VARIABILITY OF *BIPOLARIS SOROKINIANA* (SACC.) SHOEMAKER AND MANAGEMENT OF LEAF BLIGHT OF WHEAT

A
DISSERTATION
SUBMITTED TO THE UNIVERSITY OF DHAKA
FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY
IN
BOTANY
(MYCOLOGY AND PLANT PATHOLOGY)

By

MST. SELINA MOMTAZ
Reg. No.: 46 (2012–2013)
Re-reg. No.172 (2016–2017)
DEPARTMENT OF BOTANY
FACULTY OF BIOLOGICAL SCIENCES
UNIVERSITY OF DHAKA

JUNE 2022

DEDICATED

To

MY BELOVED PARENTS
And
CHILDREN

DECLARATION

I do hereby declare that the work presented in this dissertation entitled **"Variability of *Bipolaris sorokiniana* (Sacc.) Shoemaker and management of leaf blight of wheat"** is the original result of my own investigation. I further declare that this dissertation has been composed by myself and no part of this thesis has been submitted anywhere in any form for any academic degree. All sources of information have been specifically acknowledged by referring to the authors.

JUNE 2022 **(Mst. Selina Momtaz)**

CERTIFICATE

This is to certify that the research work and results of this dissertation entitled ***"Variability of Bipolaris sorokiniana (Sacc.) Shoemaker and management of leaf blight of wheat"*** *is the outcome of original work carried out by Mst. Selina Momtaz at Research Laboratory of Mycology and Plant Pathology, Department of Botany, University of Dhaka, Dhaka and also at the experiment field of Plant Pathology Division, Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur under our joint supervision.*

This is further certified that this dissertation is suitable for submission in fulfilment of the requirements for the Degree of Doctor of Philosophy in Botany (Mycology and Plant Pathology).

Supervisor

Dr. Shamim Shamsi
Professor
Department of Botany
University of Dhaka
Dhaka, Bangladesh

Co-Supervisor

Dr. Tapan Kumar Dey
Senior Program Specialist (Crops)
KGF, BARC Complex, Farmgate, Dhaka
& Former Director
BARI, Gazipur,
Bangladesh

ABSTRACT

The present research work carried out to determine the variability of *B. sorokiniana*, causal agent of leaf blight of wheat (BpLB) and its management. Diseased leaf samples of twenty-one wheat varieties from eight districts of Bangladesh were collected. A total of 35 fungal species belongs to 20 genera were isolated, the frequency percentage of *B. sorokiniana* ranged from 6.67-85.71% based on varieties. It was the maximum in Joypurhat district and minimum in Kushtia district. Association of *Bipolaris cynodontis*, *Drechslera hawaiiensis* and *Pestalosiopsis guepinii* with wheat were new record. *Bispora antenata* and *Drechslera dematioadea* were new recorded fungi for Bangladesh. Total 174 isolates of *B. sorokiniana* were obtained and 150 isolates under 8 different cultural groups were evaluated for morphological, physiological and pathogenic variability. The colour of colonies varied from ash to whitish, brownish, blackish, greenish ash and black with smooth or wavy margin, and white mat colonies with few conodia. Compatibility test reveals that 53.34% confrontations were incompatible. Ten fungicides were evaluated against *B. sorokiniana* isolates *in vitro*, Tilt 250 EC and Folicur 250 EC were identified as the complete inhibiting chemical fungicides. Out of 10 leaf extracts, *Azadirachta indica* and *Citrus limon* showed best inhibition on *B. sorokiniana* isolates. The present investigation suggests that *Trichoderma harzianum* and *T. viride* showed promising inhibitory effect on *B. sorokiniana* isolates. From field experiments, effective fungicides (Tilt 250 EC/Folicur 250 EC) two spray at 65 days and 80 days after sowing, at standard dose (0.1%) will be more fruitful on reducing disease severity and increasing yield than the standard three spray schedule. Estimation of yield loss of wheat due to Bipolaris leaf blight (BpLB), the average yield loss was 28.68-29.19%, with 1000 grain weight more than 15% lower than protected plot. From the screening of wheat germplasms against Bipolaris leaf blight (BpLB), 1.63, 39.02, 54.47, 4.88 percent genotypes were selected as resistant (R), moderately resistant (MR), moderately susceptible (MS) and susceptible (S), respectively. There was no genotype as highly susceptible (HS). Seed health test reveals that all seed samples were infected by *B. sorokiniana* in various degrees and black point seeds show lower seed viability, germination and emergence percentage, vigour index etc. than apparently healthy seeds.

ACKNOWLEDGEMENT

All the praises be to Allah without whose wish, I would not have completed my thesis. Without his blessings, I would not have come this far and attained sufficient wisdom for this task.

I would like to express my deepest sense of gratitude to my supervisor Prof. Dr. Shamim Shamsi, Department of Botany, University of Dhaka for her continuous guidance, crucial advice and complete support towards me during this whole period. I also express my heartiest respect for my co-supervisor Dr. Tapan Kumar Dey, Director, BARI, Gazipur in memory of his suggestions and corrections.

I would like to express my utmost love and affection to all my lab-mates in Mycology and Plant Pathology lab for their helping hands in trying times, especially Dr. Pranami Chowdhury and Dr. Najmun Naher.

I would like to mention Bangladesh Agricultural Research Institute (BARI) for their immense cooperation and sincere service. A big shout-out to the Ministry of Science and Technology, Government of the People's Republic of Bangladesh for providing financial support in this research through NST fellowship program.

Moreover, I reminisce the memories of my departed father (May Allah grant him Jannah) who have developed me mentally and psychologically and made me who I am today. Also remembering my mother who has always paid close attention to all my needs and shortcomings. Lastly, I am extremely grateful to my kids, brothers and sisters for their unconditional support and help during the whole time.

Mst. Selina Momtaz

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

CHAPTER ONE
INTRODUCTION

CHAPTER ONE

INTRODUCTION

Wheat (*Triticum aestivum* L.), a major grain crop, is grown expansively across the world. Wheat farming already occupies more area on the planet than any other economic crop. Wheat output surpassed 772 million tons in 2020 (FAO 2021) from 240 million hectares of land, making it the second most-produced crop behind maize. Wheat is a staple food for almost two-thirds of the world's population (Majumder 1991). Wheat is grown on around one-sixth of the world's total arable land. Paddy, on the other hand, is mostly produced in Asia, whereas wheat is grown on all continents, feeding around 36% of the global population (Bhanupriya *et al.* 2014) and supplying 20% of total food calories and protein in human nutrition (Verma *et al.* 2014). China, India, Russia, the United States and France are the world's top wheat producers, accounting for more than half of global production. Wheat trading is larger than all other crops put together (FAO 2021).

Bangladesh is an over populated country and the growth rate of population is high. Sustained increases in wheat output are generally acknowledged as a means of keeping up with the growing population and ensuring food security. Wheat is one of the important winter cereal crops and considered as the second most important grain crop after rice in Bangladesh (Hossain and Teixeira 2013). The average yield of wheat in Bangladesh is lower in comparison to other countries. During 2018–19, wheat production was 1.02 million metric tons from 0.3 million ha (BBS 2019). The country needs 6.88 million metric ton of wheat, 80–85% of which are imported every year. Bangladesh spends up to USD 1.5 billion per year on wheat to accommodate rising local demand. Wheat intake is rising as a result of the country's fast urbanization and industrialization, as well as the increased usage of a variety of bread items. Bangladesh is ranked 49th out of 123 countries in terms of wheat output (from highest to lowest) (FAO 2021).

Bangladesh has transitioned from non-traditional wheat-growing regions to conventional wheat-growing regions (Klatt 1988). It accounts for around 4% of total cultivated land and 11% of rabi season cropped area (winter crops from November to February) and adds 7% to total food cereal output (Anon. 2008). Although Bangladesh is a small wheat producing country, but has made spectacular progress in increasing its production (Ahmed and Meisner 1996).

Wheat (*Triticum aestivum* L.), the first domesticated plants by human belongs to the grass family Poaceae (Graminae). The plants produce thick spikes of this tasty grain. Botanically, the wheat kernel is a type of fruit called caryopsis. The mature wheat plant's culm is a hollow jointed cylinder with 3–6 nodes and internodes. The sheath, blade, ligules and auricle make up the wheat leaf. The bottom two-thirds of the culm are generally enclosed with leaf sheaths. During the life of the wheat plant, a maximum of 5–7 seminal roots can operate. Wheat may be cultivated in a variety of climates and soil types. It produces well in clayey loam soils. Wheat demands dry weather and good sunlight and well-distributed rainfall of 40 to 110 cm. From seeding to harvest, 100–120 days are necessary, depending on the variety and weather conditions.

For the majority of Bangladeshis, rice and wheat are the primary sources of food, calories and protein. Wheat grains are abundant in calories, carbohydrates, dietary fiber, fat, protein, thiamine, riboflavin, niacin, pantothenic acid, vitamin B_6, folate, calcium, iron, magnesium, phosphorus, potassium, zinc and manganese [USDA-National Nutrient Database for Standard Reference, Release 19 (2006)]. It provides a significant amount of protein as well as a number of necessary amino acids. It has a greater protein content than maize or rice. Water 13%, protein 11.5–12%, fat 1.72–2%, carbohydrate 69.6–70%, fiber 2% and ash 1.5–2% make up the whole grain (Purseglove 1975). Wheat protein is easily absorbed by about 99% of people, with the exception of the 1% who have gluten sensitivity. Globally, wheat contributes to 20.4% of total protein supply and 18.8% of total calories for human consumption (FAOSTAT 2009).

After independence due to the high price of rice, the people of Bangladesh had to adjust their eating habits and adopt wheat as an alternative cereal source. Though most people in Bangladesh consume wheat in the form of 'chapati' (locally known as ruti), which is produced from wheat flour (locally known as ata or maida), it may be consumed in a variety of ways. Once wheat was a food for the poorer in Bangladesh, but it has now become a staple for all classes of society. The flour of hard-kernelled wheat cultivars is used to make macaroni, spaghetti, noodles and pasta. The starchy kernels of white and soft wheat types are chosen for bread, cakes, biscuits, piecrust and a variety of morning meals. In other countries, wheat is used to create whiskey and beer, and the grain, bran and vegetative plant components are important cattle and poultry feed. In our country's rural areas, straw is employed also as a fuel source. Wheat is now firmly established as a secure crop in Bangladesh, owing to consistent market prices and the involvement of two

million farmers in wheat cultivation (Karim *et al.* 2010). Wheat cultivation is easier and requires less time and irrigation than other alternative crops like Boro rice, legumes and potatoes, and it also has lower production expenses. In Bangladesh on average, crop land is harvested thrice a year that means cropping intensity of nearly 300%, maximum in the world. For land there is a competition among winter crops including wheat. In our intensive rice-based cropping systems, wheat is farmed only easily fitting with the system. More than eighty percent wheat is cultivated in a three-crop rotation, sixty percent Aush-Aman-wheat and twenty percent jute-Aman-wheat. CIMMYT (The International Maize and Wheat Improvement Center) is continuing with wheat development programmes of Bangladesh and emphasised development of disease free high yielding varieties suitable for our environment by providing a vast elite wheat germplasm. Three major diseases, poor crop management, struggle with other winter crops and late sowing due to late harvest of Aman rice are key constraints of wheat production in Bangladesh.

Diseases are a major consideration in crop productivity. Wheat may be affected by 200 different diseases globally, 42 of which are seed-borne and 35 of which are caused by fungus (Wiese 1987). Rusts, leaf spot/spot blotch, common root rot, smut, tan spot, Septoria blotch, powdery mildew, Fusarium head blight, blast and a variety of viral, nematode and bacterial diseases are among the most frequent wheat illnesses. By seedling mortality or reducing harvest about 20% of wheat is absent due to diseases every year. In Bangladesh wheat suffer from at least 20 different diseases including 12 fungi caused diseases (Talukdar 1974, Ahmed and Hossain 1985, Ahmed 1986). Bipolaris leaf blight-BpLB (*Bipolaris sorokiniana*), leaf rust (*Puccinia recondita*), seedling blight (*B. sorokiniana*), foot and root rot (*Sclerotium rolfsii*) and black point (*B. sorokiniana, Alternaria alternata*, *Curvularia lunata* and species of *Fusarium*) are the five major diseases. Another severe disease that has recently emerged in Bangladesh is blast of wheat, which has two causative agents: *Pyricularia graminis-tritici* and *Pyricularia oryzae* (Sadat and Choi 2016).

In Bangladesh's rice-wheat farming system, Bipolaris leaf blight (BpLB) has become the most devastating disease of wheat. Disease symptoms appeared as numerous small, circular to oval and grey-brown eye-shaped spots on green leaves. The centers of the spot soon faded, becoming light grey to straw coloured with distinct dark brown margins (Chowdhury *et al.* 2005). The disease is of serious concern to wheat growers and

researchers not only in Bangladesh, also in tropical and subtropical countries of the world. In Bangladesh, the disease occurs in almost all wheat growing areas with varying degrees of severity, causing substantial loss in yield and seed quality (Bazlur Rashid *et al.* 1994, Alam *et al.* 1995). The occurrence and severity of the disease are being increasing every year in Bangladesh (Alam *et al.* 1994). Mustarin *et al.* (2021) evaluated the condition of wheat disease in Bangladesh (152 farmer's fields in 36 districts) and identified three primary diseases with high incidence: Bipolaris leaf blight (100%), leaf rust (74%) and blast (40%). Bipolaris leaf blight was discovered on every variety in every area, with variable severity and 28 to 56 percent diseased leaf area (%DLA).

Bipolaris sorokiniana is principally a non-specialized foliar blight pathogen of wheat and it also causes kernel smudge or seed abortion, seedling blight, root rot, head blight and black point (Prescott *et al.* 1986). Seed borne inoculums, crop residues, soil moisture and environmental conditions play vital role in pathogen multiplication and disease development. High temperatures and relative humidity, especially in South Asia's intensive 'irrigated rice-wheat' production systems, favour the outbreak of the disease. Rice serves as a host for the leaf blight pathogen, and rice straw plays its role as a substrate for the fungi after rice harvest (Saari 1998). The disease incidence observed on stem and leaf increased across the growth stages. There was strong linear relationship between seed borne inoculums with pathogen incidence on leaf and stem up to flowering stages. Foliar blight was influenced by both seed borne as well as air-borne inoculums. The later was more responsible for developing leaf blight at advanced growth stages. Spikelets may be impacted in perfect circumstances, causing seed shriveling. If meteorological circumstances are favorable, such as prolonged rain for 5–6 days followed by higher temperatures (day average of 20–30°C), the pathogen's vulnerability improves, and a leaf blight epidemic can progress faster (Mehta 1998).

A detailed description of *Bipolaris sorokiniana* (Sacc.) Shoemaker (Sivanesan 1990) can be obtained from C.M.I.'s Sivanesan and Holliday (1981). In the older literature, several synonyms of the anamorph have been used: *Helminthosporium sorokinianum*, *Drechslera sorokiniana* and *Helminthosporium sativum* (Maraite *et al*. 1998). Shoemaker (1959) proposed the generic name *Bipolaris* for the *Helminthosporium* species with fusoid, straight, or curved conidia germinating by one germ tube from each end (bipolar germination). *B. sorokiniana* is characterized by thick-walled, elliptical conidia (60–120 µm×12–20 µm) with five to nine cells. In axenic culture, the mycelium is composed

of hyphae interwoven as a loose cottony mass and appears white or light to dark grey depending on the isolates. The fungus is differentiated from other members of the *Bipolaris* genus on the basis of morphological features of conidiophores and conidiospores. A key for distinguishing species of *Bipolaris* was described by Subramanian (1971). The ascigerous state was observed in the laboratory on natural media in the presence of opposite mating types, and was first described as *Ophiobolus sativus*. It was later renamed *Cochliobolus sativus* (Ito & Kuribayashi) Drechsler ex. Dastur 1942 (Dastur 1942). In Zambia the perfect stage was only found under natural environments (Raemaekers 1988), and it has not been reported to occur in any other areas in which the pathogen prevails. The fungus belongs to the subdivision Ascomycotina (class: Loculoascomycetes; order: Pleosporales; family: Pleosporaceae). Globose ascomata with a long cylinder like neck, obclavate cylindrical asci with helically coiled filiform ascospores–are the features of genus *Cochloibolus*. It is associated with *Bipolaris* and *Curvularia* anamorphs.

The yield loss due to this disease amounted to 23% in Bangladesh (Sarri 1998). The pathogen *B. sorokiniana* has been reported to reduce 88.7% grains/ear and produce 87.5% discoloured and black point grains. Under field trials and artificial inoculation, the disease lowered yield up to 40% and 88% compared to the control, respectively. *B. sorokiniana* under controlled condition by artificial inoculation resulted in 100% yield loss (Hossain and Azad 1994). Soil and seed borne nature, wide genetic variability and extensive host range of the pathogen have long been remained crucial factors for effective management of the leaf blight disease (Reis *et al.* 1998). Several approaches have been practiced to combat the disease, including the use of resistant varieties, cultural control, chemical control, biological control and the use of plant extracts. A number of fungicides under various groups were tested against BpLB, but the propiconazoles were found most effective in controlling the disease with significant yield increase (Malaker *et al.* 2007).

Control of plant disease by fungicides cause environmental pollution and it is hazardous both for flora and fauna. Excessive usage and misuse of agrochemicals has resulted in severe limits on chemical pesticide use, as well as the removal of the most harmful compounds from the market. As a result, alternate means and ways for controlling the disease must be discovered. Biological management of plant diseases, for example, is one of these options. *Trichoderma* species are crucial in the management of fungal plant pathogens. A limited work has been done in controlling BpLB of wheat by the application

of *Trichoderma*. Plant disease control has been demonstrated using a number of higher plants and their components (Ashrafuzzaman and Hossain 1992). Plant extract showed obvious consequence on the viability of conidia along with (Singh *et al.* 1990 and Dubey 1991) reduction in fungal growth (Khair *et al.* 1995). The use of neem based pesticide and *Trichoderma harzianum* also proved effective in reducing the leaf blight incidence (Singh *et al.* 2002). In Bangladesh, farmers are using only fungicides to combat the disease. Application of plant extract and antagonists are trialed only in the research level. So far, there were few published report on the integration of antagonist with fungicides and plant extracts in controlling BpLB of wheat in the field.

Christensen (1922) first described four biological specializations of *Helminthosporium sativum* and in 1925 he identified at least 37 races of this pathogen. Hetzler (1992) also suggested that *B. sorokiniana* differs geographically. Ahmed (2001) identified six pathotypes of *B. sorokiniana* and found no significant variation in pathotypes regarding healthy grain formation and grain yield of wheat variety Kanchan in Bangladesh. Aminuzzaman and Hossain (2005) identified twelve pathotypes of *B. sorokiniana* from the country by pathogenicity test on a twelve-member differential sets.

Most commercially farmed high yielding cultivars have been shown to be sensitive to leaf blight (*B. sorokiniana*). The reasons for lack of substantial durable resistance in the varieties may be attributed to abundance of heterogeneity in the population (Poloni *et al*. 2008). Studies on variability in the pathogen and host are crucial for identifying resistant sources. Variation in the population of *B. sorokiniana* is of great concern to develop a resistant variety. To evaluate a resistant material against a particular pathogen it is utmost important to identify the existence of physiological races and/or pathotypes of the pathogen. Considering the above fact, the present research work carried out to determine the variability of *B. sorokiniana* and management of Bipolaris leaf blight disease of wheat in Bangladesh.

