EFFECT OF BLACK POINTED SEEDS OF DIFFERENT SEVERITY GRADES (0-5) ON LEAF BLIGHT (*Bipolaris sorokiniana*) DEVELOPMENT AND YIELD OF WHEAT

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CERTIFICATE

This is to certify that the thesis entitled, "***EFFECT OF BLACK POINTED SEEDS OF DIFFERENT SEVERITY GRADES (0-5) ON LEAF BLIGHT (Bipolaris sorokiniana) DEVELOPMENT AND YIELD OF WHEAT***". submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of ***MASTER OF SCIENCE IN PLANT PATHOLOGY,*** embodies the result of a piece of bonafide research work carried out by ***Dilruba Yesmin, Registration No. 03-01167,*** under my supervision and guidance. No part of this thesis has been submitted for any other degree or diploma.

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

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ABSTRACT

"Effect of black pointed seeds of different severity grades (0-5) on germination, seedling vigour, leaf blight development and yield contributing characters of wheat" were studied in the laboratory and field condition during the period of 2007-2008. The experiment was conducted in the M.S. laboratory, Department of Plant Pathology and in the farm of Sher-e-Bangla Agricultural University, Dhaka. Seed germination and seedling emergence were significantly decreased with the increase of different severity grades of black pointed seed. The sample having grade-5 seeds (T_6) resulted maximum reduction in germination by 31.67% and 47.50% in the blotter and rolled paper towel method, respectively. Significantly highest (2219.00) and lowest (700.10) seedling Vigour Index (VI) was found in best seed (grade-0) and (grade-5), respectively. Significant variation in leaf blight severity under different severity grades of black pointed seed at all growth stages. The highest and lowest leaf blight severity was recorded in T6 (Grains are shriveled, almost completely discolored or more than ½ of grains were discolored) and T1 (Free from infection) at flag leaf, panicle initiation, flowering, milking and hard dough stages. It has been found that plant growth characters (Plant height, ear length), spikelet formation and grain formation was decreased with the increase of severity grades of black pointed seed. The highest 1000 seed weight, straw yield and grain yield was recorded under T1 (Free from infection). The T1 treatment was increased 29.69 % grain yield over control. Considering the findings of the present studies, it has been found that seed sample having grade-0 seeds (Free from infection) showed good result of germination, seedling vigour, leaf blight severity, grain formation and yield and yield contributing characters.

Chapter 1
Introduction

REVIEW OF LITERATURE

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 discoloured seeds were germinated poorly and seedlings usually developed blights.

Machacek and Greaney (1938) reported from their greenhouse and field tests that 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.

Anderson (1952) reported that the growth of *Helminthosporium sativum* mycelium and germination of conidia occurred at temperature from 4° to 36°C with the most rapid growth of the mycelium and elongation of the germ tubes occurring at 28°C. The maximum sporulation occurred at 24-28°C. No conidia were produced blow 16°C or above 28°C on agar or infected wheat heads. H also added that the temperature from 25-30°C were the most favourable for the infection and head blight development.

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

Neergaard (1962) used the term "tolerance" to denote permissible maximum degree of infection. Tolerance may be expressed in terms of: i) Percent of

infected seeds. ii) The load of pathogen eg. Sclerotia per weight unit of seed. iii) The number of pathogen units eg. Sclerotia per weight unit of seed of spores per individual seed as obtained in suspension from washing of the seed. iv) The degree of severity as developed according to a standard procedure and recorded according to a standard scale. He also reported that the capacity of a pathogen to attack seedlings and to spread efficiently in the field or to attack the crop at a late stage of its development, or to survive in the soil and infect subsequent crops were well important factors to be considered in estimating the economic significance of a seed borne pathogen.

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.

Dharma vir *et al.* (1968) stated that the average incidence of disease as based on preliminary surveys was from 5-10 percent although considerable variation had been noticed in different samples collected from various sources. Investigations were also conducted to study the effect of this disease in seed germination. The data showed that presence of black point on the seed does not in any way impair seed germination.

Kachalova and kuzmichev (1969) observed that the incidence of black embryo in the Moscow regions of U.S.S.R was low on winter wheat (1.6%) but higher on spring wheat (10.5% and more). *Alternaria tenuis (A. alternata)* attacked winter wheat more frequently, while *Helminthosporium sativum (Cochliobolus sativus)* caused more damage to spring wheat. They also observed that besides causing visible symptoms, both the pathogens reduced germination.

Adlakha and joshi (1974) reported that the whole grains become discoloured and shriveled if the infections of black points become severe.

Prabhu and Singh (1974) reported that leaf spot caused by *Helminthosporium sativum* was serious concern prevailing in almost all the wheat growing regions of the country. This disease resulted marked yield loss of wheat. It has been stated that the seed borne infection of *Helminthosporium* was responsible for blight disease of wheat, barley, oat, rice and few other crops (Vir, 1974).

Verma *et al.* (1976) stated the effects of *Cochliobolus sativus* on components of grain yield in naturally infected wheat cultivar. The number of tillers/plant, number and weight of grains/head and thousand kernel weight in each category were determined. Increasing values of all five components were consistently associated with decreasing diseases severally.

Alam (1980) reported 18 species of fungi, representing 11 genera, from the freshly harvested seeds of wheat grown in Bangladesh. Out of them *Drechslera sorokiniana* was found pathogenic, causing leaf blight of wheat.

Raemaekers and Tinline (1981) described symptoms on different above ground parts of wheat infected by *Cochliobolus sativus*. On the leaf about tillering stage the initial spots on the leaves were manifested as tiny initial interveinal chlorotic areas. As the spots extended and some coalesced, a diversity of lesion type was encountered, ranging from small chocolat brown spots with or without a gray, tan or black centre and with or without a yellowish halo to large, pale to medium brown blotches, some of which were zonate and haloed. On the glumes, awns and exposed parts of lemmas, early infections appeared as small dark brown spots with indefinite water soaked borders. As the spots expanded, mostly linearly, the centre of some became light brown. Severely infected heads were mottled green brown, sometimes with black patches. Spikelets were infected individually. Apparently depending on time of infection after flowering, a caryopsis could be totally shriveled on it could develop into normal kernel with or without black point. The seed of most cultivar's was severally shriveled. Stems were also infected, lower nodes exhibited brown or black lesions, which extended downwards, often for many

centimeters, even under the leaf sheaths. With advancing plant age spot blotch progressed fairly uniformly on the leaves.

Sinha and Thapliyal (1984) stated that Triticale seed, infected with black point when plated on PDA, yielded *Helminthosporium sativum* (57%) and *Alternaria tenuis* (35%), the remaining 8% showed some unidentified fungi. It was concluded that *H. sativum* and *A. tenuis* are the two main organisms associated with black pointed grains.

Chaudhary *et al.* (1984) studied the effect of black point disease on germination of the grains of WL 711. The germination of the diseased seeds both in blotter method and in pots were reduced to 11.6% and 16.0% 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.

Metha and Igarashi (1985) reported that the spot blotch of wheat caused by *Cochliobolus sativus* (*Bipolaris sorokiniana*, syn *Helminthosporum sativum*) as one of the most important disease in a number of countries such as Brazil, Paraguay, Bolivia, India, Bangladesh and Thailand.

Saari (1985) found that Helminthosporium leaf blight caused by *Helminthosporum sativum* also known as *Bipolaris sorokiniana* and *Drchslera sorokiniana* with the perfect stage *Cochliobolus sativus* was a serious and sometime limiting factor to wheat and barley cultivation in tropical environment Severe leaf blight, spike and the seed infections were common. The amount of spike or kernel infection by *Helminthosporum 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%.

Frank (1985) reported that winter survival of wheat and barley was reduced by root rot pathogens, particularly *Bipolaris sorokiniana* (*Cochliobolus sativus*) seed borne and soil borne inoculums along with soil fumigation and seed treatments were used to provide different levels of disease. Differences in winter survival were measured as the differences between autumn and spring stand counts. Disease was assessed as the proportion of diseased tissue on washed roots and sub crown internodes of surviving plants in the spring. Overall stand reductions, (11-29% in wheat and 27-62% in barley) were attributed to *Cochliobolus sativus*.

Mehta (1985) reported that under favorable conditions for spot blotch of wheat caused by *Bipolaris sorokiniana*, the losses in yield could be between 30 and 80%, in some cases the losses could reach 100%.

Nalli (1986) showed that plants grown from *Cochliobolus sativus* inoculated seeds produced tiller of lower height and reduced seed production. There was a marked reduction of average weight per seed after ear infection.

Saari and Prescott (1986) reported that at least three different fungi possibly causing black point of wheat seed. *Alternaria tenuis is* not harmful at all and does not affect the seed germination, only darkly discolors the embryo end of the seed. *Drechslera sorokiniana* cause some reduction in seed germination and also causes discoloration of embryo end of seed, while *Fusariun* spp. Causes whitish to pinkish discoloration of such grains.

Bazlur Rashid *et al.* (1987) determined the effect of *Drechslera* leaf blight (*Drechslera sorokiniana* (Sacs.) Subram. and Jain.) on yield components of three susceptible wheat cultivars. Inia 66, Kalyansona and MS1. Disease severities of top three leaves were estimated. The lower

leaves became significantly more infected than the flag leaves. A significant relationship between the blight severity and the yield loss were found. Infection on flag leaves caused the most yield losses and infection on third leaves caused the least. Yield loss was determined as the most on cultivar Kalyansona, less on cultivar MS1 and the least on cultivar Inia 66 respectively, 81.68%, 72.68% and 55.81% losses in grain weight per ear. Maximum loss of grain weight per ear at the maximum disease severity score was established.

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% and that of diseased grains 34.5-71%.

Shabeer and Bockus (1988) recorded the highest yield losses for inoculations at the boot and flowering stages, indicating that plants were most susceptible physiologically to losses at those stages. Losses were the result of a significant reduction in grain weight and number of grains.

Fakir (1988) stated that black point disease surveyed in Balaka, kanchan and sonalika varieties of wheat seeds in breeders, foundation and certified tiers collected in 1985-86 growing season from nine different wheat growing areas in Bangladesh varied prevalence from 4.08% to 13.80% with respect to seed tier, breeders seeds followed by foundation and certified. Five different black point fungi detected in black point affected seeds in the country were *Alternaria tenuis, Cladosporium cladosporioides, Curvularia lunata, Drechslera sorokiniana,* and *Fusarium* spp. of these; *D. sorokiniana* followed by *A. tenuis* appeared to be the most prevalent fungi. Among the black point fungi, *D. sorokiniana* and *Fusarium sp.* were found pathogenic, capable of causing germination failure/seed rot and disease to the emerged seedlings. No significant effect of sowing up to 12% black point affected seeds of Kanchan

on seed yield and development of black point in the field was observed. Seeds with 0% and 6% levels of black point infection had no effect on germination, but 12% significantly reduced the germination.

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

Duckzek *et al.* (1989) found that under field plot conditions the degree of infection by *cochliobolus sativus* was related to a reduction in the number of tillers. Symptoms were most pronounced on plants with the fewest; tillering evident early and lasted throughout the growing season. Disease tended to progress more rapidly on plants with fewest tillers. There was no compensation in either 1000-grain weight\yield\tiller in plants with reduced number of tillers.

Tomer and Maguire (1999) reported that the seed vigour which has significant influence on crop high vigoured healthy seeds germinate more synchronously than low vigoured unhealthy seeds.

