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EFFECT OF SEED INFECTION LEVELS AND POPULATION DENSITY ON SEEDLING INFECTION AND INCIDENCE OF *Bipolaris sorokiniana* OF WHEAT CV. SHATABDI

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ABSTRACT

Begum F, Islam MN, Aminuzzaman FM, Islam MR (2017) Effect of seed infection level and population density on seedling infection and incidence of *Bipolaris sorokiniana* of wheat cv. shatabdi. *Int. J. Sustain. Crop Prod.* 12(3), 22-30.

An experiment was conducted during March 2010-April 2011 to find out the influence of seed infection level by *Bipolaris sorokiniana* and population density on leaf blight development, healthy seed production and yield of wheat, cv. shatabdi. The seed infection level and the population density influenced the pathogenic activities through creating microclimate variability that modify the leaf blight/spot development by *Bipolaris sorokiniana* of wheat, six different seed infection levels *viz.*T_1= 0%, T_2 = 5.1-15%, T_3 = 15.1-25%, T_4 = 25.1-35%, T_5 = 35.1-45%, T_6 = 45.1-60% were tested. Using three different method namely blotter method, water agar method and rolled paper towel method the germination percentage, normal seedling percentage and vigor index was maximum at 0% seed infection and minimum at 45.1-60% seed infection. Number of infected seedlings and dead seed was higher at maximum seed infection level and lower at 0% seed infection level. In green house tray method seed germination and vigor index decreased with the increase of seed infection level by *Bipolaris sorokiniana.* Different seed infection increased the severity of *Bipolaris sorokiniana* up to 45.1-60% seed infection. Population density and seed infection levels had significant influence on plant growth parameters and yield and yield contributing character. Population density 300 seeds/m^2 showed best performance in case of grain and straw yield. The highest grain yield (2.64 t/ha) and straw yield (6.87 t/ha) was obtained from 300 seeds/m^2 population density. The highest grain yield (3.14 t/ha) was also produced from 0% seed infection, whereas, 45.1-60% seed infection produced the lowest grain yield (1.04 t/ha). The highest straw yield was observed with 300 seeds/m^2 and the lowest from 200 seeds/m^2. The influence of the development of leaf blight disease of wheat by *Bipolaris sorokiniana* with increased plant density attributed to favorable microclimate produced in the field condition.

Key words: *wheat, leaf blight, fungi, bipolaris, seed infection*

INTRODUCTION

In Bangladesh, wheat is considered as a good supplement of rice and it plays a vital role to meet the food demand for the increasing population of the country. It is the leading cereal crop which ranks first both in area (21360 thousand hectares) and production (676317 thousand metric ton) of the world (FAO 2011). The total world acreage of wheat was 60.43 million acres (USDA 2008) with total production of about 676 million metric ton, 3.4% up from 2010 in the world in 2008-2009 (FAO 2008). The consumption of wheat has been increasing during the last decade by about 5.6 million ton/year (Carter 2002).

Wheat has gained much popularity among the farmers of Bangladesh due to its higher nutritive value and lower cost of production than that of rice. At present, wheat is grown in an area of about 0.40 million hectares and the total production is 0.737 million metric ton in Bangladesh in 2007 (BBS 2008). The average yield of wheat is 1.84 t/ha (BBS 2008) which is 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).

The low yield of wheat may be due to various factors. Among them use of unhealthy or diseased seeds is important one. Population density can greatly influence quality of seed. For good crop and higher yield of wheat, good seed and optimum plant population is needed. Government and semi government organizations supply 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). Optimum plant density produces optimum number of plant per unit area resulting better yield contributing characters leading to better grain and straw yields of wheat (Singh 1992). In Bangladesh, higher trend of the disease was recorded with the increase in plant age under field condition (Nahar 1995). Finally the pathogen attacks wheat grains causing black point. Hossain *et al.* (1998) reported that this disease can reduce yield up to 40% in field condition whereas 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.

