EFFECT OF DIFFERENT LEVELS OF SEED INFECTION BY *Bipolaris sorokiniana* ON SEEDLING VIGOR, LEAF BLIGHT DEVELOPMENT AND QUALITY SEED PRODUCTION OF WHEAT

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

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Dedicated To

My Beloved Parents and Younger Brother

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The Author

EFFECT OF DIFFERENT LEVELS OF SEED INFECTION BY *Bipolaris sorokiniana* ON SEEDLING VIGOR, LEAF BLIGHT DEVELOPMENT AND QUALITY SEED PRODUCTION OF WHEAT

BY
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ABSTRACT

An investigation of the effect of different levels of seed infection by *Bipolaris sorokiniana* on seedling vigor, leaf blight development and quality seed production of wheat was carried out in the Seed Health Laboratory, Department of Plant Pathology during the period of July,2007 to September, 2007 and in the farm of Sher-e-Bangla Agricultural University, Dhaka, Bangladesh during the period from November, 2007 to April, 2008 with six 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). 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 and number of infected 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 healthy spikelets/ear (30.87) were also found in T_1, while the maximum number diseased spikelets/ear (5.34) was 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. Weight of grains/ear and weight of healthy grains/ear were significantly increased with the decrease of seed infection. In respect of grains/ear of different severity grades (0-5), the maximum Grade-0 seeds were found in T_1 and the maximum Grade-5 seeds were recorded 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 increase up to 38.69% over T_6. Highly positive linear relationship was found between seed infection levels and incidence of *Bipolaris sorokiniana* on harvested seeds. Considering all this, the minimum level of seed infection by *Bipolaris sorokiniana* i.e. T_1 (0% seed infection) showed promising results in producing high quality (Grade-0) seeds of wheat.

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LIST OF ABBREVIATED TERMS AND SYMBOLS

ABBREVIATION		FULL NAME
AEZ	=	Agro-Ecological Zone
Anon.	=	Anonymous
BARI	=	Bangladesh Agricultural Research Institute
BAU	=	Bangladesh Agricultural University
BBS	=	Bangladesh Bureau of Statistics
BSMRAU	=	Bangabandhu Sheikh Mujibur Rahman Agricultural Univercity
CEC	=	Cation Exchange Capacity
CIMMYT	=	International Maize and Wheat Improvement Centre
cm	=	Centimeter
cv.	=	Cultiver (s)
DAS	=	Days After Sowing
DMRT	=	Duncuns Multiple Range Test
e.g.	=	Example
et al.	=	*et alibi* (and others)
etc.	=	et cetera (and so on)
FAO	=	Food and Agriculture Organization
Fig	=	Figure
g	=	Gram
ha	=	Hectare
HLB	=	Helminthosporium Leaf Blight
hr	=	Hour
i.e.	=	id est (that is)
ISTA	=	International Seed Testing Agency
K	=	Potassium
Kg	=	Kilogram
kg/ha	=	Kilogram per hectare
LSD	=	Least Significant Difference
m	=	Meter
m^2	=	Square meter
ml	=	Milliliter
mm	=	Millimeter
MP	=	Muriate of Potash
N	=	Nitrogen
No.	=	Number

LIST OF ABBREVIATED TERMS AND SYMBOLS (cont'd)

ABBREVIATION		FULL NAME
P	=	Phosphorus
ppm.	=	Parts per million
PDA	=	Potato Dextrose Agar
r	=	Correlation coefficient
RCBD	=	Randomized Complete Block Design
S	=	Sulphur
SAU	=	Sher-e-Bangla Agricultural University
T	=	Treatment
t/ha	=	Ton per hectare
TSP	=	Triple Super Phosphate
U.S.A.	=	United States of America
UN	=	United Nations
UNDP	=	Unites Nations Development Program
USDA	=	United States Department of Agriculture
viz.	=	Videlict (namely)
WFP	=	World Food Program
@	=	At the rate
^{o}C	=	Degree Celsius
P^H	=	Hydrogen ion potentiality
$\leq$	=	Less than or equal
$\geq$	=	More than or equal
%	=	Percent

Chapter 1
Introduction

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the largest contributor with nearly 30% of the world grain production and 50% of the world grain trade. It is considered as the staple food crop of about two third of the world's population (Majumder, 1991) in as many as 43 countries and provides about 20% of the total food calories (Anon., 1986). The total world acreage of wheat was 60.43 million acres (USDA, 2008) with total production of about 608.1 million metric tones in the world in 2007-2008 (FAO, 2008). The consumption of wheat has been increasing during the last decade by about 5.6 million tons/year (Carter, 2002). Wheat grain supplies carbohydrate, protein, minerals and certain vitamins (Anon., 1990). It is pioneer of the grains domesticated by human by 5000 BC (Anon., 2008).

In the rice based cropping system like Bangladesh, wheat is considered as the second most important cereal crop (Razzaque and Hossain, 1990). Though the crop has been introduced in 1961 in the country (former East Pakistan), its popularity has been gained after 1975. Since initiation of HYV wheat expansion program in 1974, the acreage as well as grain yield has increased manifold (Ahmed and Meisner, 1996). Bangladesh has moved from the rank of nontraditional wheat growing countries to traditional wheat growing countries (Klatt, 1988). It has gained much popularity among the farmers of Bangladesh due to its higher nutritive value and lower cost of production than that of rice. As a result, wheat cultivation extended in an area from 0.1 million ha to 0.85 million ha at the end of 70th decade in 20th century. But due to brief span of winter, competition with other lucrative crops and lack of healthy seeds, wheat cultivation has been diminished to 0.48 million ha in the 21st century (Uddin, 2008). At present, wheat was grown in an area of about 0.40 million hectares and the total production was 0.737 million metric tones in Bangladesh in 2007 (BBS, 2008). The average yield of wheat was 1.84 t/ha (BBS, 2008) which was lower than that of other countries in the world like U.K. (7.34 t/ha), Germany (7.10 t/ha), Netherlands (7.07 t/ha), China (4.78 t/ha) and Japan (3.9 t/ha) (FAO, 2008).

There are many constraints responsible for the low yield of wheat in Bangladesh. Among the different factors that affect the production of wheat, use of unhealthy or diseased seeds is one of major constrains. For good crop good seed is needed that means the seed should be pure, viable and healthy. Government and semi government organizations supplied only 22.8% of the total requirement of wheat seed during 1998-1999 (Fakir, 1998). Those seeds are treated as quality seeds in Bangladesh. The rest 77.2% of the seeds produced traditionally by the farmers with no or little care even for purity and germination remain out of the scope of certification. As a result, a huge crop loss is incurred every year in wheat due to seed borne diseases in the country (Hossain, 2000).

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 from as many as 200 diseases of which 50 were routinely important (Wiese, 1985) and damaging ones are seed borne (Noble and Richardson, 1968). Wheat suffers from as many as 26 seed borne pathogens causing 14 seed borne diseases (Fakir, 1999). Diseases reduce wheat yields approximately 15-20% equivalent to 20-30 million tons annually in developing countries (Hanson *et al.*, 1982). In Bangladesh, about 10% yield reduction was reported due to diseases (Miah, 1985). Among them, leaf spot/blight caused by *Bipolaris sorokiniana* is the major and devastating disease of wheat in Bangladesh (Hossain and Azad, 1992). *Bipolaris sorokiniana* (Sacc. in Sorok.) Shoemaker, *Drechslera sorokiniana* (Sacc.) Subram. & Jain. syn., *Helminthosporium sorokinianum* Sacc. ex Sorok., *Helminthosporium sativum* Pammel, King & Bakke, *Helminthosporium californicum* Mackie & Paxton, Perfect stage *Cochlibolus sativus* (Ito & Kurib.) Drechsl. ex Dastur is worldwide in distribution (Sprague, 1950; Dubin and Ginkel, 1991). The pathogen is seed borne and seed transmitted in nature and may exist in different parts of the seeds (Bazlur Rashid, 1998). Bipolaris Leaf Blight is a serious disease of wheat in the warmer areas of South Asia where spring wheat is grown during the winter season, November - April (Dubin and Duveiller, 2000). Fakir *et al.* (1977) first indicated the possibility of transmission of the pathogen through wheat seeds in Bangladesh. General observations indicate that Bipolaris leaf blight appears at the seedling stage (Alam *et al.*, 1994). In Bangladesh, higher trend of the disease was recorded with the increase in plant age under field condition (Nahar, 1995; Bazlur Rashid, 1997).The highest disease incidence has been reported to occur at the later stages of plant growth, particularly between flowering and grain filling (Wolf and Hoffman, 1994). Finally the pathogen attacks wheat grains causing black point. Hossain *et al.* (1998) reported that this disease reduced yield up to 40% in field condition whereas Bazlur Rashid and Fakir (1998) estimated 57.6 and 64.5% yield reduction of wheat due to Bipolaris Leaf Blight in cvs. Kanchan and Sonalika, respectively. In severe condition, 100% yield loss of wheat may result (Hossain and Azad, 1994). Ahmed and Hossain (2005) found 43.75% yield loss in an inoculated wheat field. Gilchrist *et al.* (1992) reported that the level of black point infection was independent of infection on leaves, spikes and nodes. No cultivars or genotypes have so far been found to possess high degree of resistance to black point disease caused particularly by *Bipolaris sorokiniana* (Anon. 2002).

Black point is a brownish or black discoloration of wheat kernels, localized mainly at the embryo end, but that can also extend into the surrounding area and in crease (Fernandez *et al.*, 1994). The disease was first described in the United States in 1913 (Watkins and Prentice, 1999). According to 'Grain Grading Guide' (Canadian Grain Commission, 2001), the discoloration is considered "smudge" if more than one half of the kernel is discolored, or if the discoloration extends into the crease. If the discoloration penetrates and extends throughout the endosperm, it is referred to as "penetrated smudge". The disease is reported to have deteriorating effect on quality of the seed. Black point incidence exceeding 10% results in downgrading of the grain (Canadian Grain Commission, 1983). *Bipolaris sorokiniana* causes seed rot, reduces seedling emergence and yield of subsequent crop (Aulakh *et al.*, 1988; Chaudhary *et al.*, 1984; Gill and Tyagi, 1970; Machacek and Greaney, 1938; Nestrov, 1981). Black pointed seeds give seedlings with reduced vigor (Rahman and Islam, 1998). The disease renders milled flour unacceptable due to black discoloration of affected grains (Hanson & Christensen, 1953a; King *et al.*, 1981) and causes reduction in nitrogen, fatty acids, gluten, protein, potassium, calcium, zinc and manganese (Chaudhary *et al.*, 1984; Zilling, 1932). Bangladesh Agricultural Development Corporation (BADC) has been facing serious problem in producing and distributing wheat seeds due to discoloration of the grains as affected by black point pathogens (Fakir, 1988) and the organization has to reject considerable amount of seeds every year inflicting appreciable economic loss (Hossain and Hossain, 2001).

Wheat underpins global food security and, therefore, its improvement has the potential to contribute substantially to the first Millennium Development Goal (MDG) of halving hunger and poverty by 2015 (Dixon *et al.*, 2007). The food security of many developing countries depends heavily on wheat, which accounts for 99.6 million of the total 446 million hectares of cereals in the developing world (FAO, 2007.) It is forecast that that by 2020, the world will need to produce 760 million tons of wheat per year (Rosegrant *et al.* 2001). In Bangladesh, about 28 million people are suffering from prolonged food insecurity and severe malnutrition (Alam and Hossain, 2007). Again, according to census, the population of Bangladesh will be 165 million at the end of 2025 and the demand of food will be 30.85 million tones (Rashid and Sattar, 2007). However, the present food grain production of Bangladesh is 28.05 million tones (BBS, 2008) and the annual food grain deficit is 2.0 million tones (WFP, 2008). So, the country has to cope with the challenge of producing an additional 2.80 million tones of food grain. Again, our cultivable land is only 8.4 million hectares which is decreasing at the rate of 1% per year. At the same time, population is increasing at the rate of 1.54% per year (Islam, 2007). So, to attain sustainability in food production in this 21st century, there is no alternative but to produce more food supplemented by the production of wheat by the use of high quality healthy seeds.

For more wheat production, diversified research program has to be taken along with the consideration of the leaf blight disease caused by *Bipolaris sorokiniana* as the number one constraint which is instrumental of lowering wheat yield in the country (Alam *et al.,* 1994). Around 20% crop yield can be increased through the use of healthy seed and proper management of seed borne diseases (Fakir, 1999). Bazlur Rashid and Fakir (2001) reported that the health standards of seed and seed crop were very important aspects for the development of seed borne diseases. They also opined that the field level officers can never certify a seed crop without having these standards fixed by proper authority. While they defined the standards as (i) the maximum amount of inoculum associated with the seed or seed lot that results in the development of disease in the field under favorable environmental condition with the acceptable limit referring as to the seed health standard and (ii) the maximum prevalence of a disease under the same type of favorable environmental condition and sustains the health of seeds with the association of the pathogen of acceptable limit referring as to the field health standard. But there is no such fixed seed and field health standards or tolerance levels for *Bipolaris sorokiniana*, causing of leaf blight of wheat in the country.

Considering the above facts, the present study was, therefore, designed to achieve the following objectives:

(i) To determine the effect of different levels of seed infection by *Bipolaris sorokiniana* on germination, seedling infection and seedling vigor of wheat,

(ii) To evaluate the effect of different levels of seed infection by *Bipolaris sorokiniana* on subsequent leaf blight development in the field and yield of wheat,

(iii) To determine the effect of different levels of seed infection by *Bipolaris sorokiniana* on subsequent grain infection.

Chapter 2
Review of Literature

REVIEW OF LITERATURE

Leaf blight is a destructive foliar disease of wheat caused by seed transmitted *Bipolaris sorokiniana* (Sacc. in Sorok.) Shoemaker, *Drechslera sorokiniana* (Sacc.) Subram. & Jain. syn., *Helminthosporium sorokinianum* Sacc. ex Sorok. It affects different plant parts causing leaf spot, leaf blight, spot blotch, black point, shriveled and destroyed grain etc. *Bipolaris sorokiniana* has been reported to be an acute problem to both wheat growers and researchers (Bazlur Rashid *et al.*, 1987; Fakir, 1988). Abundant works on this pathogen have been reported by many researchers but the literature on the influence of seed to plant to seed transmission of the pathogen in wheat with different rates of seed infection is very scanty. Available literature on the effect of different levels of seed infection on subsequent disease incidence and severity and seed infection in the field are compiled and presented in this chapter.

Smith (1929) reported that the mycelium of *Bipolaris sorokiniana* in the outer seed layers could remain viable for at least two years and under the damp conditions of germination produced conidia and fresh hyphae. These hyphae were the effective agents in penetrating the primary sheaths i.e., the coleorhiza (root sheath) and the coleoptile (plumule sheath). Penetration of the coleorhiza, or of the rootlets which arose from it, resulted in rotting, stunting, or at least impaired efficiency of the root system, which was a contributory cause of sickliness in the plant.

Turner and Millard (1931) demonstrated that the viability of mycelium within the pericarp was well marked and extended over a long period.

Therefore, it was probable that the mycelium was of much greater importance in carrying the disease to next years crop than the spores adhering to the outside of the grains.

Dastur (1932) reported that the value of crop was appreciably reduced when grain infected with *Helminthosporium sativum* and used as seed. He found that the discolored seed germinated poorly and seedlings usually developed blights.

Machacek and Greaney (1938) reported from their greenhouse and field trials that seeds infected with *Helminthosporium sativum* produced only 24.8% plant stand and seedling disease rating on the plants revealed that when seed infected with *H. sativum* resulted 80% seedling infection.

Hanson and Christensen (1953a) observed over 1000 samples of wheat seeds of different varieties obtained from Minnesota and adjoining states of U.S.A. during 1943 to 1953 and reported that the percentage of black point grains ranged from 0 to 35. According to them, pre-dominant fungal organisms associated with discolored seeds were *Helminthosporium* and *Alternaria* spp. *Fusarium* was also common in many lots but these have not been shown to cause black point of wheat. *Helminthosporium* or *Fusarium* was responsible for much the infection and significant decrease in stand with more seedling blight was common.

Hanson and Christensen (1953b) reported that wheat kernels infected with *H. sativum* commonly germinated poorly and that in most cases, diseased seedlings resulted from such seed. Seeds infected with *H. sativum* in the field produced 24% stands and seedling disease 80.6% for the plants from the seed containing *H. sativum*. They also stated that the discolored seeds might produce just as good stands as seed that was not discolored. This was happened when the discoloration was due to fungi which were not pathogenic to seedlings. On the other hand, there might be highly significant reductions in stand when discolored lots were infected with fungi that were pathogenic to seedlings.

Becker (1957) reported that infection with *H. sativum* at the milk stage prevents the grain from reaching the maximum size.

De tempe (1964) stated that the mycélial infection of wheat pericarp by *Drechslera sorokiniana* could persist more than a year.

Parashar and Chohan (1967) reported that *Alternaria tenuis* and *Helminthosporium sativum* associated with black pointed wheat grains reduced germination by 3.40% in the laboratory and caused 41.07% yield loss in the field.

Christensen and Kaufmann (1968) described that *Helminthosporium* was common in many lots of cereal seeds especially if the weather just before harvest had been moist. It might cause discoloration of the seed, death of the germinating seed or young seedling or root rots and blights of mature plant, but caused no loss in storage.

Vir (1974) reported that the seed borne infection of *Helminthosporium* was responsible for blight disease of wheat, barley, oat, rice and few other crops.