Zhang *et al.* (1990) reported that 1000 grain-wt of wheat grains affected by black point disease in Shaanxi Province, China which was 1.95-13.5% lower than for uninfected grains. The level of infection depended on environmental factors, cultivar and location. Germination percentage and rate of seedling emergence were also reduced in infected seeds. The predominant pathogen was identified as *Bipolaris sorokiniana* [*Cochliobolus sativus*] (in 21-54.4% of grains) and its pathogenicity was confirmed. *Alternatia tenuis* [*A. alternata*] was present in 8.8-35.7% of grains but was not an important pathogen of black point disease unless associated with other fungi.

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Razzaque and Hossain (1991) estimated yield loss in wheat cultivar Kanchan as 14% due to leaf blight disease caused by *Bipolaris sorokiniana* in Bangladesh.

Alam and Saha (1991) reported that leaf rust (*Puccinia recondita*) was considered to be the most serious disease of wheat in Bangladesh. But now Helminthosporium leaf blight (HLB) caused by *Helminthosporium sativum* (Bipolaris leaf blight caused by *Bipolaris sorokiniana*) has become the number one problem of wheat in Bangladesh and the severity of the disease has increased to an alarming proportion. They found all the recommended wheat cultivars susceptible to HLB. They also reported that less than 50% of the fields had HLB at seedling stage with less than 5% leaf area damaged. At booting stage 100% of the fields had HLB with the damage of 50% leaf area of lower foliage and 10-15% leaf area of upper foliage. At grain filling stage 100% fields found severely infected with HLB. They indicated that damaging levels of HLB mainly occurred late in the season when the wheat crop was approaching maturity.

Talukder and fakir (1993) reported that the development of black point of wheat and the occurrence of black point fungi, *Drechslera sorokiniana* being predominant indicating that the fungi might not be the absolute cause of black point disease during grain development were found to be influenced by rain. Highly significant effect of seed borne infections by *Bipolaris sorokiniana* on the germination of seeds of wheat cvs. Kanchan and Sonalika were recorded by rolled paper towel germination test as well as in pot experiment. At the maximum seed-borne infection level (90%) both the cultivars yielded the maximum germination of 30.25% and 26.50% respectively.

Agarwal *et al.* (1993) reported that grains of wheat demonstrating 3 categories of symptoms of disease were examined to establish the causal fungal pathogen. Grains with black brown to black discoloration, generally restricted to the area

around the embryo (typical black-point symptoms), and showed 100% infection by *A. alternate*. Grains with light brown to dark brown discrete lesions and a dull white spherical or elliptical area in the centre (typical 'eye-spot' symptoms) demonstrated infection by *Drechslera sorokiniana* [*Cochliobolus sativus*] alone. Grains with a creamy white or pinkish colour, mostly shriveled and lighter in weight were infected by *Fusarium graminearum* [*Gibberella zeae*].

Alam *et al.* (1994) reported that Bipolaris leaf blight (*Bipolaris sorokiniana*) or black spot is currently the number one diseases of wheat in Bangladesh. The disease starts at the seedling stage and increases with age. Almost all commercial cultivars are either moderately susceptible or susceptible or susceptible to this disease. During 1991-92 they estimated 29% yield loss in kanchan.

Alam *et al.* (1995) estimated the yield loss of wheat cultivar Kanchan due to Bipolaris leaf blight at the farmer's fields of four different locations. The average yield losses of wheat due to Bipolaris leaf blight disease at Dinajpur, Jessore, Jamalpur and Ishurdi were 13.9, 16.2, 14.8 and 14.5 percent, respectively. The average losses of grains were 14.9 percent over the locations.

Nagarajan and kumar (1997) studied the foliar blights of wheat caused by *Bipolaris sorokiniana* and found that the pathogen is capable of causing damage from the primary leaf stage, though the plant tends to become more susceptible after flowering.

Bazlur Rahsid (1997) reported that highly significant effect of seedborne infection by *Bipolaris sorokiniana* on the germination of seeds of wheat cvs. Kanchan and sonalika was recorded by rolled paper towel germination test as well as in pot experiment. At the maximum seedborne 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 in seedborne infention in both the cultivars. The maximum germination reductions were found as 71.50% and 68.00% in cv.Sonalika and cv. Kanchan respectively.

Rahman and Islam (1998) studied that 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 vigour in respect of germination and shoot and root growth into five different grades (Grade-0.1,2,3 and 4) 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 3 and grade 4 infected seeds. Germination percentage decreased sharply with the increased severity of infection increased.

Hossain *et al.* (1998) reported that leaf blight disease caused by *Bipolares sorokiniana* reduced yield up to 40% and 88% over the control (untreated) under natural field condition and artificial inoculation respectively.

Hossain (2000) reported that in blotter method seed germination was sharply decreased as the severity of black pointed infection increased. Maximum (95.0%) & (0.00%) germination was found in seeds of grade-0 (free from infection, the best seeds, apparently healthy) & grade-5 (grains are shriveled, almost completely discolored or more than ½ of grains discolored respectfully). Seeds of grade-5 & grade-0 resulted highest (92.50%) & lowest (40.50%) incidence of *Bipolaris sorokiniana*, respectively.

Reza (2001) conducted an experiment to evaluate the effect of different levels of seeds and plants infection by *Bipolaris sorokiniana* on wheat. He found that the maximum of 15.73% seed rot / seedling mortality followed by subsequent leaf blight severity of 75.4% was recorded as a result of sowing of 30%

infected seeds. While the minimum of 5% infected seeds resulted respectively 3.1% and 57.53% of the disease.

Malaker (2003) reported that when black pointed affected seeds were sown, than reduction in emergence, seedling vigour, plant growth & grain yield and increased in post-emergence mortality, severity of Bipolaris leaf blight and incidence of black point in harvested grains was increased. Such decreasing or increasing trends of the respective parameters were related with the decrease or increase in severity of black point infection.

MATERIALS AND METHODS

3.1) LABORATORY EXPERIMENT:-

The experiment was conducted in the Seed Pathology Laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka. for the study of "Effect of black pointed seeds of different severity grades (0-5) on leaf blight (*Bipolaris sorokiniana*) development and yield of wheat".

3.1.1) Collection of seeds:-

Wheat seeds of kanchon variety were collected from a farmer named Md. Asraf Ali of village- Bosuya, Thana-Boyoalia, District-Rajshahi. After collection the seeds were kept in a plastic container with air tight lid and the container was stored in normal room temperature in seed pathology laboratory, Sher-e-Bangla Agricultural University.

3.1.2) Treatments:-

The collected seed sample was divided into different grade according to Gilchrist (1985). Each graded seeds considered as treatment and this has been done physical sorting of seeds. Six treatments were used in this experiment. These treatments were as follows:

T_1=Free from infection.
T_2=Only embryo blackish.
T_3=Embryo and its adjacent are slightly infected.
T_4=Embryo and less than ¼ of grains are discolored.
T_5= Embryo and ½ of grains are infected.
T_6= Embryo are shriveled almost completely discolored or more than 1/2 of grains are discolored.

3.1.3) Seed health study:-

For studying the seed health different methods were used. These are as follows.-

(A) Blotter method:-

Incidence of seed borne *Bipolaris sorokiniana* was recorded by using the Blotter method. In this method 3 layers of blotter were soaked in sterilized water and placed at the bottom of the glass Petridish. Then 25 seeds were plated on the blotting paper in a petridish maintaining equal distance and covered with lid. 400 seeds were tested for each treatment maintaining three replications. The petridish were incubated at 25±1°C under 12/12 hrs light and darkness cycle for 7 days. After 7 days of incubation the seeds were observed for the presence of seed-borne pathogen of *Bipolaris sorokiniana* under stereo binocular microscope following the key of Mathur and Kongsdal (2003). Germination of the seeds was also recorded.

(B) Paper towel method (Seedling infection & seedling vigour Test):-

Seedling infection, germination and seedling vigour test were done in rolled paper towel method. (Warcham,1990). For this method, 150 seeds were randomly taken from each grade of black pointed seed and 50 seeds were placed between a pair of moist paper towels. The towels were rolled and the ends were closed by threads as the moist couldn't removed easily. Then the rolled papers containing seeds were placed in up right position. After finishing the work, it covered by polyethylene paper for 10 days at room temperature under normal 12/12 light. After incubation period observations pertaining to (a) number of normal seedlings, (b) number of abnormal seedlings, (c) number of dead seeds were recorded. The number of normal and abnormal seedlings were recorded to ISTA rules (1995).

Normal seedlings were categorized by following points:

(i) Intact seedling with all essential structures well developed, complete in proportion and healthy.
(ii) Seedlings with slight defects of their essential structures provided they show 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 for 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 root missing/stunted or broken and decayed due to primary infection.
(ii) Coleoptile missing/ split and deformed or bent over.
(iii) Shoot system (the mesocolyl if developed) broken/ decayed.
(iv) Leaf missing/ extending less than half-way up the coleoptile, shredded or deform.
(v) Seedlings as a whole deformed spindly, discoloured or decayed as a result of primary infection.
(vi) Blackened dead or decayed seed.

The number of seeds that produced normal seedlings were counted and the percentage calculate over the number of seeds placed for the test.
For determination of seedlings vigour 30seedlings (normal /abnormal) were randomly selected from each paper and their individual shoot and root length was measured. Length of shoot was measured from the base of the stem up to the growing point of the youngest leaf. Similarly, length of root was measured from the starting point of the root to the largest available lateral root apex. The

shoot and root portions were blotted dry with fine tissue paper and fresh weight was taken before the materials could get desiccated. Vigour of the seedling was determined by the following formula (Baki and Anderson, 1972):

Vigour Index = (mean of root length+ mean of shoot length)×percentage of seed germination.

Data were collected on the following parameters:

Germination

No. of healthy seedling.

No. of infected seedling.

Shoot length.

Root length.

Total weight.

Vigor index.

3.2) FIELD EXPERIMENT:-

3.2.1) Experimental site and experimental period:

The experiment was conducted at Sher-e-Bangla Agricultural University Farm, Dhaka-1207 during the period December 2007 to April 2008.

3.2.2) Soil:

The soil of the experimental field belongs to the Tejgaon series under the Agro Ecological Zone, Madhupur Tract (AEZ-28) and the general soil type is Shallow, Red Brown, and Terrace Soils.

3.2.3) Climate of experimental site:

The experimental area belongs to the subtropical climate was characterized by the high rainfall, high humidity, high temperature, relatively long day during April to September and scanty rainfall, low humidity, low temperature and

short day period during October to March. The later period is favorable for wheat cultivation.

3.2.4) Soil properties:

The description of the Agro-ecological Zone (UNDP and FAO, 1988) and soil properties of the experimental soil properties of the experimental site was as follows:-

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

3.2.5) Fertility status of the soil:

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

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

3.2.6) Design of the experiment:

The experiment was laid out in Randomized Complete Block Design (RCBD) comprising three replications for each treatment. The unit plot size was (3m× 3m). Distance between block and between the plots will be 1m and 1m, respectively. So, 18 plots were used for this research.

3.2.7) Land preparation:

The experimental field was thoroughly ploughed and cleaned prior to seed sowing and application of fertilizers and manure in the field.

3.2.8) Application 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 (BARI, Krishi Projucti Handbook, 2000). 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.

3.2.9) Sowing of seeds:

Wheat seeds were sown in the field on 6th December 2006 at the rate of 120 kg/ha. The seeds were sown in lines at a depth of 5 cm and covered by soil with the help of hand. The distances between lines were 25 cm in every plot.

3.2.10) Intercultural operation:

Irrigation was done once after 25 days and another after 45 days of sowing. Irrigation was generally followed the each weeding of the crops. Weeding was performed twice during the growing period of the crop for better soil aeration and conservation of soil moisture.

