EFFECT OF MANUAL SEED SORTING, SEED SOLARIZATION AND SEED TREATMENT WITH VITAVAX AND HOT WATER ON LEAF SPOT (*Bipolaris sorokiniana*) AND GRAIN YIELD OF WHEAT

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CERTIFICATE

This is to certify that the thesis entitled "**EFFECT OF MANUAL SEED SORTING, SEED SOLARIZATION AND SEED TREATMENT WITH VITAVAX AND HOT WATER 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 bonafide research work carried out by **Registation No.24912/00407**, under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

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

Dated: 30.08.06
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Dr. F. M. Aminuzzaman
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DEDICATED
TO MY
BELOVED PARENTS

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ABSTRACT

In vitro and in vivo experiment were carried out to determine the effect of manual seed sorting, seed solarization and seed treatment with Vitavax and hot water on leaf spot (*Bipolaris sorokiniana*) and grain yield of wheat during the period from November-2005 to April, 2006 at the farm of Sher-e-Bangla Agricultural University, Dhaka. The experiment was laid out in the Randomized Complete Block Design (RCBD) with three replications. The highest reduction of incidence of seed-borne *Bipolaris sorokiniana* was obtained by manually sorted healthy looking seeds treated with Vitavax-200 (T_4). Significant reduction of incidence of same fungus was achieved by treating seeds with hot water and solar heat. Treatment of apparently healthy seed with Vitavax-200 (0.4%) was found best in reducing the leaf infection, increasing seed germination and increased seed yield which was followed by treatment of unclean seeds with Vitavax-200 (0.4%). Out of four physical seed treatment methods, solar heat treatment of apparently healthy seeds was found to be the best in reducing leaf infection, increasing seed germination and seed yield. Apparently healthy seed treated with hot water increased seed germination, seed yield and also reduced the leaf infection. Apparently healthy seeds showed more or less similar performances to that of apparently healthy seed treated with hot water. The rest of the treatments have some remarkable effect in controlling the disease and increasing seed yield of wheat.

CHAPTER 1

INTRODUCTION

1. INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most important cereal crops in the world. It is the second most staple food crop next to rice in Bangladesh that plays a vital role in the national economy largely by reducing the volume of import of cereals (Razzaque *et al.*,1992). The average yield of wheat in our country is 2.23 tons/ha and it is too low 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). About 642.1 thousand hectares of land was covered by wheat cultivation with the annual production of 1253 thousand tons (1.95 t/ha) in Bangladesh (BBS, 2005).

Many factors are associated with the lowering of yield of wheat in Bangladesh. Among them disease is the most important one. Wheat plants at all growth stages are prone to the attack of numerous diseases. The crop is known to suffer from as many as 200 diseases of which the most important and damaging one's are seed borne (USDA, 1960). Seed borne infections of fungal pathogens are important not only due to the association with the seeds that cause germination failure, and/or causing disease to the newly emerged seedlings or growing plants, but also contaminate the soil by establishing its inocula permanently. Wheat suffers from as many as 26 seed borne pathogens causing 14 seed borne diseases. Among them leaf spot and black point caused by *Bipolaris sorokiniana* has become a serious concern in the recent years in Bangladesh (Azhar *et al.*,1972 ; Fakir, 1988).

A rough estimate shows that an annual crop loss (including storage loss) of TK.1400 millions is occurred due to seed borne diseases in Bangladesh (Fakir, 2000). The yield loss in wheat due to leaf blight/leaf spot/leaf blotch disease has been reported to be 20% in Sonalika, whereas 14% and 8% in Akbar and Kanchan, respectively (Razzaque and Hossain, 1991). In farmers field in 1995, the yield loss was estimated 14.97% (Alam *et al.*, 1995) whereas up to 29% yield reduction was estimated during 1991-1992 in Kanchan (Alam *et al.*, 1994). Rashid and Fakir (1998) estimated yield reduction of wheat due to *Bipolaris sorokiniana* as high as 57.6% and 64.5% in cvs. Kanchan and Sonalika, respectively at maximum disease incidence. In case of severe attack it may result even 100% yield loss (Hossain and Azad, 1994).

The most acceptable method for controlling of this disease is sowing of pathogen free seeds. Therefore along with routine seed health testing, seed treatment before sowing is necessary. Treatment of seed with seed-dressing fungicides was found to improve germination and decrease infection of seedling growth from the black pointed seeds. Indiscriminate using of chemicals are creating health hazard and environment pollution. Use of alternate methods instead of seed treating chemicals is of great concern now a days to save our environment.

Therefore, it is judicious to explore less expensive, less risky non-chemical components to treat seeds for freedom from the seed-borne pathogens. In this perspect, use of solar heat, seed treatment with hot water and manual seed sorting to obtain apparently healthy seed in controlling seed borne fungal pathogen.

Considering this view, attempt has been taken to evaluate the efficacy of manual seed sorting, seed solarization and seed treatment with Vitavax and hot water on leaf spot (*Bipolaris sorokiniana)* and grain yield of wheat with the following objectives:

1) To determine the effect of manual seed sorting, seed solarization and seed treatment with Vitavax-200 and hot water in controlling leaf spot (*Bipolaris sorokiniana)* of wheat in the laboratory.

2) To evaluate the effect of manual seed sorting, seed solarization and seed treatment with Vitavax-200 and hot water on leaf spot severity (*Bipolaris sorokiniana)* of wheat in the field.

3) To determine the effect of manual seed sorting, seed solarization and seed treatment with Vitavax-200 and hot water on yield and yield contributing characters of wheat.

CHAPTER 2

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1. Physical seed treatment by cleaning and sorting

Hossain and Doullah (1998) reported that seed cleaning and seed washing of farmer's seed reduced the seedling disease and increased yield up to 53.87% and 14.77%, respectively over the unclean farmers saved seed.

Kalknnavar *et al.* (1989) graded wheat seeds into 4 catagories (a) 2.782.39; (b)2.38-1.97; (c) 1.98-2.37 (d) 1.58-1.97 mm length. Percent germination in seeds of (a) and (b) grades were similar and higher than grade (c) and (d), Percent germination was higher in the heavy seeds than in light seeds. Root length and seedling dry weight decreased with the decreasing of seed size. Heavy seeds were superior to light seeds in seedling dry weight and vigour index.

Mia *et al.* (2000) reported that rice seed treated with Vitavax-200 showed the best performance followed by manual seed sorting against *Bipolaris oryzae*. Significant reduction of brown spot and seedlings with lesion in coleoptile was noted in Vitavax treated and and manually sorted seed. Seed cleaning also increased the number of tiller and effective tiller/hill significantly.

2.2. Seed Treatment with chemicals

In controlling seed borne infection of *Helminthosporium sativum, Bipolaris sorokiniana* and *Fusarium* spp., Pidoplichko and Andreeva (1980)

Hyder-Ali and Fakir (1993) conducted an experiment by treating seed with Dithane M-45, Granosan M, Homai 80 WP, Panoctine CG/450, Vitavax-200 and Vitavax-300 to control seed borne fungi of wheat. They observed that all the fungicides reduced seed borne infection of *Alternaria tenuis, Aspergillus flavus, Bipolaris sorokiniana, Curvularia lunata* and *Fusarium semitectum* but complete control of *A. tenuis, B. sorokiniana, C. lunata* and *F. semitectum* were obtained by Vitavax-200,Vitavax-300 and Panoctine CG/450 when used @ of 0.5% of seed weight. Vitavax-200 and Dithane M-45 increased germination of seeds.

Uddin (2005) reported that seed borne pathogens were significantly reduced by treating seeds with Vitavax-200 followed by garlic extract, brine solution, hot water and physically sorted seeds in Lentil. The highest reduction of seed borne fungal flora were observed in case of Vitavax-200 followed by garlic extract, brine solution, hot water and physically sorted seeds. In the field condition, germination percentage was higher in physically sorted seeds.

Hossien (2002) reported that farmer's clean seed, washed farmer's seed, washed clean seed and seed treated with Vitavax-200 increased 16.62%, 16.45%, 23.39% and 26.6% grain yield, respectively over farmer's saved seeds of rice (BR 11).

2.3. Seed treatment with hot water

Resting plant tissues like seeds and pathogens on seed are sensitive to humid heat. It is possible to develop a seed treatment based on the heat giving to the seed borne pathogens without affecting the seed vigor. This has been materialized in the traditional hot water treatment by soaking the seeds into hot water. By the correct combination of time and temperature, this treatment has effect on all known pathogens in cereals (Piorr, 1991,Winter *et al.* 1994, Nielsen *et al.* 2000).

Willium Nesmith (2003) at Ohio State University found hot water treatment effective against the major seed borne diseases of vegetables. He found effective temperature of 122°F (49.95°C) for 25 min for brussels sprouts, cabbage, eggplant, tomato and spinach; 122°F (49.95°C) for 20 min for broccoli, cauliflower, Chinese cabbage, carrot, kale, kohlrabi and turnips; 122°F (49.95°C) for 1-5 min for mustard and radish; 125°F (51.6°C) for 30 min for peppers and 118°F (47.73°C) for 30 min for lettuce and celery.

Gaur (2003) evaluated twenty one fungicides combined with hot water treatment in the field against seed borne inoculums of *Ascochyta rabiei* in chickpea. Four-hour seed dip in 0.2% thiabendazole solution significantly controlled seed-borne infection of *A. rabiei* with no deleterious effect on germination (88.6%). This treatment gave minimum number of diseased plant (2.9%) at flowering stages.

Jiskani (2002) reported that the brown spot or blight of rice is a much more wide spread and a common disease in almost all rice growing area of the world. He prescribed that brown spot or blight of rice caused by *Helminthosporium oryzae* effectively controlled by hot water seed treatment at 54° C for 10 minutes.

Fallik *et al.* (2002) studied the effectiveness of a short pre-storage hot water rinsing and brushing on resistance to decay development and chilling injury on pink tomato cv. 189 fruit that were kept for 15 days at 5 or 12° C plus three days at 22°C. He suggested the alternative method of a very short (15 S) HWRB (Hot Water Rinsing & Brushing) at 52°C for desirable tomatoes. This treatment extended storability well over three weeks at 5°C by minimizing CI (Chilling Injury) and enhancing resistance against pathogen during storage.