Nema and Joshi (1974) showed that *Helminthosporium sativum* was abled to infect wheat seedlings at first leaf stage of maturity and susceptibility increased with the age of the plant. Plant beyond the flower stage of growth was more susceptible than those younger in age.

Fakir *et al.* (1977) established the seed to plant transmission of *Drechslera sorokiniana* in wheat. They stressed the importance and introduction of seed health testing program for wheat in Bangladesh.

Neergaard (1979) reported that *D. sorokiniana* causing seedling blight, foot rot, ear blight were highly seed transmitted.

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 of 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.

Wu and Le (1983) reported that the conidia of *B. sorokiniana* remained viable for more than 281 days in dry soil and less than 80 days in water soaked soil, infected seeds being the main source of primary inoculum.

Sharma *et al.* (1983) found the correlation co-efficient for adult plant reaction (leaf susceptibility) to *Cochliobolus sativus* and percentage of seed infection was 0.35. Although the correlation co-efficient for adult plant susceptibility and percent seed infection for the pathogen was low.

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 WL 711. 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 pathogen.

Frank (1985) and Lin (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% in 71 cvs. and 10.1 to 30% in 54 cvs. 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 on plant stand, yield and transmission of the disease to the subsequent crop.

Nalli (1986) stated that plants grown from *Bipolaris sorokiniana* inoculated seeds produced tiller of lower height and reduced seed production.

Khanum *et al.* (1987) stated that black point is 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 blackpoint of wheat did not affect germination, plant emergence, yield and intensity of root rot in the subsequent crop. But *Helminthosporium* type of blackpoint 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 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 seeds 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 37.08%, respectively.

Hossain and Azad (1992) reported that higher age of crop plant resulted higher incidence of 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) and Bazlur Rashid and Fakir (1998) 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 *et al.* (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 of 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 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 colonized grains may serve as potential source for the survival of *B. sorokiniana* resulting germination failure, seedling mortality and spot blotch development of wheat.

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 (75.4%) 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. He also found that 65.36 percent disease severity interning the corresponding 17.42 percent seed infection.

Chapter 3
Materials and Methods

MATERIALS AND METHODS

Experiments were conducted in the Seed Health Laboratory and in the farm field allotted for the Department of Plant Pathology, Sher-E-Bangla Agricultural University, Dhaka to find out the effect of different levels of seed infection by *Bipolaris sorokiniana* on seedling vigor, leaf blight development and quality seed production of wheat.

3.1. Laboratory experiment

The experiment was conducted in the Seed Health Laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka during the period of July 2007 to September 2007.

3.1.1. Planting material

The test crop was wheat (*Triticum aestivum* L.) and variety 'Kanchan', the most widely cultivated variety in the country.

3.1.2. Collection of seed sample

Wheat seed samples of variety Kanchan was collected from a local farmer named Md. Asraf Ali of Village- Bosuya, Thana- Boyoalia, District-Rajshahi. The collected samples were stored in paper bag covered with polythene and stored in normal room temperature in Seed Health Laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka.

3.1.3. Treatment used in the experiment

The collected seed sample was physically sorted out to separate apparently healthy looking seeds with bold golden color and black pointed seeds to prepare different rates of seed infection. The seed sample with 0.00% infection was graded as T_1 (Treatment 1). The collected seed sample was divided into sub samples containing different levels of black pointed seed. Each sub sample considered as different treatment. There were altogether six treatments which were as follows:

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

T_6 = 45.1 - 60% seed infection

3.1.4. 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 20°C 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 stereo binocular microscope. Germination of the seeds was also recorded.

3.1.5. Seedling infection and seedling vigor test

Seedling infection and seedling vigor test was done by following the Rolled Paper Towel Method (Warham, 1990). In this method, one hundred and fifty seeds were randomly taken from each treatment. 50 seeds were placed uniformly between a pair of moist paper towels. The towels were rolled and the two ends were closed with rubber bands. Then the rolled papers containing seeds were placed in an upright position for 7 days at room temperature under normal 12/12 light and darkness cycle. After incubation period, observations pertaining to germination and seedling infection were recorded. Twenty seedlings were randomly selected from each paper and their individual shoot and root length were measured. The shoot and root portions were blotted dry with fine tissue paper and fresh weight was taken. Vigor of the seedling was determined by the following formula (Baki and Anderson, 1972):

Vigor Index = (Mean of root length + Mean of shoot length) × % Seed germination.

3.1.6. Water Agar Test tube Seedling Symptom test

The water agar test tube seedling symptom test developed by Khare *et al.* (1977) 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°C. The water agar in the test tube was solidified at an angle of 60° so that the seeds could be placed on the slanted agar conveniently and record of pathogens could be taken easily. Thirty seeds from each treatment were taken and one seed per test tube were placed on solidified water agar slant at the rate of one seed per tube. The seeded tubes were closed with cotton plugs and arranged in plastic racks. The tubes were then incubated at erect condition in an air cooled room (temp. 22°C) under fluorescent day light tube. The cotton plugs were 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). The normal seedlings were categorized by following points:

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.

During recording the abnormalities of 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) Blackened dead or decayed seed

3.2. Field experiment

3.2.1. Experimental site and Experimental period

The experiment was conducted at the Farm field allotted for the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka. The location of experimental site is shown in Fig 1. In previous season, the experimental field was occupied by rice. The experiment was conducted during the *Rabi* season from November 2007 to April 2008.

3.2.2. Climate of experimental site

The experimental area was under the subtropical climate which is characterized with the comparatively high rainfall, high humidity, high temperature, relatively long day during April to September and scanty rainfall, low humidity, low temperature and short day period during October to March. The later period (October-March) is favorable for wheat cultivation. Monthly air temperature, relative humidity (%) and total rainfall (mm) and average sunshine hour of site during the experimental period have been shown in Appendix –I.

3.2.3. Treatments

There were six treatments as described in laboratory experiment (3.1.3.).

3.2.4. Soil

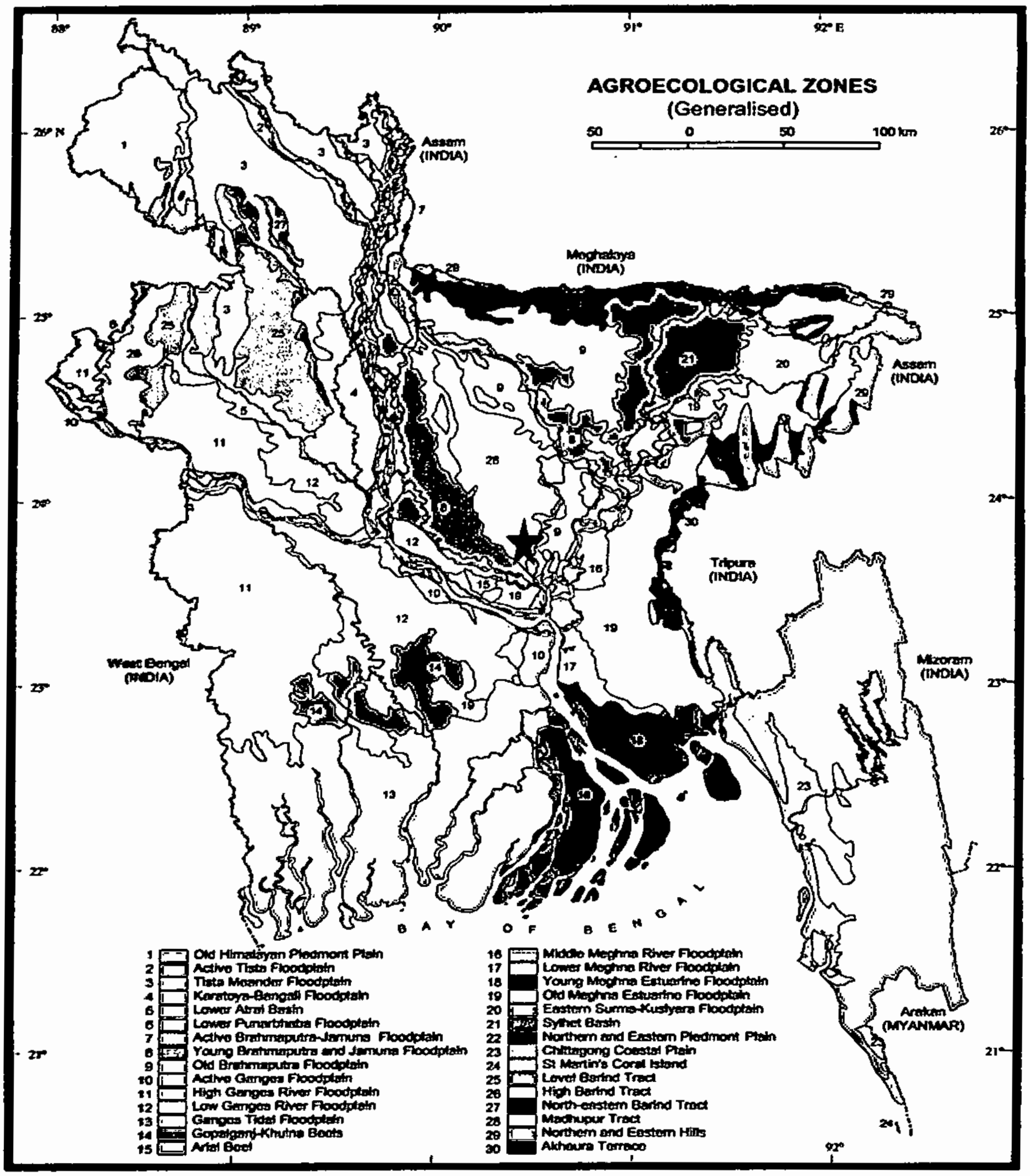
The soil of the experimental site belongs to the agro – ecological region of Madhupur Tract, AEZ – 28. It was Deep Red Brown Terrace Soil and belongs to "Nodda" cultivated soil series. The soil was silty clay loam in texture. Organic matter content was very low (0.82%) and soil pH was 5.55. The physical and chemical characteristics of soil have been shown in Appendix –II.

3.2.5. Experimental design and layout

The experiment was laid out in Randomized Complete Block Design (RCBD) comprising three replications for each treatment. The unit plot size was (3m x 3m), each plot was separated by 0.75 m wide drain. Distances between block and between plots were 1m. Thus, there were altogether 18 plots for the study. Different treatments were assigned randomly to the unit plot. The layout of the experiment has been shown in Appendix –III.

3.2.6. Land Preparation

The experimental field was thoroughly ploughed and cleaned prior to seed sowing and application of fertilizers and manure was done in the field. The experimental field was prepared by thorough ploughing followed by laddering to have a good tilth. Finally the land was properly leveled before seed sowing.



★ Indicates experimental site (AEZ - 28)

Source: www.fao.org

Fig. 1. Location of experimental field

3.2.7. Applications of fertilizers

The field was fertilized at the rate of 220 Kg Urea, 180 Kg TSP, 50 Kg MP, 120 Kg Gypsum and 10 tons Cow dung per hectare (Krishi Projukti Hatbooi, 2005). Two third of Urea, full dose of TSP, MP, Gypsum and Cow dung was applied at the time of final land preparation. Remaining one third of Urea was applied at 21 days after seed sowing.

3.2.8. Sowing of seeds

Wheat seeds were sown in the field on 6th December, 2007 at the rate of 120 kg/ha. The seeds were placed continuously in lines properly at a depth of 5cm and were covered by soil with the help of hand. The distance between lines was 25cm which made 11 rows in each unit plot.

3.2.9. Collection of data on disease incidence at 21 DAS

Data on number of seedlings/m^2 and number of seedlings/m^2 were recorded at 21 days after sowing.

3.2.10. Intercultural operations

Weeds were controlled thoroughly. Three weedings were done at 28, 54 and 78 DAS. The weeds were controlled by nirani (hand hoe). After sowing, light irrigation was given for proper germination. Then flood irrigation was given at crown root initiation (20 DAS), heading (50 DAS) and grain filling (70 DAS) stages. During irrigation, care was taken so that water could not flow from one plot to another or overflow the boundary of the plots.

3.2.11. Plant protection activities

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

3.2.12. Tagging and data collection

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

3.2.13. 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 second. The cut pieces were then washed in water at three times and were placed onto PDA media in petridish. The plates were then incubated at 25±1°C for 7 days. Later the pathogen was purified using hyphal tip culture method and grown on PDA media at 25±1°C for two weeks and identified as *Bipolaris sorokiniana* with the help of relevant literature (CMI Description).

Plate 1. Leaf blight symptom of wheat under field condition

Plate 2. *Bipolaris sorokiniana* on seed under Stereomicroscope (45X)

Plate 3. Pure culture of *Bipolaris sorokiniana*

Plate 4. Mycelia and conidia of *Bipolaris sorokiniana* under Compound microscope (100X)

3.2.14. Evaluation of leaf blight severity

Leaf blight severity of flag leaf and 2nd leaf was determined in five growth stages of plant viz. flag leaf stage, panicle initiation stage, flowering stage, milking stage and hard dough stage. The leaf blight severity of the disease was recorded following 0-5 grade (Hossain and Azad, 1992) (Plate 5). 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 $\leq$10% of the leaf area (Moderately resistant)

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

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

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

3.2.15. Harvesting of crop

The crop was harvested at full ripening stage on 30 March, 2008.

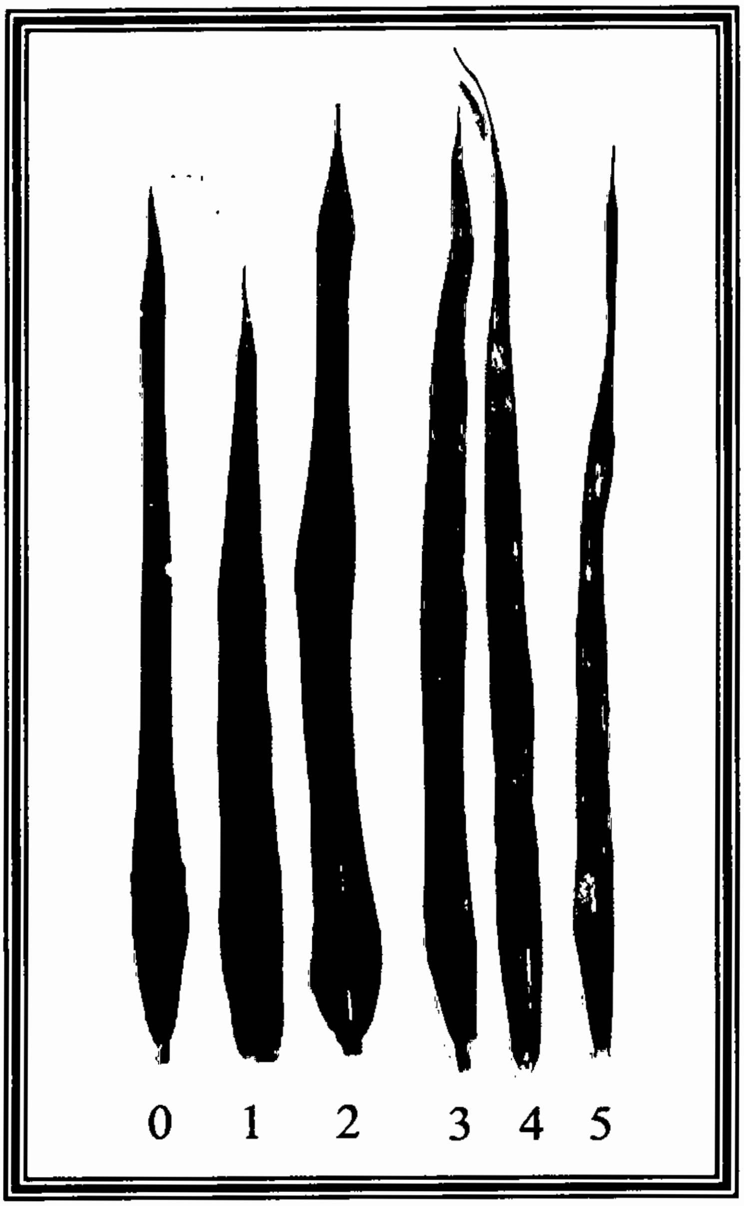


Plate 5. Leaves showing 0-5 rating scale of leaf blight severity

3.4.16. Collection of data on yield and contributing characters:

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

i) Plant height (cm)
ii) Length of ear (cm)
iii) Distance between the point of flag leaf initiation and base of ear (cm)
iv) Number of spikelets/ear
v) Number of healthy spikelets/ear
vi) Number of diseased spikelets/ear
vii) Number of grains/ear
viii) Number of healthy grains/ear
ix) Number of diseased grains/ear
x) Weight of grains/ear
xi) Weight of healthy grains/ear (g)
xii) Weight of diseased grains/ear (g)
xiii) Grading of seeds/ear (0-5)
xiv) 1000 grain weight (g)
xv) Straw yield/plot (kg)
xvi) Straw yield (t/ha)
xvii) Grain yield/plot (kg)
xviii) Grain yield (t/ha)

3.2.17. Grading of seeds

The grading of seeds will be done following the 0-5 rating scale (Plate 6). 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 ½ of grain are infected

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

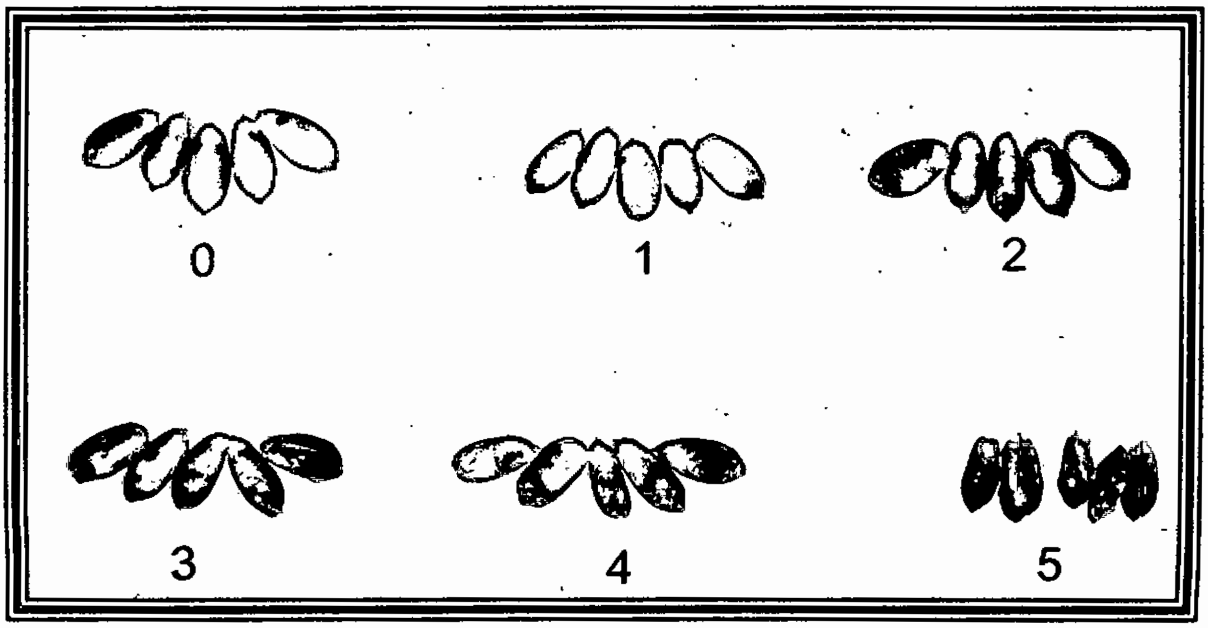


Plate 6. Wheat seeds showing different (0-5) grades

3.3. Laboratory experiment (after harvesting)

3.3.1. Seed health test of harvested seeds

Two hundred seeds were randomly selected from each harvested samples and incubated for collection of data on germination and incidence of *Bipolaris sorokiniana* following Blotter method (ISTA, 1996) as described in case of seed samples used for sowing in 3.1.4.