Fakir *et al.* (1977) first indicated the possibility of transmission of the pathogen through wheat seeds. In Bangladesh, higher trend of the disease was recorded with the increase in plant age under field condition (Nahar 1995; Rashid 1997). Optimum seed rate securing good yield of wheat. Seed rate was found to be influenced yield and yield contributing characters of wheat (Singh and Singh, 1987). As the winter is very short in Bangladesh, use of increasing seed rate can attain proper vegetative growth (Singh 1992). 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 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. Higher seed rate than the recommended one

generally increases plant population resulting intra- crop competition thereby affecting the yield. Tiller mortality is greater at high planting density, and the number of fertile spikelets per spike, along with the yield components are mostly, affected by planting density (Saradon *et al.* 1988). 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). Decreasing the planting density increases the amount of photosynthetic assimilation and provides a canopy structure which gives increased physiological activities after anthesis leading to a decreased rate of photosynthesis, increased total photosynthetic assimilation and increased sink effect on grain yield (Zhenhua and Yuyog, 1995). Lower seed rate may reduce the yield drastically. Population densities significantly affect the yield of wheat (Fernandez *et al.* 1994). The 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). Leaf spot/blight is an important fungal disease of wheat worldwide. The disease can cause yield losses of up to 60% where as higher seed infection level present and population density was also higher. Incidence and severity of leaf blight were found to be correlated with plant density at all growth stages. The percentages of seed infection increase in different plant density at all growth stages have a significant relation to determine the leaf blight incidence and severity caused by *B. sorokiniana*. Seed infection level had a significant effect on disease incidence and severity at different growth stages like flag leaf stages, panicle initiation stages, flowering stages, milking stages and hard dough stages (Shah *et al.* 1995).These factors can change the morphology and physiology of the growing plant and influence the growth and yield of the plant either directly or by the leaf blight/spot development of wheat (Ansar *et al.* 1996; Endres and Joba, 1989).

Under this situation the present experiment was conducted to assess the effect of different seed infection level and population density on leaf blight development and yield of wheat.

MATERIALS AND METHODS

The experiment was conducted during March 2010 to April 2011 in three steps. At first seed health test was done in the laboratory. Then leaf blight severity was examined in respect to different seed infection level and planting density in the field laboratory of the department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka and finally seed health study was done after harvesting in the laboratory.

Wheat cv. Shatabdi was used in this study were collected from the farm of Sher-e-Bangla Agricultural University. After collection the seeds were kept in a plastic container with air tight lid and the container was stored in normal room temperaturein seed pathology laboratory. The seed sample was physically sorted out to prepare different level of seed infection. Six level of seed infection was prepared to assess the development of leaf blight/spot by *B. sorokiniana* of wheat.

At first seed sample was collected and then sorted out of healthy looking seeds with bold golden color separated from black pointed seeds. Then, different level of seed infection was prepared by mixing of healthy seeds and black pointed seeds through laboratory test and counting the infection percentage. T_1 (0.00% infection of seeds) grade was prepared by treating healthy looking seeds with Provax-200. Then T_2, T_3, T_4, T_5 and T_6 seed sample with 5.1-15% seed infection, 15.1-25% seed infection, 25.1-35% seed infection, 35.1-45% seed infection and 45.1-60% seed infection was prepared, respectively, by mixing apparently healthy seeds with different levels of black pointed seed. The collected seed sample was divided into different grade and considered as treatment which has been done by physical sorting of seeds. Six treatments were used in this experiment. These treatments were- 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).

For studying the seed health different methods were used. Incidence of seed borne *B. 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 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 *B. sorokiniana* under stereo binocular microscope following the key of Mathur and Kongsdal (2003) (Germination of the seeds was also recorded).

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 indiameter and 15 cm in length) and then sterilized in autoclave for 15 minute under 15 Ibs 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. One hundred 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.

Seedling infection and seedling vigor test was done in the Rolled Paper Towel Method (Warham 1990). In this method, 400 seeds were randomly taken from each treatment with three replications 50 seeds were placed uniformly between a pair of moist paper towels. The towels were rolled and the two ends were closed with rubber band as the moist could not remove easily. Then the rolled papers containing seeds were placed in an upright position for 7-10 days at room temperature under normal 12/12 light and darkness cycle. After incubation number of normal seedlings, number of abnormal seedlings and number of dead seeds were recorded. The number of normal and abnormal seedlings was recorded to ISTA rules (ISTA 1996). The shoot and root portions were blotted dry with fine tissue paper and fresh weight was taken before the materials could get desiccated. 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. Vigor of the seedling was determined by the following formula (Baki and Anderson, 1972).