3.2.11) Tagging and data collection:

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

3.2.12) Evaluation of leaf blight severity:

Leaf blight severity of flag leaf and penultimate leaf was recorded in three growth stages of plant viz. flowering stage, milking stage and hard dough stage. The severity of leaf blight was recorded following 0-5 scale (Plate-1) of Hossain and Azad (1992). The grades are given below:-

0= No infection (Highly resistant)

1= Few minute lesions on leaves (Resistant)

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

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

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

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

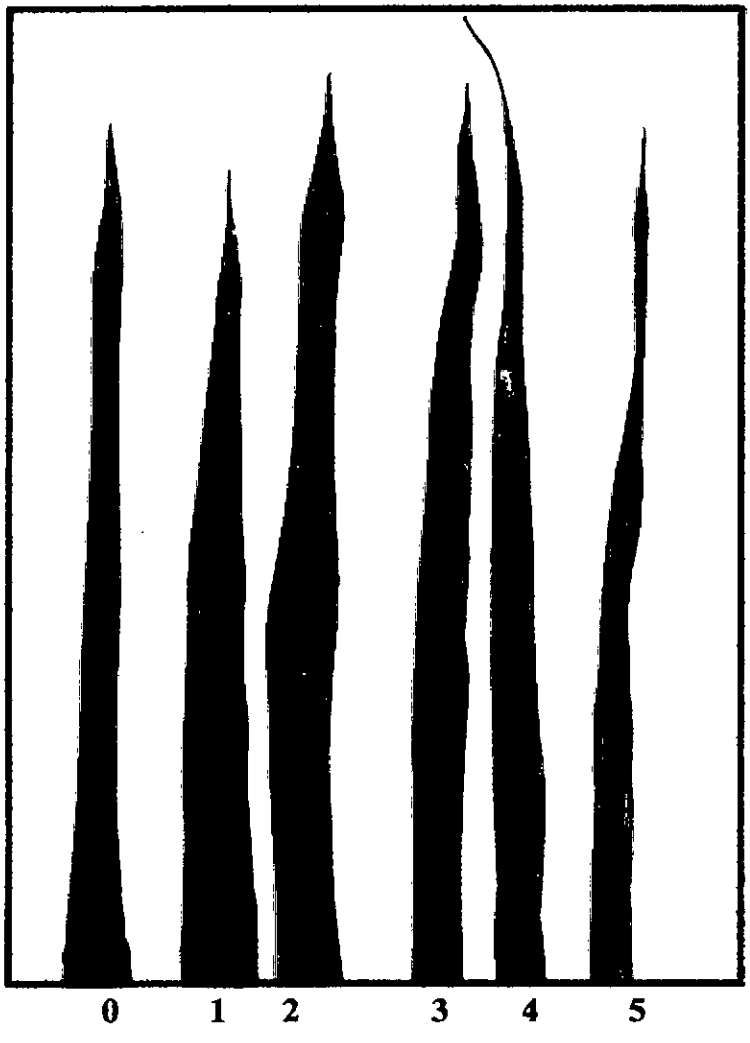


Plate 1: Leaf blight (*Bipolaris sorokiniana)* severity of wheat showing (0-5) rating scale.

3.2.13) Harvesting:

The crop was harvested at full ripening stage on 1st weak of April, **2008.**

3.2.14) Isolation and identification of *Bipolaris sorokiniana* :

To identify the pathogen, the diseased seeds were collected from the field & were taken to the laboratory in a air tight plastic container. The diseased sample (seed) was collected and surface sterilized by dipping in 0.01% Mercuric chloride ($HgCl_2$) solution for 30 seconds. The seeds were then washed in sterilized water at three times and were placed on sterilized blotting paper for absorbing excess moisture. After placing on blotting paper then the seeds were placed into PDA media in sterilized petridish with the help of sterilized forceps & incubated at 25±1°C for 7 days. Then the organism that grew freshly into culture were isolated by means of hyphal tip culture method aseptically and were cultured again in PDA to have pure culture. The PDA plate was incubated at 25±1°C for a week and identified. The colour of the culture was found in blackish. Then a slide was prepared from that culture & observed under compound microscope and the fungus was identified as *Bipolaris sorokiniana* .

3.2.15) Collection of data on yield and yield contributing characters:

Data of plant growth and yield contributing characters was 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) Length 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 (gm)

xi) Weight of healthy grains/ ear (gm)

xii) Weight of diseased grains/ ear (gm)

xiii) 1000 grains weight (gm)

xiv) Grading of seeds /ear (0-5)

xv) Grain yield /plot (Kg/plot)

xvi) Straw yield /plot (Kg/plot)

xvii) Grain yield (t/ha)

xviii) Straw yield (t/ha)

3.2.16) Grading of seeds:

The grading of seeds (Plate 2) were done following the 0-5 rating scale of CIMMYT (Gilchrist, 1985). 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 then 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 were discolored.

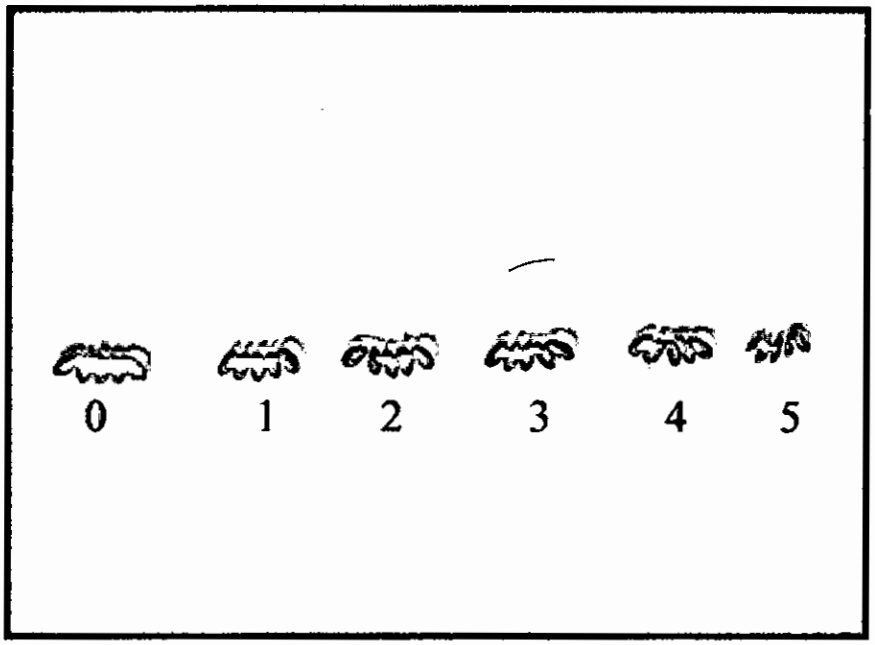


Plate 2: Different grades of wheat seed as affected by *Bipolaris sorokiniana*

3.3) Analysis of data:-

The data on various characters were statically analysed using analysis of variance to find out the variations resulting from experimental treatments. Treatment means were compared by DMRT (Duncan's Multiple Range Test).

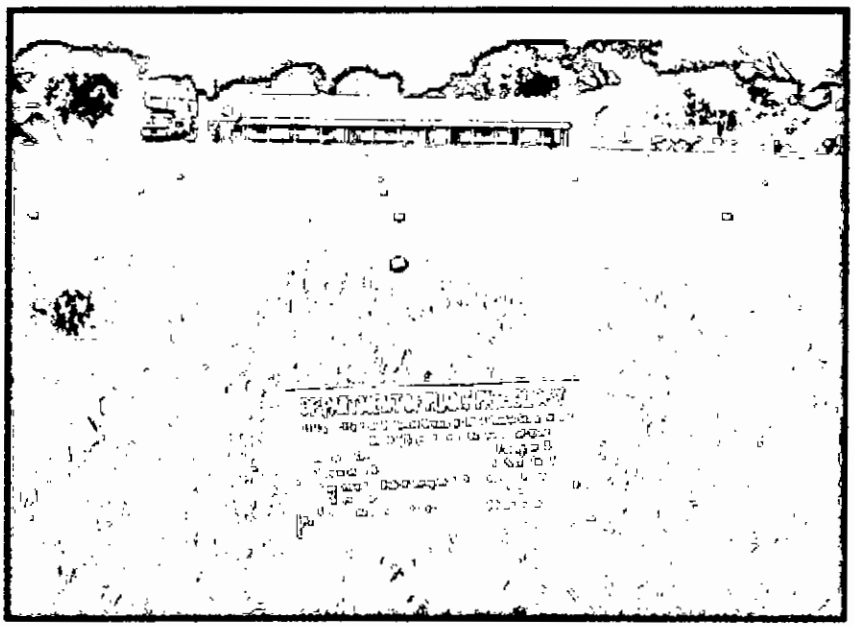


Plate 3: Experimental view of wheat at SAU research field

Plate 4: Leaf blight symptom of wheat caused by *Bipolaris sorokiniana* in the experimental field.

Plate 5: Dark brown conidial growth on the germinating wheat seed

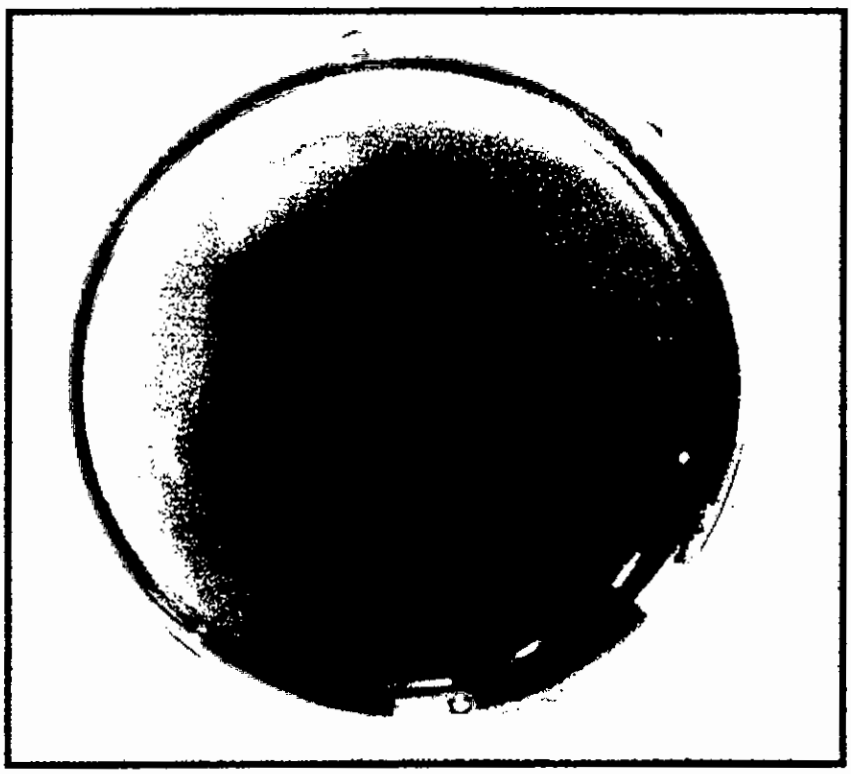


Plate 6: Pure culture of *Bipolaris sorokiniana* on PDA media

A B

Plate 7: Mycelia and Conidia (two poles germinated, A) of *Bipolaris sorokiniana* under compound microscope (X 40).