Specific objectives of the research work

1. Collection of infected leaf sample from different agro-ecological zones of Bangladesh.
2. Isolation, purification, characterization, identification and preservation of *B. sorokiniana* isolates associated with infected leaves of different varieties.
3. To determine the presence of variability of *Bipolaris sorokiniana* isolates at morphological, pathological and physiological level.
4. To assess the loss incurred due to Bipolaris leaf blight of wheat.
5. To develop management practices against the disease.

CHAPTER TWO
REVIEW OF LITERATURES

CHAPTER TWO

REVIEW OF LITERATURES

Bipolaris sorokiniana, the pathogen of leaf blight disease of wheat is a very common and important pathogen all over the world. In Bangladesh, the disease is very devastating and influenced on the wheat production of the country. The world literatures on leaf blight of wheat are discussed here, relevant to the current study. The components of the existent investigation concerning variation in *B. sorokiniana* and disease management strategies using chemical, bio-control agents have already been studied by several workers. For precise and better presentation only the most important and relevant literatures have been collected under the following heads.

2.1 Prevalence and distribution

Bipolaris sorokiniana is a pathogen that thrives in areas with high relative humidity and warmth, as well as low soil fertility, such as South Asia, South America, Africa and Australia (Duveiller and Sharma 2009). It may also be found in non-traditional subtropical lowland wheat regions of Brazil, North-East Argentina and Paraguay, as well as comparable settings on the African continent in Tanzania and rain-fed wheat growing areas of Zambia and Madagascar (Hetzler *et al.* 1991).

Dubin and van Ginkel (1991) investigated the situation of wheat diseases in wormer countries such as Bangladesh, Brazil, Burundi, China, India, Indonesia, Madagascar, Somalia, Tanzania, Thailand, Vietnam, Zambia and Zimbabwe, and concluded that *B. sorokiniana* was the most commercially significant leaf pathogen. They also claimed that *B. sorokiniana* is the most important biotic constraint to wheat in South Asia. Due to rising wheat consumption in these places, it has been expanded into areas where wheat farming is not traditionally practiced (North-west India and Pakistan).

Small wheat producing regions in Thailand, Philippines and Indonesia, as well as high rainfall and hot wheat growing areas in Southern China, had comparable growth circumstances in South East Asia (van Ginkel and Rajaram 1998). Leaf blight has been described as a dangerous disease in several states in the United States (Wegulo *et al.* 2009), as well as different parts of Europe (Duveiller and Altamirano 2000) and including west-north Russian Federation. The pathogen's capacity to acclimate to cold, which allows increased inoculum survival even in freezing winter temperatures, may explain its

presence in cooler parts of the world (east Europe, north-west China, north-west Africa and North America). The fungus' inoculum is ubiquitous and persistent (Duveiller *et al.* 2005). Although leaf blight, common root rot and black point are all caused by the same pathogen and can co-occur, depending on the environmental circumstances, one disease type typically dominates over the others (Duveiller and Altamirano 2000). Wheat production is also endangered in areas of China due to the substitution of land-race varieties with high-yielding, rust-resistant genotypes (Chang and Wu 1998).

Goel *et al.* (1999) oversaw wheat spot blotch research at the National Centre for Foliar Diseases in Faizabad, which indicated that *Helminthosporium sativum* was India's most common pathogen. Over the course of a decade, Singh *et al.* (2001) studied wheat leaf blight pathogens in 16 Indian states: Bihar, Delhi, Gujarat, Haryana, Himachal Pradesh, Jammu & Kashmir, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Orissa, Punjab, Rajasthan, Tamilnadu, Uttar Pradesh and West Bengal. They stated that *B. sorokiniana* was the most common cause of leaf blight in Bihar and West Bengal. They also reported about the disease in neighboring countries of Bangladesh and Nepal (Singh *et al.* 1997). Dubin and van Ginkel (1991) report that in the recent years the largest area of Bangladesh, Nepal and India are facing the problem. In Bangladesh, leaf blight of wheat was first documented in 1977 by Fakir. Among all the wheat diseases, leaf blight (*Drechslera sorokiniana*) and leaf rust (*Puccinia recondita*) were the most serious. Both the diseases occurred widely on almost all the varieties grown in the country and cause considerable losses to the crop.

A four years (1976–80) long experiment conducted by Bazlur Rashid *et al.* (1983) on the leaf blight of wheat in seven districts of Bangladesh caused by *B. sorokiniana* and disease occurance was 100% irrespective of variety or location. Alam and Saha (1991) reported that once leaf rust (*Puccinia recondita*) was measured to be the most notorious wheat disease in the country. But now Helminthosporium leaf blight (HLB) caused by *H. sativum* (Bipolaris leaf blight caused by *B. sorokiniana*) has become the top problematic disease of wheat and the occurrence 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 infected. All the fields had HLB with the infection of 50% leaf area of lower foliage and 10–15% leaf area of upper foliage at booting stage. 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. Alam *et al.* (1994a) reported that leaf blight caused by *B. sorokiniana* is the most serious disease of wheat in the country.

Leaf blight (HLB) is a major disease of wheat in Nepal, caused by *B. sorokiniana* and *Pyrenophora tritici-repentis* alone or in combined. Leaf blight caused by *B. sorokiniana* was a serious disease of wheat in hot and moist parts of the world, including South East Asian nations such as India, Nepal and Bangladesh, according to Chowdhary *et al.* (2013). The disease was widespread, particularly in India's North Eastern Plains Zone (NEPZ) (Bahadar *et al.* 2016). Under hot and moist circumstances, leaf blight is thought to lead to a considerably lower average wheat crop output.

Recently a country wide survey on wheat diseases of Bangladesh (Mustarin *et al.* 2021) focused on three major diseases, out of ten from 152 fields in thirty-six districts are– Bipolaris leaf blight, leaf rust and blast. Bipolaris leaf blight showed highest prevalence with 100 percent incidence and 28–56% diseased leaf area (DLA). None of the variety or field was free from leaf blight symptoms. With significant variation of disease severity in varieties and fields, leaf rust prevailed in seventy-four percent fields. As a new threaten blast was found forty percent of fields with few infections. North-western part (main wheat producing districts) of the country was free from blast. The maximum and the minimum blast severity was noted in districts Bhola and Naogaon.

2.2 Nomenclature of pathogen

Helminthosporium sativum is the common term for the fungus that create wheat leaf blight disease. Shoemaker (1959) proposed the anamorph names for graminicolous *Helminthosporium* species, which include the fungus linked with wheat leaf blight, as an illicit orthographic version of *Helmisporium* Link ex SF Gray. Plant pathologists have a profound attachment to the word *Helminthosporium*, which is tied to significant textbook diseases and, on the other hand, to the struggling and dragging to keep up with systematists' never-ending arguments and revisions in taxonomy and nomenclature. However, some of the modifications have become widely adopted and it is prudent to achieve an agreement on utilizing the same terminology in order to promote mutual understanding.

Link developed the genus *Helminthosporium* in 1809 and SF Gray confirmed it in 1821. The species type *H. velutinum* Link ex SF Gray is essentially different from the

graminicolous *Helminthosporium*. Conidia are generated singly through an aperture at the conidiophore's tip, and development is resumed by sympodial extension from the sub-apical area. Geniculate conidiophores form at the conidiogenesis site as a result of this. The aperture via which the conidium is generated is surrounded by a scar. Conidiophores of *H. velutinum* are straight or flexuous and conidia are produced through tiny holes in the walls of distal and intercalary cells. While the conidiophores elongate at the tip, the conidia develop laterally, typically in verticals below septa. Conidia have a prominently darkened hilum, but there are no scars on the conidiophores at the conidium production sites (Alcorn 1988). The graminicolous *Helminthosporium* species has been renamed due to these changes.

On the basis of the morphology and germination pattern of conidium, Nisikado (1928) split the genus *Helminthosporium* into two sub genera, *Cylindro-Helminthosporium* and *Eu-Helminthosporium*. *Cylindro-Helminthosporium* has cylindrical conidia that germinate from all cells, while *Eu-Helminthosporium* has fusoid conidia that germinate only from one end and is represented by *H. sativum*. Ito (1930) renamed the *Cylindro-Helminthosporium* subgenus to *Drechslera*.

Sorokin initially reported *Helminthosporium sativum* in 1890 from infected wheat and rye spikes in Russia and termed it *Helminthosporium sorokinianum* Sacc. in Sorokin, Trans. Soc. Nat. Univ. Kazan 22:15 (1890). (Sivanesan 1987). Without considering the preceding description, the name *Helminthosporium sativum* Pammel, King and Baker (1910) was given. *Drechslera sorokiniana* was the name given to the causative organism by Drechsler in 1923. *Drechslera* was proposed by Ito (1930) for the graminicolous species in which any or all conidia cells germinated.

Shoemaker (1959) suggested the term *Bipolaris* for *Helminthosporium* species with fusoid, straight, or curved conidia, which were previously classified as *Eu-Helminthosporium*. The term Bipolar comes from the two ends of the conidia, from which germination occurs in a bipolar pattern. However, the hyphomycetes genus *Drechslera* was taken up until's the in-depth taxonomic research of the hyphomycetes genus *Drechslera* by Shoemaker (1959, 1962). It was stated that our understanding of the systematics of two distinct genera, *Drechslera* and *Bipolaris*, was insufficient (Hawksworth 1986). As a result, the wheat leaf blight pathogen *Bipolaris sorokiniana* (Sacc.) Shoem. was given a new name. This is a popular name right now.

However, Subramaniun and Jain (1966) grouped all graminicolous *Helminthosporia* into a single genus *Drechslera*, which was adopted by Ellis (1971) in the Commonwealth Mycological Institute's book "Dematiaceous Hyphomycetes." *Bipolaris sorokiniana* (Sacc.) Shoem. is synonymized with *H. acrotheciodes* Lindfors, *H. californicum* Mackie & Paxton, and *Drechslera sorokiniana* (Sacc.) Subram. and Jain. The fungus varies from *Drechslera,* which was discovered by Ito in 1930, in that it produces fusoid conidia that only germinate from one end. *Drechslera* conidia, on the other hand, are cylindric and germinate from all cells. Both genera generate conidia apically, which distinguishes them from *Helminthosporium*, which produces conidia simultaneously at the apex and sides of conidiophores, as described by Link (1824).

The teleomorphic condition was initially discovered in the laboratory on normal medium in the company of opposing mating types and *Ophiobolus sativus* was named after it. Drechsler ex. Dastur, 1942 termed it *Cochliobolus sativus* (Ito & Kuribayashi) (Dastur 1942). The ascigerous state has only been observed in natural settings in Zambia (Raemaekers 1988) and it has not been recorded in any other regions where the disease thrives. The fungus belonged to the Ascomycotina subdivision (class: Loculoascomycetes; order: Pleosporales; family: Pleosporaceae). The globose ascomata with a long cylinder like collar, obclavate-cylindric asci, and helically coiled filiform ascospores distinguish the genus *Cochliobolus*. Anamorphs of *Bipolaris* and *Curvularia* are seen in this genus.

In the 1980s, more research was done on the classification of this genus, taking into consideration additional factors such as the kind of hilum, the spore wall, and the known or unknown teleomorph, but Shoemaker's nomenclature for the graminicolous *Helminthosporium* has remained unchanged (Sivanesan 1987, Alcorn 1988). The nomenclature of the graminicolous hyphomycetes included in the genus *Helminthosporium* and related teleomorphs was reviewed by Sivanesan and Alcorn.

Bipolaris was the biggest genus, according to Sivanesan (1987), with 52 species. Alcorn (1988) established that the type species for the anamorph genera *Bipolaris*, *Drechslera*, and *Exserohilum* were each associated with an altered ascomycetes genus, namely *Cochliobolus*, *Pyrenophora* and *Setosphaeria*. Genus *Helminthosporium* has been divided into four genera: *Drechslera*, *Bipolaris*, *Exserohilum* and *Helminthosporium*, causal agents of family Gramineae. Isolates were isolated from rice, wheat and maize infected with *Helminthosporium*. Luttrel (1955) and Alcorn (1988) developed keys to compare

their morphological properties. *Bipolaris maydis*, *Bipolaris oryzae*, *Bipolaris sorokiniana*, *Drechslera* sp., *Exserohilum rostratum*, *E. holmai* and *Helminthosporium torulosum* were among the isolates detected. Ellis has classified *Exserohilum* as a synonym for *Drechslera* (1976). However, many authors consequently used *Drechslera* in the broader logic, both for earlier defined and new texa.

The ICBN (International Code of Botanical Nomenclature) allows various stages of fungus with pleomorphic life cycles to be given unique names; if a teleomorph is present, the name refers to that morph even if the anamorph is present as well (Hawksworth *et al.* 1995). *Cochliobolus sativus* (Ito and Kurib.), Drechsler ex Dastur, anamorph: *Bipolaris sorokiniana* (Sacc.) Shoem. are the right names for the pathogen of Helminthosporium leaf blight of wheat.

2.3 Leaf blight complex

Leaf blight, commonly known as Helminthosporium leaf blight (HLB), is a complicated condition caused by many pathogens (Misra 1973, Joshi *et al.* 1978, Singh and Srivastava 1997, Ruckstuhl 1998, Singh *et al.* 1997). The leaf blight syndrome is related with *Drechslera sorokiniana*, *P. tritici-repentis*, *D. tetramera*, *Alternaria triticina* and *Alternaria alternata*, *H. spiciferum* and *Curvularia* species (Joshi *et al.* 1974).

The National Centre for Foliar Blight (Faizabad, UP) monitored the disease and found that *D. sorokiniana* impacted wheat crops more frequently than other fungi (Anon. 1995). The major pathogen responsible for the leaf blight syndrome was *B. sorokiniana* (Singh *et al.* 1980, Singh *et al.* 1998). Although *P. tritici-repentis* has been found in India, it has never been deemed a serious fungus. Prior to 1989-1990, studies in Nepal's Tarai area suggested that *P. tritici-repentis* was the most common spot blotch fungus (Karki 1981). *D. sorokiniana* has emerged as the most important spot blotch fungus in Nepal during the 1990-91 wheat seasons (Mahto and Bimb 1993, Singh *et al.* 1997, Anon. 1999). According to previous research, the Helminthosporium leaf blight (HLB) complex, which is generated by a combination infection of *B. sorokiniana* and *P. tritici-repentis*, is the most common wheat leaf blight condition in Nepal's plains (Sharma *et al.* 2003).

In a study of 360 wheat foliar blight specimens taken from Bangladesh, China, India, Nepal, Vietnam, Morocco, South Africa, Mexico, Bolivia, and Argentina between 1993 and 1996, Maraite *et al.* (1998) discovered that *D. sorokiniana* was present in 81% of the specimens and was related with foliar blight in warm and moist places.

Singh *et al*. (1980) reported that out of 350 leaf blight samples, 259 samples were reported pathogenic involving *B. sorokiniana*, *A. triticina*, *D. tetramera* and *D. sorokiniana*, which involved 74 per cent samples, appeared to be a major pathogen and was prevalent in sub-mountainous region of northern India**.** In 2001–05 growing period, India's eastern state of Uttar Pradesh, Singh *et al.* (2005) investigated spot blotch infections. With an 85 percent frequency incidence, *B. sorokiniana* was the most common cause of wheat leaf blight. Shazia and Iftikhar (2005) performed a research on foliar blight of wheat, in the major rice-wheat farming regions of Pakistan. They identified *Alternaria alternata*, *Pyrenophora tritici-repentis*, *B. sorokiniana*, *Stemphylium* spp. and *Cladosporium* spp.

Bipolaris leaf blight affected leaves of twenty-one wheat genotypes in Bangladesh, and 35 fungal species belongs to 20 genera were implicated (Momtaz *et al.* 2019). The most predominant fungi, in order of prevalence, were *Bipolaris sorokiniana*, *Alternaria alternata*, *Curvularia lunata*, *Cladosporium cladosporioides* and *Fusarium semitectum* (Momtaz *et al.* 2018). Association of five species of *Bipolaris* and two species of *Drechslera* were reported from BpLB infected wheat leaves (Momtaz *et al.* 2019).

2.4 Symptoms

Leaf blight infection may occur at any stage of development of the host plant or any part of the host plants, including roots, crown or lower stem by soil or seed-borne conidia or fungal mycelium present in plant debris (Christensen 1922). In the soil, conidia may remain viable for a long time (Chinn *et al.* 1960). At the early growth stages, the infected wheat plants display seedling blight and common root rot (CRR) symptoms, whereas mature stages show leaf blight/foliar blight, foot and root rot, head blight and black point severity. Variations in weather and environment factors have a significant impact on disease symptoms (Naito and Yousan 1997). General observations indicate that BpLB involves at the early stages and improved with the advance of plant age and lastly the pathogens attack the seeds causing black point (Alam *et al.* 1994).

Chowdhury *et al.* (2005), conveyed that during the survey of the wheat field in west Bengal, India, during January-February, 2003-04, typical symptoms of zonate eyespot were observed along with spot blotch symptom (*B. sorokiniana*). A lot of tiny, round to elliptical, dark-brown lens-shaped lesions formed on healthy foliage as illnesses. The spot's cores faded quickly, turning light brown to straw in hue with clear dark brown

edges. They remained small but the tissue between them became bleached. Similar symptoms were observed on the spikelet at the later stages of crop growth.

Leaf blight appears 5–8 weeks after wheat seeding and in the case of *B. sorokiniana*, symptoms appear after booting and spread quickly. At early growth stages, the initial symptom appears as a lot of chlorotic lesions with light brown dots, mainly on the lower foliage of the plant. A full-figured lesion is often elliptic, eye-shaped and sporulate-rich, covering more than 22% of the leaf area (Mehta 1981a). At first, the lesions on the foliage are typically round, linear, or elliptic (Mehta 1993). Eventually, as the plant grows, the spots on the upper leaves become more irregular in shape and size, with brown necrotic patches in the centers surrounded by chlorotic borders. The blotches continue to consolidate with one another, leading the leaves to dry up. As a result, all of the foliage dry out before the milk stage and the entire field seems blighted.

B. sorokiniana infection has a negative impact on seed germination and root system development, as well as causing pre-and post-emergence damping off (Mehta 1993, Dubin and Bimb 1994). Under perfect circumstances, the entire ear, including the awns, is highly sick and the seeds are extensively infected and shriveled, resulting in a reduction in seed size and a decrease in seed production. When the pathogen infects the spike, it affects the seeds, creating a light brown to blackish discoloration surrounding the seed's germination point, known as the black point. Due to shriveled and discoloured grains, the selling price decreases as well (Mahto and Bimb 1996).

2.5 Host range of *Bipolaris sorokiniana*

The fungus attacks a wide range of hosts and is mostly common on wheat and barley and is capable of causing economic losses on oats, rice and maize, vegetables and grass weeds and causes seedling blight, root and foot rot and leaf spot diseases (Dickson 1956). Christensen (1922) reported that *H. sativium* could infect seven species of *Triticum*, seven species of *Avena*, three species of *Hordium*, *Secale cereale* L., *Zea mays* L. and more than one hundred species of grasses. *B. sorokiniana* infects little kernel cereals-wheat and barley, while rye is less vulnerable and oats are rarely diseased (Zillinsky 1983). A good number of different grasses play as prospective hosts.

During 1994-95, fungi on weeds in wheat fields and adjacent regions were monitored in Faizabad (Eastern UP) and *B. sorokiniana* was discovered on *Phalaris minor* (Singh *et al.* 1995), indicating *Phalaris minor* as an alternative host of the leaf blight disease.