3.4. Statistical Analysis

The collected data for different parameters were compiled and tabulated in proper form. Appropriate statistical analysis was made by MSTAT Computer Package program. The data were subjected to arcsine transformation when needed. The treatment means were compared by Duncan's Multiple Range Test (DMRT).

Chapter 4

Results

RESULTS

4.1. Laboratory Experiment

4.1.1. Effect of different levels of seed infection by *Bipolaris sorokiniana* on germination and incidence of *Bipolaris sorokiniana* on wheat seeds before sowing (Blotter method)

In Blotter method, the results showed that seed infection by *Bipolaris sorokiniana* had injurious effect on seed germination (Table 1 and Plate 7 and 8). There was a general tendency of lessening seed germination with higher levels of seed infection by *Bipolaris sorokiniana* in the seed samples. Significant variations were observed among the treatments. The maximum seed germination (97.00%) was found in T_1 (0% seed infection) followed by T_2 (92.00%), T_3 (87.00%) and T_4 (80.00%). On the other hand, the minimum seed germination (69.00%) was recorded in T_6 (45.1-60% seed infection) preceded by T_5 (75.00%).

Seed health test also indicated that sample with increased percentage of seed infection usually had higher percentage of *Bipolaris sorokiniana*. Incidence of seed borne *Bipolaris sorokiniana* for all treatments differed from 0.00 to 55.00%, where treatment T_1 (0% seed infection) was found completely free from the pathogen preceded by T_2 (12.00%) and T_3 (20.00%). On the contrary, the highest incidence (55.00%) was observed in T_6 (45.1-60% seed infection) followed by T_5 (41.00%) and T_4 (32.00%).

Table 1. Effect of different levels of seed infection by *Bipolaris sorokiniana* on germination and incidence of *Bipolaris sorokiniana* of wheat seeds before sowing (Blotter method)

Treatments	% Germination	% *Bipolaris sorokiniana*
T_1	97.00 a	0.00 (3.49) f
T_2	92.00 b	12.00 (20.22) e
T_3	87.00 c	20.00 (26.54) d
T_4	80.00 d	32.00 (34.43) c
T_5	75.00 e	41.00 (39.80) b
T_6	69.00 f	55.00 (47.85) a
LSD (0.01)	**4.666**	**3.117**

Data in parenthesis indicate arcsine transformed value.

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

Plate 7. Seeds of T_1 (0% seed infection) in Blotter method

Plate 8. Seeds of T_6 (45.1-60% seed infection) in Blotter method

4.1.2. Effect of different levels of seed infection by *Bipolaris sorokiniana* on germination and seedling infection of wheat in the laboratory before sowing (Rolled paper towel method)

The impact of seed borne infection of *Bipolaris sorokiniana* on germination and seedling infection of wheat in rolled paper towel method was shown in Table 2 and Plate 9, 10 and 11. The germination percentages of wheat seeds decreased with the increase of seed infection by *Bipolaris sorokiniana* in the samples. The treatments were found to differ significantly from one another and the results varied from 96.00 to 72.67%. The highest germination (96.00%) was counted in T_1 (0% seed infection) followed by T_2 (92.67%), T_3 (89.33%) and T_4 (84.67%) and the lowest germination (72.67%) was observed in T_6 (45.1-60% seed infection) preceded by T_5 (78.00%).

Again, contrariety was found in respect of seedling infection. The seedling infection was increased with the increasing levels of seed infection by *Bipolaris sorokiniana* in the lots. Significant variations were found among the treatments and the results varied from 2.67 to 49.33%. The maximum (49.33%) count of seedling infection was found in T_6 (45.1-60% seed infection) followed by T_5 (38.00%) as well as T_4 (26.67%) and the minimum (2.67%) count of seedling infection was recorded in T_1 (0% seed infection) preceded by T_2 (8.00%) and T_3 (18.67%), respectively.

Table 2. Effect of different levels of seed infection by *Bipolaris sorokiniana* on germination and seedling infection of wheat in the laboratory before sowing (Rolled paper towel method)

Treatment	% Germination	% Seedling Infection
T_1	96.00 a	2.67 (9.26) f
T_2	92.67 b	8.00 (16.42) e
T_3	89.33 c	18.67 (25.58) d
T_4	84.67 d	26.67 (31.08) c
T_5	78.00 e	38.00 (38.04) b
T_6	72.67 f	49.33 (44.60) a
LSD (0.01)	**3.111**	**2.403**

Data in parenthesis indicate arcsine transformed value.

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

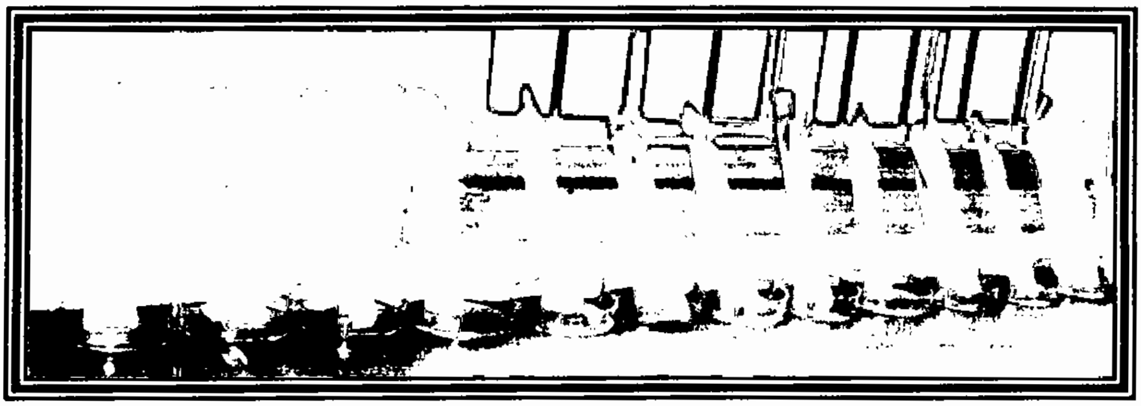


Plate 9. Rolled Paper Towel Method

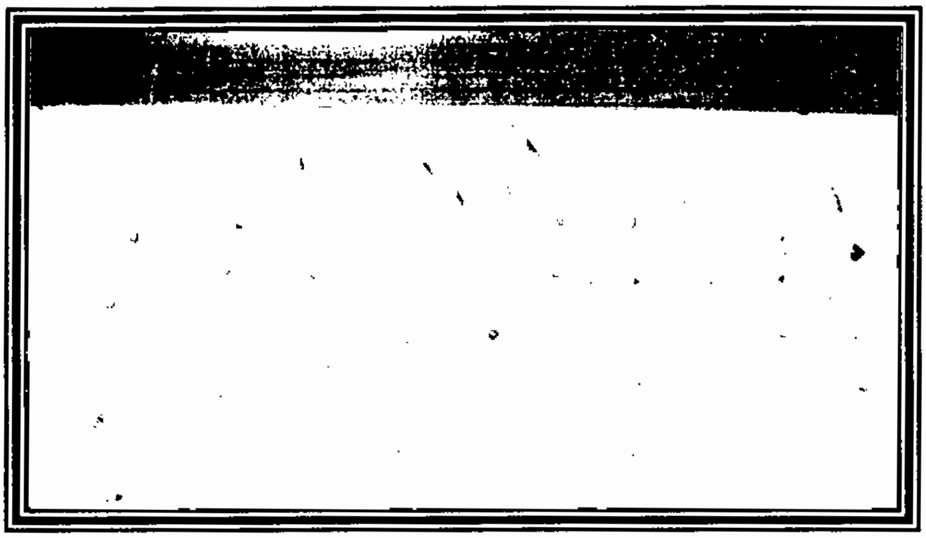


Plate 10. Seedlings of T_1 in rolled paper towel method

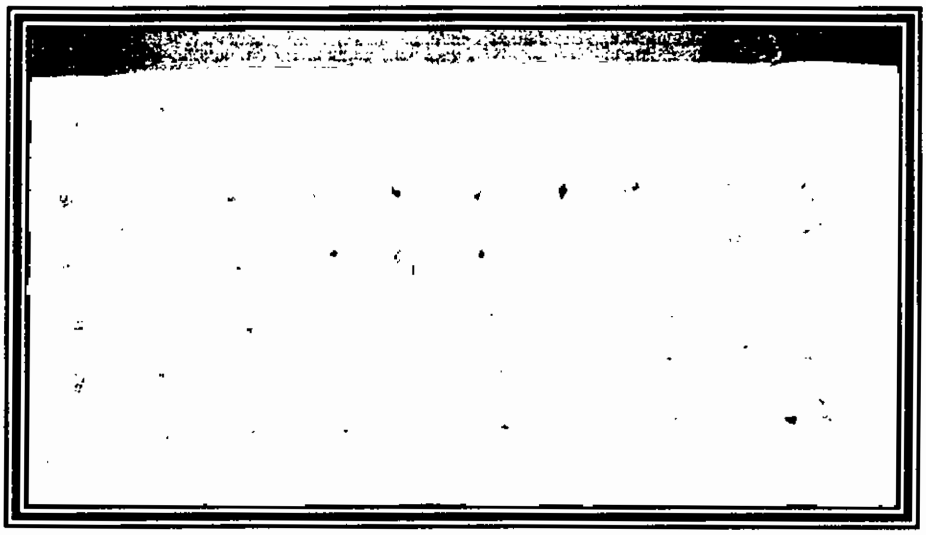


Plate 11. Seedlings of T_6 in paper towel method

4.1.3. Effect of different levels of seed infection by *Bipolaris sorokiniana* on Vigor Index of 7 days old seedlings of wheat in the laboratory before sowing (Rolled paper towel method)

The results showed that the treatments significantly influenced shoot length (cm), root length (cm), seedling weight (g) and Vigor Index of 7 days old seedlings (Table 3). The shoot lengths varied from 6.53 to 3.45 cm. The maximum shoot length (6.53 cm) was recorded in T_1 (0% seed infection) followed by T_2 (5.62 cm) as well as T_3 (4.97cm) and the minimum (3.45cm) result was found in T_6 (45.1-60% seed infection) which was statistically similar with T_5 (3.93 cm) as well as preceded by T_4 (4.34 cm).

In case of root length, the values ranged from 12.56 to 5.44 cm, where the highest (12.76 cm) root length was found in T_1 (0% seed infection) followed by T_2 and T_3 with 9.40 cm and 8.50 cm root length, respectively. On the other hand, the lowest (5.44 cm) value was observed in T_6 (45.1-60% seed infection) preceded by T_5 (6.86 cm) and T_4 (7.13 cm).

Considering seedling weight, the values ranged from 3.67 to 2.65 g. The lowest count (2.65 g) was found in T_6 (45.1-60% seed infection) which was statistically similar with T_3 (15.1-25% seed infection), T_4 (25.1-35% seed infection) and T_5 (35.1-45% seed infection). The highest count (3.67 g) was found in T_1 (0% seed infection) and T_2 (5.1-15% seed infection) showed the second highest count (3.08 g).

Vigor Index (VI) for all the treatments differed significantly with a range of 1851.51 to 669.36. The maximum Vigor Index (1851.51) was recorded in seedlings under T_1 (0% seed infection) and the minimum (669.36) was counted in T_6 (45.1-60% seed infection).

Table 3. Effect of different levels of seed infection by *Bipolaris sorokiniana* on "Vigor Index" of 7 days old seedlings of wheat in the laboratory before sowing (Rolled paper towel method)

Treatment	Shoot Length (cm)	Root length (cm)	Seedling weight (g)	Vigor index
T_1	6.53 a	12.76 a	3.67 a	1851.81 a
T_2	5.62 b	9.40 b	3.08 b	1392.02 b
T_3	4.97 c	8.50 c	2.73 c	1203.55 c
T_4	4.34 d	7.13 d	2.68 c	971.16 d
T_5	3.93 de	6.86 e	2.67 c	841.62 e
T_6	3.45 e	5.44 f	2.65 c	669.36 f
LSD (0.01)	**0.558**	**0.835**	**0.284**	**93.00**

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

4.1.4. Effect of different levels of seed infection by *Bipolaris sorokiniana* on germination and seedling health of wheat in the laboratory before sowing (Water agar test tube seedling symptom test)

Wheat seeds samples having different levels of seed infection by *Bipolaris sorokiniana* had significant effect on seed germination and seedling health of wheat in Water Agar Test tube Seedling Symptom Test (Table 4 and Plate 12, 13, 14 and 15). From the results, it was revealed that the gradual decrease of seed germination occurred with the increase in seed infection level in the seed samples. The germination varied from 96.67 to 62.67%. The highest germination (96.67%) was found in T_1 (0% seed infection) followed by T_2 (5.1-15% seed infection) and T_3 (15.1-25% seed infection) with 93.33 % and 86.00%, respectively. The lowest (62.67%) was in T_6 (45.1-60% seed infection) preceded by T_5 (70.67%) and T_4 (77.33%).

The treatments showed significant difference from one another regarding percent normal seedlings and the results for all the treatments ranged from 93.33 to 10.00%, where the maximum counts (93.33%) were found in T_1 (0% seed infection) followed by T_2 (83.33%) and T_3 (64.67%) and the minimum counts (10.00%) were found in T_6 (45.1-60% seed infection) preceded by T_5 (28.67%) and T_4 (46.67%).

The treatment also showed significant differences regarding percent abnormal seedlings. The number of abnormal seedlings was found to be increased with the increase of black pointed seeds. In case of abnormal seedlings, the percent ranged from 3.33 to 52.67%, where the highest (52.67%) percent was observed in T_6 (45.1-60% seed infection) followed by T_5 (42.00%) as well as T_4 (30.67%) and the lowest (3.33%) percent was found in T_1 (0% seed infection) preceded by T_2 (10.00%) and T_3 (21.33%).

Table 4. **Effect of different levels of seed infection by *Bipolaris sorokiniana* on germination and seedling health of wheat in the laboratory before sowing (Water agar test tube seedling symptom test)**

Treatments	%Germination	%Normal seedling	%Abnormal seedling	%Dead seed
T_1	96.67(79.57)a	93.33(75.04)a	3.33(10.38)f	3.33(10.40)f
T_2	93.33(75.04)b	83.33(65.89)b	10.00(18.43)e	6.67(14.92)e
T_3	86.00(68.00)c	64.67(53.51)c	21.33(27.49)d	14.00(21.96)d
T_4	77.33(61.10)d	46.67(43.07)d	30.67(33.61)c	23.33(28.87)c
T_5	70.67(57.19)e	28.67(32.36)e	42.00(40.38)b	29.33(32.78)b
T_6	62.67(52.32)f	10.00(18.37)f	52.67(46.51)a	37.33(37.65)a
LSD (0.01)	**2.735**	**2.806**	**2.383**	**2.735**

Data in parenthesis indicate arcsine transformed value.