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

In tray method technique, plastic tray was used to test the germination, infection, dead seed and vigor of seedling in the soil. In this method, 400 seeds were randomly taken from each treatment maintaining there replications. There was 18 number of plastic tray for each treatment. Three tray soil was used to place 400 seeds in every tray. The tray was filled with mixed soil (soil + cow dung + sand) and then treated with formalin spray and covered with polythene sheet for 2 days. After that the soil was opened for five days under the bright sunlight to remove the toxicity of fumigation. Then 5 lines were made in each tray where 80 seeds were sown in each line. After that proper care was taken like irrigation was given properly. After 7-10, days data were recorded on the basis of different parameters. For determination of seedlings vigor, Fifty (50) seedlings were randomly selected from each tray and their individual shoot and root length were measured.

Data were recorded on germination percentage, number of healthy seedling, number of infected seedling, number of dead seed, shoot length, root length and seedling weight following ISTA rules (1996) and Vigor index was determined following Baki and Anderson (1972).

Laboratory experiment (after harvesting)

Two hundred seeds were randomly selected from each harvested samples and incubated for collection of data on germination and incidence of *B. sorokiniana* following Blotter method (ISTA 1996). The grading of seeds was done following the 0-5 rating scale of CIMMYT (Gilchrist 1985). The rating scale is: 0= Free from infection, 1= Only embryo blackish, 2= Embryo and its adjacent area slightly infected, 3= Embryo and less than ¼ of grains are discolored, 4= Embryo and ½ of grains are infected, 5= Grains are shriveled almost completely discolored or more than ½ of grains are discolored.

The recorded data for different parameters were compiled and tabulated in proper form the data were subjected to arcsine transformation when needed. The treatment means were compared by Duncan's Multiple Range Test (DMRT), following Gomez and Gomez (1984).

RESULTS

In Blotter method, the results showed that seed infection by *B. sorokiniana* had negative effect on seed germination (Table 1). The effect of the treatments was significantly different regarding germination percentage and incidence of *B. sorokiniana* of wheat. The maximum seed germination (85.63%) was found in T_1 (no infection) followed by T_2 (83.85%), T_3 (78.46%), T_4 (68.46%) and T_5 (59.23%) and T_6 (52.55%).

The incidence of *B. sorokiniana* of wheat was observed highest in T_6 (49.23%) which was significantly different as compared to the other treatments. The infection of *B. sorokiniana* ranged from 0.77-49.23%, where the treatment T_1 (0.77%) was found to have minimum infection preceded by T_2 (14.37%), T_3 (23.85%). On the contrary, the highest incidence (49.23%) was observed in T_6 (45.1-60% seed infection) followed by T_5 (41.29%) and T_4 (30.52%).

Water agar test tube seedling symptom test of seeds of different levels of seed infection was found significant in respect of germination normal and abnormal seedling production and dead seed percentage of wheat. The increased in different level of seeds infection was resulted gradual decrease of seed germination and deterioration of seedling health status of wheat (Table 2). The germination varied from 90.25%-54.68%. The highest germination (90.25%) 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 87.45 and 80.52%, respectively. The lowest (54.68%) was in T_6 (45.1-60% seed infection) preceded by T_5 (61.54%) and T_6 (59.60%).

The treatments showed significant difference from one another regarding percent normal seedlings and the results for all the treatments ranged from 81.29-27.17%, where the maximum counts (81.29%) were found in T_1,

(0% seed infection) followed by T_2 (75.91%) and T_3 (62.83%) and the minimum counts (2.17%) were found in T_6 (45.1-60% seed infection) preceded by T_5 (36.68%).

The treatments showed significant differences regarding percent abnormal seedlings. The number of abnormal seedlings was found to be increased with the increase of black pointed seeds. Abnormal seedlings ranged from 8.98-27.48%, where the highest (27.48%) percent was observed in T_6 (45.1-0% seed infection) followed by T_5 (24.86%) as well as T_4 (22.92%) and the lowest (8.98%) percent was found in T_1 (0% seed infection) preceded by T_2 (11.54%) and T_3 (17.69%).

Similar trend was found in case of dead seed. It varied from 44.37-11.70%. Dead seed was found to be minimum 11.70% in T_1 (0% seed infection) which was statistically similar to T_2 (5.1-15% seed infection). T_3 and T_4 were also statistically similar but T_5 and T_6 showed non-significant results.