According to Bakonyi *et al.* (1997), monocotyledonous hosts of *Bipolaris sorokiniana* include cereals such as *Triticum aestivum, Secale cereale, Hordeum vulgare, H. murinum, Avena sativa* and grasses- *Agropyron pectinatum, A. repens, Alopecurus pratensis, Beckmannia eruciformis, Bromus erectus, B. inermis, Dactylis glomerata, Festuca heterophylla, F. ovina, Lolium perenne, Pennisetum villosum, Poa pratensis, Setaria virdis.*

According to Iftikhar *et al.* (2009), *B. sorokiniana* may be found globally on a variety of plant species other than wheat. Fifteen crops were evaluated against a local isolate of *B. sorokiniana*, including *Arachis hypogea*, *Avena sativa*, *Brassica compestris*, *Cicer arientenum*, *Glycine max*, *Helianthus annuus*, *Hordeum vulgare*, *Lens culinaris*, *Oryza sativa*, *Pennisetum amaricanum*, *Sesamum indicum*, *Sorghum bicolor*, *Vigna mungo*, *Vigna radiata* and *Zea mays*.

Bahadar *et al*. (2016) studied the host variation of *B. sorokiniana* among monocot plants which are little kernel cereals like *Triticum aestivum*, *Hordeum vulgare*, *Avena sativa*, *Sorghum bicolour* and many other grasses. Numerous plant species other than monocots with *Brassicae compestris*, *Glycine max*, *Lens culinaris*, *Vigna radiata*, *Sesamum indicum*, *Vigna mungo* and *Pennisetum americanum* were known as hosts of *B. sorokiniana*–leaves, stem, roots and inflorescence.

Nagarajan and Kumar (1998) revealed from host studies that *D. sorokiniana* infected not only wheat, but also numerous grasses that co-existed in a region. It could infect abundant host plants as well as paddy sprouts (Nelson and Kline 1966) and occurred on *Andropogon bicornis*, *Aristida pallens*, *Cynodon dactylon*, *Echinochloa crusgalli* and *Paspalum notatum* (Reis *et al*. 1985). Several cereals and *Paspalum notatum* were reported as new hosts of *B. sorokiniana.* Lapis (1985) noted that grasses and wide leaf weeds such as *Commelina diffusa*, *Chloris barbata*, *Dactylactenium aegypticum*, *Eleusine indica*, *Cyperus difformis, C. fimbricatus*, *Imperata cylindrical*, *Cynodon dactylon*, *Paspalum conjugatum*, *Leptochloa chinensis*, *Rottboellia exalata*, *Brachiaria distachya*, *B. mutica* and *Echinochloa colona* grew all year in the Philippines and might colonize the fungus.

2.6 Severity of leaf blight of wheat

Bipolaris sorokiniana is nearly exclusively had long been considered as an important pathogen of cereals and grasses (Huguelet and Kiesling 1973, Razzaque 1982, Zhang *et*

al. 1990, Tinline *et al.* 1994). Christensen (1926) demonstrated that the *Helminthosporium* disease of cereal, especially of wheat and barley caused by *H. sativum* was prevalent and destructive every season in Minnesota. Infections of the leaf blade and sheath appear as discrete circular to oblong light to dark brown spots. The pathogen may invade host tissue intercellularly without producing obvious harm when exposed to low light intensity. At later phases of development, fungal infection accelerates leaf senescence (Dehne and Oerke 1985). Temperature and humidity play a big role in disease severity, especially a few days before blooming.

Leaf infections (blade and sheath) grow as individual elliptical to oblong light to dark brown splotches. When light intensity is little, the fungus may settle host tissue intercellularly without producing obvious harm. At later phases of development, the infection speed up leaf senescence (Dehne and Oerke 1985). Disease severity is deeply related to temperature and humidity, specifically hot and humid weather a few days before blooming.

In hot and moist conditions, Mehta (1993) found that leaf blight of wheat resulting by *Cochliobolus sativus* was the most extensively spread and dangerous pathogen. Even spot blotch outbreaks are common in India, Bangladesh, Philippines, Bolivia, Brazil, Paraguay and Zambia. Dubin and van Ginkel (1991) reported that the severity of the infection was more in the warmer areas than in temperate zone. The disease severity depends on the resistant level of the genotype as well as on the climatic factors, especially temperature.

Hossain and Azad (1992) in an experiment found that wheat plants are more vulnerable to *H. sativum* at different growth stage on the environmental condition. The disease has been increased in a frightening percentage in the country. Rahman and Islam (1998) investigated the influence of black point wheat grains on quantifiable characteristics such as 1000 seed weight and seedling shoot vigor in respect of germination. They found that as the severity of the disease increased, so did the percentage of seeds that germinated.

Bazlur Rashid (1998) studied the effect of seed borne *B. sorokiniana* on the development and existence of wheat sprouts and found that the infection severity amplified meaningfully with the increase in seedling stage. Jahan *et al.* (1998) discovered that the intensity of the infection grew as the plant grew older. Mathur and Cunfer (1993) also reported that the disease is worldwide. Kaur and Aulakh (1988) recorded significant incidence of *D. sorokiniana* in normal looking grains of the Punjab State.

2.7 Yield loss

The effect of *B. sorokiniana* on grain filling of wheat is enormous and dangerous. Reduction of yield due to leaf blight of wheat are significant in fields with minimum efforts and under late-sown conditions. The references on these aspects are compiled and presented below-

Parashar and Chohan (1967) reported that *Helminthosporium sativum* associated with *Alternaria tenuis*, black pointed wheat grains, reduced germination by 3.4% in the laboratory and caused 41.07% losses in the field. About 60% losses of wheat yield owing to *A. triticina* and *H. sativum* was told by Prabhu and Singh (1974) in the cultivar NP 830.

Nema and Joshi (1971) studied the flag leaf susceptibility of wheat to *H. sativum* in relation to seed weight of cultivars Sonora-64 and NP-884. In these varieties, reduction in grain weight was correlated with the no. of blotches and infection intensity per unit area of flag leaf and estimated 3-20 percent losses in yield.

The effects of *Cochliobolus sativus* on factors of seed yield in naturally diseased wheat were reported by Verma *et al.* (1976). In each category, no. of tillers/plant, no. and weight of seeds/spike, weight of spike and thousand grain weights were calculated. Growing the values of all the factors was linked to a reduction in infection severity.

The biggest production loss for inoculation was found by Shabeer and Bockus (1988) at the boot and blooming phases, showing that crops were more biologically vulnerable to losses at those periods. The losses were caused by a large fall in seed weight and number of seeds per head, rather than a decrease in the amount of heads per plant. According to Raemaekers (1988), yield loss estimates determined under very favorable conditions was 85 percent, with yearly yield loss vary from 30–80%.

Zhang *et al.* (1990) reported that in Shaanxi province, China, the thousand seed weight of wheat infected by black point disease was inferior by 1.95–13.5% than uninfected grains. Dubin and van Ginkel (1991) stated that major yield reduction is affected by spot blotch in South Asia. Wildermuth *et al.* (1992) prepared a calculation of yield reduction on 8 varieties and lines varying in vulnerability to *B. sorokiniana.* Seed yield, tiller and no. of seed but not weight of seed declined as disease severity amplified.

B. sorokiniana causes leaf blight of wheat all over the world, according to Mehta (1993), but massive losses occur in Bangladesh, Bolivia, Brazil, Paraguay, and Zambia. The

pathogen infects all plants and can result in productivity of up to 100%. That most economic varieties are either moderately resistant or susceptible to the pathogen, according to Duveiller and Gilchrist (1994a). In 1991-92, yield reduction up to 29% was calculated. Worldwide spot blotch is considered a hazard to wheat farming.

In Bangladesh, Razzaque and Hossain (1990) showed that leaf blight affected 4–21% profit fall in seed yield in viable cultivars like Sonalica (20%), Akber (14%), Kanchan (8%) and Aghrani (4%). Bazlur Rashid *et al.* (1994) showed the effect of spot blotch on some yield parameters and grain features of wheat *viz.* spike size, no. of seed/spike and 1000 seed weight. The infection of *B. sorokiniana* on flag leaf implicated maximum crop damage, whereas disease on third leaf produced minimum loss. When assessed on the parameters *viz.* no. of seed/spike, seed weight/spike and thousand seed weight, production failure was highest in Kalyansona (81.68%).

During Rabi season at three locations of grower's field, yield reduction was estimated due to spot blotch of wheat (Anon. 1994). It was observed that Tilt 100 EC sprayed from the arrival of infection four times at fifteen days' gap, reduced percent blighted leaf area and loss in grain yield. The average yield loss was 12.86%. The 1000 grain weight of non-sprayed and sprayed plots varied from 37.4 to 41.3 g and 43.0 to 46.0 g, respectively. Alam *et al.* (1995) assessed the crop reduction of wheat (Kanchan) owing to spot blotch at cultivator's field at four locations–Dinajpur, Jessore, Jamalpur and Ishwardi. Propiconazole was sprayed at 0.04% that significantly decreased the severity. They reported the mean reduction of seeds owing to spot blotch at Dinajpur, Jessore, Jamalpur and Ishwardi were 13.9, 16.2, 14.8 and 14.5% in that order. Yield loss was 14.97% in average over the locations.

Hossain and Azad (1994) reported that the artificial inoculation of wheat plant with *B. sorokiniana* at flag leaf stage decreased the no. of grain/spike and thousand seed weight by 7–100 percent and 12–100 percent. They used nutrient element particularly micro elements Cu, B and Mo as a substitute technique of spot blotch management. On the other hand, use of B improved kernel maturation and productivity. Under natural field conditions and controlled inoculation, Hossain *et al.* (1998) found that spot blotch induced by *B. sorokiniana* decreased production up to 40 percent and 88%, respectively, over control (untreated).

Villareal *et al.* (1995) tested twenty-five spring bread wheat varieties for crop production and other characteristics for two years in Poza rica, Mexico, fungicide-protected or unprotected, under natural evidence of leaf blight. Fungicide-protected plot yields were 43.2% higher than unprotected. Singh *et al.* (1997) estimated that yield loss 21.72% and 1000 grain weight loss being 9.8% in wheat cultivars HP-1633 was caused by leaf blight pathogen, whereas cultivar UP-262 showed 18.34% yield loss and 9% loss in 1000 grain. *B. sorokiniana* is expected to cause annual yield reductions of 10–15%. Wheat plants suffer the most harm in their blooming and milk stages. Saari (1998) summarized a variety of regional research from South Asia and concluded that leaf blights caused a 20% output decline in general. At the greatest disease incidence, Bazlur Rashid and Fakir (1998) assessed a yield drop of 57.6 and 64.5% in variety Kanchan and Sonalika, accordingly, owing to *B. sorokiniana*.

Mahto (1999) reported that in Nepal during 1994-95, yield drop for Helminthosporium leaf blight (HLB) of wheat, causing 16.19 to 28.99% and the 1000 seed weights were reduced by 6.97 to 15.78%. 1000 seed weight, plant height, days to booting and crop yield were negatively interrelated with AUDPC. Zhang *et al.* (1999) investigated the loss of wheat production in a field experiment in Heilongjiang province, China and discovered that the fungi (*B. sorokiniana*) that causes foliar blight and head blight during the ripening stage lowered the crop yields of wheat substantially. They also found the relationship between head blight and diseased grains which led to a loss in seed quality. They found that the diseased grains increase with the spreads of head blight.

Spot blotch caused the most output loss (15.5%) in UP 2338, according to Pandey and Tiwari (2001). In UP 2338 and UP 262, the decline in test weight was 5.76 and 7.2%, accordingly. In comparison to UP 115, HD 2329, HUW 895 and Kalyansona (yield loss 18.7–33.5%), the damage owing to spot blotch in five wheat varieties (HUW 206, UP 262, UP 2121, PBW 299, and UP 2338) was smaller since these experienced less loss in yield (8.5–15.8%) and test weight (7.3–15.1%) despite similar disease severity (58.5–67.6%). They summarized grain losses due to spot blotch of wheat (*B. sorokiniana*) from 24–27% in highly susceptible cultivars.

Siddique *et al.* (2006) conducted repeated trials in a warm area of Bangladesh during the 2003 and 2004 winter season at two places: grower's farm and research centre. Leaf blight impacted 60% of the crop, resulting in crop losses of 2–22 percent. Krishnendu *et*

al. (2011) saw that great grain loss happened when flag leaf and the leaf below the flag leaf become diseased before the arrival of spike.

2.8 Variation of *Bipolaris sorokiniana* isolates

Considerable number of investigation work have been conducted by the researchers worldwide on the variability of the fungus, *B. sorokiniana* in respect of cultural, physiological and pathogenic specialization. Christensen (1922) found four biologic specializations of *H. sativum.* These forms differed physiologically as is showed by the degree and characters of their growth on the similar or dissimilar medium. They showed variation in width, length and number of septation of conidia. The spores of these forms make diverse grades of disease symptoms on the same cereals and grasses. Efforts to recognize the races in *H. sativum* were made earlier in 1925 (Christensen 1925) and no less than 37 races of this pathogen were acknowledged. Variation among the monoconidial cultures of the same isolates and even among the hyphal tip culture from a single germinating spore of *H. sativum* were studied. Luttrell (1955) set up diverse measurements of spores in different isolates of *B. sorokiniana*.

Shoemaker (1959) described that the conidia of *B. sorokiniana* are fusoid, straight or cuved with bipolar germination and characterized by dense-walled, oblique conidia (60–120 µm ×12–20 µm) with 4–8 septa. Ashworth *et al.* (1960) tested wheat, oats and sorghum for differential susceptibility to single conidium derived strains of *H. sativum.* They classified the isolates from wheat into six pathogenic groups. Tinline (1962) stated the variation in the asexual populations of the fungus *B. sorokiniana* was supposed to be because of parasexual recombination.

Wood (1962) described that the different isolates of *B. sorokiniana* differed strictly in respect of their parasitic compatibilities, irrespective of the plant source or the ecological origin. But simultaneously it may also be taken into deliberation that the pathogen is tremendously variable and its offsprings from a spore may be different in pathogenicity. Continuous mutation and salvation are further complications which make race identification quite impossible.

Harding (1975) reported that initial pH and sucrose concentration of the medium markedly affected sporulation and conidial characteristics specially septation in four *Bipolaris* species. *Bipolaris sorokiniana* and *B. zeicola* produced spore high at all pH levels; *B. setariae* and *B. maydis* created rather less spore. Maximum isolates of *B.*

sorokiniana made conidia huge at all sucrose levels; spore formation by *B. zeicola* and *B. maydis* was less, specially at more sucrose concentrations.

Mehta (1981a) tried race identification on a primary set of 96 single spore isolates achieved from 41 municipal areas including five diverse states and fifty-one differential varieties in Brazil and documented the presence of 32 races of *B. sorokiniana*. These races, when tested on adult plants, varied amazingly from one another with regard to sporulation and blighted area. In addition, the reaction pattern of races was differed in general after the isolates had been preserved for 8 to 10 months (Mehta 1981b). For this cause, the term 'strain' for *B. sorokiniana* has been questioned. On the other hand, with the formation of the typical differential set of varieties, it is not impossible to detect the races with diverse virulence whenever by new single spore isolates.

Sivanesan and Holliday (1981) described relatively similar type of spore of *B. sorokiniana* having straight to bent, 3–12 septa with olivaceous brown colour. Misra *et al.* (1981) observed that isolates from Dholi (Bihar) and Bhubaneshwar (Orissa) differ in their pathogenicity on eight wheat cultivars. The Dholi isolates were more aggressive than the Bhubaneshwar individual. They later reported that similar differences also occurred in five isolates of the pathogen from wheat, barley and triticale from diverse locations. They also recognized leaf isolates of *B. sorokiniana* were more aggressive than others.

Harding (1984) isolated the spore masses of *B. sorokiniana* from naturally diseased sub crown internodes of barley and wheat. On normal medium the mycelium looked as white, brown or grey in colour. There were noticeable variances in colony characters on a range of media having differences in protein hydrolysates. The spore formed a toxin, that inhibited seed viability of wheat and all were pathogenic on sub crown internodes of Neepawa wheat. Dehne and Oerke (1985) reported a biotrophic stage in the course of disease on cereal foliage support the indication that physiological differentiation and racial specialization presents in *C. sativus* populations regionally.

In an experiment on the colony characters and spore formation of *B. sorokiniana*, Raguchander *et al.* (1988) found oatmeal and PDA giving the superior radial colony diameter and spore formation of the pathogen among 12 media tested. Conner and Atkinson (1989) isolated *C. sativus* from wheat and demonstrated that isolates were

highly virulent on their original host species, but weakly virulent on the alternative host species.

Ahmed AU (1989) tested sixteen spring wheat cultivar for their resistance to the various diseases caused by *C. sativus* by five Montana isolates and four Bangladesh isolates. Ten cultivars were resistant to root rot, eight cultivars were resistant to foliar spot blotch, and six cultivars were resistant to head blight or black point. Some cultivars were resistant to all isolates from both Bangladesh and the USA. The isolates tested differed significantly in pathogenicity. Isolates obtained from roots were able to attack foliage/heads and vice versa. The isolates from Bangladesh did not have a higher temperature requirement than the USA isolates. The maximum disease development for root rot, foliar and head blight/black point phases occurred at 30°C with a 72-hour exposure to moist conditions. Hossain (1991) reported that wheat germplasm/line/cultivar(s) varied from one another in respect to their disease reaction against the different isolates of *H. sativum.*

Hetzler *et al.* (1991) studied the pathogenicity with a universal assemblage of *C. sativus* single spore isolates on a divergence set of eleven wheat cultivars and lines, using detached leaf tests which revealed 15 pathotypes. It was calculated from several nations isolate collection that 1–2% variance was due to interaction and it was assumed that several genes for virulence were operating in the pathosystem.

Pascual and Raymundo (1995) observed cultural variability among 200 isolates of *H. sativum* when cultured on potato dextrose agar, wheat extract agar and V-8 juice agar. They found a different variation in growth characters among 112, 16 and 118 isolates in PDA. They also stated that the isolates varied in aggressiveness as expressed by incubation time, no. of spot/3 cm^2 leaf area and spot diameter in a commercial wheat variety Trigo 3. Of the 200 isolates assessed 16 and 140 were most aggressive but 118 showed the minimum aggressiveness.

Hetzler (1992) recommended an ecological strain diversity of *B. sorokiniana* as he found a affinity for isolates from hot and dry areas to be minimum infectious and maximum infectious isolates came from southern and central Africa. Bazlur Rashid *et al.* (1992) saw little disparity in the prevalence of seed borne *B. sorokiniana* on wheat with respect to cultivar and geographic location but marked variation was found with respect to seed sources. Hossain and Azad (1992) gathered 83 isolates of *C. sativus* from 7 areas of

Bangladesh and they identified differential colony growth parameters and spore formation capability of the isolates on potato dextrose agar medium regarding area.

Bakonyi *et al.* (1993) found the variation among the isolates of *Bipolaris* spp. in respect of pathogenicity in artificial inoculation experiment. Valim *et al.* (1997) obtained 10 isolates of *B. sorokiniana* from 4 wheat growing areas in Brazil and tested all the isolates for pathogenicity. The isolates showed pathogenic and morphologic variability.

Valjavec-Gratain and Steffenson (1997) studied 33 isolates of *C. sativus*, including one from wheat root, on three differential barley genotypes for their virulence. Based on infection response of the genotypes, 3 pathotypes were identified and were designated 0, 1 and 2 to the coded triplet system of nomenclature. Pathotype 0 (low virulence on the 3 genotypes), Pathotype 2 (high).

