TRANSMISSION OF *Bipolaris sorokiniana* FROM SEED TO PLANT TO SEED OF WHEAT

MD. REZAUL ISLAM

DEPARTMENT OF PLANT PATHOLOGY
SHER-E-BANGLA AGRICULTURAL UNIVERSITY
DHAKA 1207

DECEMBER, 2009

TRANSMISSION OF *Bipolaris sorokiniana* FROM SEED TO PLANT TO SEED OF WHEAT

BY

MD. REZAUL ISLAM

REG. NO. : 03-01078

A Thesis
Submitted to the Department of Plant Pathology
Sher-e-Bangla Agricultural University, Dhaka
in partial fulfillment of the requirements
for the degree
of

MASTER OF SCIENCE (MS)
IN
PLANT PATHOLOGY

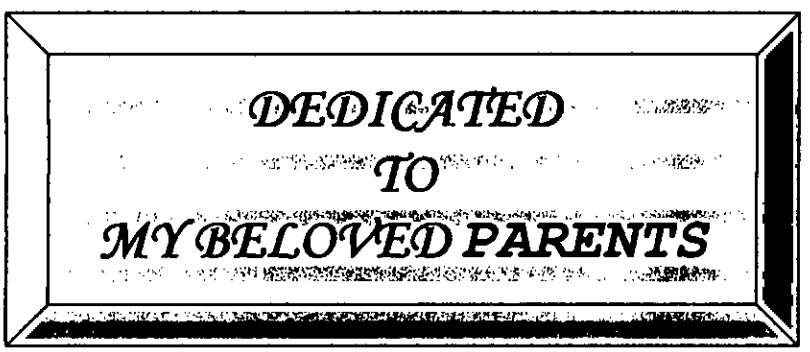
SEMESTER: JULY-DECEMBER, 2009

APPROVED BY:

Dr. M. Salahuddin M. Chowdhury
Associate Professor
Department of Plant Pathology
Sher-e-Bangla Agricultural University
Supervisor

Dr. F. M. Aminuzzaman
Assistant Professor
Department of Plant Pathology
Sher-e-Bangla Agricultural University
Co-Supervisor

Dr. M. Salahuddin M. Chowdhury
Chairman
Examination Committee


DEDICATED
TO
MY BELOVED PARENTS

DEPARTMENT OF PLANT PATHOLOGY

Sher-e-Bangla Agricultural University

Sher-e-Bangla Nagar, Dhaka-1207

Memo No: SAU/Plant Pathology

CERTIFICATE

This is to certify that the thesis entitled "**Transmission of *Bipolaris sorokiniana* from Seed to Plant to Seed 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 **Md. Rezaul Islam**, Registration number: **03-01078** 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 duly been acknowledged.

Dated: 31.12.2009
Dhaka, Bangladesh

Dr. M. Salahuddin M. Chowdhury
Department of Plant Pathology
Sher-e-Bangla Agricultural University
Dhaka-1207
Supervisor

SHER-E-BANGLA AGRICULTURAL U...

ACKNOWLEDGEMENTS

All praises are due to Almighty Allah, the Great, Gracious and Merciful, Whose blessings enabled the author to complete this research work successfully.

The author likes to express his deepest sense of gratitude to his respected supervisor Dr. M. Salahuddin M. Chowdhury, Associate Professor and Chairman Department of Plant Pathology, Sher-e-Bangla Agricultural University (SAU), Dhaka, Bangladesh for his scholastic guidance, support, encouragement and valuable suggestions and constructive criticism throughout the study period and gratuitous labor in conducting and successfully completing the research work and in the preparation of the manuscript writing.

The author also expresses his gratefulness to his respected Co-Supervisor, Dr. F. M. Aminuzzaman, Assistant Professor, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka for his scholastic guidance, helpful comments and constant inspiration, inestimatable help, valuable suggestions throughout the research work and in preparation of the thesis.

The author also expresses heartfelt thanks to all the teachers of the Department of Plant Pathology, SAU, for their valuable suggestions, instuctions, cordial help and encouragement during the period of the study.

The author expresses his sincere appreciation to his brother, sisters, relatives, well wishers and friends for their inspiration, help and encouragement throughout the study period.

The Author

TRANSMISSION OF *Bipolaris sorokiniana* FROM SEED TO PLANT TO SEED OF WHEAT

ABSTRACT

The experiments were conducted in the Seed Health Laboratory and Experimental Field of Plant Pathology Department, Sher-e-Bangla Agricultural University, Dhaka-1207, during the period from October 2008 to April 2009 to find out the transmission of *Bipolaris sorokiniana* from seed to plant to seed of wheat. There were altogether eight samples that used as treatment for different experiment which are as follows: T_1 = 0% seed infection (Bavistin treated @ 0.25% of seed weight); T_2 = 0% seed infection (Provax treated @ 0.25% of seed weight); T_3 = 0.1-10% seed infection; T_4 = 10.1-15% seed infection; T_5 = 15.1-20% seed infection; T_6 = 20.1-25% seed infection; T_7 = 25.1-40% seed infection and T_8 = 50.1-55% seed infection. Disease severity showed significant variation at flag leaf stage, panicle initiation stage, flowering stage, milking stage and hard dough stage for without and with chemical control measure. For without chemical control measure, at flag leaf stage, in an average the lowest disease severity (0.09) was recorded in T_1 and the highest (0.45) in T_8. At panicle initiation stage, in an average the lowest disease severity (0.15) was observed in T_1, whereas the highest (1.06) in T_8. At flowering stage, in an average the lowest (0.43) was found in T_1 and T_2, whereas the highest (1.32) was found in T_8. At milking stage, in an average, the lowest disease severity (1.14) was recorded in T_1 and the highest (2.10) in T_8. At hard dough stage, in an average the lowest disease severity was recorded in T_1 (2.30) and the highest in T_8 (3.81). Considering plant height the longest plant (89.35 cm) was recorded in T_1 whereas the shortest (80.80 cm) in T_8. For grain yields per hectare the highest (3.67 tonnes) were recorded in T_1 while the lowest yield per hectare (2.25 tonnes) in T_8. In field condition with chemical control measures at flag leaf stage, in an average the lowest disease severity (0.07) was recorded in T_1 and the highest (0.38) in T_8. At panicle initiation stage, in an average the lowest disease severity (0.13) was observed in T_1 whereas the highest (0.92) was recorded in T_8. At flowering stage, in an average the lowest (0.39) was recorded in T_1 while the highest (1.25) in T_8. At milking stage, in an average the lowest disease severity (0.97) was observed in T_1 and the highest (1.96) in T_8. At hard dough stage, in an average the lowest disease severity (1.70) was recorded in T_1 and the highest (3.56) in T_8. In respect of plant height, the longest plant (90.10 cm) was recorded in T_1 while the shortest (81.37 cm) in T_8. In respect of grain yields per hectare the highest (3.71 tonnes) was recorded in T_1 while the lowest (2.29 tonnes) in T_8.There is a linear relationship between number of infected seedlings/m^2 and incidence of *Bipolaris sorokiniana* and the relationship was highly positive for without and with chemical control measures.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

CHAPTER 1

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most important cereal crops as well as staple food all over the world. About two third of the world's population consume wheat as staple food (Majumder, 1991). It supplies mainly carbohydrate (69.60%) and also protein (12%), fat (1.72%) and minerals (16.20%) (BARI, 1997). Dubin and Ginkel (1991) reported that the largest area of wheat cultivation in the warmer climates exists in the South-East Asia including Bangladesh, India and Nepal. In Bangladesh it is the second most important cereal crops next to rice that contribute to the national economy by reducing the volume of import of cereals for fulfilling the food requirements of the country (Razzaque *et al.*, 1992). Besides these, wheat and straw are also used as animal feed. Wheat straw is also used as fuel or house material of the poor man of Bangladesh.

Wheat is a well adapted cereal crop for its vegetative growth and development in our native climatic condition. Though the crop was introduced in Bangladesh during the former East Pakistan in 1967, its reputation increased after 1975. Now the popularity of wheat as staple food is rising day by day in our country. Wheat cultivation has been increased manifolds to meet up the food shortage in the country. But, in spite of its importance, the yield of the crop in our country is low (2.2 t ha^{-1}) in comparison to other countries of the world, where average yield estimated 2.69 t ha^{-1} (FAO, 1997). The area, production and yield of wheat have been increasing dramatically during the last two decades, but its present yield is too low in comparison to some developed countries like Japan, France, Germany and UK producing 3.76, 7.12, 7.28, and 8.00 t ha^{-1}, respectively (FAO, 2000). At present about 707.56 thousand hectares of land in Bangladesh is covered by wheat with the annual production of 1578 thousand tons (BBS, 2008).

All the above and below ground parts of wheat plants at all growth stages are prone to the attack of numerous diseases which play a major role among the

various factors responsible for lowering yields of the crop. Wheat suffers as many as 120 different diseases, out of which 42 are seed borne and seed transmitted including 35 diseases caused by fungi alone. Among them, seedling blight, leaf spot, bipolaris leaf blight (BLB), leaf blotch, head blight & black point caused by *Bipolaris sorokiniana* has become a serious concern in the recent years (Fakir, 1988).

In Bangladesh, yield loss due to leaf blight diseases has been reported to be 20%, in Sonalika, whereas 14% & 8% in Akbar & Kanchan, respectively (Razzaque & Hossain, 1991). In farmer's field, the yield loss was estimated to be 14.97% (Alam *et al.*, 1995), whereas 29% yield reduction was estimated during 1991-92 in Kanchan (Alam *et al.*, 1994). The diseases now days are devastating & common in the country. It may result even 100% yield loss of wheat if the plants attacked severely (Hossain and Azad, 1994).

Apart from being air borne and soil borne, the pathogen is highly seed borne and seed transmitted in wheat (Fakir *et al.*, 1977). Fakir *et al.* (1977) first indicated the possibility of transmission of the pathogen through wheat seeds in Bangladesh. Ali and Fakir (1992) reported that *B. sorokiniana* is the pathogenic to the germinating seeds and seedlings. Bazlur Rashid (1992) identified 27 fungal species associated with wheat seeds of which *B. sorokiniana* was most predominant pathogen. Prevalence of the pathogen in seed, however, depends largely on storage conditions and durations of storage. *B. sorokiniana* also colonizes in crop residues and may remain viable in soil for a long period. After sowing, as soon as the seeds start germination, the resting mycelium remaining in the pericarp and testa and in the embryo start to grow and produces inocula sufficient to invade the young seedling through the coleoptile and subsequently penetrate the upper foliage parts of the plant (Elekes, 1983). Like *Drechslera graminea* causing barely leaf stripe, *Biopolaris sorokiniana* is believed to penetrate directly from the coleoptile into the sheathing base of the first leaf and from the first leaf into the second leaf in contact with it and thus the penetration process from outer leaf to the inner leaf is repeated for all the subsequent leaves as

well as the ear. Raemaekers (1988) observed a significant correlation among vertical spread of Bipolaris leaf blight, head blight and black point incidence. The level of black point infection was dependent on infection of leaves, spikes and nodes.

The present food production of Bangladesh is 20.0 million tones and the annual food deficit around 2.0 million tones (BBS, 1999). By the end of 2010, the country will face to meet up 153 million people by producing 25.6 million tones of food. So, the country has to cope with the challenge of producing an additional 5.6 million tones of food. To attain sustainability in food production in the 21st century, there is no alternative, but use of high quality healthy seeds. But virtually it is impossible to obtain wheat seed production with a 0.0% level of black point infection which should be attainable acceptable in respect of germination, seedling vigor, plant stand and quality of seed (Hossain, 2000).

Considering the present context the study was designed with the following objectives:

1. To determine the rate of transmission of *Bipolaris sorokiniana* from seed to plant to seed,
2. To evaluate the effect of different levels of black pointed seeds on subsequent leaf spot development by *Bipolaris sorokiniana* in the field with and without chemical control measures and yield of wheat,
3. To determine the effect of different levels of black pointed seeds on subsequent grain infection by *Bipolaris sorokiniana*.

CHAPTER 2

REVIEW OF LITERATURE

CHAPTER 2

REVIEW OF LITERATURE

Black point or kernel discoloration is an important disease of wheat seeds in Bangladesh. The disease occurs in almost all wheat production areas in the country at varying degrees of severity, depending on the cultivar, location and environmental conditions. Among the various fungi associated with the disease, *Bipolaris sorokiniana* [*Cochliobolus sativus*] and *Alternaria alternata* have been the most predominant. The disease causes significant reduction in seed germination, seedling vigour, plant population and grain yield, especially when *B. sorokiniana* remains the major causal pathogen. The level of reduction in these parameters increases with the increase in the incidence and severity of infection. Very few research works related to the transmission of *Bipolaris sorokiniana* from seed to plant to seed have been carried out. The research work so far done in Bangladesh is not adequate and conclusive. However, some of the important and informative works and research findings related to the transmission and control measures of *Bipolaris sorokiniana*, so far been done at home and abroad on this crop, have been reviewed in this chapter.

Hampton (1980) found that wheat seeds were affected with *Drechslera sorokiniana* and 35-37% infection from certified wheat seeds were recorded which were apparently healthy looking. Shaner (1981) reported that the contaminated seeds could be the primary source inoculum for the *Drechslera* leaf blight on small grains, and the level of primary inoculum depends directly on the development of disease on the preceding crop and on conditions for infection during seed formation.

Rana and Gupta (1982) investigated the incidence of black point in different varieties of wheat and the effect of the disease on the germination and root and shoot growth of wheat seedlings. Incidence of black point was 3-4%, which was caused by *Helminthosporium sativum*. Black point infections greatly affected not

only seed germination but also the root and shoot growth of the seedlings. The effect was very prominent on root growth.

Elekes (1983) carried out microscopic examination on the outer layer of wheat pericarp after total peripheral stripping and staining with trypan blue to detect absolute hyphal infection of wheat seed samples. Incubation on filter paper and Papavizas agar was used to determine the degree of absolute living fungal infection. *Helminthosporium sativum* infection was found in 3-6% of the samples examined. In the incubation experiments, fungal colonies developed on 85-100% of seeds with inhibited germination.

Chaudhary *et al.* (1984) studied the effect of black point disease on germination of the grain of WL711. The germination of the diseased seeds both in blotter method and in pots was reduced to 11.6% and 16%, respectively. The invasion of pathogen on plumule and coleoptile might be impairing the germination, as lesions have been noticed in the young plumule and protruding out from diseased seeds. Reduction in germination to 44.67% has been observed in some cases. Sinha and Thapliyal (1984) found maximum reduction (38%) in germination of wheat seed infested with black pointed pathogens.

Frank (1985) recorded 6.2-29% reduction in seedling stand of winter wheat due to seed borne infection of *B. sorokiniana* alone. Saari (1985) reported that the amount of spike or kernel infection by *Helminthosporium sativum* in the tropics can be significant. If severe leaf infection is present and some rain occurs after heading, the percentage of grain infection may exceed 50%. The high level of kernel infection has major implications on seedling blight, or damping off if the grain is used for seed. Both pre and post emergence damping off will be at a high level.

Randhawa and Sharma (1985) made a survey to record the distribution of *Cochliobolus sativus,* the cause of root rot, spot blotch, leaf blight, seedling blight and black point of wheat in the Punjub state of India. Out of 263 wheat seed

samples tested, they found 211 samples carrying *C. sativus*. The level of infection in most of the samples was below 10 percent. They found significant proportion of the samples ranging 11-20 percent infection level. A few samples were found to fall in the range of 21-30 percent and very few in 31- 40 percent level of infection.

Lin (1985) stated that *H. sativum (Cochliobolus sativus)* persisted in the seed and soil. So, wheat crowns, leaves and heads could be infected at any growing stage. Seed infection rate recorded 3.4 to 60.5% and 10.1 to 30%. Black embryo seeds gave 6.2 to 10.2% lower rate of seedling survival in a plot test.

Schmidt (1986) reported that in Bangladesh, sowing of black pointed wheat seeds at various infestation levels had no effect of plant stand, yield and transmission of the disease to the subsequent crop. Nalli (1986) stated that plant grown from *Bipolaris sorokiniana* inoculated seeds produced tiller of lower height and reduced seed production.

Khanum *et al.* (1987) stated that black point responsible for the failure of germination of a high percentage of grains in the field. Visual observations indicated that natural infection of grains of the cultivars Lyp-73, Pari-73 and Pak-81 were 50%, 35% and 15%, respectively. The germination of healthy grains was 55-96.5% and that of diseased grains 34.5-71%.

Tanner (1988) reported that *Helminthosporium sativum* infected all plant parts of wheat resulting leaf spots, head blights and seed infections; and the pathogen had been considered as the major obstacle to successful rainfed wheat production in Africa. Raemaekers (1988) elucidated that in Zambia, the major constraints to wheat production under rainfed conditions was the high disease pressure caused by *Helminthosporium sativum* (*Cochliobolus sativus*). Seedling blight, stunting and root rot due to the pathogen were observed in wheat field and the infections were probably due to a combination of seed and soil borne inoculum of the pathogen.

Fakir (1988) observed that in Bangladesh, no significant effect of sowing 0.6 and 12% black point affected seeds on the yield, incidence of seedling blight or leaf blight and development of black point in the harvested grains. However, he showed that reduction in germination of black point affected seeds was directly related with the severity of infection. According to him, *D. sorokiniana* was responsible to cause more diseases to the germinating seeds and seedlings than other black point fungi.

Singh *et al.* (1989) reported that *Alternaria* type of black point of wheat did not affect germination, plant emergence, yield and intensity of root rot in the subsequent crop. But *Helminthosporium* type of black point reduced germination, seedling emergence and yield and increased the intensity of root rot in the subsequent crop.

Viedma (1990) found that the seedling infection was related to seed infection and there was a clear evidence that *D. sorokiniana* reduced the growth of wheat in the early stage. Zhang *et al.* (1990) observed that 1000 grain weight of black pointed grains infected by *Bipolaris sorokiniana* was 1.95-13.50% lower than uninfected grains.

Reis (1991) reported that the main sources of inoculum of *Bipolaris sorokiniana,* cause of spot blotch of wheat were infected seeds, infected crop residues, soil having free dormant conidia and secondary hosts. These also represented the survival mechanisms of the pathogen. He also reported that the incidences of spot blotch caused by *Bipolaris sorokiniana* in early growth stages of wheat were lower in the regions where wheat was not previously grown. It was often observed that the infected seed being the sole source of inoculum and the transmission rate of the fungus from infected seed to the coleoptile was about 2:1.

Hetzler *et al.* (1991) found the spot blotch of wheat caused by *Cochliobolus sativus* was transmitted by seed. Talukder and Fakir (1991) reported that the disease symptoms of leaf blight of wheat first appeared on the floret grains at the

hard dough stage and it become pronounced at the dead ripe stage. The percentages of black point affected grains observed at the full and dead ripe stage were 6.25 and 37.8%, respectively. Hossain and Azad (1992) reported that higher age of crop plant resulted higher incidence leaf spot (*B. sorokiniana).*

Bazlur Rashid *et al.* (1994) reported that the relationship of leaf blight incidence with the seed quality. They reported that seed quality deterioration is positively associated with the incidence of leaf blight caused by *B. sorokiniana* under field conditions. Orsi *et al.* (1994) found a positive correlation between *Drechselera sorokiniana (Cochliobolus sativus)* and black point incidence.

Bazlur Rashid (1996) reported that development of black point infection in the field was due to seed to plant to seed transmission of black point pathogen.

Bazlur Rashid (1997) reported a highly significant effect of seed borne infection by *Bipolaris sorokiniana* on the germination of seeds of wheat cvs. Kanchan and Sonalika recorded by rolled paper towel germination test as well as pot experiment. At the maximum seed borne infection level (90%) both the cultivars yielded the minimum germination of 30.25% and 26.50%, respectively. Relationship between the levels of seed borne infection and present seed germination showed gradual reduction in germination of seed with the increase of infection level. There was a trend of decrease in seed germination with the increase in seed borne infection in both the cultivars. The maximum germination reductions were found as 71.50% and 68.00% in cv. Sonalika and cv. Kanchan, respectively.

Hossain *et al.* (1998) observed that leaf infection at flowering stages has direct effect on the reduction of formation of healthy grains with the increase in number of black pointed as well as discolored grains. Rahman and Islam (1998) reported the effect black point seeds of wheat on its qualitative characters, such as weight of 1000 grains, total crude protein, total crude fiber, total ash dry matter and seed shoot vigor in respect of germination and shoot and root growth into five different

grades (Grade-0, I, II, III and IV) on the basis of level of black point infection. All the qualitative parameters except total crude proteins decreased significantly with the increase of black point infection. The decrease was more pronounced in grade III and Grade IV infected seeds. Germination percentage decreased sharply with the increased severity of infection of the disease, while shoot and root growth also decreased as the grade of infection increased.

Bazlur Rashid and Fakir (1998) reported that shriveled grain and black pointed kernel symptoms have been recorded as the effect of seed to plant to seed transmission of *B. sorokiniana.* Zhimin *et al.* (1998) reported that seed germination and seedling growth decreased with the increase in susceptibility of a variety to black point infection.

Hossain (2000) reported that seed germination and seedling emergence were significantly decreased with the increase in number of black pointed seed. The sample having 28% black pointed seed resulted maximum reduction in germination by 20.20%and 42.69% in the blotter and rolled paper towel method and emergence by 34.27% and 40.74% in the field and in the pot, respectively. The rate of reduction of growth was the maximum by 28% black pointed seeds as recorded root length was 57.21% and for shoot length was 41.40%. Significantly the highest (1582.61) and the lowest (433.16) seedling Vigor Index (VI) were recorded while the best seed treated with Vitavax and in 28% black pointed seed and seed samples 28% having black pointed seeds, respectively. Significant decrease in plant stand in pot and field has been observed with the increase in number of black pointed seeds in seed samples. Higher the level of seed borne fungal infection, there will be higher primary inoculum level in the field resulted higher infection in the field and maximum infection severity was attained at hard dough stage. The higher level of black point infection in the seed sample incited more disease to the crop plants resulting formation of higher number of diseased seed in the field.

Mondal (2000) reported that infected seeds and soils infested either with conidial suspension or colonozed grains may serve as potential source for the survival of *B. sorokiniana* resulting germination failure, seedling mortality and spot blotch development of wheat.

Successful transmission of plant diseases over long distances through the atmosphere depends on the reproductive rate of the pathogens, on the carrying capacity of the source locality, on atmospheric turbulence, stability, and wind speed, and on the survival of spores during exposure to inhospitable temperature and humidity and to UVB radiation from the sun was reported by Aylor (2003). These interacting factors were incorporated into a model to estimate the rate and extent of seasonal incursions of disease from southern into northern areas of the United States. The model indicates a practical limit for long-distance dispersal (LDD) of a plant pathogen, which depends strongly on its fecundity and on its ability to survive in the atmosphere. Two classic plant diseases, stem rust of wheat and tobacco blue mold, are used to illustrate the model. Both diseases appear to spread northward on average at about the same rate as the seasonal advance of the "green wave" of available susceptible host tissue. The near concordance of the disease wave and the green wave underscores two important points. First, it suggests that disease spread over long distances may be limited more often by pathogen establishment than by LDD. The green wave also reduces the stochastic variability and speed of disease spread by presenting a barrier to potential long-distance, low-probability dispersal events. Second, it helps to focus attention on alternative pathways for disease spread and on possible unappreciated niches for over seasoning, both of which can have important implications for disease control strategies.