Sadek *et al.* (2001) stated that hot water treatment at 10°C for 10 minutes with potassium permanganate (1%) or copper sulfate (1%) application effectively controlled the pathogen in infected seeds of tomato, tobacco, cowpea, bean and pepper. By this treatment irregular necrotic spots were overcome finely.

Muniz (2001) stated that the dry heat treatment on the control of seed transmitted pathogens and its effects on the viability of tomato seeds treated at 70°C for 12 days eradicated fungi associated with tomato seeds. But in hot water treatment at 50°C for 30 minutes under laboratory research the associated fungi in tomato seeds were eradicated.

Winter *et al.* (2001) stated that the incidence of common bunt *(Tilletia caries)* in winter wheat was strongly reduced by a seed treatment with skim milk powder and warm water. The combined seed treatment with warm water at 45°C for 2hours and skim milk powder (160 g/litre water) controlled the seed-borne infection of *Tilletia caries* (common bunt), *Garlachia nivalis*, (Snowmould), *Fusarium graminearum* and *Septoria nodorum* (damping off) in winter wheat.

Nega *et al.* (2000) stated that five important vegetable crops (carrot, cabbage, celery, parsley lamb's lettuce) and their most important seed-borne pathogens (*Alternaria* spp., *Phoma* spp., *Septoria* spp., *Xanthomonas* spp., *Peronspora valerianellae*) have been investigated in laboratory with hot water treatments at 40°C & 50°C to 55°C for 10 to 30 min, in some cases to 60 min and found no infected seeds from those vegetables. Seed-borne pathogens could be reduced without significant losses of germination by hot water treatments at 50°C for 20 to 30 min up to 53°C for 10 to 30 min.

Satvinder and Kahur (2000) reviewed some physical techniques such as dry heat, hot water, solar heat, washing, radiation, microwave treatment, ultrasonic waves and forced air circulation for the management of plant disease including post harvest disease.

Karunaratne (1999) reported that the effect of hot water treatments (different temperature, time combination) of tomatoes, cucumbers and *Momordica charantia* (55°C for 1 min), *Capsicum annuum* (Chillis), carrots(50°C for 1 min), *Phaseolus vulgaris* (50°C for 30S) and okras (52°C for 30S) on the shelf life of each commodity at room temperature, (27±3°C) and relative humidity (65±5%). No disease symptoms was developed from the treated seeds.

Hermansen *et al.* (1999) studied the effect of hot water treatments of carrot seeds on seed-borne fungi, germination, emergence and yield treatment at 44°C to 59°C for 20 min was employed for controlling seed-borne pathogen *Alternaria dauci.* Hot water treatment of carrot seeds at 44, 49 and 54°C generally improved germination of infected seeds and reduced the incidence of *Alternaria dauci.* They recommended hot water treatment as an alternative to fungicides was used to eradicate seed-borne pathogens in carrots in organic farming system.

Fallik *et al.* (1999) stated that hot water treatment qualified sweet pepper in storage condition after treating with 55±1°C for 12±2 S. This treatment significantly improved the general appearance of the fruits, reduced decay and maintained fruit firmness. The respiration rate of rinsed and cleaned fruits was significantly lower than that of untreated fruits during storage and shelf-life simulation.

Lurie *et al.* (1998) studied a pre-storage dry heat treatment and a hot water dip at 30°C for 48 to 72 h and 50 to 53°C for 2 to 3min, respectively for reducing storage rots on capsicum bell peppers and tomatoes. Under these conditions *in vitro* germination and growth of *Alternaria alternata* and *Botrytis cinerea* were weakened or prevented.

Ranganna *et al.* (1998) stated that hot *water treatment at* 57.5°C for 20-30 min for controlling storage pathogen like *Fusariun solani and Erwinia carotovora* was effectively done for potatoes.

Khaleduzzaman (1996) studied hot water treatment of wheat seeds at 49°C, 52°C,55°C and 61°C, respectively for 5 and 10 min in controlling seed borne infection. Hot water treatment at 52°C-55°C for 10 min gave highest control of *Alternaria tenuis, Aspergillus flavus, Aspergillus niger, Bipolaris sorokiniana, Curvularia luanata, Fusarium* spp. and *Penecillium* spp. and increased seed germination.

Hadojo (1993) stated that ratoon stunting and chlorotic streak of sugarcane were controlled by treating setts in hot water at 52°C for 20 min or at 50°C for 2 hrs.

Jindal *et al.*(1991) reported that hot water treatment of bean seeds at 52°C for 10 min was found most effective for controlling *Xanthomonas campestris* pv. *phaseoli* .

According to Zhang *et* al. (1992), seed transmitted *Phytophthora boehmeriae,* the pathogen of ball rot of cotton was killed by exposure to 55°C for 5 min seed treatment with hot water.

Strandberg and White (1989) studied the tolerance of carrot seeds to heat treatments in order to eradicate seed borne pathogens. They observed that germination and emergence of seedlings from seeds treated in hot water at 35, 40, 45 and 55°C from 4-20 min were not affected, but seeds treated at 60°C for 8 min or more were affected adversely. Prolonged treatment and the higher temperatures were particularly effective in reducing populations of seed borne *Alternaria dauci.*

According to IRRI (1983) *Bipolaris oryzae* caused brown spot of rice as a seed transmitted fungus effectively controlled by the hot water seed treatment at 53-54°C for 10-12 minutes. This treatment controlled primary infection at the seedling stages. Presoaking the seed in cold water for 8 hours increased effectivity of the treatment.

Singh (1983) reported the method of hot water treatment as soaking of eggplant seeds in water at 20 - 30°C for 4-6 hr then dipping in water at 49°C for 2 min, followed by drying before planting. There are chances of reduction in germination if there is an increase in either temperature or duration of soaking of the seed. Because of the inherent problems in the method and in general the fact that only smaller quantities of seed can be treated.

Daniels (1983) observed that *Fusarium moniliforme* was eliminated from corn seeds when it was treated at 60°C for 5 min. According to him the seeds remained viable and neither the seeds nor aseptically germinated seedlings yielded the pathogen when plated on komaga agar.

Lambat *et al.* (1974) reported eradication of *Phoma betae* in sugarbeet seed by hot water treatment at 50°C for 30 min. Hot water seed treatment has also been reported to eradicate certain bacterial pathogens like black-rot pathogen (*Xanthomonas campestris pv. campestris*) in crucifer seeds at 50°C for 30 min; bacterial blight of cluster bean (*X. campestris* pv. *cyamopsidis*) at 56°C for 10 min (Srivastava and Rao, 1963).

Prabhu and Prasada (1970) controlled alternaria leaf blight of wheat caused by *Alternaria triticina* by soaking seed in water at 52 to 54°C for 10 min. Lowest dead seeds (9.9%) recorded at temperature 51-52°C, which was lower than dead seeds (12.8%) at control condition. Increase in temperature above 51- 52°C, there observed a continuous significant increase in dead seeds indicating the negative effect of heat against viability of seeds. The highest dead seeds (53.6%) recorded at temperature 58-59°C.

Winter *et al.* (1994) compared hot water treatment of certified wheat seeds dipped in water at 45°C for 2hrs, then air-dried at 40^0C for 5hrs with chemical seed treatment with fenpiclonil and carboxin at 400 ml/kg seed for control of seed-borne fungi of wheat. According to them hot water treatment was equally or more effective than the fungicides in controlling *Gerlachia nivalis* and *Septoria nodorum*.

Winter *et al.* (1996) found that hot water treatment of barley seeds at 52° C for 5 or 10 minutes was partially effective against seed borne *Drechslera teres* and *Helminthosporium sativum.* However, hot water treatment at 52° C for 10 min sometimes reduced germination and field emergence but the effect was less with 5 min treatment.

2.4. Seed treatment by solar heat

Guldhe *et al.* (1985) tested a physical methods against *Ustilago tritici* (*U. nuda*) associated with wheat seeds collected from infected field. They found that modified solar heat treatment was the best but gave only 46.9% control.

Mohinder *et al.*(1994) conducted a field experiment at Hisar, India, to study the efficacy of solar heat treatment for controlling loose smut of wheat caused by *Ustilago tritici* (*U. segetum* var *tritici*). The disease was completely controlled by solar heat. Jahan (1996) demonstrated that solar heat treatment of jute seed effectively inhibited seed-borne fungi.

Haque (1997) conducted an experiment to evaluate the solar heat treatment for 3 hours to control major seed-borne fungal pathogens of chilli. He found solar heat treatment significantly inhibited the growth of all the major seed-borne fungi, in chilli seeds as compared to the control. Treated seed yielded 3.75%, 4.25%, 6.25% and 8.50% *Alternaria tenuis, Colletotrichum capsici, Curvularia lunata* and *Fusarium* spp., respectively. In the control treatment infection percentage were 14.0%, 12.75%, 12.00%, 20.25% for *A. tenuis, C. capsici, C. lunata and Fusarium* spp., respectively. Mahfuzul (1997) reported solar heat treatment as an effective method in reducing seed-borne infection of chilli compared to control.

Fakir and Jahan (1998) carried out an experiment to control seed-borne, fungal pathogens of jute by seed treatment with solar heat. Solar heat treatment effectively reduced 91.3% seed-borne infections and increased 9.0% seed germination.

CHAPTER 3

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1. Laboratory experiment

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

3.1.1. Treatments

There were ten treatments namely :

T_1 = Farmer's saved seed.

T_2 = Apparently healthy seed.

T_3 = Farmer's saved seed treated with Vitavax-200 (0.4%).

T_4 = Apparently healthy seed treated with Vitavax-200 (0.4%).

T_5 = Sundrying of farmer's saved seed.

T_6 = Sundrying of apparently healthy seed.

T_7 = Hot water treatment of farmer's saved seed.

T_8 = Hot water treatment of apparently healthy seed.

T_9 = Polythene solarization of farmer's saved seed.

T_{10} = Polythene solarization of apparently healthy seed.