Table 1. Effect of different levels of infection by *Bipolaris sorokiniana* on germination and incidence of *Bipolaris sorokiniana* of wheat

Treatments	**Germination (%)**	***Bipolaris sorokiniana* (%)**
T_1= (0%)	85.63a	0.77e
T_2= (5.1-15%)	83.85a	14.37d
T_3= (15.1-25%)	78.46a	23.85c
T_4= (25.1-35%)	68.46b	30.52c
T_5= (35.1-45%)	59.23bc	41.29b
T_6= (45.1-60%)	52.55c	49.23a
CV%	7.53	14.08

In a column, figure having same letter(s) do not differ significantly at 5% or 1% level by DMRT

Table 2. Effect of different levels of seed infection by *Bipolaris sorokiniana* on germination, normal and abnormal seedling, dead seed of wheat (Water agar test tube)

Treatments	**Germination (%)**	**Normal seedling (%)**	**Abnormal seedling (%)**	**Dead seed (%)**
T_1= (0%)	90.25a	81.29a	8.98c	11.78e
T_2= (5.1-15%)	87:45a	75.91ab	11.54c	15.14de
T_3= (15.1-25%)	80.52a	62.83b	17.69bc	21.78cd
T_4= (25.1-35%)	59.60b	36.68c	22.92ab	25.63c
T_5= (35.1-45%)	61.54b	36.68c	24.86ab	35.14b
T_6= (45.1-60%)	54.68b	27.17c	27.48a	44.37a
CV%	9.55	14.36	25.69	15.00

In a column, figure having same letter(s) do not differ significantly at 5% or 1% level by DMRT

Different levels of seed infection of *B. sorokiniana* by germination and seedling infection of wheat in rolled paper towel method are shown in Table 3. The germination of wheat seeds decreased with the increase of seed infection by *B. sorokiniana* in the samples. The treatments were found to differ significantly from one another and the results varied from 81.29 to 45.14%. The highest germination (81.29%) was counted in T_1 (0% seed infection) followed by T_2 (72.83%), T_3 (67.94%) and T_4 (63.60%) and the lowest germination (45.14%) was observed in T_6 (45.1-60% seed infection) proceeded by T_5 (61.20%).

Reverse trend was found in respect of seedling infection. The seedling infection was increased with the increasing levels of seed infection by *B. sorokiniana* of wheat. Significant variations were found among the treatments and the results varied from 21.29 to 57.45%. The maximum (57.45%) seedling infection was found in T_6 (45.1-60% seed infection) followed by T_5 (45.63%), T_4 (38.98%) and the minimum (21.29%) seedling infection was recorded in T_1 (0% seed infection) preceded by T_2 (29.75%) and T_3 (34.62%).

Table 3. Effect of different levels of infection by *Bipolaris sorokiniana* on germination, seedling growth, vigor index and seedling infection of wheat (Rolled paper towel method)

Treatments	**Shoot length(cm)**	**Root length (cm)**	**Seedling weight (g)**	**Germination (%)**	**Vigor index**	**Seedling infection (%)**
T_1= (0%)	14.82a	12.99a	11.92a	81.29a	2457.11a	21.29d
T_2= (5.1-15%)	14.28ab	13.29a	11.33ab	72.83ab	2074.11ab	29.75cd
T_3= (15.1-25%)	12.61be	12.08ab	10.74ab	67.94bc	1870.13bc	34.62c
T_4= (25.1-35%)	12.57be	12.33ab	9.63be	63.60c	1566.81cd	38.98bc
T_5= (35.1-45%)	11.36cd	10.26be	8.42cd	61.02c	1376.20de	45.63b
T_6= (45.1-60%)	9.67d	9.50c	7.51d	45.14d	1035.02e	57.45a
CV%	7.71	11.72	10.14	7.34	14.19	13.39

In a column, figure having same letter(s) do not differ significantly at 5% or 1% level by DMRT

The different levels of seed infection differed significantly in respect of shoot length (cm), root length (cm), seedling weight (g) and Vigor Index of 7 days old seedlings (Table 3). The highest shoot length (142.82 cm) was recorded in T_1 (0% seed infection) followed by T_2 (14.28 cm), T_3 (12.61 cm) and the minimum (12.57 cm)

was found in T_6 (45.1-60% seed infection) which was statistically similar to T_5 (11.36 cm) as well as proceeded by T_4 (12.61 cm). Length varied from 14.82 to 9.67 cm. The maximum shoot length (14.82 cm).