Ahmed *et al*. (1997) observed physiological and morphological diversity amongst twenty-seven isolates of *C. sativus* composed from fourteen districts of wheat cultivating areas in Bangladesh. Colony colour were ash brown, olive green, light green or dark green with regular or wavy margins, fluffy, spread or velvety texture and with or without sector. No. of cells/spore differed from 3–10 and length and breadth of spore differed from 35–270 µm and 15–65 µm depending on isolates. They assembled 27 isolates into 4 individual groups. Among them three belonged to cluster I, six to cluster II, fourteen to cluster III and four to cluster IV. Alam *et al.* (1997) investigated the variation of twenty-seven isolates of *B. sorokiniana* and found 7 dissimilar morphological and physiological variances among isolates.

Debnath (1997) evaluated the incidence of chromogenic variant in *B. sorokiniana* and he nominated pigment producing and non-pigment producing isolates of *B. sorokiniana* as chromogenic and non-chromogenic, correspondingly. The colony colour of non-chromogenic isolates were brown to black, compact, relatively regular margined and formed more or less small conidia than that of chromogenic one, while the chromogenic ones were fluffy, cottony white, more or less irregular and formed comparatively larger conidia.

Maraite *et al*. (1998) experienced the twenty-seven isolates of *B. sorokiniana* in diverse colony colour on normal medium from white to light pink and dark green. The dark coloured colony exhibited a solid association with virulence of the fungi. They stated that great alterations in host reaction after inoculation wheat with isolates from separate

ecological regions, presenting a large number of potential gene-for-gene interactions joining both horizontal and vertical resistance types. They also stated that most of the isolates were capable to overcome promising sources of resistance under organized environments, proposing the threat of pathogen acclimatization to resistant genotypes and identification of extra specified or more virulent pathotypes.

Adhikary (2000) evaluated the diversity among 122 monoconidial isolates of *B. sorokiniana* assembled from all the main wheat producing locations of Bangladesh. He categorized the isolates into four clusters based on major factor analysis. The cluster I, II, III and IV confined 22, 8, 14 and 78 isolates, separately. Eight isolates of cluster II were found to be most aggressive and 78 isolates of cluster IV were the least aggressive.

Ahmed (2001) composed 262 isolates of *B. sorokiniana* from 16 wheat cultivating districts of Bangladesh and he classified these isolates into 13 physiological groups based on their cultural variability *viz.* colour, shape and compactness of the colony on PDA. Out of 262 isolates, he tested 43 isolates on five china and six Brazil differential varieties and identified six pathotype.

Mahto *et al*. (2002) described the pathogenic nature of predominant isolates of *B. sorokiniana* gathered from diverse agro ecological zones on wheat cv. Wafaq-2001. *Bipolaris sorokiniana* has a huge phenotypic diversity, the genetic variability of the pathogen has not been wholly investigated. Assessment have been made of isozyme polymorphisms among isolates (Oliveira *et al.* 2002).

Chand *et al*. (2003) assessed the variation in normal populations of spot blotch fungi (*B. sorokiniana*) and categorized the isolates into 5 groups on the basis of morphological variation of colony. They found that the most of the isolates (44.63%) of black suppressed type in the regular population were of most virulent and was acknowledged as the epidemic population as compared to the lowest frequency of the isolates (4.96%) of white coloured having very few conidia. In most of the locations in next year study, the black coloured cultures were found more as compared to previous year may be due to nonstop cultivating of similar susceptible variety and vital launch of this seed and soil borne pathogen.

Iram and Ahmad (2004) categorized isolates of *B. sorokiniana* along with their virulence activities based on disease severity scale. A tree was created based on the pattern of bands

which highlighted the correlation between morphological, virulence and genetic disparities of *B. sorokiniana.*

Aminuzzaman *et al.* (2005) composed isolates of *B. sorokiniana* from seventeen wheat varieties of 18 district of Bangladesh. Sixty-five physiological races compromising 12 pathotype were identified. 12 pathotypes were identified from each of Dhaka and Rajshahi division, whereas 9 from Mymensingh and a single pathotype was identified in each of Feni, Kishorgonj, Kushtia, Sirajgonj and Thakurgoan. The greatest dangerous pathotype was found in Jamalpur and Pabna.

Pandey *et al.* (2005) described the aggressiveness, morphological and physiological variation of thirty-five *B. sorokiniana* isolates together from diverse geographical areas in Brazil and other countries. The isolates were assessed for their morphological variation, considering mycelial colour, sector development and growth rate. Based on these morphological features, the isolates were clustered in five different morphological groups. The consequences found from inoculation of seeds and seedlings exposed that isolates from the similar geographic area and morphological group had diverse aggressiveness.

Singh (2006) stated that colony shape of *B. sorokiniana* isolates from different parts of wheat and barley were observed in terms of colony colour and diameter, and shape and size of conidiophores and conidia. Barley isolates had maximum and wheat isolates minimum colony diameter.

Iftikhar *et al*. (2006) detected 4 different colours of the colony of the *B. sorokiniana*. Among these the black cultures spore formed abundantly and had suppressed type of colony. Most were showing grayish to brownish color and few were of albino (whitish) type with few spore. The measurement of the spore of some isolates in 2005, have more breadth and length with fewer number of septa than the isolates during 2004. They stated the spore of isolates of 2004 were to some extent bent with brown to olive brown while in 2005 collection the conidia were dark brown, slender and mildly bent; few were straight and light brown to brown.

Jaiswal *et al*. (2007) evaluated one hundred fifty-five isolates of *B. sorokiniana* of wheat for their morpho-pathological classification. These isolates were clustered in 5 classes– black, brown/dull black, gray cottony growths, dull white/greenish black and white on the basis of their growth behavior. The incidence of the black suppressed type was highest

(45.63%), while the white isolate showed minimum occurrence (6.96%) in the regular population.

Poloni *et al.* (2008) conveyed that *B. sorokiniana* has a great physiological and morphological variation that creates a hard job to manage the pathogen. They designated twenty-one isolates of *B. sorokiniana* and from them polysporic and monosporic cultures were achieved. The morphological features such: colouration, edge, apparent texture, above medium mycelium and colour and size of the sectors and the growth pattern of the isolates were investigated in four altered media: potato dextrose agar (PDA), Sabouraud maltose, Sabouraud galactose and Sabouraud glucose. The monosporic cultures did not show major variance in the growth pattern among the diverse media. Though, a minor morphologic variability, plus on the replications of the isolates in the identical medium was gotten. Polysporic cultures exhibited a great morphologic variation amongst the 4 medium.

Pandey *et al*. (2008) composed isolates of *B. sorokiniana* from the foliage and kernels of naturally cultivated wheat at 4 different locations in eastern Gangetic plains of India. Eighty-six clonal isolates resulting from a single isolate (gray with white patches, Group III), which separated in an equivalent amount of parental and nonparental forms, were studied. The isolates showed morphologic variation to be correlated to the pathologic diversity.

Poloni *et al.* (2009a) evaluated the aggressiveness, morphologic and physiologic variation of thirty-five *B. sorokiniana* isolates together from diverse geographical areas in Brazil and other countries. The isolates were assessed for their morphologic variation, allowing for colony colour, sector development and growth frequency. Based on these morphological features, the isolates were clustered in five diverse morphological clusters.

Poloni *et al.* (2009b) studied the diversity of isolates of *B. sorokiniana* by dint of vegetative incompatibility. Thirty-five isolates of *B. sorokiniana* from diverse ecological states in Brazil and other countries were studied. The vegetative incompatibility among the isolates and the effects of altered culture media on these responses were assessed. Eighteen of 31 oppositions exhibited vegetative incompatibility.

Iftikhar *et al.* (2009) gathered fungi from leaf samples of wheat of diverse agrological regions was categorized on the basis of culture/colony colour and texture, spore morphology and pathogenic features. The normal size ranged from 38.3–65.8 µm x 12.3–

25 µm with a little curved, brown to olive brown with 2–13 septation. More or less isolates had comparatively long and broad slender spore, whereas some were consistently straight and cylinder-shaped and light brown in colour.

Asad *et al*. (2009) categorized *B. sorokiniana* isolates into four groups having black, grayish black, brown and albino (whitish) colony colour with abundant spore and suppressed type of colony to fluffy and fewer sporulation type. All the isolates did not display variance in pathogenicity performance by making the symptoms on leaves but their response wide-ranging in terms of virulance.

Aggarwal *et al*. (2009) also stated diverse pathogenic variation among isolates of *B. sorokiniana* when inoculated on a set of fourteen different hosts (wheat). On the basis of average infection index and disease response on different hosts, five pathotypes were recognized. Pathotype 1 was minimum aggressive having the highest amount of isolates and pathotype 5 was greatest aggressive which showed 'S' to 'HS' response on most of the difference hosts.

Srinivas *et al*. (2009) conveyed great variation in 103 isolates composed from diverse geographic regions of India, on the basis of their morphologic features, pathogenic behavior and DNA fingerprinting. Based on their growth performance the isolates were characterized into 5 clusters. The incidence of the off white/greenish black colony type was the highest (38.83%), while both black, suppressed type and white fluffy type colonies exposed the lowest rate (11.65%) in the fungi. Total 40 isolates from five recognized clusters were more studied for growth percentage, spore formation, pathogenicity and molecular variation. Colony diameter after seven days of inoculation extended from 20.3–63 mm, spore formation range was ($10x10^7$/colony) to ($1.0x10^7$/colony). No spore produced in one isolate even after15 days of inoculation.

Aminuzzaman *et al*. (2010) evaluated 86 isolates of *B. sorokiniana* isolated from foliage and kernels of 17 cultivars of wheat composed from 18 main wheat producing areas of Bangladesh. The isolates diverged considerably regarding colony growth, sporulation ability, size, septa, shape and colour of spore. The isolates were categorized into nine cultural groups based on colony characters. The highest number of 34 isolates formed effuse black regular colony with an occurrence of 39.53%, while 29 isolates formed effuse black irregular colony with an occurrence of 33.72%. Cultural group 6 having

blackish whitish irregular colony (EBWI) seems to be more virulent than others resulting seedling inoculation and detached leaf assay.

Knight *et al.* (2010) discovered variances inside Australian *B. sorokiniana* populations by cluster analysis of amplified fragment length polymorphisms in genomic DNA of 48 *B. sorokiniana* isolates brought from the northern cropping areas of Australia. Cluster analysis of the phenotypic infection reaction scores grouped isolates into three pathogenicity clusters demonstrating low, intermediate or high pathogenicity. The consequences of this experiment recommend variance within Australian populations of *B. sorokiniana* in relation to host tissue specificity.

Sharma *et al*. (2011) acknowledged eight single spore isolates (I_1-I_8) of *B. sorokiniana* from foliar blight of barley leaves on colony growth, colour, sporulation and conidial size. The regular length and breadth of spore of several isolates differed from 62.0–85.25×25.57–27.90 mm with 3–8 no. of septa.

Chauhan *et al*. (2017) studied morphologic variation and pathogenicity in *B. sorokiniana* affecting leaf blight of wheat (*Triticum aestivum*, *T. durum*, *T. dicoccum*) in India. Kumar and Rai (2018) stated among nine culture medium, for radial growth and biomass production of *B. sorokiniana* oat meal medium was best, but the highest numbers of spores/ml were recorded in potato dextrose agar media.

Singh *et al.* (2018) ten isolates of *B. sorokiniana* was studied on cultural, morphological and pathogenic variability. The variation was, colony colour–grey to dark brown and white to light grey with regular to irregular margins, length, breadth and septation of conidia 35.07–60.53µm, 13.20–17.60µm and 3.9–6.3. During pathogenic variation two were highly virulent, two were least virulent and rest were categorized as moderately virulent.

Momtaz *et al.* (2019) studied cultural variation of the particular isolates of *B. sorokiniana* through hyphal compatibility. Cultural variability was considered on the basis of colony colour and quality on PDA medium. They were–Black Mat (B-M), Black Fluffy (B-F), Ash Mat (A-M), Brownish Ash Fluffy (BrA-F), Blackish Ash Mat (BlA-M), Whitish Ash Mat (WA-M), Greenish Ash Fluffy (GA-F) and Pinkish White Mat (PW-M). The percent of compatible, partially compatible and incompatible confrontation was 26%, 19% and 55%.

2.9 Management of leaf blight of wheat

2.9.1 *In vitro* evaluation of fungicides against *Bipolaris sorokiniana*

Singh and Chauhan (1995) evaluated the effectiveness of Dithane M-45 (0.25% and 0.30%), Tilt 25 EC (0.025% and 0.05%) and Topsin M (0.05% and 0.10%) against *H. sativum*, foliar blight pathogen of wheat inside petri plates and also in fields. Tilt (500 ppm) delivered important resistor of pathogen at 24 and 28 hrs, interval of incubation, *in vitro*.

Giri *et al.* (2001) reported that diseased grains grown up on damp cotton in test tubes yielded brown necrotic spots on leaf blade and sheath of the emergent sprouts within 10 to 15 days. Diseased cells when isolated on PDA, produced culture of *B. sorokiniana*. Among the fungicides used (carbendazim at 1 g/kg and captan, thiram and mancozeb each at 3g/kg seed), mancozeb was the highest efficient (90.5%) to protect kernels from infection, followed by thiram (84.2%). Germination performance was also better in mancozeb (34.65%), followed by thiram (34.15%). carbendazim failed to seed borne infection.

Hasan *et al*. (2008) evaluated the effect of five fungicides namely hexaconazole, carbendazim, mancozeb, difenoconazole+propiconazole and propiconazole at different concentrations (100, 200, 300, 400 and 500 ppm) on the radial colony growth of *B. sorokiniana*. 100% colony diameter reduction with the use of propiconazole, Hexaconazole and difenoconazole+propiconazole at all application was succeeded.

Kavita *et al*. (2017), using food poison technique studied 15 fungicides on radial hyphal growth of *B. sorokiniana* on barley at 0.05, 0.5, 0.1 and 2% concentration. Propiconazole (0.1%) and (0.05%) inhibited radial mycelial growth by 87.77 % and 81.57% followed by hexaconazole (0.2%) and (0.1 %) by 77.98% and 70.37%.

In Nepal, Angdembe *et al.* (2020) evaluated four fungicides (mancozeb 75% wp, copper oxychloride 50% WP, carbendazim 50% WP and thiram 75% WS) with three concentrations (100, 200 and 400 ppm) against spot blotch pathogen *B. sorokiniana* of barley. All concentrations of copper oxychloride and 400 ppm of mancozeb were capable to prevent more than 50% colony diameter of the fungus.

Magar *et al.* (2020) eight fungicides *viz.* Sectin, Curex, Bavistin, Vacomil plus, Saaf, Sajha, Criptan and Tilt at different application levels (25, 50 and 100 ppm) excluding Curex (50, 100 and 200 ppm) were used to calculate the influence on the colony diameter

and reduction percentage of the fungus. Tilt was the most efficient chemical that totally constrains the hyphal progression at all applications. As well, fungicides *viz.* Sajha (79.78%), Saaf (73.59%) and Sectin (70%), at 100 ppm were operative in controlling the pathogen than others. Hyphal growth reduction of the fungus was found to be amplified with the increase in ppm of chemicals.

2.9.2 *In vitro* evaluation of leaf extracts against *Bipolaris sorokiniana*

Ashrafuzzaman and Hossain (1992) assessed pudina (*Mentha viridis*) extract against *B. sorokiniana* and detected the extract reduced hyphal growth and germination of conidia. They also found that extract of castor (*Ricinus communis*) and danta kalash (*Leucas aspera*) were also found protective against *B. sorokiniana.*

Hossain and Schlosser (1993) reported excellent antifungal effect of neem (*Azadirachta indica*) leaf extracts in regulating *B. sorokiniana* infection on wheat seed. They also studied the possibility of using neem (*A. indica*) seed extract against *B. sorokiniana* of wheat. On wheat leaves, both extracts suppressed pathogen development and decreased pathogenesis. After treatment with neem seed extract, the germination percentage of wheat seed rose.

Ganguly (1994) demonstrated *in vitro* antifungal efficacy of aqueous extracts of *Catharanthus roseus*, *Lantana camara*, *Ocimum sanctum*, *Solanum melongena*, *Azadirachta indica*, *Polyalthia longifolia*, *Aegle marmelos* and *Datura metel* foliage towards *Pyricularia oryzae* and *Helminthosporium oryzae*. *C. roseus* extract inhibited the most colony development and sporulation in both fungi, followed by *P. longifolia* and *A. indica* extracts. Extracts of *D. metel* had lowest antifungal activity. Ahmed and Islam (2000) used neem, garlic, onion and bishkatali (*Polygonum hydropiper*) extracts, among them neem and garlic extracts were efficacious towards *Bipolaris oryzae* at 1:1 dilution.

According to Khaleduzzaman (1996), garlic was shown to be the most effective in lowering seed-borne infections and improving seed germination %, followed by ginger, neem and bishkatali extract. The seeds were treated with crude extract and dilution extracts (1:1 v/v) of the specified plant species. Greater outcomes were obtained with neem bark, garlic cloves and bishkatali. The use of alcoholic extracts of neem bark and garlic clove to suppress seed-borne fungus in wheat yielded better outcomes. The accuracy achieved using diluted alcohol extracts of gagra and garlic clove.

Bishkatali, garlic, ginger, and Neem extracts were shown to be beneficial against seed-borne diseases of *Alternaria alternata*, *B. sorokiniana*, *Curvularia lunata* and *Fusarium* spp. of wheat by Rahman *et al.* (1999). Garlic, however, outperformed the other extracts, next by ginger and neem.

Hossain *et al.* (2005) found that extracts from bishkatali (*Polygonum hydropiper*), vatpata (unknown scientific name), garlic, gagra (*Xanthium italicum*), bitter gourd (*Momordica charantia*) and neem were tried towards fungi allied with wheat seeds using standered blotter method. Five different fungi were detected in wheat seeds, i.e. *B. sorokiniana*, *A. alternata*, *C. lunata*, *Fusarium* spp. and *Aspergillus* spp. All extracts showed significant activity against these fungi, with crude extracts being more effective than dilluted extracts. Neem, followed by garlic, bishkatali and vatpata, exhibited the greatest fungicidal activities.

Hasan *et al.* (2005) investigated the efficacy of ten plant extracts obtained from *Zingiber officinale* rhizomes, *Allium sativum* and *Allium cepa* bulbs, *Adhatoda vasica* leaves, *Lawsonia alba*, *Azadirachta indica* and *Achyranthes aspera* leaves, *Cuscuta reflexa* stems, *Vinca rosea* roots, and *Nigella sativa* seeds against seed-borne pathogens of wheat. All plant extracts decreased the occurrence of seed-borne fungus, improved seed germination, increased the number of healthy seedlings and boosted the vigour index. The intensity of *B. sorokiniana* was entirely suppressed by alcoholic neem and garlic extracts.

On conidial germination of *Bipolaris sorokiniana*, Akhter *et al.* (2006) evaluated eight ethanolic plant extracts, ten aqueous plant extracts in conjunction with cow dung and five aqueous plant extracts in connection with cow urine. Conidial germination was fully suppressed by ethanolic extracts of *Adhatoda vasica* (leaf) and *Zingiber officinale* (rhizome) at a concentration of 2.5 percent. After treating with *Vinca rosea*, Piper betle and *Azadirachta indica* extracts in conjunction with cow dung, 100% suppression of conidial germination was observed, with *Rauwolfia serpentine* (30%) extract showing the lowest inhibition at the same dose. At a 2.5 percent concentration of *Calotropis procera* extracts in conjunction with cow urine, there was a 91 percent inhibition on conidial germination. *Ocimum sanctum* extract had a lower inhibitory impact in most situations.

