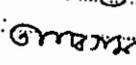


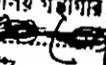
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EFFICACY OF SELECTED PLANT EXTRACTS ON LEAF SPOT (*Bipolaris sorokiniana*) AND GRAIN YIELD OF WHEAT

MD. AMINUR ISLAM

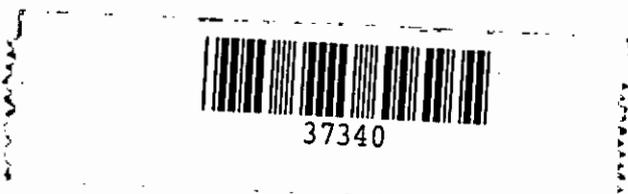
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**EFFICACY OF SELECTED PLANT EXTRACTS ON
LEAF SPOT (*Bipolaris sorokiniana*) AND GRAIN
YIELD OF WHEAT**

BY
MD. AMINUR ISLAM
Registration No. 25105/00292

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

**MASTER OF SCIENCE
IN
PLANT PATHOLOGY
SEMESTER: JANUARY - JUNE, 2006**

Approved by:



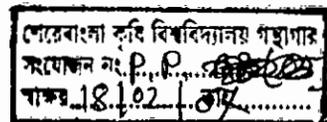
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This is to certify that the thesis entitled, "*EFFICACY OF SELECTED PLANT EXTRACTS ON LEAF SPOT (*Bipolaris sorokiniana*) AND GRAIN YIELD OF WHEAT*" submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment 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. Aminur Islam, Roll No. 00292, Registration No. 25150/00292*, 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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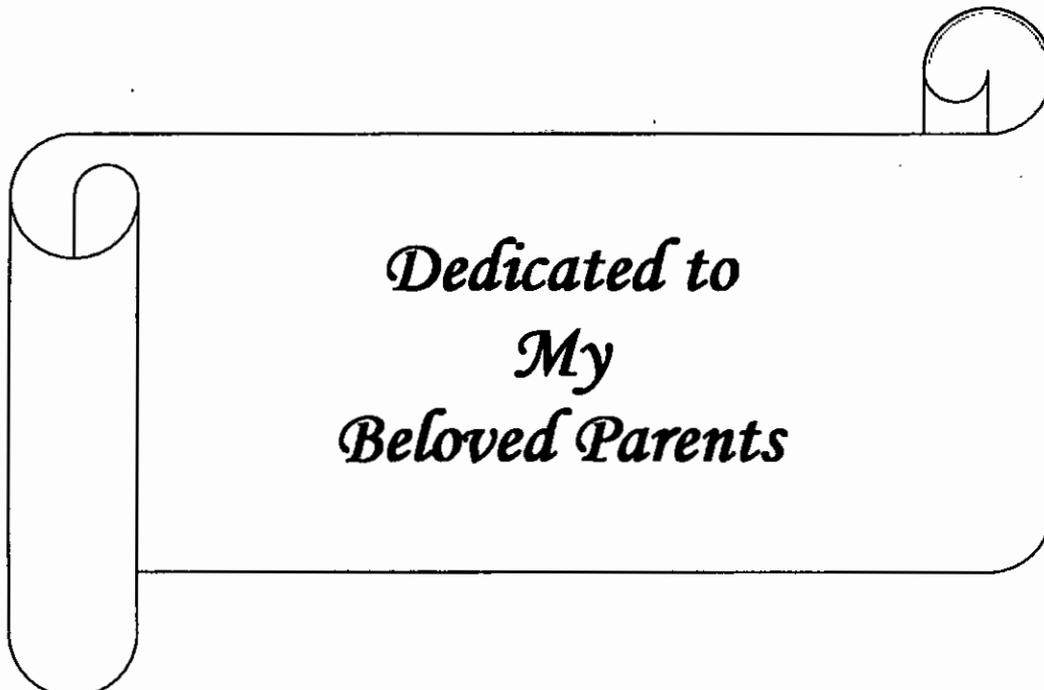
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*Dedicated to
My
Beloved Parents*

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The Author

EFFICACY OF SELECTED PLANT EXTRACTS ON LEAF SPOT (*Bipolaris sorokiniana*) AND GRAIN YIELD OF WHEAT

BY

MD. AMINUR ISLAM

ABSTRACT

Efficacy of selected plant extracts was studied on the leaf spot (*Bipolaris sorokiniana*) and grain yield of wheat during December, 2005 to April, 2006. The experiment was conducted in the laboratory and in the farm of the Sher-e-Bangla Agricultural University, Dhaka. Ten different treatments viz. T₁ (control), T₂ (Vitavax 200 @ 0.3 %), T₃ (Onion bulb extract 1:2 w/v), T₄ (Garlic clove extracts 1:2 w/v), T₅ (Kalizira extracts 1:2 w/v), T₆ (Zinger Rhizome extracts 1:2 w/v), T₇ (Allamanda leaf extract 1:2w/v), T₈ (Bishkatali extracts 1:2 w/v), T₉ (Neem leaf extract 1:2 w/v) and T₁₀ (Mehedi leaf extracts 1:2 w/v). Were explored in the experiment in the laboratory the plant extracts were used in three concentrations i.e. 1:1, 1:2 and 1:4 w/v. All the plant extracts except Mehedi increased seed germination in the laboratory in compared to untreated control. The highest incidence of seed borne *Bipolaris sorokiniana* was recorded under control (T₁). A remarkable reduction of the incidence of fungi was achieved by treating seeds with botanicals. The leaf spot severity of flag leaf and penultimate leaf and 3rd leaf differed significantly from one treatment to another at flag leaf, flowering, milking and hard dough stages. The lowest severity of flag leaf in every stage was found in treatment T₂ followed by treatment T₈ and treatment T₄. The highest disease severity of flag leaf, penultimate leaf and third leaf in all growth stages was recorded under the treatment T₁ (untreated control). Significantly the highest plant height (85.77 cm) and distance between the point of flag leaf initiation and base of ear (15.62 cm) were found in the treatment T₄, ear length (19.98cm) in treatment T₈, number of tillers/plant (6.48) in treatment T₁₀, number of spikes/plot (513.66) were found under the treating seeds with botanicals. But the highest number of grains/ear (43.98), weight of grains/ear (1.88g), grain yield (3.52 t/ha) and straw yield (6.85 t/ha) was found in T₂. Extract of Onion, Garlic, Kalizira, Zinger, Bishkatali and Neem resulted statistically similar grain yield as of seed treatment with Vitavax-200 (T₂).

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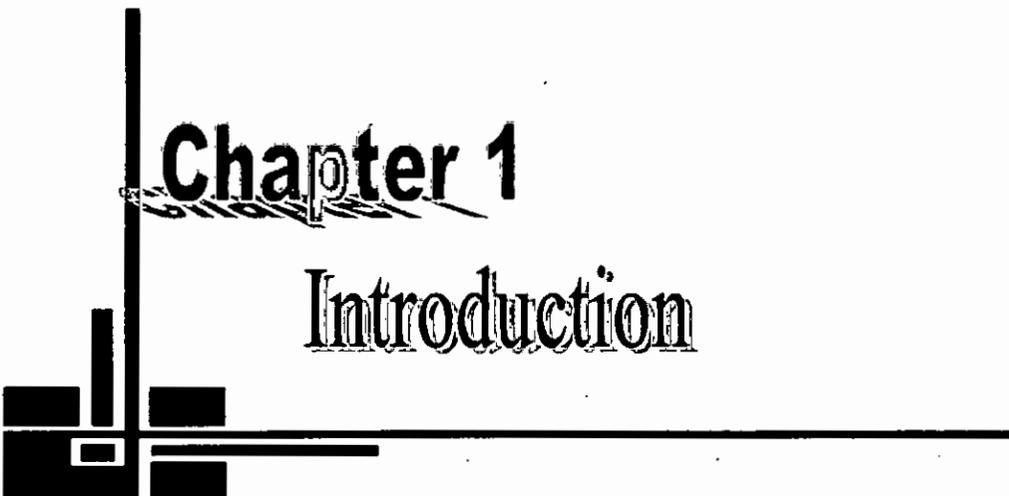
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ABBREVIATIONS USED

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
Fig.	=	Figure
GY	=	Grain yield
ha	=	Hectare
HLB	=	<i>Helminthosporium</i> leaf blight
i.e.	=	That is
K	=	Potassium
Kg	=	Kilogram
lb	=	Pound
LSD	=	Least significant difference
m	=	Meter
mm	=	Millimeter

MP	=	Muriate of potash
N	=	Nitrogen
NS	=	Not significant
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
ton /ha	=	Ton per hectare
TSP	=	Triple Super Phosphate
UNDP	=	United Nation Development Program
wt.	=	Weight
w/v	=	weight/volume
Zn	=	Zinc
°C	=	Degree Centigrade
%	=	Percent



Chapter 1

Introduction

1. INTRODUCTION

Wheat (*Triticum aestivum L.*) is one of the most popular staple foods in the world. It is the second most important cereal crop next to rice in Bangladesh. About 706.86 thousand hectares of land in Bangladesh is covered by wheat cultivation with the annual production of 1570 thousand tons (BBS, 2005). Wheat is well adapted crop in our native climate and can play a vital role in recovering our food deficit. Though the crop has been introduced in 1967 in the country (East Pakistan), but its popularity increased after 1975. Now it is cultivating throughout the country and well accepted by the farmer. The average yield of wheat was 2.20 tons/ha in 1999-2000 (BBS, 2000), where the average yields of wheat in India was 2.65 and world average is 2.75 tons/ha (FAO, 1999). Wheat suffers from as many as 26 seed-borne pathogens causing 14 seed-borne diseases (Fakir, 1999). Among them leaf blight and black point caused by *Bipolaris sorokiniana* has become a serious concern in Bangladesh. (Fakir,1998a). Though the average production and yield rate of wheat have been increasing dramatically during the last decade, the wheat yield in Bangladesh is too low (2.2 t/ ha) 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, respectively (FAO, 2000)

In the farmer's field, the yield loss in wheat due to leaf blight / spot disease in the country have been reported to be 14.97 % (Alam *et al.*, 1995). In case of severe attack, it may result 100% yield loss (Hossain and Azad, 1994). Considering 10 % production loss of wheat caused by this disease, approximately 175000 tons of wheat worth more than Tk 1400 million is lost annually in Bangladesh (Fakir, 2000). The yield loss in wheat due to leaf blight

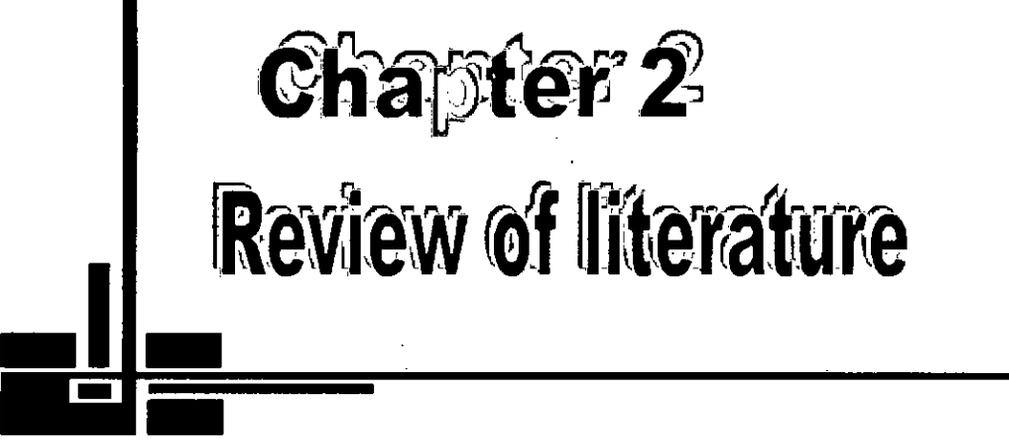
/ leaf blotch disease in the country has been reported to be 20% in var Sonalika, where 14% and 8% in Akbar and Kanchan, respectively (Razzaque and Hossain, 1991). There are several approaches that have been practiced for controlling leaf blight disease, such as use of resistant variety, cultural control, chemical control, biological control, use of plant extract etc. The most acceptable method for controlling the disease is cultivation of resistant variety. But none of the wheat varieties in the country is found resistant against this disease (Hossain and Azad, 1992). Farmer's of the country are still not aware of their seed health. Seed treatment with botanicals may be a nice option in controlling seed borne pathogens as well as seed transmitted disease in the field. Controlling leaf spot of wheat with botanicals have been successfully practiced by many researchers. (Hossain and Schlosser, 1993; Ashrafuzzman and Hossain, 1992).