Experiments were conducted by Luz (2003) in vitro and in the field, in Passo Fundo, Rio Grande do Sul, Brazil, in 1999 and 2000 in order to test the combination of bioprotector and fungicide against wheat (*Triticum aestivum*) seed pathogens: *Fusarium graminearum* [*Gibberella zeae*], *Bipolaris sorokiniana* [*Cochliobolus sativus*], *Drechslera tritici-repentis* [*Pyrenophora tritici-repentis*]

and *Aspergillus* sp. The following products and doses per 100 kg of seeds were used: Paenibacillus macerans (Embrapa 144), 13 g; Paenibacillus macerans + difenoconazole 15 FS (13+200 ml); Paenibacillus macerans + difenoconazole (13 g+100 ml); Paenibacillus macerans + difenoconazole 15 FS (6.5 g+100 ml); difenoconazole 15 FS, 200 ml. A nontreated check was maintained in the experiment. All chemical and biological treatments effectively controlled the wheat seed pathogens. The combination *Paenibacillus macerans* (Embr. 144) (13.0 g) + difenoconazole 15 FS (200 ml) completely or almost completely eradicated seed pathogens. Powdery mildew was effectively controlled only by seed treatments containing difenoconazole 15 FS, from 35 up to 40 days after sowing. All fungicide treatments significantly improved plant stand and grain yield. The beneficial effects were greater when *Paenibacillus macerans* (Embr. 144) was applied in combination with difenoconazole 15 FS, even when the fungicide dose was reduced to half.

Investigations were conducted by Vlasenko *et al.* (2004) during 2001-02 in the Ob' river region, Siberia, Russia, to study the effect of N fertilizer on diseases and yield of spring wheat. The spring medium-early wheat cv. Novosibirskaya 22 was grown after fallow following wheat in the rotation. Ammonium nitrate was applied at 90 and 120 kg/ha before sowing. Raxil [tebuconazole] was used for seed treatment and Folicur [tebuconazole] for spraying to control root and aerial infections. Seed infection with Fusarium and *Bipolaris sorokiniana* [*Cochliobolus sativus*] reached 23 and 14%, and 18 and 10% in 2001 and 2002, respectively. Data are tabulated on percentage infection of spring wheat with *Helminthosporium* and *Fusarium* root rot depending on development stage of plants and the use of chemicals compared with untreated control variants. Data are tabulated on percentage infection with powdery mildew [*Erysiphe graminis*], brown rust [*Puccinia recondita*] and Septoria leaf spots depending on fertilizer rate. Yield increases resulting from the use of the fertilizer and fungicides are outlined. It is concluded that the higher fertilizer rate increased the occurrence of plant diseases without significantly increasing yields.

Barley cultivars RD 57 and RD 2503 were used to estimate the losses due to leaf spot (*Bipolaris sorokiniana* [*Cochliobolus sativus*]) and net blotch (*Helminthosporium teres* [*Pyrenophora teres*]) in a field experiment in Karnal, Haryana, India conducted by Singh (2004). Fungicide treatments included tebuconazole (Raxil2 DS) at 1 and 1.5 g/kg, and carboxin (Vitavax 75 WP) at 2 and 2.5 g/kg, both as dry seed treatments and seed soaking solutions. Dry seed treatment with tebuconazole was repeated soaking was conducted with 60 ml of the solutions for 100 kg seeds, for 16 h. Tebuconazole dry seed treatment was repeated in the second year along with Captan at 3 g/kg. Seed soaking was not conducted during the second year. Dry seed treatments with captan, tebuconazole and carboxin were combined with spraying with propiconazole (Tilt) at 0.1% (one or 2 sprays). The maximum increase in yield was obtained with dry seed treatment with tebuconazole at 1 g/kg combined with 2 foliar sprays of propiconazole, in RD 57 during the first year and in RD 2503 during the second year. Dry seed treatment with captan + 2 sprays of propiconazole resulted in 29.3 and 13.5% increase in the number of grains/ear head and 1000-grain weight, respectively.

The efficacy of aerated steam treatment for decontamination of wheat, barley and oat seed from seed-borne diseases was tested in field trials by Forsberg *et al.* (2005) using highly infested/infected seed. For seed-borne *Fusarium* spp. and Tilletia caries in wheat, *Drechslera teres*, *D. graminea* and *Bipolaris sorokiniana* in barley and for *D. avenae* and *Ustilago avenae* in oats the pathogen control obtained from the treatment and crop yield obtained from treated seed, also in cases with slightly reduced field emergence, was equivalent with chemical seed dressing. For *Ustilago nuda* in barley the thermal treatment effect was not at the same level as reached by the chemical fungicides. Most of the treatments were performed using a large-scale treatment device with a treatment capacity of up to two tons of seed/hour. The obtained results indicate that aerated steam treatment has a potential to be a competitive alternative to chemical seed dressing for the control of seed-borne diseases in cereals.

The incidence of blackpoint (caused by *Alternaria alternata* and *Bipolaris sorokiniana* [*Cochliobolus sativus*]) was evaluated in 2 bread wheat cultivars (Buck Poncho and *B. Charrua*) by Sisterna and Sarandon (2005) grown in Buenos Aires, Argentina, during 1995 under various tillage methods (mouldboard plough, chisel and no-tillage) and N fertilizer rates (0 and 90 kg N ha-1 applied as urea at sowing or as split dressings during sowing and at the end of tillering). At harvesting, 30 ears per plot were randomly collected and hand-threshed. Natural disease incidence (percentage of discoloured grains) was determined and the grains were classified visually as healthy (normal) or discoloured grains, then weighed. The natural level of the disease was mainly affected by the cultivar and N fertilizer rate, but not by the tillage system. Discoloured grains were heavier than normal grains. Susceptibility differences associated with the morphophysiological features and nutritional status of the cultivars are discussed.

Presence of fungi in inert matter of rice and wheat seed samples was investigated by Khokon *et al.* (2005). Sixty seven seed samples representing three rice and one wheat cultivars collected from nine seed processing centers and four contact grower zones in 13 locations of Bangladesh were analyzed for the presence of inert matter and infestation of seeds by fungi. The inert matter separated from seeds was grouped into three categories: I, broken seeds, husk and awn; II, sand and soil and III, pieces of brick, stone, fiber, hair and insects. Fungi associated with inert matter were detected following centrifugation of inert matter and subsequently plating on Agar plates both the supernatant and the sediment. Inert matter accounted for 0.28% of total weight of rice seed samples with 60% in Group I category and fungi detected were *Bipolaris oryzae*, *Fusarium* spp., *Aspergillus flavus*, *A. niger*, *A. ochraceus* and *Penicillium* sp. Upon analyses by standard Blotter Method, samples of rice seed yielded *B. oryzae*, *Pyricularia oryzae*, *Alternaria padwickii* and *Fusarium* spp. with variable frequencies. Inert matter in wheat seeds accounted for 0.294% of total weight of seed samples analyzed. Fungi detected in inert matter of wheat seeds included *Fusarium moniliforme*, *B. sorokiniana*, *A. flavus*, *A. niger*, *A. candidus*, *A. ochraceaus* and

Panicillium sp. Similarly, samples of wheat seeds analyzed by the Blotter Method yielded the same fungi but in different frequencies. Also like rice, Group I category of inert matter dominated the wheat seeds samples and this component of the inert matter carried the important pathogenic fungi *B. oryzae* and *B. sorokiniana* respectively. The number of fungal colonies distinctly varied among the three categories of inert matter and was positively correlated with the total weight of inert matter. Germination of seeds was negatively correlated to seed infection and significantly affected by seed infection by *Bipolaris* sp.

Reza *et al.* (2006) reported on the effect of different levels of seed and plant infection by *Bipolaris sorokiniana* on wheat determined under induced field condition. The maximum seed rot/seedling mortality (15.73%) followed by subsequent leaf blight severity (75.40%) was recorded as a result of sowing 30% infected seeds while the minimum (5%) infected seeds resulted in 3.1% and 57.53% of seed rot/seedling mortality and leaf blight severity, respectively. They also found that 65.36% disease severity interning the corresponding 17.42% seed infection.

An experiment was conducted by Kabir *et al.* (2007) in Dhaka, Bangladesh, during December 2005-April 2006 to investigate the effects of physical and chemical seed treatments on leaf spot (*B. sorokiniana* [*Cochliobolus sativus*]) and yield of wheat. There were 10 treatments, comprising farmer's stored seeds (T_1, control), apparently healthy seeds (T_2), farmer's stored seeds treated with Vitavax 200 [carboxin+thiram] at 0.4% (T_3), apparently healthy seeds treated with Vitavax 200 at 0.4% (T_4), farmer's stored seeds washed with water (T_5), apparently healthy seeds washed with water (T_6), farmer's stored seeds treated with brine solution at 2% (T_7), apparently healthy seeds treated with brine solution at 2% (T_8), washed farmer's stored seeds treated with brine solution at 2% (T_9) and washed apparently healthy seeds treated with brine solution at 2% (T_{10}). The physical seed treatments increased the seed germination and suppressed the incidence of *B. sorokiniana* in the laboratory. The lowest germination (84.20 and 86.08%, respectively, at 10 and 15 days after sowing, DAS) was recorded in T_4 and the lowest (49.84 and 55.54%

at 10 and 15 DAS, respectively) was recorded in T_1. The lowest disease severity at panicle initiation, flowering, milking and hard dough stages was recorded in T_4 (vitavax treated seed), which were 0.05, 0.19, 0.36 and 0.84, respectively. On the other hand, the highest disease severity at panicle initiation, flowering, milking and hard dough stages was recorded in T_1, which were 0.55, 1.21, 1.69 and 2.52, respectively. The highest grain yield (3.5 t/ha) was obtained in T_4, which was 75% increased over the control. Thus, physically sorted apparently healthy seeds treated with Vitavax 200 may be recommended to suppress leaf spot, with increasing grain yield of wheat.

Laboratory and field experiments were carried out to determine the effect of manual seed sorting, seed solarization and seed treatment with Vitavax-200 [carboxin + thiram] and hot water on black point (*Bipolaris sorokiniana* [*Cochliobolus sativus*]) and seed yield and yield contributing characters of wheat from November 2005 to April 2006 at the farm of Sher-e-Bangla Agricultural University, Dhaka, Bangladesh by Zobaer *et al.* (2007). The highest reduction of seedborne *Bipolaris sorokiniana* incidence was recorded by manually sorted healthy looking seeds treated with Vitavax-200 (T_4). Significant reduction of incidence of the same fungus was achieved by treating with hot water and solar heat. Seed treatment of apparently healthy seeds with Vitavax-200 (at 0.4%) reduced the pathogen incidence, and recorded the highest seed germination and increased seed yield, followed by the seed treatment of unclean seeds with Vitavax-200 (at 0.4%). Only manually sorted seeds reduced the incidence of *Bipolaris sorokiniana* (66.15%) over the untreated control. Sun-dried sorted seeds and hot water-treated sorted seeds had reduced incidence of *Bipolaris sorokiniana* (76.79% and 73.16%, respectively) over the untreated control.

An experiment was carried out by Zamal *et al.* (2007) to determine the comparative efficacy of Vitavax-200 [carboxin + thiram] (0.4%), Tilt [propiconazole]-250 EC (0.1%), Bavistin [carbendazim]-50 WP (0.1%) and Pencozeb [mancozeb]-80 WP (0.2%) in controlling leaf blight of wheat caused by *Bipolaris sorokiniana* [*Cochliobolus sativus*] under field conditions. The single

effects of Vitavax-200, Tilt-250 EC, Bavistin-50 WP and Pencozeb-80 WP were found less effective than that of combined effect of Vitavax-200 with Tilt-250 EC, Vitavax-200 with Pencozeb-80 WP and Vitavax-200 with Bavistin-50 WP. The effect of seed treatment with Vitavax-200 followed by subsequent three times foliar spray with Tilt-250 EC (T_8) has been found significantly more effective than the other single and combined effects in reducing leaf blight severity of wheat. Maximum grain yield (4.05 t/ha) was found under the treatment T_8 (Vitavax-200 with three sprays of Tilt-250 EC) which was 44.64% increased grain yield over the untreated control. However, treatment T_8 had a statistically similar effect to that of treatment T_7 (Vitavax-200 with two sprays of Tilt-250 EC) in reducing leaf blight severity and increasing wheat grain yield over the control.

Malaker *et al.* (2008) reported that black point or kernel discoloration is an important disease of wheat seeds in Bangladesh. The disease causes significant reduction in seed germination, seedling vigour, plant population and grain yield, especially when *B. sorokiniana* remains the major causal pathogen. The level of reduction in these parameters increases with the increase in the incidence and severity of infection. Cultivars and advanced lines of wheat in Bangladesh have been evaluated for their genetic resistance against this disease. However, none of these genotypes were completely free from the disease. Only a few lines showed less than 5% infection, while the others exhibited susceptibility at various degrees. Attempts to control the disease through seed dressing and foliar spray with different fungicides have also been made. Among the seed treating fungicides used, Vitavax-200 [carboxin + thiram] was the most effective, significantly increasing seedling emergence and grain yield. Of the spray fungicides tested, propiconazole was the most effective in reducing black point incidence in harvested seeds.

An investigation of the effect of different levels of black pointed seeds on seedling vigor, leaf blight (*Bipolaris sorokiniana*) development and quality seed production of wheat was carried out by Shukti (2008) in the Seed Health Laboratory, Department of Plant Pathology with seven treatments viz., T_1 (0%

seed infection), T_2 (5.1-15% seed infection), T_3 (15.1-25% seed infection), T_4 (25.1-35% seed infection), T_5 (35.1-45% seed infection) and T_6 (45.1-60% seed infection) and found that under *in vitro* condition, seed germination and seedling vigor were lessened, whereas seedling infection was increased with the gradual increase of seed infection. Germination of seeds and vigor of seedlings were the highest in T_1 and the lowest in T_6, respectively. The maximum and the minimum seedling infection were recorded in T_6 and T_1, respectively. In the field condition, significant variations were found considering number of seedlings/m^2. The highest (122.19) and the lowest number of seedlings/m^2 (90.20) were recorded in T_1 and T_6, respectively, whereas the maximum (25.73) and the minimum (8.54) number of infected seedlings/m^2 was found in T_6 and T_1, respectively. Leaf blight severity (0-5 scale) on flag leaf and penultimate leaf was recorded in flag leaf stage, panicle initiation stage, flowering stage, milking stage and hard dough stage and positive co-relationship was found between seed infection levels and the average disease severity. Average disease severity on leaf was found the highest in T_6 and the lowest in T_1, respectively in all growth stages. The highest plant height (89.67 cm) and spike length (15.95 cm) were observed in T_1. The maximum number of spikelets/ear (31.24) and number of health spikelets/ear (5.34) were found in T_6. Number of grains/ear and number of healthy grains/ear were the highest in plots of T_1, while number of diseased grains/ear was the highest in T_6. The highest grain yield (3.67 t/ha) and straw yield (6.41 t/ha) were recorded in T_1, where grain yield was increased upto 38.69% over T_6. Highly positive linear relationship was found between seed infection levels and incidence of *Bipolaris sorokiniana* on harvested seeds.

Autoinfection (within host inoculum transmission) allows plant pathogens locally to increase their density on an infected host was reported by Lannou *et al.* (2008). Estimating autoinfection is of particular importance in understanding epidemic development in host mixtures. More generally, autoinfection influences the rate of host colonization by the pathogen, as well as pathogen evolution. Despite its importance in epidemiological models, autoinfection has not yet been directly

quantified. It was measured here on wheat (*Triticum aestivum*) leaves infected by a pathogenic fungus (*Puccinia triticina*). Autoinfection was measured either on inoculated leaves or by assessing the local progeny of spontaneous infections, and was described by a model of the form y = micro × alpha, where alpha accounts for host saturation and micro represents the pathogen multiplication rate resulting from autoinfection. It was shown that autoinfection resulted in typical patterns of disease aggregation at the leaf level and influenced lesion distribution in the crop during the first epidemic stages. The parameter micro was calculated by taking overdispersion of the data and density dependence into account. It was found that a single lesion produced between 50 and 200 offspring by autoinfection, within a pathogen generation.

Uttam *et al.* (2009) reported that spot blotch caused by *Bipolaris sorokiniana* is a destructive disease of wheat in warm and humid wheat growing regions of the world. The development of disease resistant cultivars is considered as the most effective control strategy for spot blotch.

CHAPTER 3

MATERIALS AND METHODS

CHAPTER 3

MATERIALS AND METHODS

The experiments were conducted in the Seed Health Laboratory and Experimental Field of Plant Pathology Department, Sher-e-Bangla Agricultural University, Dhaka-1207, during the period from October 2008 to April 2009 to find out the transmission of *Bipolaris sorokiniana* from seed to plant to seed of wheat. This chapter presenting a brief description of the location of the experiment, climatic condition, test crop and its characteristics, collection of seeds, seed processing for grading, treatments, experimental design, experimental operations, data collection procedures and analysis of different parameters under the following headings-

3.1 Description of the experiment

The details experimental description was presented below:

3.1.1 Location of the experiment

The experiments were carried out in the Seed Health Laboratory and experimental field of Plant Pathology Department, Sher-e-Bangla Agricultural University, Dhaka-1207. The experimental site was located at $90^0$22′E longitude and $23^0$41′N latitude at an altitude of 8.6 meters above the sea level in the agro-ecological region of "Madhupur Tract" (AEZ No. 28). Details characteristics of the soil presented in Appendix I.

3.1.2 Climatic condition

The experimental area under the sub-tropical climate that is characterized by high temperature, high humidity and heavy rainfall with occasional gusty winds in kharif season (April-September) and less rainfall associated with moderately low temperature during the rabi season (October-March). The weather data regarding temperature, rainfall, relative humidity and sunshine hour were collected from the weather station, Agargaon, Dhaka during the study period at the experimental site is presented in Appendix II.

3.1.3 Test crop and its characteristics

The test crop was wheat (*Triticum aestivum L.*) and variety was 'Shatabdi' the most widely cultivated variety in the country. Shatabdi (BARI Gom 21) is a modern wheat variety released by BARI in 2000. It is semi-dwarf (95-100 cm) with good tillering ability. It produces generally 5-6 tillers per plant. The leaves are broad, recurved and light green in color. Flag leaves are also broad and droopy. The plants are light green in color with very weak glaucosity in the spike, culm and flag leaf sheath. Lower glume beak is long and the lower glume shoulder shape is elevated. The total life duration ranges from 105-112 days and the vegetative phase (66-75 days) is longer than any other variety so far released in our country. The grains are white and large with weight of 1000-grains ranges from 46-48 g. The variety is resistant to leaf rust and highly tolerant to *Bipolaris* leaf blight. The variety has good level of tolerance to heat stress and is best suited both under optimum and late planting for rice-wheat cropping system. Under normal environmental condition, the variety yields 3600-5000 kg/ha.

3.1.4 Collection of seeds

Wheat seed samples of variety Shatabdi was collected from BADC in Gazipur district of Bangladesh. The collected samples were stored in paper bag covered with polythene in normal temperature in Seed Pathology Laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka.

3.1.5 Seed Processing for Grading

The collected seed sample was physically sorted out to separate apparently healthy looking seeds with bold golden color and black pointed seeds to classify the seeds in different grades containing different level of black pointed seed. Healthy seeds treated with Bavistin and Provax (@ o.25% of seed weight) were graded as T_1 and T_2, respectively. Each sub-samples considered as different treatments that were used in different laboratory and field experiments. There were altogether eight samples that used as treatments for different experiments which are as follows:

T_1 = 0% seed infection (Bavistin treated @ 0.25% of seed weight)

T_2 = 0% seed infection (Provax treated @ 0.25% of seed weight)

T_3 = 0.1-10% seed infection

T_4 = 10.1-15% seed infection

T_5 = 15.1-20% seed infection

T_6 = 20.1-25% seed infection

T_7 = 25.1- 40% seed infection and

T_8 = 50.1-55% seed infection

3.1.6 Experimental details

Consequently four experiments were conducted to find out the transmission of *Bipolaris sorokiniana* from seed to plant to seed of wheat. Among the experiment 1 and 4 were conducted in laboratory condition and experiment 2 and 3 were conducted in field condition at same time. The title of the experiments presented below:

Experiment-1: Effect of different levels of black pointed seeds on germination and health status before sowing in blotter method and water agar test tube seedling symptom test

Experiment-2: Effect of different levels of black pointed seeds on disease incidence, leaf blight severity and quality seed production of wheat

Experiment-3: Effect of different levels of black pointed seeds after chemical control measures on disease incidence, leaf blight severity and quality seed production of wheat

Experiment-4: Effect of different levels of black pointed seeds on germination and health status of harvested seeds of wheat in blotter method both for the seeds of without and with chemical treatment

3.1.7 Experimental design and layout

All of the laboratory experiments were laid out in Complete Block Design (CBD) with three replications and all of the field experiments were laid out in Randomized Complete Block Design (RCBD) with three replications.