Islam *et al.* (2006) used eight plant extracts and Vitavax-200 to treat wheat blight disease (*B. sorokiniana*) and found that onion, garlic, kalijira, ginger, biskatali and neem extract yielded crop production that were significantly equal to seed treatment with Vitavax-200.

Hasan *et al*. (2008) evaluated plant extracts and fungicides against *B. sorokiniana*. The effect of five botanical extracts, namely garlic, onion, ginger, neem and black cumin, on the mycelial development of *B. sorokiniana* at various composition (5, 10 and 15%), revealed that garlic extract had the highest percent suppression of colony growth by 67.5% at 15% concentration. Among the five fungicides Tilt-250 EC considerably reduced the hyphal growth of *B. sorokiniana*.

Hasan (2013) examined the efficacy of plant extracts in reducing seed-borne fungi related with wheat seeds. Garlic bulb, margosa leaf and ginger rhizome extracts decreased seed-borne fungal infections as well as the abundance of identified target fungus *B. sorokiniana*, *F. graminearum*, *A. flavus* and *A. alternata* among 10 plant extracts. Allicin is the most significant physiologically active ingredient in *A. sativum* crude extract, according to Perello *et al.* (2013), and it has been proven to be highly active against several fungal species. It may have hindered radial colony development and spore germination in *P. tritici-repentis*, *B. sorokiniana* and *Septoria tritici* on agar plates.

Katooli *et al.* (2014) performed an *in vitro* experiment to determine the effectiveness of eucalyptus essential oil towards *Pythium ultinum*, *Rhizoctonia solani* and *B. sorokiniana*, three plant pathogenic fungus. Only in *P. ultinum* and *R. solani* did eucalyptus essential oil in all concentrations fully block mycelial development. *B. sorokiniana* demonstrated perfect inhibition for the first 5 days, but thereafter mycelial development and non-inhibition.

When Yadav *et al.* (2015) tested extracts of eucalyptus leaf, garlic clove, neem leaf and neem cake in wheat plant, they discovered that two applications of aqueous eucalyptus leaf extract at the tillering and boot leaf stages led to better wheat production than other botanical extracts.

Magar *et al.* (2020) evaluated the impact of five botanicals, namely neem, garlic, eucalyptus, bojho and asuro, at three different doses (5, 10 and 15%), on the pathogen's radial colony growth and mycelial growth inhibition percentage. The application of garlic clove extract (52.85%) at a concentration of 15% inhibited the most mycelial growth, following by bojho (52.48%) at a concentration of 15%. The fungus's mycelial growth inhibition was observed to increase as botanical concentrations rose.

2.9.3 *In vitro* evaluation of antagonists against *Bipolaris sorokiniana*

Pesticides were widely used, which harmed soil quality, water quality, and ecological balance, as well as the socio-economic situation. Pesticide resistance is also one of the country's most concerning issues. The only way to solve these issues is to use a biological approach to sustainable agriculture.

B. sorokiniana has a good antagonistic capacity against *Cercospora* species, according to Zhang and Pfender (1993). *B. sorokiniana* has a same antagonistic capacity against the wheat parasite *P. tritici-repentis*. *Stenotrophomonas maltophilia* strain C_3 was estimated for control leaf spot on tall fescue (*Festuca arundinacea*) caused by *B. sorokiniana* (Zhang and Yuen 1999). Mandal *et al.* (1999) reported that inhibitory effect of *Trichoderma reesei*, *T. pseudokoningii*, *T. hamatum*, *Talaromyces flavus*, *Chaetomium globosum* and *Trichothecium roseum* on radial colony growth of *D. sorokiniana* was witnessed. Culture filtrates of these fungi inhibited spore germination equal to 92 percent. Dal Ballo *et al.* (2003) reported the biocontrol efficiency of *Epicoccum purpurascens*, *Gliocladium roseum*, three strains of *Bacillus subtilis* and *Pseudomonas fluorescens*, isolated from the rhizosphere of wheat plants, was evaluated regarding seedling blight affected by *B. sorokiniana.* Dal Bello *et al.* (2008) reported that all *Trichoderma* spp. inhibited significantly the mycelial growth of *B. sorokiniana* between 51 to 71%. However, four *Bacillus cereus* strains and one sample of *Stenotrophomonas maltophilia* showed the most inhibition.

Salehpour *et al.* (2005) founded that *Trichoderma viride* isolates T112 and MO and *T. harzianum* isolates M and T194 were used as prospective biological agents for the control of common root rot caused by *B. sorokiniana*. Cell free and antifungal metabolites created by all the *Trichoderma* isolates inhibited the growth of *B. sorokiniana*. Etebarian and Mohammadifar (2009) reported a tough antagonistic capability of widespread biocontrol means *Trichoderma viride* and *T. harzianum* against *B. specifera* under *in vitro* and micro plot conditions. Hasan (2013) conducted experiment under *in vitro* and *in vivo* conditions to estimate the effectiveness of *Trichoderma harzianum* in controlling leaf blight of wheat. The outcomes of these experiments obviously revealed that *T. harzianum* isolate RUT103 reduced radial growth of *B. sorokiniana* by 45.45 per cent after an incubation period of 8 days.

2.9.4 Chemical control of Bipolaris leaf blight of wheat

Singh and Singh (1971) observed that leaf blight of wheat was effectively controlled by six applications of Dithane Z-78 (Zineb) and Dithane M-45 (Mancozeb) with corresponding increase in yield. Ashok *et al.* (1989) reported the most effective and economic treatment of Mancozeb was three sprays, applied at 10 days intervals followed by three sprays at 15 days intervals. The yield increased by using three treatments was more than double than of only single spray.

Dithane M-45 (Mancozeb) 0.2%, Rural (0.2%), Tilt 250 EC (0.1%) and G 698 (0.2%) were evaluated controlling leaf blight disease of wheat. Three spraying were done at an interval of 15 days. Both Rural and Tilt were highly effective and reduced disease incidence equally and increased grain yield (Anon. 1989).

Experiments were done in three wheat sites to measure yield loss due to Bipolaris leaf blight, with four sprays of Tilt 100 EC @ 0.125 percent applied at 15-day intervals starting with the first manifestation of disease signs. Sprays were said to have decreased the amount of leaf blight disease, with a 25 percent reduction in grain production anticipated (Anon. 1992a). Tilt application with seed treatment (three times) resulted in less yield loss than the other application. Tilt application during the post-anthesis stage boosted yield by 17%, which was much higher than at the booting stage. Three Tilt treatments, with or without seed treatment, were comparable to post-anthesis treatments (Anon. 1992b). The average yield loss was 24%. The 1000 grain weight of non-sprayed and sprayed plots varied from 39.6 to 42.59g and 43.3 to 47.39, respectively (Anon. 1993).

Malaker *et al.* (1994) observed severity of disease at four locations under conditions of natural infection by *H. sativum* in each location there were two treatments sprayed with Tilt 100 EC @ 0.125% and unsprayed (control). Tilt 100 EC was effective against leaf blight (HLB) and disease severity were significantly different 3.04 and 3.44 for unsprayed and sprayed plots respectively. In compared to unsprayed plots, the use of Tilt 25 EC as a foliar spray resulted in increased grain number per plot, 1000 grain weight, and big seeds, resulting in a better grain yield.

During 1991-92 and 1992-93, Mondal *et al.* (1994) tested four commercial fungicides for their efficiency in controlling Bipolaris leaf blight of wheat under natural epiphytotic environments. Tilt 25 EC (0.05%) was the most effective and lucrative, having the

highest profit margin. Dithane M-45 (0.2%) and Mencozeb (0.15%) both decreased disease severity and provided a lucrative yield, but Rural (0.2%) was determined to be uneconomic and provided the lowest gross margin.

In vitro and *in vivo* studies with Dithane M-45 (0.25 and 0.30%), Tilt 25 EC (0.025 and 0.05%), and Topsin M (0.05 and 0.10%) against Helminthosporium leaf blight of wheat were conducted by Singh and Chauhan (1995). With a cost benefit ratio of 1:2:6:9, Tilt (500 ppm) gave considerable pathogen control after 24 and 28 hours of incubation, whereas Tilt (0.05%) provided substantial control as foliar treatment in the field after three sprays at a 10-day interval.

Singh *et al.* (1995) employed four fungicides to treat wheat foliar blight: Mancozeb, Tilt 25 EC, Topsin M and Rhizolex. With three sprayings of Tilt (500 ml/ha) at disease manifestation, greatest yield, grain weight, and least disease were identified among the four tested fungicides. In terms of productivity, grain weight, and disease severity, three Tilt sprays were as good as four. Mancozeb (2.5 kg/ha) was the second most effective fungicide, with four treatments at disease onset.

These treatments produced 79.5, 81.5, 82.5, 78.0 and 81.0 percent emergence in green house tests, compared to 74.5 percent for the control and 2.5, 0.6, 0.0, 5.1 and 11.1 percent plants with *B. sorokiniana*, compared to 27.5 percent for the control. Seed treatment fungicides can be used to minimize *B. sorokiniana* and other seed and soil-borne fungi main inoculum (Ahmed and Meisner 1996).

Leaf blight (HLB) is a serious wheat disease in Nepal, producing yield losses of 3.1 to 23.8 percent. Under field conditions, three applications of Tilt (propiconazole) at two-week intervals were shown to be successful. These interactions show that HLB may be handled using an integrated approach that includes host resistance, cultural practices and appropriate fungicidal treatment (Mahto and Bimb 1996). Singh *et al.* (2011) investigated the efficacy of propiconazole and tebuconazole in controlling leaf blight in wheat variety HD 2329 under field conditions in India. Wheat grain yields increased considerably with both propiconazole (5.24 ton/ha) and tebuconazole (5.08 ton/ha) treatments.

Tilt (propiconazole) and Folicur (tebuconazole) were given to FSD-85, LU-26, and Pak-81 during growth stages 10.1 (heading) and 10.5 (anthesis) in the spring of 1996 to see how they affected *B. sorokiniana* of wheat in Pakistan. A single spray of Tilt or Folicur at

the crop's heading stage significantly reduced leaf blight development and reduced the AUDPC.

Picinini *et al*. (1996) assessed for a long period the efficacy of Propiconazole applied @ 125 g ai/ha against foliar blight of wheat and the disease control was achieved with two sprays (at booting and flowering stages). Yield differences between treated and untreated plots varied from 18% in 1991 to 203% in 1982. The average yield of treated plots was 3734 kg/ha, which was 44.61 percent (1152 kg/ha) greater than the yield of untreated control plots. Tilt 25 EC (0.1 percent) was shown to be highly efficacious as a foliar spray than the impact of seed treatment with Vitavax-20 in reducing Bipolaris leaf blight of wheat in an experiment conducted by Rahman (1998).

Pandey and Tewari (2001) used Tilt 25 EC (propiconazole), Folicur 250 EC (tebuconazole), and Bayleton 25 WP (triadimefon) to suppress *B. sorokiniana*. Folicur was shown to be the most effective at controlling foliar blight, followed by Tilt and Bayleton. Folicur (16.26 percent) had the best grain production increase over check, followed by Tilt (13.47 percent) when sprayed @ 750 ml/ha. Mahto (1999) noticed that the presentation of Tilt at 125 ml ai/ha with three sprays at two weeks interval gave excellent protection upon testing various fungicides *viz*., Blitox, Captan, Dithane M-45, Thiram, Folicur, Tilt, Bayleton and Contaf against *B. sorokiniana*.

Singh *et al*. (2005) reported that the efficacy of Vitavax 200 WS (Carboxin; 2.0, 2.5 or 3.0 g/kg of seeds), Vitavax 75 WP (2.5 g/kg) and Thiram 75 WS (3.0 g/kg), applied to seeds at 24 h before sowing, against *B. sorokiniana* and *A. triticina* in wheat (cultivars PBW 373 and HUW 234) was evaluated in Karnal and Cooch Behar, Haryana, India, during 2003-04. Vitavax 200 WS at all rates completely eradicated *B. sorokiniana* and *A. triticina* in Karnal and meaningfully reduced the frequency of both pathogens in Cooch Behar.

According to Malaker and Mian (2009), foliar treatment of Tilt 250 EC (0.05 percent) under all spray schedules except 70 and 90 DAS effectively reduced disease severity compared to control. Bazlur Rashid *et al.* (2001) previously observed that foliar spraying with Tilt 250 EC at 0.1 percent concentration effectively suppressed *B. sorokiniana* infection on foliage and reduced black point occurrence in grains.

Yadav *et al.* (2015) investigated the effects of fungicides (propiconazole, carbendazim and hexaconazole) on wheat seed production and spot blotch disease. The highest

decrease in disease was achieved with two sprays of carbendazim (0.1 percent) during the tillering and boot leaf stages, followed by two applications of propiconazole at the tillering and boot leaf stages. Propiconazole, carbendazim, and hexaconazole were also found to be helpful in lowering disease levels and increasing crop output.

Selvakumar *et al.* (2015) used artificial epiphytotic field conditions to test various fungicides (captan 50% WP, carboxin 37.5% WP + thiram 37.5% WP, propiconazole 25% EC, tubeconazole 25% EC and mancozeb 75% WP) as seed treatments or foliar sprays and in combination. The best control of leaf blight under severe circumstances was seed treatment with carboxin 37.5% WP + thiram 37.5% WP @ 1.5 g/kg seed, followed by spraying with propiconazole 25% EC @ 0.1 percent at boot leaf stage.

2.9.5 Evaluation of Germplasms

Hossain (1991) screened 533 wheat germplasm from both local and exotic sources for *B. sorokiniana* infection in the field and found that none was devoid of contamination. Hossain and Khan (1993) tested the wheat materials under field condition that resulted resistant to moderately resistant reaction against *B. sorokiniana* after artificial inoculation. Out of 37 materials, 33 and 28 were found to be resistant at heading stage and soft dough stage, respectively. Under field condition, 9 and 21 materials from Brazil were recorded to be highly resistant and resistant, respectively at seedling stage. Under natural infection in the field 10 and 25 wheat material from Japan were found to be highly resistant and resistant, respectively. In flag leaf stage, only 1 and 16 materials were recorded to be highly resistant and resistant, respectively.

Singh *et al.* (1995) found that only 15 out of 257 genotypes were persistently resistant to *H. sativum*, 47 were moderately resistant, 158 were moderately susceptible, 33 were susceptible and 4 were highly susceptible during field inoculation experiments. Infection was found in all genotypes.

Ragiba *et al.* (2001) screened 567 wheat entries for resistance to Helminthosporium leaf blight in the areas of high of Pusa, Bihar and discovered 57 resistant (R), 123 moderately resistant (MR), 291 moderately susceptible (MS), 88 susceptible (S) and 8 highly susceptible lines. Chirya-3 and Mayoor from CIMMYT, Mexico, were the most resistant lines.

Molan *et al.* (2001) used a green house to test the sensitivity of 18 wheat genotypes and two wheat varieties (Yecora Rojo and West bred) to *B. sorokiniana*. Spore suspension

containing 4×10^5 spore/ml of fungus were applied on seedlings (14 days old). Disease severity was assessed 21 days after application. None were resistant to *B. sorokiniana*, although 12 were moderately resistant, including Yecora Rojo and West bred, while 8 were susceptible genotypes.

24 wheat genotypes were put in Petri-dishes and infected with spore suspensions of *H. sativum* isolates by Akram and Singh (2001). They discovered five genotypes that were resistant. Sharma and Duveiller (2003) suggested that selection for HLB resistant wheat lines with high grain yield and grain weight was possible using selection index.

Akram and Singh (2003) reported that out of 148 genotypes, none was immune to the leaf blight disease, 26 genotypes which showed a disease leaf area coverage between 1 and 10% on the flag leaf, were rated as resistant and 33 genotypes as moderately resistant (10-30%) under greenhouse condition. The response of the genotypes to *B. sorokiniana* varied depending on the environmental conditions and different inoculum loads. In the following year, 24 wheat genotypes were shown in field (UP-India) and under greenhouse conditions. Out of 24 genotypes retested for resistant genotypes showed higher incubation period, lesser number of spores, low disease severity and low sporulation on the flag leaf comparatively and were rated as moderately resistant in the field. Only two were resistant due to low number of spots, low disease severity and low sporulation on the flag leaf. The incubation period varied between 6-13 days in the field and 5 days in the green house for all the genotypes.

During 2001-02, Reza *et al.* (2004) tested thirty wheat genotypes in Dinajpur, Bangladesh, for resistance to Bipolaris leaf blight. Four genotypes were resistant, three were moderately resistant, six were moderately susceptible, twelve were susceptible and five were highly susceptible.

Mikhalova *et al.* (2004) reported that *Triticum durum* and *Triticum tauschii* (*Aegilops tauschii*) of CIMMYT were evaluated *in vitro* and *in vivo* for resistance to leaf blight by *B. sorokiniana*. Wheat genotypes with high and moderate resistance were identified. Ibeagha *et al*. (2005) examined the interaction of resistant wheat genotypes with *B. sorokiniana*. Wheat genotypes Yangmai 6, M3(W7976), Shanghai 4, Chirya 7 were highly resistant to susceptible control variety Sonalika.

Singh *et al.* (2007) described a wheat genetic stock known as 'Harit 1' (M 3) that is resistant to *B. sorokiniana*. It was registered by the Plant Germplasm Registration

Committee (PGRC) of the Indian Council of Agricultural Research (ICAR) in 2004 and given the registration number INGR-04023 and the national identity number 427810.

In Dinajpur, Bangladesh, Ahmed (2007) tested 385 wheat genotypes for leaf blight in 2004-05. The pathogen was resistant to moderately susceptible in fifty genotypes. During 2005-06, the entries were again evaluated in a pot experiment utilizing susceptible variety Kanchan as control and artificial inoculation. 13 genotypes were resistant to infection, with up to 5% leaf area infection. Moderately resistant, moderately susceptible, and susceptible were found to be 28, 7 and 2 genotypes, respectively. Kanchan were highly susceptible to *B. sorokiniana* infection (more than 60 percent leaf area infection).

Khan and Chowdhary (2011) evaluated 422 spring wheat germplasm in natural epiphytotic conditions against spot blotch and found that no genotype was immune, while 52 were resistant, 180 moderately susceptible, 171 susceptible and 19 highly susceptible; Chirya 3, Chirya 7 and Mayoor from CIMMYT were highly resistant both in field and polyhouse.

Singh *et al.* (2018) tested sixty-two wheat genotypes for resistance to leaf blight and found that eight of them were resistant, with disease severity of 34.26 to 35.0 percent and a AUDPC value of 330.90–402.80. The disease severity of 24 genotypes ranged from 39.45 to 57.0%, while the rest of the wheat genotypes were classified as moderately susceptible or susceptible.

2.10 Seed health test of wheat seed

A number of plant pathogenic fungi, bacteria, viruses, nematodes and even angiospermic parasites are transported by seeds. In most of the cases, infected seeds show a range of symptoms on it. These symptoms are seed rot, shriveling of grain, seed necrosis and seed discolouration. According to Neergaard (1979), noticeable brown, grey or black necrotic stain on the seed coat of many seeds are caused by the infection of many parasitic seed-borne fungi. Brown to dark brown or black discolouration mainly limited to the embryonic area of wheat seed is recognized as black point. Sometimes, the discolouration can be seen near the brush end, in the ventral crease, or on any other part of the grain. When the diacolouration affects more than one-half of the grain it is interpreted as kernel smudge. In severe infection the whole grain may be discoloured, shriveled and undersized. The discolouration may be light to dark and fairly uniform in colour or it may appear as light coloured lesions with dark margins. This type of seed abnormalities is

produced predominantly by *B. sorokiniana* and *Alternaria alternata* (Fakir *et al.*1989; Dey *et al.* 1992; Mathur and Cunfer 1993). Misra *et al.* (1969) stated *B. sorokiniana* as the most commercially significant seed-borne and phylloplane fungus of some duram and aestivum wheat. According to Vir (1974), the seed borne infection of *H. sativum* causes blight disease in wheat, barley, oat, rice and various crops.