Though chemical treatment are most effective for controlling the disease, but continuous use of chemicals results in accumulation of harmful chemical residues in soil as well as in the plant products causing serious health hazard. Chemical fungicides also pollute the environment and develop tolerance of pathogen. The chemical fungicides are also costly that is sometimes burden for the poor farmers. In addition, their harmful effect is responsible for air, soil and water pollution.

Use of plant extracts in controlling pathogens is now successfully used against certain fungal pathogens (Assadi and Behroozin, 1987; Miah *et al.*, 1990; Fakir and Khan, 1992; Suratuzzaman, 1995; Hossain *et al.* 1997). Hossain and Schlosser (1993) have been reported promising fungicidal effect of Neem (*Azadirachta indica*) extracts against *Bipolaris sorokiniana*.

Considering the above facts, the present research program has been designed with the following objectives:

1. To determine the efficacy of selected plant extracts as seed treating agent in controlling seed borne *Bipolaris sorokiniana* in laboratory.
2. To evaluate the efficacy of selected plant extracts as seed treating agent in controlling leaf spot caused by *Bipolaris sorokiniana* 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

Management of plant diseases with eco-friendly approaches, i.e. alternative to chemicals is now getting top priority. Use of botanicals instead of chemical fungicides in controlling disease is one of the recent approaches for plant disease control. Literatures related to control of leaf spot of wheat caused by *Bipolaris sorokiniana* with plant extracts is presented in this chapter.

2.1 Seed treatment with plant extract

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*.

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*. According to them, jute seeds treated with garlic paste increased the rate of 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 oxysporum* 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.

Some researcher compared the antifungal properties of plant extracts with fungicides. 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.s. niviem* 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) obtained garlic extract have significant effect in controlling seed-borne *Drechslera oryzae*. Evaluation of plant extracts against seed-borne infection of fungus and increased the germination ability of the treated seeds (Alice and Rao, 1987).

Assadi and Behroozin (1987) conducted an experiment with bulb extracts of onion and garlic. They demonstrated that the effect of these extract 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) observed that the inhibition of mycelial growth of *Gibberella zeae* by means of treatments with garlic extracts compared to chemical fungicide Captafol. All treatments inhibited mycelial growth of *Gibberella zeae*.

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 botanical extracts to control leaf blight of mustard. They systematically recorded the reduced number of diseased leaves per plant, percent leaf area diseased, pods per plant and number of spots per pod in mustard var. Sonalika, Sampad and Sambal that received spray of either mustard leaf extract of Sambal leaves brought 34.0 and 68.2% reduction of leaf area diseased and percent pod infection respectively over control.

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

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* (Shetty *et al.* 1989).

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 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*.

Tariq and Magee (1990) observed that the effect of volatiles from garlic bulb extracts on *Fusarium oxysporum* f. sp. *lycopersici*. Volatile component of crude aqueous extracts of garlic bulb inhibited 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 the level of micro conidia formation was unaffected.

After conducting experiments with aqueous garlic extracts (10, 20 and 30 g per 100 ml), Managamma and Sreeramula (1991) observed that extract was toxic to *Xanthomonas campestris* pv. *vescatoria* isolated from infected tomatoes but were inactive after sterilization in an autoclave. Tomato seeds soaked in a culture suspension of *X. campestris* pv. *vescatoria* were treated with 30% aqueous garlic extracts and 15-35 days after soaking, plant had 85% decreased in the number of leaf spot as compared to control. They conclude that garlic extracts might be used as seed treatment agent to control seed-borne infection by *X. campestris* pv. *vescatoria*.

Zhang *et al.* (1990) reported that 1000 grain weight of grains affected by black point disease in Shaanxi province, China was 1.95-13.5 % lower than uninfected grains.

Dubey and Dwivedi (1991) found that fungistatic 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.

Studies 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 (Thakhur *et al.*, 1991).

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 *et al.* (1992) evaluated 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. The main fungal species isolated were *Fusarium moniliforme* (*Gibberella zeae*), *F. oxysporum* and *F. semitectum* (*F. pallidoroseum*). They treated seeds with 80 ppm neem (*Azadirachta indica*) extract and 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.

Achimu and Schlosser (1992) demonstrated that the effects of Neem seeds extract against downy mildew (*Plasmopara viticola*) of grapevine. They found that the raw Neem seed extracts and commercial Neem product (Margo-san-o, Neem oil and Neem- azal-s) had high (80-90%) antifungal properties against *Plasmopara viticola*.

Rovesti *et al.* (1992) also found aqueous Neem extract effective against some important diseases of different herbaceous crops.

Wildermuth *et al.* (1992), made an assessment of yield loss on 8 cultivars and lines of wheat differing susceptibility to *Bipolaris sorokiniana*.

Hossain and Schlosser (1993) observed that the possibility of using Neem plant (*Azadirachta indica*) extract as a means of controlling *Bipolaris sorokiniana* of

wheat. Neem seed extracts /cake was found 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 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 *Rhizoctonia solani* and *Bipolaris sorokiniana*. Among the test extract, *A. sativum* completely inhibited the mycelial growth at dilution ratio of 1:4 (w/v). In another study, 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.

Suratuzzaman *et al.* (1994) obtained that good effect of garlic extract in controlling *Pyricularia oryzae* and *Curvularia lunata*.

Hossain *et al.* (1995) reported that extract of Mehedi (*Lawsonia alba*) was found to be effective against *Bipolaris sorokiniana*.

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

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.

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.

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.

According to Khaleduzzaman (1996) the effect of plant extracts viz Bishkatali (*Polygonum hydropiper*), Garlic (*Allium sativum*), Ginger (*Zingiber officinale*) and Neem (*Azadirachta indica*) and a seed dressing chemical Vitavax-200 (Carboxin) for controlling seed-borne infection 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.

Hossain *et al.* (1997) demonstrated that the extract of *Allium sativum* and *Lawsonia alba* showed marked effect in controlling the spore germination and mycelial growth of *Bipolaris sorokiniana* and pathogenecity to wheat leaves

and *Nigella sativa* showed positive antifungal activity in reducing the pathogenicity of *Bipolaris sorokiniana* of wheat leaves.

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) studied that the activity of Neem oil (*Azadiracta 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 laboratory, 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 Binasail and TN1 variety.

Rahman (1998a) demonstrated that the single effects of Vitavax –200 and garlic extract (1:2) as foliar spray which was significantly less effective than the combination use the Vitavax –200 and Tilt 250 EC to control leaf blight of wheat crop under field condition.

Hossain *et al.* (2005) reported that different plant part viz. Bishkatali, Vatpata, Garlic, Gagra, Bitter Guard and Neem were tested against fungi associated with wheat seed. After dipping in the crude extract and alcoholic extract both in undiluted and diluted form for 24 hours wheat seed were investigated and found that five different fungi viz. *Bipolaris sorokiniana*, *Alternaria tenuis*, *Curvularia lunata*, *Fusarium* spp and *Aspergillus* spp were reduced significantly by plant extracts seed and increased seed germination. Crude extracts were superior to the dilution extract. Out of 6 plant species, Neem extract was turned up as superior among the selected extracts followed by Garlic, Bishkatali and Vatapta.

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 50⁰c, 52⁰c, 54⁰c, 56⁰c and 58⁰c 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.



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 6 kg seed sample of wheat cv Kanchan was collected from a farmer of Village Domkandi, Upazilla-Sariakandi, District-Bogra. After collection, the seeds were kept in an air tight plastic container and stored at normal room temperature in MS laboratory of the Department of Plant Pathology, Sher-e-Bangla Agricultural University.

3.1.2. Collection of botanicals and preparation of extract

Botanicals were collected from different places (Plate 1 and Plate 2). Clove of Garlic, rhizome of Zinger, seed of Kalizira and bulb of Onion were collected from the Agargoan market Tejgaon, Dhaka. Stem and leaf of Bishkatali, leaves of Neem, Allamanda and Mehedi were collected from Sher-e-Bangla Agricultural University campus. The extracts were prepared by using the method of Hossain *et al.* (2005). For preparation of extracts, collected leaves were weighted in an electric balance and then washed in 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 3).

For getting 1:1(w/v) ratio, 100 ml of distilled water was added with 100 g plant parts. Similarly to get 1:2 (w/v) and 1:4 (w/v) ratios, 200 and 400 ml distilled water was

added in 200g and 400g plant parts respectively (Plate 4). The particulars of the botanicals used for the experiment are cited below:

Sl no	Common name	Botanical name	Plant parts used
1	Allamanda	<i>Allamanda cathartica</i>	Leaf
2	Bishkatali	<i>Polygonum hydropiper</i>	Leaf & stem
3	Neem	<i>Azadirachta indica</i>	Leaf
4	Garlic	<i>Allium sativum</i>	Clove
5	Onion	<i>Allium cepa</i>	Bulb
6	Ginger	<i>Zingiber officinalis</i>	Rhizome
7	Mehedi	<i>Lawsonia alba</i>	Leaf
8	Kalizira	<i>Nigella sativa</i>	Seed