RESULTS

4.1. LABORATORY EXPERIMENT

4.1.1. Effect of black pointed seeds of different severity grades (0-5 Scale) on germination and incidence of *Bipolaris sorokiniana* on wheat seeds by blotter method in laboratory (Before sowing)

The effect of black pointed seeds of different severity grades (0-5 Scale) on germination and incidence of *Bipolaris sorokiniana* on wheat seeds in blotter method was found significant variations (Table-1). The highest seed germination (92.33%) was recorded in T_1 (Free from infection) treatment that is statistically similar to T_2 (only embryo blackish). The lowest seed germination (63.00%) was found in T_6 (Grains are shriveled, almost completely discolored or more than ½ of grains were discolored). Incidence of seed borne *Bipolaris sorokiniana* on wheat seed for all treatments ranged from 4.33% to 25.33%, where treatment T_6 showed the highest incidence (25.33%) of *Bipolaris sorokiniana* and the lowest incidence (4.33%) of *Bipolaris sorokiniana* was recorded in T_1 treatment that is statistically similar to T_2 (only embryo blackish) treatment. The treatment T_1 decreased 82.91% of *Bipolaris sorokiniana* decreased over control.

Table 1. Effect of black pointed seeds of different severity grades (0-5 Scale) on germination and incidence of *Bipolaris sorokiniana* on wheat seeds by blotter method in laboratory (Before sowing)

Treatments	Germination (%)	*Bipolaris sorokiniana* on seed surface (%)	Decrease (%) of *Bipolaris sorokiniana* over control
T_1	92.33 a	4.33 e	82.91
T_2	89.67 ab	6.67 e	73.67
T_3	86.33 bc	11.00 d	60.52
T_4	84.67 c	14.33 c	43.43
T_5	74.00 d	18.00 b	28.94
T_6	63.00 e	25.33 a	-----
LSD (0.05)	4.30	3.089	-----

T_1= Free from infection

T_2= only embryo blackish

T_3= Embryo and its adjacent area slightly infected

T_4= Embryo and less then ¼ of grains are discolored

T_5= Embryo and ½ of grain are infected

T_6= Grains are shriveled, almost completely discolored or more than ½ of grains were discolored.

4.1.2. Effect of black pointed seeds of different severity grades (0-5 scale) on germination and seedling infection of wheat at 10 days old seedlings (Paper towel method)

Wheat seed having different level of black pointed seed had significant effect on seed germination in Rolled Paper Towel (Table -2). There was a general tendency of decreasing of seed germination with the increase in severity grades of black pointed seeds in the seed sample. The treatments were found to differ significantly from one another. The percent germination of the different treatments ranged from 90.00 % to 47.50%, where maximum (90.00 %) and minimum (47.50 %) germination were found in treatment T_1 (Free from infection) and T_6 (Grains are shriveled, almost completely discolored or more than ½ of grains were discolored), respectively. Incase of normal seedlings, the highest percent of normal seedling 83.30 % and the lowest percent of normal seedling 25.50% were recorded in treatment T_1 and T_6, respectively. The opposing trend was recorded incase of percent of abnormal seedlings and percent dead seed under different treatments. The number of abnormal seedlings and dead seeds increased with the increase of black pointed infection level in the seed sample. Incase of abnormal seedlings, highest (22.00%) and lowest (6.70%) were counted in T_6 and T_1 treatment. Incase of dead seed same case was recorded, the highest (52.50%) and lowest (10.00%) were counted in T_6 and T_1 treatment, respectively.

Table 2. Effect of black pointed seeds of different severity grades (0-5 scale) on germination and seedling infection of wheat at 10 days old seedlings (Paper towel method)

Treatments	%Germination	% Normal seedlings	% Abnormal seedlings	% Dead seed
T_1	90.00 a	83.30 a	6.70 f	10.00 e
T_2	86.67 ab	76.50 b	10.17 e	13.33 d
T_3	84.67 b	72.33 c	12.33 d	15.33 d
T_4	79.50 c	64.00 d	15.50 c	20.50 c
T_5	69.33 d	51.33 e	18.00 b	30.67 b
T_6	47.50 e	25.50 f	22.00 a	52.50 a
LSD (P=0.05)	3.440	3.134	1.086	3.330

T_1= Free from infection

T_2= only embryo blackish

T_3= Embryo and its adjacent area slightly infected

T_4= Embryo and less then ¼ of grains are discolored

T_5= Embryo and ½ of grain are infected

T_6= Grains are shriveled, almost completely discolored or more than ½ of grains were discolored.

Plate 10: Experimental view of different severity grades of wheat seed on Paper Towel Method.

Plate 11: Normal seedling, abnormal seedling and dead seed of wheat on Paper towel method.

4.1.3. Effect of black pointed seeds of different severity grades (0-5 Scale) on shoot length, root length, shoot weight, root weight and Vigor index of wheat at 10 days old seedlings (Paper towel method).

The treatments were found to differ significantly in respect of shoot length (cm), root length (cm), fresh root and shoot weight (gm) and vigour index of 10 days old seedlings (Table-3). The shoot length at 10 days old seedlings for treatment ranged from 10.37 to 5.73 (cm) while the highest value (10.37cm) was recorded in T_1 (Free from infection) that was statistically similar to T_2 & T_3 treatments. The lowest value (5.73 cm) was recorded in T_6 (Grains are shriveled, almost completely discolored or more than ½ of grains were discolored) and it was also statistically similar to T_5 treatment. The root length at 10 days old seedlings for different treatment ranged from 14.28 to 9.01 (cm), while the highest (14.28cm) was recorded in T_1 that was statistically similar to T_2 and the lowest (9.01 cm) was recorded in T_6, respectively. The fresh (root + shoot) weight of 10 days old seedlings for different treatments ranged from 11.27 to 4.17 (gm), while the highest value (11.27 cm) was recorded in T_1 and the lowest value (4.17 gm) was recorded in T_6. The vigour index ranged from 2219.00 to 700.10 where the lowest and highest vigour index was recorded under the treatment in T_6 & T_1, respectively.

Table 3. Effect of black pointed seeds of different severity grades (0-5 Scale) on shoot length, root length, shoot weight, root weight and Vigor index of wheat at 10 days old seedlings (Paper towel method).

Treatments	Shoot length (cm)	Root length (cm)	(Shoot+ Root) weight (gm)	*Vigor index
T_1	10.37 a	14.28 a	11.27 a	2219.00 a
T_2	9.65 a	13.32 a	9.83 ab	1991.00 b
T_3	8.90 ab	11.88 ab	9.33 a-c	1759.00 c
T_4	8.00 bc	12.73 ab	8.67 bc	1648.00 d
T_5	7.27 cd	11.60 ab	7.50 c	1308.00 e
T_6	5.73 d	9.01 b	4.17 d	700.10 f
LSD (P=0.05)	1.583	3.735	2.088	87.45

T_1= Free from infection

T_2= only embryo blackish

T_3= Embryo and its adjacent area slightly infected

T_4= Embryo and less then ¼ of grains are discolored

T_5= Embryo and ½ of grain are infected

T_6= Grains are shriveled, almost completely discolored or more than ½ of grains were discolored.

* Vigour Index = (mean of root length + mean of shoot length)×percentage of seed germination.

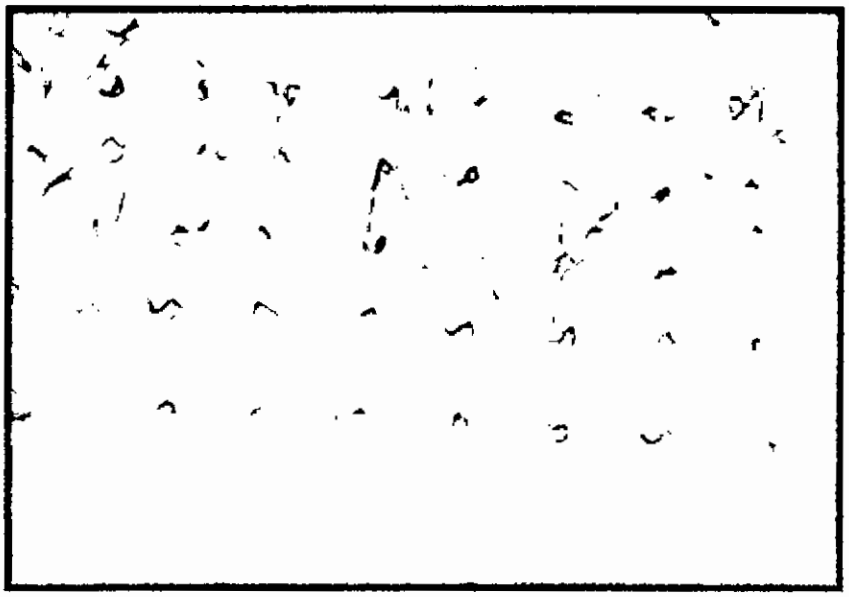


Plate 12: Germination and Vigour Index Test at 10 days old seedlings on Rolled paper Towel Method under treatment T_1 (Free from infection).

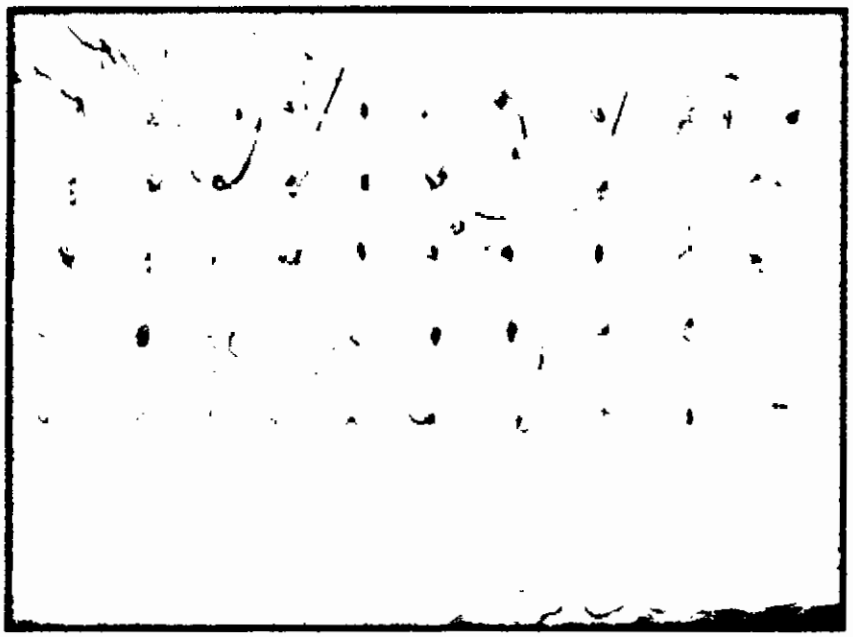


Plate 13: Germination and Vigour Index Test at 10 days old seedlings on Rolled paper Towel Method under treatment T_6 (Grains are shriveled, almost completely discolored or more than ½ of grains were discolored).

4.2. FIELD EXPERIMENT

4.2.1. Effect of black pointed seeds of different severity grades (0-5 Scale) on leaf blight severity of wheat at flag leaf stage and panicle initiation stage

Leaf blight severity at flag leaf stage and panicle initiation stage was recorded on flag leaf and penultimate leaf infection of wheat and was found statistically significant among different treatments (Table-4). In the flag leaf stage, the lowest disease severity on flag leaf and penultimate leaf was recorded in the treatment T_1 (Free from infection) that was statistically similar to T_2 (only embryo blackish) & T_3 (Embryo and its adjacent area slightly infected). On the other hand the highest disease severities 0.14 and 0.17 were recorded in T_6 (Grains are shriveled, almost completely discolored or more than ½ of grains were discolored) on flag leaf and penultimate leaf of wheat, respectively. The average disease severity of flag leaf and penultimate leaf under different treatments at flag leaf stage ranged from 0.00 to 0.16 where the highest and lowest disease severity was recorded under the treatment T_6 and T_1, respectively. In panicle initiation stage, the lowest disease severities on flag leaf (0.23) and penultimate leaf (0.35) were recorded in the treatment T_1 . On the other hand, the highest disease severity on flag leaf (1.33) and penultimate leaf (1.69) were obtained under the treatment T_6. The average disease severity at panicle initiation stage under different treatments ranged from 0.29 to 1.51 where the lowest and highest disease severity was recorded under the treatment T_1 and T_6, respectively.