Sharma *et al.* (1983) found a 0.35 percent correlation co-efficient between adult plant leaf susceptibility to *C. sativus* and the percentage of seed infection, implying a link between pathogen infection at the adult plant stage and seed infection. Saari (1985) found that if there is significant leaf infection and some rain during heading, the proportion of seed infection can reach 50%.

Between 1985 and 1990, a five-year research on seed-borne mycoflora of 1267 wheat seed samples from 25 cultivars was conducted in Pakistan. *D. sorokiniana* was the most common pathogen, followed by *Fusarium moniliforme* and *Cephalosporium acremonium*. *F. semitectum* (*F. pallidoroseum*), *Penicilium*, *Alternaria* spp., and *Aspergillus* sp. were among the pathogens found (Khan and Bhutta 1994). Reis (1998) reported that infected seeds were the only source of *B. sorokiniana* inoculums in regions where wheat had not been planted before.

Fakir *et al.* (1977) proven *D. sorokiniana* seed to plant transmission in wheat. They emphasized the significance of establishing a wheat seed health testing program in Bangladesh. Neergaard (1979) reported that *D. sorokiniana* causing seedling blight, foot rot, ear blight was seed transmitted.

A number of mycoflora were testified to be allied with black point affected wheat grains in Bangladesh (Fakir *et al.* 1987, Islam *et al.* 1991, Dey *et al.* 1992, Mahmud 2005). Hossain (2000), Malaker and Mian (2002) detected *B. sorokiniana*, *A. alternata*, *Curvularia lunata* and *Fusarium* spp. and observed that the incidence of *B. sorokiniana* increased with the increase in severity of black point infection. Malaker *et al.* (2007) identified 22 fungi representing 17 genera from black point infected seeds collected from major wheat growing areas of Bangladesh. The most predominant fungus was *B. sorokiniana*, which was followed by *A. alternata* and *C. lunata*. Among the fungi associated with the black point disease of wheat seeds in Bangladesh, *Bipolaris sorokiniana* (Sacc.) Shoemaker (syn. *Helminthosporium sativum*, *Drechslera sorokiniana*) appeared to be the most predominant one, which also causes seed rot or

germination failure, seedling blight, leaf blight and head blight in wheat (Dey *et al.* 1992, Hossain 2000, Misra *et al.* 2001, Malaker and Mian 2002).

In Bangladesh, black point disease of wheat occurs in almost all wheat growing areas with varying degrees of severity. The prevalence of the disease was found to vary from 2–15% in some selected commercial wheat cultivars (Ahmed 1986b). In a comprehensive survey, Rahman *et al.* (1988) recorded 4–14% black point affected seed with respect to seed tier, variety and seed source. Dey *et al.* (1992) reported 5–55% black point infection depending on different varieties grown in the major wheat areas of the country. These observations indicate an increasing trend in prevalence of the disease over the years.

In Bangladesh, the adverse effects of black point on seed germination and seedling vigor have been documented by many workers (Fakir 1988, Islam *et al.* 1991, Dey *et al.* 1992, Rahman and Islam 1998, Hossain 2000, Malakar and Mian 2002, Siddique 2003, Rashid 2005, Tonu 2006). The emergence and root and shoot growth of seedlings decreased with the increase in incidence and severity of black point infection. Plant stand and grain yield were also reduced when black point affected seeds were used for sowing (Malakar and Mian 2002). Grain weight of wheat was reported to be reduced by 42% when the grains were severely infected with the black point disease (Rahman and Islam 1998).

Malakar (2003) discovered that when black point grains were planted, seedling emergence and vigour, plant development and yield output were decreased, whereas post-emergence mortality, *B. sorokiniana* disease severity and black point incidence were all boosted. According to Özer (2005), black point has been linked to *Alternaria*, *Bipolaris*, *Fusarium*, *Cladosporium* and *Sclerotium* species of fungus.

Reza et al. (2006) conducted a study on the impact of varying degrees of B. sorokiniana infection on wheat grains and crops. He discovered that planting 30 percent contaminated grains resulted in a maximum of 15.73 percent seed rot/seedling mortality, following a severity of 75.4 percent leaf blight. While lowest of 5% contaminated grains resulted both 3.1% and 57.53% of the disease.

Hossain (2000) reported that seed germination and seedling emergence were significantly decreased with the increase in number of black pointed seed. The sample having 28% black pointed seed resulted maximum affected grain observed at the full and dead ripe stage 6.25 and 37.08%. According to Hossain (2000), as the quantity of black point seed increased, percentage of germination and seedling emergence fell dramatically. At the full

and dead ripe states, the sample with 28 percent black point seed had the most damaged grain (6.25 and 37.08 percent, correspondingly).

Singh *et al.* (2012) conducted an experiment in 5 districts of Agra zone, namely Agra, Mathura, Firozabad, Mainpuri and Etah, and discovered that *B. sorokiniana* is the most common wheat fungi, followed by *A. triticina*. *A. triticina*, on the other hand, has a smaller impact. *B. sorokiniana* and *A. triticina* had average incidence of 62 percent and 43 percent at wheat ripeness, accordingly, although *A. alternata* had an average incidence of 14.4% in April.

Islam *et al.* (2015) investigated the effect of several seed-borne pathogen on germination percentage and the efficacy of chemical treatment. During germination, he isolated *B. sorokiniana*, *A. tenuis*, *C. lunata*, *Fusarium* spp., *Aspergillus* spp., *Penicillium* spp., and *Rhizopus* spp from seed. Germ ination percentage of treated seeds (Vitavax 200, Bavistin, and Captan) was substantially greater than that of untreated seeds, and the greatest infection of *B. sorokiniana* was found in the control, at 7.75 percent. Seed treatment using Vitavax 200, Bavistin, and Captan, *B. sorokiniana* was found to be 0.25, 1.75, and 3.00 percent, respectively.

Pathak and Zaidi (2013) isolated *Fusarium moniliforme*, *Rhizopus* spp., *Mucor* spp., *Alternaria alternata, Aspergillus niger, A. flavus, Curvularia lunata*, *Drechslera* spp., *Alternaria* spp. and *Penicillium* spp. from the cultivar HD264. The optimum media for isolating fungi, whether external or internal, was determined to be the blotter technique.

CHAPTER THREE
MATERIALS AND METHODS

CHAPTER THREE

MATERIALS AND METHODS

3.1 Experiments

All the experiments were done during the periods (2011–2019) in laboratory and field, presented in Table 1.

Table 1. List of the experiments performed during the research tenure

Sl. No.	Name of the experiments	Condition
1	Isolation, identification, purification and preservation of isolates of *Bipolaris sorokiniana* collected from the leaf and seed samples	Laboratory
2	Growth study and grouping of isolates of *Bipolaris sorokiniana* leads to cultural variability	Laboratory
3	Study of morphological variability due to colony characters and conidial morphology	Laboratory
4	Pathogenic variability of *Bipolaris sorokiniana* isolates	Laboratory
5	Physiological variability of *Bipolaris sorokiniana* isolates	Laboratory
6	Compatibility test or colony interaction of *Bipolaris sorokiniana* isolates	Laboratory
7	Molecular identification of *Bipolaris sorokiniana*	Laboratory
8	*In vitro* screening of fungicides against *Bipolaris sorokiniana*	Laboratory
9	*In vitro* screening of leaf extracts against *Bipolaris sorokiniana*	Laboratory
10	*In vitro* screening of biocontrol agents against *Bipolaris sorokiniana*	Laboratory
11	Determination of number of fungicide sprays to minimize Bipolaris leaf blight disease of wheat	Field
12	Standardization of doses of fungicide named Tilt and Folicur against Bipolaris leaf blight disease of wheat	Field
13	Estimation of yield loss of wheat due to Bipolaris leaf blight	Field
14	Screening of germplasms against *Bipolaris sorokiniana*	Field
15	Quality analysis of seeds obtained from field experiments	Laboratory
16	Seed health test of different wheat varieties	Laboratory

3.1.1 Laboratory Experiments

The laboratory experiments were carried out in the Mycology and Plant Pathology Laboratory, Department of Botany, University of Dhaka, Dhaka, during the year 2013–2019.

3.1.2 Field Experiments

The field experiments of the present investigation on leaf blight of wheat and its management were conducted at the experiment field of Plant Pathology Division, Bangladesh Agriculture Research Institute (BARI), Joydebpur, Gazipur, during two successive years 2010-11 and 2011-12.

3.2 Materials

The present study is based on leaf blight infected wheat leaves and black point infected wheat seeds, which were collected during the period of March, 2011 to March, 2014.

3.3 Experiment 1: Collection, isolation, identification, purification and preservation of fungi from collected leaf and seed samples.

3.3.1 Collection of samples

Bipolaris leaf blight (BpLB) infected wheat leaf samples were collected from different wheat growing areas of Bangladesh. Seed samples were collected from Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur.

3.3.1.1 Collection of leaf samples

Wheat leaf samples infected with *B. sorokiniana* were gathered from various locations of eight districts of Bangladesh. The districts are–Dhaka, Gazipur, Dinajpur, Joypurhat, Sirajgonj, Pabna, Chuadanga and Kushtia. The samples of diseased leaves were together from twenty-one different wheat varieties (Table 2). Leaf samples were obtained from the BARI research station as well as farmer fields during the grain filling stage. The diseased leaves were cut from the plant with a scissor, put into a brown paper envelope and labeled properly with variety name, collection date and information about disease symptoms. After that, each collection's brown paper envelops were brought to the laboratory and were subjected to the process of isolation. All the 21 varieties used in the experiments from different locations were tabulated as under with symbolic/short name for easily use in the designation of *B. sorokiniana* isolates.

3.3.1.2 Collection of seed samples

The wheat seed samples were obtained from the Bangladesh Agricultural Research Institute (BARI) at Joydebpur, Gazipur. The seeds were gathered using cotton bags shortly after harvest, sun dried and stored in the refrigerator at 5°C until utilized for *B. sorokiniana* isolation. All the seed samples from six newly released wheat varieties and Kanchan were used in the experiments (Table 3). They were tabulated as under with symbolic/short name for easily use in the designation of *B. sorokiniana* isolates.

Table 2. Leaves of different wheat varieties used in this study

Sl. No.	Name of the Variety	Symbol	Year of release	BpLB reaction
1	**Ananda (BAW 18)**	An	1983	
2	**Aghrani**	Ag	1987	
3	**Akbar**	Ak	1983	
4	**Balaka**	Bl	1979	
5	**BARIGom-25**	BG-25	2010	Tolerant
6	**BARIGom-26**	BG-26	2010	Tolerant
7	**Barkat**	Bk	1983	
8	**Bijoy (BARIGom-23)**	Bj	2005	
9	**Ciano-T-79 (Mexican variety)**	C		Susceptible
10	**Gaurab (BARIGom-20)**	G	1998	
11	**Inia-66**	I	1974	
12	**Kalyansona (Indian variety)**	Kl	1968	
13	**Kanchan**	Kn	1983	Susceptible
14	**Kheri**	Kh		Land race
15	**Prodip (BARIGom-24)**	Pd	2005	
16	**Protiva**	Pv	1993	
17	**Saurav (BARIGom-19)**	Sv	1998	
18	**Seri-82 (Mexican variety)**	Sr		
19	**Shatabdi (BARIGom-21)**	St	2000	
20	**Sonalica**	Sl	1974	Susceptible
21	**Sonora-64**	Sn	1974	

Table 3. Seeds of different wheat varieties used in this study

Sl. No.	Name of Variety	Symbol	Year of release	BpLB reaction
1	**Kanchan**	Kn	1983	Susceptible
2	**BARI GOM-25**	BG-25	2010	Tolerant
3	**BARI GOM-26**	BG-26	2010	Tolerant
4	**BARI GOM-27**	BG-27	2012	Tolerant
5	**BARI GOM-28**	BG-28	2012	Tolerant
6	**BARI GOM-29**	BG-29	2014	Tolerant
7	**BARI GOM-30**	BG-30	2014	Tolerant

3.3.2 Isolation of fungi associated with leaves and seeds

3.3.2.1 Glassware cleaning

Glassware (Borasil brand) was placed in a cleaning solution containing 60 g potassium dichromate ($K_2Cr_2O_7$) and 60 ml concentrated sulphuric acid (H_2SO_4) diluted in 1000 ml distilled water for 24 hours for laboratory research. The glassware was then cleaned with a detergent solution, rinsed with tap water, and then sterilized with distilled water (Riker and Riker 1936, Tuite 1969).

3.3.2.2 Media for isolation of fungi

The associated fungi were isolated and maintained on potato dextrose agar (PDA) medium. The physiological variation studies were conducted on PDA, carrot dextrose agar (CDA), tomato dextrose agar (TDA), host extract agar (HEA) and water agar (WA) media, as listed in Appendix-1.

3.3.2.3 Sterilization of glassware

For subsequent usage, washed glassware was disinfected in a hot air oven at 160°C for 2 hours. The medium prepared in the laboratory were disinfected at 15 lbs per square inch (psi) at 121ºC for 15 minutes in an autoclave.

3.3.2.4 Preliminary examination of the disease symptoms

A sterile teasing needle was used to scrape the wheat leaf showing typical leaf blight symptoms. The scraped parts were transferred to a clean glass slide, dipped in cotton blue, covered with a cover slip and inspected under a microscope for the presence of mycelium/spores, in order to provide a provisional diagnosis of the pathogen linked with the clinical symptoms.

3.3.2.5 Preparation for the isolation of fungi

On PDA medium, the fungi were isolated from leaf samples using the "Tissue planting/Agar plate" technique (CAB 1968) and from seed samples following the both “Blotter paper” method (ISTA 1976) and “Tissue planting/Agar plate” method (CAB 1968), which will be discussed again in seed health testing experiment.

3.3.2.5.1 Tissue planting method

The surface of the working table was sanitized with alcohol (95%) before beginning the surface sterilization of the leaf samples. The diseased leaf samples were then placed on the worktop. The samples of diseased leaves were washed in running tap water. Small pieces (2^2 mm) of diseased leaf area with typical symptoms also with healthy tissues were cut into pieces using a sterile blade and the surfaces of the pieces were sanitized by soaking them in a 1% chlorine solution (10% sodium hypochlorite-Clorox) for 2 minutes.

The inocula were then rinsed three times in sterile water. Finally, excess surface water was removed by placing the inocula into the folds of sterile blotting paper. Inocula were transferred to medium under aseptic conditions utilizing laminar air flow after being blot-dried. The inocula were put in Petri plates with sterilized potato dextrose agar (PDA) medium. Each Petri plate included 15 ml of PDA media and 1 drop (ca.0.03 ml) of lactic acid for bacterial growth monitoring. For each sample, a total of 50 inocula were put into 10 Petri plates. The inoculated plates were then incubated for seven days at room temperature (25±2º C). The colonies of fungus were checked for mycelial growth on the seventh day. The colonies of fungus were inspected on the eighth day for mycelial development, colony colour and type, sporulation, pigmentation, and zonations.

3.3.2.5.2 Blotter method

Clorox solution (1:10) was used for surface sterilization of the the collected seed samples. The surface of the working table was disinfected with alcohol (95%) before beginning the surface sterilization of the seed samples. The diseased seed samples were then placed on the work surface. The seed were soaked in water for 30 minutes in a beaker before treating with Clorox solution for surface sterilization. The presoaked seeds were transferred to a watch glass containing Clorox solution for 5 minutes. The seed was then rinsed three times in sterile water before being put in the moist chamber. In case of isolation from seed, moist chambers were created by putting three layers of wet filter paper on the bottom of the Petri plate, covering with the upper plate and then autoclaved at 15 lbs pressure/inch and 121ºC temperature for 20 minutes. In each Petri plate, surface sterilized inocula were placed on the filter paper. A maximum of 25 inocula were transplanted in per Petri plate and if necessary, were moistened with sterilized water. For seven days, petri plates were incubated at room temperature with 12 hour cycles of light and darkness. Based on 400 seeds per test, the growth characteristics of fungus as well as the percentage of infection were measured on the eighth day.

3.3.3 Identification of fungi associated with leaves and seeds

Following incubation, the samples were analyzed and the infected and healthy samples were counted under a stereo binocular microscope to record the incidence of various fungus. If required, temporary slides of the fungal colony were made and examined under a compound microscope. Different species grew on seeds in a blotter test and colonies on agar were documented in some cases. After examining the morphology of conidia and conidiophores, the identification was verified. There were also diagrammatic illustrations of sporulating structures generated. Slides mounts in water were used to determine

conidial size, and 20–25 spores were chosen at random to be measured. For each species, detailed notes were taken on colour, shape, hilum nature and other distinguishing characteristics. For fungus identification appropriate keys (Barnett and Hunter 1972, Benoit and Mathur 1970, Booth 1971, Chidambaram *et al.* 1973, Ellis 1971, 1976, Ellis and Ellis 1997, Raper and Thom 1949, Thom and Raper 1945, Subramanian 1971, Sutton 1980) were referenced. For each pathogen, the findings were provided as a percent incidence.

3.3.4 Purification of fungi associated with leaves and seeds

With the use of a sharp and sterile needle, the mycelial tips of each organism were gently extracted and put to the center of PDA in Petri plates. The Petri plates were incubated following the same procedure as mentioned before. The fungi growing out of the inocula were checked and if possible, identified before being transferred to PDA slants after 7–10 days. The isolates were purified using the dilution plate technique (Anon. 1968), kept on PDA slants and stored in an incubator at (5±0.5ºC) for future research.

3.3.5 Pure culture and Preservation of the fungi

Hyphal tip culture was used to create a pure fungus culture. To acquire pure culture of the pathogen, a 5 mm mycelial disk was excised from the border of the actively developing colonies and placed to Petri plates with PDA. Mycelial tips were used to grow the fungus, which were then transferred to the PDA slant using hyphal tip culture and stored at 5°C for future investigation.

3.3.6 Calculation of frequency percentage

On the seventh day after inoculation, readings of fungal colonies produced from the inocula were obtained and continued for two weeks, depending on the medium and the fungal organism connected with the inocula. Purr and Welty (1972) used the following calculation to compute the percentage frequency of the presence of the fungal isolates.

$$\text{Frequency (\%)} = \frac{\text{No.of inocula from which fungal isolates were raised}}{\text{total number of inocula culture}} \times 100$$

3.4 Study of isolates of *Bipolaris sorokiniana*

Isolated *B. sorokiniana* were recorded carefully for their variability studies on different parameters.

3.4.1 Number of *Bipolaris sorokiniana* isolates

Total 174 *B. sorokiniana* isolates were obtained from leaves and seeds of twenty-five wheat varieties. Distribution of total isolates was shown in the table 5.

3.4.2 Designation of *Bipolaris sorokiniana* isolates

According to Aminuzzaman *et al.* (2010), *B. sorokiniana* isolates were classified based on their location and source. For example, an isolate with the designation JSVBjL-1 was isolated from the district Joypurhat (J), upazila Joypurhat Sadar (S), village Vutiapara (V), and variety Bijoy (Bj), plant component utilized leaf (L). The number 1 signifies the collection's serial number.