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

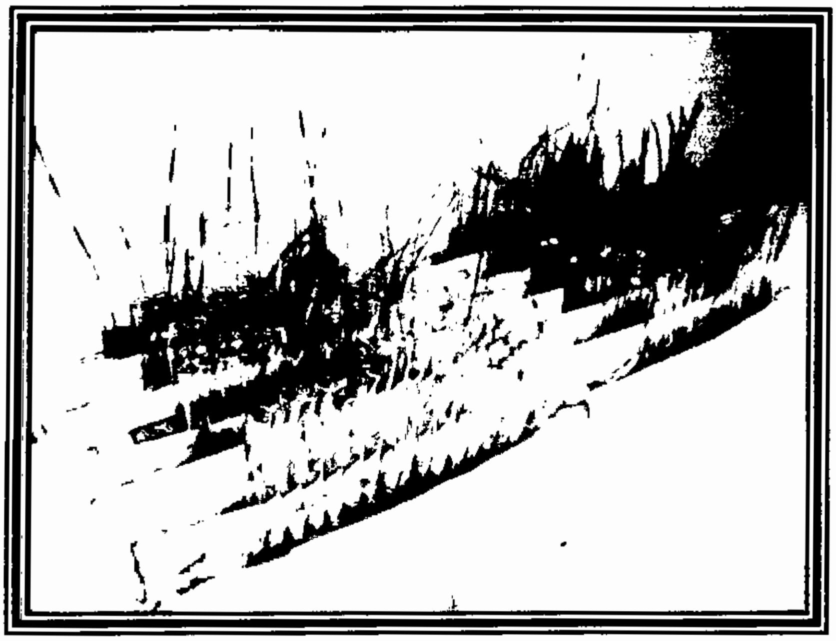


Plate 12. Water Agar Test Tube Seedling Symptom Test

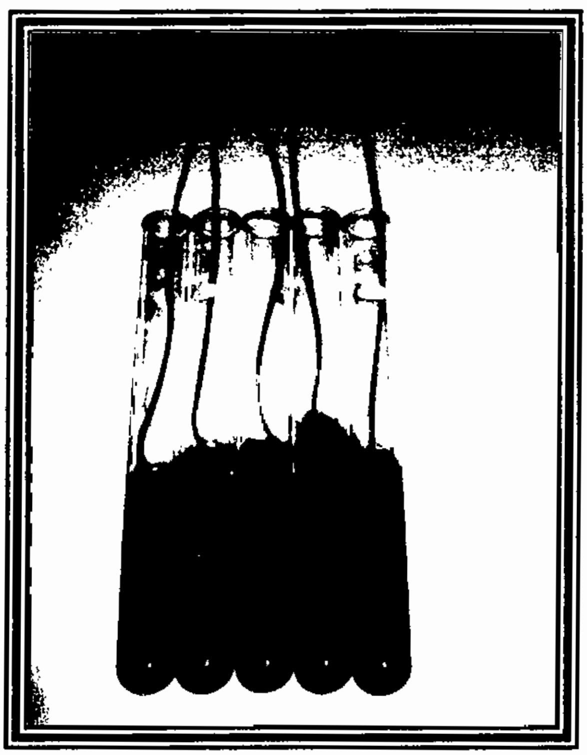


Plate 13. Normal Seedlings in Water Agar

Test tube Seedling Symptom test

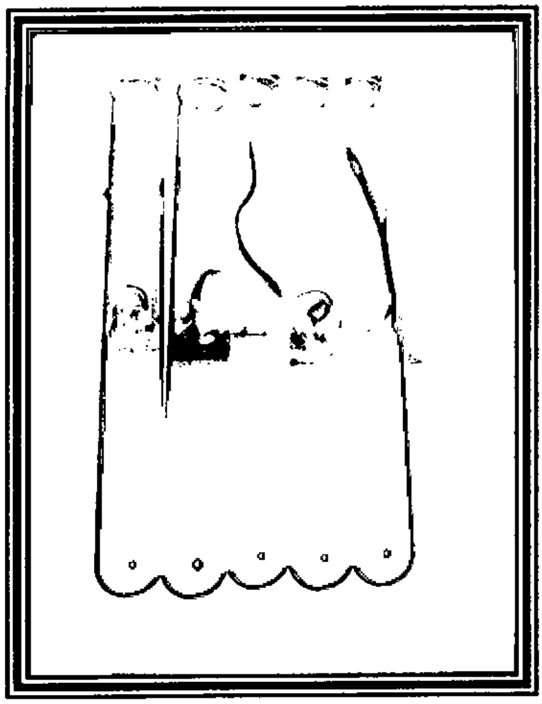


Plate 14. Abnormal Seedlings in water ...

Test tube Seedling Symptom test

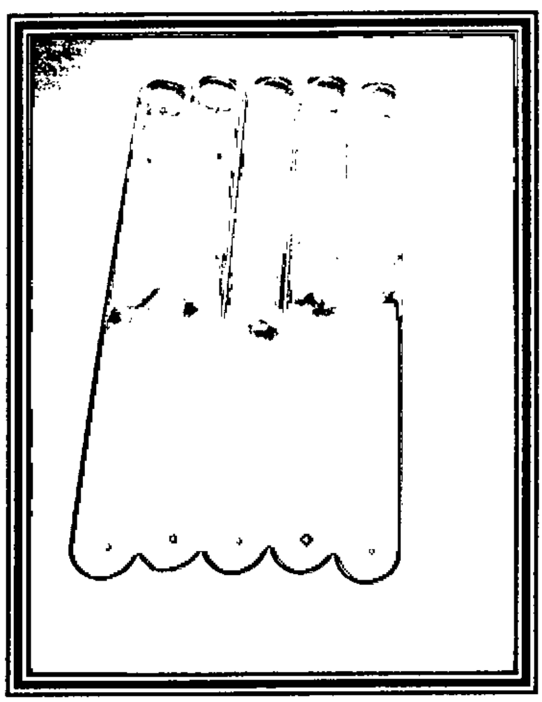


Plate 15. Dead Seed/Seed rot in Water Agar

Test tube Seedling Symptom test

Similar trend of result was found in case of percent dead seed. The results varied from 3.33 to 37.33%. Percent dead seed was found the minimum (3.33%) in T_1 (0% seed infection) which was preceded by T_2 (5.1-15% seed infection) and T_3 (15.1-25% seed infection) with 6.67% and 14.00% dead seed, respectively and the maximum (37.33%) dead seed was recorded in T_6 (45.1-60% seed infection) followed by T_5 (29.33%) and T_4 (23.33%).

4.2. Field Experiment

4.2.1. Effect of different levels of seed infection by *Bipolaris sorokiniana* on disease incidence at 21 DAS of wheat in the field

Considering number of seedlings/m^2, the treatments differed significantly (Table 5 and Plate 16, 17 and 18). The values ranged from 90.20 to 122.19, where the highest number of plants/m^2 was found in T_1 (0% seed infection) followed by T_2 (115.05), T_3 (108.37) and T_4 (100.94) and the lowest number of plants/m^2 was recorded in T_6 (45.1-60% seed infection) preceded by T_5 (94.68).

In case of number of infected seedlings/m^2, significant variations were found among the treatments. The maximum number of infected plants/m^2 (25.73) was found in T_6 (45.1-60% seed infection) followed by T_5 (23.38) and T_4 (19.25). On the other hand, the minimum number of infected plants/m^2 (8.54) was found in T_1 (0% seed infection) preceded by T_2 (11.28) and T_3 (15.41).

Table 5. Effect of different levels of seed infection by *Bipolaris sorokiniana* on disease incidence at 21 DAS of wheat in the field

Treatment	Number of seedlings/m^2	Number of infected seedlings/m^2
T_1	122.19 a	8.54 f
T_2	115.05 b	11.28 e
T_3	108.37 c	15.41 d
T_4	100.94 d	19.25 c
T_5	94.68 e	23.38 b
T_6	90.20 f	25.73 a
LSD (0.01)	**2.440**	**0.6124**

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

Plate 16. Experimental field showing seedlings at 21 DAS

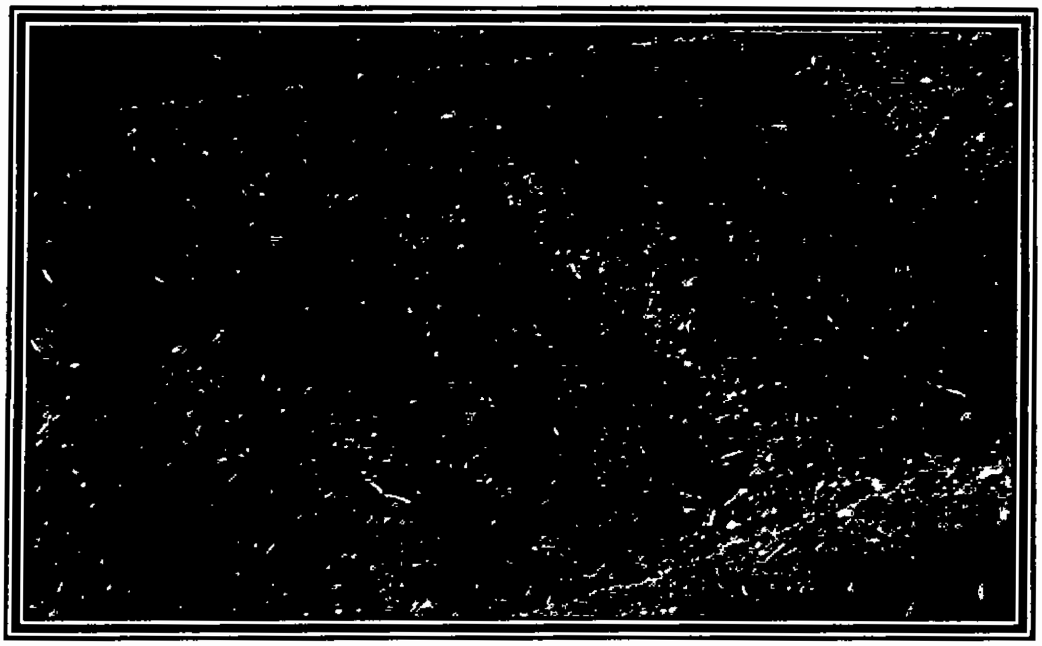


Plate 17. Unit plot showing seedlings of
T_1 (0% seed infection) at 21 DAS

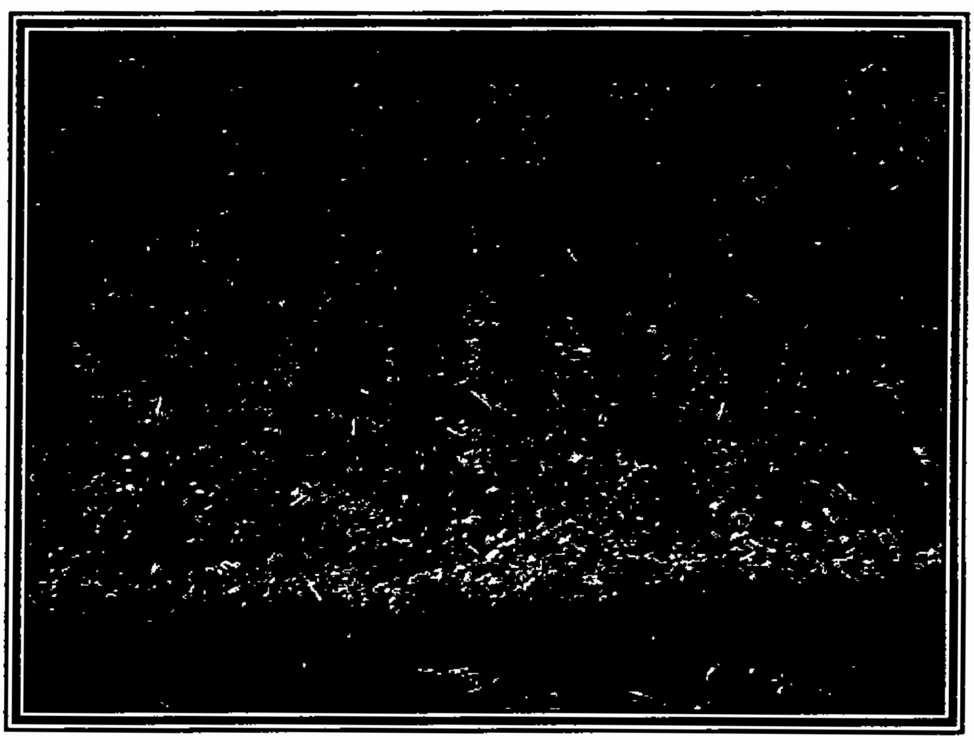


Plate 18. Unit plot showing seedlings of
T_6 (45.1-60% seed infection) at 21 DAS

4.2.2. Effect of different levels of seed infection by *Bipolaris sorokiniana* on leaf blight severity (0-5 scale) of wheat at flag leaf stage

Leaf blight severity of wheat at flag leaf stage on flag leaf and penultimate leaf was recorded and presented in Table 6. In flag leaf stage, there was no significant variation of disease severity on flag leaf among the treatments though the highest severity (0.08) was found in T_6 (45.1-60% seed infection) and the lowest severity (0.01) was found in T_1 (0% seed infection).

In case of penultimate leaf, the disease severity was found the maximum (0.30) in T_6 (45.1-60% seed infection) and the minimum (0.08) in T_1 (0% seed infection) which did not differ significantly with the other treatments.

The treatments showed significant variation of the average leaf blight severity of flag leaf and penultimate leaf .The average disease severity ranged from 0.05 to 0.19, where the highest severity (0.19) was found in T_6 (45.1-60% seed infection) and the lowest severity (0.05) was found in T_1 (0% seed infection). The other treatments did not show any significant difference with T_1 (0% seed infection).

Table 6. Effect of different levels of seed infection by *Bipolaris sorokiniana* on leaf blight severity of wheat at flag leaf stage

Treatments	Disease severity (0-5 scale)		
	Flag leaf	**Penultimate leaf**	**Average**
T_1	0.01	0.08b	0.05c
T_2	0.03	0.09b	0.06bc
T_3	0.04	0.12b	0.08bc
T_4	0.06	0.14b	0.10bc
T_5	0.07	0.18b	0.13b
T_6	0.08	0.30a	0.19a
LSD (0.01)	**NS**	**0.1157**	**0.04146**

NS= Not significant

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

4.2.3. Effect of different levels of seed infection by *Bipolaris sorokiniana* on leaf blight severity (0-5 scale) of wheat at panicle initiation stage

In panicle initiation stage, leaf blight severity of flag leaf and penultimate leaf was statistically significant among the treatments (Table 7 and Plate 19). In flag leaf, the lowest disease severity (0.56) was recorded in T_1 (0% seed infection) which was statistically similar with T_2 (5.1-15% seed infection) preceded T_3 (0.67). On the other hand, the highest disease severity (0.98) was observed in T_6 (45.1-60% seed infection) which was statistically similar with T_5 (35.1-45% seed infection) on flag leaf followed by T_4 (0.78).

Similar results were found in case of penultimate leaf, where the maximum disease severity (1.56) was observed in T_6 (45.1-60% seed infection) which was statistically similar with T_5 (35.1-45% seed infection). On the other hand, the minimum disease severity (1.04) was recorded in T_1 (0% seed infection). T_2 (5.1-15% seed infection) and T_3 (15.1-25% seed infection) were statistically similar.

The average leaf blight severity of flag leaf and penultimate leaf ranged from 0.80 to 1.27, where the highest (1.27) and the lowest (0.80) counts were made in T_6 (45.1-60% seed infection) and T_1 (0% seed infection), respectively. Results of Treatment T_1 (0% seed infection) and T_2 (5.1-15% seed infection) were statistically similar. Treatment T_4 (25.1-35% seed infection) and T_5 (35.1-45% seed infection) also differed significantly.

Table 7. Effect of different levels of seed infection by *Bipolaris sorokiniana* on leaf blight severity (0-5 scale) of wheat at panicle initiation stage

Treatments	Disease severity (0-5 scale)		
	Flag leaf	Penultimate leaf	Average
T_1	0.56d	1.04d	0.80d
T_2	0.61cd	1.20c	0.91cd
T_3	0.67c	1.26bc	0.97c
T_4	0.78b	1.40b	1.09b
T_5	0.92a	1.51a	1.21a
T_6	0.98a	1.56a	1.27a
LSD (0.01)	**0.08183**	**0.1404**	**0.1157**

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

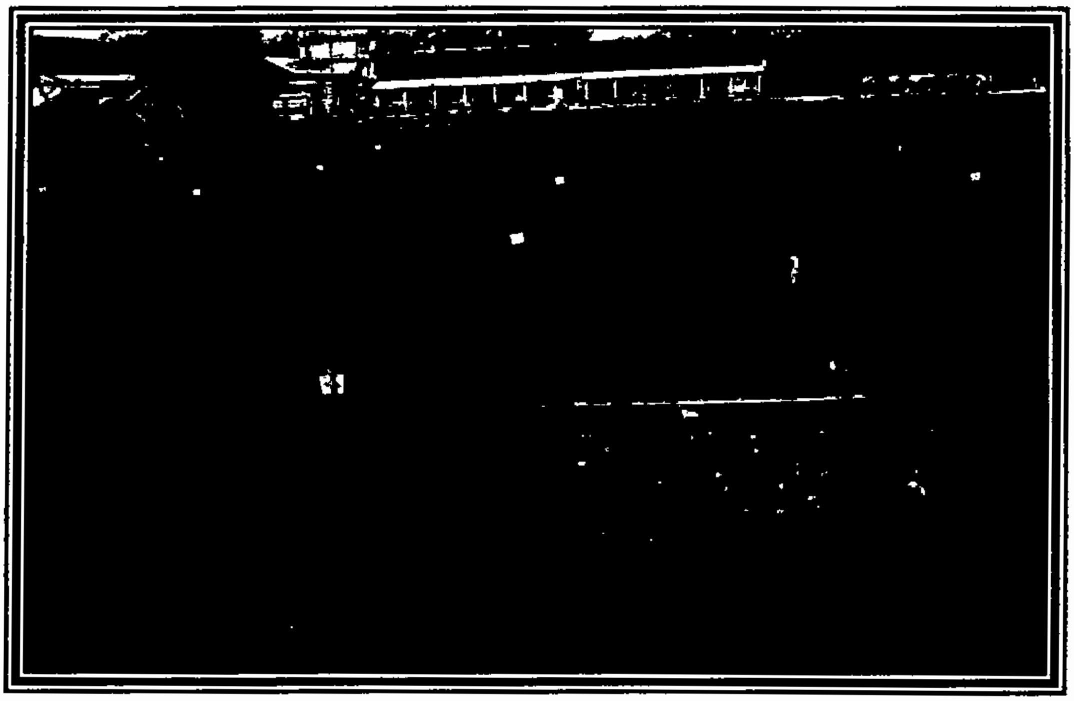


Plate 19. Experimental Field at Panicle initiation stage

4.2.4. Effect of different levels of seed infection by *Bipolaris sorokiniana* on leaf blight severity (0-5 scale) of wheat at flowering stage

Significant effect of different levels of seed infection by *Bipolaris sorokiniana* on leaf blight severity at flowering stage on flag leaf and penultimate leaf was observed (Table 8). In the flag leaf, the leaf blight severity ranged from 1.09 to 1.83. The maximum (1.83) value was found in T_6 (45.1-60% seed infection) followed by T_5 (1.70) as well as T_4 (1.52). On the contrary, T_1 (0% seed infection) showed the minimum (1.09) disease severity. Treatments T_1 (0% seed infection) and T_2 (5.1-15% seed infection) showed statistically similar results preceded by T_3 (1.29).