In case of root length, the values ranged from 13.29 to 9.55 cm, where the highest (13.29 cm) root length was found in T_2 (5.1-15% seed infection) followed by T_1 and T_3 with 12.99 and 12.08 cm root length, respectively. On the other hand, the lowest (9.50 cm) value was recorded in T_6 (45.1-60% seed infection) proceeded by T_5 (10.26 cm) and T_4 (12.33 cm).Considering seedling weight, the values ranged from 11.92 g to 7.51 g. The lowest weight (7.51g) was found in T_6 (45.1-60% seed infection) which was statistically similar to T_5 (15.1-25% seed infection), T_4 (25.1-35% seed infection) and T_3 (35.1-45% seed infection). The highest weight (11.92 g) was found in T_1 (0% seed infection). The treatment T_2 (5.1-15% seed infection) showed the second highest weight (11.3 g) which was statistically similar to T_1. Vigor Index (VI) for all the treatments differed significantly with a range of 2457.11-1035.02. The maximum Vigor Index (2457.11) was recorded in seedlings under T_1 (0% seed infection) and the minimum (1035.02) was counted in T_6 (45.1-60% seed infection).

The impact of seed borne infection of *B. sorokiniana* on germination and seedling infection of wheat in tray method is shown in Table 4. The germination of 7 and 10 days old seedling of wheat decreased with the increase of seed infection by *B. sorokiniana* in the samples. The treatments were found to differ significantly from one to another and the results varied from 86.17 -57.58% (7 days old seedling). The highest germination (86.17%) was counted in T_1, (0% seed infection) followed by T_2 (84.50%), T_3 (81.25%) and T_4 (78.67%) and the lowest germination 57.58% was observed in T_6 (45.1-60% seed infection). After 10 days old seedlings the results varied from 86.33-69.67%. The highest germination (86.33%) was counted in T_1, (0% seed infection) followed by T_2, T_3, T_4 and T_5 showed the statistically similar results. The lowest germination (69.67%) was observed in T_6 (45.1-60% seed infection) which was not statistically similar to all other treatments.

In respect of seedling infection, significant variations were found among the treatments and the results varied from 0.0%-1.67%. The maximum (1.67%) count of seedling infection was found in T_6 followed by T_5 (1.42%) as well as T_4 (1.08%) and the minimum (0.00%) count of seeding infection wasrecorded in T_1 (0% seed infection) preceded by T_2 (0.67%) and T_3 (0.75%) respectively.

Table 4. Effect of different levels of seed infection by *Bipolaris sorokiniana* on germination, seedling growth, vigor index and seedling infection of wheat

Treatments	Shoot length (cm)	Root length (cm)	Seeding weight (g)	Germination (%)		Vigor index	Seeding infection (%)	Dead seed (%)
				7 DAS	10 DAS			
T_1= (0%)	21.50a	12.82	9.17a	86.17a	86.33a	2735.38a	0.00d	14.67c
T_2= (5.1-15%)	20.39ab	11.91	9.17a	84.50a	84.92a	2493.36ab	0.67c	15.08c
T_3= (15.1-25%)	19.66abc	11.78	9.10a	81.25a	82.00a	2305.66ab	0.75c	18.00bc
T_4= (25.1-35%)	18.63be	10.97	8.66ab	78.67a	82.17a	2250.44b	1.08 be	17.83bc
T_5= (35.1-45%)	18.05cd	10.96	7.89be	77.08a	78.50a	2239.65b	1.42ab	21.50b
T_6= (45.1-60%)	16.73d	10.33	6.79c	57.58b	69.67b	1657.13c	1.67a	30.33a
CV%	5.27	8.66	7.38	6.47	5.14	13.66	33.51	13.21

In a column, figure having same letter(s) do not differ significantly at 5% or 1% level by DMRT

The significant variation was found in respect of shoot length (cm), seedling weight (g) and Vigor Index of 7 and 10 days old seedlings (Table 4). The shoot length varied from 21.50-16.73 cm. The maximum shoot length (21.50 cm) was recorded in T_1 (0% seed infection) followed by T_2 (20.39 cm) as well as T_3 (19.66 cm) and the minimum (16.73 cm) result was found in T_6 which was statistically similar to T_5 (18.05 cm) as well as preceded by T_4 (18.63 cm).