3.1.2. Preparation of seeds for different treatments :

3.1.2.1. Seed sorting

Apparently healthy seeds were obtained by manual seed sorting, eliminating inert matter, varietal mixture, other crop seeds, weed seeds, crop residues and black pointed seeds.

3.1.2.2. Seed treatment with Vitavax-200

Seeds were taken in a beaker and the specific amount of chemical was added into the seeds. The chemical was mixed thoroughly by a stick. Both apparently healthy seeds and original farmers saved seeds were treated with Vitavax-200 (0.4%).

3.1.2.3. Seed treatment with hot water

Apparently healthy and farmer's saved seed were treated with hot water at 50°C for 5 minutes.

3.1.2.4. Seed treatment by sundrying

Apparently healthy and farmer's saved seed were sundried for 14 hours before sowing.

3.1.2.5. Seed Treatment by polythene solarization

Apparently healthy and farmer's saved seed were covered by transparent polyethylene paper and sundried for 14 hours before sowing.

3.1.3. Seed health study

Health status of the treated and untreated seeds was done following ISTA rules. In this method 3 layers of blotter were soaked in sterilized water and placed at the bottom of the glass petridish. Then 25 seeds were set up on the blotting paper in a petridish maintaining equal distance and covered with the lid. Seeds thus plated were incubated in an air cooled room at about 20°c temperature for 7 days in seed Pathology Laboratory, Sher-e-Bangla Agriculture University, Dhaka. After 7 days of incubation the seeds were observed for the presence of seed-borne *Biopolaris sorokiniana* fungi under stereo binocular microscope following the key of Mathur and germination of the seeds was also recorded.

3.2. Field experiment

3.2.1. Experimental site and cropping history of the field

The experiment was conducted at the Farm of Sher-e-Bangla Agricultural University, Dhaka. The location of experimental site is shown in Fig-1. The field was covered by rice in the previous crop season.

3.2.2. Experimental period

The experiment was carried out during the period from November 2005 to April 2006.

3.2.3. Climate of experimental site

The experimental area was under the subtropical climate which characterized with the comparatively high rainfall, high humidity, high temperature, relatively long day during April to September and scanty rainfall, low humidity, low temperature and short day period during October to March. The later period is favorable for wheat cultivation.

3.2.4. Crop

The wheat (*Triticum aestivum* L.) cultivar Kanchan, collected from a farmer of Shirajgonj district was used in this study. Kanchan is a popular variety which is commonly cultivated in different areas of the country.

3.2.5. Treatments

There were ten treatments as described in laboratory experiment (3.1.1).

3.2.6. Soil properties

Soil properties of the Experimental site was as follows:-

Agro-ecological region	: Madhupur Tract (AEZ – 28).
Land Type	: Medium high land.
General soil type	: Non-Calcareous Darkgray floodplain soil
Soil series	: Tejgaon
Topography	: Up land
Elevation	: 8.45
Location	: SAU Farm, Dhaka.
Field level	: Above flood level.
Drainage	: Fairly good.
Firmness (consistency)	: Compact to friable when dry.

3.2.7. Fertility status of the soil

The soil of experimental site was analysed in Soil Resource Development Institute (SRDI), Dhaka and found as silty clay with pH 6.0 and the soil contains

1. Total N (%)	0.078
2. Organic matter (%)	0.88
3. Phosphorous (%)	0.0015
4. Potassium (%)	0.0053
5. Sulphur (%)	0.0017

Doses of fertilizer was used considering existing nutrient of the field soil mentioned below as BARC fertilizer recommendation guide:

Fertilizer	Doses
Cowdung	10 tons/ha
Urea	220 Kg/ha
TSP	180 Kg/ha
MP	50 Kg/ha
Gypsum	120 Kg

3.2.8. Design of experiment

The experiment was laid out in Randomized Complete Block Design (RCBD) comprising three replications for each treatment. The unit plot size was (2m X 1m). Distance between block and between the plots was 1m and 0.5m, respectively. Thus, there were altogether 30 plots for the study. Different treatments were assigned randomly to the unit plot.

3.2.9. Land preparation

The experimental field was thoroughly ploughed and cleaned prior to seed sowing and application of fertilizers and manure in the field. The experimental field was well prepared by thorough ploughing followed by laddering to have a good tilth and for proper leveling before sowing of seeds.

3.2.10. Fertilizer application

Soil was fertilized with Nitrogen (in the form of urea), Phosphorus (in the form of Triple Super Phosphate-TSP), Calcium and Sulphur (in the form of Zypsum) and cowdung during final land preparation. Whole quantity of TSP, MP and two third of Urea was applied at final land preparation. Remaining Urea was applied at 21 days after sowing of seed.

3.2.11. Preparation of seeds for different treatments

Preparation of seeds for different treatments were done as of laboratory experiment described in 3.1.2.

3.2.12. Sowing of seeds

The seeds were sown in the field on December 2, 2005 at the rate of 120 kg/ha. Amount of seeds for each plot was confined before sowing. Seeds were placed continuously in lines properly and were covered by soil with the help of hand. Row to row distance was 20 cm which made 5 rows in each unit plot.

3.2.13. Collection of data on seeding emergence

Data on seedling emergence was recorded at 10 and 15 days after sowing of seeds.

3.2.14. Intercultural operation

Irrigation was done once after 25 days and another after 45 days of sowing. Irrigation was generally followed the each weeding of the crops. Weeding was performed twice during the growing period of the crop for better soil aeration and conservation of soil moisture. The common weeds were *Cynodon dactylon* L (Durba grass), *Cyperus rotundus* L. (Mutha) and *Chenopodium album* L. (Bathua).Weeding was done carefully keeping the delicate young plants undisturbed.

3.2.15. Plant protection activities

Special care was taken for 12 days after sowing to protect the crop from birds especially at sowing and germination stages and at the ripening stage of the crop.

3.2.16. Tagging and data collection

Randomly five plants were selected from each row of the plot and tagged. So, 25 plants/plot were tagged for rating and mean values were determined to get rating score of the material of each treatment.

3.2.17. Collection of data on number of tillers/plant

The data on number of tillers/plant were collected at tillering stage from 25 tagged plants of each plot.

3.2.18. Evaluation of leaf spot severity

Leaf spot severity of flag leaf, penultimate (next to flag leaf) leaf and third leaf (next to penultimate leaf) were recorded in four growth stages of plant viz. Panicle initiation stage, flowering stage, milking stage and hard dough stage. The severity of leaf spot disease was recorded following 0-5 grade (plate-1) of Hossain and Azad (1992). The grades are given below: -

0 = No infection (Highly resistant)

1 = Few minute lesions on leaves (Resistant)

2 = Black lesion with no distinct chlorotic halos covering ≤10% of the leaf area (Moderately resistant)

3 = Typical lesions surrounded by distinct chlorotic halos covering 10-50 % of the leaf area (Moderately susceptible)

4 = Severe lesions on leaves with ample necrotic zones drying over part of the leaf, covering ≥ 50% of the leaf area (susceptible)

5= Severe infection , drying of the leaf spike infected to some extend (Highly susceptible)

3.2.19. Recording data on number of spike / m^2

Data on number of spike / m^2 was taken at the time of ripening stage.

3.2.20. Harvesting

The crop was harvested at full ripening stage on 21^{th} March 2006.

3.2.21. Isolation and identification of pathogen

Isolation and identification of pathogen were made in two ways –

a) By direct inspection

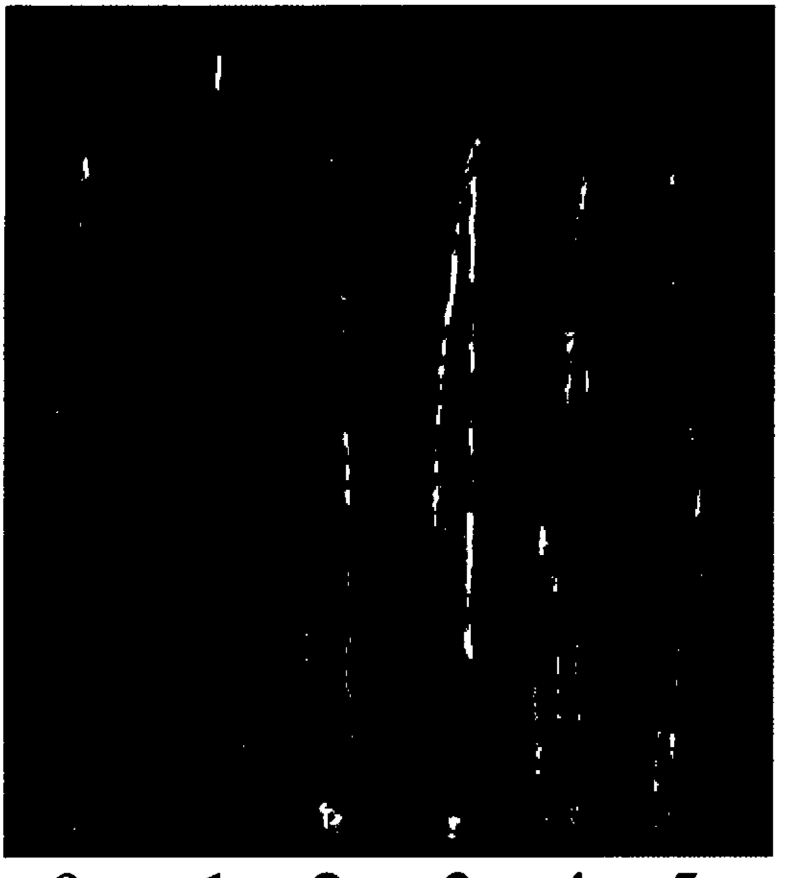
b) By inoculating sample tissue on PDA medium.

(a) By direct observation

The diseased leaves of wheat plants were collected and kept in polythene bags and tagged. The samples were then taken to the laboratory. Then slides were prepared from the diseased samples, observed under microscope and the pathogen was identified according to CMI description.