Table 4. Effect of black pointed seeds of different severity grades (0-5 Scale) on leaf blight severity of wheat at flag leaf stage and panicle initiation stage

Treatments	Disease severity at flag leaf stage			Disease severity at Panicle initiation stage		
	Flag leaf	Penultimate leaf	Average	Flag leaf	Penultimate leaf	Average
T_1	0.00 d	0.00 c	0.00 c	0.23 e	0.35 f	0.29 f
T_2	0.01 cd	0.03 bc	0.02 bc	0.32 d	0.42 e	0.37 e
T_3	0.03 b-d	0.05 bc	0.04 bc	0.85 c	0.92 d	0.89 d
T_4	0.07 b	0.08 b	0.08 b	1.10 b	1.15 c	1.13 c
T_5	0.06 bc	0.07 b	0.07 b	1.37 a	1.47 b	1.42 b
T_6	0.14 a	0.17 a	0.16 a	1.33 a	1.69 a	1.51 a
LSD (P=0.05)	0.054	0.054	0.054	0.054	0.054	0.054

T_1= Free from infection

T_2= only embryo blackish

T_3= Embryo and its adjacent area slightly infected

T_4= Embryo and less then ¼ of grains are discolored

T_5= Embryo and ½ of grain are infected

T_6= Grains are shriveled, almost completely discolored or more than ½ of grains were discolored.

4.2.2. Effect of black pointed seeds of different severity grades (0-5 Scale) on leaf blight severity of wheat at flowering stage and milking stage

Significant variation was observed in disease severity of leaf blight on flag leaf and penultimate leaf at flowering stage and milking stage of wheat plant when different treatments were applied (Table-5). In the flowering stage, the lowest disease severity were recorded in the treatment T_1 (Free from infection) which was 1.27 & 2.11 on flag leaf and penultimate leaf, respectively. On the other hand, then highest disease severities 1.67 and 2.80 were recorded under the treatment T_6 (Grains are shriveled, almost completely discolored or more than ½ of grains were discolored) on flag leaf and penultimate leaf of wheat, respectively. The average disease severity of flag leaf and penultimate leaf under different treatments ranged from 1.69 to 2.24 where the lowest and highest disease severity was recorded under the treatment T_1 and T_6, respectively. In milking stage, the lowest disease severities on flag leaf (1.70) and penultimate leaf (2.73) were recorded in the treatment T_1. On the other hand, the highest disease severities on flag leaf (2.11) and penultimate leaf (4.20) were recorded in the treatment under the treatment T_6. The average disease severity at milking stage under different treatments ranged from 2.33 to 3.16 where the lowest and highest disease severity was recorded under the treatment T_1 and T_6, respectively.

Table 5. Effect of black pointed seeds of different severity grades (0-5 Scale) on leaf blight severity of wheat at flowering stage and milking stage

Treatments	Disease severity at flowering stage			Disease severity at milking stage		
	Flag leaf	Penultimate leaf	Average	Flag leaf	Penultimate leaf	Average
T_1	1.27 d	2.11 f	1.69 e	1.70 d	2.73 e	2.33 e
T_2	1.29 cd	2.27 c	1.78 d	1.84 c	3.33 d	2.59 d
T_3	1.33 c	2.35 d	1.84 c	1.88 c	3.57 cd	2.73 c
T_4	1.30 cd	2.45 c	1.88 c	1.89 c	3.63 c	2.76 c
T_5	1.41 d	2.67 d	2.04 b	1.99 b	3.90 b	2.97 b
T_6	1.67 a	2.80 a	2.24 a	2.11 a	4.20 a	3.16 a
LSD (P=0.05)	0.054	0.054	0.054	0.054	0.261	0.054

T_1= Free from infection

T_2= only embryo blackish

T_3= Embryo and its adjacent area slightly infected

T_4= Embryo and less then ¼ of grains are discolored

T_5= Embryo and ½ of grain are infected

T_6= Grains are shriveled, almost completely discolored or more than ½ of grains were discolored.

4.2.3. Effect of black pointed seeds of different severity grades (0-5 Scale) on leaf blight severity of wheat at hard dough stage

Leaf blight severity at hard dough stage was recorded on flag leaf and penultimate leaf of wheat and was found statistically significant effect among different treatments (Table-6). The lowest disease severities were recorded in the treatment T_1 (Free from infection) which was 2.72 and 4.33 on flag leaf and penultimate leaf, respectively. On the other hand, the highest disease severities 3.33 and 5.00 on flag leaf and penultimate leaf was recorded under the treatment T_6 (Grains are shriveled, almost completely discolored or more than ½ of grains were discolored). The average disease severity at hard dough stage under different treatments ranged from 3.55 to 4.17, where the lowest and highest disease severity were recorded under the treatment T_1 and T_6, respectively.

Table 6. Effect of black pointed seeds of different severity grades (0-5 Scale) on leaf blight severity of wheat at hard dough stage

Treatments	Disease severity at hard dough stage		
	Flag leaf	Penultimate leaf	Average
T_1	2.72 e	4.33 e	3.55 f
T_2	2.80 d	4.60 d	3.70 e
T_3	2.78 d	4.80 c	3.79 d
T_4	2.99 c	4.87 b	3.93 c
T_5	3.20 b	4.95 a	4.08 b
T_6	3.33 a	5.00 a	4.17 a
LSD (P=0.05)	0.054	0.054	0.054

T_1= Free from infection

T_2= only embryo blackish

T_3= Embryo and its adjacent area slightly infected

T_4= Embryo and less then ¼ of grains are discolored

T_5= Embryo and ½ of grain are infected

T_6= Grains are shriveled, almost completely discolored or more than ½ of grains were discolored.

4.2.4. Effect of black pointed seeds of different severity grades (0-5 Scale) on plant growth and spikelets formation of wheat cv. Kanchan

The effect of black pointed seeds of different severity grades on plant height, ear length and distance between the flag leaf initiation and base of the ear was found statistically significant variation (Table 7). Incase of plant height the highest result (88.83 cm) was found in T_1 (Free from infection) and that was statistically similar (86.63 cm) to T_2 (only embryo blackish). The lowest result (74.10 cm) was found in T_6 (Grains are shriveled, almost completely discolored or more than ½ of grains were discolored) that was statistically similar to T_5 (Embryo and ½ of grain are infected) & T_4 (Embryo and less then ¼ of grains are discolored), respectively. Incase of ear length, maximum ear length (15.80 cm) was recorded in $T_1$and minimum ear length (10.00 cm) was recorded in T_6. The maximum distance between the flag leaf initiation and base of the ear (15.83 cm) was observed in T_1 and minimum result was (9.00 cm) observed in T_6, respectively.

Number of spikelets/ear, number of healthy spikelets/ear, number of diseased spikelets/ear varied significantly in respect of different severity grades of black pointed seed (Table-8). The highest number of spikelets per ear (25.50) was observed in T_1 and lowest number of spikelets per ear was (15.97) observed in T_6 that was statistically similar to T_2, T_3, T_4 & T_5, respectively. The highest number of healthy spikelets per ear (22.03) was observed in T_1 & the lowest number of healthy spikelets per ear was (9.30) found in T_6, respectively. The highest number of diseased spikelets per Ear (6.67) was observed in T_6 that was statistically similar to T_4 & T_5, respectively. The lowest number of diseased spikelets per ear was (3.47) found in T_1 treatment, which was statistically similar to T_2 treatment.

Table 7. Effect of black pointed seeds of different severity grades (0-5 Scale) on plant growth and spikelets formation of wheat cv. Kanchan

Treat ments	Plant height (cm)	Ear length (cm)	Distance between the flag leaf initiation and base the ear(cm)	Number of Spikelets/ ear	Number of healthy spikelets/ ear	Number of diseased spikelets/ ear
T_1	88.83 a	15.80 a	15.83a	25.50 a	22.03 a	3.47 d
T_2	86.63 ab	14.03b	13.50 b	17.54 bc	15.54 b	4.33 cd
T_3	82.87 b	13.60 d	13.60 b	19.20 bc	13.86 c	5.34 bc
T_4	78.00 c	12.50 c	12.00 bc	18.47 bc	12.49 d	5.98 ab
T_5	76.50 c	11.50 c	11.50 c	18.00 bc	11.89 d	6.11 ab
T_6	74.10 c	10.40 d	9.00 d	15.97 c	9.30 e	6.67 a
LSD (P=0.05)	3.921	0.574	1.576	4.159	1.351	1.141

T_1= Free from infection

T_2= only embryo blackish

T_3= Embryo and its adjacent area slightly infected

T_4= Embryo and less then ¼ of grains are discolored

T_5= Embryo and ½ of grain are infected

T_6= Grains are shriveled, almost completely discolored or more than ½ of grains were discolored.

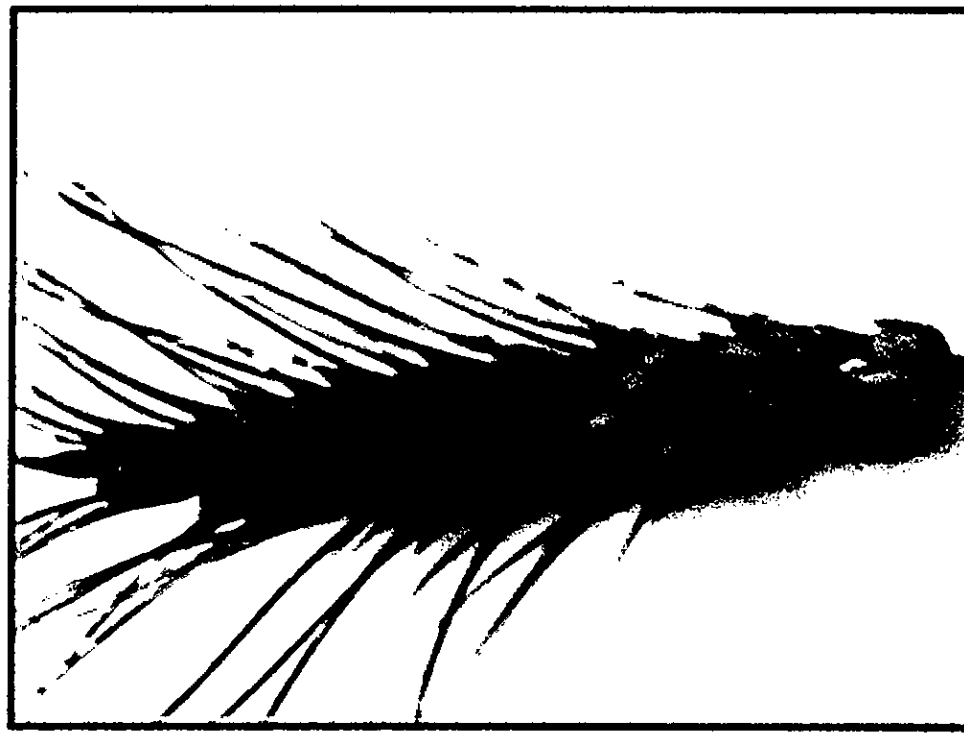


Plate 14: Healthy spikelet of wheat cv. kanchan.