Leaf blight severity on penultimate leaf varied from 2.07 to 2.87. The lowest severity (2.07) was recorded in T_1 (0% seed infection) which was statistically indifferent with T_2 (5.1-15% seed infection) and T_3 (15.1-25% seed infection) and the highest severity (2.87) was found in T_6 (45.1-60% seed infection) which was statistically similar with T_5 (35.1-45% seed infection) followed by T_4 (25.1-35% seed infection.

In case of average disease severity, the values were found in a range of 1.58 to 2.35. T_6 (45.1-60% seed infection) and T_1 (0% seed infection) showed the maximum (2.35) and the minimum (1.58) counts.

Table 8. Effect of different levels of seed infection by *Bipolaris sorokiniana* on leaf blight severity (0-5 scale) of wheat at flowering stage

Treatments	Disease severity (0-5 scale)		
	Flag leaf	Penultimate leaf	Average
T_1	1.09e	2.07c	1.58e
T_2	1.18e	2.15c	1.66e
T_3	1.29d	2.39bc	1.84d
T_4	1.52c	2.56b	2.04c
T_5	1.70b	2.71a	2.20b
T_6	1.83a	2.87a	2.35a
LSD (0.01)	**0.1157**	**0.2062**	**0.1237**

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

4.2.5. Effect of different levels of seed infection by *Bipolaris sorokiniana* on leaf blight severity(0-5 scale) of wheat at milking stage

It was revealed that different levels of seed infection by *Bipolaris sorokiniana* had significant but different effects on leaf blight severity on flag leaf and penultimate leaf at milking stage (Table 9). The disease severity was found in range of 1.57 to 2.48 on flag leaf. The minimum (1.57) count was found in T_1 (0% seed infection) preceded by T_2 (1.72) and T_3 (1.93). On the other hand, the maximum (2.48) count was observed in T_6 (45.1-60% seed infection) followed by T_5 (2.35) and T_4 (2.06).

Considering penultimate leaf, the disease severity varied from 3.17 to 4.28. The highest (3.17) and the lowest (4.28) severities were found in T_6 (45.1-60% seed infection) and T_1 (0% seed infection), respectively.

The average disease severity ranged from 2.37 to 3.38. The maximum (3.38) and the minimum (2.37) values were recorded in T_6 (45.1-60% seed infection) and T_1 (0% seed infection), respectively. Treatments T_5 and T_6 showed statistically indifferent results. On the other hand, the results of T_2 (5.1-15% seed infection), T_3 (15.1-25% seed infection) and again T_3 and T_4 were similar.

Table 9. Effect of different levels of seed infection by *Bipolaris sorokiniana* on leaf blight severity (0-5 scale)of wheat at milking stage

Treatments	Disease severity (0-5 scale)		
	Flag leaf	Penultimate leaf	Average
T_1	1.57f	3.17c	2.37d
T_2	1.72e	3.47bc	2.60c
T_3	1.93d	3.54bc	2.74bc
T_4	2.06c	3.75b	2.91b
T_5	2.35b	4.21a	3.28a
T_6	2.48a	4.28a	3.38a
LSD (0.01)	**0.1157**	**0.3901**	**0.2055**

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

4.2.6. Effect of different levels of seed infection by *Bipolaris sorokiniana* on leaf blight severity (0-5 scale) of wheat at hard dough stage

Significant variations were found in leaf blight severity in hard dough stage on flag leaf and penultimate leaf (Table 10 and Plate 20). In case of flag leaf, the disease severity ranged from 2.63 to 3.78, where the maximum (2.63) and the minimum (3.78) values were found in T_6 (45.1-60% seed infection) and T_1 (0% seed infection), respectively. The result of T_1 was preceded by T_2 (2.86) and T_3 (3.13). T_5 (35.1-45% seed infection) and T_6 (45.1-60% seed infection) were statistically similar followed by T_4 (3.36).

Considering disease severity on penultimate leaf, the severities ranged from 3.95 to 4.96. The lowest severity (3.95) was found in T_1 (0% seed infection). On the other hand, the highest severity (4.96) was observed in T_6 (45.1-60% seed infection). T_2 (5.1-15% seed infection) and T_3 (15.1-25% seed infection) were statistically indifferent. The results of T_5 (35.1-45% seed infection) and T_6 (45.1-60% seed infection) were statistically similar followed by T_4 (25.1-35% seed infection).

From the average disease severity, it was revealed that, treatment T_6 (45.1-60% seed infection) showed the maximum severity (4.37), where the minimum severity (3.29) was recorded in T_1 (0% seed infection). The results of T_2 (5.1-15% seed infection) and T_3 (15.1-25% seed infection) were variable but statistically indifferent. T_5 (35.1-45% seed infection) and T_6 (45.1-60% seed infection) showed statistically similar results followed by T_4 (3.99).

Table 10. Effect of different levels of seed infection by *Bipolaris sorokiniana* on leaf blight severity (0-5 scale)of wheat at hard dough stage

Treatments	Disease severity (0-5 scale)		
	Flag leaf	Penultimate leaf	Average
T_1	2.63e	3.95d	3.29d
T_2	2.86d	4.38c	3.62c
T_3	3.13c	4.42c	3.78c
T_4	3.36b	4.62b	3.99b
T_5	3.71a	4.91a	4.31a
T_6	3.78a	4.96a	4.37a
LSD(0.01)	**0.1637**	**0.08183**	**0.1637**

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

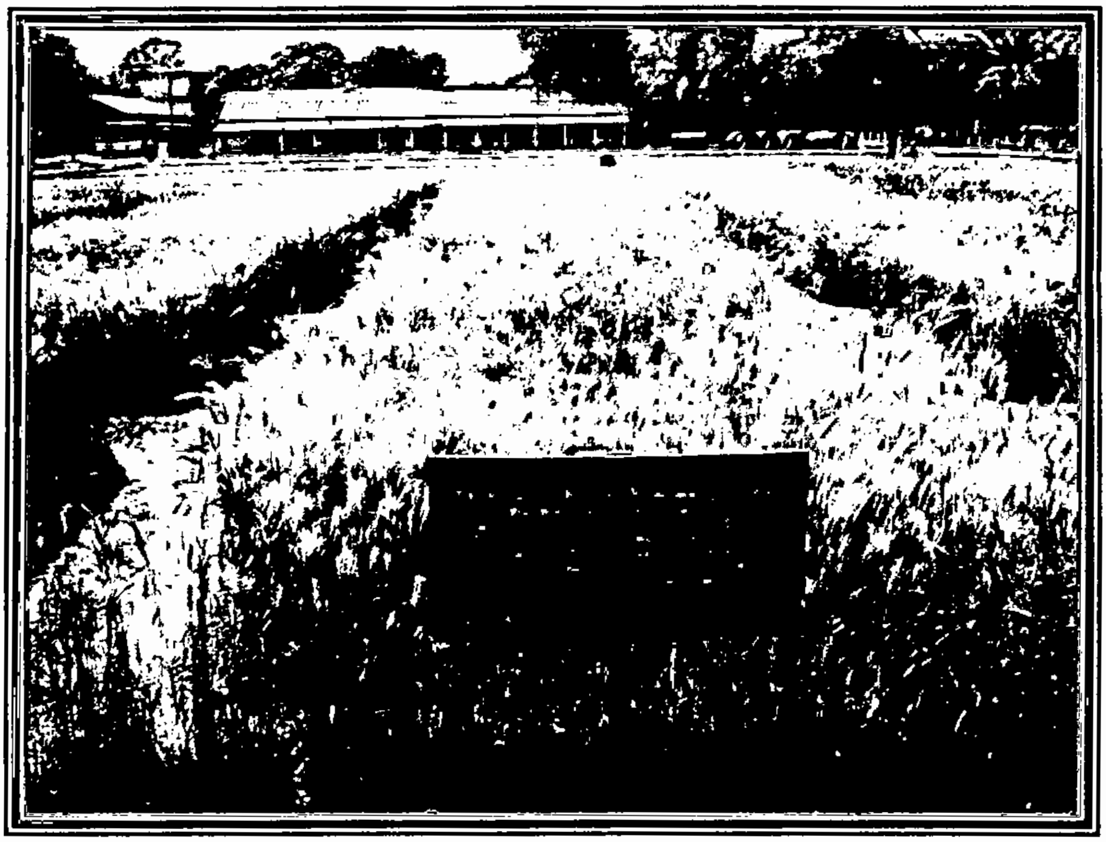


Plate 20. Experimental field at hard dough stage

4.2.7. Effect of different levels of seed infection by *Bipolaris sorokiniana* on plant growth of wheat in the field

The treatments were found to differ significantly in respect of plant height (cm) and spike length (cm) but in case of distance between the point of flag leaf initiation and base of ear (cm), no significant variation was found (Table 11).

The plant height ranged from 89.67 to 85.42 cm, where the highest plant height (89.67 cm) was found in T_1 (0% seed infection) and the lowest plant height (85.42 cm) was found in T_6 (45.1-60% seed infection) which was statistically indifferent with T_4 (25.1-35% seed infection) and T_5 (35.1-45% seed infection). T_2 (5.1-15% seed infection) and T_3 (15.1-25% seed infection) showed statistically similar results.

In respect of spike length, the treatments showed significant difference. The spike lengths lied in a range of 15.95 to 14.80 cm, where the maximum (15.95 cm) count was found in T_1 (0% seed infection) which was statistically similar with T_2 (5.1-15% seed infection).The minimum spike length (14.80 cm) was found in T_6 (45.1-60% 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) showed statistically similar effect on spike length.

In case of distance between the point of flag leaf initiation and base of ear (cm), there was no significant difference, though the highest (14.25 cm) and lowest (13.25 cm) values were found in T_1 (0% seed infection) and T_6 (45.1-60% seed infection), respectively.

Table 11. Effect of different levels of seed infection by *Bipolaris sorokiniana* on plant growth of wheat in the field

Treatment	Plant height (cm)	Spike length (cm)	Distance between the point of flag leaf initiation and base of ear (cm)
T_1	89.67a	15.95a	14.25
T_2	88.05b	15.50ab	13.87
T_3	87.93bc	15.30bc	13.73
T_4	86.00cd	15.16bc	13.70
T_5	85.72d	14.96bc	13.57
T_6	85.42d	14.80c	13.45
LSD (0.01)	**1.219**	**0.5957**	**NS**

NS= Not significant

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

4.2.8. Effect of different levels of seed infection by *Bipolaris sorokiniana* on spikelet formation of wheat

It was observed that the treatments differed significantly in respect of no. of spikelets/ear, no. of healthy spikelets/ear and no. of diseased spikelets/ear (Table 12).

Number of spikelets/ear was ranged from 31.24 to 20.65. The maximum value was found in T_1 (0% seed infection) followed by T_2 (5.1-15% seed infection) with 27.84 spikelets/ear. On the other hand, the minimum value was found in T_6 (45.1-60% seed infection). Treatments T_3 (15.1-25% seed infection), T_4 (25.1-35% seed infection) and T_5 (35.1-45% seed infection) showed statistically similar results.

Number of healthy spikelets/ear was varied from 30.87 to 15.31, where the highest (30.87) count was made in T_1 (0% seed infection) followed by T_2 (26.22) and T_3 (22.60) and the lowest (15.31) count was made in T_6 (45.1-60% seed infection). The results of Treatment T_4 (25.1-35% seed infection) and T_5 (35.1-45% seed infection) were statistically indifferent.

In case of number of diseased spikelets/ear, the values were found lying in a range of 0.77 to 5.34. The maximum number of diseased spikelets/ear (5.34) was recorded in T_6 (45.1-60% seed infection) followed by T_5 (35.1-45% seed infection) and T_4 (25.1-35% seed infection) with the values 4.98 and 3.94, respectively. The minimum no. of diseased spikelets/ear (0.77) was observed in T_1 (0% seed infection) preceded by T_2 (1.62) and T_3 (2.42).

Table 12. Effect of different levels of seed infection by *Bipolaris sorokiniana* on spikelet formation of wheat

Treatments	Number of spikelets/ear	Number of healthy spikelets/ear	Number of diseased spikelets/ear
T_1	31.24a	30.87a	0.77f
T_2	27.84b	26.22b	1.62e
T_3	25.02c	22.60c	2.42d
T_4	24.14c	20.20d	3.94c
T_5	23.96c	18.98d	4.98b
T_6	20.65d	15.31e	5.34a
LSD (0.01)	**1.244**	**1.853**	**0.1157**

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

4.2.9. Effect of different levels of seed infection by *Bipolaris sorokiniana* on grain formation and grain weight of wheat

Considering grain formation and grain weight of wheat, the treatments differed significantly (Table 13). In respect of number of grains, the results ranged from 38.68 to 31.98. The maximum number of grains (38.68) was found in T_1 (0% seed infection) followed by T_2 (36.65) and the minimum number of grains (31.98) was found in T_6 (45.1-60% seed infection) which was statistically similar with T_5 (35.1-45% seed infection). Treatments T_3 (15.1-25% seed infection) and T_4 (25.1-35% seed infection) showed statistically indifferent results.

In the formation of healthy grains, the values varied from 33.25 to 24.10. The highest number of healthy grains/ear (33.25) was found in T_1 (0% seed infection) followed by T_2 (5.1-15% seed infection) and T_3 (15.1-25% seed infection) with 30.89 and 28.40 healthy grains/ear, respectively. The lowest number of healthy grains/ear (24.10) was recorded in T_6 (45.1-60% seed infection) preceded by T_5 (24.98) and T_4 (27.40).

A significant variation was recorded among the treatments under the present trial in number of diseased grains/ear. The number of diseased grains/ear ranged from 5.43 to 7.95. The maximum count (7.95) was made in T_6 (45.1-60% seed infection) which showed statistically similar result with T_5 (35.1-45% seed infection).The minimum count (5.43) was made in T_1 (0% seed infection) which was statistically indifferent with T_2 (5.1-15% seed infection). Statistically similar results also found in Treatments T_3 (15.1-25% seed infection) and T_4 (25.1-35% seed infection).

Table 13. Effect of different levels of seed infection by *Bipolaris sorokiniana* on grain formation and grain weight of wheat

Treatments	Number of grains /ear	Number of healthy grains /ear	Number of diseased grains /ear	Weight of grains /ear(g)	Weight of healthy grains /ear(g)	Weight of diseased grains /ear(g)
T_1	38.68a	33.25a	5.43d	1.67a	1.51a	0.23
T_2	36.65b	30.89b	6.03d	1.45ab	1.22ab	0.24
T_3	35.13c	28.40c	6.73c	1.39ab	1.13b	0.26
T_4	34.20c	27.40d	7.16bc	1.36ab	1.08b	0.28
T_5	32.46d	24.98e	7.48ab	1.29b	0.99b	0.30
T_6	31.98d	24.10f	7.95a	1.27b	0.96b	0.32
LSD (0.01)	**1.208**	**0.5957**	**0.6124**	**0.295**	**0.3567**	**NS**

NS= Not significant

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

In case of weight of grains, the values varied from 1.67 to 1.27g, where the highest (1.67g) and the lowest (1.27) values were found in T_1 (0% seed infection) and T_6 (45.1-60% seed infection) which was statistically similar with T_5 (35.1-45% seed infection), respectively. T_1 (0% seed infection), T_2 (5.1-15% seed infection), T_3 (15.1-25% seed infection) and T_4 (25.1-35% seed infection) showed statistically similar results.

In respect of weight of healthy grains, the results ranged from 1.51 to 0.96g, where the maximum weight of healthy grains (1.51) was found in T_1 (0% seed infection) which was statistically similar with T_2 (5.1-15% seed infection). On the other hand, the minimum weight of healthy grains (0.96g) was found in T_6 (45.1-60% seed infection). Treatments 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) showed statistically similar results.