In case of root length, no significant results was found but the values ranged from 12.82-10.33 cm, where the highest (12.82 cm) root length was found in T_1 (0% seed infection) followed by T_2 and T_3. On the other hand, the lowest (10.33 cm) value was observed in T_6. Considering seedling weight, the values ranged from 9.17-6.79 g. The lowest count (6.79 g) was found in T_6 which was statistically similar to T_5 (15.1-25% seed infection) and T_4 (25.1-35% seed infection). The highest weight (9.17 g) was found in T_1 (0% seed infection) and T_2 (5.1-15% seed infection), T_3 (15.1-25% seed infection) showed statistically similar result.Vigor Index (VI) for all the treatments differed significantly with a range of 2735.38-1657.13. The maximum Vigor Index (2735.38) was recorded in seedlings under T_1 (0% seed infection) and the minimum (1657.13) was calculated in T_6 (45.1-60% seed infection). In case of percent dead seed, the results varied from 14.67-30.33%. Percent dead seed was found the maximum 30.33% in T_6 which was statistically different from all other treatments. The minimum 14.67% was found in T_1, which was statistically similar to T_2, T_3 and T_4.

Laboratory experiment (After harvesting)

In harvested seeds, the germination percentage and incidence of *B. sorokiniana* were found to differ significantly among the treatments (Table 5). Considering germination percentage, the values ranged from 93.78-74.36%, where the maximum (93.78%) counts were made in T_1 (0% seed) infection) followed by T_2 and T_3. There was a significantly similar relationship between T_2 and T_3 treatment and T_4 and T_5 treatment but T_6

(45.1-60%) showed significant results than other treatments. In respect of infection percentage, the values varied from 35.78-13.17%, where the highest presence (35.78%) was found in T_6 (45.1-60% seed infection) which was followed by T_5 (33.06%) and T_4 (30.61%). The lowest incidence of the fungus (13.17%) was recorded in T_1 (0% seed infection) Proceeded by T_2 (5.1-15% seed infection) and T_3 (15.1-25% seed infection with 28.22% and 29.11%, respectively.

Table 5. Effect of different levels of seed infection by *B. sorokiniana* on germination and incidence of *B. sorokiniana* of harvested seed of wheat (Blotter method)

Population density (seeds/m^2)	Germination (%) after harvest	Infection (%) after harvest
200	83.76b	28.86c
300	84.96a	31.06a
400	83.56c	25.06b
CV%	9.48	4.53
Seed infection (%)		
T_1= (0%)	93.78a	13.17e
T_2= (5.1-15%)	86.89b	28.22d
T_3= (15.1-25%)	85.42b	29.11d
T_4= (25.1-35%)	83.67bc	30.61c
T_5= (35.1-45%)	80.44c	33.06b
T_6= (45.1-60%)	74.36d	35.78a
CV%	9.48	4.53

In a column, figure having same letter(s) do not differ significantly at 5% or 1% level by DMRT

Population density showed significant influence on germination and incidence of infection percentage of wheat after harvest in the laboratory (Blotter method). In germination, the value ranged from 84.96-83.56. Where the maximum value recorded 84.96% at 300 seeds/m^2 and minimum value recorded 83.56 at 400 seeds/m^2 which was a non- significant relation with 200 seeds/m^2 seed rate of wheat i.e 83.76% seed germination. The incidence of *B. sorokiniana* the values varied from 31.06-25.06%, where the maximum values 31.06% at 300 seeds/m^2 and the minimum value recorded 25.06% at 400 seeds/m^2.

There was no significant combined interaction on germination percentage of wheat (Table 6). The maximum no of germination was found 96.67% at 400 seeds/m^2 and T_1 (0% seed infection), and the minimum was found 74.08 at 300 seeds/m^2 and T_6 (45.1-60%). But there was a significant variation among the infection (%) of wheat. The highest results was recorded 36.00% at 400 seeds/m^2 and T_6 (45.1-60% seed infection) and the lowest results obtained 8.67% at 400 seeds/m^2 and T_1 (0% seed infections).

Table 6. Interaction effect of different levels of seed infection and population density on germination and incidence of *B. sorokiniana* on harvested seeds