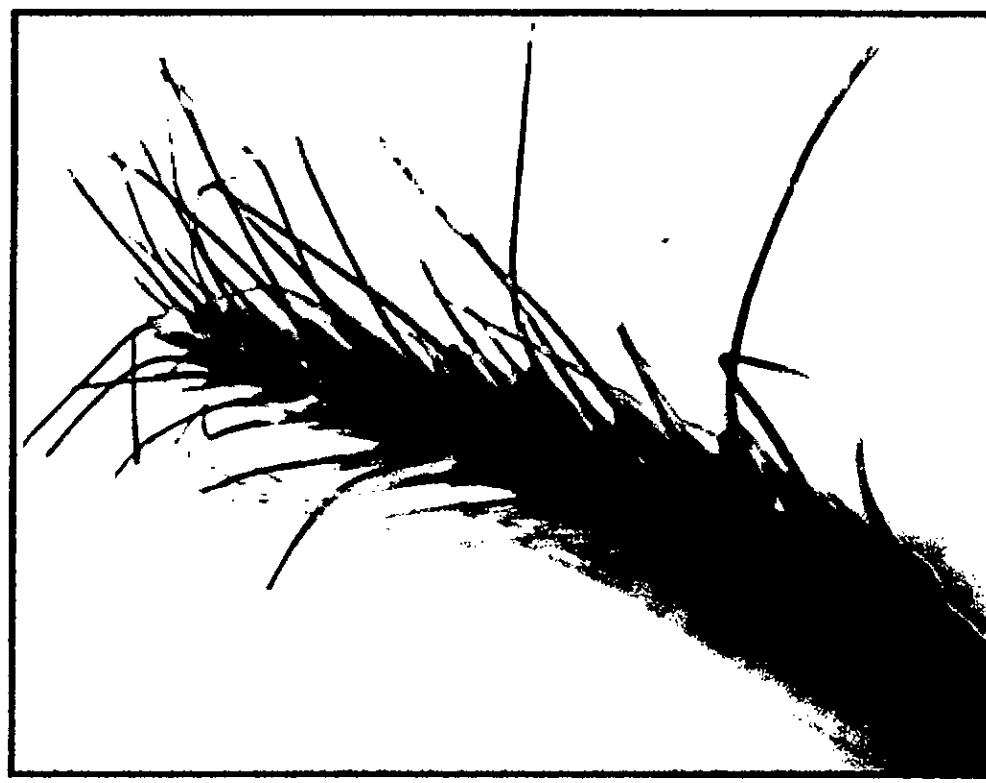


Plate 15: Diseased spikelet of wheat cv. kanchan.

4.2.5. Effect of black pointed seeds of different severity grades (0-5 Scale) on grain formation and grain weight of wheat cv. Kanchan in the field

It has been found that the effect of black pointed seeds of different severity grades on grain formation and grain weight differed significantly (Table 8). The highest number of grains/ear (50.61) was recorded in T_1 (Free from infection) which was closely followed by T_2 (only embryo blackish). The lowest number of grains per ear (35.67) was found in T_6 (Grains are shriveled, almost completely discolored or more than ½ of grains were discolored) treatment. The highest number of healthy grains/ear (47.27) was recorded in T_1 treatment & the lowest number of healthy grains per Ear (25.50) was recorded in T_6 treatment, respectively. The highest number of diseased grains/ear (12.17) was recorded in T_6 & the lowest number of diseased grains per ear (3.33) was found in T_1, respectively.

Incase of grain weight, the highest weight of grains/ear (1.80 gm) was recorded in T_1 which was closely followed by T_2. The lowest weight of grains/ear was recorded (0.66 gm) in T_6 treatment. The highest weight of healthy grains/ear was recorded (1.62 gm) in T_1 treatment. The lowest weight of healthy grains/ear was recorded (0.20 gm) in T_6. The lowest weight of diseased grains/ear was recorded (0.18 gm) in T_1 that was closely followed by T_2 and T_3. The highest weight of diseased grains/ear was recorded (0.46 gm) in T_6 treatment and that was statistically similar to T_4 (Embryo and less then ¼ of grains are discolored).

Table 8. Effect of black pointed seeds of different severity grades (0-5 Scale) on grain formation and grain weight of wheat cv. Kanchan in the field

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	50.61 a	47.27 a	3.33 e	1.80 a	1.62 a	0.18 c
T_2	48.43 a	43.33 b	5.10 d	1.68 a	1.27 b	0.21 c
T_3	45.00 b	38.10 c	6.90 c	1.44 b	1.20 b	0.24 bc
T_4	42.17 bc	34.74 d	7.33 c	1.22 b	0.81 c	0.41 a
T_5	39.90 c	29.97 e	9.93 b	0.80 c	0.50 d	0.30 b
T_6	35.67 d	25.50 f	12.17 a	0.66 c	0.20 e	0.46 a
LSD (P=0.05)	3.102	0.944	1.205	0.238	0.144	0.077

T_1= Free from infection

T_2= only embryo blackish

T_3= Embryo and its adjacent area slightly infected

T_4= Embryo and less then ¼ of grains are discolored

T_5= Embryo and ½ of grain are infected

T_6= Grains are shriveled, almost completely discolored or more than ½ of grains were discolored.

4.2.6. Effect of black pointed seeds of different severity grades (0-5 Scale) on number of grains/ear of different grades of wheat cv. Kanchan.

Seeds were graded into 0-5 rating scale where grade-0 indicates healthy seeds and grade-5 indicate maximum diseased symptom showing seeds. The different treatments were found to show significant effect on seeds formation of different grades (Table-9). The highest percentage of grade-0 seeds (93.40%) were recorded in T_1 (Free from infection) and lowest percentage of grade-0 seeds (65.88%) were recorded in T_6 (Grains are shriveled, almost completely discolored or more than ½ of grains were discolored). The highest percentage of grade-1 seeds (11.87%) were found in T_6 from others treatments. The lowest percentage of grade-1 seeds (2.67%) were found in T_1 treatment. Incase of grade-2, grade-3 grade-4 & grade-5, the highest percentage of seeds were found in T_6 and lowest percentage of seeds were found in T_1 treatment, respectively.

Table 9. Effect of black pointed seeds of different severity grades (0-5 Scale) on number of grains/ear of different grades of seeds of wheat cv. Kanchan in the field.

Treatments	% Grains under different grades (0-5 scale)					
	0	1	2	3	4	5
T_1	93.40 a	2.67 d	1.90 f	1.63 d	0.10 e	0.30 e
T_2	89.47 b	4.17 c	2.87 d	2.00 d	0.47 d	1.02 d
T_3	84.67 c	5.01 c	2.50 e	3.17 c	0.47 d	2.16 c
T_4	82.38 d	6.50 b	3.33 c	4.57 b	1.10 c	2.08 c
T_5	74.50 e	7.50 b	4.17 b	6.00 a	2.33 b	5.50 b
T_6	65.88 f	11.87 a	6.17 a	6.40 a	3.33 a	6.40 a
LSD (P=0.05)	1.917	1.171	0.322	0.582	0.272	0.545

T_1= Free from infection

T_2= only embryo blackish

T_3= Embryo and its adjacent area slightly infected

T_4= Embryo and less then ¼ of grains are discolored

T_5= Embryo and ½ of grain are infected

T_6= Grains are shriveled, almost completely discolored or more than ½ of grains were discolored.

4.2.7. Effect of black pointed seeds of different severity grades (0-5 Scale) on germination and incidence of *Bipolaris sorokiniana* on wheat seeds by blotter method in laboratory (After harvesting)

In blotter method (After harvesting), Seed germination was also decreased with the increase of black pointed and discoloured seeds in the seed lot (Table-11). The highest seed germination (90.00%) was found in T_1 (Free from infection) treatment that was statistically similar to in T_2 (only embryo blackish) & in T_4 (Embryo and less then ¼ of grains are discolored) treatments. The lowest seed germination (46.67%) was recorded in T_6 (Grains are shriveled, almost completely discolored or more than ½ of grains were discolored) treatment.

Incidence of seed borne *Bipolaris sorokiniana* on wheat seed for all treatments ranged from 11.00% to 31.67%, where treatment T_6 showed the highest incidence (31.67%) of *Bipolaris sorokiniana* and T_1 showed the lowest incidence (11.00%) of *Bipolaris sorokiniana* that was statistically similar to T_2 treatment. The treatment T_1 decreased 65.27% *Bipolaris sorokiniana* over control.

Table 10. Effect of black pointed seeds of different severity grades (0-5 Scale) on germination and incidence of *Bipolaris sorokiniana* on wheat seeds by blotter method in laboratory (After harvesting)

Treatments	Germination (%)	*Bipolaris sorokiniana* on seed surface (%)	Decrease (%) of *Bipolaris sorokiniana* over control
T_1	90.00 a	11.00 e	65.27
T_2	88.67 a	14.33 de	54.75
T_3	80.00 bc	18.00 cd	43.16
T_4	84.33 ab	20.33 c	35.81
T_5	76.00 c	25.67 b	18.95
T_6	46.67 d	31.67 a	-----
LSD (0.05)	4.30	3.089	-----

T_1= Free from infection

T_2= only embryo blackish

T_3= Embryo and its adjacent area slightly infected

T_4= Embryo and less then ¼ of grains are discolored

T_5= Embryo and ½ of grain are infected

T_6= Grains are shriveled, almost completely discolored or more than ½ of grains were discolored.

4.2.8. Effect of black pointed seeds of different severity grades (0-5 Scale) on 1000 seed weight and yield of wheat cv. Kanchan

It has been found that the effect of black pointed seeds of different severity grades on 1000 seed weight & yield of wheat was differed statistically (Table-10). The highest 1000-seed weight (36.40gm) was recorded in the treatment T_1 (Free from infection) that is statistically similar to T_2-T_5 treatments and the lowest 1000-seed weight (30.20gm) was recorded in the treatment T_6 (Grains are shriveled, almost completely discolored or more than ½ of grains were discolored). The highest straw yield (Kg/plot, ton/ha) was obtained in the treatment T_1 that was statistically similar to T_2 and T_3. On the other hand, the lowest straw yield (Kg/plot, ton/ha) was obtained in the treatment T_6 that was statistically similar to T_4 and T_5. The highest grain yield (Kg/plot, ton/ha) was recorded in the treatment T_1 .On the other hand, the lowest grain yield (Kg/plot, ton/ha) was obtained in the treatment T_6 .The treatment T_1 29.69% grain yield increased over control.

Table 11. Effect of black pointed seeds of different severity grades (0-5 Scale) on 1000 seed weight and yield of wheat cv. Kanchan

Treatments	1000- seed weight (gm)	Straw yield (kg/plot)	Straw yield (t/ha)	Grain yield (kg/plot)	Grain yield (t/ha)	% Grain yield increased over control
T_1	36.40 a	5.45 a	6.05a	2.88 a	3.20 a	29.69
T_2	36.00 a	5.37 a	5.97 a	2.54 b	2.87 b	26.38
T_3	35.50 a	4.73 ab	5.25 ab	2.33 c	2.59 c	13.13
T_4	33.30 a	4.51 b	5.01 b	2.22 d	2.47 d	8.91
T_5	34.00 a	4.38 b	4.87 b	2.12 e	2.35 e	4.25
T_6	30.20 b	4.19 b	4.65 b	2.03 f	2.25 f	-----
LSD (P=0.05)	2.988	0.054	0.892	0.054	0.054	-----

T_1= Free from infection

T_2= only embryo blackish

T_3= Embryo and its adjacent area slightly infected

T_4= Embryo and less then ¼ of grains are discolored

T_5= Embryo and ½ of grain are infected

T_6= Grains are shriveled, almost completely discolored or more than ½ of grains were discolored.