Weight of diseased grains did not show any significant variation among the treatments, though the highest value (0.32g) and lowest value (0.23g) were found in T_6 (45.1-60% seed infection) and T_1 (0% seed infection), respectively.

4.2.10. Effect of different levels of seed infection by *Bipolaris sorokiniana* on number of grains/ear of different severity grades (0-5) of harvested seeds of wheat

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 effects of the treatments were found on the formation of seeds under Grade-0 (free from infection i.e. apparently healthy seeds), Grade-2 (Embryo and its adjacent area slightly infected), Grade-3 (embryo and less than ¼ of grains are discolored), Grade-4 (embryo and ½ of grain are infected) and Grade-5 (grains are shriveled, almost completely discolored or more than ½ of grains were discolored) and presented in Table 14.

The number of grains under Grade-0 was found in a range of 24.10 to 33.25. The maximum (33.25) count of healthy seeds was observed in T_1 (0% seed infection) followed by T_2 (5.1-15% seed infection) and T_3 (15.1-25% seed infection) with the number 30.89 and 28.40, respectively. The minimum (24.10) count of healthy seeds was recorded in and T_6 (45.1-60% seed infection) preceded by T_5 (24.94) and T_4 (27.04).

No significant variation was observed among the treatments in respect of Grade-1 seeds (only embryo blackish), though the highest (3.82) number was found in T_6 (45.1-60% seed infection) and the lowest (3.32) number was found in T_1 (0% seed infection).

Considering Grade-2 seeds, the results varied from 0.64 to 0.98. The minimum value (0.64) was found in T_1 (0% seed infection) and the maximum value (0.98) was found in T_6 (45.1-60% seed infection) which was statistically indifferent with T_4 (25.1-35% seed infection) and T_5 (35.1-45% seed infection). T_1 (0% seed infection) and T_2 (5.1-15% seed infection) showed statistically similar effects on formation of grains under Grade-2.

Table 14. Effect of different levels of seed infection by *Bipolaris sorokiniana* on number of grains/ear of different severity grades (0-5) of harvested seeds of wheat

Treatments	Grading of seeds					
	0	1	2	3	4	5
T_1	33.25 a	3.22	0.64 d	0.45 c	0.33 c	0.85 f
T_2	30.89 b	3.37	0.72 cd	0.46 c	0.36 c	1.08 e
T_3	28.40 c	3.36	0.86 b	0.56 b	0.68 ab	1.30 d
T_4	27.04 d	3.49	0.91 ab	0.58 b	0.74 ab	1.44 c
T_5	24.94 e	3.61	0.97 a	0.63 ab	0.66 b	1.60 b
T_6	24.10 f	3.82	0.98 a	0.69 a	0.76 a	1.71 a
LSD (0.01)	**0.5957**	**NS**	**0.09015**	**0.08183**	**0.08183**	**0.08183**

NS= Not significant

0 = Free from infection

1 = Only embryo blackish

2 = Embryo and its adjacent area slightly infected

3 = Embryo and less than ¼ of grains are discolored

4 = Embryo and ½ of grain are infected

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

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

The highest Grade-3 seeds formation (0.69) was observed in T_6 (45.1-60% seed infection) which showed statistically indifferent result with T_5 (35.1-45% seed infection) and T_4 (25.1-35% seed infection). The lowest number of seeds of Grade-3 was found in T_1 (0% seed infection). The effects of T_1 and T_2 were statistically similar and they differed from T_3.

In case of number of seeds under Grade-4, the values ranged from 0.33 to 0.76, where the maximum value (0.76) was found in T_6 (45.1-60% seed infection) and followed by T_5 (0.66) and the minimum value (0.33) was recorded in T_1 (0% seed infection) and T_2 (5.1-15% seed infection) were statistically similar which differed from T_3 and T_4.

Regarding to the formation of Grade-5 seeds, the treatments varied from 0.85 to 1.71. The lowest (0.85) grains under Grade-5 were observed in T_1 (0% seed infection) preceded by T_2 (1.08) and T_3 (1.30). On the contrary, the highest (1.71) grains under Grade-5 were recorded in and T_6 (45.1-60% seed infection) followed by T_5 (35.1-45% seed infection) and T_4 (25.1-35% seed infection) with 1.60 and 1.44 grains under Grade-5.

4.2.11. Effect of different levels of seed infection by *Bipolaris sorokiniana* on 1000 seed weight and yield of wheat

Highly significant variations were observed among the treatments in respect of 1000 seeds weight, grain yield and straw yield of wheat cv. Kanchan (Table 15). The 1000 seeds weight (g) of wheat varied from 32.98 to 40.23g. The highest (40.23g) weight of 1000 seeds was found in T_1 (0% seed infection) followed by T_2 (5.1-15% seed infection) and T_3 (15.1-25% seed infection) which provide 36.18g and 35.26g weight of 1000 seeds. On the contrary, the lowest (32.98g) weight of 1000 seeds was recorded in T_6 (45.1-60% seed infection). Statistically similar but variable results were obtained in T_5 (35.1-45% seed infection) and T_6 (45.1-60% seed infection) preceded by T_4 (25.1-35% seed infection) with 34.11 g weight of 1000 seeds.

It was revealed that the maximum straw yield (5.77 kg/plot) was recorded in T_1 (0% seed infection) followed by T_2 (5.24 kg/plot) and T_3 (4.91kg/plot). The minimum straw yield (4.14 kg/plot) was observed in T_6 (45.1-60% seed infection) preceded by T_5 (4.37 kg/plot) and T_4 (4.63 kg/plot). Considering straw yield (t/ha), the yields ranged from 4.60 to 6.41 t/ha, where the highest (6.41 t/ha) yield was found in the plot of T_1 (0% seed infection) followed by T_2 (5.82 t/ha) and T_3 (5.45 t/ha). On the other hand, the lowest (4.60 t/ha) yield was in T_6 (45.1-60% seed infection) preceded by T_5 (4.86 t/ha) and T_4 (5.15 t/ha).

In case of yield of wheat (kg/plot), the treatments had profound effects. All the treatments differed significantly from one to another. The values ranged from 2.03 to 3.30 kg/plot. The highest grain yield (3.30 kg/plot) was found in T_1 (0% seed infection) which was followed by T_2 (5.1-15% seed infection) and T_3 (15.1-25% seed infection) with grain yield 2.94 kg/plot and 2.72 kg/plot. The lowest grain yield (2.03 kg/plot) was found in T_6 (45.1-60% seed infection) preceded by T_5 (2.30 kg/plot) and T_4 (2.57 kg/plot). Considering grain yield (t/ha), treatment T_1 (0% seed infection) resulted the maximum yield (3.67 t/ha) followed by T_2 (3.27 t/ha) and T_3 (3.02 t/ha).On the other hand, minimum grain yield (2.25 t/ha) was obtained in the plot of

Table 15. Effect of different levels of seed infection by *Bipolaris sorokiniana* on 1000 seeds weight and yield of wheat

Treatments	1000 seeds weight (g)	Straw yield (kg/plot)	Straw yield (t/ha)	Grain yield (kg/plot)	Grain yield (t/ha)	% Grain yield decreased over T_1
T_1	40.23a	5.77a	6.41a	3.30a	3.67a	-
T_2	36.18b	5.24b	5.82b	2.94b	3.27b	10.90
T_3	35.26c	4.91c	5.45c	2.72c	3.02c	17.71
T_4	34.11d	4.63d	5.15d	2.57d	2.86d	22.07
T_5	33.37e	4.37e	4.86e	2.30e	2.56e	30.25
T_6	32.98e	4.14f	4.60f	2.03f	2.25f	38.69
LSD (0.01)	**0.06124**	**0.1830**	**0.2004**	**0.1157**	**0.1417**	-

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

T_6 (45.1-60% seed infection) preceded by T_5 (35.1-45% seed infection) and T_4 (25.1-35% seed infection) with 2.56 t/ha and 2.86 t/ha, respectively. The treatments decreased grain yield (t/ha) over T_1 (0% seed infection) ranged from 10.90 to 38.69% which was found in T_6 (45.1-60% seed infection).

4.3. Laboratory experiment (after harvesting)

4.3.1. Effect of different levels of seed infection by *Bipolaris sorokiniana* on germination and incidence of *Bipolaris sorokiniana* of harvested seeds of wheat (Blotter method)

In harvested seeds, the germination percentage and incidence of *Bipolaris sorokiniana* were found to differ significantly among the treatments (Table 16 and Plate 21). Considering germination percentages, the values ranged from 63.00 to 90.00%, where the maximum (90.00%) counts were made in T_1 (0% seed infection) which was followed by the treatment T_2 (85.00%). The minimum (63.00%) germination was counted in T_6 (45.1-60% seed infection) preceded by T_5 (35.1-45% seed infection), T_4 (25.1-35% seed infection) and T_3 (15.1-25% seed infection) with 68.00%, 71.00% and 78.00%, respectively.

In case of incidence of seed borne *Bipolaris sorokiniana*, the values varied from 8.30 to 58.38%, where the highest presence (58.38%) was found in T_6 (45.1-60% seed infection) which was followed by T_5 (45.43%) and T_4 (39.15%). The lowest presence of the pathogen (8.30) was recorded in T_1 (0% seed infection) preceded by T_2 (5.1-15% seed infection) and T_3 (15.1-25% seed infection) with 18.27% and 27.75%, respectively.

Table 16. Effect of different levels of seed infection by *Bipolaris sorokiniana* on germination and incidence of *Bipolaris sorokiniana* of harvested seeds of wheat (Blotter method)

Treatments	%Germination	% *Bipolaris sorokiniana*
T_1	90.00 a	8.30 (17.06) f
T_2	85.00 b	18.27 (25.30) e
T_3	78.00 c	27.75 (31.30) d
T_4	71.00 d	39.15 (38.64) c
T_5	68.00 e	45.43 (42.13) b
T_6	63.00 f	58.38 (49.60) a
LSD (0.01)	**1.811**	**3.567**

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

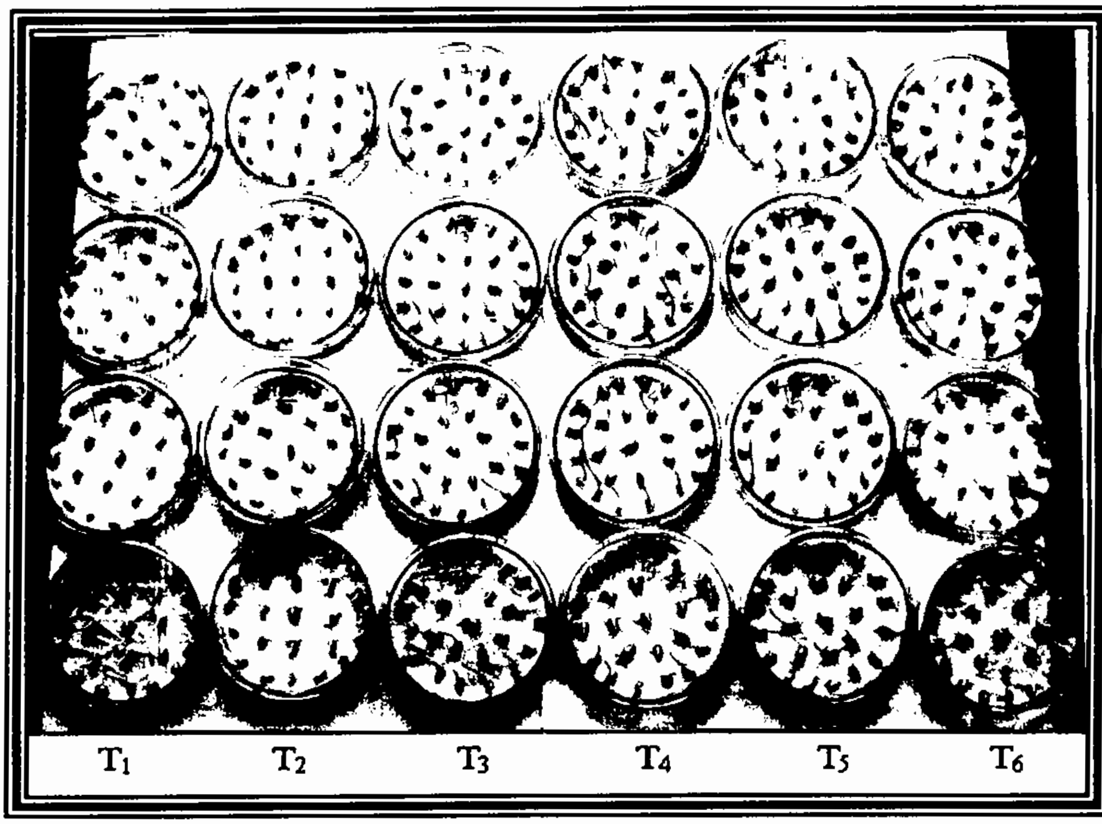


Plate 21. Evaluation of harvested seeds of the different levels of seed infection by *Bipolaris sorokiniana* in Blotter method

4.4. Correlation Regression Study

4.4.1. Relationship between different levels of seed infection by *Bipolaris sorokiniana* and average leaf blight severity (0-5 scale) at flag leaf stage

Relationship between seed infection by *Bipolaris sorokiniana* and leaf blight severity of wheat at flag leaf stage has been shown in Figure 2. The regression equation y = 0.002x + 0.033 and the straight line plotted in the figure indicate that there is a linear relationship between different levels of seed infection and average leaf blight severity at flag leaf stage with highly positive correlation (r =0.908**).

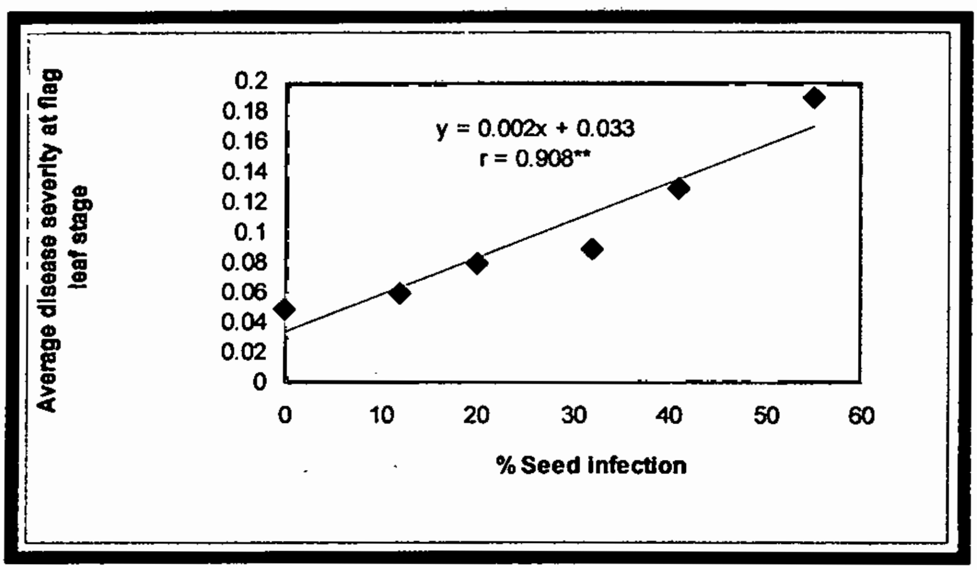


Figure 2. Relationship between different levels of seed infection by *Bipolaris sorokiniana* and average leaf blight severity (0-5 scale) at flag leaf stage

4.4.2. Relationship between different levels of seed infection by *Bipolaris sorokiniana* and average leaf blight severity (0-5 scale) at panicle initiation stage

A linear relationship with the regression equation y = 0.008x + 0.808 between the levels of seed infection by *Bipolaris sorokiniana* and average leaf blight severity at panicle initiation stage was found under field condition with a highly positive correlation (r = 0.963**) (Figure 3).

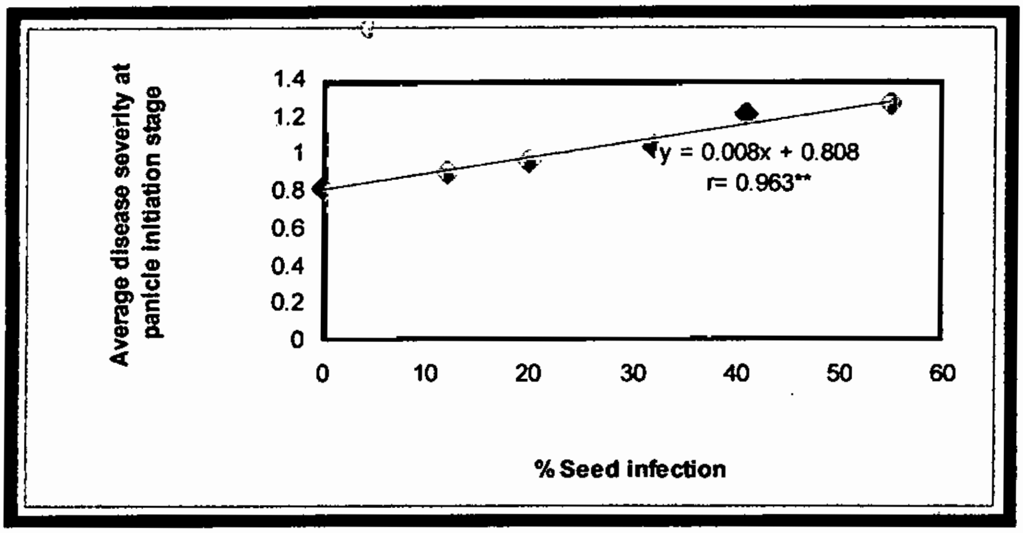


Figure 3. Relationship between different levels of seed infection by *Bipolaris sorokiniana* and average leaf blight severity (0-5 scale) at panicle initiation stage

4.4.3. Relationship between different levels of seed infection by *Bipolaris sorokiniana* and average leaf blight severity (0-5 scale) at flowering stage

Relationship between different levels of seed infection by *Bipolaris sorokiniana* with average leaf blight severity at flowering stage was presented in Figure 4. It observed that the levels of seed infection and leaf blight severity was found positively correlated (r=0.983**) and their relationship is expressed by y=0.015x + 1.543.