Plate 1: Botanicals used for seed treatment



Onion bulbs



Mehedi leaves



Neem leaves with branch



Zinger Rhizome

Plate 2. Botanicals used for seed treatment

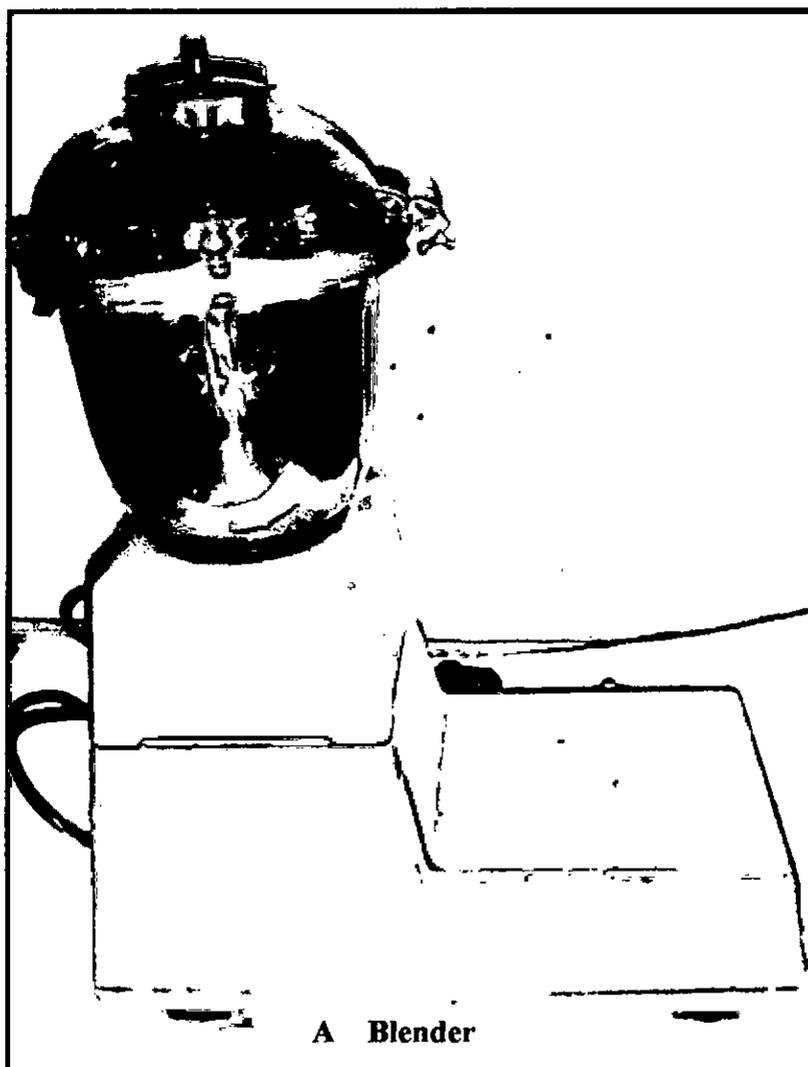


Plate 3. Extract preparing blender

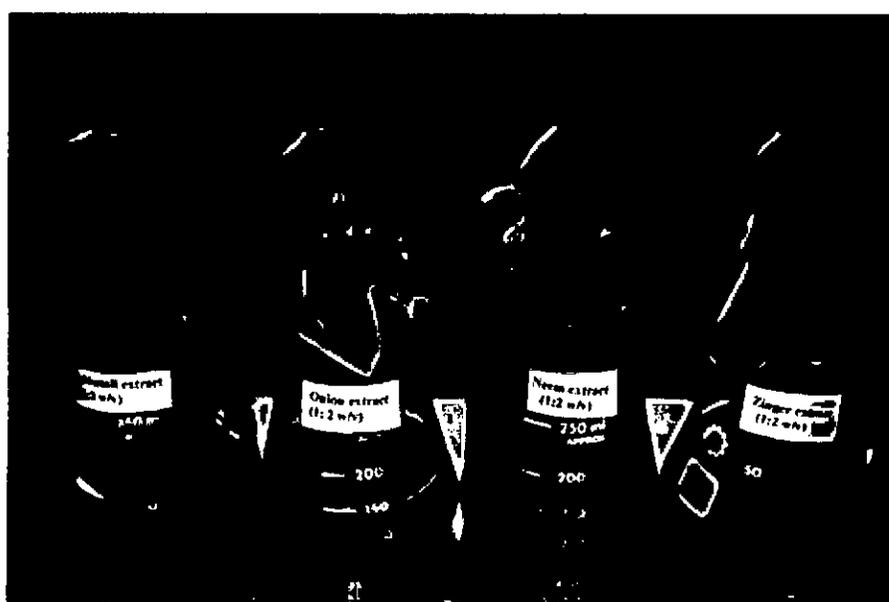
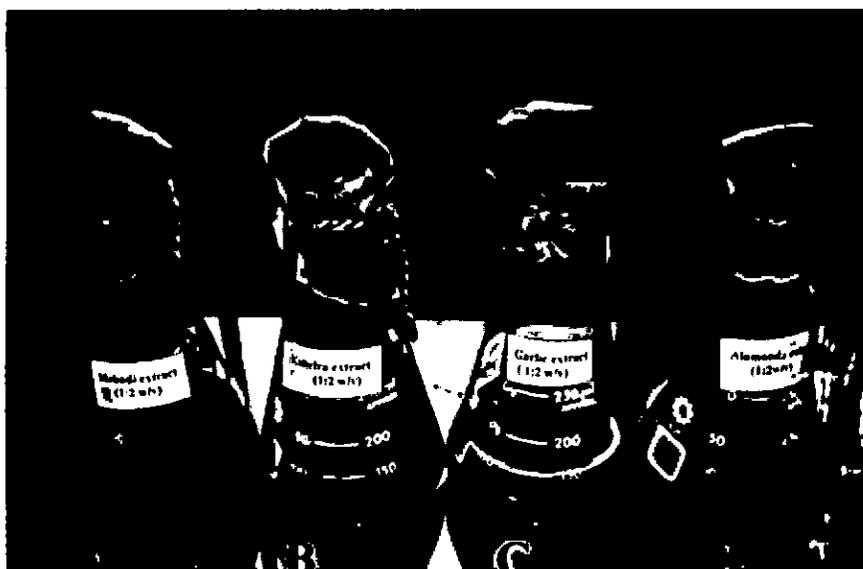


Plate 4. Plant extracts used for seed treatment. (A = Mehedi extracts, B = Kalizira Extract, C = Garlic extract, D = Allamanda extract, E = Biskatali extract, F = Onion extract, G = Neem extract, H = Zinger extract)

3.1.3. Seed treatment

Seeds were treated by following dipping method separately in different extracts. Different plant extracts with different concentrations were taken in different sterilized petridishes as per requirement. Then 400 seeds were dipped in the solution for 30 minutes. The treated seeds were then taken out of the extract and kept in blotting paper to remove excess moisture from seed surface.

3.1.4. Detection of seed-borne *Bipolaris sorokiniana*

Incidence of seed-borne *Bipolaris sorokiniana* was recorded 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 no 1) in a petridish maintaining equal distance and covered with the lid. The petridishes were incubated in an air cooled room at about 20⁰C temperature for 7 days maintaining 12 /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 seeds were observed for the presence of seed-borne *Bipolaris sorokiniana* under stereobinocular microscope (plate 5). Germination of the seeds was also recorded. Identification of *Bipolaris sorokiniana* under 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).



Plate 5. Habit character of *Bipolaris sorokiniana* on incubated wheat under stereomicroscope(X 45).

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, 2005 to March, 2006.

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: Domkandi, Upazilla: Sariakandi, District: Bogra.

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 10 unit plots in each. Each unit plot size was 2m × 1m. Block to block and plot to plot distance was 1m and 1m each (Appendix I)

3.2. 6. Treatments

There were ten different treatments as follows:

T₁ = Control

T₂ = Vitavax-200 (0.03%)

T₃ = Onion bulb extract (1: 2 w /v)

T₄ = Garlic clove extract (1: 2 w /v)

T₅ = Kalizira seeds extract (1: 2 w /v)

T₆ = Zinger rhizome extract (1: 2 w /v)

T₇ = Allamanda leaf extract (1: 2 w /v)

T₈ = Bishkatali leaf extract (1: 2 w /v)

T₉ = Neem leaf extract (1: 2 w /v)

T₁₀ = Mehedi leaf extract (1: 2 w /v)

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. Cowdung 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 Hossain *et al.* (2005) as described in laboratory experiment (3.1.2.)

3.2. 10. Seed treatment procedure:

Seed treatment was done by dipping seed in different plant extracts for 30 minutes using the method of Hossain *et al.* (2005) as described in laboratory experiment (3.1.3.).

3.2. 11. Sowing of Seeds

The seeds were sown in the field on 2nd December, 2005 at the rate of 120 kg / ha. Seeds were placed continuously in lines at the depth of 5 cm and covered by soil with the help of hand. The distance between lines was 20 cm in every plot.

3.2. 12. Irrigation

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

3.2. 13. Intercultural operations

Various intercultural operations such as thinning, weeding and mulching were accomplished whenever required to keep the plant healthy and the field weed free. The plants were kept under careful observation. Weeding was done thrice during the whole experimental period at 30, 45 and 55 days after sowing. Mulching and thinning were done at 30 DAS to get the suitable tiller and healthy plant.

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3.2.14. Isolation and identification of *Bipolaris sorokiniana*

The diseased leaves were collected by using polythene bag and taken to the laboratory of the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka. Then the diseased leaves were cut into small pieces (0.5 cm) and these were surfaced sterilized with Chlorox (1:1000) for one minute. Then the cut pieces were washed with sterilized water thrice, placed on PDA. The petridish containing leaves pieces (three pieces per plate) were incubated at $25\pm 1^{\circ}\text{C}$ for 7 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 6 and plate 7) following the key of Brooth, 1971 and then preserved at 4°C in the refrigerator for future use.



Plate 6. Pure culture of *Bipolaris sorokiniana* on PDA

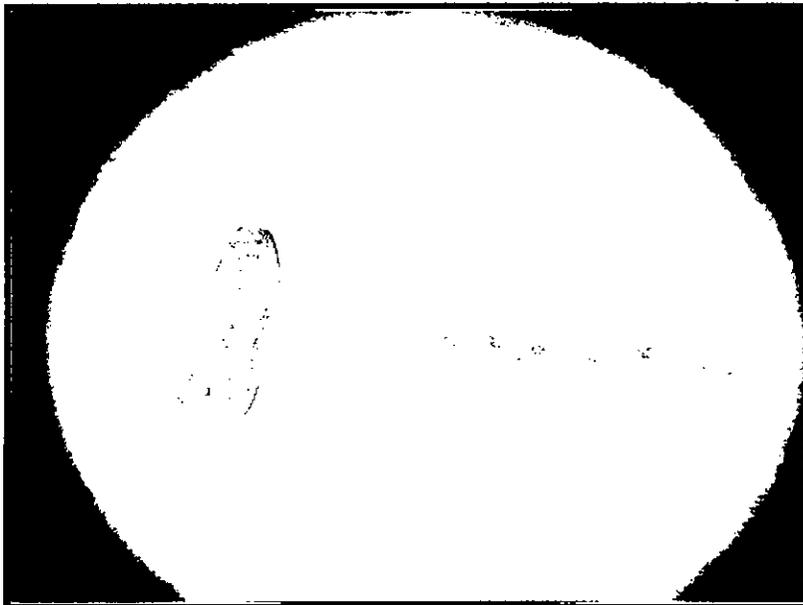


Plate 7. Conidia of *Bipolaris sorokiniana* (×400) under compound microscope.

3.2. 15. Recording of disease severity:

Leaf spot severity was recording from 25 selected plants per plot (Five plants per line on flag leaf, penultimate leaves (2nd leaf from top) and 3rd leaf from top of the plant (Plate 8). The data were recorded at flag leaf, flowering, milking and hard dough stages. The disease severity was recorded following 0-5 rating scale (Plate 9) as used by Hossain and Azad (1992) and the rating scale is given below:

0 = No infection

1 = Few minute lesion on leaves

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

3= Typical lesions surround by distinct chlorotic halos covering 10-50% of leaf area.

4 = Severe lesion on leaves with ample necrotic zones drying over a part of the leaf covering $\geq 50\%$ of the leaf area.

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



Plate 8. Wheat plant showing leaf blight symptom.