(b) By growing on PDA medium

The diseased leaves were collected and were taken to the laboratory. The leaves were then cut into small pieces (about 0.5 cm) with diseased portion and surface sterilized with $HgCl_2$ solution (0.01%) for 30 second. The cut pieces were then washed in water at three times and were placed onto PDA media in petridish. The plates were then incubated at 25 ± 1^0C for 7days. Later the



0 1 2 3 4 5

Photo Plate : 1. Disease severity grades (0-5) of spotted leaves of wheat

pathogen was purified using hyphal tip culture method and grown on PDA media at $25\pm1^{0}C$ for 2 weeks and identified as *Bipolaris sorokiniana* with the help of relevant literature (CMI Description).

3.2.22. Collection of data on yield and yield contributing characters :

Data of plant growth and yield contributing characters were recorded from the randomly selected 25 tagged plants of each unit plot on the following parameters.

i. Plant height (cm).
ii. Length of ear (cm).
iii. Distance between the point of flag leaf initiation and base of ear (cm).
iv. Number of spikelets/ear.
v. Number of healthy spikelet/ear.
vi. Number of diseased spikelet/ear.
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 grain weight (g).
xiv. Grading of seeds/ear (0-5).
xv. Grain yield/plot (kg).
xvi. Grain yield (t/ha).
xvii. Straw yield/plot (kg).
xviii. Straw yield (t/ha).

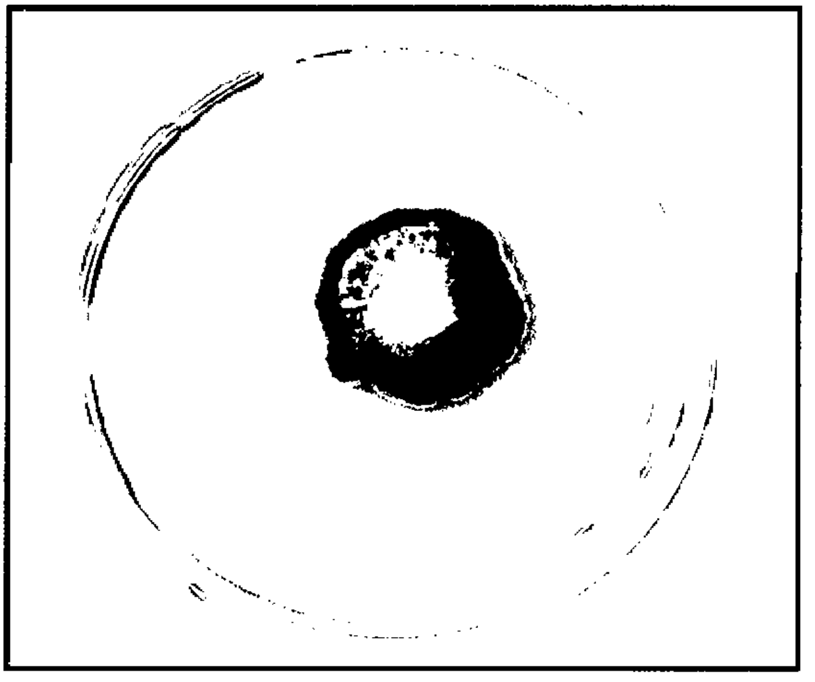


Plate: 2. Pure culture of *Bipolaris sorokiniana*

Plate: 3. Mycelia and conidia of *Bipolaris sorokiniana* as observed under compound microscope (X100)

3.2.23. Grading of seeds

The grading of seeds were done following the 0-5 rating scale (plate-4). The rating scale is as follows:

0	=	Free from infection
1	=	Only embryo blackish
2	=	Embryo and its adjacent are slightly infected
3	=	Embryo and less than ¼ of grains are discolored
4	=	Embryo and ½ of grains are infected
5	=	Grains are shriveled almost completely discolored or more than ½ of grains discolored.

3.2.24. Analysis of data

The data on various parameters were analyzed using analysis of variance to find out variation obtained from different treatments. Treatment means were compared by DMRT (Duncan's Multiple Range Test).

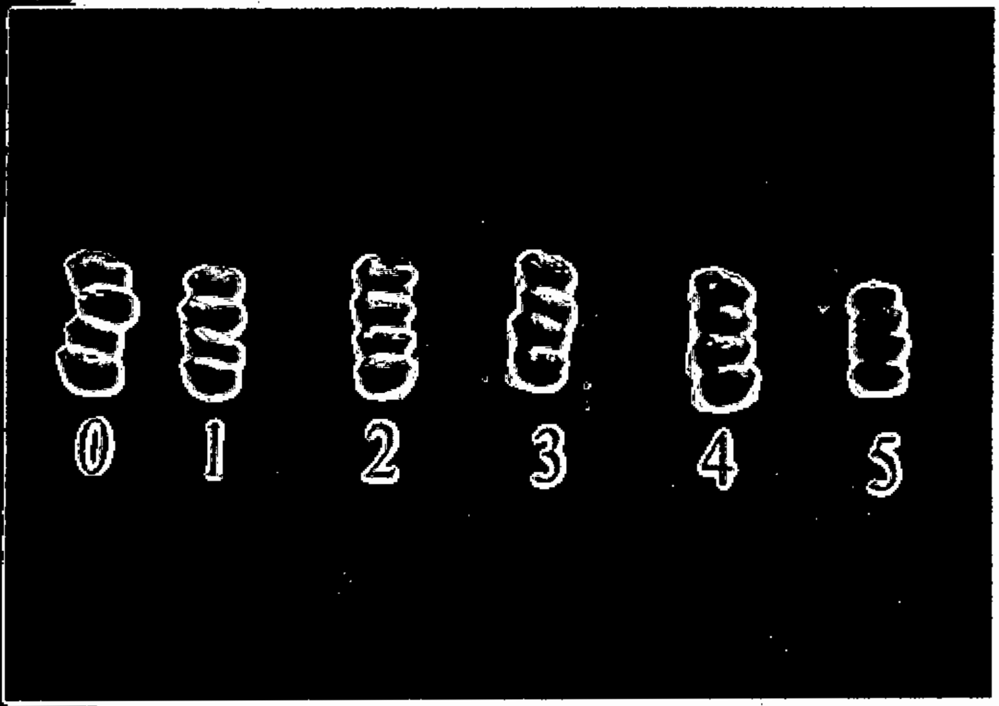


Plate : 4. Wheat seeds of different grade (0-5)

CHAPTER 4

RESULTS

4. RESULTS

4.1. Laboratory experiment

Under *in vitro* investigation, results on the effect of chemicals and other physical seed treatments in controlling seed borne infection of *Bipolaris sorokiniana* and seed germination on the blotting paper are presented in Table-1.The highest (92.25 %) seed germination was recorded in the treatment T_4 (apparently healthy seeds treated with Vitavax) which was statistically identical (89.72 %) to treatment T_3 (farmer's saved seeds treated with Vitavax). The lowest seed germination (76.21%) was recorded in farmer's saved seed. Among the other physical seed treatment methods, T6 (Sundrying of apparently healthy seed) gave germination 88.56% which was followed by T_8 (87.93%), T_7 (86.18%), T_2 (85.41%), T_{10} (83.99%) and T_9 (81.88%). The prevalence of *Bipolaris sorokiniana* was varied significantly depending on the different seed treatment methods.

The incidence of *Bipolaris sorokiniana* recorded heighest in the untreated seeds (25.38%) which differed significantly from all other treatments. Among all the treatments, T_4 (apparently healthy seed treated with Vitavax) yielded significantly the lowest prevalence (1.50%) of *Bipolaris sorokiniana* which was 94.08% reduction from untreated control and it was statistically similar with treatment T_3 (2.95 %). Incidence of *Bipolaris sorokiniana* was also reduced over control when farmer's seed as well as physically sorted seeds were treated with sundrying, hot water and polythene solarization. Apparently healthy seeds obtained by manual seed sorting reduced 66.15% incidence of *Bipolaris sorokiniana* over untreated control. Apparently healthy seed and polythene solarised seeds also gave good results than control.

Table.1. Effect of seed treatments on seed germination and seed yielding *Bipolrais sorokiniana* of wheat in the laboratory.

Treatments	%Germination	% incidence of *Bipolrais sorokiniana*	% incidence of *Bipolaris sorokiniana* decreased over control
T_1	76.21 e	25.38 a	------
T_2	85.41 bcd	8.59 cd	66.15
T_3	89.72 ab	2.95 g	88.37
T_4	92.25 a	1.50 g	94.08
T_5	83.81 cd	7.21 def	71.59
T_6	88.56 abc	5.89 f	76.79
T_7	86.18 bcd	8.30 cde	67.29
T_8	87.93 abc	6.81 ef	73.16
T_9	81.88 d	11.03 b	56.54
T_{10}	83.99 cd	9.61 bc	62.13
$LSD_{(0.01)}$	5.059	1.535	----

T_1 = Farmer's saved seed.

T_2 = Apparently healthy seed.

T_3 = Farmer's saved seed treated with Vitavax-200 (0.4%).

T_4 = Apparently healthy seed treated with Vitavax-200 (0.4%).

T_5 = Sundrying of farmer's saved seed.

T_6 = Sundrying of apparently healthy seed.

T_7 = Hot water treatment of farmer's saved seed.

T_8 = Hot water treatment of apparently healthy seed.

T_9 = Polythene solarization of farmer's saved seed.

T_{10} = Polythene solarization of apparently healthy seed.

4.2. Field experiment

4.2.1. Effect of seed treatment on seedling emergence and plant growth of wheat

The emergence of wheat seedling was counted at 10 and 15 days after sowing (DAS) of seeds. All the treatments increased germination compared to control (Table-2). The range of soil temperature and moisture were same for all the treatments. The results showed that the highest germination was counted 83.89% and 87.95% at 10 and 15 DAS in the treatment T_4 (apparently healthy and Vitavax treated seed) which was statistically identical to treatment T_3 (farmer's saved seed treated with Vitavax). Among the other physical seed treatment methods, T_6 (Sundrying of apparently healthy seed) gave moderate germination followed by T_5 , T_2 , T_8, T_7 , T_{10} and T_9. The treatment T_2, T_5, T_6, T_7 and T_8 resulted statistically similar effect on field emergence of seedlings.

