios and it reduced 52.26% mycelial growth even at concentration ratio of 1:8.

## 5.2. Field experiment

Efficacy of seed treatment with plant extracts in controlling leaf blight (*Bipolaris sorokiniana*) of wheat var. Kanchan was studied under the field condition. Extracts of thirteen plants were selected for seed treatment by in vitro test against *Bipolaris sorokiniana*. From the present study it is evident that the treatments showed significant effect in respect of disease severity (0-5 scale) on flag leaf and penultimate leaf at flowering, milking and hard dough stages. It has been observed that garlic clove extract (T<sub>2</sub>) and turmeric rhizome extract (T<sub>12</sub>) resulted remarkable reduction of leaf spot severity over untreated control. Many scientist such as Rovesti *et al.* (1992); Hossain and Schlosser (1993); Khan and Hossain (1993); Bisht and Khulbe (1995); Hossain *et al.* (1997); Rahman (1998); Islam *et al.* (2006) had done their research with plant extracts to evaluate their antifungal potentiality. Hossain



and Schlosser (1993) reported that neem (*Azadirachta indica*) extract had a potential ability for controlling *Bipolaris sorokiniana* of wheat. They also found that the neem extract inhibited the growth of *Bipolaris sorokiniana* in wheat seeds and reduced its pathogenicity on wheat leaves. Khan and Hossain (1993) informed that extracts of *Allium cepa*, *A. sativum*, *Datura stramonium*, *D. plumeiri*, *Lawsonia alba*, *Ricinus communis*, *Leomurus sibiricus* and *Mentha viridis* completely inhibited spore germination of *B. sorokiniana* at 1:3 (w/v) dilution ratio. Bisht and Khulbe (1995) studied the efficacy of leaf extract of *Allium sativum* in controlling the growth of *Drechslera oryzae* and reported that *Allium sativum* significantly reduced the mycelial growth compared to control.

Hossain *et al.* (1997) evaluated that the extracts of *Allium sativum* (Garlic) and *Lawsonia alba* (Mehedi) showed remarkable effect in reducing the mycelial growth of *Bipolaris sorokiniana* and its pathogenicity to wheat leaves. A remarkable reduction of leaf blight severity of wheat by spraying botanicals such as *Allium sativum*, *Nigella sativa*, *Lawsonia alba* and *Cymbopogon citratus* have been reported by Rahman (1998). The findings of the present study is well supported by Islam *et al.* (2006) who found a

remarkable reduction of leaf blight severity of wheat by seed treatment with garlic (*Allium sativum*) and bishkatali (*Polygonum hydropiper*) extracts.

In the present experiment, it has been found that seed treatment with plant extracts showed a significant effect on plant height, ear length, length between the point of flag leaf initiation and base of ear and number of spikelets/ear. The highest plant height was observed in the seed treated with garlic (*Allium sativum*) clove extract where as the lowest plant height was recorded in untreated control. Islam *et al.* (2006) stated that influence of plant growth in case of spraying garlic extract might be due to the increase of nitrogen uptake efficiency as well as protein quality resulting positive effect in vegetative growth such as stem elongation. The similar results were also found by Wildermuth *et al.* (1992) who made an assessment of yield loss on 8 cultivars and lines differing susceptibility to *Bipolaris sorokiniana*.

The number of grains/ear, number of healthy grains/ear, weight of grains/ear and weight of healthy grains/ear were increased by seed treatment with plant extracts. Kalijira (*Nigella sativa*) seed extract, garlic (*Allium sativum*) clove extract, allamanda (*Allamanda cathartica*) leaf extract and turmeric (*Curcuma longa*) rhizome extract showed an excellent performance to

increase the number and weight of healthy grains. The findings of the present study corroborates with the findings of Rahman (1998) who reported that the extracts of *Cymbopogon citratus*, *Lawsonia alba*, *Nigella sativa*, and *Allium sativum* significantly increased number of grains/ear and healthy grains/ear over untreated control. Islam *et al.* (2006) also agreed with present study. They reported that garlic, ginger, bishkatali and neem extract resulted statistically similar effect as of seed treatment with Vitavax-200 regarding number of grains/ear and number of healthy grains/ear. This result indicates plant extracts as an excellent alternative to chemicals for seed treatment.

From this study it is evident that the highest percentage of healthy grain was found in turmeric rhizome extract ( $T_{12}$ ) and the lowest in untreated control ( $T_1$ ). The highest percentage of black pointed seeds of grade-2, grade-3, and grade-4 was counted under the treatment  $T_1$ .