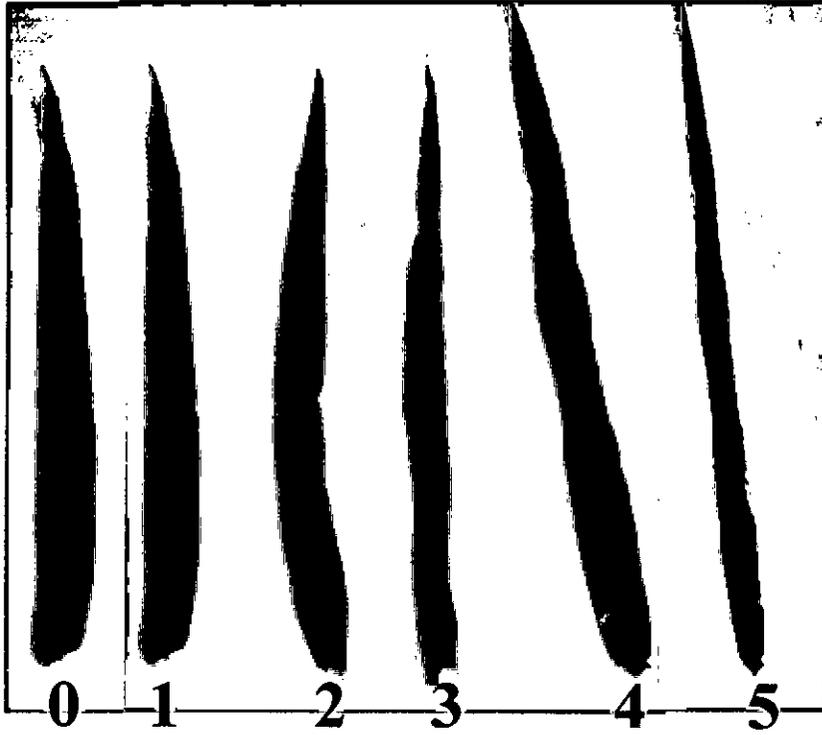


Plate 9. Leaf spot severity of wheat showing 0-5 rating scale.

3.2. 16. Harvesting and recording data on plant growth and yield attributes

The crop was harvested on 24th march, 2006 at full ripening stage. The data on the following parameters were recorded from the randomly selected tagged plants of each unit plot:

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

3.2. 17. Grading of seeds:

The grading of seeds was done using 0-5 rating scale (Plate12) 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

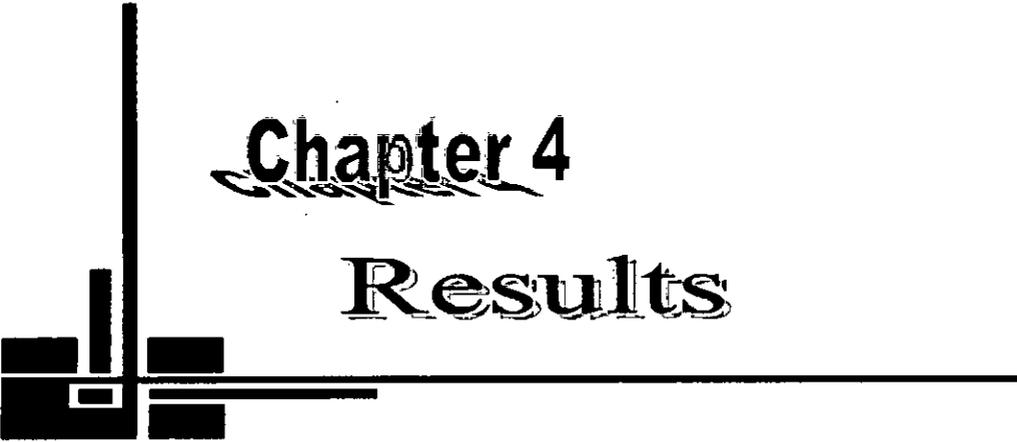
5 = Grains are shriveled, almost completely discolored or more than $\frac{1}{2}$ of grains are discolored.

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).

3.2. 19. Weather report:

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



Chapter 4

Results

4. RESULTS

4.1. Laboratory experiment.

4.1.1. Efficacy of seed treatment with plant extracts on germination of wheat seeds in the laboratory

Effects of different plant extracts on germination of wheat seeds are shown in figure 1. It was observed that the highest germination (91%) was found while seeds were treated with Kalizira extract, (1:2 w/v) and the lowest germination (68%) was found with Mehedi leaf extracts (1:1 w/v). The Garlic clove extract, Neem extract and Bishkatali extract were also increased seed germination remarkasly over untreated control.

4.1.2. Efficacy of plant extract on the incidence of seed-borne *Bipolaris sorokiniana* on wheat seeds.

Remarkable reduction in incidence of seed-borne *Bipolaris sorokiniana* was achieved by treating seeds with different plant extracts (Figure 2). All the plant extracts reduced the incidence of *Bipolaris sorokiniana* over untreated control. Highest reduction was found in case of treating seeds with Bishkatali.

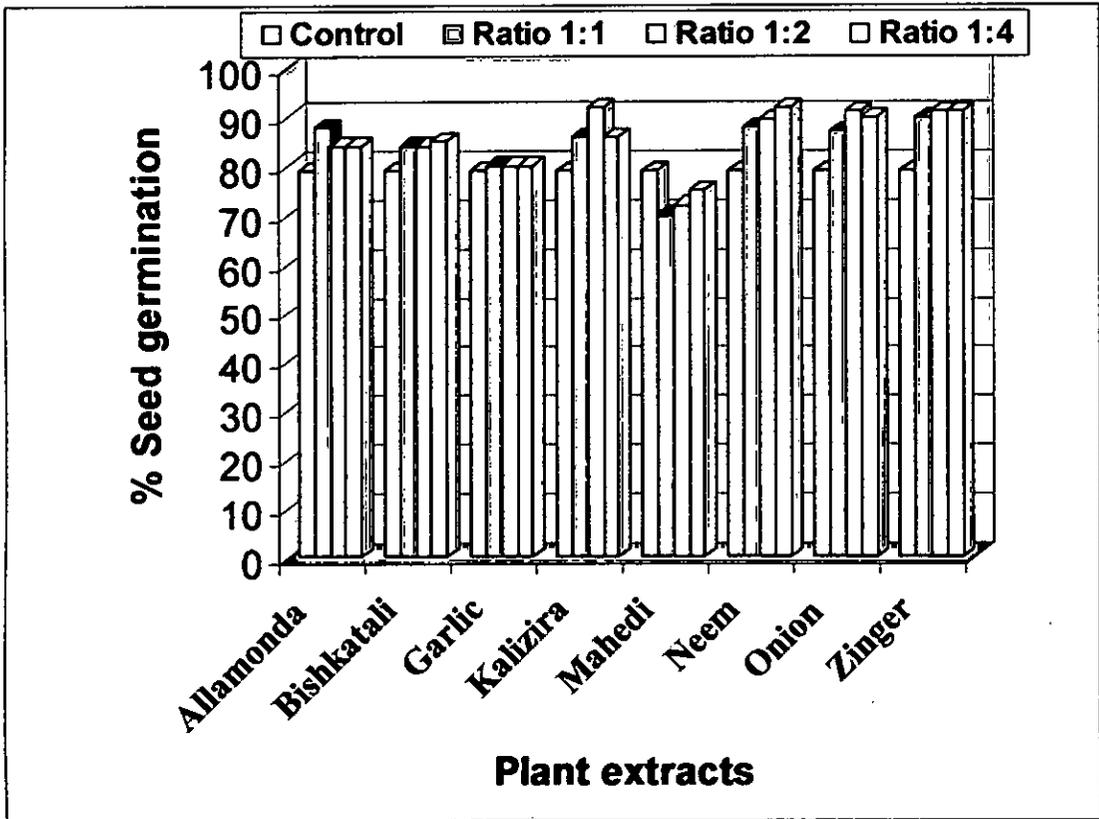


Figure 1. Effect of seed treatment with plant extracts on germination of wheat seeds

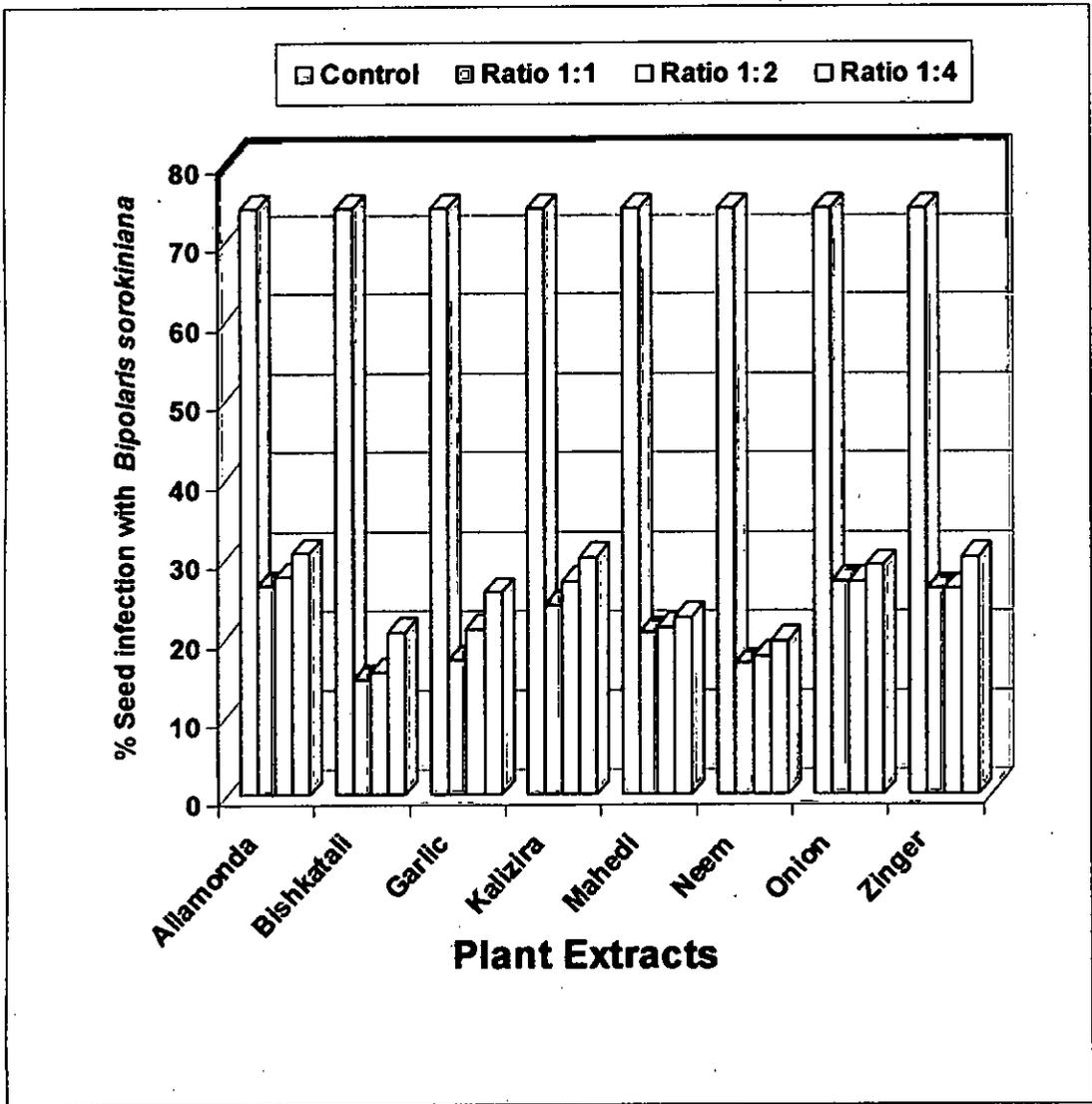


Figure 2. Effect of seed treatment with plant extracts on incidence of seed-borne *Bipolaris sorokiniana* on wheat seeds.