3.2 Laboratory experiment

Experiment 1 and 4 were conducted in laboratory condition for seed health status and incidence of *Bipolaris sorokiniana*. Details were presented below:

3.2.1 Seed health status of the selected samples

To determine the seed health status, the blotter method (ISTA, 1996) was used. In this method, three layers of blotter paper were soaked in sterilized water and placed at the bottom of each sterilized glass petridish. Then, twenty five seeds were plated on the blotter paper in a petridish maintaining equal distance and covered with lid. The seeds on petridishes were incubated in an air cooled room at about 22^0C temperature under 12/12 hr light and darkness cycle for 7 days in Seed Health Laboratory. Sterilized water was added time to time to maintain the moisture. After 7 days of incubation, the seeds were observed for the presence of seed-borne *Bipolaris sorokiniana* under sterio-binocular microscope. Germination of the seeds was also recorded. All of the samples are tested separately by blotter method and the following data was recorded:

3.2.1.1 Germination (%)

The germination of seedlings in the petridishes was recorded on the basis of visible emergence of seedlings. The number of seedling was counted starting from 6 days after plated (DAP) and continued upto 10 DAP. Percentage of germination was calculated by using the following formula:

$$\text{Germination (\%)} = \frac{\text{Number of germinated seeds}}{\text{Number of plated seeds}} \times 100$$

3.2.1.2 Incidence of *Bipolaris sorokiniana* (%)

The incidence of *Bipolaris sorokiniana* was counted from the germinated seedlings in the petridishes was recorded on the basis of visible infection of emerged seedlings. The number of infected seedling was counted starting from 6 days after plated (DAP) and continued upto 10 DAP. Percentage of incidence was calculated by using the following formula:

$$\text{Incidence of } \textit{Bipolaris sorokiniana} \ (\%) = \frac{\text{Number of Infected seedlings}}{\text{Number of germinated seedlings}} \times 100$$

3.2.2 Water Agar Test tube Seedling Symptom Test

The Water Agar Test tube Seedling Symptom test was used in the present evaluation. In this technique, test tube slants were prepared by pouring 10 ml of 1% water agar in each test tube (2 cm in diameter and 15 cm in length) and then sterilized in autoclave for 6-7 minutes under 15 lbs pressure at 121^0C. The water agar in the test tube was solidified at an angle of 60^0 so that the seeds could be placed on the slanted agar conveniently and record of pathogens could be taken easily. One hundred seeds from each treatment were taken and one seed per test tube were placed on the solidified water agar slant at the rate of one seed per tube. The tubes were closed with cotton plugs and arranged in plastic racks. The tubes were then incubated at erect condition in an air cooled room (22^0C) under fluorescent day light tube. The cotton plug was then removed when the seedlings reached the rim of the test tube. Data on germination, number of normal seedlings, number of abnormal seedlings and number of dead seeds were recorded. The normal and abnormal seedlings were categorized according to ISTA rules (1996).

3.2.2.1 Normal seedlings

The normal seedlings were categorized by considering the following points individually or combinedly:

i. Intact seedling with all essential structures, well developed, complete in proportion of all structures and healthy.

ii. Seedlings with slight defects of their essential structures and otherwise satisfactory and balanced development comparable to that of intact seedling in the same test.

iii. Seedlings with secondary infection that would have fallen into categories i or ii but found infection by fungi or bacteria from sources other than the parent seed.

3.2.2.2 Abnormal seedlings

During recording the abnormalities the germinating seeds and seedlings, the following points were considered:

i. Seminal roots missing/stunted or broken and decayed due to primary infection.

ii. Coleoptile missing/split and deformed or bent over.

iii. Shoot system (the mesocotyl if developed) broken/decayed.

iv. Leaf missing/extending less than half-way up the coleoptile, shredded or deformed.

v. Seedling as a whole deformed spindly, discolored or decayed as a result of primary infection.

vi. Partially blackened dead or decayed seed.

3.2.2.3 Dead seeds

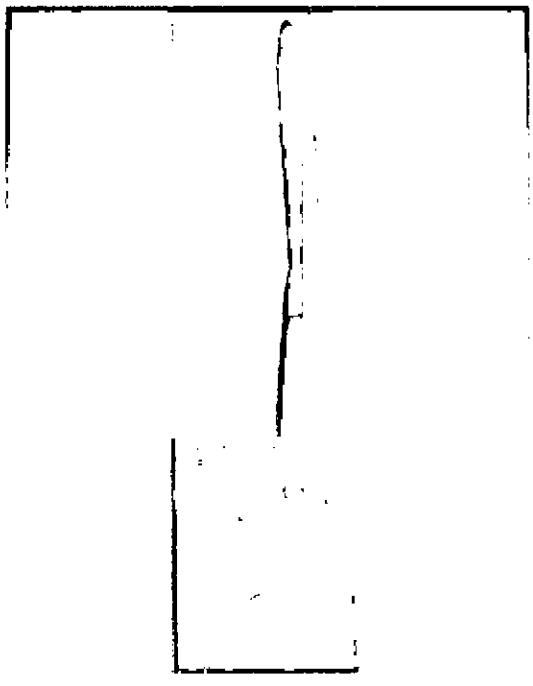
During recording the abnormalities the germinating seeds and seedlings, the following points were considered:

i. Seeds with appearing any seedlings i.e. no germination

ii. Blackened dead or decayed seed

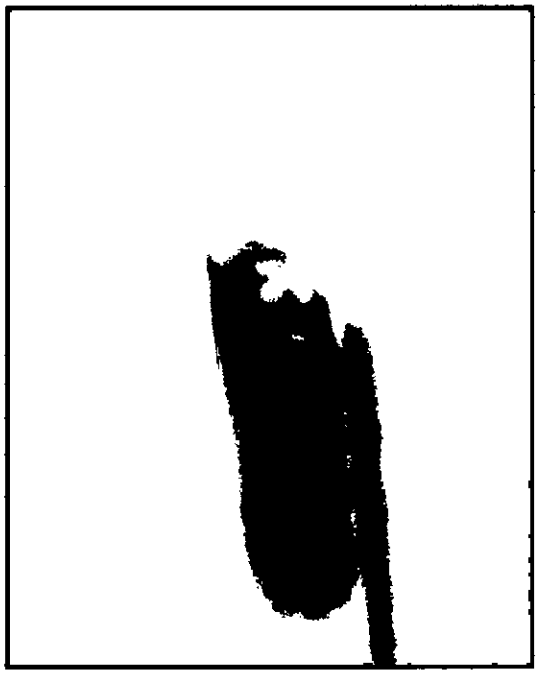
The dead seedlings were calculated by using the following formula:

Dead seeds = 100 – (Normal seedlings + Abnormal seedlings)



Normal seedling

Abnormal Seedling



Dead seeds

Plate 1. Photograph showing normal and abnormal seedling

3.3 Field experiment

Experiment 2 and 3 were conducted at a same time in field condition to evaluate the effect of different levels of black pointed seeds on disease incidence, leaf blight severity and quality seed production of wheat without and with chemical treatment. Details were presented below:

3.3.1 Soil

The farm belongs to the general soil type, Shallow Red Brown Terrace Soils under Tejgaon Series. The land was above flood level and sufficient sunshine was available during the experimental period. Soil samples from 0-15 cm depths were collected from experimental field. The analyses were done at Soil Resource and Development Institute (SRDI), Dhaka. The physicochemical properties of the soil are presented in Appendix I.

3.3.2 Growing of crops

The followings initiatives were undertaken for growing of wheat crops of these field experiments:

3.3.2.1 Preparation of the main field

The experiment plot was opened in the first October 2008 with a power tiller, and was exposed to the sun for a week, after which the land was harrowed, ploughed and cross-ploughed several times followed by laddering. Weeds and stubble were removed, and finally obtained a desirable tilth and properly leveled for seed sowing. The main plot was sub-divided into 48 unit plots with sized 2 m × 1.5 m.

3.3.2.2 Fertilizers and manure application

The fertilizers N, P, K, S, Zn and B in the form of Urea, TSP, MP, Gypsum, Zinc sulphate and borax, respectively were applied. The entire amount of TSP, MP, Gypsum, Zinc sulphate and borax were applied during the final preparation of land. Urea was applied in two equal installments at final land preparation and before flowering after 45 days of seeds sowing. The dose and method of application of fertilizer are shown in Table 1.

Table 1. Dose and method of application of fertilizers in wheat field

Fertilizers	Dose (kg/ha)	Application (%)	
		Basal	1st installment (21 DAS)
Urea	200 kg	67	33
TSP	180 kg	100	--
MP	50 kg	100	--
Gypsum	120 kg	100	--
Cowdung	10 tons	100	--

Source: BARI 2005, Joydebpur, Gazipur

3.3.2.3 Sowing of seeds

Wheat seeds were sown in the field on 5th December, 2008 at the rate of 120 kg/ha. The seeds were placed continuously in lines properly at a depth of 5 cm and were covered with the help of hand. The distance between lines was 25 cm that made 6 rows in unit plot.

3.3.2.4 After care

3.3.2.4.1 Irrigation and drainage

Irrigation was done once after 25 days and another 45 days of sowing. Irrigation was generally followed the each weeding of the crops. It was arranged well drained facilities as prevention process of removing rain water if any.

3.3.2.4.2 Weeding

Weeding was performed twice during the growing period of the crops for better soil aeration and conservation of soil moisture. Weeding was done carefully keeping the delicate young plants undisturbed.

3.3.2.4.3 Top dressing

After basal dose, the remaining doses of urea were top-dressed at 21 days after sowing before flowering. The fertilizers were applied on both sides of the rows with the soil.

3.3.2.4.4 Plant protection activities

Special cares were taken to protect the crop from birds especially at sowing and germination stages and at the ripening stage of the crop. In case of chemical control measures (Experiment-3) Tilt 250 EC 0.05% were applied as foliar spray when diseases were firstly appeared and 2nd spray was done at 15 days after 1st spray.

3.3.2.4.5 Tagging and data collection

Randomly five plants were selected from each row of the plot and tagged. There were six rows in each plot. So, 30 plants/plot was tagged for ratting and mean values were determined to get rating score of each treatment.

3.3.3 Isolation and identification of pathogen

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 and healthy portion and surface sterilized with $HgCl_2$ solution (0.01%) for 30 seconds. The cut pieces were then washed in water at three times and were placed on to PDA media in petridish. The plate is then incubated at 25 ± 1^0C for 7 days. Later the pathogen was purified using hyphal tip culture method and grown on PDA media at 25 ± 1^0C for 2 weeks and identified as *Bipolaris sorokiniana* with the help of relevant literature (CMI Description) (Mathur and Kongsdal, 2003).

3.3.4 Evaluation of leaf blight severity

Leaf blight severity of 1st and 2nd leaf was recorded in five growth stages of plant viz. flag leaf stage, panicle initiation stage, flowering stage, milking stage and hard dough stage. The severity of leaf spot disease will be recorded following 0-5 grade 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)

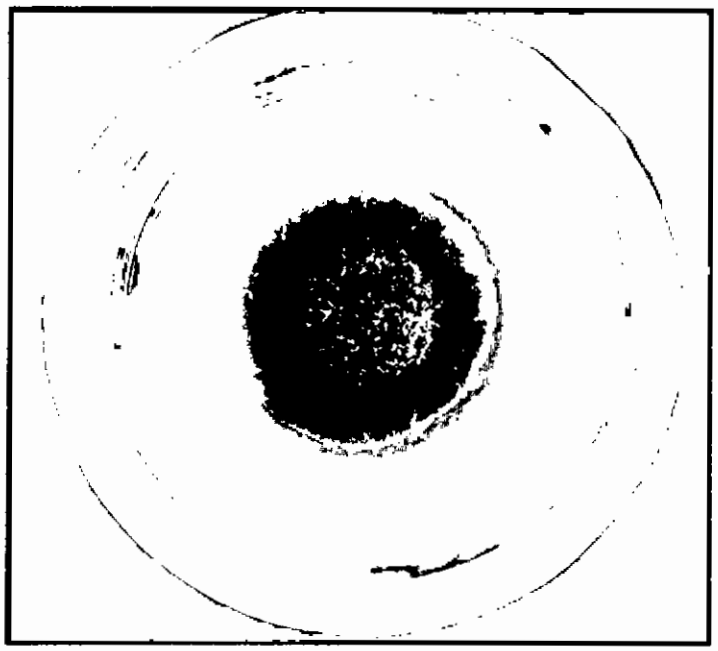


Plate 2. Photograph showing pure culture of *Bipolaris sorokiniana*

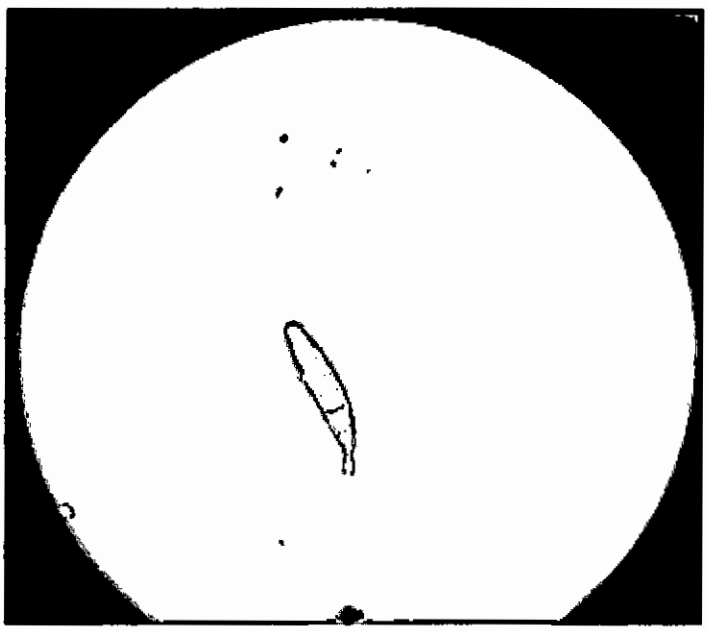


Plate 3. Photograph showing conidium of *Bipolaris sorokiniana* (100x)

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).

3.3.5 Harvesting, threshing and cleaning

The wheat was harvested at the maturity of plant at 29th March, 2009 and harvesting was done manually from each plot. The harvested crop of each plot was bundled separately, properly tagged and brought to threshing floor. Enough care was taken for harvesting, threshing and also cleaning of wheat grains. The grains were cleaned and finally the weight was calculated and converted into hectare yield.

3.3.6 Collection of data on yield and contributing characters

Data of plant growth and yield contributing characters were recorded from the randomly selected 30 tagged plants of each unit plot on the following parameters.

1. Plant height (cm)
2. Spike length (cm)
3. Distance between flag leaf and base of ear (cm)
4. Number of healthy spikelets/ear
5. Number of infected spikelets/ear
6. Number of total spikelets/ear
7. Number of healthy grains/ear
8. Number of diseased grains/ear
9. Number of total grains/ear
10. Weight of healthy grains/ear (g)

11. Weight of diseased grains/ears (g)
12. Weight of total grains/ears (g)
13. Weight of 1000 seeds (g)
14. Grain yield /plot (kg)
15. Grain yield (t/ha)
16. Straw yield/plot (kg)
17. Straw yield (t/ha)
18. Grading of seeds/ear (0-5 scale)

3.3.7 Grading of seeds

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

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 ¼ of grain are infected

5 = Grains are shivered, almost completely discolored or more than ½ of grains were discolored.

3.4 Statistical analysis

The collected data were complied and analyzed to find out the statistical significance among the treatments. The collected data were analyzed by MSTAT–C software. The means for all recorded data were calculated the analyses of variance of all characters were performed. The mean differences were evaluated by Duncan's Multiple Range Test (DMRT) at 0.01 level of probability (Gomez and Gomez, 1984).

CHAPTER 4

RESULTS AND DISCUSSION

CHAPTER 4

RESULTS

The experiment was conducted to find out the transmission of *Bipolaris sorokiniana* from seed to plant to seed of wheat. Consequently four experiments were conducted. Data on seed health status, disease incidence, leaf blight severity, yield contributing characters, yield and quality seed were recorded. The analyses of variance (ANOVA) of the data on different parameters are given in Appendix III-XXII. The results have been presented under the following headings:

4.1 Experiment-1: Effect of different levels of black pointed seeds on germination and health status before sowing in blotter method and water agar test tube seedling symptom test

A. In Blotter Method

In Blotter method before sowing wheat seeds germination showed significant difference for different treatment. There was a general tendency of increasing seed germination percentage with lower level of black pointed seeds in the seeds samples (Table 2). The maximum seed germination (98.00%) was found in T_1 (0% seed infection) which was statistically identical with T_2 (97.50%), T_3 (97.00%) and T_4 (95.00%). On the other hand, the minimum seed germination (78.00%) was recorded in T_8 (50.1-55% seed infection) which was statistically similar with T_7 (85.00%), T_6 (87.00%) and T_5 (92.00%).

Seed health test also indicated that seed samples showed significant difference and higher percentage of *Bipolaris sorokiniana* with increased percentage of black pointed seeds. Incidence of seed borne *Bipolaris sorokiniana* varied from 0.00% to 57.00% (Table 2). Among the different treatments T_1 and T_2 performed completely free from the pathogen and the lowest (2.50%) incidence was recorded from T_3 (0.1 -10% seed infection) which was followed by T_4 (12.50%). Again, the highest incidence (57.00%) was recorded in T_8 (50.1-55% seed infection) which was closely followed by T_7 (33.50%), T_6 (22.50%) and T_5 (18.00%).

Table 2. Effect of different levels of black pointed seeds on germination and incidence of *Bipolaris sorokiniana* of wheat seeds before sowing in blotter method

Treatment	Germination (%)	Incidence of *Bipolaris sorokiniana* (%)
T_1	98.00 a	0.00 e
T_2	97.50 a	0.00 e
T_3	97.00 a	2.50 e
T_4	95.00 a	12.50 d
T_5	92.00 ab	18.00 c
T_6	87.00 ab	22.50 c
T_7	85.00 ab	33.50 b
T_8	78.00 b	57.00 a
$LSD_{(0.01)}$	14.26	5.006
CV(%)	6.56	11.50

In a column mean values having similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly at 0.01 level of probability

T_1: 0% seed infection (Bavistin treated)

T_2: 0% seed infection (Provax treated)

T_3: 0.1-10% seed infection

T_4: 10.1-15% seed infection

T_5: 15.1-20% seed infection

T_6: 20.1-25% seed infection

T_7: 25.1-40% seed infection

T_8: 50.1-55% seed infection

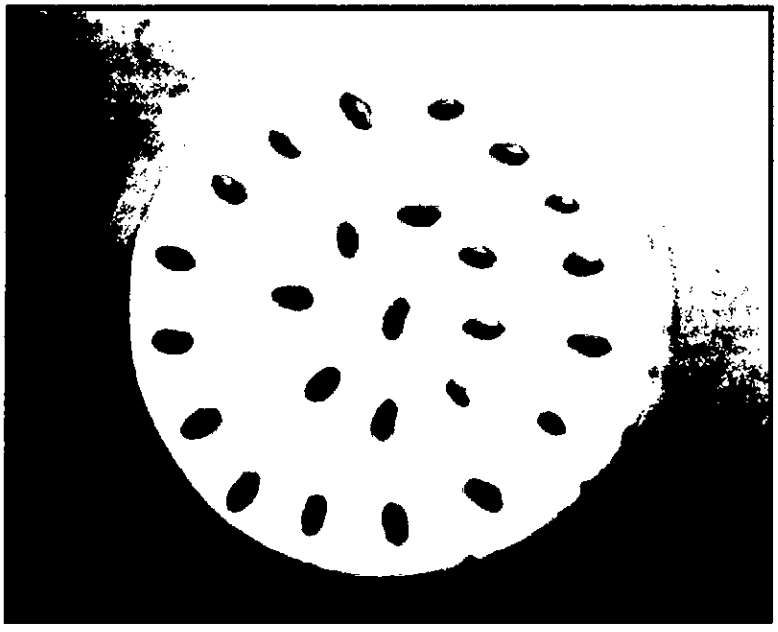


Plate 4. Photograph showing plating of wheat seed for germination

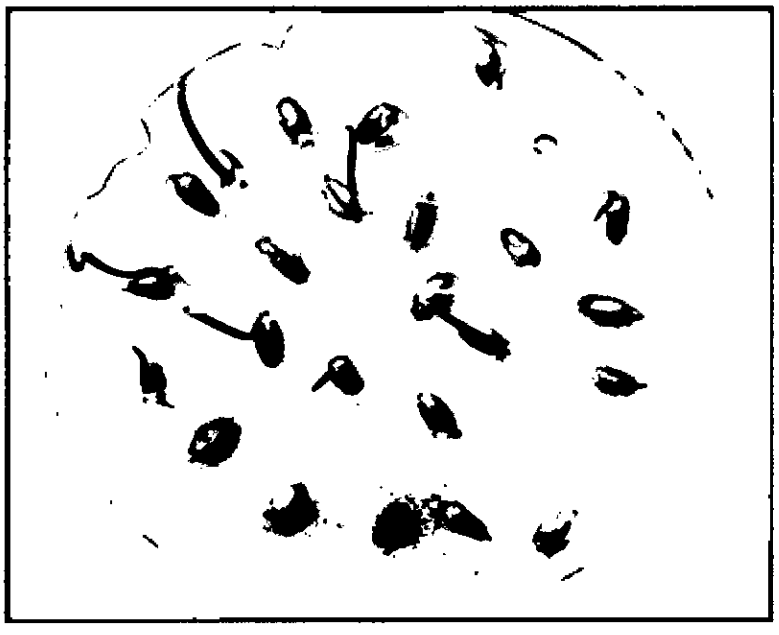


Plate 5. Photograph showing germination of wheat seed in plating

B. Water Agar Test Tube Seedling Symptom Test

Different levels of black pointed seeds before sowing showed significant effect on seeds germination and seedling health of wheat seeds in water agar test tube seedling symptom test. There was a gradual tendency of decreasing seed germination percentage with the increase in black pointed seeds in the seeds samples (Table 3). The maximum seed germination (96.00%) was recorded in T_1 and T_2 (0% seed infection) which was statistically identical with T_3 (95.50%) and T_4 (91.50%), T_5 (86.50%) and T_6 (84.00%), while the minimum seed germination (64.00%) was recorded in T_8 (50.1-55% seed infection) which was statistically similar with T_7 (73.50%), T_6 (84.00%) and T_5 (86.50%).

Normal seedlings showed significant differences among different treatment under the present trial and the values ranged from 27.50% to 93.00% (Table 3). Among the treatments the highest normal seedlings (93.00%) was observed in T_1 (0% seed infection) which was statistically identical with T_2 (92.50%) and T_3 (92.00%) and closely followed by T_4 (79.00%) again, the lowest normal seedlings (27.50%) was recorded in T_8 (50.1-55% seed infection) which was closely followed by T_7 (40.50%) and T_6 (45.50%) but they were statistically identical.

In case of abnormal seedling significant variation was observed (Table 3). The lowest abnormal seedlings (3.00%) was observed in T_1 (% seed infection) which was statistically identical with T_2 (3.50%) and T_3 (3.50%) and followed by T_4 (12.50%) and T_5 (18.0%), whereas the highest abnormal seedlings (38.50%) was found in T_8 (50.1-55% seed infection) which was closely followed by T_7 (33.00%).

Significant differences were recorded for different black pointed seeds before sowing for dead seeds (Table 3). The lowest dead seeds (4.00%) was recorded in T_1 and T_2 (% seed infection) which was statistically identical with T_3 (4.50%) and closely followed by T_4 (8.50%), again the highest (34.00%) was recorded in T_8 (50.1-55% seed infection) which was followed by T_7 (26.50%).