Plant height varied from 77.91 to 88.32 cm. The highest plant height (88.32 cm) was recorded under the treatment T_4 (apparently healthy seeds treated with Vitavax) which was statistically similar with the treatment T_3 (farmer's saved seed treated with Vitavax). On the other hand the lowest plant height (77.91 cm) was recorded in the treatment T_1 (farmer's saved seed). The results showed that treatment T_6 (86.29cm) was statistically similar to that of treatment T_8 (85.94 cm), T_5 (85.04 cm), T_2 (84.40 cm), T_7 (84.02 cm),T_{10} (83.54 cm) and T_9 (80.41 cm). No significant variation among the treatments was found regarding spike length though the highest and lowest spike length was obtained under the treatments T_4 (14.39 cm) and T_1 (13.26 cm), respectively.

Considering distance between the point of flag leaf initiation and the base of ear, it has been found that there was no significant variation among the

Table.2. Effect of seed treatment on germination, plant height, spike length and length between panicle initiation and tip of spike of wheat

Treatments	Germination (%)		Plant height (cm)	Spike length (cm)	Distance between the point of flag leaf initiation and base of ear (cm)
	10 DAS	15 DAS			
T_1	49.22 c	58.36 g	77.91 c	13.26	13.28
T_2	67.35 b	74.81 cd	84.40 ab	13.56	13.45
T_3	80.13 a	84.34 ab	87.62 a	14.03	14.27
T_4	83.89 a	87.95 a	88.32 a	14.39	14.75
T_5	68.94 b	75.19 cd	85.04 ab	13.57	13.99
T_6	70.98 b	79.35 bc	86.29 ab	13.86	14.13
T_7	63.59 b	68.91 de	84.02 ab	13.74	13.68
T_8	68.76 b	74.90 cd	85.94 ab	13.76	13.52
T_9	52.92 c	60.19 fg	80.41 bc	13.45	13.37
T_{10}	53.90 c	65.36 ef	83.54 ab	13.83	13.78
$LSD_{(0.01)}$	6.844	5.865	5.085	NS	NS

T_1 = Farmer's saved seed.

T_2 = Apparently healthy seed.

T_3 = Farmer's saved seed treated with Vitavax-200 (0.4%).

T_4 = Apparently healthy seed treated with Vitavax-200 (0.4%).

T_5 = Sundrying of farmer's saved seed.

T_6 = Sundrying of apparently healthy seed.

T_7 = Hot water treatment of farmer's saved seed.

T_8 = Hot water treatment of apparently healthy seed.

T_9 = Polythene solarization of farmer's saved seed.

T_{10} = Polythene solarization of apparently healthy seed.

treatments though the highest (14.75 cm) and lowest (13.28 cm) distance were recorded under the treatments T_4 (14.75 cm) and T_1 (13.28 cm), respectively.

4.2.2. Effect of seed treatment on leaf spot severity of wheat at panicle initiation stage.

Leaf spot severity of wheat at panicle initiation stage on flag leaf, second leaf and third leaf varied significantly and ranged from 0.00 to 0.08, 0.02 to 0.32 and 0.03 to 0.67, respectively. The average disease severity of flag leaf, second leaf, and third leaf ranged from 0.02 to 0.35 where the highest and lowest disease severity was recorded under the treatments T_1 and T_4, respectively.The treatments T_5, T_6, T_7, T_8, T_9 and T_{10} also significantly reduced leaf spot severity over untreated control. Moreover, manually sorted apparently healthy seeds (T_2) significantly reduced leaf spot severity over untreated control (Farmer's saved seed, T_1).

4.2.3. Effect of seed treatment on leaf spot severity of wheat at flowering stage

Leaf spot severity of wheat at flowering stage on flag leaf, second leaf and third leaf varied significantly and ranged from 0.03 to 0.13, 0.05 to 0.23 and 0.07 to 0.70, respectively. The average disease severity of flag leaf, second leaf, and third leaf ranged from 0.05 to 0.70 where the highest and lowest disease severity was recorded under the treatments T_1 (0.70) and T_4 (0.05), respectively. Rèst of the treatments also significantly reduced leaf spot severity over untreated control. Moreover, sundried and hot water treated apparently healthy seeds significantly reduced leaf spot severity over untreated control (Farmer's saved seed, T_1).

Table.3. Effect of seed treatment on leaf spot severity of wheat at panicle initiation stage

Treatments	Disease severity at panicle initiation stage			
	Flag leaf	2nd leaf	3rd leaf	Average
T_1	0.08 a	0.23 a	0.67 a	0.35 a
T_2	0.03 cd	0.08 cd	0.42 d	0.18 d
T_3	0.01 ef	0.05 e	0.06 g	0.04 f
T_4	0.00 f	0.02 f	0.03 h	0.02 g
T_5	0.03 d	0.08 cd	0.34 e	0.15 e
T_6	0.02 de	0.07 d	0.32 f	0.14 f
T_7	0.03 cd	0.09 cd	0.47 c	0.20 c
T_8	0.03 cd	0.08 cd	0.41 d	0.17 d
T_9	0.06 b	0.13 b	0.50 b	0.23 b
T_{10}	0.05 bc	0.09 c	0.49 b	0.21 bc
$LSD_{(0.01)}$	0.017	0.017	0.017	0.017

T_1 = Farmer's saved seed.

T_2 = Apparently healthy seed.

T_3 = Farmer's saved seed treated with Vitavax-200 (0.4%).

T_4 = Apparently healthy seed treated with Vitavax-200 (0.4%).

T_5 = Sundrying of farmer's saved seed.

T_6 = Sundrying of apparently healthy seed.

T_7 = Hot water treatment of farmer's saved seed.

T_8 = Hot water treatment of apparently healthy seed.

T_9 = Polythene solarization of farmer's saved seed.

T_{10} = Polythene solarization of apparently healthy seed.

Table.4. Effect of seed treatment on leaf spot severity of wheat at flowering stage

Treatments	Disease severity at flowering stage			
	Flag leaf	2nd leaf	3rd leaf	Average
T_1	0.13 b	0.32 a	1.04 a	0.70 a
T_2	0.08 cde	0.15 c	0.68 de	0.30 de
T_3	0.04 f	0.08 e	0.09 h	0.07 g
T_4	0.03 f	0.05 f	0.07 i	0.05 h
T_5	0.10 c	0.17 b	0.72 b	0.32 c
T_6	0.06 e	0.10 d	0.53 g	0.23 f
T_7	0.08 cd	0.16 bc	0.69 cd	0.31 de
T_8	0.07 de	0.15 c	0.67 e	0.30 e
T_9	0.20 a	0.12 d	0.59 f	0.47 b
T_{10}	0.09 c	0.17 bc	0.70 c	0.32 cd
$LSD_{(0.01)}$	0.017	0.017	0.017	0.017

T_1 = Farmer's saved seed.

T_2 = Apparently healthy seed.

T_3 = Farmer's saved seed treated with Vitavax-200 (0.4%).

T_4 = Apparently healthy seed treated with Vitavax-200 (0.4%).

T_5 = Sundrying of farmer's saved seed.

T_6 = Sundrying of apparently healthy seed.

T_7 = Hot water treatment of farmer's saved seed.

T_8 = Hot water treatment of apparently healthy seed.

T_9 = Polythene solarization of farmer's saved seed.

T_{10} = Polythene solarization of apparently healthy seed.

4.2.4. Effect of seed treatment on leaf spot severity of wheat at milking stage

Leaf spot severity of wheat at milking stage on flag leaf, second leaf and third leaf varied significantly and ranged from 0.07 to 0.74, 0.10 to 1.61 and 0.30 to 2.69, respectively. The average disease severity of flag leaf, second leaf, and third leaf ranged from 0.16 to 1.68 where the highest and lowest disease severity was recorded under the treatments T_1 (1.68) and T_4 (0.16), respectively. The second lowest severity was recorded in treatment T_3 (0.28) followed by T_6, T_{10}, T_8, T_2, T_5, T_7, T_9 and T_{10}.

4.2.5. Effect of seed treatment on leaf spot severity of wheat at hard dough stage

Leaf spot severity of wheat at hard dough stage on flag leaf, second leaf and third leaf varied significantly and ranged from 0.18 to 1.50, 0.59 to 2.34 and 1.21 to 3.59, respectively. The average disease severity of flag leaf, second leaf, and third leaf ranged from 0.66 to 2.47 where the highest and lowest disease severity was recorded under the treatments T_1 and T_4, respectively. The treatments T_3, T_5, T_6, T_7, T_8, T_9 and T_{10} also significantly reduced leaf spot severity over untreated control. Moreover, sundried and hot water treated apparently healthy seeds significantly reduced leaf spot severity over untreated control (Farmer's saved seed, T_1).

Table.5. Effect of seed treatment on leaf spot severity of wheat at milking stage

Treatments	Disease severity at milking stage			
	Flag leaf	2nd leaf	3rd leaf	Average
T_1	0.74 a	1.61 a	2.69 a	1.68 a
T_2	0.10 e	0.88 e	1.68 f	0.89 e
T_3	0.09 e	0.17 i	0.58 i	0.28 i
T_4	0.07 f	0.10 j	0.30 j	0.16 j
T_5	0.13 d	0.72 g	1.63 g	0.82 g
T_6	0.07 f	0.70 h	1.50 h	0.75 h
T_7	0.10 e	1.00 d	1.78 de	0.96 d
T_8	0.09 e	0.76 f	1.70 de	0.85 f
T_9	0.20 b	1.32 b	1.96 b	1.16 b
T_{10}	0.185 c	1.21 c	1.82 c	1.07 c
LSD 0.001	0.017	0.017	0.017	0.017

T_1 = Farmer's saved seed.

T_2 = Apparently healthy seed.

T_3 = Farmer's saved seed treated with Vitavax-200 (0.4%).

T_4 = Apparently healthy seed treated with Vitavax-200 (0.4%).

T_5 = Sundrying of farmer's saved seed.

T_6 = Sundrying of apparently healthy seed.

T_7 = Hot water treatment of farmer's saved seed.

T_8 = Hot water treatment of apparently healthy seed.

T_9 = Polythene solarization of farmer's saved seed.

T_{10} = Polythene solarization of apparently healthy seed.