4.2. Field experiment

4.2.1. Effect of seed treatment with plant extracts on leaf spot (*Bipolaris sorokiniana*) severity of wheat at flag leaf stage

The effect of plant extracts and chemical on disease severity of leaf spot of flag leaf stage of wheat plants was found significant (Table 1). Vitavax-200 (0.3%) was used here as a stander check. In flag leaf stage the lowest disease severity was found in the treatment T₂ (0.02) which was statistically similar with all the treatment except T₁ and T₃. On the other hand the highest severity (0.18) was found under treatment T₁ where no plant extracts were used. In the case of penultimate leaf, the lowest disease severity was found treatment T₄ (0.04) which was statistically similar with T₂ and T₈ treatments, whereas the highest severity (0.21) was found in T₁ treatment. In case of 3rd leaf, the lowest disease severity was found in treatment T₂ (0.12) which was statistically similar with T₄ and T₈ treatments and the highest severity (0.22) was found with T₁.

Table 1. Effect of seed treatment with plant extracts on leaf spot (*Bipolaris sorokiniana*) severity of wheat at flag leaf stage

Treatments	Disease severity (0-5 scale)		
	Flag leaf	Penultimate leaf	3 rd leaf
T ₁ = Control	0.18a	0.21a	0.22a
T ₂ = Vitavax-200 (0.3%)	0.02c	0.04e	0.12d
T ₃ = Onion extract (1:2 w/v)	0.09b	0.15bc	0.15b-d
T ₄ = Garlic extract (1:2 w/v)	0.03c	0.04e	0.12d
T ₅ = Kalizira extract (1:2 w/v)	0.06bc	0.14bc	0.17a-d
T ₆ = Zinger extract (1:2 w/v)	0.05bc	0.13cd	0.15b-d
T ₇ = Allamanda extract (1:2 w/v)	0.06bc	0.18a-c	0.20ab
T ₈ = Bishkatali extract (1:2 w/v)	0.03c	0.05e	0.12d
T ₉ = Neem extract (1:2 w/v)	0.04bc	0.07de	0.14cd
T ₁₀ = Mehedi extract (1:2 w/v)	0.04bc	0.19ab	0.20a-c
LSD (P = 0.05)	0.05841	0.05841	0.05841

4.2.2. Effect of seed treatment with plant extracts on leaf spot (*Bipolaris sorokiniana*) severity of wheat at flowering stage

Significant variation was observed in disease severity of leaf spot of flag leaf at flowering stage of wheat plants when different treatments (plant extracts and chemical) were applied (Table 2). The highest performance was found in chemical treatment (0.10), that was T₂ treatment and nearly similar result was found in T₈ and T₄ treatment, which was Garlic clove extract and Bishkatali leaf and stem extract, respectively. On the other hand the lowest performance was found in T₁ (0.20) which was control and the second highest performance was found in T₃ (0.13) and T₉ (0.14) treatments that was Onion extract and Neem leaf extract. Similar result was found in the Penultimate leaf at flowering stage that the lowest disease severity was observed in T₂ (.103) and the highest disease severity was demonstrated in control treatment (T₁). The moderate disease severity was found in T₄ (0.11) and T₈ (0.11) treatment.

In case of 3rd leaf at flowering stage, the lowest disease severity was observed in the T₄ (0.18) treatment and the second highest performance were observed in T₂ (0.18) treatment and remarkable effect was observed in T₈ (0.21). The highest disease severity was found in control treatment.

Table 2. Effect of seed treatment with plant extracts on leaf spot (*Bipolaris sorokiniana*) severity of wheat at flowering stage

Treatments	Disease severity (0-5 scale)		
	Flag leaf	Penultimate leaf	3 rd leaf
T ₁ = Control	0.25a	0.31a	0.51a
T ₂ = Vitavex-200 (0.3%)	0.10e	0.10d	0.18e
T ₃ = Onion extract(1:2 w/v)	0.13cde	0.18bc	0.29b-d
T ₄ = Garlic extract(1:2 w/v)	0.10de	0.11d	0.18e
T ₅ = Kalizira extract(1:2 w/v)	0.16b-d	0.15b-d	0.31bc
T ₆ = Zinger extract(1:2 w/v)	0.16b-d	0.14cd	0.25c-e
T ₇ = Allamanda extract(1:2 w/v)	0.19ab	0.19bc	0.35b
T ₈ = Bishkatali extract(1:2 w/v)	0.10de	0.11d	0.21de
T ₉ = Neem extract(1:2 w/v)	0.14bc-e	0.15b-d	0.24c-e
T ₁₀ = Mehedi extract(1:2 w/v)	0.16bc	0.20b	0.30bc
LSD (P = 0.05)	0.05841	0.05841	0.05841

4.2.3. Effect of seed treatment with plant extracts on leaf spot (*Bipolaris sorokiniana*) severity of wheat at milking stage

Wheat plant showed significant variation in the disease severity at milking stage. It was observed that the lowest disease severity was showed in the treatment at T₂ (0.12) of flag leaf, penultimate and 3rd leaf at milking stage, that was statistically similar with the T₄ (0.12) and T₈ (0.12). The highest disease severity was recorded in the control treatment which was 0.312 in flag leaf, 0.78 in penultimate leaf and 1.02 in 3rd leaf. (Table 3)

Table 3. Effect of seed treatment with plant extracts on leaf spot (*Bipolaris sorokiniana*) severity of wheat at milking stage

Treatments	Disease severity (0-5 scale)		
	Flag leaf	Penultimate leaf	3 rd leaf
T ₁ = Control	0.32a	0.78a	1.02a
T ₂ = Vitavax-200(0.3%)	0.12e	0.36d	0.41e
T ₃ = Onion extract (1:2 w/v)	0.18d	0.69b	0.92ab
T ₄ = Garlic extract (1:2 w/v)	0.12e	0.40d	0.42e
T ₅ = Kaliziraextract(1:2 w/v)	0.29ab	0.51c	0.78c
T ₆ = Zinger extract (1:2 w/v)	0.21cd	0.50c	0.64d
T ₇ = Allamanda extract (1:2 w/v)	0.25bc	0.74ab	0.89bc
T ₈ = Bishkatali extract (1:2 w/v)	0.12e	0.41d	0.45e
T ₉ = Neem extract (1:2 w/v)	0.24bcd	0.50c	0.61d
T ₁₀ = Mehedi extract (1:2 w/v)	0.29ab	0.69b	0.98ab
LSD(P=0.05)	0.05841	0.05841	0.1168

4.2.4. Effect of seed treatment with plant extracts on leaf spot (*Bipolaris sorokiniana*) severity of wheat at hard dough stage

Significant variation of disease severity of leaf spot of wheat at hard dough stage was observed when different plant extracts were applied (Table 4). The highest performance was shown by the T₂ treatment (Vitavax-200) in all the three categories of leaves at hard dough stage. The second highest performance was shown by the garlic extracts (T₄) at flag leaf but in case of penultimate and 3rd leaf, the second highest performance was shown by in the treatment T₈ (Bishkatali extracts). The lowest performance was obtained in control treatment that was 1.37 in flag leaf, 2.923 in penultimate leaf and 4.65 in 3rd leaf. The Chemical (Vitavax-200), Garlic, Neem and Bishkatali extracts shown more or less similar result in this observation.

Table 4. Effect of seed treatment with plant extracts on Leaf spot (*Bipolaris sorokiniana*) severity of wheat at hard dough stage

Treatments	Disease severity (0-5 scale)		
	Flag leaf	Penultimate leaf	3 rd leaf
T ₁ = Control	1.37a	2.92a	4.65a
T ₂ = Vitavax-200(0.3%)	0.37e	1.25f	2.84e
T ₃ = Onion extract(1:2 w/v)	0.78b	2.19bc	3.79bc
T ₄ = Garlic extract(1:2 w/v)	0.40e	1.57d-f	2.92e
T ₅ = Kalizira extract(1:2 w/v)	0.82b	1.92b-d	3.96b
T ₆ = Zinger extract(1:2 w/v)	0.52c-e	1.82b-e	3.40d
T ₇ = Allamanda extract(1:2 w/v)	0.66b-d	2.23bc	3.59cd
T ₈ = Bishkatali extract(1:2 w/v)	0.46de	1.32ef	2.87e
T ₉ = Neem extract(1:2 w/v)	0.73bc	1.68c-f	3.75bc
T ₁₀ = Mehedi extract(1:2 w/v)	0.76b	2.29b	3.73bc
LSD (P = 0.05)	0.2185	0.5633	0.3035

4.2.5. Efficacy of seed treatment with plant extracts on number of tillers/plant, plant height, ear length, distance between the point of flag leaf initiation and base of ear, number of spikes/plot and number of spikelets /ear

Seed treatments with plant extracts were found to differ significantly in respect of number of tillers /plant, ear length and number of spikelets /ear (Table 5). But the plant height, distance between the point flag leaf initiation and base and number of ear/plot were not significant in respect of seed treatment with different plant extract (Table 5). In case of tillers/plant, the highest numbers were observed in the treatment T₁₀ (6.48) where as the lowest number (4.05) of tillers were recorded in control (T₁). The highest plant height was observed in the treatment T₄ and the lowest plant height was found in the control treatment (T₁). The length of ear ranged from 14.42 to 19.98 cm (Plate 10) where the highest ear length was found in T₈ (19.98cm) and the lowest ear length was found in control (T₁) treatment. The treatments T₂, T₃, T₄, T₉ and T₁₀ resulted statistically similar ear length and differed significantly from untreated control (T₁).

The distance between the point of the flag leaf initiation and the ear ranged from 13.00 to 15.62 where the highest (15.81cm) and the lowest (13.00) distances were observed under the treatments T₃ and T₁, respectively. Considering the number of spikes/plot, the highest number of spikes was in the treatment T₉ (513.66) and the lowest counts was observed in the T₁₀ treatment (379.33). It has been found that the number of spikelets/ear ranged from 15.17 to 19.28 where the highest and the lowest counts were made under the treatments T₂ and T₁, respectively.