Table 4. Prevalence of *Bipolaris sorokiniana* isolates from different locations and sources in Bangladesh

Division	District	Upazilla	Village/Area	Source	Name of Variety
Dhaka	Dhaka	DU	Carzon Hall	Leaf	BARI Gom-25, BARI Gom-26, BARI Gom-27, BARI Gom-28
	Gazipur	Joydebpur	BARI	Leaf	Kalyansona, Kanchan, Kheri, Seri-82, Sonora-64
				Seed	BARI Gom-25, BARI Gom-26, BARI Gom-27, BARI Gom-28, BARI Gom-29, BARI Gom-30, Kanchan
Rangpur	Dinajpur	BARI	WRC	Leaf	Aghrani, Akber, Ananda, Balaka, Barkat, Bijoy, Ciano-79, Kanchan, Prodip, Shatabdi, Sonalika
Rajshahi	Joypurhat	Sadar	Vutiapara	Leaf	Bijoy, Prodip, Saurav, Shatabdi
	Pabna	Bera	Kazir Hat Kashinathpur	Leaf	Bijoy, Shatabdi
		Sujanagar	Matighara Vatikaya		
	Sirajgonj	Sadar	Binoypur Vennabari	Leaf	Bijoy, Prodip, Saurav, Shatabdi
Khulna	Kushtia	Veramara	Farakpur	Leaf	Bijoy, Shatabdi
			Khemirdiar	Leaf	Bijoy, Prodip
		Mirpur	Kodalipara	Leaf	Prodip
	Chuadanga	Sadar	Farmpara	Leaf	Shatabdi
4	8	10	14	02	25

Table 5. Designation of *Bipolaris sorokiniana* isolates based on collection sites and sources with date of collection

Sl. No.	Designation of Isolates	Location			Host		Date of collection
		District	Upazila	Village	Variety	Plant part	
1	GJBKhL-01	Gazipur	Joydebpur	BARI	Kheri	Leaf	10.03.11
2	GJBKhL-08	Gazipur	Joydebpur	BARI	Kheri	Leaf	10.03.11
3	GJBKhL-16	Gazipur	Joydebpur	BARI	Kheri	Leaf	10.03.11
4	GJBKhL-18	Gazipur	Joydebpur	BARI	Kheri	Leaf	10.03.11
5	GJBSnL-01	Gazipur	Joydebpur	BARI	Sonora-64	Leaf	10.03.11
6	GJBKlL-01	Gazipur	Joydebpur	BARI	Kalyansona	Leaf	10.03.11
7	GJBKlL-09	Gazipur	Joydebpur	BARI	Kalyansona	Leaf	10.03.11
8	GJBKlL-12	Gazipur	Joydebpur	BARI	Kalyansona	Leaf	10.03.11
9	GJBSrL-01	Gazipur	Joydebpur	BARI	Seri-82	Leaf	15.03.11
10	GJBKnL-01	Gazipur	Joydebpur	BARI	Kanchan	Leaf	15.03.11
11	DiWRSlL-01	Dinajpur	Nashipur	WRC	Sonalica	Leaf	08.03.12
12	DiWRBlL-01	Dinajpur	Nashipur	WRC	Balaka	Leaf	08.03.12
13	DiWRAnL-01	Dinajpur	Nashipur	WRC	Ananda	Leaf	08.03.12
14	DiWRStL-01	Dinajpur	Nashipur	WRC	Shatabdi	Leaf	08.03.12
15	DiWRBjL-01	Dinajpur	Nashipur	WRC	Bijoy	Leaf	08.03.12
16	DiWRBjL-03	Dinajpur	Nashipur	WRC	Bijoy	Leaf	08.03.12
17	DiWRKnL-01	Dinajpur	Nashipur	WRC	Kanchan	Leaf	08.03.12
18	DiWRPdL-02	Dinajpur	Nashipur	WRC	Prodip	Leaf	08.03.12
19	DiWRAgL-01	Dinajpur	Nashipur	WRC	Aghrani	Leaf	08.03.12
20	DiWRAkL-01	Dinajpur	Nashipur	WRC	Akber	Leaf	08.03.12
21	DiWRBkL-02	Dinajpur	Nashipur	WRC	Barkat	Leaf	08.03.12
22	DiWRCL-10	Dinajpur	Nashipur	WRC	Ciano-79	Leaf	08.03.12
23	GJBKnL-04	Gazipur	Joydebpur	BARI	Kanchan	Leaf	12.03.12
24	GJBEnL-02	Gazipur	Joydebpur	BARI	En-113	Leaf	12.03.12
25	GJBEnL-03	Gazipur	Joydebpur	BARI	En-113	Leaf	12.03.12
26	GJBEnL-04	Gazipur	Joydebpur	BARI	En-45	Leaf	12.03.12
27	SSVStL-01	Sirajgonj	Sadar	Vennabari	Shatabdi	Leaf	29.01.13
28	SSVStL-02	Sirajgonj	Sadar	Vennabari	Shatabdi	Leaf	29.01.13
29	SSVStL-03	Sirajgonj	Sadar	Vennabari	Shatabdi	Leaf	29.01.13
30	SSVStL-04	Sirajgonj	Sadar	Vennabari	Shatabdi	Leaf	29.01.13
31	SSVStL-05	Sirajgonj	Sadar	Vennabari	Shatabdi	Leaf	29.01.13
32	SSVStL-06	Sirajgonj	Sadar	Vennabari	Shatabdi	Leaf	29.01.13
33	SSVStL-07	Sirajgonj	Sadar	Vennabari	Shatabdi	Leaf	29.01.13
34	SSVSvL-01	Sirajgonj	Sadar	Vennabari	Saurav	Leaf	29.01.13
35	SSVSvL-02	Sirajgonj	Sadar	Vennabari	Saurav	Leaf	29.01.13
36	SSVSvL-03	Sirajgonj	Sadar	Vennabari	Saurav	Leaf	29.01.13
37	SSVSvL-04	Sirajgonj	Sadar	Vennabari	Saurav	Leaf	29.01.13
38	SSVSvL-05	Sirajgonj	Sadar	Vennabari	Saurav	Leaf	29.01.13
39	SSVSvL-06	Sirajgonj	Sadar	Vennabari	Saurav	Leaf	29.01.13
40	SSVSvL-07	Sirajgonj	Sadar	Vennabari	Saurav	Leaf	29.01.13
41	SSVSvL-08	Sirajgonj	Sadar	Vennabari	Saurav	Leaf	29.01.13

Sl. No.	Designation of Isolates	Location			Host		Date of collection
		District	Upazila	Village	Variety	Plant part	
42	SSVPdL-01	Sirajgonj	Sadar	Vennabari	Prodip	Leaf	29.01.13
43	SSVPdL-02	Sirajgonj	Sadar	Vennabari	Prodip	Leaf	29.01.13
44	SSVBjL-01	Sirajgonj	Sadar	Binoypur	Bijoy	Leaf	29.01.13
45	SSVBjL-02	Sirajgonj	Sadar	Binoypur	Bijoy	Leaf	29.01.13
46	SSVBjL-03	Sirajgonj	Sadar	Binoypur	Bijoy	Leaf	29.01.13
47	SSVBjL-04	Sirajgonj	Sadar	Binoypur	Bijoy	Leaf	29.01.13
48	SSVBjL-05	Sirajgonj	Sadar	Binoypur	Bijoy	Leaf	29.01.13
49	DUC25L-01	Dhaka	DU	Carzon hall	BARI Gom-25	Leaf	22.02.13
50	DUC25L-02	Dhaka	DU	Carzon hall	BARI Gom-25	Leaf	22.02.13
51	DUC25L-03	Dhaka	DU	Carzon hall	BARI Gom-25	Leaf	22.02.13
52	DUC25L-04	Dhaka	DU	Carzon hall	BARI Gom-25	Leaf	12.02.14
53	DUC25L-05	Dhaka	DU	Carzon hall	BARI Gom-25	Leaf	12.02.14
54	DUC26L-01	Dhaka	DU	Carzon hall	BARI Gom-26	Leaf	22.02.14
55	DUC26L-02	Dhaka	DU	Carzon hall	BARI Gom-26	Leaf	22.02.13
56	DUC26L-03	Dhaka	DU	Carzon hall	BARI Gom-26	Leaf	12.02.13
57	DUC26L-04	Dhaka	DU	Carzon hall	BARI Gom-26	Leaf	12.02.13
58	DUC26L-05	Dhaka	DU	Carzon hall	BARI Gom-26	Leaf	12.02.13
59	DUC27L-01	Dhaka	DU	Carzon hall	BARI Gom-27	Leaf	12.02.13
60	DUC27L-02	Dhaka	DU	Carzon hall	BARI Gom-27	Leaf	12.02.13
61	DUC28L-01	Dhaka	DU	Carzon hall	BARI Gom-28	Leaf	12.02.13
62	DUC28L-02	Dhaka	DU	Carzon hall	BARI Gom-28	Leaf	12.02.13
63	DUC28L-03	Dhaka	DU	Carzon hall	BARI Gom-28	Leaf	12.02.13
64	KVFStL-01	Kushtia	Veramara	Farakpur	Shatabdi	Leaf	17.03.12
65	KVKBjL-01	Kushtia	Veramara	Khemirdiar	Bijoy	Leaf	17.03.12
66	KMKPdL-01	Kushtia	Mirpur	Kodalipara	Prodip	Leaf	17.03.12
67	CSFStL-01	Chuadanga	Sadar	Farmpara	Shatabdi	Leaf	19.03.12
68	CSFBjL-01	Chuadanga	Sadar	Farmpara	Bijoy	Leaf	19.03.12
69	PBKStL-01	Pabna	Bera	Kazirhat	Shatabdi	Leaf	24.03.12
70	PBKBjL-01	Pabna	Bera	Kazirhat	Bijoy	Leaf	24.03.12
71	PSMBjL-01	Pabna	Sujanagar	Matigara	Bijoy	Leaf	24.03.12
72	PSVStL-01	Pabna	Sujanagar	Vatikaya	Shatabdi	Leaf	24.03.12
73	PBCBjL-01	Pabna	Bera	Char	Bijoy	Leaf	24.03.12
74	JSDStL-01	Joypurhat	Sadar	Doripara	Shatabdi	Leaf	04.04.13
75	JSDStL-02	Joypurhat	Sadar	Doripara	Shatabdi	Leaf	04.04.13
76	JSDStL-03	Joypurhat	Sadar	Doripara	Shatabdi	Leaf	04.04.13
77	JSDStL-04	Joypurhat	Sadar	Doripara	Shatabdi	Leaf	04.04.13
78	JSDStL-05	Joypurhat	Sadar	Doripara	Shatabdi	Leaf	04.04.13
79	JSDStL-06	Joypurhat	Sadar	Doripara	Shatabdi	Leaf	04.04.13
80	JSDStL-07	Joypurhat	Sadar	Doripara	Shatabdi	Leaf	04.04.13
81	JSDStL-08	Joypurhat	Sadar	Doripara	Shatabdi	Leaf	02.03.14
82	JSDStL-10	Joypurhat	Sadar	Doripara	Shatabdi	Leaf	02.03.14
83	JSDStL-11	Joypurhat	Sadar	Doripara	Shatabdi	Leaf	02.03.14
84	JSDStL-12	Joypurhat	Sadar	Doripara	Shatabdi	Leaf	02.03.14
85	JSDBjL-01	Joypurhat	Sadar	Doripara	Bijoy	Leaf	04.04.13

Sl. No.	Designation of Isolates	Location			Host		Date of collection
		District	Upazila	Village	Variety	Plant part	
86	JSDBjL-02	Joypurhat	Sadar	Doripara	Bijoy	Leaf	04.04.13
87	JSDBjL-03	Joypurhat	Sadar	Doripara	Bijoy	Leaf	04.04.13
88	JSDPdL-01	Joypurhat	Sadar	Doripara	Prodip	Leaf	04.04.13
89	JSDPdL-02	Joypurhat	Sadar	Doripara	Prodip	Leaf	04.04.13
90	JSDPdL-03	Joypurhat	Sadar	Doripara	Prodip	Leaf	04.04.13
91	JSDPdL-04	Joypurhat	Sadar	Doripara	Prodip	Leaf	04.04.13
92	JSDPdL-05	Joypurhat	Sadar	Doripara	Prodip	Leaf	04.04.13
93	JSDPdL-06	Joypurhat	Sadar	Doripara	Prodip	Leaf	04.04.13
94	JSDPdL-07	Joypurhat	Sadar	Doripara	Prodip	Leaf	04.04.13
95	JSDPdL-12	Joypurhat	Sadar	Doripara	Prodip	Leaf	02.03.14
96	JSDPdL-13	Joypurhat	Sadar	Doripara	Prodip	Leaf	02.03.14
97	JSDPdL-14	Joypurhat	Sadar	Doripara	Prodip	Leaf	02.03.14
98	JSDPdL-16	Joypurhat	Sadar	Doripara	Prodip	Leaf	02.03.14
99	JSDSvL-01	Joypurhat	Sadar	Doripara	Saurav	Leaf	04.04.13
100	JSDSvL-02	Joypurhat	Sadar	Doripara	Saurav	Leaf	04.04.13
101	JSDSvL-03	Joypurhat	Sadar	Doripara	Saurav	Leaf	04.04.13
102	JSDSvL-04	Joypurhat	Sadar	Doripara	Saurav	Leaf	04.04.13
103	JSDSvL-05	Joypurhat	Sadar	Doripara	Saurav	Leaf	04.04.13
104	JSDSvL-06	Joypurhat	Sadar	Doripara	Saurav	Leaf	04.04.13
105	JSDSvL-07	Joypurhat	Sadar	Doripara	Saurav	Leaf	04.04.13
106	JSDSvL-09	Joypurhat	Sadar	Doripara	Saurav	Leaf	04.04.13
107	JSDSvL-10	Joypurhat	Sadar	Doripara	Saurav	Leaf	04.04.13
108	JSDSvL-14	Joypurhat	Sadar	Doripara	Saurav	Leaf	04.04.13
109	JSDSvL-15	Joypurhat	Sadar	Doripara	Saurav	Leaf	04.04.13
110	JSDSvL-18	Joypurhat	Sadar	Doripara	Saurav	Leaf	04.04.13
111	JSDSvL-19	Joypurhat	Sadar	Doripara	Saurav	Leaf	04.04.13
112	JSDSvL-20	Joypurhat	Sadar	Doripara	Saurav	Leaf	04.04.13
113	JSDSvL-21	Joypurhat	Sadar	Doripara	Saurav	Leaf	02.03.14
114	JSDSvL-22	Joypurhat	Sadar	Doripara	Saurav	Leaf	02.03.14
115	JSDSvL-23	Joypurhat	Sadar	Doripara	Saurav	Leaf	02.03.14
116	JSDSvL-24	Joypurhat	Sadar	Doripara	Saurav	Leaf	02.03.14
117	JSDSvL-24	Joypurhat	Sadar	Doripara	Saurav	Leaf	02.03.14
118	JSDSvL-26	Joypurhat	Sadar	Doripara	Saurav	Leaf	02.03.14
119	JSDSvL-27	Joypurhat	Sadar	Doripara	Saurav	Leaf	02.03.14
120	JSDSvL-28	Joypurhat	Sadar	Doripara	Saurav	Leaf	02.03.14
121	JSVStL-01	Joypurhat	Sadar	Vutiapara	Shatabdi	Leaf	02.04.13
122	JSVStL-02	Joypurhat	Sadar	Vutiapara	Shatabdi	Leaf	02.04.13
123	JSVStL-03	Joypurhat	Sadar	Vutiapara	Shatabdi	Leaf	04.04.13
124	JSVBjL-01	Joypurhat	Sadar	Vutiapara	Bijoy	Leaf	04.04.13
125	JSVBjL-02	Joypurhat	Sadar	Vutiapara	Bijoy	Leaf	04.04.13
126	JSVBjL-07	Joypurhat	Sadar	Vutiapara	Bijoy	Leaf	04.04.13
127	JSVBjL-08	Joypurhat	Sadar	Vutiapara	Bijoy	Leaf	02.03.14
128	JSVBjL-09	Joypurhat	Sadar	Vutiapara	Bijoy	Leaf	02.03.14
129	JSVBjL-10	Joypurhat	Sadar	Vutiapara	Bijoy	Leaf	02.03.14

Sl. No.	Designation of Isolates	Location			Host		Date of collection
		District	Upazila	Village	Variety	Plant part	
130	JSVPdL-01	Joypurhat	Sadar	Vutiapara	Prodip	Leaf	04.04.13
131	JSVPdL-02	Joypurhat	Sadar	Vutiapara	Prodip	Leaf	04.04.13
132	JSVPdL-03	Joypurhat	Sadar	Vutiapara	Prodip	Leaf	04.04.13
133	JSVPdL-04	Joypurhat	Sadar	Vutiapara	Prodip	Leaf	04.04.13
134	JSVPdL-05	Joypurhat	Sadar	Vutiapara	Prodip	Leaf	04.04.13
135	JSVPdL-06	Joypurhat	Sadar	Vutiapara	Prodip	Leaf	04.04.13
136	JSVPdL-07	Joypurhat	Sadar	Vutiapara	Prodip	Leaf	04.04.13
137	JSVPdL-08	Joypurhat	Sadar	Vutiapara	Prodip	Leaf	04.04.13
138	JSVPdL-09	Joypurhat	Sadar	Vutiapara	Prodip	Leaf	02.03.14
140	JSVPdL-10	Joypurhat	Sadar	Vutiapara	Prodip	Leaf	02.03.14
141	JSVPdL-11	Joypurhat	Sadar	Vutiapara	Prodip	Leaf	02.03.14
142	JSVPdL-12	Joypurhat	Sadar	Vutiapara	Prodip	Leaf	02.03.14
143	JSVPdL-13	Joypurhat	Sadar	Vutiapara	Prodip	Leaf	02.03.14
144	JSVPdL-14	Joypurhat	Sadar	Vutiapara	Prodip	Leaf	02.03.14
145	JSVPdL-15	Joypurhat	Sadar	Vutiapara	Prodip	Leaf	02.03.14
146	JSVPdL-16	Joypurhat	Sadar	Vutiapara	Prodip	Leaf	02.03.14
147	JSVPdL-17	Joypurhat	Sadar	Vutiapara	Prodip	Leaf	02.03.14
148	JSVPdL-18	Joypurhat	Sadar	Vutiapara	Prodip	Leaf	02.03.14
149	JSVSvL-01	Joypurhat	Sadar	Vutiapara	Saurav	Leaf	04.04.13
150	GJBKnL-02	Gazipur	Joydebpur	BARI	Kanchan	Leaf	29.03.13
151	GJBKnL-03	Gazipur	Joydebpur	BARI	Kanchan	Leaf	29.03.13
152	GJBEnL-01	Gazipur	Joydebpur	BARI	En-113	Leaf	29.03.13
153	GJB25S-01	Gazipur	Joydebpur	BARI	BARI Gom-25	Seed	13.06.14
154	GJB25S-02	Gazipur	Joydebpur	BARI	BARI Gom-25	Seed	13.06.14
155	GJB26S-02	Gazipur	Joydebpur	BARI	BARI Gom-26	Seed	13.06.14
156	GJB26S-03	Gazipur	Joydebpur	BARI	BARI Gom-26	Seed	13.06.14
157	GJB28S-01	Gazipur	Joydebpur	BARI	BARI Gom-28	Seed	13.06.14
158	GJB28S-02	Gazipur	Joydebpur	BARI	BARI Gom-28	Seed	13.06.14
159	GJB28S-03	Gazipur	Joydebpur	BARI	BARI Gom-28	Seed	13.06.14
160	GJB28S-04	Gazipur	Joydebpur	BARI	BARI Gom-28	Seed	13.06.14
161	GJB28S-05	Gazipur	Joydebpur	BARI	BARI Gom-28	Seed	13.06.14
162	GJB28S-06	Gazipur	Joydebpur	BARI	BARI Gom-28	Seed	13.06.14
163	GJB28S-07	Gazipur	Joydebpur	BARI	BARI Gom-28	Seed	13.06.14
164	GJB28S-08	Gazipur	Joydebpur	BARI	BARI Gom-28	Seed	13.06.14
165	GJB28S-09	Gazipur	Joydebpur	BARI	BARI Gom-28	Seed	13.06.14
166	GJB29S-01	Gazipur	Joydebpur	BARI	BARI Gom-29	Seed	13.06.14
167	GJB29S-02	Gazipur	Joydebpur	BARI	BARI Gom-29	Seed	13.06.14
168	GJB29S-03	Gazipur	Joydebpur	BARI	BARI Gom-29	Seed	13.06.14
169	GJB29S-04	Gazipur	Joydebpur	BARI	BARI Gom-29	Seed	13.06.14
170	GJB29S-05	Gazipur	Joydebpur	BARI	BARI Gom-29	Seed	13.06.14
171	GJB29S-06	Gazipur	Joydebpur	BARI	BARI Gom-29	Seed	13.06.14
172	GJB29S-07	Gazipur	Joydebpur	BARI	BARI Gom-29	Seed	13.06.14
173	GJB30S-01	Gazipur	Joydebpur	BARI	BARI Gom-30	Seed	13.06.14
174	GJB30S-02	Gazipur	Joydebpur	BARI	BARI Gom-30	Seed	13.06.14

3.5 Experiment 2: Cultural variability of *Bipolaris sorokiniana* isolates

The colony development patterns of several *B. sorokiniana* isolates were used to investigate cultural variability.