DISCUSSION

Comparative effect of Black pointed seeds of different severity grades (0-5) on germination, seedling vigour, and growth characters; leaf blight *(Bipolaris sorokiniana)* development and yield of wheat cv. Kanchan were studied in vitro and in vivo condition. Different severity grades (0-5) of black pointed seeds were used in this study & each grade of black pointed seeds were considered as treatment. Therefore, there were six treatments viz. T_1= Free from infection, T_2= only embryo blackish, T_3= Embryo and its adjacent area slightly infected, T_4= Embryo and less then ¼ of grains are discolored, T_5= Embryo and ½ of grain are infected, T_6= Grains are shriveled, almost completely discolored or more than ½ of grains were discolored. The effect of the black pointed seeds of different severity grades (0-5 Scale) on germination and incidence of *Bipolaris sorokiniana* on wheat seeds before sowing and after harvest in blotter method was found significant variations. From the present study, it is evident that black point infection greatly affected seed germination and seedling emergence of wheat and percent reduction in germination become higher with the increase of level of black pointed seed in the seed sample used for sowing. The highest seed germination (92.33% and 90.00%) in blotter method and paper towel method was recorded in T_1 (Free from infection) treatment & the lowest seed germination (63.00% and 47.50%) was found in T_6, respectively. Treatment T_6 showed the highest incidence (25.33%) of *Bipolaris sorokiniana* on seed surface and T_1 showed the lowest incidence (4.33%) of *Bipolaris sorokiniana* which was 82.91% decreased Bipolaris sorokiniana on seed surface over control.

These finding is in accordance with earlier reports of other workers. Chaudhary *et al.* (1984) studied the effect of black point disease on germination of the grains of WL 711. The germination of the diseased seeds both in blotter method and in pots was reduced to 11.6% and 16.0%, 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. Khanom *et al.* (1987) found 55-96% and 34.5-71% germination for healthy and diseased grains, respectively. Zhimin *et al.* (1998) also reported that seed germination decreased with the increased in susceptibility of a variety to black point infection. The decreased in seed germination and seedling emergence with increasing severity of black point infection has also reported by several workers (Fakir 1988, Dhruj 1991, Rahman and Islam, 1998). Hossain (2000) reported that seed germination in blotter method was sharply decreased as the severity of black pointed infection increased. Maximum (95.0%) & (0.00%) germination was found in seeds of grade-0 (free from infection, the best seeds, apparently healthy) & grade-5 (grains are shriveled, almost completely discolored or more than ½ of grains discolored respectfully). Seeds of grade-5 & grade-0 resulted highest (92.50%) & lowest (40.50%) incidence of *Bipolaris sorokiniana.* Malaker (2003) reported that maximum 99.00% germination was found on blotter when apparently healthy seeds under grade-0 were sown and minimum 55.00% germination was recorded under grade-5 seeds.

Significant decrease in plant stand in pot and in the field has been observed with the increased in number of black pointed seed in seed sample. This might be due to poor germination or failure of seedling emergence as well as pre and post emergence death of the seedlings. The seed borne black point fungi might be responsible for causing seed rot as well as both pre and post emergence seedlings rot, seedling blight and death of the seedling and finally resulted poor plant stand. This has been supported by other researchers. Machacek and Greaney (1938) repored from their green house and field test that seed infected with *Helminthosporium sativum* produced only 248 % plant stand and resulted 80.6% seedling infection Hanson and Christensen (1953) reported *Helminthosporium* and *Fusarium* were responsible for black point infection,

significant decrease in plant stand with more seedling blight. Fakir (1988) stated that among the black point fungi *Drechslera sorokiniana* and *fusarium* sp. causes germination failure/seed rot and disease to the emerged seedlings.

Relationship between black point infection grade and growth of seedlings was investigated was observed that shoot and root length of the seedlings decreased with the increase in infection grade of the seeds though little effect on weight of seedling has been recorded. The rate of reduction of growth was maximum by T_7(28% black pointed seeds) at recorded root length was 57.21% and for shoot length was 41.40%. Vigour Index (VI) was found decrease with the increase in seed infection level where maximum reduction (72.63%) infection was resulted by the seedlings of T_7(28% black pointed seeds). The findings of the present studies are supported by others. Rana & gupta (1982) reported that black point infection greatly affected root & shoot growth of the seedlings, the effect being very prominent on root growth. Rahman & Islam (1998) observed significant reduction in seedling vigour, germination, shoot & root length with the increase of black point infection. Bazlur Rashid (1998) reported that shoot length, root length decreased with the increase of infection grade of seed transmitted *Bipolaris sorokiniana*. He also mentioned that the seedlings that developed from such seed were usually poor vigoured. Hossain (2000) found that the percent of normal seedlings decreased but abnormal seedlings & dead seeds increased with the increase of black point infection level in the seed sample. He also observed that shoot length, root length, weight & vigour index were decreased with the increase of black point infection level in the seed sample.

Significant variation in leaf blight severity were found among the treatments used in the present study at flag leaf, panicle initiation, flowering, milking and hard dough stages under field condition. Lower level of leaf blight (*Bipolaris sorokiniana*) infection was recorded in plots where best seeds were used. This is supported by other researchers. Leaf blight development is a consequence of

the seed of the plant to seed transmission of the pathogen (*Bipolaris sorokiniana*) under field condition (Bazlur Rashid, 1996; Bazlur Rashid & Fakir, 1998). Moreover, higher the level of seed borne fungal infection, there will be higher primary inoculum level in the field. So, higher grade of black pointed seed resulted higher infection in the field. Through the leaf blight severity was found to increase at a significantly higher rate as they grew older, which indicated that maximum infection severity was attained at hard dough stage. Nema & 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 the crop plant resulted higher incidence of leaf spot (*Bipolaris sorokiniana*). Temperature plays also an important factor for leaf blight incidence. Temperature range 25-28°C is favorable for this disease epidemic. In Bangladesh Temperature 25-28°C normally presents in march when wheat plant turn to soft dough to hard dough stage. Therefore, maximum leaf blight disease incidence occurs at that time. Hossain (2000) reported that the disease severity of leaf blight (*Bipolaris sorokiniana*) of wheat in all the plots under different treatments was increased with the increase in age of the plants.

The effect of black pointed seeds of different severity grades on plant height, ear length and distance between the flag leaf initiation and base of the ear was found statistically significant. The result of the study showed that plant height, ear length, distance between the flag leaf initiation and base of the ear, number of spikelets/ear & number of healthy spikelets/ear were decreased with the increase the infection level of leaf blight (*Bipolaris sorokiniana*) severity of wheat. Numbers of diseased spikelets/ear were increased with the increase the infection level of leaf blight (*Bipolaris sorokiniana*) severity of wheat. These findings were supported by Bazlur Rashid *et al.* (1994). They mentioned the relationship of leaf blight incidence with the seed quality. They also reported that seed quality deterioration is positively associated with the incidence of leaf blight caused by *Bipolaris sorokiniana* under field conditions. Hossain *et al.*

blight intensity with grain infection. Seeds from the plot T_6(Grains are shriveled, almost completely discolored or more than ½ of grains were discolored) resulted (31.67%) highest incidence of *B. sorokiniana* on the seed surface. The lowest incidence of *B. sorokiniana* on the seed surface was found in T_1 which was 65.27% decreased *B. sorokiniana* over control.

It has been found that the effect of black pointed seeds of different severity grades on 1000 seed weight & yield of wheat was differed statistically. The highest 1000-seed weight, straw yield (Kg/ha, ton/ha) & grain yield (Kg/ha, ton/ha) were obtained in the treatment T_1 and the lowest result were observed in the treatment T_6. The results indicated that there was a decreasing trend in yield and yield contributing characters with the increase in severity of black point infection of seeds using for sowing. Yield reductions due to sowing of black point infected seeds have also been reported by other workers (Parashar and Chohan 1967, Zwatz 1975, Sznuics and Sznuics 1981, Nalli 1986). Nalli (1986) stated that *Bipolaris sorokiniana* inoculated seeds produced tiller of lower height and reduced seed production. Malaker (2003) found maximum 1000-seed weight & grain yield (Kg/plot, ton/ha) in grade-0 seeds and minimum results in grade-5 seeds.

SUMMARY AND CONCLUSION

"Effect of black pointed seeds of different severity grades (0-5) on leaf blight development and yield of wheat" was studied in the laboratory and field condition during the period of Dec. 2007-April. 2008.

Seed germination and seedling emergence were significantly decreased with the increase of severity of black pointed seed under different treatments used for sowing but seed germination and emergence of seedlings were found to increased when treatment T_1(Free from infection) was used. Black point infection significantly affected shoot growth, root growth and fresh weight of the seedling. Significantly (2219.00) and lowest (700.10) seedling vigour index (VI) were recorded incase of seedlings under treatment T_1 and T_6, respectively.

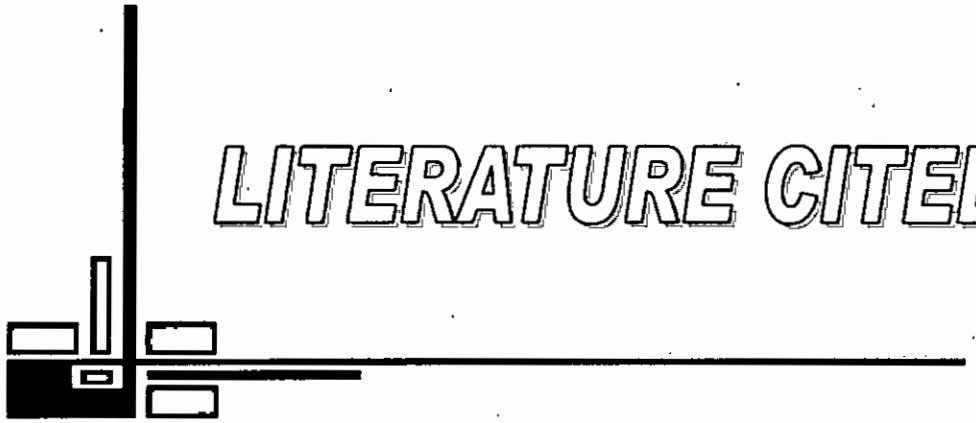
Leaf blight severity was increased with the increase in plant age of the plants under different treatments used but lower disease severity was found in plots where grade-0 (T_1) seeds were sown. All the treatments differed significantly on disease severity at flag leaf stage to hard dough stages. In all stages, the highest disease severity were recorded in T_6 and lowest disease severity were recorded in T_1, respectively.

The treatments differ significantly in respect of plant height and ear length. Both these parameters gave highest performance (88.83cm, 15.80cm) in T_1treatment and lowest (74.10cm, 10.40cm) in T_6, respectively. The treatments significantly produced highest (25.50) spikelets/ear and (22.03) healthy spikelets/ear in T_1 treatment. The lowest (15.97) spikelets/ear and (9.30) healthy spikelets/ear was recorded in T_6 treatment. The highest spikelets/ear (25.50) and (22.03) healthy spikelets/ear was recorted in T_1 treatment. The lowest diseased spikelets/ear (6.67) was recorded in T_6 treatment and (3.47) in T_1treatment.