Table.6. Effect of seed treatment on leaf spot severity of wheat at hard dough stage

Treatments	Disease severity at hard dough stage			
	Flag leaf	2^{nd} leaf	3^{rd} leaf	Average
T_1	1.50 a	2.34 a	3.59 a	2.47 a
T_2	0.70 d	1.68 e	2.63 d	1.67 e
T_3	0.20 h	0.73 i	1.53 g	0.82 e
T_4	0.18 i	0.59 j	1.21 h	0.66 j
T_5	0.59 f	1.53 g	2.40 f	1.51 g
T_6	0.52 g	1.42 h	2.38 f	1.44 h
T_7	0.76 c	1.73 d	2.80 c	1.76 d
T_8	0.61 e	1.60 f	2.52 e	1.58 f
T_9	1.01 b	1.90 b	2.94 b	1.95 b
T_{10}	0.75 c	1.83c	2.85 c	1.81 c
$LSD_{(0.01)}$	0.017	0.017	0.054	0.017

T_1 = Farmer's saved seed.

T_2 = Apparently healthy seed.

T_3 = Farmer's saved seed treated with Vitavax-200 (0.4%).

T_4 = Apparently healthy seed treated with Vitavax-200 (0.4%).

T_5 = Sundrying of farmer's saved seed.

T_6 = Sundrying of apparently healthy seed.

T_7 = Hot water treatment of farmer's saved seed.

T_8 = Hot water treatment of apparently healthy seed.

T_9 = Polythene solarization of farmer's saved seed.

T_{10} = Polythene solarization of apparently healthy seed.

4.2.6. Effect of seed treatments on number of tillers/ plant and number of spikelets/ ear of wheat

Considering the number of tillers/plant the effects were differed significantly among the treatments (Table-7). The lowest number (6.12) of tiller was recorded in T_1 treatment (farmer's saved seed) which was statistically similar to that of T_9 treatment (6.25). The highest number of tiller (8.89) was recorded in the treatment T_4 (apparently healthy seed treated with Vitavax -200, 0.4%) followed by T_3, T_6, T_5, T_2 T_8, T_{10} and T_8, respectively. Result obtained from number of spikelets/ear indicated that there were some differences among the treatment (Table-7). Treatment T_4 scored the maximum spikelets (28.36) that was followed by treatment T_3 (26.29) whereas control gave minimum value (20.08).

4.2.7. Effect of seed treatments on grain formation and grain weight of wheat

It was found that grain formation and weight of grains of wheat cv. Kanchan differed significantly among the treatments (Table-8). The lowest number of grains/ear (31.11) was recorded under the treatment T_1 (farmer's saved seed). On the other hand the highest number of grains/ear (38.19) was recorded in the treatment T_4 (apparently healthy seed treated with Vitavax-200). From these results it was observed that Farmer's saved seed gave the lowest grain and chemically treated seed gave the highest grains. Though apparently healthy seeds (T_2) and farmer's saved seed (T_1) produced statistically similar number of grains/ear but the result differed significantly when sundrying of apparently healthy seeds (T_6) were done.

Table.7. Effect of seed treatment on number of tillers/plant and number of spikelets/ear of wheat.

Treatments	Number of tillers/plant	Number of spikelets/ear
T_1	6.12 f	20.08 e
T_2	7.30 c	24.00 bcd
T_3	7.96 b	26.29 ab
T_4	8.89 a	28.36 a
T_5	7.32 c	24.88 bc
T_6	7.88 b	25.41 bc
T_7	6.83 e	23.13 cd
T_8	7.22 cd	24.84 bc
T_9	6.25 f	21.62 de
T_{10}	6.93 de	22.59 cde
$LSD_{(0.01)}$	0.292	2.586

T_1 = Farmer's saved seed.

T_2 = Apparently healthy seed.

T_3 = Farmer's saved seed treated with Vitavax-200 (0.4%).

T_4 = Apparently healthy seed treated with Vitavax-200 (0.4%).

T_5 = Sundrying of farmer's saved seed.

T_6 = Sundrying of apparently healthy seed.

T_7 = Hot water treatment of farmer's saved seed.

T_8 = Hot water treatment of apparently healthy seed.

T_9 = Polythene solarization of farmer's saved seed.

T_{10} = Polythene solarization of apparently healthy seed.

A significant variation was recorded among the treatment under the present trial considering number of healthy grains/ear. (Table-8). The lowest healthy grains/ear (28.55) was recorded in the treatment T_1 (farmer's saved seed). On the other hand the highest healthy seed (37.89) was recorded in the treatment T_4 (apparently healthy seed treated with Vitavax -200) which appeared statistically similar to that of T_3 and T_6. From these results it was observed that Farmer's saved seed gave the lowest healthy grain in wheat and chemically treated seeds and physically treated, like sundrying and hot water treated seeds gave the highest number of healthy grains.

A significant variation was recorded among the treatment under the present trial in number of diseased grain (Table-8). The highest diseased grains/ear (2.56) was recorded under the treatment T_1 (farmer's saved seed). On the other hand the lowest diseased seed (0.30) was recorded in the treatment T_4 (apparently healthy seed treated with Vitavax -200) followed by T_3, T_6, T_5, T_8, T_2, T_7, T_{10}, T_9 and control (T_1). From these results it was observed that Farmer's saved seed gave the highest diseased grains/ear of wheat. On the other hand chemically treated seed and physical treated, like sundrying and hot water treated seed also gave the lowest diseased grains.

Considering weight of grains/ear it was found that the different methods of seed treatment varied significantly among them (Table-8). The minimum weight of grains (1.21 g) was recorded in the treatment T_1 (farmer's saved seed) and the maximum weight of grains (1.52 g) was recorded in the treatment T_4 (apparently healthy seed treated with Vitavax -200). Considering the weight of healthy grains/ear seed treatments of wheat differed significantly among them (Table-8). The lowest weight of healthy grains/ear (1.14 g) was recorded in the

Table.8. Effect of seed treatment on grain formation and weight of grains of wheat

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_1	31.11 c	28.55 e	2.56 a	1.21 e	1.14 e	0.10 a
T_2	34.30 abc	33.89 bc	1.24 e	1.37 abc	1.35 b	0.04 cd
T_3	36.65 a	36.03 ab	0.62 h	1.46 ab	1.44 ab	0.02 ef
T_4	38.19 a	37.89 a	0.30 i	1.52 a	1.51 a	0.01 f
T_5	34.11 abc	33.01 bcd	1.10 f	1.36 bcde	1.32 bcd	0.04 cd
T_6	35.77 ab	34.98 ab	0.79 g	1.43 ab	1.39 ab	0.03 de
T_7	31.74 bc	30.22 cde	1.52 d	1.26 cde	1.20 cde	0.06 c
T_8	34.84 abc	33.67 bc	1.17 ef	1.39 abcd	1.34 bc	0.04 cd
T_9	31.15 c	29.18 de	1.97 b	1.24 e	1.16 e	0.07 b
T_{10}	31.73 bc	30.02 cde	1.71c	1.26 de	1.20 de	0.05 c
$LSD_{(0.01)}$	3.714	3.670	0.133	0.133	0.133	0.017

T_1 = Farmer's saved seed.

T_2 = Apparently healthy seed.

T_3 = Farmer's saved seed treated with Vitavax-200 (0.4%).

T_4 = Apparently healthy seed treated with Vitavax-200 (0.4%).

T_5 = Sundrying of farmer's saved seed.

T_6 = Sundrying of apparently healthy seed.

T_7 = Hot water treatment of farmer's saved seed.

T_8 = Hot water treatment of apparently healthy seed.

T_9 = Polythene solarization of farmer's saved seed.

T_{10} = Polythene solarization of apparently healthy seed.

treatment T_1 (farmer's saved seed) and the highest weight of healthy grains/ear (1.51 g) was recorded in the treatment T_4 (apparently healthy seed treated with Vitavax -200).

Considerable variation was recorded among the treatments under the present piece of experiment in weight of diseased grains/ear. (Table-8). The highest weight of diseased grains/ear (0.10g) was recorded in the treatment T_1 (farmer's saved seed), which was followed by T_9, T_{10} and T_7 treatment. The lowest weight of diseased grains/ear (0.01g) was recorded in the treatment T_4 (apparently healthy seed treated with Vitavax-200) that was followed by T_3 (0.02g) treatment (farmer's saved seed treated with Vitavax-200). From the results it was observed that farmer's saved seed gave the highest weight of diseased grains/ear.

4.2.8. Effect of seed treatment on the formation of grain's of different grades of wheat

It was found that grading of seeds (0-5 scale) of wheat varied significantly among the treatments (Table-9). The highest number of grains under grade-0, was recorded in T_4 (apparently healthy seed treated with Vitavax-200, @ 0.4%), which was 37.89, and the lowest was recorded in T_1 (farmer's saved), which was 28.55 followed by T_9, T_{10} and T_7. The highest number of grade-1 grains/ear (0.83) was recorded in T_1 treatment and the lowest number of grade-1 grains/ear (0.25) was recorded in T_4 treatment (apparently healthy treated with Vitavax -200), which was followed by T_3 (farmer's saved seed treated with Vitavax -200.) The highest infected seed of this grade (0.64) was recorded in T_1 treatment (farmer's saved seed) and the lowest infected seed (0.02) was recorded in T_4 treatment (apparently healthy with Vitavax-200).

Table.9. Effect of seed treatments on the formation of grains of different grades of wheat cv. Kanchan.

Treatments	Grading of seeds (0-5 scale)					
	0	1	2	3	4	5
T_1	28.55 d	0.83 a	0.64 a	0.36 a	0.16 a	0.57 a
T_2	32.90 bc	0.52 d	0.18 g	0.16 e	0.08 d	0.30 e
T_3	36.03 ab	0.38 e	0.12 h	0.05 h	0.06 e	0.01 g
T_4	37.89 a	0.25 f	0.02 i	0.00 i	0.03 f	0.00 g
T_5	33.01 bc	0.50 d	0.20 f	0.13 f	0.05 e	0.22 f
T_6	34.98 ab	0.40 e	0.12 h	0.09 g	0.00 g	0.18 f
T_7	30.22 cd	0.59 c	0.26 d	0.20 d	0.11 c	0.36 d
T_8	33.67 b	0.40 e	0.24 e	0.16 e	0.09 d	0.28 e
T_9	29.18 d	0.65 b	0.37 b	0.30 b	0.14 b	0.51 b
T_{10}	30.02 cd	0.60 bc	0.30 c	0.26 c	0.13 b	0.42 c
$LSD_{(0.01)}$	3.010	0.054	0.017	0.017	0.017	0.054

0 = free from infection
1 = only embryo blackish
2 = embryo and its adjacent area slightly infected
3 = embryo and less than 1/4 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 discolored.