Table 5. Efficacy of seed treatments with plant extracts on number of tillers/ plant, plant height, ear length, distance between the point flag leaf initiation and base of ear number of ears/plot and number of spikelets /ear

Treatments	Number of tillers/ plant	Plant height (cm)	Ear length (cm)	Distance between the point of flag leaf initiation and base of ear (cm)	Number of spikes / plot	Number of Spikelets /ear
T ₁ = Control	4.053e	81.84	14.42c	13.00	500.66	15.17d
T ₂ = Vitavax-200 (0.3%)	4.920b-e	82.47	15.90bc	14.80	485.00	19.28a
T ₃ = Onion extract (1:2 w/v)	4.833c-e	85.77	15.00bc	15.81	499.66	17.28c
T ₄ = Garlic extract (1:2 w/v)	5.843ab	87.87	16.78b	15.62	465.66	18.43ab
T ₅ = Kalizira extract (1:2 w/v)	5.123b-d	84.00	14.76c	13.73	492.66	18.19b
T ₆ = Zinger extract (1:2 w/v)	5.070b-d	84.20	14.85c	14.04	445.00	18.33b
T ₇ = Allamanda extract (1:2 w/v)	4.343de	84.86	14.59c	15.43	457.66	18.32b
T ₈ = Bishkatali extract (1:2 w/v)	5.337bc	85.47	19.98a	14.62	484.00	18.91ab
T ₉ = Neem extract (1:2 w/v)	4.467c-e	84.24	15.80bc	14.56	513.66	18.37b
T ₁₀ = Mehedi extract (1:2 w/v)	6.480a	82.56	15.99bc	14.70	379.33	17.29c
LSD (P = 0.05)	0.9472	NS	1.846	NS	NS	0.8877

NS = Not Significant

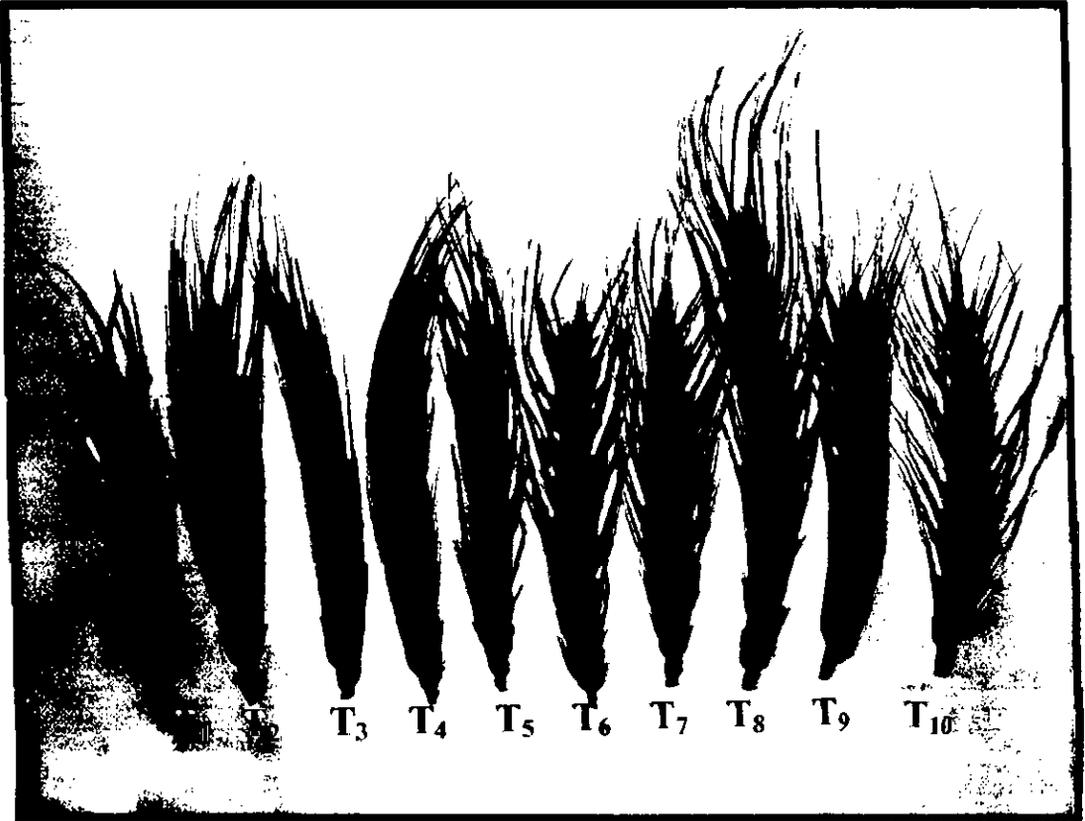


Plate 10. Length of ear under different treatment.

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

The treatments effects were found to differ significantly in respect of grain formation as well as grain weight of wheat cv. Kanchan (Table 6). The number of grains/ear ranged from 37.67 to 43.98, where the highest and the lowest number of grains/ear were recorded under the treatments T₂ and T₁. The treatments T₂, T₄, T₆, T₈ and T₉ were resulted similar effect regarding number of grains/ear. The number of healthy grains/ear ranged from 34.90 to 42.88 where the highest and the lowest counts were made under the treatments T₂ and T₁, respectively. The treatments T₂, T₄, T₆, T₈ and T₉ resulted statistically similar effect regarding healthy grains/ear. On the other hand the highest number of diseased grains/ear (2.74) was recorded under the treatments T₁. Seed treatment with botanicals resulted significantly lower number of diseased grains/ear. The treatment T₂ (Vitvax-200) and T₄ (Garlic extract) gave statistically similar result regarding number of diseased grains/ear. Considering weight of grains/ear, it has been found that the highest (1.88g) and the lowest (1.18g) weight of grains/ear were obtained under the treatments T₂ and T₁, respectively. Among the botanicals, the maximum weight of grains/ear (1.69g) was found in seeds treated with Garlic extract (T₄) which was statistically similar to the result obtained by treating seeds with Vitavax-200 (T₂).

The weight of healthy grains/ear ranged from 1.84 to 1.03 where the highest and lowest counts were made under the treatments T₂ and T₁, respectively. The treatments T₂, T₄, and T₈ resulted statistically similar effect regarding weight of healthy grains/ear. On the other hand, the highest weight of diseased grains/ear (0.15) was recorded under the treatments T₁. Seed treatment with botanicals resulted significantly lower weight of diseased grains/ear. The treatment T₂ (Vitvax-200) and T₄ (Garlic extract) and T₈ (Bishkatali extract) gave statistically similar resulted regarding weight of diseased grains/ear.

Table 6. Efficacy of seed treatment with plant extracts on grain formation and grain weight/ear of wheat cv. Kanchan

Treatments	Number of grains /ear	Number of healthy grains /car	Number of diseased grains /ear	Weight of grain/ ear (g)	Weight of healthy grains/ ear (g)	Weight of diseased grains/ ear (g)
T ₁ = Control	37.67d	34.90e	2.74a	1.18d	1.03e	0.15a
T ₂ = Vitavax-200 (0.3%)	43.98a	42.88a	1.10c	1.88a	1.84a	0.04d
T ₃ = Onion extract (1:2 w/v)	39.64b-d	38.79b-d	1.60b	1.44b-d	1.35b-d	0.08b-d
T ₄ = Garlic extract (1:2 w/v)	43.43a	41.91ab	1.55bc	1.69ab	1.63ab	0.05d
T ₅ = Kalizira extract (1:2 w/v)	39.56b-d	35.72de	1.74b	1.37cd	1.24de	0.012a-c
T ₆ = Zinger extract (1:2 w/v)	42.96ab	41.28a-c	1.70b	1.49bc	1.41b-d	0.07b-d
T ₇ = Allamanda extract (1:2 w/v)	38.98cd	37.10de	1.88b	1.46bc	1.39b-d	0.074cd
T ₈ = Bishkatali extract (1:2 w/v)	43.93a	42.27ab	1.67b	1.61a-c	1.55a-c	0.06d
T ₉ = Neem extract (1:2 w/v)	42.41a-c	40.61a-c	1.72b	1.57bc	1.50b-d	0.070b-d
T ₁₀ = Mehedi extract (1:2 w/v)	39.89b-d	38.27c-e	1.62b	1.42b-d	1.29c-e	0.13ab
LSD (P = 0.05)	3.434	3.485	0.4562	0.2740	.2801	0.05841

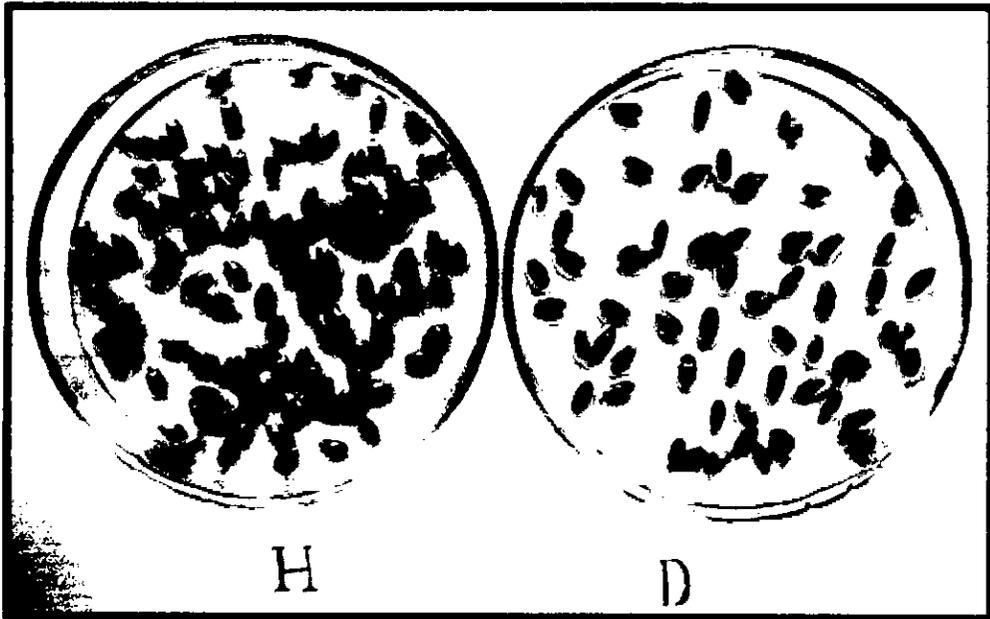


Plate 11: Healthy seeds (H) and Diseased seeds (D) of wheat

4.2.7. Effect of seed treatment with plant extract on the formation of grains of different grades (0-5 scale) of wheat cv. Kanchan

Seed were graded in 0-5 rating scale where zero indicates healthy seeds and 5 indicate maximum diseased symptom showing seeds. The treatment was found to show significant effect on formation of grade 0 grains /ear where the highest (42.88) and lowest (34.90) counts were made under the treatments T₂ and T₁, respectively.

In case of grade 1, the highest number of grains/ear (1.29) was found in treatment T₁ which was followed by T₉ (0.99), T₆ (0.96), T₁₀ (0.95), T₇ (0.94), T₃ (0.92), T₅ (0.88) T₄ (0.85) and T₈ (0.85). The lowest number of grade 1 grains/ ear was observed in the treatment T₂ (0.64).

In case of grade 2, the highest number of diseased grains/ ear was observed in the treatment T₁ but the lowest disease grains of grade 2 seeds were found in the treatment T₆. Treatments T₂, T₄ and T₁₀ resulted statistically similar effect on regarding grade-2 grain/ear.

Considering grade 3 grains/ear the different treatments showed remarkable variation. Maximum grade 3 seeds were found in T₈ (0.28) and the lowest in T₄. In grade 4, diseased seeds did not found in the treatment T₅. But the highest disease grains/ ear were found in the T₄ treatment.

In case of grade 5, there were no diseased grains/ear were found in the treatment T₂, T₄, T₆, T₈ and T₁₀. On the other hand the highest diseased grain/ ear of grade-5 were found in treatment T₇ (0.19).

Table 7. Effect of seed treatment with plant extracts on the formation of grains of different grades (0-5 scale) of wheat cv. Kanchan.