Table 3. Effect of different levels of black pointed seeds on germination and seedling health of wheat seeds in the laboratory before sowing in water agar test tube seedling symptom test

Treatment	Germination (%)	Normal Seedling (%)	Abnormal Seedling (%)	Dead Seeds (%)
T_1	96.00 a	93.00 a	3.00 f	4.00 e
T_2	96.00 a	92.50 a	3.50 f	4.00 e
T_3	95.50 a	92.00 a	3.50 f	4.50 de
T_4	91.50 a	79.00 b	12.50 e	8.50 d
T_5	86.50 ab	55.00 c	18.00 d	13.50 c
T_6	84.00 ab	45.50 d	22.50 c	16.00 c
T_7	73.50 bc	40.50 d	33.00 b	26.50 b
T_8	64.00 c	27.50 e	38.50 a	34.00 a
$LSD_{(0.01)}$	13.54	8.640	4.000	4.022
CV(%)	6.61	5.52	9.98	12.15

In a column mean values having similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly at 0.01 level of probability

T_1: 0% seed infection (Bavistin treated)

T_2: 0% seed infection (Provax treated)

T_3: 0.1-10% seed infection

T_4: 10.1-15% seed infection

T_5: 15.1-20% seed infection

T_6: 20.1-25% seed infection

T_7: 25.1-40% seed infection

T_8: 50.1-55% seed infection

4.2 Experiment-2: Effect of different levels of black pointed seeds on disease incidence, leaf blight severity and quality seed production of wheat

A. Number of seedlings/m^2

Different level of black pointed seeds in field condition without any chemical control measures showed significant variations in terms of number of healthy seedlings/^{2}. With the increase of black pointed seeds in the seeds samples number of healthy seedlings/m^2 decreased which varied from 88.33 to 132.33 (Table 4). The maximum number of healthy seedlings/m^2 (132.33) was recorded in T_1 which was statistically identical with T_2 (129.33), T_3 (122.67), T_4 (115.00). On the other hand, the minimum number of healthy seedlings/m^2 (88.33) was recorded in T_8 (50.1-55% seed infection) which was statistically similar with T_7 (90.67), T_6 (94.33), and T_5 (107.67).

Significant differences were recorded for different black pointed seeds in field condition without any chemical control measures for infected seedlings/^{2}. Number of infected seedlings/m^2 increased with the increase of black pointed seeds in the seeds samples (Table 4). The minimum number of infected seedlings/m^2 (1.00) was recorded in T_1 which was statistically identical with T_2 (1.33) and closely followed by T_3 (10.00), T_4 (12.33) and T_5 (14.33), while the maximum (27.67) was recorded in T_8 (50.1-55% seed infection) which was closely followed by T_7 (20.33) and T_6 (15.67).

B. Leaf blight severity

Leaf blight severity (0-5 grade) was measured in 1st and 2nd leaf and average also calculated treatment wise at flag leaf, panicle initiation, flowering, milking and hard dough stage and significant variation was recorded (Table 5, Table 6 and Table 7).

At flag leaf stage, in case of 1st leaf the lowest disease severity (0.05) was recorded in T_1 (0% seed infection) which was closely followed by T_2 (0.08) again

Table 4. Effect of different levels of black pointed seeds on disease incidence at 21 DAS of wheat in the field

Treatment	Number of seedlings/m^2	
	Healthy	Infected
T_1	132.33 a	1.00 e
T_2	129.33 a	1.33 e
T_3	122.67 ab	10.00 d
T_4	115.00 ab	12.33 cd
T_5	107.67 bc	14.33 cd
T_6	94.33 c	15.67 bc
T_7	90.67 c	20.33 b
T_8	88.33 c	27.67 a
$LSD_{(0.01)}$	18.14	4.703
CV(%)	6.78	15.08

In a column mean values having similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly at 0.01 level of probability

T_1: 0% seed infection (Bavistin treated)

T_2: 0% seed infection (Provax treated)

T_3: 0.1-10% seed infection

T_4: 10.1-15% seed infection

T_5: 15.1-20% seed infection

T_6: 20.1-25% seed infection

T_7: 25.1-40% seed infection

T_8: 50.1-55% seed infection

Plate 6. Photograph showing *Bipolaris sorokiniana* infected leaf

the highest disease severity (0.38) was found in T_8 (50.1-55% seed infection) and closely followed by T_7 (0.25). In 2nd leaf, the lowest disease severity was found in T_1 (0.12) which was statistically similar with T_2 (0.14) and closely followed by T_3 (0.18) and T_4 (0.20), again, the highest disease severity was recorded in T_8 (0.52) which was closely followed by T_7 (0.38). In an average, the lowest disease severity was recorded in T_1 (0.09) which was closely followed by T_2 (0.11) and the highest was obtained in T_8 (0.45) which was closely followed by T_7 (0.32).

At panicle initiation stage, in case of 1st leaf the lowest disease severity (0.09) was recorded in T_1 and T_2 (0% seed infection) which was statistically identical with T_3 (0.14) and closely followed by T_4 (0.24) and T_5 (0.31), whereas the highest disease severity (0.57) was found in T_8 which was closely followed by T_7 (0.45). In 2nd leaf, the lowest disease severity was found in T_1 (0.21) which was statistically similar with T_2 (0.28), T_3 (0.42) and T_4 (0.47) and closely followed by T_5 (0.57), while the highest disease severity was recorded in T_8 (1.56) which was closely followed by T_7 (0.86) and T_6 (0.71). In an average, the lowest disease severity was observed in T_1 (0.15) which was statistically similar with T_2 (0.18) and closely followed by T_3 (0.28) and T_4 (0.36), whereas the highest was found in T_8 (1.06) which was closely followed by T_7 (0.66).

At flowering stage, in case of 1st leaf the lowest disease severity (0.28) was found in T_1 and T_2 which was statistically identical with T_3 (0.36), T_4 (0.38) T_5 (0.42) and closely followed by T_6 (0.51), while the highest disease severity (0.77) was found in T_8 which was closely followed by T_7 (0.66). In 2nd leaf, the lowest disease severity was found in T_1 (0.58) which was statistically similar with T_2 (0.58) and closely followed by T_3 (1.04), T_4 (1.04) and T_5 (1.32). On the other hand, the highest disease severity was recorded in T_8 (1.87) which was statistically similar with T_7 (1.61) and closely followed by T_6 (1.49). In an average, the lowest disease severity (0.43) was found in T_1 and T_2 which was closely followed by T_3 (0.70) and T_4 (0.71), whereas the highest was found in T_8 (1.32) which was closely followed by T_7 (1.14) and T_6 (1.00).

Table 5. Effect of different levels of black pointed seeds on leaf blight severity of wheat at flag leaf and panicle initiation stage

Treatment	Disease severity (0-5 grade) at flag leaf stage			Disease severity (0-5 grade) at panicle initiation stage		
	1st leaf	2nd leaf	Average	1st leaf	2nd leaf	Average
T_1	0.05 h	0.12 f	0.09 h	0.09 e	0.21 e	0.15 f
T_2	0.08 g	0.14 f	0.11 g	0.09 e	0.28 e	0.18 f
T_3	0.11 f	0.18 e	0.15 f	0.14 e	0.42 de	0.28 e
T_4	0.14 e	0.20 e	0.17 e	0.24 d	0.47 cde	0.36 e
T_5	0.18 d	0.26 d	0.22 d	0.31 cd	0.57 cd	0.44 d
T_6	0.21 c	0.32 c	0.27 c	0.36 c	0.71 bc	0.53 c
T_7	0.25 b	0.38 b	0.32 b	0.45 b	0.86 b	0.66 b
T_8	0.38 a	0.52 a	0.45 a	0.57 a	1.56 a	1.06 a
$LSD_{(0.01)}$	0.024	0.024	0.018	0.077	0.255	0.078
CV(%)	11.73	6.82	4.62	10.35	16.44	10.86

In a column mean values having similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly at 0.01 level of probability

T_1: 0% seed infection (Bavistin treated)

T_2: 0% seed infection (Provax treated)

T_3: 0.1-10% seed infection

T_4: 10.1-15% seed infection

T_5: 15.1-20% seed infection

T_6: 20.1-25% seed infection

T_7: 25.1-40% seed infection

T_8: 50.1-55% seed infection

Table 6. Effect of different levels of black pointed seeds on leaf blight severity of wheat at flowering and milking stage

Treatment	Disease severity (0-5 grade) at flowering stage			Disease severity (0-5 grade) at milking stage		
	1st leaf	2nd leaf	Average	1st leaf	2nd leaf	Average
T_1	0.28 e	0.58 d	0.43 e	0.80 f	1.47 b	1.14 g
T_2	0.28 e	0.58 d	0.43 e	1.00 ef	1.57 b	1.29 f
T_3	0.36 de	1.04 c	0.70 d	1.13 de	2.14 a	1.64 e
T_4	0.38 de	1.04 c	0.71 d	1.28 cd	2.14 a	1.71 de
T_5	0.42 cd	1.32 bc	0.87 c	1.38 bcd	2.23 a	1.81 cd
T_6	0.51 c	1.49 b	1.00 bc	1.43 bc	2.33 a	1.88 bc
T_7	0.66 b	1.61 ab	1.14 b	1.57 ab	2.38 a	1.98 ab
T_8	0.77 a	1.87 a	1.32 a	1.77 a	2.43 a	2.10 a
$LSD_{(0.01)}$	0.109	0.317	0.147	0.266	0.352	0.147
CV(%)	8.91	10.93	10.14	8.33	6.87	5.00

In a column mean values having similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly at 0.01 level of probability

T_1: 0% seed infection (Bavistin treated)

T_2: 0% seed infection (Provax treated)

T_3: 0.1-10% seed infection

T_4: 10.1-15% seed infection

T_5: 15.1-20% seed infection

T_6: 20.1-25% seed infection

T_7: 25.1-40% seed infection

T_8: 50.1-55% seed infection

At milking stage, in case of 1st leaf the lowest disease severity (0.80) was recorded in T_1 which was statistically identical with T_2 (1.00) and closely followed by T_3 (1.13), T_4 (1.28) and T_5 (1.38), whereas the highest disease severity (1.77) was found in T_8 which was statistically similar with T_7 (1.57) and closely followed by T_6 (1.43). In 2nd leaf, the lowest disease severity was obtained in T_1 (1.47) which was statistically similar with T_2 (1.57) and the highest was recorded in T_8 (2.43) which was statistically similar with T_7 (2.38), T_6 (2.33), T_5 (2.23), T_4 (2.14) and T_3 (2.14). In an average, the lowest disease severity (1.14) was recorded in T_1 which was closely followed by T_2 (1.29) and the highest was found in T_8 (2.10) which was statistically similar with T_7 (1.98) and followed by T_6 (1.88).

At hard dough stage, in case of 1st leaf the lowest disease severity (1.76) was recorded in T_1 which was statistically identical with T_2 (1.86), T_3 (1.92), T_4 (2.03) and closely followed by T_5 (2.21), whereas the highest disease severity (3.27) was found in T_8 which was statistically similar with T_7 (3.07) and closely followed by T_6 (2.64). In 2nd leaf, the lowest disease severity was recorded in T_1 (2.84) which was statistically similar with T_2 (2.92) and closely followed by T_3 (3.50), T_4 (3.60) and T_5 (3.70) and the highest disease severity was recorded in T_8 (4.35) which was statistically similar with T_7 (3.90) and T_6 (3.84). In an average, the lowest disease severity (2.30) was recorded in T_1 which was statistically similar with T_2 (2.39) and closely followed by T_3 (2.71), T_4 (2.82) and T_5 (2.96) and the highest was found in T_8 (3.81) which was followed by with T_7 (3.49) and T_6 (3.24).

C. Growth parameters

Growth parameters plant height, spike length and distance between flag leaf and base of ear showed significant variation for different treatment in field condition without any chemical control measures (Table 8).

Considering plant height, the highest plant height (89.35 cm) was recorded in T_1 which was statistically identical with T_2 (87.55 cm), T_3 (86.20 cm), T_4 (86.67 cm), T_5 (85.65 cm), T_6 (84.50 cm) and T_7 (83.80 cm) whereas the lowest plant height (80.80 cm) was found in T_8 (50.1-55% seed infection).

Table 7. Effect of different levels of black pointed seeds on leaf blight severity of wheat at hard dough stage

Treatment	Disease severity (0-5 grade) at hard dough stage		
	1st leaf	2nd leaf	Average
T_1	1.76 d	2.84 c	2.30 d
T_2	1.86 cd	2.92 c	2.39 d
T_3	1.92 cd	3.50 b	2.71 c
T_4	2.03 cd	3.60 b	2.82 c
T_5	2.21 c	3.70 b	2.96 c
T_6	2.64 b	3.84 ab	3.24 b
T_7	3.07 a	3.90 ab	3.49 b
T_8	3.27 a	4.35 a	3.81 a
$LSD_{(0.01)}$	0.384	0.521	0.266
CV(%)	6.79	5.98	5.13

In a column mean values having similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly at 0.01 level of probability

T_1: 0% seed infection (Bavistin treated)

T_2: 0% seed infection (Provax treated)

T_3: 0.1-10% seed infection

T_4: 10.1-15% seed infection

T_5: 15.1-20% seed infection

T_6: 20.1-25% seed infection

T_7: 25.1-40% seed infection

T_8: 50.1-55% seed infection

Table 8. Effect of different levels of black pointed seeds on yield contributing characters and yield of wheat

Treatment	Plant height (cm)	Spike length (cm)	Distance between flag leaf and base of ear	Spikelets per ear (No.)		
				Healthy	Infected	Total
T_1	89.35 a	16.43 a	14.80 a	31.20 a	1.13 f	32.33 a
T_2	87.55 ab	15.75 ab	14.65 ab	30.85 a	1.60 ef	32.45 a
T_3	86.20 ab	15.60 ab	14.55 ab	30.00 ab	2.05 e	32.05 a
T_4	86.67 ab	15.25 ab	14.30 ab	28.20 abc	2.80 d	31.00 ab
T_5	85.65 ab	14.70 ab	14.05 ab	27.50 abc	3.20 d	30.70 ab
T_6	84.50 ab	14.50 ab	13.80 ab	26.20 bcd	4.10 c	30.30 ab
T_7	83.80 ab	14.30 ab	13.45 ab	25.30 cd	4.70 b	30.00 ab
T_8	80.80 b	13.57 b	13.05 b	23.10 d	5.40 a	28.50 b
$LSD_{(0.01)}$	6.539	2.031	1.460	4.077	0.504	2.842
CV(%)	6.14	5.56	4.27	8.04	6.64	5.82

In a column mean values having similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly at 0.01 level of probability

T_1: 0% seed infection (Bavistin treated)

T_2: 0% seed infection (Provax treated)

T_3: 0.1-10% seed infection

T_4: 10.1-15% seed infection

T_5: 15.1-20% seed infection

T_6: 20.1-25% seed infection

T_7: 25.1-40% seed infection

T_8: 50.1-55% seed infection

In context of spike length the longest spike (16.43 cm) was recorded in T_1 which was statistically similar with T_2 (15.75 cm), T_3 (15.60 cm), T_4 (15.25 cm), T_5 (14.70 cm), T_6 (14.50 cm) and T_7 (14.30 cm), while the shortest spike (13.57 cm) was obtained in T_8.

In case of distance between flag leaf and base of ear the highest length (14.80 cm) was recorded in T_1 which was statistically similar with T_2 (14.65 cm), T_3 (14.55 cm), T_4 (14.30 cm), T_5 (14.05 cm), T_6 (13.80 cm) and T_7 (13.45 cm), while the lowest (13.05 cm) was recorded in T_8.

D. Yield contributing characters

Yield contributing characters of wheat differ significantly for different treatments in field condition without any chemical control measures under the present trial (Table 9 and Table 10).

In case of healthy spikelets per ear the maximum (31.20) was recorded in T_1 which was statistically similar with T_2 (30.85), T_3 (30.00), T_4 (28.20) and T_5 (27.50). On the other hand the minimum number of healthy spikelets per ear (23.10) was observed in T_8 which was statistically identical with T_7 (25.30) and T_6 (26.20). For infected spikelets per ear the minimum number of infected spikelets per ear (1.13) was recorded in T_1 which was statistically similar with T_2 (1.60) and closely followed by T_3 (2.05) again, the maximum (5.40) was obtained in T_8 which was closely followed by T_7 (4.70). In case of total spikelets per ear the maximum (32.33) was recorded in T_1 which was statistically similar with T_2 (32.45), T_3 (32.05), T_4 (31.00), T_5 (30.70) T_6 (30.30) and T_7 (30.00). On the other hand, the minimum number of total spikelets per ear (28.50) was found in T_8.

In context of number of healthy grains per ear the maximum (42.20) was recorded in T_1 which was statistically similar with T_2 (40.90), T_3 (37.00), T_4 (36.20) and closely followed by T_5 (33.40). On the contrary, the minimum number of healthy grains per ear (23.00) was observed in T_8 which was statistically identical with T_7 (28.00) and closely followed by T_6 (30.20). In case of diseased grains per ear the

Table 9. Effect of different levels of black pointed seeds on number and weight of grains per ear of harvested seeds of wheat

Treatment	Number of grains per ear			Weight of grains per ear (g)		
	Healthy	Diseased	Total	Healthy	Diseased	Total
T_1	42.20 a	2.80 f	45.00 a	1.88 ab	0.08 f	1.96 ab
T_2	40.90 a	2.70 f	43.60 ab	1.92 a	0.07 f	1.99 a
T_3	37.00 ab	4.40 e	41.40 abc	1.66 abc	0.13 e	1.79 ab
T_4	36.20 abc	4.80 de	41.00 abc	1.63 bc	0.16 d	1.79 ab
T_5	33.40 bcd	6.00 cd	39.40 abc	1.53 cd	0.21 c	1.74 abc
T_6	30.20 cd	7.20 bc	37.40 bcd	1.39 cd	0.34 b	1.73 abc
T_7	28.00 de	8.40 ab	36.40 cd	1.29 de	0.38 a	1.67 bc
T_8	23.00 e	8.80 a	31.80 d	1.06 e	0.39 a	1.45 c
$LSD_{(0.01)}$	5.767	1.478	6.198	0.266	0.024	0.277
CV(%)	7.01	10.79	6.46	7.22	7.58	6.39

In a column mean values having similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly at 0.01 level of probability

T_1: 0% seed infection (Bavistin treated)

T_2: 0% seed infection (Provax treated)

T_3: 0.1-10% seed infection

T_4: 10.1-15% seed infection

T_5: 15.1-20% seed infection

T_6: 20.1-25% seed infection

T_7: 25.1-40% seed infection

T_8: 50.1-55% seed infection

minimum number of diseased grains per ear (2.70) was recorded in T_2 which was statistically similar with T_1 (2.80) and closely followed by T_3 (4.40) and T_4 (4.80), whereas, the maximum (8.80) was found in T_8 which was statistically identical with T_7 (8.40) closely followed by T_6 (7.20). In case of total grains per ear the maximum (45.00) was recorded in T_1 which was statistically similar with T_2 (43.60), T_3 (41.40), T_4 (41.00) and T_5 (39.40), while, the minimum number of total grains per ear (31.80) was recorded in T_8 which was statistically similar with T_7 (36.40) and T_6 (37.40).

Considering the weight of healthy grains per ear the maximum (1.92 g) was recorded in T_2 which was statistically similar with T_1 (1.88 g) and T_3 (1.66 g) and closely followed by T_4 (1.63 g), whereas the minimum weight of healthy grains per ear (1.06 g) was found in T_8 which was statistically identical with T_7 (1.29 g) and closely followed by T_6 (1.39 g) and T_5 (1.53 g). For diseased grains per ear the minimum weight of diseased grains per ear (0.07 g) was found in T_2 which was statistically similar with T_1 (0.08 g) and closely followed by T_3 (0.13 g), while, the maximum (0.39 g) was found in T_8 which was statistically identical with T_7 (0.38 g) closely followed by T_6 (0.34 g). In case of total weight of grains per ear, the maximum was recorded in T_2 (1.99 g) which was statistically similar with T_1 (1.96 g), T_3 (1.79 g), T_4 (1.79 g), T_5 (1.74 g) and T_6 (1.73 g), while, the minimum weight of total grains per ear (1.45 g) was obtained in T_8 which was statistically similar with T_5 (1.74 g), T_6 (1.73 g) and T_7 (1.67 g).

E. Grain and Straw yield

In case of weight of 1000 grains, the highest was obtained in T_1 (44.85 g) which was statistically similar with T_2 (43.02 g), T_3 (42.54 g), T_4 (41.55 g), T_5 (41.47 g), T_6 (41.10 g) and T_7 (40.53 g), while, the lowest weight of 1000 grains (38.03 g) was observed in T_8 (Table 10).

Grain and straw yield of wheat differ significantly for different treatment in field condition without application of any chemical control measures (Table 10).

Table 10. Effect of different levels of black pointed seeds on yield of wheat

Treatment	Weight of 1000 seeds (g)	Grain yield		Straw yield	
		Plot (kg)	Hectare (tons)	Plot (kg)	Hectare (tons)
T_1	44.85 a	1.10 a	3.67 a	1.91 a	6.37 a
T_2	43.02 a	1.07 a	3.58 a	1.84 a	6.13 ab
T_3	42.54 ab	1.05 a	3.50 ab	1.64 b	5.46 bc
T_4	41.55 ab	1.00 a	3.33 abc	1.59 b	5.30 cd
T_5	41.47 ab	0.90 b	3.00 bcd	1.57 bc	5.23 cd
T_6	41.10 ab	0.85 b	2.83 cd	1.41 cd	4.70 cde
T_7	40.53 ab	0.81 b	2.70 de	1.37 d	4.57 de
T_8	38.03 b	0.67 c	2.25 e	1.31 d	4.37 e
$LSD_{(0.01)}$	4.393	0.096	0.4800	0.166	0.749
CV(%)	5.34	6.34	6.34	5.85	5.85

In a column mean values having similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly at 0.01 level of probability

T_1: 0% seed infection (Bavistin treated)

T_2: 0% seed infection (Provax treated)

T_3: 0.1-10% seed infection

T_4: 10.1-15% seed infection

T_5: 15.1-20% seed infection

T_6: 20.1-25% seed infection

T_7: 25.1-40% seed infection

T_8: 50.1-55% seed infection

Considering the grain yields per plot the highest was recorded in T_1 (1.10 kg) which was statistically similar with T_2 (1.07 kg), T_3 (1.05 kg) and T_4 (1.00 kg) and closely followed by T_5 (0.90 kg), while, the lowest yield per plot (0.67 kg) was obtained in T_8 which was statistically similar with T_7 (3.24 kg) and closely followed by T_6 (3.40 kg). In context of grain yields per hectare the highest was recorded in T_1 (3.67 tonnes) which was statistically similar with T_2 (3.58 tonnes), T_3 (3.50 tonnes) and T_4 (3.33 tonnes) and closely followed by T_5 (3.00 tonnes), while, the lowest yield per hectare (2.25 tonnes) was recorded in T_8 which was statistically similar with T_7 (2.70 tonnes) and closely followed by T_6 (2.83 tonnes).