T_1 = Farmer's saved seed.

T_2 = Apparently healthy seed.

T_3 = Farmer's saved seed treated with Vitavax-200 (0.4%).

T_4 = Apparently healthy seed treated with Vitavax-200 (0.4%).

T_5 = Sundrying of farmer's saved seed.

T_6 = Sundrying of apparently healthy seed.

T_7 = Hot water treatment of farmer's saved seed.

T_8 = Hot water treatment of apparently healthy seed.

T_9 = Polythene solarization of farmer's saved seed.

T_{10} = Polythene solarization of apparently healthy seed.

The grade-3 grains/ear ranged from 0.00 to 0.36 where the highest and lowest counts were made under the treatments T_1 (0.36) and T_4 (0.00) respectively.

Considering grade-4 grains/ear it was found that the highest number of grains (0.16) was recorded in T_1 treatment and the lowest counts (0.03) was recorded in T_4.

It was found that grade-5 grains (shrivelled and completely discolored) differed significantly among the treatments.The highest number of grade-5 grains (0.57) was recorded in T_1 treatment (farmer's saved seed), which was closely followed by T_9 (0.51). The lowest infected seed, which was 0.00, recorded in T_4 treatment (apparently healthy seed treated with Vitavax-200) and also in T_3 (farmer's saved seed treated with Vitavax-200).

4.2.9. Effect of seed treatments on 1000 grain weight and yield of wheat cv. Kanchan.

Effect of seed treatments on 1000 grain weight and yield of wheat cv. Kanchan is presented in Table-10. The 1000-grain weight did not differ significantly where the highest (38.08) and lowest (33.37) 1000-grain weight were recorded under the treatments T_4 and T_1, respectively.

Considering the straw yield of wheat a significant variation was recorded among the treatments. Straw yield under the treatment varied from 6.13 to 4.53 t/ha. The highest straw yield was recorded 6.13 t/ha in T_3 treatment (farmer's saved and Vitavax-200 treated seed), which was statistically identical (6.09 t/ha) to the treatment T_4 (apparently health with Vitavax -200). On the other hand the lowest straw yield (4.53 t/ha) was recorded in the treatment T_1 (farmer's saved

Table.10. Effect of seed treatment on 1000 seed weight and yield of wheat

Treatments	1000 seeds weight (g)	Straw yield (t/ha)	Grain yield (t/ha)	% Grain yield increased over control
T_1	33.37	4.53 c	2.16 e	--
T_2	35.40	5.40 d	2.75 bc	27.31
T_3	37.93	6.13 a	3.31 a	53.24
T_4	38.08	6.09 a	3.49 a	61.57
T_5	35.60	5.58 bc	2.50 cd	15.74
T_6	36.41	5.71 b	2.83 b	31.01
T_7	34.94	5.23 e	2.31 de	6.94
T_8	35.26	5.48 cd	2.78 bc	28.70
T_9	34.40	4.83 f	2.25 e	4.16
T_{10}	34.80	5.11 e	2.39 de	10.64
$LSD_{(0.01)}$	NS	0.144	0.277	-----

T_1 = Farmer's saved seed.

T_2 = Apparently healthy seed.

T_3 = Farmer's saved seed treated with Vitavax-200 (0.4%).

T_4 = Apparently healthy seed treated with Vitavax-200 (0.4%).

T_5 = Sundrying of farmer's saved seed.

T_6 = Sundrying of apparently healthy seed.

T_7 = Hot water treatment of farmer's saved seed.

T_8 = Hot water treatment of apparently healthy seed.

T_9 = Polythene solarization of farmer's saved seed.

T_{10} = Polythene solarization of apparently healthy seed.

seed) that was closely followed by T_9, T_7, T_{10} and T_5 treatment. From these results it was found that chemically treated seed gave the highest straw yield.

Grain yield varied from 2.16 to 3.49 t/ha. The highest grain yield was found 3.49 t/ha in T_4 treatment (apparently health and Vitavax -200 treated), which was statistically similar (3.31 t/ha) to the treatment treatment T_3 (farmer's saved seed treated with Vitavax -200). On the contrary the lowest grain yield (2.16t/ha) was recorded in the treatment T_1 (farmer's saved seed). The treatments T_4 resulted maximum 61.57% increased grain yield over untreated control. In the present study the manually sorted seeds T_2 (apparently healthy looking) and sundrying of manually sorted seeds (T_6) increased 27.31% and 31.01% grain yield over untreated control (farmer's saved seed).

CHAPTER 5

DISCUSSIONS

5. DISCUSSION

In the present study different kind of seed treatments were used for controlling leaf spot of wheat. The effect of manual seed sorting, sundrying, seed treatment with hot water and Vitavax-200 (0.4%) on germination, disease incidence in the field as well as on yield of wheat cv. Kanchan was studied. There were ten treatments in the study namely farmer's saved seed (T_1), apparently healthy seed (T_2), farmer's saved seed treated with Vitavax-200 (0.4%) (T_3), apparently healthy seed treated with Vitavax-200 (0.4%) (T_4), sundrying of farmer's saved seed (T_5), sundrying of apparently healthy seed (T_6), hot water treatment of farmer's saved seed (T_7), hot water treatment of apparently healthy seed (T_8), polythene solarization of farmer's saved seed (T_9) and polythene solarization of apparently healthy seed (T_{10}).

The effect of different treatments were differed significantly among leaf spot severity of wheat. Under *in vitro* test the lowest seed germination was recorded (76.21%) in the treatment T_1 (farmer's saved seed) where the highest germination was counted (92.25%) under the treatment T_4 (apparently healthy seed treated with Vitavax-200) which is closely followed by the treatment T_3 (farmer's saved seed treated with Vitavax-200). Increase of seed germination by Vitavax-200 treated seed have been reported by different workers (Nene and Saxena, 1971; Dang and Tyagi, 1973; Hyder-Ali and Fakir, 1993).

In respect of incidence of *Bipolaris sorokiniana*, the highest incidence was counted (25.38%) under the treatment T_1 (farmer's saved seed) and the lowest incidence was counted (1.50%) under the treatment T_4 (apparently healthy seed treated with vitavax-200). The treatment T_4 reduced 94.08%

incidence of *Bipolaris sorokiniana* over control (farmer's saved seed). Moreover untreated farmer's saved seed treated with vitavax-200 reduced 88.37% disease incidence over control. Most of the researchers found Vitavax-200 either most effective or controlled completely the seed borne infections of damaging pathogens of wheat. Mironova (1991) observed that Vitavax-200 was most effective in reducing seed borne infection of *Bipolars sorokiniana* and *Fusarium* spp. Similar reduction of seed borne *Drechslera sp. (syn Bipolaris sorokiniana)* with Vitavax-200 was also reported by Dey *et al.*, (1992).

The rest of the treatments also perfomed good in comparison to untreated farmer's saved seed. From the physically treated seeds, the treatment T_6 (sundrying of apparently healthy seed) reduced 76.79% and the treatment T_8 (apparently healthy seed treated with hot water) reduced 73.16% disease incidence over control. These results closely agreed with the report of Fakir and Jahan (1988), Mohindar *et al.* (1994) and Guldhe *et al.* (1985).

From the results it was observed that the highest germination was counted 83.89% and 87.95% at 10 and 15 DAS in the treatment T_4 (apparently healthy seed treated with vitavax-200) followed by the treatment T_3 (farmer's saved seed treated with vitavax-200). On the other hand the lowest germination was 49.22% and 58.36% at 10 and 15 DAS, respectively recorded in the treatment T_1 (farmer's saved seed). From this result it was observed that farmer's saved seed have the lowest germination percentage, this will be happened due to lack of proper storage facilities, germination inhibitory fungus or other microorganism. On the other hand chemically treated seeds gave the highest germination percentage, which was the result of destroying germination inhibitory fungus or other microorganisms.

Seed sorting (apparently healthy seed) followed by sundrying also gave higher germination percentage 70.98% and 79.35% at 10 and 15 DAS, respectively. The findings of the present study corroborates with the findings of Fakir and Jahan (1998) who reported that solar heat treatment increased 9.0% seed germination. Hot water treatment of wheat seeds at 52°C for 5 min increased seed germination by 68.76% and 74.90% at 10 and 15 DAS, respectively that was statistically similar to that of the treatment T_7 (Hot water treatment of farmer's saved seed) and T_2 (apparently healthy seeds). Uddin (2005) reported that germination percentage was higher in physically sorted lentil seeds over control (untreated seeds).

It was also observed that the the minimum plant height was found in the treatment T_1 (77.91 cm) and the highest plant height was found in the treatment T_4 (88.32 cm) and it was closely followed by treatment T_3, T_6, T_8, T_5, T_7, T_{10} and T_9.

Regarding the leaf spot severity of wheat it was found that the first onset of infection & preliminary disease development was more or less similar for all the treatments but in different growth stages the disease severity appeared to be distinct in comparison to control. It has been found that the farmer's saved seed always performed highest average disease severity at panicle initiation (0.35%) flowering (0.70%), milking (1.68%) and hard dough (2.47%) stage. The treatment T_4 (apparently healthy seed treated with vitavax-200) resulted minimum disease severity at all growth stages and it was closely followed by the treatment T_3 (farmer's saved seed treated with vitavax-200). Hyder-Ali and

Fakir (1993) observed that Vitavax-200 completely controlled seed borne infection of *A. tennis, Bipolaris sorokiniana, C. lunata* and *F. semitectum* when the seeds were treated with higher doze of chemical @ 0.5% seed weight.