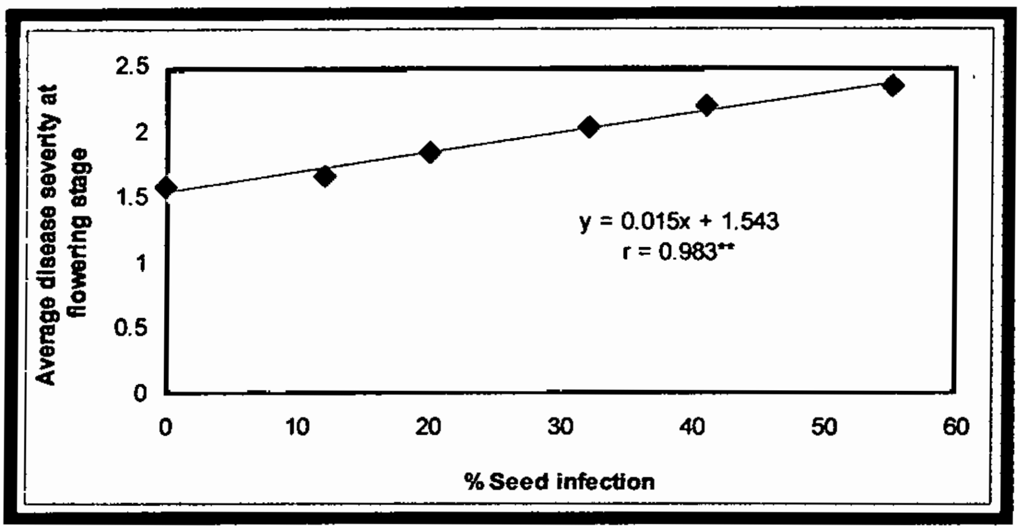


Figure 4. Relationship between different levels of seed infection and average leaf blight severity (0-5 scale) at flowering stage

4.4.4. Relationship between different levels of seed infection by *Bipolaris sorokiniana* and average leaf blight severity (0-5 scale) at milking stage

The regression equation y = 0.018x + 2.384 and the straight line in the figure indicated the linear relationship between different levels of seed infection by *Bipolaris sorokiniana* and average leaf blight severity at milking stage which was with highly positive correlation (r = 0.949**) (Figure 5).

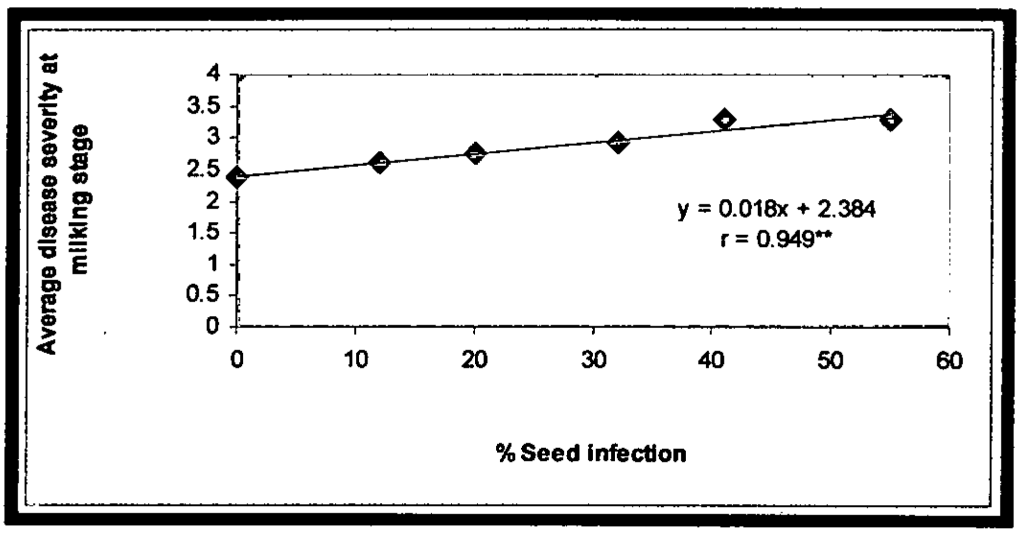


Figure 5. Relationship between different levels of seed infection by *Bipolaris sorokiniana* and average leaf blight severity (0-5 scale) at milking stage

4.4.5. Relationship between different levels of seed infection by *Bipolaris sorokiniana* and average leaf blight severity (0-5 scale) at hard dough stage

The linear relationship between different levels of seed infection by *Bipolaris sorokiniana* and average leaf blight severity at hard dough stage in vivo was found in Figure 6 with regression equation y = 0.020x + 3.350 and highly positive correlation (r = 0.964**).

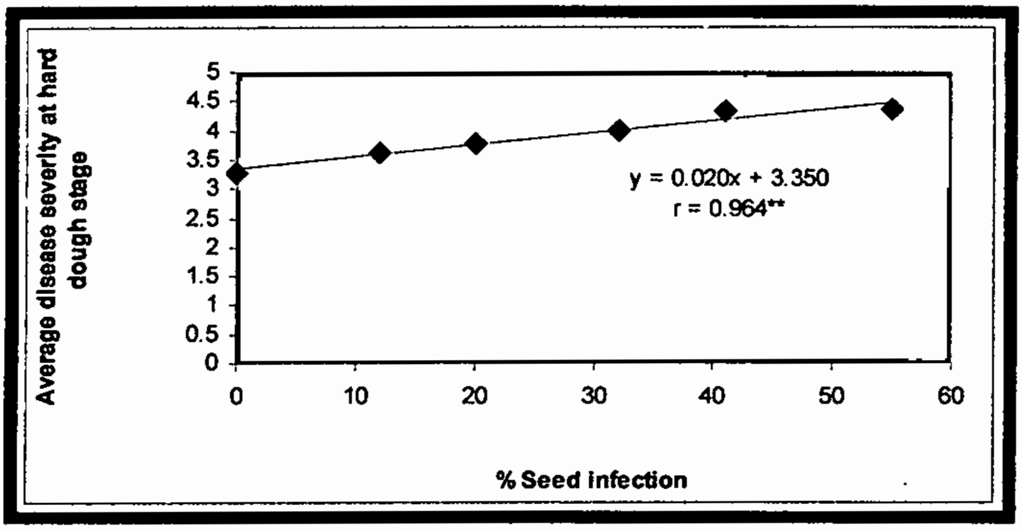


Figure 6. Relationship between different levels of seed infection by *Bipolaris sorokiniana* and average leaf blight severity (0-5 scale) at hard dough stage

4.4.6. Relationship between different levels of seed infection by *Bipolaris sorokiniana* and incidence of *Bipolaris sorokiniana* in harvested seeds

There was a strong relationship of different levels of seed infection by *Bipolaris sorokiniana* with the incidence of *Bipolaris sorokiniana* in harvested seeds found and presented in Figure 7. The regression equation was y = 0.916x + 8.439 with highly positive correlation (r = 0.997**).

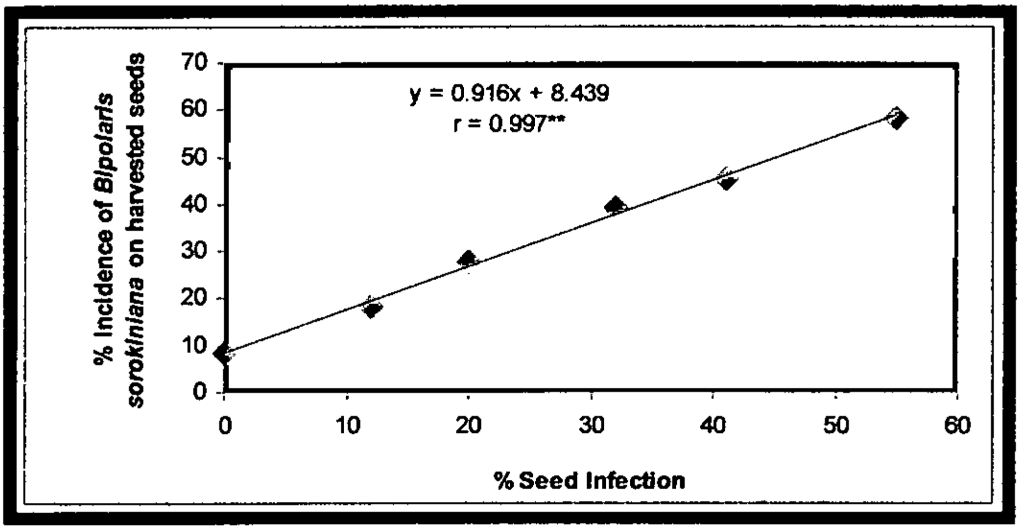


Figure 7. Relationship between different levels of seed infection by *Bipolaris sorokiniana* and incidence of *Bipolaris sorokiniana* in harvested seeds

Chapter 5

Discussion

DISCUSSION

In the present study, significant effect of different levels of seed infection by *Bipolaris sorokiniana* on seedling vigor, leaf blight development and quality seed production of wheat was found both under laboratory and field condition. There were six treatments with different levels of seed infection by *Bipolaris sorokiniana* were used in the study viz. T_1 = 0% seed infection, T_2 = 5.1-15% infection, T_3 = 15.1-25% infection, T_4 = 25.1-35% infection, T_5 = 35.1-45% infection and T_6 = 45.1-60% infection.

5.1. Laboratory experiment

Significant variations were observed among the treatments in respect of seed germination in blotter method, rolled paper towel method and water agar test tube seedling symptom test. It was evident that the maximum seed germination was found in seeds which were free from infection (T_1). With the increase of seed infection, remarkable lessening of the seed germination was recorded and lowest germination was found in seeds with the maximum (45.1-60%) infection (T_6). The minimum germination percentages were 69.00, 72.67 and 62.67 in blotter method, rolled paper towel method and water agar test tube seedling symptom test, respectively found in T_6 (45.1-60% infection).

The present findings are well supported by others (Hanson and Christensen, 1953a; Choudhary *et al.*, 1984; Khanum *et al.*, 1987; Hossain, 2000). Similar trend of variation in germination of *Helminthosporium sativum* infected wheat seeds was reported by Hanson and Christensen (1953a).

They observed that seed germination as 66% and 62% from the seeds having respectively 81% and 74% infection with *H. sativum*. Choudhary *et al.* (1984) reported that germination of the diseased (black pointed) seeds both in blotter and pot soil was found to decrease by 11.6% and 16.0%, respectively. Khanum *et al.* (1987) found 55-96% and 34.5-71% germination for healthy grains and diseased grains, 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 other previous workers (Parashar and Chohan, 1967; Rana and Gupta, 1982; Sinha *et al.*, 1984 and Zhang *et al.*, 1990).

From the present study, it was revealed that different levels of seed infection by *Bipolaris sorokiniana* had significant relationship with seedling infection as well as seedling health. Seedling infection increased with the increasing level of seed infection in rolled paper towel method and water agar test tube seedling symptom test. The highest infection of seedlings was recorded in T_6 (45.1-60% infection) which showed contrariety with the minimum seedling infection of T_1. The shoot length, root length, seedling weight and also Vigor Index (VI) were decreased with the increasing levels of seed infection.

The findings of the present study are supported by the earlier reports of other workers (Rana and Gupta, 1982; Rahman and Islam, 1998; Bazlur Rashid, 1998; Hossain, 2000). Rana and Gupta (1982) found that blackpoint infection greatly affected root and shoot growth of the seedlings, the effect being very prominent on root growth. Rahman and Islam (1998) observed significant reduction in seedling vigor in respect of germination, shoot and root length with the increase of black point infection. Bazlur Rashid (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 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%. He also mentioned that Vigor Index (VI) was found with maximum reduction (72.63%) resulted by the seedlings of 28% black pointed seeds.

5.2. Field experiment

Under in vivo condition, it was well exposed that number of seedlings/m^2 and number of infected seedlings/m^2 were significantly varied due to the effect of different levels of seed infection. The maximum number of seedlings/m^2 (122.19) was found in seeds which were free from infection (T_1) while the minimum number of seedlings/m^2 (90.20) were recorded in T_6 (45.1-60% seed infection). The highest number of infected seedlings/m^2 (25.73) was recorded in T_6 with maximum i.e. 45.1-60% infections whereas, the lowest number of infected seedlings/m^2 (8.54) were found in T_1 (0% seed infection).

These findings were well supported by other researchers (Machacek and Graney, 1938; Hossain, 2000). Machacek and Graney (1938) who reported from their green house and field test that seed infected with *Helminthosporium sativum* produced only 24.8% plant stand and resulted 80.6% seedling infection. Hossain (2000) also stated that significant decrease in plant stand in pot and field has been observed with the increase in number of black pointed seeds in seed samples.

From the present study, it was evident that the effect of different levels of seed infection subsequently was very significant with the increasing trend of leaf blight severity (0-5 scale) at flag leaf stage, panicle initiation stage, flowering stage, milking stage and hard dough stage. The minimum leaf blight severity was recorded in the plots of T_1 (0% seed infection) in every growth stages recorded. 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. It has been found that there were linear relationships with highly positive correlations (r = 0.908**, 0.963**, 0.983**, 0.949** and 0.964**) between different levels of seed infection by *Bipolaris sorokiniana* and disease severity at different growth stages.

The present findings were well supported by other researchers (Nema and Joshi, 1974; Hossain and Azad, 1992; Hossain, 2000; Reza *et al.*, 2006). 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 the favorable temperature range 25-28°C for disease epidemy 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.

The effect of different levels of seed infection was highly significant in respect of plant height and spike length but little effect was found in case of distance between the point of flag leaf initiation and base of ear. The highest plant height and spike length were found in 0.00% seed infection (T_1) where lowest values were found in the maximum i.e. 45-60% seed infection.

The treatments varied significantly considering spikelet formation i.e. number of spikelets/ear, number of healthy spikelets/ear and number of diseased spikelets/ear. The maximum seed infection (T_6) attained the lowest number of spikelets/ear and healthy spikelets/ear but the highest number of diseased spikelets/ear. The opposing trend was observed in T_1 (0% seed infection).

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.

These findings were well supported by other previous researchers (Bazlur Rashid *et al.*, 1994; Hossain *et al.*, 1998; Bazlur Rashid and Fakir, 1998; Hossain, 2000). Bazlur Rashid *et al.* (1994) also 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. 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. 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 incited more disease to the crop plants resulting formation of higher number of diseased seed in the field.

On the other hand, weight of grains/ear and weight of healthy grains/ear were significantly increased with the decrease of level of seed infection though there was no significant variation among the effect of different levels of seed infection on weight of diseased grains/ear.

Considering number of grains/ear of different severity grades (0-5) of harvested seeds, the treatments showed significant variations. Treatment T_1 (0% seed infection) attained the highest amount of seeds under Grade-0 (free from infection i.e. apparently healthy seeds) whereas, Treatment T_6 (45.1-60% seed infection) obtained Grade-2 (Embryo and its adjacent area slightly infected), Grade-3 (embryo and less than ¼ of grains are discolored), Grade-4 (embryo and ½ of grain are infected) and Grade-5 (grains are shriveled, almost completely discolored or more than ½ of grains were discolored). No significant effect of the treatments was found on Grade-1 seeds (only embryo blackish). From the findings, it was found that with the higher levels of seed infection by *Bipolaris sorokiniana* resulted the higher number of harvested seeds with black point.

These findings were well supported by Bazlur Rashid, 1996; Bazlur Rashid and Fakir, 1998; Hossain *et al.*, 1998; Reza *et al.*, 2006). Bazlur Rashid (1996) and Bazlur Rashid and Fakir (1998) reported that development of black point infection in the field was due to seed to plant to seed transmission of black point 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 percent disease severity interning the corresponding 17.42 percent seed infection.

Significant variations in respect of 1000 seeds weight and both grain and straw yield indicated the significant effect of different levels of seed infection on the above. 1000 seeds weight was found the highest in the plots under T_1 (0% seed infection) and the lowest in T_6 (45.1-60% seed infection) which was statistically similar with T_5 (35.1-45% seed infection). Grain yield as well as straw yield were found in maximum amount in T_1 (0% seed infection) whereas, the minimum yields were obtained in T_6 having maximum seed infection.

5.3. Laboratory experiment (after harvesting)

The germination and incidence of *B. sorokiniana* in blotter method of harvested seeds were found significant among the treatments. The germination was found to be lessened and the incidence of *B. sorokiniana* was found to be increased with the increase of the level of black pointed seeds in the seed samples that had been used for sowing. There was a linear relationship between the seed infection levels and incidence of *B. sorokiniana* recorded on harvested seeds.

These findings were well supported by Orsi *et al.* (1994). He found a positive correlation between *Drechselera sorokiniana* (*Cochliobolus sativus*) and black point incidence.