For straw yields per plot the highest was recorded in T_1 (1.91 kg) which was statistically similar with T_2 (1.84 kg) and closely followed by T_3 (1.64 kg), whereas, the lowest straw yield per plot (1.31 kg) was observed in T_8 which was statistically similar with T_7 (5.48 kg) and T_6 (5.64 kg). In case of straw yields per hectare the highest was recorded in T_1 (6.37 tonnes) which was statistically similar with T_2 (6.13 tonnes) and closely followed by T_3 (5.46 tonnes), while, the lowest straw yield per hectare (4.37 tonnes) was obtained in T_8 which was statistically similar with T_7 (4.57 tonnes) and T_6 (4.70 tonnes).

F. Grading of seeds

Grading of seeds was done in 0-5 rating scale where '0' indicates apparently healthy seeds i.e. showing the minimum disease symptom and '5' indicates seeds with the maximum disease symptom. Significant variation was recorded for grading of seeds in field condition without applying any chemical control measures (Table 11).

In case of grade-0, the maximum value was recorded in T_1 (42.20) which was statistically similar with T_2 (41.20), T_3 (37.00) and closely followed by T_4 (36.20), while, the minimum (23.00) was obtained in T_8 which was statistically similar with T_7 (28.00) and closely followed by T_6 (30.20) and T_5 (33.40). Considering grade-1, the minimum values was found in T_2 (1.53) which was statistically

Table 11. Effect of different levels of black pointed seeds on different severity grades (0-5) of harvested seeds of wheat

Treatment	Grading of seeds					
	G_0	G_1	G_2	G_3	G_4	G_5
T_1	42.20 a	1.55 d	0.40 fg	0.20 d	0.18 e	0.47 e
T_2	41.20 ab	1.53 d	0.33 g	0.18 d	0.20 de	0.45 e
T_3	37.00 abc	2.58 c	0.55 ef	0.47 c	0.26 de	0.54 de
T_4	36.20 bc	2.68 c	0.64 de	0.51 c	0.31 d	0.65 de
T_5	33.40 cd	3.23 bc	0.74 cd	0.68 b	0.44 c	0.91 cd
T_6	30.20 d	3.92 ab	0.86 c	0.81 ab	0.57 b	1.05 bc
T_7	28.00 de	4.39 a	1.05 b	0.92 a	0.73 a	1.33 ab
T_8	23.00 e	4.33 a	1.22 a	0.88 a	0.80 a	1.56 a
$LSD_{(0.01)}$	5.540	0.893	0.154	0.154	0.109	0.352
CV(%)	6.72	12.15	8.46	10.42	9.39	16.60

In a column mean values having similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly at 0.01 level of probability

T_1: 0% seed infection (Bavistin treated)

T_2: 0% seed infection (Provax treated)

T_3: 0.1-10% seed infection

T_4: 10.1-15% seed infection

T_5: 15.1-20% seed infection

T_6: 20.1-25% seed infection

T_7: 25.1-40% seed infection

T_8: 50.1-55% seed infection

similar with T_1 (1.55) and followed by T_3 (2.58) and T_4 (2.68), while, the maximum values (4.39) was found in T_7 which was statistically similar with T_8 (4.33) and T_6 (3.92) and closely followed by T_5 (3.23). In respect of grade-2, the minimum values was recorded in T_2 (0.33) which was statistically similar with T_1 (0.40) and closely followed by T_3 (0.55) and T_4 (0.64), while, the maximum values (1.22) was recorded in T_8 which was closely followed by T_7 (1.05). For grade-3, the minimum values was found in T_2 (0.18) which was statistically similar with T_1 (0.20) and closely followed by T_3 (0.47) and T_4 (0.51) they were statistically identical, while the maximum values (0.92) was found in T_7 which was statistically similar with T_8 (0.88) and T_6 (0.81) and closely followed by T_5 (0.68). In case of grade-4, the minimum values was observed in T_1 (0.18) which was statistically similar with T_2 (0.20) and T_3 (0.26) and closely followed by T_4 (0.31), whereas the maximum values (0.80) was found in T_8 which was statistically similar with T_7 (0.73) and closely followed by T_6 (0.57). In seed grade-5, the minimum values was obtained in T_2 (0.45) which was statistically similar with T_1 (0.47), T_3 (0.54) and T_4 (0.65), while the maximum values (1.56) was recorded in T_8 which was statistically similar with T_7 (1.33) and closely followed by T_6 (1.05).

4.3 Experiment-3: Effect of different levels of black pointed seeds after chemical control measures on disease incidence, leaf blight severity and quality seed production of wheat

A. Number of seedlings/m^2

Different black pointed seeds in field condition with chemical control measures varied significantly for number of healthy seedlings/m^2. With the increase of black pointed seeds in the seeds samples number of healthy seedlings/m^2 were also decreased and varied from 91.00 to 132.67 (Table 12). The maximum number of healthy seedlings/m^2 (132.67) was found in T_1 which was statistically identical with T_2 (129.33), T_3 (123.00), T_4 (117.00). Again, the minimum number of healthy seedlings/m^2 (91.00) was obtained in T_8 (50.1-55% seed infection) which was statistically similar with T_7 (92.33), T_6 (96.67) and closely followed by T_5 (109.33).

Number of infected seedlings/^{2} showed significant differences for different black pointed seeds in field condition with chemical control measures. Number of infected seedlings/m^2 increased with the increased of black pointed seeds in the seeds samples (Table 12). The minimum number of infected seedlings/m^2 (0.67) was recorded in T_1 which was statistically identical with T_2 (1.00%) and closely followed by T_3 (8.33). On the other hand, the maximum (24.67) was found in T_8 (50.1-55% seed infection) which was closely followed by T_7 (17.67).

B. Leaf blight severity

In field condition leaf blight severity (0-5 grade) was measured in 1st and 2nd leaf and average also calculated treatment wise with the application of chemical control measures at flag leaf, panicle initiation, flowering, milking and hard dough stage and significant variation was observed (Table 13, Table 14 and Table 15). Where '0' represent no disease and grade '5' indicate the maximum disease severity.

Table 12. Effect of different levels of black pointed seeds after chemical control measure at field level on disease incidence at 21 DAS of wheat in the field

Treatment	Number of seedlings/m^2	
	Healthy	Infected
T_1	132.67 a	0.67 f
T_2	129.33 a	1.00 f
T_3	123.00 ab	8.33 e
T_4	117.00 ab	11.33 d
T_5	109.33 bc	13.00 cd
T_6	96.67 cd	14.67 c
T_7	92.33 d	17.67 b
T_8	91.00 d	24.67 a
$LSD_{(0.01)}$	15.90	2.825
CV(%)	5.87	10.18

In a column mean values having similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly at 0.01 level of probability

T_1: 0% seed infection (Bavistin treated)

T_2: 0% seed infection (Provax treated)

T_3: 0.1-10% seed infection

T_4: 10.1-15% seed infection

T_5: 15.1-20% seed infection

T_6: 20.1-25% seed infection

T_7: 25.1-40% seed infection

T_8: 50.1-55% seed infection

At flag leaf stage, in respect of 1st leaf the lowest disease severity (0.04) was recorded in T_1 (0% seed infection) which was statistically similar with T_2 (0.07) and T_3 (0.10), while the highest disease severity (0.35) was found in T_8 (50.1-55% seed infection) and closely followed by T_7 (0.23), T_6 (0.20) and T_6 (0.16). In the 2nd leaf, the lowest disease severity was found in T_1 (0.10) which was statistically similar with T_2 (0.13), T_3 (0.17) and T_4 (0.18), again, the highest was recorded in T_8 (0.41) which was statistically identical with T_7 (0.35) and closely followed by T_6 (0.30). In an average, the lowest disease severity was recorded in T_1 (0.07) which was closely followed by T_2 (0.10) and the highest was obtained in T_8 (0.38) which was closely followed by T_7 (0.29).

At panicle initiation stage, considering the 1st leaf the lowest disease severity (0.08) was recorded in T_1 and T_2 which was identical with T_3 (0.13) and followed by T_4 (0.21) and T_5 (0.29), whereas the highest disease severity (0.52) was found in T_8 which was closely followed by T_7 (0.42). For 2nd leaf, the lowest disease severity was found in T_1 (0.18) which was closely followed by T_2 (0.26) and T_3 (0.32) and the highest was recorded in T_8 (1.32) which was closely followed by T_7 (0.74). In an average, the lowest disease severity was observed in T_1 (0.13) which was statistically similar with T_2 (0.17) and followed by T_3 (0.23), whereas the highest was recorded in T_8 (0.92) which was closely followed by T_7 (0.58).

At flowering stage (Table 14), in context of 1st leaf the lowest disease severity (0.25) was found in T_2 which was statistically identical with T_1 (0.26), T_3 (0.33), T_4 (0.35) and T_5 (0.39), while the highest disease severity (0.72) was obtained in T_8 which was statistically identical with T_7 (0.60) and closely followed by T_6 (0.48). In 2nd leaf, the lowest disease severity was found in T_1 (0.52) which was statistically similar with T_2 (0.55) and closely followed by T_3 (0.97), T_4 (1.04) and T_5 (1.19). On the other hand, the highest disease severity was found in T_8 (1.77) which was statistically similar with T_7 (1.51) and closely followed by T_6 (1.43). In an average, the lowest (0.39) was recorded in T_1 which was statistically similar with T_2 (0.40) and closely followed by T_3 (0.65) and T_4 (0.70), while the highest was found in T_8 (1.25) which was closely followed by T_7 (1.06) and T_6 (0.95).

Table 13. Effect of different levels of black pointed seeds after chemical control measure at field level on leaf blight severity of wheat at flag leaf and panicle initiation stage

Treatment	Disease severity (0-5 grade) at flag leaf stage			Disease severity (0-5 grade) at panicle initiation stage		
	1st leaf	2nd leaf	Average	1st leaf	2nd leaf	Average
T_1	0.04 f	0.10 e	0.07 h	0.08 e	0.18 f	0.13 g
T_2	0.07 ef	0.13 e	0.10 g	0.08 e	0.26 e	0.17 g
T_3	0.10 def	0.17 de	0.13 f	0.13 e	0.32 e	0.23 f
T_4	0.13 cde	0.18 de	0.16 e	0.21 d	0.44 d	0.33 e
T_5	0.16 bcd	0.24 cd	0.20 d	0.29 cd	0.49 d	0.39 d
T_6	0.20 bc	0.30 bc	0.25 c	0.33 c	0.64 c	0.49 c
T_7	0.23 b	0.35 ab	0.29 b	0.42 b	0.74 b	0.58 b
T_8	0.35 a	0.41 a	0.38 a	0.52 a	1.32 a	0.92 a
$LSD_{(0.01)}$	0.077	0.077	0.018	0.077	0.077	0.055
CV(%)	14.09	12.18	9.49	9.23	6.99	5.97

In a column mean values having similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly at 0.01 level of probability

T_1: 0% seed infection (Bavistin treated)

T_2: 0% seed infection (Provax treated)

T_3: 0.1-10% seed infection

T_4: 10.1-15% seed infection

T_5: 15.1-20% seed infection

T_6: 20.1-25% seed infection

T_7: 25.1-40% seed infection

T_8: 50.1-55% seed infection

At milking stage, in case of 1st leaf the lowest disease severity (0.76) was recorded in T_1 which was statistically identical with T_2 (0.87) and T_3 (1.07) and followed by T_4 (1.18), T_6 (1.20) and T_5 (1.28), whereas the highest disease severity (1.64) was recorded in T_8 which was statistically similar with T_7 (1.44) and closely followed by T_6 (1.20). In 2nd leaf, the lowest disease severity was found in T_1 (1.17) which was statistically similar with T_2 (1.27) and T_3 (1.71) and the highest was recorded in T_8 (2.28) which was statistically similar with T_7 (2.25), T_6 (2.21), T_5 (2.07) and T_4 (1.96). In an average, the lowest disease severity (0.97) was observed in T_1 which was statistically identical with T_2 (1.07) and closely followed by T_3 (1.39) and T_4 (1.57) and the highest was found in T_8 (1.96) which was statistically similar with T_7 (1.84) and followed by T_6 (1.71) and T_5 (1.68).

At hard dough stage, in case of 1st leaf the lowest disease severity (1.31) was recorded in T_1 which was closely followed by T_2 (1.69), T_3 (1.72) and T_4 (1.95), while the highest disease severity (3.05) was obtained in T_8 which was statistically similar with T_7 (2.92) and closely followed by T_6 (2.31). In 2nd leaf, the lowest disease severity was found in T_1 (2.10) which was statistically similar with T_2 (2.62) and closely followed by T_3 (3.23) and T_4 (3.30) and the highest disease severity was recorded in T_8 (4.08) which was statistically similar with T_7 (3.58), T_6 (3.57) and T_5 (3.43). In an average, the lowest disease severity (1.70) was recorded in T_1 which was closely followed by T_2 (2.16) and the highest was recorded in T_8 (3.56) which was closely followed by with T_7 (3.25). (Table 15)

C. Growth parameters

Growth parameters plant height, spike length and distance between flag leaf and base of ear showed significant variation for different treatment in field condition with chemical control measures (Table 16).

In respect of plant height, the longest plant (90.10 cm) was recorded in T_1 which was statistically identical with T_2 (88.03 cm), T_3 (86.73 cm), T_4 (87.43 cm), T_5 (87.23 cm) and T_6 (85.37 cm) which was closely followed by T_7 (83.53 cm) while the shortest plant (81.37 cm) was found in T_8.

Table 14. Effect of different levels of black pointed seeds after chemical control measure at field level on leaf blight severity of wheat at flowering and milking stage

Treatment	Disease severity (0-5 grade) at flowering stage			Disease severity (0-5 grade) at milking stage		
	1st leaf	2nd leaf	Average	1st leaf	2nd leaf	Average
T_1	0.26 d	0.52 d	0.39 e	0.76 d	1.17 b	0.97 e
T_2	0.25 d	0.55 d	0.40 e	0.87 d	1.27 b	1.07 e
T_3	0.33 d	0.97 c	0.65 d	1.07 cd	1.71 ab	1.39 d
T_4	0.35 cd	1.04 c	0.70 cd	1.18 bc	1.96 a	1.57 cd
T_5	0.39 cd	1.19 bc	0.79 c	1.28 bc	2.07 a	1.68 bc
T_6	0.48 bc	1.43 b	0.95 b	1.20 bc	2.21 a	1.71 bc
T_7	0.60 ab	1.51 ab	1.06 b	1.44 ab	2.25 a	1.84 ab
T_8	0.72 a	1.77 a	1.25 a	1.64 a	2.28 a	1.96 a
$LSD_{(0.01)}$	0.133	0.317	0.124	0.298	0.533	0.235
CV(%)	12.48	11.77	9.04	10.24	11.75	8.76

In a column mean values having similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly at 0.01 level of probability

T_1: 0% seed infection (Bavistin treated)

T_2: 0% seed infection (Provax treated)

T_3: 0.1-10% seed infection

T_4: 10.1-15% seed infection

T_5: 15.1-20% seed infection

T_6: 20.1-25% seed infection

T_7: 25.1-40% seed infection

T_8: 50.1-55% seed infection

Table 15. Effect of different levels of black pointed seeds after chemical control measure at field level on leaf blight severity of wheat at hard dough stage

Treatment	Disease severity (0-5 grade) at hard dough stage		
	1st leaf	2nd leaf	Average
T_1	1.31 e	2.10 d	1.70 f
T_2	1.69 d	2.62 cd	2.16 e
T_3	1.72 d	3.23 bc	2.48 d
T_4	1.95 cd	3.30 bc	2.63 d
T_5	2.08 bc	3.43 ab	2.76 cd
T_6	2.31 b	3.57 ab	2.94 c
T_7	2.92 a	3.58 ab	3.25 b
T_8	3.05 a	4.08 a	3.56 a
$LSD_{(0.01)}$	0.326	0.704	0.271
CV(%)	6.29	8.96	5.82

In a column mean values having similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly at 0.01 level of probability

T_1: 0% seed infection (Bavistin treated)

T_2: 0% seed infection (Provax treated)

T_3: 0.1-10% seed infection

T_4: 10.1-15% seed infection

T_5: 15.1-20% seed infection

T_6: 20.1-25% seed infection

T_7: 25.1-40% seed infection

T_8: 50.1-55% seed infection

Table 16. Effect of different levels of black pointed seeds after chemical control measure at field level on yield contributing characters of wheat

Treatment	Plant height (cm)	Spike length (cm)	Distance between flag leaf and base of ear	Spikelets per ear (No.)		
				Healthy	Infected	Total
T_1	90.10 a	16.62 a	15.07 a	32.07 a	1.11 g	33.18 a
T_2	88.03 ab	15.87 ab	14.85 ab	31.35 ab	1.53 f	32.88 a
T_3	86.73 ab	15.77 ab	14.72 ab	30.50 abc	1.97 e	32.47 a
T_4	87.43 ab	15.50 ab	14.48 abc	28.83 abcd	2.53 d	31.37 ab
T_5	87.23 ab	14.93 ab	14.20 abcd	27.93 bcd	3.07 c	31.00 ab
T_6	85.37 abc	14.73 ab	13.88 bcd	26.87 cde	4.00 b	30.87 ab
T_7	83.53 bc	14.55 ab	13.57 cd	25.83 de	4.28 b	30.12 ab
T_8	81.37 c	13.80 b	13.18 d	23.52 e	5.00 a	28.52 b
$LSD_{(0.01)}$	4.427	1.929	0.978	3.428	0.392	3.525
CV(%)	9.11	5.21	6.83	4.97	5.53	4.63

In a column mean values having similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly at 0.01 level of probability

T_1: 0% seed infection (Bavistin treated)

T_2: 0% seed infection (Provax treated)

T_3: 0.1-10% seed infection

T_4: 10.1-15% seed infection

T_5: 15.1-20% seed infection

T_6: 20.1-25% seed infection

T_7: 25.1-40% seed infection

T_8: 50.1-55% seed infection

In consideration of spike length the longest spike (16.62 cm) was recorded in T_1 which was statistically similar with T_2 (15.87 cm), T_3 (15.77 cm), T_4 (15.50 cm), T_5 (14.93 cm), T_6 (14.73 cm) and T_7 (14.55 cm), while the shortest spike (13.80 cm) was recorded in T_8.

In case of distance between flag leaf and base of ear the highest length (15.07 cm) was recorded in T_1 which was statistically similar with T_2 (14.85 cm), T_3 (14.72 cm), T_4 (14.48 cm) and T_5 (14.20 cm), while the lowest (13.18 cm) was found in T_8 which was statistically identical with T_7 (13.57 cm) and T_6 (13.88 cm).

D. Yield contributing characters

Yield contributing characters of wheat differ significantly for different treatment in field condition with chemical control measures under the present trial (Table 16 and Table 17).

In respect of healthy spikelets per ear the maximum (32.07) was recorded in T_1 which was statistically similar with T_2 (31.35), T_3 (30.50) and T_4 (28.83), while the minimum number of healthy spikelets per ear (23.52) was found in T_8 which was statistically identical with T_7 (25.83) and T_6 (26.87). In consideration of infected spikelets per ear, the minimum number of infected spikelets per ear (1.11) was found in T_1 which was closely followed by T_2 (1.53) again, the maximum (5.00) was found in T_8 which was closely followed by T_7 (4.28) and T_6 (4.00). In case of total spikelets per ear the maximum (33.18) was recorded in T_1 which was statistically similar with T_2 (32.88), T_3 (32.47), T_4 (31.37), T_5 (31.00), T_6 (30.87) and T_7 (30.12). On the other hand, the minimum number of total spikelets per ear (28.52) was recorded in T_8.

In respect of number of healthy grains per ear the maximum (43.50) was recorded in T_1 which was statistically similar with T_2 (41.90), T_3 (38.67) and closely followed by T_4 (37.27). Again, the minimum number of healthy grains per ear (24.17) was found in T_8 which was closely followed by T_7 (30.00) and T_6 (31.30). For diseased grains per ear the minimum number of diseased grains per ear (2.60)

Table 17. Effect of different levels of black pointed seeds after chemical control measure at field level on number and weight of grains per ear of harvested seeds of wheat

Treatment	Number of grains per ear			Weight of grains per ear (g)		
	Healthy	Diseased	Total	Healthy	Diseased	Total
T_1	43.50 a	2.60 e	46.10 a	1.97 a	0.08 f	2.06 a
T_2	41.90 ab	2.67 e	44.57 a	1.95 a	0.09 f	2.04 a
T_3	38.67 abc	4.10 d	42.77 ab	1.70 b	0.14 e	1.84 ab
T_4	37.27 bc	4.63 d	41.90 ab	1.68 b	0.16 d	1.84 ab
T_5	35.00 cd	5.83 c	40.83 ab	1.56 bc	0.23 c	1.79 b
T_6	31.30 d	7.05 b	38.35 bc	1.43 cd	0.36 b	1.79 b
T_7	30.00 d	7.83 ab	37.83 bc	1.32 d	0.39 a	1.71 bc
T_8	24.17 e	8.80 a	32.97 c	1.11 e	0.41 a	1.52 c
$LSD_{(0.01)}$	5.184	1.130	5.361	0.188	0.024	0.217
CV(%)	6.06	8.54	5.42	4.95	8.05	4.96

In a column mean values having similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly at 0.01 level of probability

T_1: 0% seed infection (Bavistin treated)

T_2: 0% seed infection (Provax treated)

T_3: 0.1-10% seed infection

T_4: 10.1-15% seed infection

T_5: 15.1-20% seed infection

T_6: 20.1-25% seed infection

T_7: 25.1-40% seed infection

T_8: 50.1-55% seed infection

was recorded in T_1 which was statistically similar with T_2 (2.67) and closely followed by T_3 (4.10) and T_4 (4.63), whereas, the maximum (8.80) was recorded in T_8 which was statistically identical with T_7 (7.83) and closely followed by T_6 (7.05). In case of total grains per ear the maximum (46.10) was recorded in T_1 which was statistically similar with T_2 (44.57), T_3 (42.77), T_4 (41.90) and T_5 (40.83) while, the minimum number of total grains per ear (32.97) was obtained in T_8 which was statistically similar with T_7 (37.83) and T_6 (38.35).

Considering the weight of healthy grains per ear the maximum (1.97 g) was recorded in T_2 which was statistically similar with T_1 (1.95 g) and closely followed by T_3 (1.70 g) and T_4 (1.68 g) and they were statistically similar, while the minimum weight of healthy grains per ear (1.11 g) was found in T_8 which was closely followed by T_7 (1.32 g) and T_6 (1.43 g). In respect of diseased grains per ear the minimum weight of diseased grains per ear (0.08 g) was observed in T_1 which was statistically similar with T_2 (0.09 g) and closely followed by T_3 (0.14 g), while the maximum (0.41 g) was found in T_8 which was statistically identical with T_7 (0.39 g) closely followed by T_6 (0.36 g). In case of total weight of grains per ear, the maximum was recorded in T_1 (2.04 g) which was statistically similar with T_2 (2.04 g), T_3 (1.84 g) and T_4 (1.84 g) and closely followed by T_5 (1.79) and T_6 (1.79) while, the minimum weight of total grains per ear (1.52 g) was obtained in T_8 which was statistically similar with T_7 (1.71 g).