Regarding 1000 grain weight under different treatments the highest weight was recorded in the treatment  $T_2$  (garlic clove extract) which was statistically similar with the treatment  $T_{12}$  (turmeric rhizome extract). The lowest weight of 1000 grains was recorded in the control treatment  $T_1$ . The finding of the present study is supported by Rahman (1998). He obtained

increased 1000 grains weight over untreated control by treating wheat seeds with garlic extract. In another study Rahman (1998) further reported that the extracts of *Cymbopogon citratus*, *Lawsonia alba*, *Nigella sativa* and *Allium sativum* increased 1000 seed weight of wheat over control. Islam *et al.* (2006) reported that considering 1000 grain weight (g), seed treatment with plant extracts resulted statistically similar effect as of seed treatment with Vitavax-200.

In the present study, it is well exposed that seed treatment with plant extracts increased grain yield over untreated control. Seed treated with turmeric rhizome extract (T<sub>12</sub>) resulted the highest yield (3.60 t/ha) and treatment T<sub>1</sub> resulted the lowest yield (2.18 t/ha). Rahman (1998) reported that *Cymbopogon citratus*, *Lawsonia alba*, *Nigella sativa* and *Allium sativum* increased 0.96 %, 2.24 %, 4.17 % and 8.01 % grain yield over untreated control. Islam *et al.* (2006) also reported that seed treatment with bishkatali extract increased 29.74 % grain yield over untreated control. In the present study it has been found that turmeric rhizome extract increased 39.44 % grain yield over untreated control.

The results of present investigation confirmed the promising fungicidal effect of plant extracts against *Bipolaris sorokiniana*. Among the botanicals, garlic clove extract; turmeric rhizome extract; kalijira seed extract and neem leaf extract are very effective in controlling leaf spot in the field. The findings of the study have ventilated an opportunity to manage the leaf blight of wheat with the increase in yield by using plant extracts. However, more investigations need to pursue including more plant extracts as seed treating agent for consecutive year to confirm the result or to have more potential plant extracts against leaf blight (*Bipolaris sorokiniana*) of wheat.



## Chapter 6

# Summary and Conclusion

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## 6. SUMMARY AND CONCLUSION

Wheat (*Triticum aestivum* L.) is considered as the second most important cereal in Bangladesh. Leaf blight caused by *Bipolaris sorokiniana* is a limiting factor of wheat production in the warmer areas. The fungus reduces the yield and quality of seeds. The research programme were undertaken to study the efficacy of some selected plant extracts against leaf blight (*Bipolaris sorokiniana*) of wheat in the field.

Use of plant extracts against plant diseases is relatively a recent approach. The present experiment was carried out in the seed health laboratory, Department of Plant Pathology and at the farm of Sher-e-Bangla Agricultural University, Dhaka 1207 during January-2006 to April-2007.

In the present study, all the tested botanicals significantly reduced radial mycelial growth of the fungus in vitro. Among 33 botanicals, significantly lowest radial growth of *Bipolaris sorokiniana* was recorded in allamanda leaf extract and the highest growth was recorded in untreated control. It has been also found that all the botanicals have strong inhibitory capability to produce inhibition zone against *Bipolaris sorokiniana* in culture media. Among them Onion bulb extract produced the largest inhibition zone where as no inhibition zone found in untreated



control. Garlic clove extract, durba leaf extract, naglingam leaf extract, periwinkle leaf extract, kalijira seed extract, ginger rhizome extract, mutha leaf and root extract, bel leaf extract, turmeric rhizome extract, eucalyptus leaf extract and neem leaf extract also resulted significantly lower radial growth and larger inhibition zone than control. Thirteen botanicals were selected on the basis of their antifungal activity against *Bipolaris sorokiniana* in vitro and their extracts were used as seed treating materials for controlling leaf blight in the field.

From the present study it is revealed that the treatments showed significant effect in respect of disease severity (0-5 scale) of flag leaf and penultimate leaf at flowering, milking and hard dough stages. It has been observed that garlic clove extract (T<sub>2</sub>) and turmeric rhizome extract (T<sub>12</sub>) resulted remarkable reduction of leaf spot severity over untreated control.

Seed treatment with plant extracts showed a significant effect on plant height, ear length, length between the point of flag leaf initiation and base of ear and number of spikelets/ear. The highest plant height was observed in the seed treated with garlic (*Allium sativum*) clove extract where as the lowest plant height was recorded in untreated control. The present study showed that number of grains/ear, number of healthy grains/ear, weight of grains/ear and weight of healthy grains/ear were increased by using

seeds treated with plant extracts. Kalijira (*Nigella sativa*) seed extract, garlic (*Allium sativum*) clove extract, allamanda (*Allamanda cathartica*) leaf extract and turmeric (*Curcuma longa*) rhizome extract showed an excellent performance to increase the number and weight of healthy grains. In respect of the formation of healthy as well as black pointed grains, it was found that neem leaf extract (T<sub>14</sub>) and eucalyptus leaf extract (T<sub>13</sub>) performed the best result by producing the highest percentage of healthy grains and lowest number of black pointed grains.