The highest number of grains/ear (50.61) was recorded in T_1 & the lowest number of grains per ear (35.67) was found in T_6 treatment. The highest number of healthy grains/ear (47.27) was recorded in T_1 treatment & the lowest number of healthy grains per ear (25.50) was recorded in T_6 treatment, respectively. The highest number of diseased grains/ear (12.17) was recorded in T_6 & the lowest number of diseased grains per ear (3.33) was found in T_1, respectively. Incase of grain weight, the highest weight of grains/ear, healthy grains/ear (1.80 gm & 1.62 gm) was recorded in T_1 and the lowest weight (0.66 gm & 0.20 gm) grains/ear, healthy grains/ear was recorded in T_6 treatment. The lowest weight of diseased grains/ear was recorded (0.18 gm) in T_1 & the highest weight of diseased grains/ear was recorded (0.46 gm) in treatment

In the field formation of highest (93.40%) and lowest (65.88%) of grade-0 seeds was recorded from the plot under treatment T_1 and T_6, respectively. On the other hand, highest (6.40%) and lowest (0.30%) of grade-5 seeds was recorded from the plot under treatment T_6 and T_1, respectively. It has been found that the effect of black pointed seeds of different severity grades on 1000 seed weight & yield of wheat was differed statistically. The highest 1000-seed weight, straw yield (Kg/plot, ton/ha) & grain yield (Kg/plot, ton/ha) were obtained in the treatment T_1 and the lowest result were observed in the treatment T_6. The results indicated that there was a decreasing trend in yield and yield contributing characters with the increase in severity of black point infection of seeds using for sowing.

Considering the findings of the present studies it has been found that the seed sample T_1 seeds showed good results in respect of germination, seedling vigour, plant growth characters and production of best seed as grade-0. So, wheat seeds of treatment T_1 may be considered as a standard one for sowing in the field for good seed production. But the research findings need to confirm by carrying out same research for another 2-3 years under different agro-climatic zones of Bangladesh.



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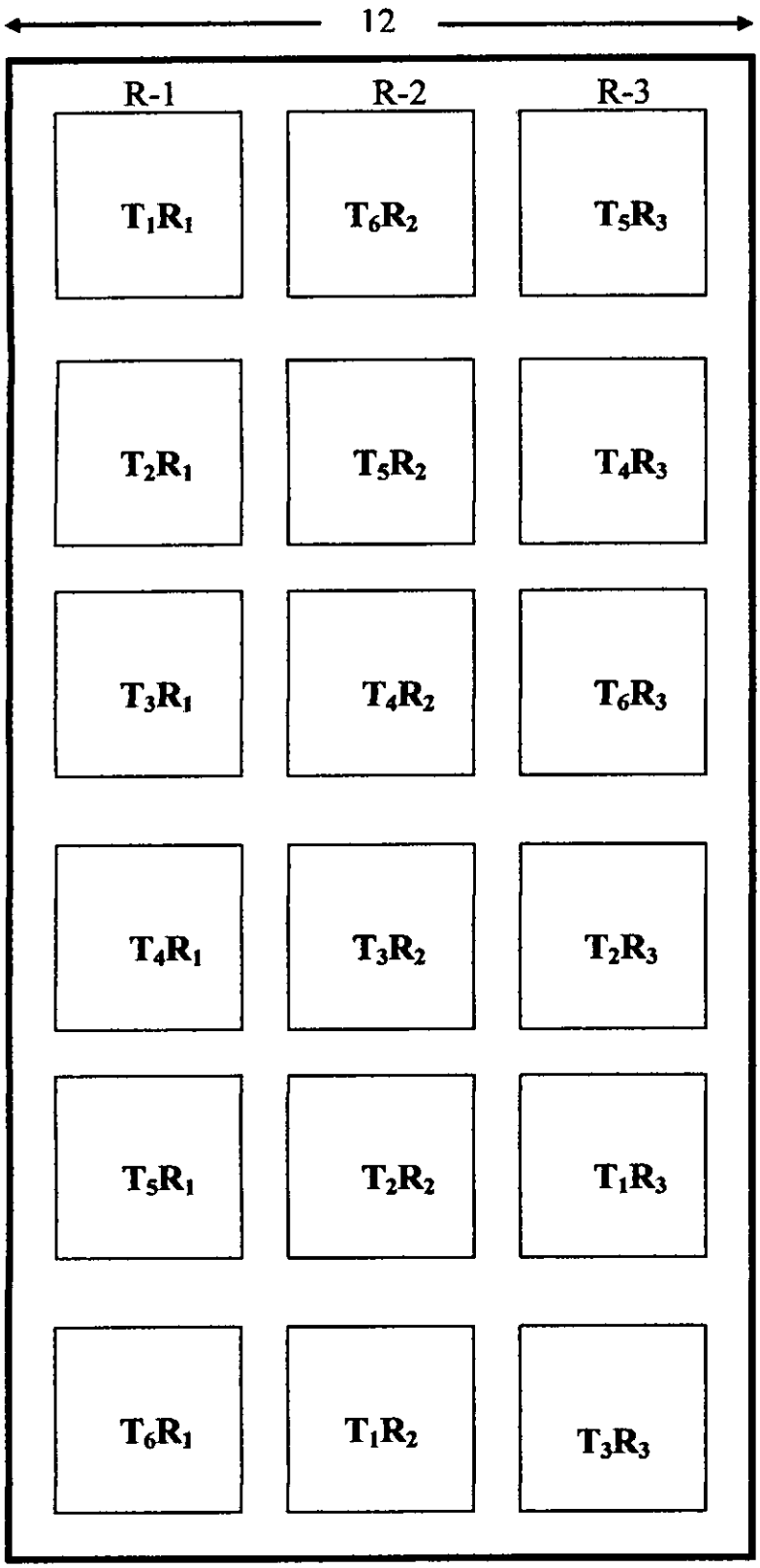
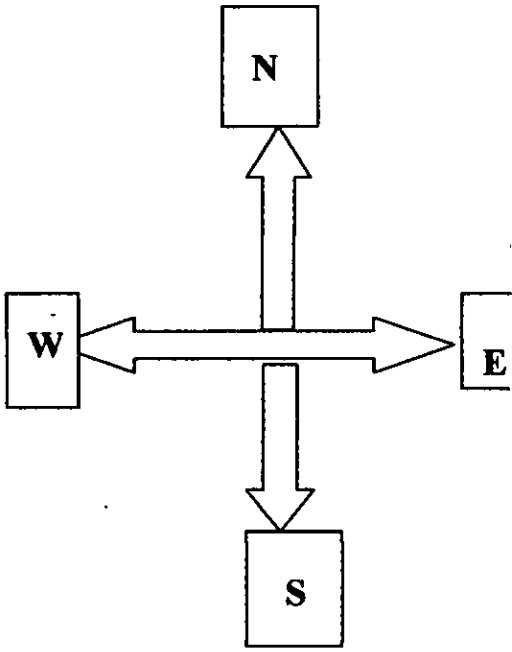
Appendices





Plot size → 3m X 3m
Spacing → 25 cm
Plot spacing → 1m
Row to Row distance → 1

Figure : Field layout in The Randomized Complete Black Design (RCBD)

APPENDICES

Appendix I. Records of meteorological information (monthly) of the experimental site during the period from November 2007-April 2008.

Month (2007-08)	Average Temperature (° C)		Rainfall (mm)
	Maximum	Minimum	
November, 2007	29.03	19.90	423
December, 2007	25.87	15.10	0
January, 2008	24.57	14.53	23
February, 2008	26.65	15.10	54
March, 2008	31.15	21.45	38
April, 2008	34.35	24.50	81

Source: Agronomy division of Bangladesh Jute Research Institute (BJRI), Dhaka.

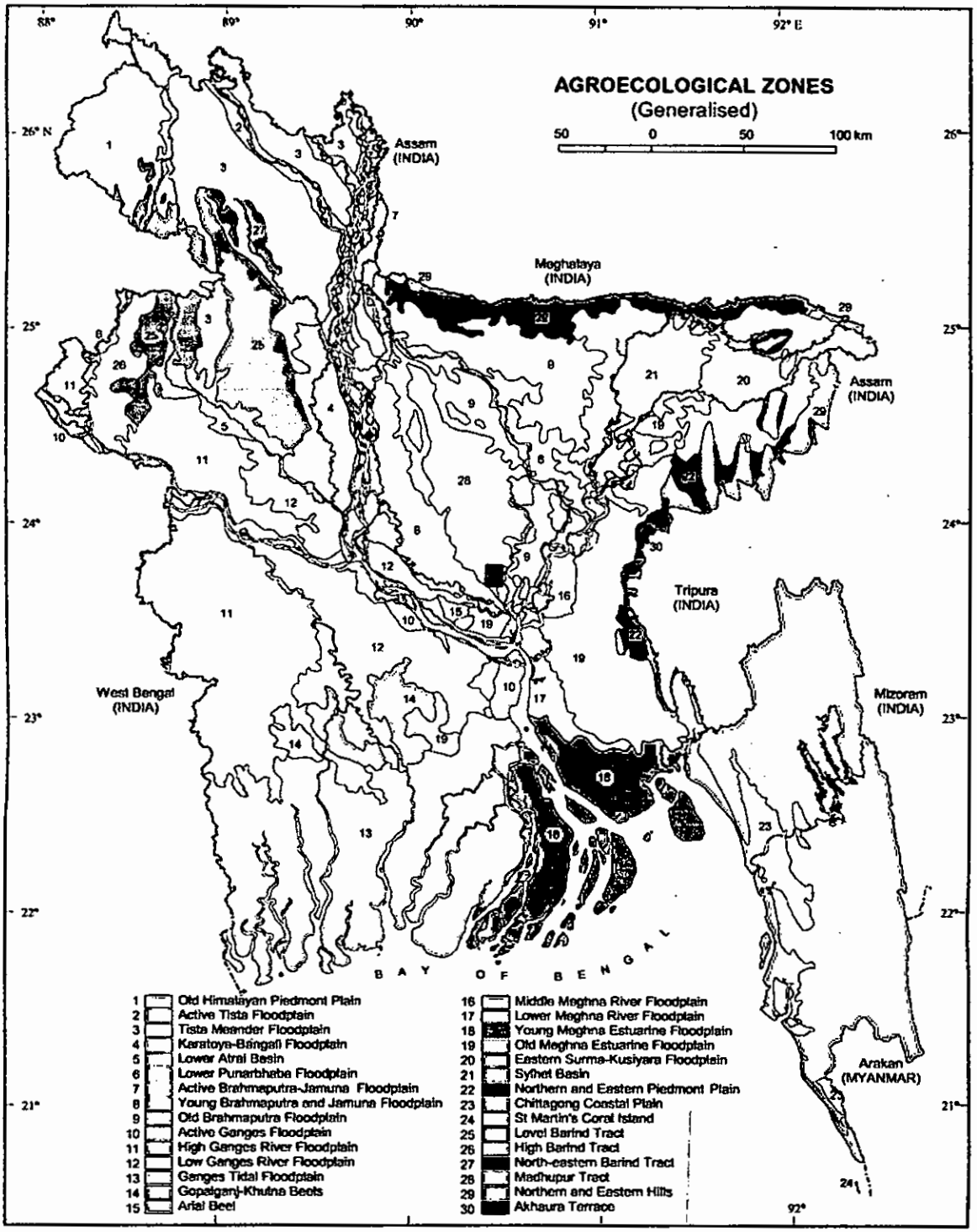


Fig: Location of experimental site under study