In respect of weight of 1000 grains, the highest was recorded in T_1 (45.58 g) which was statistically similar with T_2 (43.50 g) and closely followed by T_3 (42.37 g), T_4 (41.88 g), T_5 (41.94 g), T_6 (41.50 g) and T_7 (41.00 g) while, the lowest weight of 1000 grains (38.37 g) was found in T_8. (Table 18)

E. Grain and Straw yield

Grain and straw yield of wheat differ significantly for different treatment in field condition with the application chemical control measures (Table 18).

Table 18. Effect of different levels of black pointed seeds after chemical control measure at field level on yield of wheat

Treatment	Weight of 1000 seeds (g)	Grain yield		Straw yield	
		Plot (kg)	Hectare (tons)	Plot (kg)	Hectare (tons)
T_1	45.58 a	1.11 a	3.71 a	1.90 a	6.32 a
T_2	43.50 ab	1.09 a	3.64 a	1.86 a	6.19 ab
T_3	42.37 b	1.06 a	3.54 ab	1.67 b	5.56 abc
T_4	41.88 b	1.03 ab	3.42 abc	1.62 b	5.41 bcd
T_5	41.94 b	0.92 bc	3.05 abc	1.59 bc	5.30 cd
T_6	41.50 b	0.87 c	2.90 bcd	1.43 cd	4.78 cde
T_7	41.00 bc	0.84 c	2.81 cd	1.40 d	4.65 de
T_8	38.37 c	0.69 d	2.29 d	1.33 d	4.42 e
$LSD_{(0.01)}$	2.750	0.136	0.624	0.166	0.780
CV(%)	5.69	8.08	8.08	6.02	6.02

In a column mean values having similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly at 0.01 level of probability

T_1: 0% seed infection (Bavistin treated)

T_2: 0% seed infection (Provax treated)

T_3: 0.1-10% seed infection

T_4: 10.1-15% seed infection

T_5: 15.1-20% seed infection

T_6: 20.1-25% seed infection

T_7: 25.1-40% seed infection

T_8: 50.1-55% seed infection

Considering the grain yields per plot the highest was recorded in T_1 (1.11 kg) which was statistically similar with T_2 (1.09 kg), T_3 (1.06 kg) and T_4 (1.02 kg) while, the lowest yield per plot (0.69 kg) was found in T_8 which was statistically similar with T_7 (3.38 kg) and T_6 (3.48 kg). In respect of grain yields, the highest was recorded in T_1 (3.71 t ha^{-1}) which was statistically similar with T_2 (3.64 tonnes), T_3 (3.54 tonnes), T_4 (3.42 tonnes) and T_5 (3.05 tonnes) while, the lowest yield (2.29 t ha^{-1}) was recorded in T_8 which was statistically similar with T_7 (2.81 tonnes) and T_6 (2.90 tonnes).

In respect of straw yields per plot the highest was recorded in T_1 (1.90 kg) which was statistically similar with T_2 (1.86 kg), whereas, the lowest straw yield per plot (1.33 kg) was found in T_8 which was statistically similar with T_7 (5.58 kg) and T_6 (5.73 kg). In respect of straw yields per hectare the highest was recorded in T_1 (6.32 tonnes) which was statistically similar with T_2 (6.19 tonnes) and T_3 (5.56 tonnes) while, the lowest straw yield (4.42 t ha^{-1}) was found in T_8 which was statistically similar with T_7 (4.65 tonnes) and T_6 (4.78 tonnes).

F. Grading of seeds

Grading of seeds was done in 0-5 rating scale where '0' indicates apparently healthy seeds i.e. showing the minimum disease symptom and '5' indicates seeds with the maximum disease symptom. Significant variation was recorded for grading of seeds in field condition with chemical control measures under the present trial (Table 19).

Considering of grade-0, the maximum value was found in T_1 (43.73) which was statistically similar with T_2 (42.93) and closely followed by T_3 (38.33) and T_4 (37.87), while, the minimum (23.73) was found in T_8 which was closely followed by T_7 (29.67) and T_6 (31.87). In respect of grade-1, the minimum values was recorded in T_2 (1.51) which was statistically similar with T_1 (1.56) and closely followed by T_3 (2.68), T_4 (2.81) and T_5 (3.37) while, the maximum values (4.43) was recorded in T_8 which was statistically similar with T_7 (4.26) and T_6 (4.02).

Table 19. Effect of different levels of black pointed seeds after chemical control measure at field level on different severity grades (0-5) of harvested seeds of wheat

Treatment	Grading of seeds					
	G_0	G_1	G_2	G_3	G_4	G_5
T_1	43.73 a	1.51 d	0.43 f	0.23 d	0.21 e	0.45 d
T_2	42.93 ab	1.56 d	0.43 f	0.21 d	0.22 de	0.44 d
T_3	38.33 bc	2.68 c	0.57 e	0.49 c	0.28 de	0.52 d
T_4	37.87 bc	2.81 c	0.67 de	0.51 c	0.33 cd	0.69 d
T_5	35.07 cd	3.37 bc	0.77 cd	0.67 b	0.43 bc	0.95 c
T_6	31.87 de	4.02 ab	0.88 c	0.77 ab	0.53 b	1.17 bc
T_7	29.67 e	4.26 a	1.09 b	0.89 a	0.68 a	1.36 b
T_8	23.73 f	4.43 a	1.25 a	0.91 a	0.76 a	1.64 a
$LSD_{(0.01)}$	5.032	0.688	0.109	0.133	0.109	0.255
CV(%)	5.85	9.20	5.49	9.38	10.32	11.88

In a column mean values having similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly at 0.01 level of probability

T_1: 0% seed infection (Bavistin treated)

T_2: 0% seed infection (Provax treated)

T_3: 0.1-10% seed infection

T_4: 10.1-15% seed infection

T_5: 15.1-20% seed infection

T_6: 20.1-25% seed infection

T_7: 25.1-40% seed infection

T_8: 50.1-55% seed infection

In case of grade-2, the minimum values was recorded in T_2 and T_1 (0.43) and closely followed by T_3 (0.57) and T_4 (0.67) while, the maximum values (1.25) was recorded in T_8 which was closely followed by T_7 (1.09). In grade-3, the minimum values was recorded in T_2 (0.21) which was statistically similar with T_1 (0.23) and closely followed by T_3 (0.49) and T_4 (0.51) they were statistically identical, while the maximum values (0.91) was recorded in T_8 which was statistically similar with T_7 (0.89) and T_6 (0.77). In case of grade-4, the minimum values was recorded in T_1 (0.21) which was statistically similar with T_2 (0.22) and T_3 (0.28) and closely followed by T_4 (0.33), whereas the maximum values (0.76) was found in T_8 which was statistically similar with T_7 (0.68) and closely followed by T_6 (0.53). In respect of seed grade-5, the minimum values was recorded in T_2 (0.44) which was statistically similar with T_1 (0.45), T_3 (0.52) and T_4 (0.69), while the maximum values (1.64) was obtained in T_8 which was closely followed by T_7 (1.36) and T_6 (1.17).

4.4 Experiment-4: Effect of different levels of black pointed seeds on germination and health status of harvested seeds of wheat in blotter method both for the seeds of without and with chemical treatment

A. Without chemical treatment

In Blotter method after harvested wheat seeds without any chemical treatment germination showed significant differences and the germination percentage varied from 67.83% to 92.00% (Table 20). The maximum seed germination (92.00%) was found in T_1 (0% seed infection) which was statistically similar with T_2 (91.00%), T_3 (89.00%), T_4 (85.00%) and T_5 (81.00%). On the other hand, the minimum seed germination (67.83%) was recorded in T_8 (50.1-55% seed infection) which was statistically identical with T_7 (72.00%) and T_5 (75.17%).

Seed health test of harvested seeds also indicated that seed samples usually significant and higher percentage of *Bipolaris sorokiniana* incidence was recorded with increased percentage of black pointed seeds without chemical treatment (Table 20). Among the different treatments T_1 showed the lowest (8.00%) incidence of *Bipolaris sorokiniana* which was statistically similar with T_2 (9.17%), T_3 (12.17%) and closely followed by T_4 (19.50%). Again, the highest incidence (60.67%) was recorded in T_8 (50.1-55% seed infection) which was closely followed by T_7 (40.33%), T_6 (30.17%) and T_5 (26.33%).

B. With chemical treatment

In Blotter method after harvested wheat seeds with chemical treatment germination percentage showed significant differences under the present trial. There was a gradual tendency of decreasing seed germination percentage with the increase in black pointed seeds in the seeds samples (Table 21). The maximum seed germination (96.00%) was recorded in T_1 (0% seed infection) which was statistically similar with T_2 (95.50%), T_3 (94.00%), T_4 (92.67%), T_5 (92.33%), T_6 and T_7 (90.33%) while, the minimum seed germination (85.33%) was recorded in T_8 (50.1-55% seed infection).

Table 20. Effect of different levels of black pointed seeds on germination and incidence of *Bipolaris sorokiniana* of harvested seeds of wheat in blotter method

Treatment	Germination (%)	Incidence of *Bipolaris sorokiniana* (%)
T_1	92.00 a	8.00 e
T_2	91.00 a	9.17 e
T_3	89.00 a	12.17 e
T_4	85.00 ab	19.50 d
T_5	81.00 abc	26.33 c
T_6	75.17 bcd	30.17 c
T_7	72.00 cd	40.33 b
T_8	67.83 d	60.67 a
$LSD_{(0.01)}$	11.31	5.887
CV(%)	5.81	9.57

In a column mean values having similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly at 0.01 level of probability

T_1: 0% seed infection (Bavistin treated)

T_2: 0% seed infection (Provax treated)

T_3: 0.1-10% seed infection

T_4: 10.1-15% seed infection

T_5: 15.1-20% seed infection

T_6: 20.1-25% seed infection

T_7: 25.1-40% seed infection

T_8: 50.1-55% seed infection

Table 21. Effect of different levels of black pointed seeds after chemical control measure at field level on germination and incidence of *Bipolaris sorokiniana* of harvested seeds of wheat in blotter method

Treatment	Germination (%)	Incidence of *Bipolaris sorokiniana* (%)
T_1	96.00 a	6.83 d
T_2	95.50 a	7.27 d
T_3	94.00 ab	10.00 d
T_4	92.67 ab	14.00 c
T_5	92.33 ab	17.00 c
T_6	90.33 ab	21.67 b
T_7	90.33 ab	24.33 b
T_8	85.33 b	33.00 a
$LSD_{(0.01)}$	8.258	3.353
CV(%)	3.76	8.39

In a column mean values having similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly at 0.01 level of probability

T_1: 0% seed infection (Bavistin treated)

T_2: 0% seed infection (Provax treated)

T_3: 0.1-10% seed infection

T_4: 10.1-15% seed infection

T_5: 15.1-20% seed infection

T_6: 20.1-25% seed infection

T_7: 25.1-40% seed infection

T_8: 50.1-55% seed infection

Seed health test of harvested seeds also performed that seed samples usually significant and higher percentage of *Bipolaris sorokiniana* incidence was recorded with increased percentage of black pointed seeds with chemical treatment in field condition (Table 21). Data reveled that among the different treatments with chemical treatment in field condition T_1 showed the lowest (6.83%) incidence of *Bipolaris sorokiniana* which was statistically similar with T_2 (7.27%) and T_3 (10.00%) and closely followed by T_4 (14.00%) and T_5 (17.00) and they statistically identical. Again, the highest incidence (33.00%) was found in T_8 (50.1-55% infection) which was closely followed by T_7 (24.33) and T_6 (21.67%) and they showed statistically significant differences.

4.5 Comparison and relationship between without and with chemical control measures

Comparison between number of infected seedlings/m^2, disease severity at flag leaf stage, panicle initiation stage, flowering stage, milking stage and hard dough stage and relationship between number of infected seedlings/m^2 and incidence of *Bipolaris sorokiniana* was observed for without and with chemical control measure in different samples with different level of wheat seed infection level.

4.5.1 Number of infected seedlings /m^2

In consideration of average number of infected seedlings/m^2 was calculated and found that the average number of infected seedlings varied for different infection level (Figure 1). Average number of infected seedlings 12.83/m^2 was recorded for without chemical control measures whereas with chemical control measures it was 11.42/m^2. The average number of infected seedlings/m^2 increased 12.39% for without control measures than with control measures for different infection level in field condition.

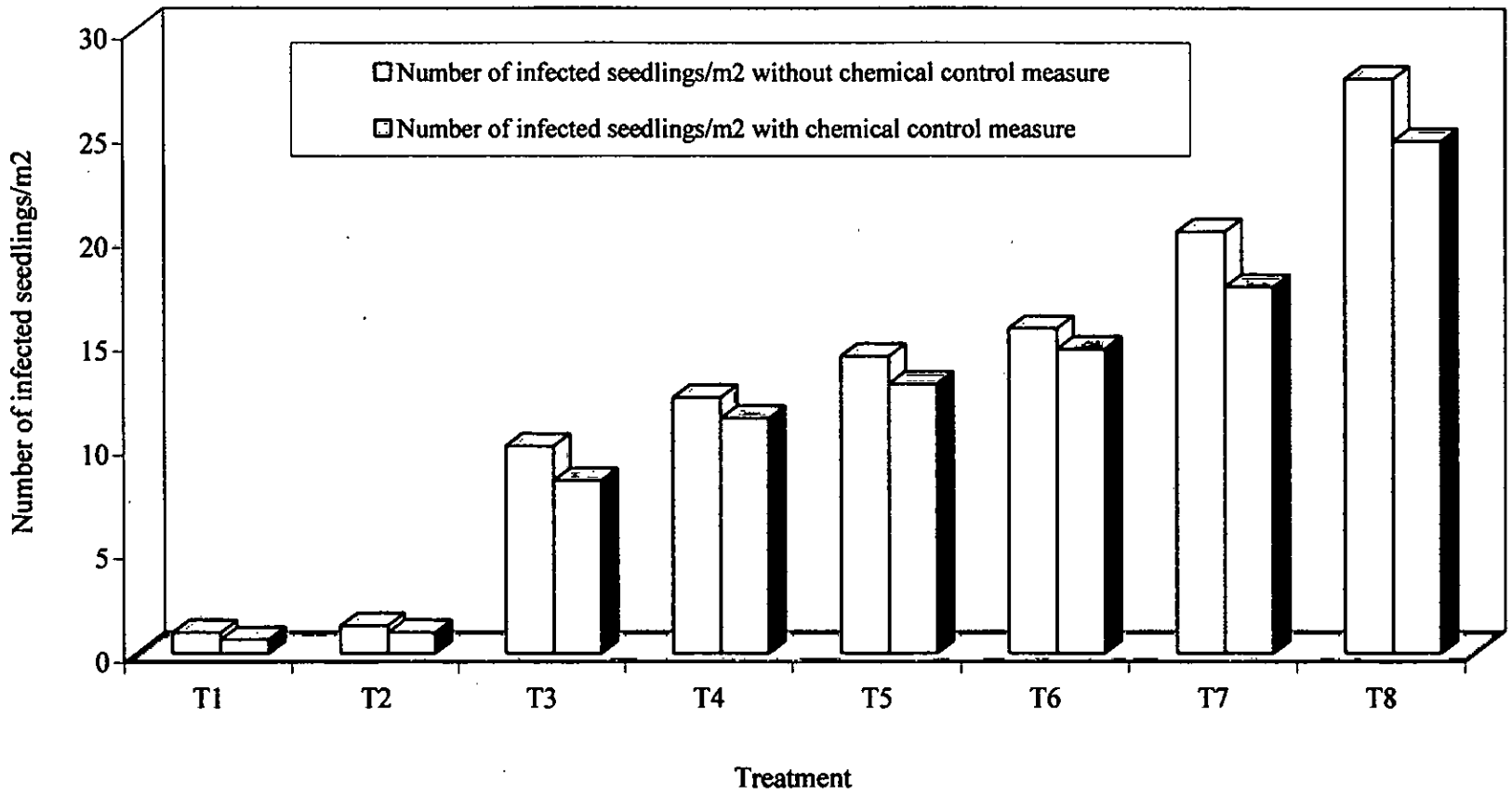


Figure 1. Number of infected seedlings/m2 of wheat without and with chemical control measure

4.5.2 Disease severity at different stage

Disease severity (0-5 grade) was recorded for without and with chemical control measure at flag leaf stage, panicle initiation stage, flowering stage, milking stage and hard dough stage and found that the highest severity was recorded for without control measures than the with control measure for all the recorded growth stage (Figure 2). At flag leaf stage, average disease severity 0.22 was recorded for without chemical control measure, whereas disease severity 0.20 was recorded for with chemical control measures and it was 12.66% increased in case of without chemical control measure than the control measure. At panicle initiation stage, average disease severity 0.46 was recorded for without chemical control measure, while disease severity 0.41 was recorded for with chemical control measures and it was 12.96% increased in case of without chemical control measure than the control measure. At flowering stage, average disease severity 0.83 was recorded for without chemical control measure, while disease severity 0.77 was recorded for with chemical control measures and it was 6.62% increased in case of without chemical control measure than the control measure. At milking stage, average disease severity 1.69 was recorded for without chemical control measure, while disease severity 1.52 was recorded for with chemical control measures and it was 11.16% increased in case of without chemical control measure than the control measure. At hard dough stage, average disease severity 2.97 was recorded for without chemical control measure, whereas disease severity 2.69 was recorded for with chemical control measures and it was 10.43% increased in case of without chemical control measure than the control measure.

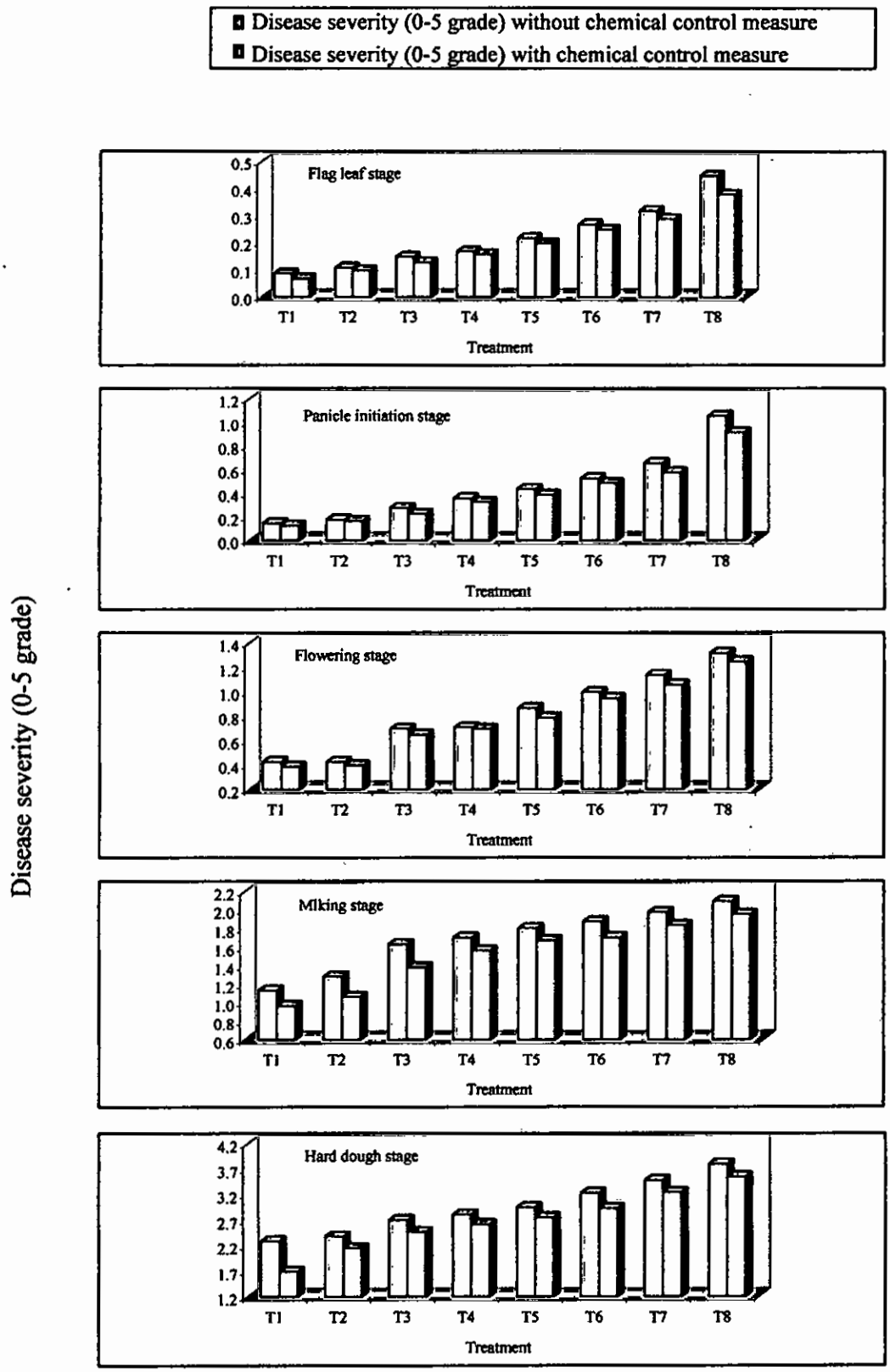


Figure 2. Disease severity (0-5 grade) at different growth stages of wheat without and with chemical control measures

4.5.3 Relationship between number of infected seedlings/m^2 and incidence of *Bipolaris sorokiniana*

Relationship between number of infected seedlings/m^2 and incidence of *Bipolaris sorokiniana* for without and with chemical control measures has been shown in Figure 3. In case of with control measures the regression equation was y = 1.9096x + 1.2881 and the straight line plotted in the Figure indicate that there is a linear relationship between number of infected seedlings/m^2 and incidence of *Bipolaris sorokiniana* and the relationship was highly positive (r = 0.956**). In case of without control measures the regression equation was y = 0.9858x + 4.1121 and the straight line plotted in the Figure indicate that there is a linear relationship between number of infected seedlings/m^2 and incidence of *Bipolaris sorokiniana* and the relationship was highly positive (r = 0.969**).