It was found that sundried of physically sorted seeds also reduced the leaf spot severity at all growth stages of wheat. The possible explanation of decrease of this (*B. sorvkiniana*) fungi may be due to the seeds dried for long time, firstly the temperature acted upon primarily on the fungal propagules lying on the surface of the seeds as contaminants and with the increasing of temperature it penetrated within the seed and killed the fungal parts embedded deeper and deeper in the seeds. So the present findings showed that growth of seed-borne fungi (*B. sorvkiniana*) significantly inhibited by solar heat treatment. These results closely agreed with the report of Fakir and Jahan (1998), Mohindar *et al.* (1994) and Guldhe *et al.* (1985). According to Fakir and Jahan (1998), solar heat was most effective against major seed-borne pathogens of jute and reduced 91.3% infections. It has been reported that solar heats completely eradicate the loose smut pathogens of wheat (Luthra, 1941; Mohindar *et al.*, 1994; Guldhe *et al.*, 1985).

Results also showed that apparently healthy seeds treated with hot water at 52°C for 5 min reduced greatly the leaf spot severity over control. The findings of the present study corroborates with the study of Prabhu and Prasada (1970) who reported the elimination of *Alternaria triticina* at 52-54°C for 10 min while seed borne infection of loose smut was eliminated at 55.5°C for 10 minutes. (Bever,1951; Bedi ,1957; and Dean,1969).

It was found that apparently healthy seeds reduced the leaf spot severity in panicle initiation (0.18%), flowering (0.30%), milking (0.89%) and hard dough (1.67%) stage in comparison to control. The findings are in agreement with Hossain and Doullah (1998), who found cleaning and seed washing of farmer's seed reduced the seedling disease and increased yield up to 53.87% and 14.77% respectively over the unclean farmers saved seed.It was observed that the the treatment T_4 (apparently healthy seeds treated with vitavax-200) resulted the highest number of grains/ear (38.19) and healthy grains /ear (37.89) and the lowest diseased grains/ear (0.30) which was closely followed by farmer's saved seed treated with vitavax-200 (T_3) and sundried apparently healthy (T_6)seeds and hot water treated apparently healthy seeds. Farmer's saved seed (T_1) always resulted significantly the lowest number of grains/ear (31.11), healthy grains/ear (28.55) and diseased grains/ear (2.56). Rahman *et al.* (2000) reported that seed treatment with Vitavax-200 and manually sorted seeds produced the highest number of healthy grains.

Regarding wheat seed yield, significant increase in grain yield was obtained in all the treatments over control. The highest grain yield (3.49 ton/ha) was recorded in the treatment T_4 (apparently healthy seeds treated with Vitavax-200) and it was significantly similar to that of treatment T_3. The lowest yield was recorded in the treatment T_1 (farmer's saved seed). It was observed that grain yield was increased 4.16%, 6.94%, 10.64%, 15.74%, 27.31%, 28.70% and 31.01% for the treatments T_9, T_7, T_{10}, T_5, T_2, T_8 and T_6 , respectively. Hossein (2002) reported that farmer's clean seed, washed farmer's seed, washed clean seed and seed treated with Vitavax-200 increased grain yield by 16.62%, 16.45%, 23.39% and 26.60%, respectively over farmer's saved seed of rice (cv. BR11).

CHAPTER 6

SUMMARY AND CONCLUSION

6. SUMMARY AND CONCLUSION

An experiment was conducted in the farm of Sher-e-Bangla Agricultural University, Dhaka, during the period of November 2005 to April, 2006 to study on the management of leaf spot of wheat caused by *Bipolaris sorokiniana*. The experiment was laid out in the Randomized Complete Block Design with three replications. Wheat variety Kanchan was used. Farmer's saved seed (T_1), apparently healthy seed (T_2), farmer's saved seed treated with Vitavax-200 (0.4%) (T_3), apparently healthy seed treated with Vitavax-200 (0.4%) (T_4), sundrying of farmer's saved seed (T_5), sundrying of apparently healthy seed (T_6), hot water treatment of farmer's saved seed (T_7), hot water treatment of apparently healthy seed (T_8), polythene solarization of farmer's saved seed (T_9) and polythene solarization of apparently healthy seed (T_{10}) were used to explore the possibility of controlling the leaf spot disease of wheat.

The observations were made on the effect of the treatments on percent seed germination, percent seed infection, percent leaf spot severity, seed yield, yield contributing characters and thousand seed weight. Before sowing seeds, seed germination and seed infection were investigated.

Under *in vitro* test, the highest germination (92.25%) was noted in T_4 (apparently healthy seed treated with Vitavax -200 (0.4%) which was followed by that of T_3 (farmer's saved seed seed treated with Vitavax-200 (0.4%). (89.72%), where the lowest germination was observed in control (76.21%) treatment. On the other hand, sundrying of wheat seed, hot water treated seeds and apparently healthy seeds also gave the good performance than untreated farmer's saved seed.

The lowest seed infection by *Bipolaris sorokiniana* was recorded in treatment T_4 followed by T_3. On the other hand, among the physical seed treatment, sundried apparently healthy seed gave the lowest seed infection followed by apparently healthy seed treated with hot water and apparently healthy seed. The highest percent seed infection was noted in farmer's saved seed (T_1).

The lowest percent leaf infection was observed in case of Vitavax -200 treated seed which was followed by sundrying, hot water treated, apparently healthy and polythene solarized seeds. The highest percent leaf infection was recorded in control treatment. All the treatments under this experiment were also differed in respect of seed yield in comparison to control. The seed yield was recorded highest in apparently healthy Vitavax-200 treated seed, followed by by sundried, hot water treated, apparently healthy and polythene solarized seeds. The lowest seed yield was observed in control treatment (T_1).

However, considering the overall performances of chemicals and other physical treatments in controlling leaf spot disease of wheat, Vitavax -200 was found best followed by other treatments. Seed treatment with the above chemical not only reduced the seed borne infections and increased germination of seeds, it also decreased leaf spot severity and increased seed yield. From the results it also observed that solar heat treatment of apparently healthy seeds for 14 hours gave good control of leaf spot disease and increased seed yield. Hot water treatment of apparently healthy seeds at 52°C for 5 min. gave good result that was more or less similar to that of sundrying. Uses of apparently healthy seed also performed better than untreated control. The rest of the treatments have some remarkable effect in controlling the disease.

The findings the present study revealed that Vitavax -200 treated seeds revealed to be effective for controlling leaf spot of wheat among the treatments employed in the experiments. It was also observed that uses of apparently healthy seed, sundried and hot water treated can be reduced leaf spot disease and increased seed yield. Further study is needed to determine the suitable time for sundrying of seed and also the temperature and effective time period for suppression of the seed borne *Bipolaris sorokiniana* and leaf spot in field.

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APPENDICES

APPENDIX

Appendix I. Analysis of variance of the data on germination and yield contributing characters of wheat

Sources of variation	Degrees of freedom	Mean square				
		Germination (%)		Plant height (cm)	Spike length (cm)	Length between panicle initiation and point of spike (cm)
		10 DAS	15 DAS			
Replication	2	0.365	6.400	4.048	1.849	0.036
Treatment	9	389.391**	284.783**	34.693**	0.299	0.645
Error	18	15.917	11.690	8.786	1.577	1.325

DAS: Day After Sowing

** Significant at 1% level of significance

Appendix II. Analysis of variance of the data on disease severity at panicle initiation and flowering stage of wheat

Sources of variation	df	Mean square					
		Disease severity at Panicle initiation Stage			Disease severity at Flowering Stage		
		Flag leaf	2nd leaf	3rd leaf	Flag leaf	2nd leaf	3rd leaf
Replication	2	0.001	0.001	0.001	0.001	0.001	0.0001
Treatment	9	0.002**	0.020**	0.116**	0.121**	0.196**	0.259**
Error	18	0.0001	0.0001	0.001	0.0001	0.001	0.001

DAS: Day After Sowing
** Significant at 1% level of significance

df : Degrees of freedom

Appendix III. Analysis of variance of the data on disease severity at milking and hard dough stage of wheat

Sources of variation	Degree of freedom	Mean square					
		Disease severity at Milking Stage			Disease severity at Hard dough Stage		
		Flag leaf	2^{nd} leaf	3^{rd} leaf	Flag leaf	2^{nd} leaf	3^{rd} leaf
Replication	2	0.001	0.001	0.001	0.001	0.001	0.003
Treatment	9	0.124**	0.671**	1.374**	0.435**	0.826**	1.406**
Error	18	0.001	0.001	0.001	0.001	0.000	0.001

** Significant at 1% level of significance

Appendix IV. Analysis of variance of the data on yield contributing character of wheat

Sources of variation	df	Mean square				
		Healthy spikelets/ear	Healthy grains/ear (No.)	Disease grains/ear (No.)	Healthy grains/ear (wt.)	Wt. of diseased grains/ear (g)
Replication	2	4.316	0.361	0.026	0.003	0.0001
Treatment	9	17.118**	69.09**	1.744**	0.047**	0.002**
Error	18	2.262	10.05	0.011	0.006	0.0001

** Significant at 1% level of significance

df : Degrees of freedom

Appendix V. Analysis of variance of the data on yield contributing character and yield of wheat

Sources of variation	df	Mean square				
		Healthy seed/plant	Diseased seed/plant	Straw yield (t/ha)	Grain yield (t/ha)	1000 seed weight (g)
Replication	2	0.001	0.001	0.006	0.001	13.307
Treatment	9	29.534*	1.345**	0.779**	0.615**	12.399**
Error	18	4.578	0.006	0.007	0.026	7.695

* Significant at 5% level of significance
** Significant at 1% level of significance
df : Degrees of freedom

Appendix VI. Analysis of variance of the data on grading of wheat seed

Sources of variation	Degree of freedom	Mean square				
		Grading of seeds (0-5 scale)				
		1	2	3	4	5
Replicatio n	2	0.534	0.0001	0.0001	0.0001	0.0001
Treatment	9	29.072**	0.082**	0.088**	0.038**	0.008**
Error	18	3.078	0.001	0.0001	0.0001	0.0001

** Significant at 1% level of significance

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 discolored.