Treatments	Number of grains/ear under different grades					
	0	1	2	3	4	5
T ₁ = Control	34.90 e	1.29 a	0.66 a	0.27 b	0.33 b	0.17 ab
T ₂ = Vitavax-200 (0.3%)	42.88 a	0.64 f	0.38 bc	0.03de	0.06 cd	0.00 b
T ₃ = Onion extract (1:2 w/v)	38.79 b-d	0.92 b-d	0.45 b	0.06 d	0.00 d	0.013ab
T ₄ = Garlic extract (1:2 w/v)	41.91 ab	0.85 de	0.39 bc	0.00 e	0.58 a	0.00 b
T ₅ = Kalizira extract (1:2 w/v)	35.72 de	0.88 c-e	0.46 b	0.26 b	0.00d	0.13 ab
T ₆ = Zinger extract (1:2 w/v)	41.28a-c	0.96 b	0.09 d	0.37 a	0.25 bc	0.00 b
T ₇ = Allamanda extract (1:2 w/v)	37.10 de	0.94 bc	0.34 c	0.16 c	0.32 b	0.19 a
T ₈ = Bishkatali extract (1:2w/v)	42.27 ab	0.85 de	0.36 c	0.28 b	0.18 b-d	0.00 b
T ₉ = Neem extract (1:2 w/v)	40.61 bc	0.99 b	0.36 c	0.01de	0.25 bc	0.06ab
T ₁₀ = Mehedi extract (1:2 w/v)	38.27 de	0.95 bc	0.39 c	0.25 b	0.05 cd	0.00 b
LSD (P = 0.05)	3.485	0.08260	0.086	0.0584	0.2023	0.1847

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

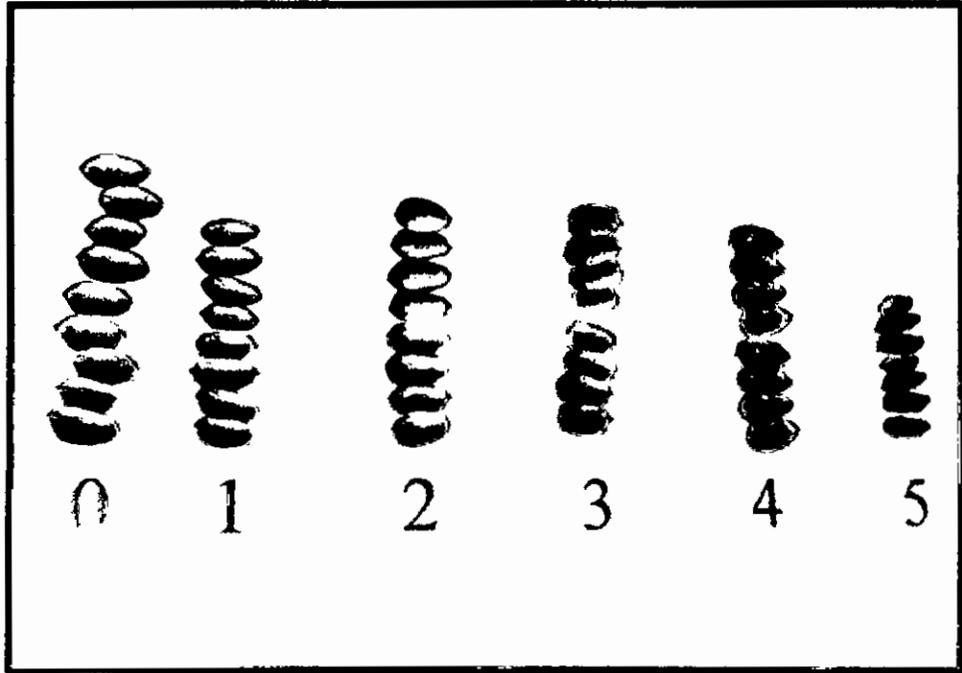


Plate 12: Grading of wheat seeds (0-5 scale)

4.2.8. Efficacy of seed treatment with plant extract on yield of wheat cv.

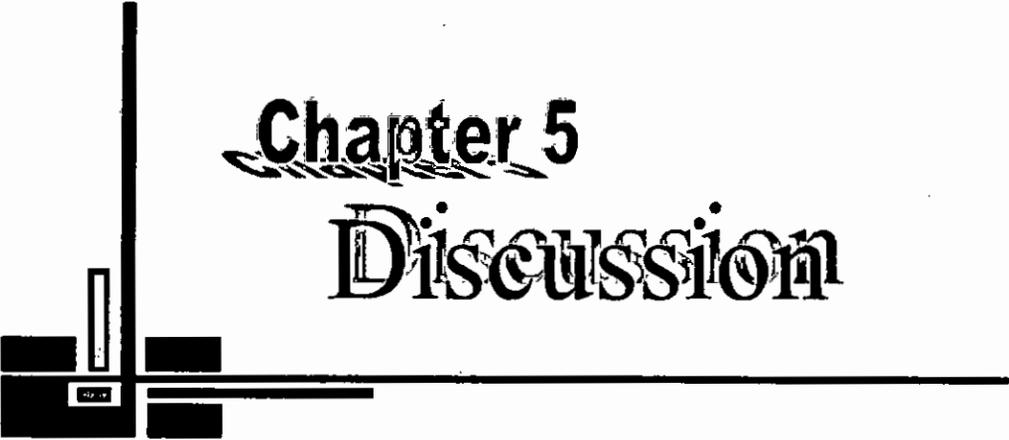
Kanchan

It has been found that the effect on 1000 grain weight, grain yield and straw yield differed significantly while seed were treated treating seeds with botanicals (Table 8). The 1000 grain weight of wheat ranged from 31.63 to 41.36g. The highest weight of 1000 grains (41.36g) was recorded in T₂ treatment. The lowest weight of 1000 grains (31.63g) was recorded in the control treatment. All the treatments except untreated control (T₁) resulted statistically similar effect on 1000 grain weight.

Straw yield under different treatments ranged from 4.33 t/ha to 6.85 t/ha where the highest and lowest straw yield was obtained under the treatments T₂ and T₁₀, respectively. Among the botanicals, seed treated with garlic extract (T₄) resulted the highest straw yield over untreated control. Considering grain yield (kg/plot and t/ha) it has been found that the treatment T₂ (Vitavax-200) resulted the highest (3.52 t/ha) grain yield which was statistically similar to that of T₃, T₄, T₅, T₆, T₈ and T₉. From the present study it has been found that seed treatment with Bishkatali extract (T₈) resulted 29.74% increased grain yield over untreated control.

Table 8. Efficacy of seed treatment with plant extracts on yield of wheat cv. Kanchan.

Treatments	1000 grain weight (g)	Straw yield (kg/plot)	Straw yield (t/ha)	Grain yield (kg/plot)	Grain yield (t/ha)	% increased (+) or decreased (-) of grain yield over control
T ₁ = Control	31.63b	1.11d	5.62d	0.54e	2.70cd	
T ₂ = Vitavax-200 (0.3%)	41.36a	1.37a	6.85a	0.70a	3.52a	30.70(+)
T ₃ = Onion extract (1:2 w/v)	36.43ab	1.21cd	6.06cd	0.64b-d	3.38ab	25.44 (+)
T ₄ = Garlic extract (1:2 w/v)	40.00a	1.36a	6.83a	0.69a-c	3.49ab	29.40 (+)
T ₅ = Kalizira extract (1:2w/v)	34.76ab	1.00e	5.02e	0.63d	3.19ab	18.22 (+)
T ₆ = Zinger extract (1:2 w/v)	34.80ab	1.26bc	6.42a-c	0.63d	3.19ab	18.44 (+)
T ₇ = Allamanda extract (1:2w/v)	37.80ab	1.25bc	6.27bc	0.62d	3.11bc	15.259(+)
T ₈ = Bishkatali extract (1:2 w/v)	40.83a	1.35ab	6.76ab	0.70ab	3.50ab	29.74 (+)
T ₉ = Neem extract (1:2w/v)	37.10ab	1.19cd	5.99cd	0.64cd	3.20ab	18.59 (+)
T ₁₀ = Mehedi extract (1:2 w/v)	35.53ab	0.86f	4.33f	0.52e	2.61d	3.33 (-)
LSD (P = 0.05)	7.853	0.102	0.490	0.0541	0.409	



Chapter 5

Discussion

5. DISCUSSION

5.1. Laboratory experiment

In the present study the highest seed germination (92 %) was recorded under the treatment T3 (Kalizira extract) followed by Neem extract (T9), Onion extract (T3) and Ginger extract (T6). Considering the incidence of seed yielding *Bipolaris sorokiniana*, it has been found that the treatment T8 (Biskatali extract) resulted the highest reduction in incidence of *Bipolaris sorokiniana* on wheat seeds. The present findings were well supported by the reports of Shami *et al.* (1986); Alice and Rao (1987); Fakir and Khan (1992); Khan and Kumar (1992); Hossain and Schlosser (1993); Khan and Fakir (1995) and Hossain *et al.* (1997).

Asrafuzzaman *et al.* (1992) reported that Bishkatali (*Polygonum hydropiper*) extract inhibited the mycelial growth and spore germination of *Rhizoctonia solani* effectively. He also reported that Neem seed extracts was found effective against *Bipolaris sorokiniana* and the extract inhibited the growth of the fungus in wheat seeds and also reduced its pathogenecity on wheat leaves. Hossain and Schlosser (1993) reported that germination rate of wheat seeds increased after treatment with extract of Neem seed and Cake. Neem oil also inhibited the growth of *Alternaria alternata*.

Hossain *et al.* (1997) found that the plant extract of *Allium sativum* and *Lawsonia alba* showed remarkable effect in controlling the spore germination and mycelial growth of *Bipolaris sorokiniana*. Shami *et al.* (1986) reported that the garlic extract inhibited spore germination and reduced the mycelial growth of *Fusarium*

oxysporum in vitro. Hossain *et al.* (2005) reported that Neem extract reduced the incidence of *Bipolaris sorokiniana* significantly and increased seed germination. He also found that out of 6 plant extracts, Neem extract was proved superior followed by Garlic, Bishkatali and Vatpata. Fakir and Khan (1992) found that garlic bulb extract was effective in controlling seed-borne fungal pathogen of jute such as *Macrophomina phaseolina* and *Fusarium spp.* on seed treatment. Alice and Rao, (1987) evaluated plant extracts against seed-borne infection of fungus and found increased germinability of the treated seeds. Khan and Kumar (1992) also observed the antifungal activity of leaf extract of Neem (*Azadirachta indica*) with different dilutions against seed mycoflora of wheat. They recorded remarkable reduction of seed mycoflora and increased seed germination of treated seeds.

5.2. Field experiment

It has been found that garlic extracts (T4) and Biskatali extracts (T8) resulted remarkable reduction of leaf spot severity over untreated control in flag leaf stage, flowering stage, milking stage and hard dough stage. The antifungal activity of botanicals as seed treating agent have been reported by Alice and Rao (1987); Rovesti *et al.* (1992); Hossain and Schlosser (1993); Hossain *et al.* (1997); Rahman (1998a); Khan and Hossain (1993); Arun *et al.* (1995); Bisht and Khulbe (1995). The finding of the present study is also supported by Rahman (1998b) who found a remarkable reduction of leaf blight severity of wheat by spraying botanicals such as *Allium sativum*, *Nigella sativa*, *Lawsonia alba* and *Cymbopogon citrus*.

Hossain and Schlosser (1993) observed the potential use of Neem (*Azadirachta indica*) extract as a means of controlling *Bipolaris sorokiniana* of wheat. They also reported that the Neem extract inhibited the growth of *Bipolaris sorokiniana* in wheat seeds and reduced its pathogenicity on wheat leaves. Hossain *et al.* (1993) evaluated that extracts of *Lawsonia alba*, *Ipomoea fistulosa*, *Allium sativum* and *Leucas aspera* against *Rhizoctonia solani* and *Bipolaris sorokiniana* where, *A. sativum* completely inhibited the mycelial growth at dilution ratio of 1:4 (w/v). Hossain *et al.* (1997) also reported 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. Khan and Hossain (1993) observed 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. Hossain *et al.* (1995) further reported that extract of Mehedi (*Lawsonia alba*) was found to be effective against *Bipolaris sorokiniana*. Arun *et al.* (1995) found that the extract of garlic bulb was effective in suppressing radial growth of the pathogens *Fusarium sp.* and *Sclerotium sp.* Bhisht 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.