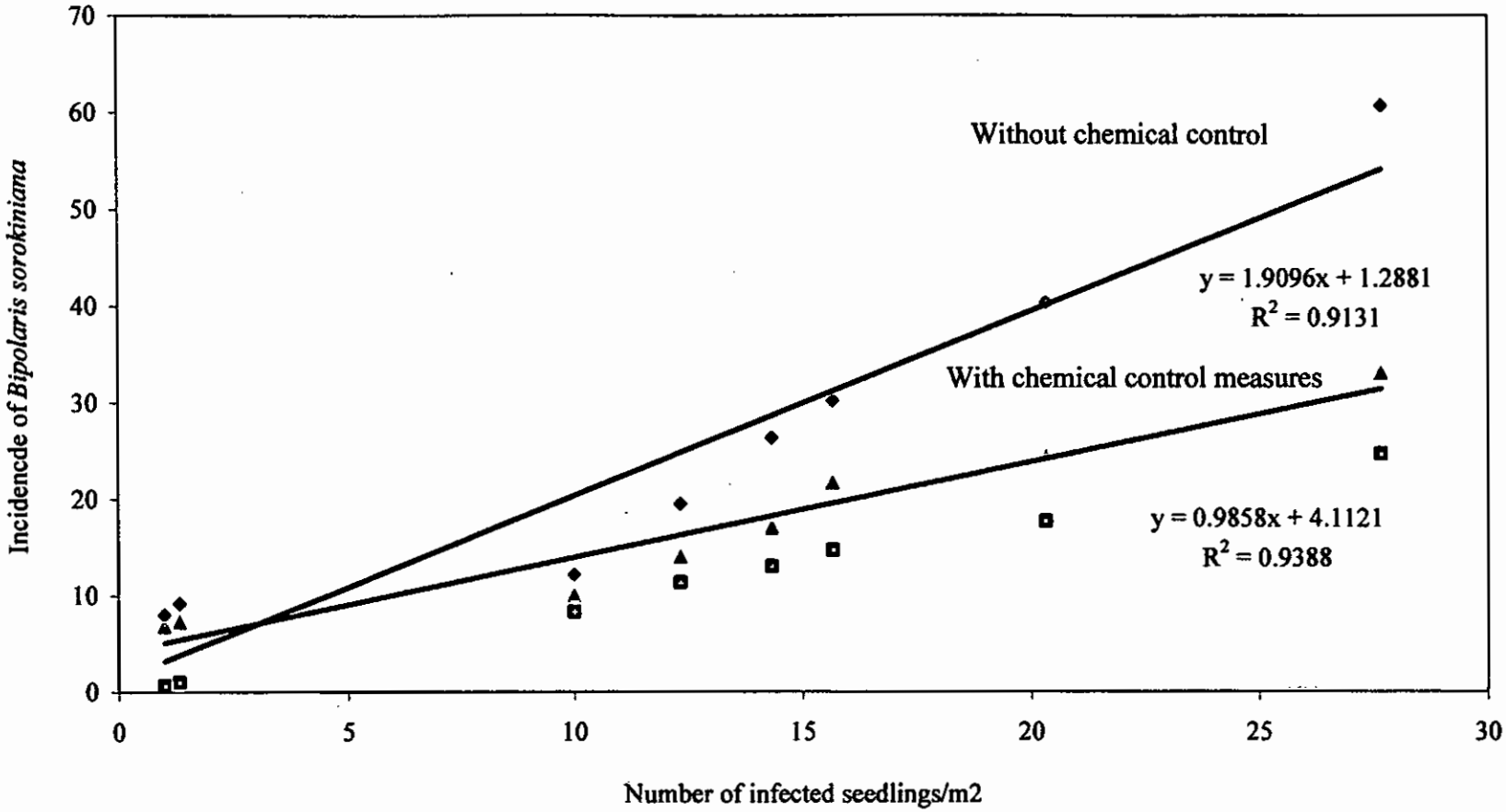


Figure 3. Relationship between number of infected seedlings/m2 and incidence of *Bipolaris sorokiniana* without and with chemical control measure

CHAPTER 5

DISCUSSION

CHAPTER 5

DISCUSSION

In the present experiment, significant effect of different levels of black pointed seeds on seed health status, disease incidence, leaf blight severity, yield contributing characters, yield and quality seed was recorded both laboratory and field condition. There would be altogether eight samples that used as treatment for different experiment which are as follows: T_1 = 0% seed infection (Bavistin treated); T_2 = 0% seed infection (Provax treated); T_3 = 0.1-10% seed infection; T_4 = 10.1-15% seed infection; T_5 = 15.1-20% seed infection; T_6 = 20.1-25% seed infection; T_7 = 25.1-40% seed infection and T_8 = 50.1-55% seed infection.

5.1 Laboratory experiment

In blotter method and water agar test tube seedling symptom test before sowing wheat seeds germination data showed significant differences for different treatments. It was evident that in blotter method, the maximum seed germination (98.00%) was found in T_1 (0% seed infection) and the minimum (78.00%) was recorded in T_8 (50.1-55% seed infection). In water agar test tube seedling symptom test method, the maximum seed germination (96.00%) was recorded in T_1 and T_2 (0% seed infection) whereas the minimum (64.00%) was recorded in T_8. The findings of the present study was supported by Choudhary *et al.*, 1984; Khanum *et al.*, 1987; Hossain, 2000). They observed that seed germination as 66.00% and 62.00% from the seeds having 81.00% and 74.00% infection, respectively with *H sativum.* Choudhary *et al.* (1984) reported that germination of the diseased (blackpointed) seeds both in *blotter* and pot soil was found to decrease by 11.60% and 16.00%, respectively. Khanum *et al.* (1987) found 55.00-96.00% and 43.50-71.00% germination, respectively. Hossain (2000) reported that maximum reduction of germination was found by 20.20% and 42.69% in blotter and rolled paper towel method, respectively in 28% black pointed seeds. Reduction in germination of wheat seeds due to black point infection was also recorded by Rana and Gupta, 1982 and Zhang *et al.*, 1990).

Seed health test also indicated that seed samples usually significant and higher percentage of *Bipolaris sorokiniana* with increased percentage of black pointed seeds. In blotter method, among the different treatments T_1 and T_2 performed completely free from the pathogen and while the highest incidence (57.00%) was recorded in T_8. In water agar test tube seedling symptom test significant differences were recorded for different black pointed seeds before sowing for dead seedlings. The lowest dead seedlings (4.00%) were recorded in T_1 and T_2 again the highest (34.00%) was recorded in T_8 (50.1-55% seed infection). Similar findings of the present study are supported by the earlier reports by Rana and Gupta, 1982; Rahman and Islam, 1998; Bazlur Rashid, 1998; Hossain, 2000). Rahman and Islam (1998) observed significant reduction in seedling vigor in respect of germination and percentage of dead seedlings with the increase of black point infection. Bazlur Rashid and Fakir (1998) reported that percent reduction in shoot and root length increased with the increase of infection grade of seed transmitted *Bipolaris sorokiniana* and the overall reductions were the highest for root length. He also mentioned that the seedlings that developed from such seed were usually poor vigorous. Hossain (2000) found that the rate reduction of growth was maximum by 28% black pointed seeds. He also mentioned that Vigor Index was found with maximum reduction (72.63%) resulted by the seedlings of 28% black pointed seeds.

5.2 Field experiment

In field condition, different black pointed seeds without or with chemical control measures showed significant variations for number of healthy seedlings/m^2. In field condition without chemical treatment the maximum number of healthy seedlings/m^2 (132.33) was recorded in T_1 and the minimum (88.33) in T_8. The minimum number of infected seedlings/m^2 (1.00) was recorded in T_1 while the maximum (27.67) was recorded in T_8. On the other hand, in field condition with chemical control measures the maximum number of healthy seedlings/m^2 (132.67) was found in T_1 again the minimum number (91.00) was obtained in T_8. The minimum number of infected seedlings/m^2 (0.67) was recorded in T_1 and the

maximum (24.67) was found in T_8. These results were well supported by the others researchers earlier. Hossain (2000) reported that significant decrease in plant stand in field has been observed with the increase in number of black pointed seeds in seed samples. Machacek and Graney (1938) also reported from their field test that seed infected with *Helminthosporium sativum* produced only 24.8% plant stand and resulted 80.6% seedling infection.

From the present study, it was evident that leaf blight severity (0-5 grade) was measured in 1st and 2nd leaf and average also calculated as treatment wise at flag leaf, panicle initiation, flowering, milking and hard dough stage and significant variation due to the effect of different levels of seed infection subsequently was very significant with the increasing trend of leaf blight severity. The minimum leaf blight severity was recorded in the plots of T_1 (0% seed infection) and the maximum severity was recorded in T_8 (50.1-55% seed infection) in every growth stages. Leaf spot/leaf blight development is a usual consequence of the seed to plant to seed transmission of the pathogen *(B. sorokiniana)* under field condition (Bazlur Rashid, 1996, Bazlur Rashid and Fakir, 1998). The disease severity was found to increase with the age of the plant and the maximum disease severity was observed in hard dough stage in all the treatments than the other stages. Nema and Joshi (1974) reported that age was one of the important factors influencing disease intensity and susceptibility of wheat plant to *H. sativum* increased with the age of the plants. Hossain and Azad (1992) reported that higher age of crop plant resulted higher incidence of leaf spot (*B. sorokiniana*). Hossain (2000) reported higher the level of seed borne fungal infection, there will be higher primary inoculum level in the field resulted higher infection in the field. He also found that the maximum infection severity was attained at hard dough stage due to favorable temperature range 25-28^{0}C for disease epidemic in March when the plants turn to soft dough to hard dough stage. Reza *et al.* (2006) found that the maximum seed infection level gave rise the highest disease severity in adult plants.

Growth parameters like as plant height, spike length and distance between flag leaf and base of ear showed significant variation for different treatment in field

condition without or with chemical control measures. In field condition without or with chemical control measures the longest plant, highest spike length and distance between flag leaf and base of ear was recorded in T_1 whereas the lowest was found in T_8. On the other hand, in field condition with chemical control measures the longest plant, highest spike length and distance between flag leaf and base of ear was recorded in T_1 whereas the lowest was found in T_8. Yield contributing characters like as healthy, infected and total spikelets per ear of wheat differ significantly for different treatment in field condition without or with chemical control measures. In field condition without or with chemical control measures the maximum number of healthy, infected and total spikelets per ear was recorded in T_1 whereas the lowest was recorded in T_8. Grain formation and grain weight were significantly differed with the different levels of seed infection. Number of grains/ear and number of healthy grains/ear were decrease with the increase of seed infection whereas number of diseased grains/ear was increased with the same pattern. Considering the weight of 1000 grains, grain and straw yield of wheat differ significantly for different treatment in field condition without or with application of any chemical control measures. The highest weight of 1000 grains, grain and straw yield was recorded in T_1 while, the lowest was obtained in T_8. These findings were supported by different researcher earlier with working in similar condition. Bazlur Rashid *et al.* (1994) reported that the relationship of leaf blight incidence with associate with the seed quality and seed quality effect number of healthy, infected and total spikelets per ear and as well as grains/ear. Hossain *et al.* (1998) observed that leaf infection at flowering stages has direct effect on the formation of healthy grains with the increase in number of black pointed seeds. Bazlur Rashid and Fakir (1998) reported that shriveled grain and black pointed kernel symptoms have been recorded as the effect of seed to plant to seed transmission of *B. sorokiniana*. Hossain (2000) found that the higher level of black point infection in the seed sample encouraged more disease to the crop plants resulting formation of higher number of diseased seed in the field condition and ultimate results was the highest grain and straw yield was obtained from the seeds with no or minimum infection.

Significant variation was recorded for grading of seeds in field condition without or with applying any chemical control measures. In case of grade-0, 1, 2, 3, 4 and 5 the maximum value was recorded in T_1 and the minimum was obtained in T_8 in most of the cases. Bazlur Rashid (1996); Hossain *et al.* (1998); Reza *et al.* (2006) and Shukti (2008) also reported similar findings in their earlier experiment. Bazlur Rashid (1996) reported that development of black pointed infection in the field was due to seed to plant to seed transmission of black pointed pathogen. Hossain *et al.* (1998) observed that leaf infection at flowering and milk ripening stages has direct effect on the reduction of formation of healthy grains with the increase in number of black pointed as well as discolored grains. Hossain (2000) also found significant relationship of leaf blight severity with grain infection. Reza *et al.* (2006) found that 65.36% disease severity interning the corresponding 17.42% seed infection.

5.3 Laboratory experiment (after harvesting)

In blotter method after harvested wheat seeds without or with chemical control measures percentage of germination showed significant differences. The maximum seed germination was found in T_1 (0% seed infection) and the minimum was recorded in T_8 (50.1-55% seed infection). Seed health test of harvested seeds also indicated that seed samples usually significant and higher percentage of *Bipolaris sorokiniana* incidence was recorded with increased percentage of black pointed seeds without or with chemical control measures. Among the different treatments T_1 showed the lowest incidence of *Bipolaris sorokiniana* again the highest incidence was recorded in T_8. These findings were supported by Orsi *et al.* (1994) and Shukti (2008). They found significant relationship between *Bipolaris sorokiniana* incidence with increased percentage of black pointed seeds.

In the view of above findings, it has been found that minimum level of black pointed seeds resulted minimum disease incidence and subsequent disease development in the field as well as for quality seed production.

5.4 Comparison and relationship between without and with chemical control measures

The average number of infected seedlings/m^2 increased 12.39% for without control measures than with control measures for different infection level in field condition. At flag leaf stage, average disease severity 12.66% increased in case of without chemical control measure than the control measure. At panicle initiation stage, average disease severity 12.96% increased in case of without chemical control measure than the control measure. At flowering stage, average disease severity 6.62% increased in case of without chemical control measure than the control measure. At milking stage, average disease severity 11.16% increased in case of without chemical control measure than the control measure. At hard dough stage, average disease severity 10.43% increased in case of without chemical control measure than the control measure. There is a linear relationship between number of infected seedlings/m^2 and incidence of *Bipolaris sorokiniana* and the relationship was highly positive for without and with control measures. Shukti (2008) reported a highly positive linear relationship between seed infection levels and incidence of *Bipolaris sorokiniana* on harvested seeds.

CHAPTER 6

SUMMARY AND CONCLUSION

CHAPTER 6

SUMMARY AND CONCLUSION

The experiments were conducted in the Seed Health Laboratory and Experimental Field of Plant Pathology Department, Sher-e-Bangla Agricultural University, Dhaka-1207, during the period from October 2008 to April 2009 to find out the transmission of *Bipolaris sorokiniana* from seed to plant to seed of wheat. There would be altogether eight samples that used as treatment for different experiment which are as follows: T_1 = 0% seed infection (Bavistin treated); T_2 = 0% seed infection (Provax treated); T_3 = 0.1-10% seed infection; T_4 = 10.1-15% seed infection; T_5 = 15.1-20% seed infection; T_6 = 20.1-25% seed infection; T_7 = 25.1-40% seed infection and T_8 = 50.1-55% seed infection.

In blotter method the maximum seed germination (98.00%) was found in T_1 and the minimum (78.00%) in T_8. Among the different treatments T_1 and T_2 performed completely free from the pathogen and the lowest incidence (2.50%) was recorded from T_3, again the highest incidence (57.00%) in T_8. In water agar test tube seedling symptom test, the maximum seed germination (96.00%) was recorded in T_1 and T_2, while the minimum (64.00%) in T_8. At flag leaf stage, in an average the lowest disease severity (0.09) was recorded in T_1 and the highest (0.45) in T_8. At panicle initiation stage, in an average the lowest disease severity (0.15) was observed in T_1, whereas the highest (1.06) in T_8. At flowering stage, in an average the lowest (0.43) was found in T_1 and T_2, whereas the highest (1.32) was found in T_8. At milking stage, in an average, the lowest disease severity (1.14) was recorded in T_1 and the highest (2.10) in T_8. At hard dough stage, in an average the lowest disease severity was recorded in T_1 (2.30) and the highest in T_8 (3.81). Considering plant height the longest plant (89.35 cm) was recorded in T_1 whereas the shortest (80.80 cm) in T_8. In case of healthy spikelets per ear the maximum (31.20) was recorded in T_1 and the minimum (23.10) in T_8. Considering the weight of healthy grains per ear the maximum (1.92 g) was recorded in T_2

whereas the minimum (1.06 g) in T_8. For grain yields per hectare the highest (3.67 tonnes) were recorded in T_1 while the lowest yield per hectare (2.25 tonnes) in T_8.

In field condition with chemical control measures the maximum number of healthy seedlings/m^2 (132.67) was found in T_1 again the minimum (91.00) was obtained in T_8. The minimum number of infected seedlings/m^2 (0.67) was recorded in T_1 and the maximum (24.67) in T_8. At flag leaf stage, in an average the lowest disease severity (0.07) was recorded in T_1 and the highest (0.38) in T_8. At panicle initiation stage, in an average the lowest disease severity (0.13) was observed in T_1 whereas the highest (0.92) was recorded in T_8. At flowering stage, in an average the lowest (0.39) was recorded in T_1 while the highest (1.25) in T_8. At milking stage, in an average the lowest disease severity (0.97) was observed in T_1 and the highest (1.96) in T_8. At hard dough stage, in an average the lowest disease severity (1.70) was recorded in T_1 and the highest (3.56) in T_8. In respect of plant height, the longest plant (90.10 cm) was recorded in T_1 while the shortest (81.37 cm) in T_8. In case of healthy spikelets per ear the maximum (32.07) was recorded in T_1 and the minimum (23.52) in T_8. Considering the weight of healthy grains per ear the maximum (1.97 g) was recorded in T_2 while the minimum (1.11 g) in T_8. In respect of grain yields per hectare the highest (3.71 tonnes) was recorded in T_1 while the lowest (2.29 tonnes) in T_8.

In blotter method, harvested wheat seeds without any chemical control measures the maximum seed germination (92.00%) was found in T_1 and the minimum (67.83%) in T_8. The lowest incidence (8.00%) and the highest incidence (60.67%) of *Bipolaris sorokiniana* was recorded in T_1 and T_8, respectively. In same method, harvested wheat seeds with chemical control measure the maximum seed germination (96.00%) was recorded in T_1 whereas the minimum (85.33%) in T_8. In same way, the lowest incidence (6.83%) and the highest incidence (33.00%) was recorded in T_1 and T_8, respectively. There is a linear relationship between number of infected seedlings/m^2 and incidence of *Bipolaris sorokiniana* and the relationship was highly positive for without and with control measures.

REFERENCES

REFERENCES

Alam K. B. Shaeed, M. A. Ahmed, A. U. and Malaker, P. K. 1994. Bipolaris leaf blight (spot blotch) of wheat in Bangladesh. In: Saunders, D.A. and G.P. Hettel (eds.). Wheat in Heat stressed Environments: Irrigated, Dry Areas and Rice Wheat Farming Systems. Mexico, D.F. CIMMYT. Pp 339-342.

Alam, K. B., Malaker, R. K., Shasheed, M. A., Ahmed, M. U., Ahmed, F. and Haque, M. S. (1995). Yield loss assessment of wheat due to Bipolaris leaf blight in Bangladesh. *J. Pl. Path.*, **11**(1-2): 35-37.

Ali, A. A. and Fakir, G. A. (1992). Control of some selected seed-borne fungal pathogens of jute by seed treatment with garlic extract. Proc. BAU, *Res. Prog.* **6:** 176-180.

Aylor, D. E. (2003). Spread of plant disease on a continental scale: role of aerial dispersal of pathogens. *Ecology*. **84**(8): 1989-1997.

BARI (Bangladesh Agricultural Research Institute). (2000). Annual Report. (1999-2000). BARI, Gazipur. 34 p.

BARI. (1997). Increase wheat cultivation and decrease irrigation cost (A folder in Bengali). Wheat Res. Centre. Bangladesh Agril. Res. Inst. Nashipur, Dinajpur. pp. 12-15.

Bazlur Rashid, A. Q. M. (1996). *Bipolaris sorokiniana* in wheat seeds of Bangladesh. Ph. D. Thesis. Dept. of Plant Pathology. BAU, Mymensingh. pp. 181-185.

Bazlur Rashid, A. Q. M. (1997). *Bipolaris sorokiniana* in wheat seeds of Bangladesh. Ph. D. Thesis. Dept. of Plant Pathology. BAU, Mymensingh. pp. 181-185.

Bazlur Rashid, A. Q. M. and Fakir, G. A. (1998). Seed borne nature and transmission of *Bipolaris sorokiniana* of wheat. In: Bangladesh Travel Report, First National Workshop on Seed Pathology and Inauguration of the Danida Seed Pathology Laboratory in Bangladesh. June 6-12, 1998, by S.B. Mathur. 10p.

Bazlur Rashid, A. Q. M. Lahiri, B, P. and Islam, T. (1994). Effect of *Bipolaris sorokiniana* on some yield components and seed quality of wheat. *Bangladesh J. Agric. Sci.* **21**: 185-192.

Bazlur Rashid, A. Q. M., (1992). Effect of *Bipolaris sorokiniana* leaf blight on some yield components and seed quality of wheat. *Bangladesh J. Agric Sci.*, 20(1): 65-69.

BBS (Bangladesh Bureau of Statistics). (1999). Statistical Year Book of Bangladesh. Bangladesh Bureau of Statistics. Statistics Division. Ministry of Planning. Government of the Peoples Republic of Bangladesh. Dhaka. pp. 121.

BBS, (2008). Summary Crop Statistics of Major Crops. http://www.bbs.gov.bd. Bangladesh Statistics division, Ministry of Planning, Govt. of Bangladesh, Dhaka.

Chaudhary,R. C. Aujia, S. S. Sharma, I. and Singh, R. (1984). Effect of black point of wheat on germination and quality characters of WL-711. *Indian Phytopath.* **37**(2): 351-353.

Dubin, H. J. and M. V. Ginkel (1991). The status of wheat diseases and disease research in warmer areas. In D. A. Saunders ed., wheat for the non traditional, warm areas, Mexico D. F. CIMMYT. 125-145 pp.

Elekes, P. (1983). Methods for detecting hyphal and living fungal infection in wheat seed samples. *Seed Sci. Tech.*, **11**: 421-433.

Fakir, G. A. (1988). Report on investigation into black point disease of wheat in Bangladesh. Seed Pathology Laboratory. Department of Plant Pathology, BAU, Mymensingh.

Fakir, G. A., Khan, A. L., Neergaard, P. and Mathur, S. B. 1977. Transmission of *Drechslera* spp. Through wheat seed in Bangladesh. Bangladesh J. Agric. 1(2): 113-118

FAO. (1997). Production Yearbook. Food and Agriculture Organization of the United Nations, Italy, Rome. 62 p.

FAO. (2000). Production Yearbook. Food and Agriculture Organization of the United Nations, Italy, Rome, 62p.

Forsberg, G., Johnsson, L. and Lagerholm, J. (2005). Effects of aerated steam seed treatment on cereal seed-borne diseases and crop yield. *J. Pl. Sci.*, **112**(3): 247-256.

Frank, J. A. (1985). Influence of root rot on winter survival and yield of winter barley and winter wheat. *Phytopath.*, **75**: 1039-1041.

Gomez, K. A. and Gomez, A. A. (1984). Statistical Procedure for Agricultural Research (2nd edn.). Int. Rice Res. Inst., A Willey Int. Sci., Pub., pp. 28-192.