Population density (seeds/m^2)	Seed infection	Germination (%) after harvest	Infection (%) after harvest
200	T_1= (0%)	93.33	11.004
	T_2= (5.1-15%)	87.33	29.33e
	T_3= (05.1-25%)	84.92	30.67de
	T_4= (25.1-35%)	83.33	31.67cde
	T_5= (35.1-45%)	79.00	34.67ab
	T_6= (45.1-60%)	74.67	35.83ab
300	T_1= (0%)	91.33	19.83h
	T_2= (5.1-15%)	88.33	31.67cde
	T_3= (15.1-25%)	87.00	32.00cd
	T_4= (25.1-35%)	85.67	33.50be
	T_5= (35.1-45%)	83.33	33.83abc
	T_6= (45.1-60%)	74.08	35.50ab
400	T_1= (0%)	96.67	8.67j
	T_2= (5.1-15%)	85.00	23.67g
	T_3= (15.1-25%)	84.33	24.67fg
	T_4= (25.1-35%)	82.00	26.67f
	T_5= (35.1-45%)	79.00	30.67de
	T_6= (45.1-60%)	74.33	36.00a
		NS	
	CV%	9.48	4.53

In a column, figure having same letter(s) do not differ significantly at 5% or 1% level by DMRT

DISCUSSION

In the present study, effect of different levels of seed infection by *B. sorokiniana* on seedling vigor, leaf blight development and healthy seed production of wheat was observed both under laboratory and field condition. Six treatments with different levels of seed infection by *B. 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). The "population density" which has a significant relationship with disease development in five growth stages (flag leaf stage, panicle initiation stage, flowering stage, milking stage and hard dough stage) and yield of wheat were also observed. Three different types of seed rates *viz.* 200 seeds/m^2, 300 seeds/m^2 and 400 seeds/m^2 were used.

In seed germination experiment significant variations was observed in blotter method, rolled paper towel method water agar test tube method and tray method. The maximum seed germination was found in those seeds which were 0% infection (T_1). With the increase of seed infection by *B. sorokiniana* remarkable reduction of the seed germination was recorded. Lowest germination was found in seeds with the maximum 45.1-60% infection (T_6). The minimum germination percentages 52.55, 54.68, 45.14 and 57.58 were found in T_6 (45.1-60% seed infection) in blotter method, rolled paper towel method and water agar test tube seedling symptom test, respectively. The present findings are well supported by other researchers (Hanson and Christensen, 1953; Choudhary *et al*. 1984; Khanum *et al*. 1987; Hossain 2000). Similar trend of variation in germination of *Helminthosporium sativum* infected wheat seeds were reported by Hanson and Christensen (1953). They reported 66 and 62% seed germination having seed infection 81 and 74% infection, respectively with *Helminthosporium sativum.* Choudhary *et al.* (1984) reported that germination of the infected (black pointed) seeds both in blotter and pot soil was found to be decreased 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 workers (Parashar and Chohan, 1967; Rana and Gupta, 1982; Sinha and Thapliyal, 1984; Zhang *et al.* 1990). Chowdhury *et al*. (2010) reported that germination of wheat seeds having different levels of seed infection ranged 69.00-97.00, 72.67-96.00 and 62.67-96.67% respectively in blotter method, rolled paper towel method and water agar method depending on levels of seed infection in wheat seeds. The findings were also similar with others (Choudhary *et al.* 1984; Khanum *et al.* 1987; Aulakh *et al.* 1988). The present study revealed that different levels of seed infection by *B. sorokiniana* has 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). The findings of the present study are supported by the earlier reports (Rana and Gupta, 1982; Rahman and Islam, 1998; Rashid and Fakir, 1998; Hossain 2000). Rana and Gupta (1982) found that black point 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. Rashid and Fakir (1998) reported that percent reduction in shoot and root length increased with the increase of infection grade of seed transmitted *B. sorokiniana* and the overall reductions were highest for root length. He also mentioned that the seedlings that developed from such seed were usually poor in vigor. 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 cm and for shoot length was 41.40 cm. He also mentioned that Vigor Index (VI) was found with maximum reduction (72.63%) resulted by the seedlings of 28% black pointed seeds. Chowdhury *et al*. (2010) found that different levels of seed infection greatly affected root and shoot growth of wheat seedlings where as Vigor index of seedling was 1851.81, 1392.02, 1203.55, 971.16, 841.62 and 669.36, respectively in case of 0, 5.1-15, 15.1-25, 25.1-35, 35.1-45 and 45.1-60% seed infection.

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 reduced 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 *Drechslera 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 healthy seed production. The higher plant density had significant effect on the development of leaf blight of wheat which attributed to favorable microclimate produced in the field condition compared to lower plant density. 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 (*B. sorokiniana*) for healthy seed production.

CONCLUSION

The present investigation demonstrated that higher level of *Bipolaris* infected seed or greater population density can significantly increase the seedling infection and incidence of *Bipolaris sorokiniana* on wheat cv. shatabdi.

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