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. However, more investigations are needed to be perused in different Agro-ecological Zones to fix a suitable seed health standard against leaf blight of wheat (*Bipolaris sorokiniana*) for quality seed production.

Chapter 6

Summary and Conclusion

SUMMARY AND CONCLUSION

Wheat (*Triticum aestivum* L.) is considered as important cereal commodity in the world and the second most important cereal crop next to rice in Bangladesh. Seed is the basic means of crop production and its quality is therefore of decisive importance for a farm's success. Black point of wheat grains causes downgrading on the seed quality which is based on the incidence of affected kernels and the severity of leaf infection.

The present study was conducted in the laboratory and in the field laboratory of the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka to find out the effect of different levels of seed infection by *Bipolaris sorokiniana* on seedling vigor, leaf blight development and quality seed production of wheat during the period of June'07 to March'08.

Under *in vitro* condition, in Blotter method, there was a general tendency of decreasing seed germination with the increase of levels of seed infection by *Bipolaris sorokiniana* in the seed samples. The highest seed germination (97.00%) was found in T_1 (0% seed infection) and the lowest seed germination (69.00%) was recorded in T_6 (45.1-60% seed infection). T_1 (0% seed infection) was found completely free from the seed borne pathogen *Bipolaris sorokiniana* and the highest incidence (55.00%) was observed in T_6 (45.1-60% seed infection).

In case of Rolled Paper Towel method, the maximum germination (96.00%) was counted in T_1 (0% seed infection) and the lowest germination (72.67%)

was observed in T_6 (45.1-60% seed infection). The highest (49.33%) and lowest (2.67%) percent of seedling infection were found in T_6 (45.1-60% seed infection) and T_1 (0% seed infection), respectively. The maximum shoot length (6.53 cm), root length (12.76 cm), seedling weight (3.67 g) and were recorded in T_1 (0% seed infection). However, the above values were found to be lessened with the increase of seed infection levels and were the minimum in T_6 (45.1-60% seed infection). Vigor Index (VI) (1851.81) was observed the highest (1851.81) in T_1 (0% seed infection) and the lowest (669.36) in T_6 (45.1-60% seed infection).

Water Agar Test tube Seedling Symptom Test indicated that the maximum seed infection T_6 (45.1-60% seed infection) attained the minimum (62.67%) seed germination and lowest normal seedlings (10.00%) but the maximum (52.67%) abnormal seedlings and dead seed (37.33). On the other hand, the opposite results were found in T_1 (0% seed infection).

Under field condition, the number of seedlings/m^2 (122.19) was the maximum in T_1 (0% seed infection) but the number of infected seedlings/m^2 were the minimum (8.54) while the lowest number of seedlings/m^2 (90.20) and highest number of infected seedlings/m^2 (25.73) were recorded in T_6 (45.1-60% seed infection) at 21 Day after Sowing (DAS).

Leaf spot /leaf blight severity were increased significantly with increase of seed infection and also with the age of the plants. The lowest disease severity was found in plots of T_1 (0% seed infection) in different growth stages recorded in the study and highest disease severity was observed in T_6 (45.1-60% seed infection). Highly positive relationship was found between different levels of seed infection by *Bipolaris sorokiniana* and average disease severity at different growth stages ($r = 0.908^{**}$, 0.963^{**}, 0.983^{**}, 0.949** and 0.964**).

Considering plant growth i.e. plant height, spike length and distance between the point of flag leaf initiation and base of ear, the maximum and the minimum performance were found in T_1 (0% seed infection) and T_6 (45.1-60% seed infection), respectively though distance between the point of flag leaf initiation and base of ear did not differ significantly among the treatments.

Spikelet formation was significantly influenced with the different levels of seed infection by *Bipolaris sorokinian.* The highest number of spikelets/ear (31.24) and number of healthy spikelets/ear (30.87) were recorded in seeds with zero infection (T_1) whereas, the lowest values were found in T_6 with the maximum seed infection (45.1-60% seed infection). Number of diseased spikelets/ear was found the maximum (0.77) and the minimum (5.34) in T_6 (45.1-60% seed infection) and T_1 (0% seed infection), respectively.

It was well exposed that the treatments had significant effect on grain formation and grain weight as well as number of grains/ear of different severity grades (0-5) of harvested seeds. The formation of grains/ear and healthy grains/ear were found the highest in T_1 (0% seed infection) and the lowest in T_6 (45.1-60% seed infection) where as the opposing trend was found in case of number of diseased grains/ear. The weight of grains/ear and weight of healthy grains/ear were the minimum in T_6 (45.1-60% seed infection) and the maximum in T_1 (0% seed infection) though no significant effect on weight of diseased grains/ear of the seed infection levels was found. The highest (33.25) and the lowest (24.10) number of grains/ear under Grade-0 were found in T_1 (0% seed infection) and T_6 (45.1-60% seed infection), respectively.

The 1000 seeds weight of harvested seeds, grain yield and straw yield were significantly varied with the different levels of seed infection by *Bipolaris sorokiniana*. The weight of 1000 seeds were found to be the highest (40.23) in T_1 (0% seed infection) and the lowest (32.98) in T_6 (45.1-60% seed infection) which was statistically similar with T_5 (35.1-45% seed infection). The maximum (6.41 t/ha) and the minimum (4.60 t/ha) straw yield were obtained in T_1 (0% seed infection) and T_6 (45.1-60% seed infection), respectively. The maximum grain yield (3.67) was found in T_1 (0% seed infection) which was 38.69% more than that of T_6 (45.1-60% seed infection).

Again, under laboratory condition, germination of harvested seeds was decreased with the increase of seed infection in the seed samples used for sowing. The incidence of *Bipolaris sorokiniana* was in a similar trend with different levels of black pointed seeds i.e. seed infection. A linear relationship was found with highly positive correlation (r = 0.997**) between seed infection levels of seeds samples that had been sown and incidence of *Bipolaris sorokiniana* on harvested seeds.

It may be concluded that, the minimum level of seed infection by *Bipolaris sorokiniana* resulted better seedling vigor, lowered disease severity in field and increased quality of seeds. Considering all these, the findings of the present study may, in future, open a new horizon in plant disease control through use of quality seeds. So, further study is needed in different AEZ to find out the seed standard for quality seed production of wheat.

Chapter 7

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Appendices

APPENDICES

Appendix I. Monthly average temperature, relative humidity, total rainfall and Sunshine (hour) of the experimental site during the period from November 2007 to April 2008

Year	Month	*Air temperature (^0C)		*Relative Humidity (%)	*Total rainfall (mm)	*Sunshine (hr)
		Maximum	Minimum			
2007	November	31.8	16.8	67	111	5.7
	December	28.2	11.3	63	0	5.5
2008	January	29.0	10.5	61.5	23	5.6
	February	30.6	10.8	54.5	56	5.8
	March	34.6	16.5	61.5	45	5.8
	April	36.9	19.6	59.5	91	8.3

*Monthly average

Source: Bangladesh Meteorological Department (Climate division), Agargaon, Dhaka - 1207

Appendix -II. Physical and Chemical characteristics of initial soil in the experimental field

A. Physical composition of the soil

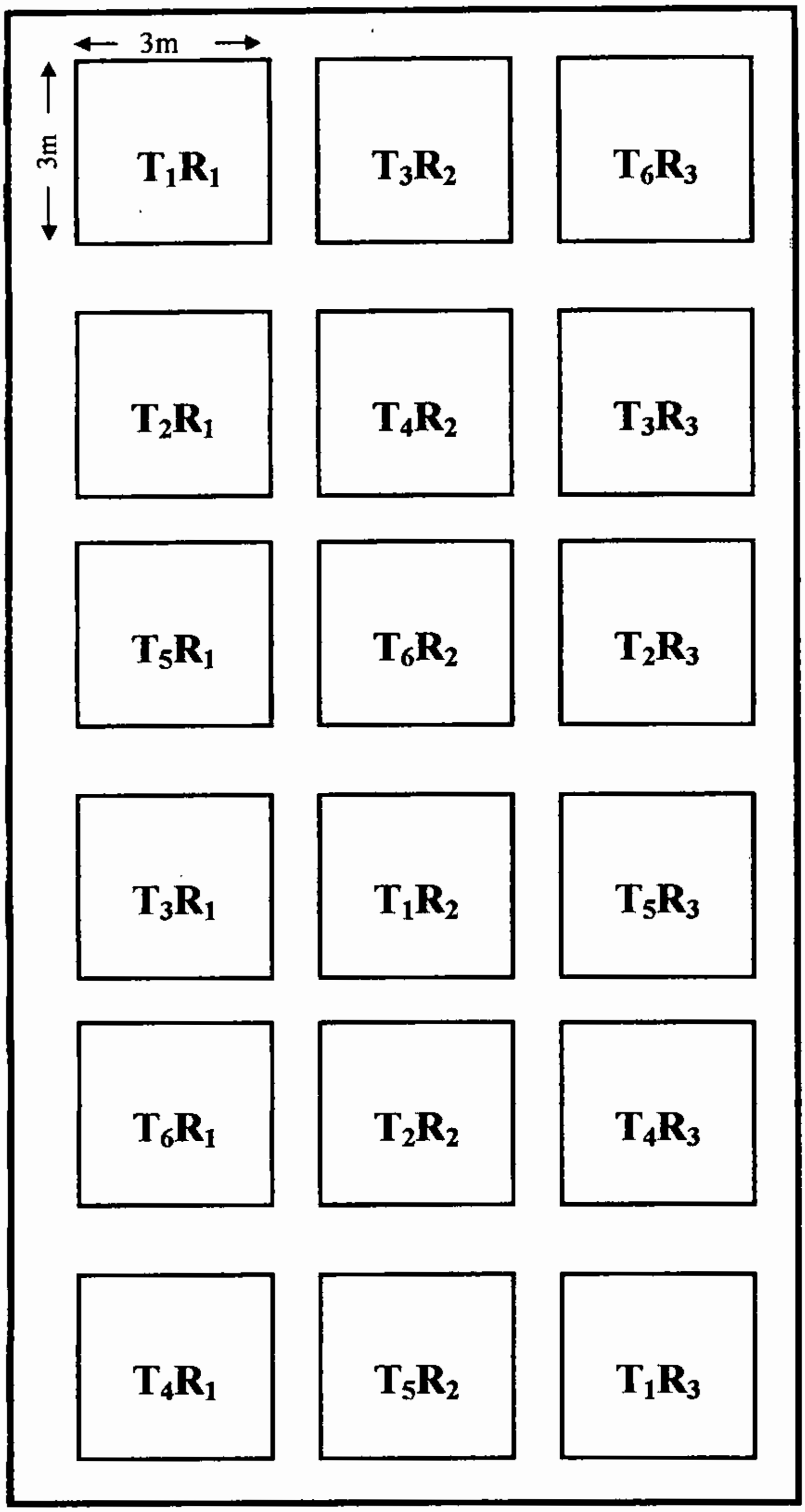
Soil separates	*(%)*	*Method employed*
Sand	36.90	Hydrometer method (Day, 1995)
Silt	26.40	-do-
Clay	36.66	-do-
Texture class	Silty clay loam	-do-

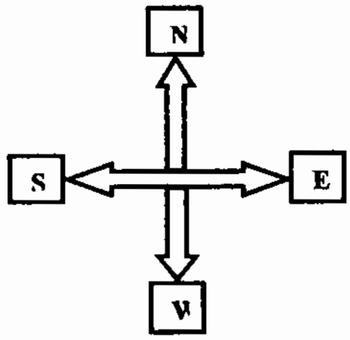
B. Chemical composition of the soil

SL No.	*Soil Characteristics*	*Analytical data*	*Method employed*
1.	Organic carbon (%)	0.82	Walkly and Black, 1947
2.	Total N (kg/ha)	1790.00	Bremner & Mulvaney, 1995
3.	Total S (ppm)	225.00	Bardsley and Lancster, 1965
4.	Total P (ppm)	840.00	Olsen and Sommers, 1982
5.	Available N (kg/ha)	54.00	Bremner, 1965
6.	Available P (kg/ha)	69.00	Olsen and Dean, 1965
7.	Exchangeable K (kg/ha)	89.50	Pratt, 1965
8.	Available S (ppm)	16.00	Hunter, 1984
9.	P^H (1:2.5 soil to water)	5.55	Jeckson, 1958
10.	CEC	11.23	Chapman, 1965

Source: Soil Resources Development Institute (SRDI), Khamarbari, Dha

Appendix III. Layout of the experimental field





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

T_6 = 45.1-60% seed infection

Plot Size: 3m x 3m

Plot to Plot distance: 1m

Block to Block Distance: 1m

Appendix IV. Analysis of variance of the data on germination and incidence of % *Bipolaris sorokiniana* (Blotter method) and germination and seedling infection of wheat (Rolled Paper Towel Method)

Sources of Variation	Degrees of freedom	Mean square			
		Blotter method		Rolled Paper Towel Method	
		% Germination	% *Bipolaris sorokiniana*	% Germination	% Seedling Infection
Treatment	5	336.800**	741.080**	238.756**	525.187**
Error	12	3.5	1.562	1.556	0.928

** Significant at 1% level of significance

Appendix V. Analysis of variance of the data on Vigor Index of 7 days old seedlings of wheat (Rolled Paper Towel Method)

SV	df	Mean square			
		Shoot Length (cm)	Root length (cm)	Seedling weight (g)	Vigor index
Treatment	5	3.891**	19.650**	0.493**	547155.054**
Error	12	0.050	0.112	0.013	1390.476

** Significant at 1% level of significance

Appendix VI. Analysis of variance of the data on germination and seedling health of wheat (Water agar test tube seedling symptom test)

SV	df	Mean square			
		% Germination	% Normal seedling	% Abnormal seedling	% Dead seed
Treatment	5	334.446**	1337.053**	549.7**	334.197**
Error	12	1.195	1.266	0.913	1.195

** Significant at 1% level of significance

Appendix VII. Analysis of variance of the data on disease incidence at 21 DAS and leaf blight severity at flag leaf stage of wheat

SV	df	Mean square				
		Disease incidence at 21 DAS		Leaf blight severity at flag leaf stage		
		No. of seedlings/m^2	No. of infected seedlings/m^2	Flag leaf	Penultimate leaf	Average
Treatment	5	468.22**	131.42**	0.002	0.019**	0.009**
Error	10	0.889	0.056	0.001	0.002	0.001

** Significant at 1% level of significance
DAS = Days after sowing

Appendix VIII. Analysis of variance of the data on leaf blight severity at panicle initiation stage and flowering stage of wheat

SV	df	Mean square					
		Panicle initiation stage			Flowering stage		
		Flag leaf	Penultimate leaf	Average	Flag leaf	Penultimate leaf	Average
Treatment	5	0.087**	0.131**	0.706**	0.258**	0.237**	0.273**
Error	10	0.001	0.006	0.002	0.002	0.014	0.004

** Significant at 1% level of significance

Appendix IX. Analysis of variance of the data on leaf blight severity at milking stage and hard dough stage of wheat

SV	df	Mean square					
		Milking stage			Hard dough stage		
		Flag leaf	Penultimate leaf	Average	Flag leaf	Penultimate leaf	Average
Treatment	5	0.37**	0.51**	0.468**	0.623**	0.45**	0.518**
Error	10	0.002	0.05	0.009	0.004	0.001	0.004

** Significant at 1% level of significance

Appendix X. Analysis of variance of the data on plant growth of wheat

SV	df	Mean square		
		Plant height (cm)	Spike length (cm)	Distance between the point of flag leaf initiation and base of ear (cm)
Treatment	5	7.944**	0.504**	0.233
Error	10	0.222	0.053	0.205

** Significant at 1% level of significance

Appendix XI. Analysis of variance of the data on spikelet formation of wheat

SV	df	Mean square		
		Number of spikelets/ear	Number of healthy spikelets/ear	Number of diseased spikelets/ear
Treatment	5	39.836**	91.936**	10.391**
Error	10	0.231	0.513	0.002

** Significant at 1% level of significance

Appendix XII. Analysis of variance of the data on grain formation and grain weight of wheat

SV	df	Mean square					
		Number of grains/ear	Number of healthy grains/ear	Number of diseased grains/ear	Weight of grains/ear	Weight of healthy grains/ear	Weight of diseased grains/ear
Treatment	5	19.415**	36.732**	2.628**	0.065**	0.119**	0.003
Error	10	0.218	0.053	0.056	0.013	0.019	0.001

** Significant at 1% level of significance

Appendix XIII. Analysis of variance of the data on number of grains/ear of different severity grades (0-5) of harvested seeds of wheat

SV	df	Mean square					
		Grading of seeds					
		0	1	2	3	4	5
Treatment	5	36.88**	0.136	0.058**	0.027**	0.11**	0.316**
Error	10	0.053	0.050	0.008	0.001	0.001	0.001

** Significant at 1% level of significance

Appendix XIV. Analysis of variance of the data on 1000 seeds weight and yield of wheat

SV	df	1000 seeds weight (g)	Straw yield (kg/plot)	Straw yield (t/ha)	Grain yield (kg/plot)	Grain yield (t/ha)
Treatment	5	21.371**	1.068**	1.135**	0.617**	0.762**
Error	10	0.056	0.005	0.006	0.002	0.003

** Significant at 1% level of significance

Appendix XV. Analysis of variance of the data on germination and incidence of *Bipolaris sorokiniana* of harvested seeds of wheat (Blotter method)

SV	df	Mean square	
		% Germination	% *Bipolaris sorokiniana*
Treatment	5	326.4**	434.232**
Error	12	1.000	2.046

** Significant at 1% level of significance