Regarding 1000 grain weight under different treatments the highest weight was recorded in the treatment T<sub>2</sub> (garlic clove extract) which was statistically similar with treatment T<sub>12</sub> (turmeric rhizome extract). The lowest weight of 1000 grains was recorded in the control treatment T<sub>1</sub>.

The findings of the present study indicate that plant extracts have promising effect against *Bipolaris sorokiniana* and there is a good chance of employing plant extracts in controlling leaf blight of wheat. The findings on the antifungal activities of plant extracts may, in future, open a new horizon in plant disease control. So, special attention in biocontrol with plant extracts should be emphasized.



Chapter 7

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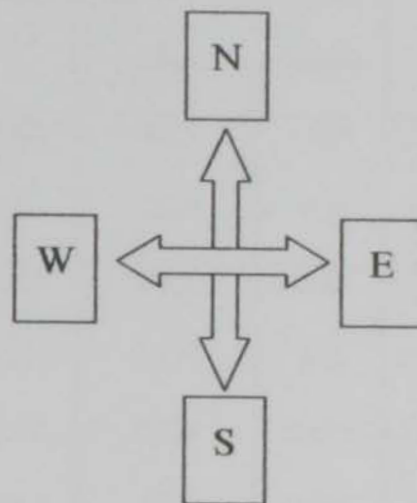
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# Appendices

R <sub>1</sub> T <sub>2</sub>	R <sub>2</sub> T <sub>5</sub>	R <sub>3</sub> T <sub>8</sub>
R <sub>1</sub> T <sub>4</sub>	R <sub>2</sub> T <sub>7</sub>	R <sub>3</sub> T <sub>3</sub>
R <sub>1</sub> T <sub>6</sub>	R <sub>2</sub> T <sub>1</sub>	R <sub>3</sub> T <sub>10</sub>
R <sub>1</sub> T <sub>9</sub>	R <sub>2</sub> T <sub>8</sub>	R <sub>3</sub> T <sub>2</sub>
R <sub>1</sub> T <sub>12</sub>	R <sub>2</sub> T <sub>14</sub>	R <sub>3</sub> T <sub>6</sub>
R <sub>1</sub> T <sub>14</sub>	R <sub>2</sub> T <sub>4</sub>	R <sub>3</sub> T <sub>11</sub>
R <sub>1</sub> T <sub>3</sub>	R <sub>2</sub> T <sub>2</sub>	R <sub>3</sub> T <sub>5</sub>
R <sub>1</sub> T <sub>13</sub>	R <sub>2</sub> T <sub>12</sub>	R <sub>3</sub> T <sub>9</sub>
R <sub>1</sub> T <sub>11</sub>	R <sub>2</sub> T <sub>3</sub>	R <sub>3</sub> T <sub>13</sub>
R <sub>1</sub> T <sub>7</sub>	R <sub>2</sub> T <sub>13</sub>	R <sub>3</sub> T <sub>14</sub>
R <sub>1</sub> T <sub>10</sub>	R <sub>2</sub> T <sub>6</sub>	R <sub>3</sub> T <sub>12</sub>
R <sub>1</sub> T <sub>1</sub>	R <sub>2</sub> T <sub>11</sub>	R <sub>3</sub> T <sub>1</sub>
R <sub>1</sub> T <sub>8</sub>	R <sub>2</sub> T <sub>10</sub>	R <sub>3</sub> T <sub>7</sub>
R <sub>1</sub> T <sub>5</sub>	R <sub>2</sub> T <sub>9</sub>	R <sub>3</sub> T <sub>4</sub>



Plot size: 2m x 1m (2 m<sup>2</sup>)

Plot to plot distance: 1 m

Block to block distance: 1m

**Appendix I.** Layout of the experimental field



**Appendix II. Monthly average of Temperature, Relative humidity, Total Rainfall and  
Sunshine hour of the experimental site during the period from November  
2006 to April 2007**

Year	Month	Air temperature ( <sup>o</sup> c)			Relative humidity (%)	Rain fall (mm)	Sun shine (hr)
		Maximum	Minimum	Mean			
2006	November	29.0	19.1	24.05	68.5	0.0	230.7
	December	26.5	16.5	21.5	70.5	0.0	213.5
2007	January	24.9	13.2	19.05	67.5	3.0	192.5
	February	28.1	17.8	22.95	61.5	4.0	220.3
	March	32.5	22.6	27.55	66.6	0155	214.5
	April	34.5	24.8	29.65	67.5	0091	251.8

Source: Bangladesh Meteorological Department (Climate division), Agargaon, Dhaka-1212.

পেপের বাংলা কৃষি বিশ্ববিদ্যালয় গজাগার  
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