Hampton, J. G. (1980). Fungal pathogens in New Zealand certified seed. *New Zealand J. Exp. Agric.* 7: 391-393.

Hetzler, J, Eyal, Z. Mehta, Y. R. Campos, L. A, Fehrmann, H, Kushnir, U, Zekaria, J. and Cohen, L. (1991). Interactions between spot blotch *(Cochliobolus sativus)* and wheat cultivars. In: Wheat for the Nontraditional Warm Areas, ed. Saunders, D.A. Mexico, D.F, CIMMYT, 146-164pp.

Hossain, I. and Azad, A. K. (1994). *Bipolaris sorokiniana*, its reaction and effect on yield of wheat. *Prog. Agric.*, **5**(2): 63-69.

Hossain, I. and Azad, A. K. (1992). Reaction of Wheat to *Helminthosporium sativum* in Bangladesh. *Hereditas*. **116**: 203-205.

Hossain, I. Bazlur Rashid, A. Q. M; Fakir, G. A. and Meah, M. B. (1998). Leaf blight of wheat: Its status and impact on grain formation. First national workshop on seed pathology. Progress and prospect of seed pathological research in Bangladesh. Dept. of Plant Pathology. BAU, Mymensingh. pp. 9-10.

Hossain, M. M. (2000). Effect of different levels of black pointed seed on germination, seedling vigor, plant stand and seed quality of wheat. M.S. Thesis. Dept of Plant Pathology. BAU, Mymensingh.

ISTA. (1996). Iternational Rules of Testing Association. Proc. *Int. Seed Test. Assoc.*, 180. p.

Kabir, M. H., Aminuzzaman, F. M., Islam, M. R. Chowdhury, M. S. M. (2007). Effect of physical and chemical seed treatments on leaf spot (*Bipolaris sorokiniana*) and yield of wheat. *World J. Agril. Sci.*, **3**(3): 306-315.

Khanum, M., Nigar, Y. and Khanzada, A. K. (1987). Effect of black point disease on the germination of wheat varieties. *Pakistan J. Agric. Res.* **8**(4): 467-473.

Khokon, M. A. R., Meah, M. B. and Fakir, G. A. (2005). Inert matter with rice and wheat seeds is source of inoculum of plant pathogens. *Seed Sci. Technol.*, **33**(1): 127-140.

Lannou, C., Soubeyrand, S., Frezal, L. and Chadoeuf, J. (2008). Autoinfection in wheat leaf rust epidemics. *New Phytologist*. 177(4): 1001-1011.

Lin, Y. Q. (1985). Damage by wheat root rot and the efficacy of control with Bay Meb 6447. *Plant Prot.* **11**(1): 2-4.

Luz, W. C. (2003). Evaluation of biological and chemical wheat seed treatments. *Fitopatologia Brasileira.* **28**(1): 93-95

Machacek, J. E. and Graney, F. J. (1938). The black point or kernel or kernel smudge diseases of cereals. *Canadian J. Res.*, **16**: 84-113.

Majumder, A. R. (1991). Assessment of yield loss caused by common root rot in wheat a cultivar in Queensland *(Bipolaris sorokiniana). Australian. J. Agril. Res.*, **13**(3): 143-151.

Malaker, P. K., Reza, M. M. A., Ahmed, F., Rahman, M. M., Rahman, M. M. and Amin, M. F. (2008). Occurrence and significance of black point disease of wheat seeds in Bangladesh: a review. *Bangladesh J. Pl. Patho.*, **23**(1/2): 43-50.

Mathur, S. B. and Kongsdal O. 2003. Common laboratory seed sheath testing methods for detecting fungi. International seed testing association, Denmark. 3rd edition, p: 47.

Mondal, H. (2000). Effect of seed and soil borne inocula of *Bipolaris sorokiniana* on seedling mortality and spot blotch of wheat. M.S. Thesis. Department of Plant Pathology, BSMRAU, Gazipur, 32p.

Nalli, R. (1986). Observations on the effects produced by *Bipolaris sorokiniana* on wheat. *Seed Abs.* 11(3): 100.

Nema, K. G. and Joshi, L. M. (1974). Spot blotch disease of wheat in relation to host age, temperature and moisture. *Indian Phytopath.* 26: 41-48.

Orsi, C., Chiusa, G. and Rossi, V. (1994). Further investigation on the relationship between the mycoflora of durum wheat kernels and the incidence of black point. *Petria.* **4**(3). 225-235.

Raemaekers, R. H. (1988). *Helminthosporium sativum:* Disease complex on wheat and sources of resistance in Zambia. In: Wheat production constraints in tropical environments, ed. Klatt, A.R. Mexico, D.F. CIMMYT, 175-186pp.

Rahman, G. M. and Islam, M. R. (1998). Effect of black point of wheat on some qualitative characters of its grain and seed vigor. *Bangladesh J. Agric. Res.*, **23**(2): 283-287.

Rana, J. P. and Gupta, P. K. S. (1982). Occurrence of black point disease of wheat in West Bengal. *Indian Phytopath.* **35**: 700-702.

Randhawa, H. S. and Sharma, H. L. (1985). Detection and distribution of *Cochliobolus sativus* on wheat seed in the Punjab State. *Indian Phytopath.* **38**: 341-343.

Razzaque, M. A. and A. B. S. Hossain. (1991). The wheat development program in Bangladesh. "Wheat for the non traditional warm areas" edited by D. A. Saunders. Proc. International conf. held in July 29 to August 3, 1990. in Foz do Iguacu, Brazil.

Razzaque, M. A. Tancald, D. and Hossain A. B. S. (1992). The wheat development programme in Bangladesh "Wheat for the nontraditional warm areas" edited by D.A. Saunders. Proc. Intl.Conf. held in July 29 to Aug. 3, 1990 in Foz dolguacu Brazil CIMMYT. pp. 44-54.

Reis, E. M. (1991). Integrated disease management-The changing concepts of controlling head blight and spot blotch. In: Wheat for the Nontraditional, Warm Areas, Mexico, D.F. CIMMYT, ed. Saunders, D.A. 165-177pp.

Reza, M. M. A. Khalequzzaman, K.M. and Bazlur Rashid, A. Q. M. (2006). Effect of different levels of seed and plant infection by *Bipolaris sorokiniana* on wheat. *Bangladesh J. Agric. Res.* **31**(2): 241-248.

Saari, E. E. (1985). Distribution and importance of root rot diseases of wheat, barley and triticale in Southand Southeast Asia. In: Wheat for More Tropical Environments. A Proceeding of the International Symposium, CIMMYT, 189-195pp.

Salehpour, M., Etebarian, H. R., Roustaei, A., Khodakaramian, G. and Aminian, H. (2005). Biological control of common root rot of wheat (*Bipolaris sorokiniana*) by Trichoderma isolates. *Pl. Patho. J.*, **4**(1): 85-90.

Schmidt, H. S. (1986). Investigation of the influence of black point infested wheat seed at various infestation levels on the cultivated crop. Bangladesh German Seed Development Project. Dhaka, Bangladesh. 23pp.

Shaner, G. (1981). Effect of environment on fungal leaf blights of small grains. *Ann. Rev. Rev. Phytopath.* **19**: 273-296.

Shukti, R. C. (2008). Effect of different levels of black pointed seeds on seedling vigor, leaf blight (*Bipolaris sorokiniana*) development and quality seed production of wheat. M. S. Thesis. Department of Plant Pathology, Shere-e-Bangla Agricultural University, Shere-e-Bangla Nagar, Dhaka, 116pp.

Singh, D. P. (2004). Losses due to leaf spots caused by *Bipolaris sorokiniana* and net blotches by *Helminthosporium teres* in barley and their effective management. *Pl. Dis. Res. Ludhiana.* **19**(2): 173-175.

Singh, D. V. Srivastava, K. D. and Joshi, L. M. (1989). Occurrence and distribution of blackpoint disease of wheat in India. *Seed Res.*, **17**(2): 164-168.

Sinha, A. P. and Thapliyal, P. N. (1984). Seed disinfection in relation to black point disease of Triticle. *Indian Phytopath.* **37**(1): 154-155.

Sisterna, M. N. and Sarandon, S. J. (2005). Preliminary studies on the natural incidence of wheat blackpoint under different nitrogen fertilization levels and tillage systems in Argentina. *Pl. Pathol. J.*, **4**(1): 26-28.

Talukder, K. A. and Fakir, G. A. (1991). Association of fungi with spikelet parts during development of wheat. Fourth Biennial Conf. Bangladesh Phytopathol. Soc. Abstr. No. 40.

Tanner, D. G. (1988). Characterization of tropical wheat environments: Identification of production constraints and progress achieved in Africa. In: Wheat production constraints in tropical environments, ed. Klatt, A. R. Mexico, D. F. CIMMYT, 35-43 pp.

Uttam, K., Joshi, A. K., Sundeep, K., Ramesh, C. and Roder, M. S. (2009). Mapping of resistance to spot blotch disease caused by *Bipolaris sorokiniana* in spring wheat. *TAG Theoretical and Applied Genetics*. **118**(4): 783-792.

Viedma, L. Q. (1990). *Drechslera sorokiniana* and other seed-borne fungi in wheat samples from Paraguay. In: Summaries of Research Projects 1967-88 conducted at the Institute of Seed Pathology for Developing Countries, Copenhagen, Denmark. 64p. ed, by S. B. Mathur.

Vlasenko, N. G., Teplyakov, B. I. and Teplyakova, O. I. (2004). Effectiveness of agrochemicals for growing of spring wheat. *Zashchita Karantin Rastenii.* **9**: 47-48.

Zamal, M. S., Aminuzzaman, F. M., Sultana, N. and Islam, M. A. (2007). Efficacy of fungicides in controlling leaf blight of wheat caused by *Bipolaris sorokiniana. Interl. J. Sustain. Agril. Tech.*, **3**(2): 1-6.

Zhang, T. Y. Wang, H. L. Xu, F. L. (1990). Effects of grain black point disease of wheat and the pathogenic fungi. *Acta Phytophylacica*. **17**(4): 313-316.

Zhimin, X. Lianfa, S. and Wenli, X. (1998). Breeding for foliar blight resistance in Heilongjang Province, China. *In:* Helminthosporium Blighrs of wheat: Spot Blotch and Tan Spot. E. Duveillier, H. J. Dubin, J. Reeves and A. McNab (eds.). Mexico, D. F.: CIMMYT. pp. 114-118.

Zobaer, A. S. M., Aminuzzaman, F. M., Chowdhury, M. S. M. and Miah, M. S. (2007). Effect of manual seed sorting, seed solarization and seed treatment with vitavax-200 and hot water on black point (*Bipolaris sorokiniana*) of wheat. *Interl. J. Sustain. Agril. Tech.*, 3(2): 54-59.

APPENDICES

APPENDICES

Appendix I. Characteristics of experimental field soil analyzed in Soil Resources Development Institute (SRDI), Khamarbari, Farmgate, Dhaka

A. Morphological characteristics of the experimental field

Morphological features	Characteristics
Location	Agronomy field , SAU, Dhaka
AEZ	Madhupur Tract (28)
General Soil Type	Shallow red brown terrace soil
Topography	Fairly leveled

B. Physical and chemical properties of the initial soil

Characteristics	Value
% Sand	27
% Silt	43
% clay	30
Textural class	Silty-clay
pH	5.6
Organic matter (%)	0.78
Total N (%)	0.03
Available P (ppm)	20.00
Exchangeable K (me/100 g soil)	0.10
Available S (ppm)	45

Appendix II. Monthly record of air temperature, relative humidity and rainfall of the experimental site during the period from August to October 2008 and April, 2009

Month	*Air temperature (°C)		*Relative humidity (%)	*Rainfall (mm) (total)
	Maximum	Minimum		
October, 2008	29.18	18.26	81	39
November, 2008	25.82	16.04	78	00
December, 2008	22.4	13.5	74	00
January, 2009	24.5	12.4	68	00
February, 2009	27.1	16.7	67	30
March, 2009	31.4	19.6	54	11
April, 2009	33.2	21.1	61	88

* Monthly average,

* Source: Bangladesh Meteorological Department (Climate & weather division) Agargoan, Dhaka.

Appendix III. Analysis of variance of the data on germination and incidence of *Bipolaris sorokiniana* of wheat seeds before sowing in blotter method due to the effect of different levels of black pointed seeds

Source of variation	Degrees of freedom	Mean square	
		Germination (%)	Incidence of *Bipolaris sorokiniana* (%)
Between	7	156.415**	1156.929**
Within	16	35.750	4.406

**: Significant at 0.01 level of probability

Appendix IV. Analysis of variance of the data on germination and seedling health of wheat seeds in the laboratory before sowing in water agar test tube seedling symptom test due to the effect of different levels of black pointed seeds

Source of variation	Degrees of freedom	Mean square			
		Germination (%)	Normal Seedling (%)	Abnormal Seedling (%)	Dead Seedling (%)
Between	7	413.518**	2120.946**	569.987**	377.518**
Within	16	32.250	13.125	2.813	2.844

**: Significant at 0.01 level of probability

Appendix V. Analysis of variance of the data on disease incidence at 21 DAS of wheat in the field due to the effect of different levels of black pointed seeds

Source of variation	Degrees of freedom	Mean square	
		Number of seedlings/m^2	
		Healthy	Infected
Replication	2	2.542	0.792
Treatment	7	922.327**	243.048**
Error	14	55.685	3.744

**: Significant at 0.01 level of probability

Appendix VI. Analysis of variance of the data on leaf blight severity of wheat at flag leaf and panicle initiation stage due to the effect of different levels of black pointed seeds

Source of variation	Degrees of freedom	Mean square					
		Disease severity (0-5 grade) at flag leaf stage			Disease severity (0-5 grade) at panicle initiation stage		
		1st leaf	2nd leaf	Average	1st leaf	2nd leaf	Average
Replication	2	0.0001	0.0001	0.0001	0.000	0.004	0.001
Treatment	7	0.034**	0.055**	0.044**	0.092**	0.553**	0.267**
Error	14	0.0001	0.0001	0.0001	0.001	0.011	0.002

**: Significant at 0.01 level of probability

Appendix VII. Analysis of variance of the data on leaf blight severity of wheat at flowering and milking stage due to the effect of different levels of black pointed seeds

Source of variation	Degrees of freedom	Mean square					
		Disease severity (0-5 grade) at flowering stage			Disease severity (0-5 grade) at milking stage		
		1st leaf	2nd leaf	Average	1st leaf	2nd leaf	Average
Replication	2	0.001	0.005	0.003	0.001	0.005	0.002
Treatment	7	0.095**	0.656**	0.306**	0.295**	0.402**	0.333**
Error	14	0.002	0.017	0.007	0.012	0.021	0.007

**: Significant at 0.01 level of probability

Appendix VIII. Analysis of variance of the data on leaf blight severity of wheat at hard dough stage due to the effect of different levels of black pointed seeds

Source of variation	Degrees of freedom	Mean square		
		Disease severity (0-5 grade) at hard dough stage		
		1st leaf	2nd leaf	Average
Replication	2	0.013	0.0001	0.003
Treatment	7	1.004**	0.757**	0.823**
Error	14	0.025	0.046	0.023

**: Significant at 0.01 level of probability

Appendix IX. Analysis of variance of the data on yield contributing characters and yield of wheat due to the effect of different levels of black pointed seeds

Source of variation	Degrees of freedom	Mean square					
		Plant height (cm)	Spike length (cm)	Distance between flag leaf and base of ear	Spikelets per ear (No.)		
					Healthy	Infected	Total
Replication	2	4.479	0.011	0.008	0.080	0.018	0.174
Treatment	7	20.077*	2.538*	1.136*	24.365**	6.934**	5.463
Error	14	7.237	0.698	0.361	2.814	0.043	3.242

**: Significant at 0.01 level of probability

Appendix X. Analysis of variance of the data on number and weight of grains per ear of harvested seeds of wheat due to the effect of different levels of black pointed seeds

Source of variation	Degrees of freedom	Mean square					
		Number of grains per ear			Weight of grains per ear (g)		
		Healthy	Diseased	Total	Healthy	Diseased	Total
Replication	2	0.566	0.039	0.309	0.006	0.000	0.006
Treatment	7	128.717**	16.765**	54.103**	0.257**	0.054**	0.085**
Error	14	5.629	0.370	6.503	0.012	0.000	0.013

**: Significant at 0.01 level of probability

Appendix XI. Analysis of variance of the data on yield of wheat due to the effect of different levels of black pointed seeds

Source of variation	Degrees of freedom	Mean square				
		Weight of 1000 seeds (g)	Grain yield		Straw yield	
			Plot (kg)	Hectare (tons)	Plot (kg)	Hectare (tons)
Replication	2	2.205	0.003	0.028	0.002	0.025
Treatment	7	11.814*	0.067**	0.744**	0.140**	1.553**
Error	14	3.267	0.003	0.039	0.009	0.095

**: Significant at 0.01 level of probability

Appendix XII. Analysis of variance of the data different severity grades (0-5) of harvested seeds of wheat due to the effect on of different levels of black pointed seeds

Source of variation	Degrees of freedom	Mean square					
		Grading of seeds					
		G_0	G_1	G_2	G_3	G_4	G_5
Replication	2	3.851	0.225	0.004	0.005	0.001	0.003
Treatment	7	130.560**	3.928**	0.287**	0.252**	0.174**	0.521**
Error	14	5.196	0.135	0.004	0.004	0.002	0.021

**: Significant at 0.01 level of probability

Appendix XIII. Analysis of variance of the data on disease incidence at 21 DAS of wheat in the field due to the effect of different levels of black pointed seeds after chemical control measure at field level

Source of variation	Degrees of freedom	Mean square	
		Number of seedlings/m^2	
		Healthy	Infected
Replication	2	10.292	1.542
Treatment	7	831.786**	197.690**
Error	14	42.768	1.351

**: Significant at 0.01 level of probability

Appendix XIV. Analysis of variance of the data on leaf blight severity of wheat at flag leaf and panicle initiation stage due to the effect of different levels of black pointed seeds after chemical control measure at field level

Source of variation	Degrees of freedom	Mean square					
		Disease severity (0-5 grade) at flag leaf stage			Disease severity (0-5 grade) at panicle initiation stage		
		1st leaf	2nd leaf	Average	1st leaf	2nd leaf	Average
Replication	2	0.001	0.0001	0.0001	0.0001	0.001	0.0001
Treatment	7	0.029**	0.036**	0.032**	0.080**	0.400**	0.205**
Error	14	0.001	0.001	0.0001	0.001	0.001	0.001

**: Significant at 0.01 level of probability

Appendix XV. Analysis of variance of the data on leaf blight severity of wheat at flowering and milking stage due to the effect of different levels of black pointed seeds after chemical control measure at field level

Source of variation	Degrees of freedom	Mean square					
		Disease severity (0-5 grade) at flowering stage			Disease severity (0-5 grade) at milking stage		
		1st leaf	2nd leaf	Average	1st leaf	2nd leaf	Average
Replication	2	0.001	0.006	0.003	0.003	0.018	0.002
Treatment	7	0.084**	0.593**	0.276**	0.246**	0.581**	0.381**
Error	14	0.003	0.017	0.005	0.015	0.048	0.018

**: Significant at 0.01 level of probability

Appendix XVI. Analysis of variance of the data on leaf blight severity of wheat at hard dough stage due to the effect of different levels of black pointed seeds after chemical control measure at field level

Source of variation	Degrees of freedom	Mean square		
		Disease severity (0-5 grade) at hard dough stage		
		1st leaf	2nd leaf	Average
Replication	2	0.034	0.011	0.011
Treatment	7	1.101**	1.136**	1.049**
Error	14	0.018	0.084	0.024

**: Significant at 0.01 level of probability

Appendix XVII. Analysis of variance of the data on yield contributing characters of wheat due to the effect of different levels of black pointed seeds after chemical control measure at field level

Source of variation	Degrees of freedom	Mean square					
		Plant height (cm)	Spike length (cm)	Distance between flag leaf and base of ear	Spikelets per ear (No.)		
					Healthy	Infected	Total
Replication	2	3.800	0.272	0.184	0.357	0.030	0.551
Treatment	7	22.545**	2.370**	1.303**	25.602**	5.841**	7.208**
Error	14	3.318	0.630	0.162	1.989	0.026	2.103

**: Significant at 0.01 level of probability

Appendix XVIII. Analysis of variance of the data on number and weight of grains per ear of harvested seeds of wheat due to the effect of different levels of black pointed seeds after chemical control measure at field level

Source of variation	Degrees of freedom	Mean square					
		Number of grains per ear			Weight of grains per ear (g)		
		Healthy	Diseased	Total	Healthy	Diseased	Total
Replication	2	0.714	0.036	0.852	0.006	0.001	0.009
Treatment	7	126.038**	16.272**	52.875**	0.271**	0.056**	0.089**
Error	14	4.549	0.216	4.865	0.006	0.0001	0.008

**: Significant at 0.01 level of probability

Appendix XIX. Analysis of variance of the data on yield of wheat due to the effect of different levels of black pointed seeds after chemical control measure at field level

Source of variation	Degrees of freedom	Mean square				
		Weight of 1000 seeds (g)	Grain yield		Straw yield	
			Plot (kg)	Hectare (tons)	Plot (kg)	Hectare (tons)
Replication	2	0.598	0.0001	0.002	0.001	0.009
Treatment	7	12.724**	0.066**	0.731**	0.130**	1.446**
Error	14	1.280	0.006	0.066	0.009	0.103

**: Significant at 0.01 level of probability

Appendix XX. Analysis of variance of the data on different severity grades (0-5) of harvested seeds of wheat due to the effect of different levels of black pointed seeds after chemical control measure at field level

Source of variation	Degrees of freedom	Mean square					
		Grading of seeds					
		G_0	G_1	G_2	G_3	G_4	G_5
Replication	2	6.361	0.146	0.001	0.000	0.000	0.001
Treatment	7	138.198**	3.946**	0.269**	0.224**	0.130**	0.620**
Error	14	4.286	0.080	0.002	0.003	0.002	0.011

**: Significant at 0.01 level of probability

Appendix XXI. Analysis of variance of the data on germination and incidence of *Bipolaris sorokiniana* of harvested seeds of wheat in blotter method due to the effect of different levels of black pointed seeds

Source of variation	Degrees of freedom	Mean square	
		Germination (%)	Incidence of *Bipolaris sorokiniana* (%)
Between	7	251.256**	970.851**
Within	16	22.490	6.094

**: Significant at 0.01 level of probability

Appendix XXII. Analysis of variance of the data on germination and incidence of *Bipolaris sorokiniana* of harvested seeds of wheat in blotter method due to the effect of different levels of black pointed seeds after chemical control measure at field level

Source of variation	Degrees of freedom	Mean square	
		Germination (%)	Incidence of *Bipolaris sorokiniana* (%)
Between	7	35.475**	251.659**
Within	16	11.990	1.977

**: Significant at 0.01 level of probability