It was found that the application of plant extracts did not show significant effect on plant height, distance between the point of flag leaf initiation and base of ear and number of spikes / plot. But the application of plant extracts as seed treatment showed significant effect on the number of tillers / plant, ear length and spikelets / ear. The highest plant height was observed in the seed treated with Garlic (*Allium*

sativum) extract treated seeds. The lowest plant height was observed in control treatment. The 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 in 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*. Grain yield, tiller and grain number but not grain weight decreased and disease severity increased.

From the present study, it has been found that number of grains/ear and number of healthy grains/ear were increased by treating seeds with plant extracts. Garlic, Zinger, 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. The findings of the present study corroborates with the findings of Rahman (1998a). He found 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.

In the present study, the lowest number of diseased grains/ear was found in the chemical treatment with Vitavax-200 (0.3 %) which was statistically similar with Garlic extract. This result is also supported by Rahman (1998b).

Considering 1000-grain weight (g), seed treatment with plant extracts resulted statistically similar effect as of seed treatment with Vitavax-200. The result is also supported by Rahman (1998a). He found increased 1000- grains weight over control by treating wheat seeds with Garlic extract. In another study Rahman

(1998b) further reported that the extracts of *Cymbopogon citratus*, *Lawsonia alba*, *Nigella sativa* and *Allium sativum* increased 1000-seed weight of wheat over control.

The highest grain yield (3.52 t/ha) was recorded under the treatment Vitavax-200 which was statistically similar to the use of extracts of Onion (3.38 t/ha), Garlic (3.49 t/ha), Kalizira (3.19 t/ha), Zinger (3.19 t/ha), Bishkatali (3.50 t/ha) and Neem (3.20 t/ha). From the present study it has been found that seed treatment with Bishkatali extract increased 29.74 % grain yield over untreated control. These findings agreed with the findings of Rahman (1998b) who found 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.

From the above findings of the study it is well exposed that Vitavax- 200 is very effective in controlling seed-borne *Bipolaris sorokiniana* and leaf spot in the field. Among the botanicals, Garlic extract and Bishkatali extract was found promising in reducing the incidence of seed-borne *Bipolaris sorokiniana* as well as controlling leaf spot in the field with the increase in yield of wheat.



Chapter 6

Summary and Conclusion

6. SUMMARY AND CONCLUSION

Wheat (*Triticum aestivum* L.) is the second most important cereal crop next to rice in Bangladesh. Wheat suffers from as many 26 seed-borne pathogen causing 14 diseases (Fakir, 1999). Leaf blight which caused by *Bipolaris sorokiniana* is a common and devastating disease of wheat in Bangladesh.

The present experiment was conducted at farm of the Sher-e-Bangla Agricultural University, Dhaka 1207 during Rabi season of 2005-2006 experimental seasons to study the efficacy of plant extracts on leaf spot (*Bipolaris sorokiniana*) and grain yield of wheat.

The highest seed germination (92 %) was recorded under the treatment T₃ (Kalizira extract) followed by Neem extract (T₉), Onion extract (T₃) and Ginger extract (T₆). The incidence of seed-borne *Bipolaris sorokiniana*, was found lowest under the treatment T₈ (Biskatali extract).

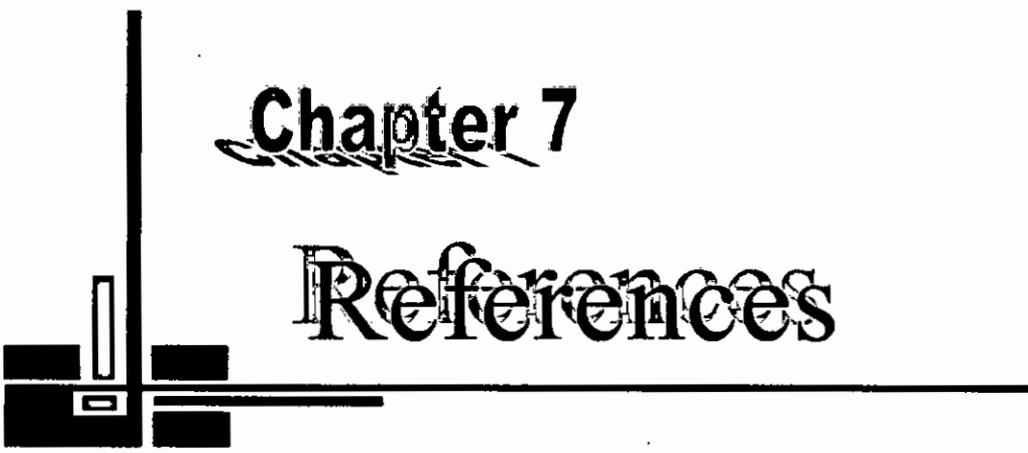
✓ A remarkable reduction of leaf spot severity over untreated control in flag leaf stage, flowering stage, milking stage and hard dough stage was achieved by seed treatment with garlic extracts (T₄) and Biskatali extracts (T₈).

Seed treatment with plant extracts did not show significant effect on plant height, distance between the point of flag leaf initiation and base of ear and number of spikes/plot. But plant extracts have been found promising on the number of tillers /plant, ear length and spikelets/ ear. The highest plant height was observed in the seed treated with Garlic (*Allium sativum*) extract whereas the lowest plant height was observed in untreated control. The influence of

plant growth in case of spraying garlic extract might be due to the increased of nitrogen uptake efficiency as well as protein quality resulting in positive effect in vegetative growth such as stem elongation. Number of grains/ear and number of healthy grains/ear were increased by treating seeds with plant extracts. Garlic, Zinger, 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. The lowest number of diseased grains/ear was found under the treatment T₂ (Vitavax-200 @ 0.03 %) which was statistically similar with Garlic extract.

Considering 1000-grain weight (g), seed treatment with plant extracts resulted statistically similar effect as of seed treatment with Vitavax-200. The highest grain yield (3.52 t/ha) was recorded under the treatment Vitavax-200 (T₂) which was statistically similar to the treatments Onion (3.38 t/ha), Garlic (3.49 t/ha), Kalizira (3.19 t/ha), Zinger (3.19 t/ha), Bishkatali (3.50 t/ha) and Neem (3.20 t/ha). Seed treatment with Bishkatali extract increased 29.74 % grain yield over untreated control.

From the findings of the study it is well exposed that Vitavax- 200 is very effective in controlling seed-borne *Bipolaris sorokiniana* and leaf spot severity in the field. Garlic extract and Bishkatali extract was also found effective in reducing the incidence of *Bipolaris sorokiniana* as well as leaf spot severity in the field. However more investigation needs to be carried out in different Agro-ecological zones for confirmation of the fitness.



Chapter 7

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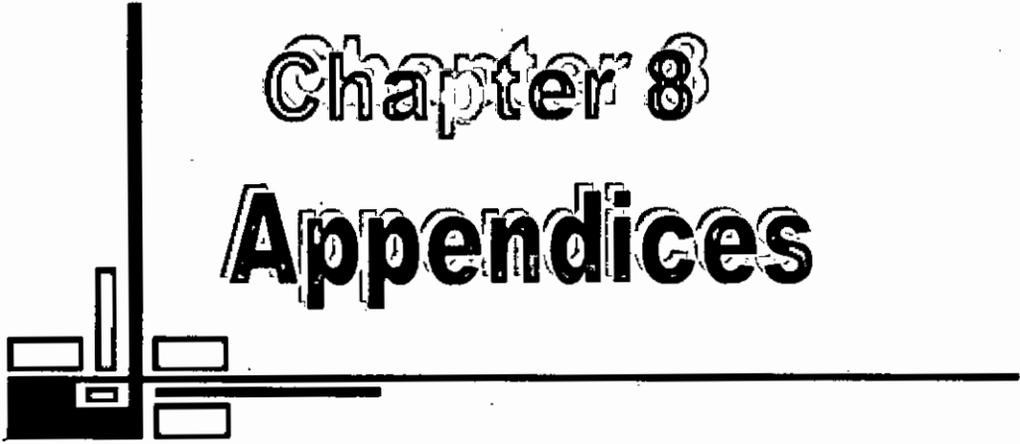
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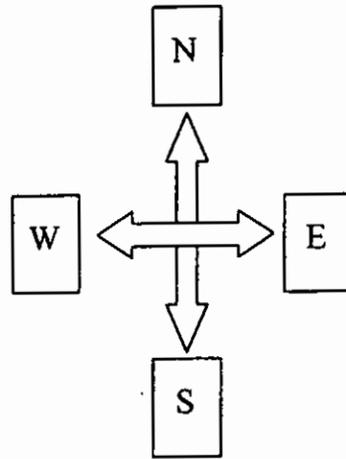
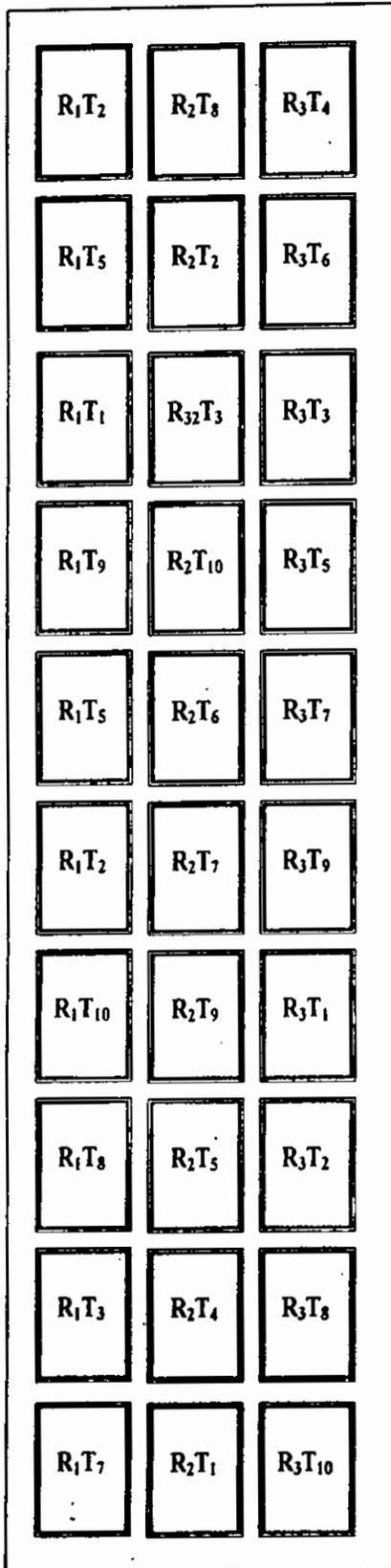
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Chapter 8

Appendices



8. APPENDICES



Plot size: 2m x 1m (2 m²)
 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 experiment site during the period from November 2005 to April 2006

Year	Month	Air temperature (^o c)			Relative humidity (%)	Rain fall (mm)	Sun shine (hr)
		Maximum	Minimum	Mean			
2005	November	29.5	18.6	24.0	69.5	0.0	233.2
	December	26.9	16.2	21.5	70.6	0.0	210.5
2006	January	24.5	13.9	19.2	68.5	4.0	194.1
	February	28.9	18.0	23.4	61.0	3.0	221.5
	March	32.14	22.39	27.27	66.67	0155	216.4
	April	34.44	24.23	29.34	67.66	0091	253.4

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

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