3.5.1 Growth study of *Bipolaris sorokiniana*

PDA was used to investigate the growth of *B. sorokiniana*. The PDA plates were inoculated with a 5 mm diameter mycelial block in the middle. The plates with *B. sorokiniana* were placed in incubator at 25±1ºC for 9 days. Then the mycelial growth, its nature of growth, colony shape, colony colour and colony compactness were studied.

3.5.2 Fungal growth measurement technique

The diameter of the colonies on the same axis was used to determine mycelial growth directly. The average of two diameters at right angles to one another was used to collect data. The colony's mycelial growth was measured in millimeters (mm) using a thin clear plastic scale.

3.5.3 Grouping of isolates of *Bipolaris sorokiniana* and selection of isolates from each group

The collecting *B. sorokiniana* isolates were grouped based on colony morphology, colony colour, colony compactness, size and shape of the colony, shape and size of the conidia, septation and colour of conidia. The colour of upside down of the colony and septation of the conidia were also evaluated. Moreover, the conidial abundance of the colony was determined by excising 5^2 mm colony area using cork borer. To remove the conidia, it was shaking vigorously after adding 1 ml water. The abundance of spore on the slide under microscope was categorized by + (sporulation scanty), ++ (sporulation moderate) and +++ (sporulation abundant). The representation isolate from each group was selected. These selected isolates from different groups were preserved for further study. As a result of the above mentioned morphological characteristics, the isolates were classified into eight cultural groups and were preserved for further study.

Table 6. Two selected isolates of *Bipolaris sorokiniana* from each cultural group used in this study

Sl. No.	Cultural Group	Isolate Name (Two isolates from each group)
1	Black Mat (B-M)	JSDSvL-01, GJBEnL-01
2	Black Fluffy (B-F)	JSVPdL-08, GJBKnL-01
3	Ash Mat (A-M)	JSDSvL-28, GJBKhL-01
4	Brownish Ash Fluffy (BrA-F)	JSDBjL-03, DiWRBjL-01
5	Blackish Ash Mat (BlA-M)	JSVPdL-10, PSVStL-02
6	Whitish Ash Mat (WA-M)	JSDSvL-26, JSVPdL-04
7	Greenish Ash Fluffy (GA-F)	JSDSvL-05, GJBKnL-03
8	Pinkish White Mat (PW-M)	JSDSvL-09, JSDStL-01

Table 7. Morphological characters and their multiple character states used for analysis of *Bipolaris sorokiniana* isolates

Sl. No.	Characters attributes	Character states
1	Colony colour (Upper side and Lower side)	Black, Blackish ash, Brownish ash, Ash, Greenish ash and Pinkish white
2	Colony texture	Mat, Fluffy
3	Colony margin	Smooth, Wavy
4	Colony growth pattern	Regular, Irregular
5	Colony growth measurement	3^{rd} day/ 5^{th} day/ 7^{th} day/ 10^{th} day & Growth per day
6	Sporulation	Abundance of spore under microscopic focus showed by + (sporulation scanty), ++ (sporulation moderate), +++ (sporulation abundant)
7	Conidiophore length/width	mm/mm
8	Spore size length/width	Lowest–highest (µm)
9	Mean spore length/width	µm/µm
10	Spore colour	Brown, Light brown, Dark brown
11	Septation	4–12
12	Spore shape	Straight/slightly curved

3.6 Experiment 3: Morphological variability of of *Bipolaris sorokiniana* isolates

To determine the identification of each isolate of *B. sorokiniana*, detailed morphological examinations were conducted. Fungal features such as mycelium, conidiophores and

conidia were scrape off the surface using a scalpel or blade, or acquired with a needle, then mounting in lacto phenol on a glass slide for microscopic viewing. A drop of aniline blue (cotton blue) was applied to the mounting fluid in the case of hyaline formations. A fresh cover slip was put over the materials, surplus fluid was socked with blotting paper, and the materials were inspected using a light microscope at 40x and 100x magnifications. A high-resolution digital camera was used to capture the tiny structural image of the fungus. For the identification of that fungus, conidia and conidiophores of major fungi were drawn with a Camera Lucida. According to Sivanesan and Holliday (1981), the conidia form and size were noticed and quantified. The Herbarium, Mycology and Plant Pathology Laboratory, Department of Botany, University of Dhaka, Bangladesh, housed all of the specimens used in this study.

3.7 Experiment 4: Pathogenic variability of *Bipolaris sorokiniana* isolates

3.7.1 Pathogenic variability of *Bipolaris sorokiniana* isolates following detached leaf assay

Using healthy matured leaves of susceptible wheat variety Kanchan, a subset of *B. sorokiniana* isolates from each cultural group were examined for pathogenic potential. The leaves were first cut into 85 mm long pieces, carefully cleaned under flowing tap water and then surface sterilized for 3 minutes in 10% chlorox. Leaves were placed on two layers of properly autoclaved filter paper on a Petri plate to remove extra chlorox. The leaves were infected with a 5 mm mycelial block of each isolate that had already been cultured on PDA media and incubation period was 7 days. The following six treatments with three replications for each isolate were used:

T_1= dorsally inoculated leaf with PDA block (control)

T_2= ventrally inoculated leaf with PDA block (control)

T_3= dorsally un-pricked inoculated leaf

T_4= ventrally un-pricked inoculated leaf

T_5= dorsally pricked inoculated leaf

T_6= ventrally pricked inoculated leaf

For incubation, the inoculated Petri plates were kept at 26–28°C. The size of the lesion and the symptom were measured after 5 days of inoculation. The data was collected using a 0–5 scale when symptoms appeared on the leaves.

0 = no symptoms,

1 = 1–5 % spots/lesion on leaves,

2 = 6–20 % spots/lesion on leaves,

3 = 21–40 % spots/lesion on leaves,

4 = 41–60 % spots/lesion on leaves and

5 = 61% and above spots/lesion on leaves (Anon. 1996).

The pathogen was re-isolated from leaf spots, and Koch's hypothesis was validated when these isolates were compared to the mother culture.

3.7.2 Pathogenicity test following seed inoculation technique

Two isolates of *B. sorokiniana* under each cultural group were selected and tested for their pathogenesis using healthy seeds of susceptible wheat cultivar Kanchan following seed inoculation technique. 10 seeds were used for each isolates. There were also control sets which were not inoculated with pathogens. The healthy seeds were properly rinsed under flowing tap water before being surface sterilized for 3 minutes with 10% chlorox. Seeds were placed on two layers of sanitized autoclaved filter paper on a Petri plate to eliminate excess chlorox. The seeds were infected with a conidial suspension of each isolate cultured on PDA medium earlier and incubated for thirty days. Within 10 to 15 days, inoculated seeds grown on water agar in test tubes formed brown necrotic patches on the developing seedlings' leaves and leaf sheath. When infected tissues were isolated on PDA, *B. sorokiniana* culture was obtained. Data were only recorded as symptoms appeared or not.

3.8 Experiment 5: Physiological variability of *Bipolaris sorokiniana* isolates

Two *B. sorokiniana* isolates under eight cultural group were tested for their physiological potentiality using different culture medium, temperature and P^H.

3.8.1 Evaluation of culture media against *Bipolaris sorokiniana* isolates

Five culture madia *viz.*, Potato dextrose agar (PDA), Carrot dextrose agar (CDA), Tomato dextrose agar (TDA), Host extract agar (HEA) and Water agar (WA) was utilized to compare the *B. sorokiniana*'s growth rate. *B. sorokiniana* mycelial discs (5 mm diameter) from a 7-day-old culture were put in the centre of each pre-poured Petri plate and incubated at 25±2º C. For each medium, four replicatons were retained. After 10 days of incubation, colony growth of the *B. sorokiniana* was measured using a scale to determine the average of two diameters at right angles to one another in mm.

3.8.2 Evaluation of Temperature against *Bipolaris sorokiniana* isolates

Five different temperature *viz.*, 10ºC, 15ºC, 20ºC, 25ºC and 30ºC was used to evaluate the growth pattern of *B. sorokiniana*. 5 mm diameter mycelial discs of *B. sorokiniana* (7 days old culture) were placed on the central of each pre-poured Petri plates containing PDA and incubated at 25±2ºC. Four replicatons kept for each temperature. The mycelial

growth of *B. sorokiniana* colony were recorded after 10 days of the incubation by measuring the average of two diameters at right angles to one another in mm with a scale.

3.8.3 Evaluation of media P^H against *Bipolaris sorokiniana* isolates

Five different P^H *viz.*, 5.5, 6.0, 6.5, 7.0 and 7.5 was used to observe the growth form of *B. sorokiniana*. From 7 days aged culture of *B. sorokiniana*, a mycelial disc of 5 mm diameter was cut and placed on the mid of each PDA containing Petri plates and incubated at 25±2ºC. For each P^H Four replications were kept. The data was taken after 10 days of the incubation by measuring the average of two diameters at right angles to one another (mm) using a scale.

3.8.4 Experimental detail of physiological variability

Treatments – 16 (Two isolates of *B. sorokiniana* from 8 cultural group)

Replications – 4

Design – CRD (Completely randomized design)

3.9 Experiment 6: Compatibility test or colony interaction of *Bipolaris sorokiniana* isolates

To acquire pure culture of *B. sorokiniana*, a 5 mm mycelial disk was excised from the border of the actively developing colonies and placed to Petri plates with PDA. Schafer and Kohn (2006) and Minghe *et al.* (2010) explained how to group mycelial compatibility. On PDA media, *B. sorokiniana* isolates were coupled at a distance of 3 cm from each other and from the border of the Petri plate. On the same Petri plate, a 5 mm mycelial disk of 7 days aged PDA culture of each isolate from the same and distinct cultural groups was put and incubated for seven days at room temperature (25±2ºC). The reactivity between each isolate pair was assessed after incubation.

3.10 Experiment 7: Molecular identification of *Bipolaris sorokiniana* isolate

Molecular identification of *B. sorokiniana* was done following Islam and Mukherjee (2011) with some modification.

3.10.1 DNA Extraction

Fungal mycelia were harvested by scraping the surface of 15 days old cultures with a sterile spatula from the test tube. One gram of fungal mycelia was taken in 1.5 ml eppendrof tube and placed in liquid nitrogen. The mycelium was immediately grinded with a homogenizer machine to get fine powder. 750 µl of lysis buffers were added in each eppendrof tube and stir with a vortex to get homogenous mixture. The tubes were shifted to 65°C preheated water bath for 30 minutes. The samples were cooled to room temperature after being removed from the water bath. 700 µl of chloroform: phenol (1:1)

mixture was added and mixed quietly. The samples were centrifuged at 12,000 rpm for 5 minutes. The aqueous phase was transferred into fresh eppendrof tube and again mixed with 700 µl of chloroform: phenol and centrifuged at 12,000 rpm for 5 minutes. The aqueous phase was transferred to new eppendrof tubes and 70 µl of NaOAc was added. Top off the eppendrof tube with 300 µl of isopropanol, gently inverted several times. DNA 'ropes' precipitate was seen here. The samples were centrifuged at 13,000 rpm for 10 minutes to form DNA pellet. The supernatant was thrown away and the pellet was washed with 70% ethanol. The pellets were air dried and dissolved in 100 µl of TE buffer mixed with RNAse A (final concentration 10 mg/ml). The samples were incubated for 30 minutes at 37°C. 10 µl of NaOAc was added to the tubes and top off the eppendrof tube with 750 µl absolute ethanol and mixed mildly. The samples were centrifuged at 13,000 rpm for 10 minutes. The supernatant was discarded completely, washed with 70% ethanol, air dried the pellet and dissolved in 100 µl TE buffer. The DNA was allowed to dissolve overnight at 4°C.

3.10.2 PCR Amplification

Molecular identification of the isolate was performed using the internal transcribed spacer (ITS) regions. PCR amplification was conducted using the ITS1 (5-TCCGTAGGTGAACCTGCGC-3) and ITS4 (5-TCCTCCGCTTATTGATATGC-3) primers for the ITS regions. The PCR was supported out in 0.2 ml PCR tube with 25 reaction volume having 2.0 µl Template DNA, 12.5 µl Master mix, 1.0 µl Forward Primer, 1.0 µl Reverse Primer and 8.5 µl MilliQ H_2O. Reaction mixture was vortexed and centrifuged in a microcentrifuge. The PCR was initiated by an initial denaturation step at 94°C for 5 minutes following 30 cycles of 94, 54 and 72°C each for 30 secs, with a final extension step of 5 min at 72°C and ended with 4°C. PCR amplified products were stored in -20°C freezer for analysis by resolving in 1% agarose gel. The gel was ready with 1.0 g agarose powder having ethidium bromide. Agarose gel electrophoresis was accompanied in 1×TAE buffer at 90 Volts and 300 mA for 40 minutes. One molecular weight marker 1kb ladder was electrophoresed alongside the ITS reactions. DNA bands were photographed by a Gel Documentation system (model: DI-HD, UK).

3.10.3 Sequencing analysis

Alcohol precipitation was used to purify PCR generated products, which were then sequenced using an automated sequencer at the University of Dhaka's Centre for Advanced Research in Sciences (CARS). To identify the genus and species of the isolates, the sequences were analyzed using the BLAST program (http://

blast.ncbi.nlm.nih.gov) of the National Certer for Biotechnology Information (NCBI, Bethesda, MD, USA) as well as BOL database.

3.11 Experiment 8: *In vitro* evaluation of fungicides against *Bipolaris sorokiniana* isolates

Eight systemic fungicides [Knowin 50 WP (carbandazim), Score 250 EC (difenconazole), Contaf 5 EC (hexaconazole), Tilt 25 EC (propiconazole), Folicur 25 EC (tebuconazole) and Ridomil Gold MZ 68 WG (metalaxyl), Dithane M-45 75WP and Secure 600 WG (mancozeb), two protectants/contact (non-systemic) fungicides Capvit 50 WP (cupper oxychloride) and sulpher (Silika 80 WG)] were tested *in vitro* for their effectiveness against *B. sorokiniana* (Table 8). All the fungicides were collected from Siddique Bazar seed market, Dhaka-1000. The fungicides were tested at 100, 200, 300, 400 and 500 ppm concentrations using poisoned food technique (Nene and Thapliyal 1993). At first, for each fungicide, a stock solution with a concentration of 10,000 ppm was created. By applying the required quantity of fungicide stock solutions to sterilized PDA media in Petri plates, the desired concentrations were achieved. Instead of a fungicide, the needed amount of water was supplemented for control. After that, 15 mL of medium was placed into each Petri plate and let to set. Following that, each plate was inoculated with a 5mm pathogen mycelial disc, i.e. fungus obtained from 7–10 day aged PDA cultures. The inoculated plates were incubated at 25±1^{0}C till the fungus, in control set filled the entire plate. In both situations, three replications were kept. At 7th day of incubation, the radial growth of control and treatment plates was assessed. The colony's radial growth was measured and the percent inhibition of each treatment was estimated using Vincent's (1927) formula:

$$I = \frac{C-T}{C} \times 100$$

Where, I = Percent growth inhibition

C = Growth in control

T = Growth in treatment

Table 8. Particulars of fungicides used in this study

Sl. No.	Coined name	Labelled name	Fungicide group	Active ingredient (s)	Chemical name	Manufacture's name
1	Carbandazim (Systemic fungicide)	Knowin 50 WP	Benzimidazoles Fungicide	Carbandazim 50%	Methyl (1*H*-1,3-benzimidazol-2-yl)carbamate	Macdonald Bangladesh Pvt. Ltd.
2	Cupper oxychloride (Protectant fungicide)	Capvit 50 WP	Copper Fungicide	Copper Oxychloride 50%	Copper Oxychloride	Jiangsu Hong Ze Chem. and Ind. Co. Ltd., China.
3	Difenconazole (Systemic fungicide)	Score 250 EC	Triazoles Fungicide	Difenoconazole 25%	Cis, trans-3-chloro-4(4-methyl-2(1H-1,2,4-triazole-1-yl methyl) 1,3dioxalan-2yl) phenyl, 4-chlorophenylether	Syngenta Bangladesh
4	Hexaconazole (Systemic fungicide)	Contaf 5 EC	Triazoles Fungicide	Hexaconazole 5%	2-(2,4-Dichlorophenyl)-1-(1*H*-1,2,4-triazol-1-yl) hexan-2-ol	Rallis India Ltd.
5	Mancozeb (Systemic fungicide)	Diathane M-45 75WP	Dithiocarbamate	Mancozeb 80%	Manganese zinc ethylene bisdithiocarbamate	Dow Argo Science, India
6	Mancozeb (Systemic fungicide)	Secure 600 WG	Dithiocarbamate	Mancozeb 50% and Phenamidon 10%	Manganese Zinc ethylenebis	Bayer Crop Science Ltd.
7	Metalaxyl (Systemic fungicide)	Ridomil Gold MZ 68 WG	Phenylamide & dithiocarbamate	4% Metalaxyl and 64% Mencozeb	a methyl N-(2,6-dimethylphenyl)-N-(methoxyacetyl)alaninate	Syngenta Bangladesh Ltd.
8	Propiconazole (Systemic fungicide)	Tilt 25 EC	Triazoles Fungicide	Propiconazole 25%	1-(2,4-dichlorophenyl)4-propyl-1-3-dioxalam-2-methyl) H-1, 4-triazole	Syngenta crop protection, Switzerland
9	Tubeconazole (Systemic fungicide)	Folicur 25 EC	Triazoles Fungicide	Tebuconazole 38.7%	a-(2-4-chlorophenyl) ethyl a-(1,1-diomethyl) 1H1, 2,4-triazole-1-ethanole	Bayer India Ltd.
10	Sulphur (Protectant fungicide)	Silika 80WG	Sulphur Fungicide	Sulpher 80%	Sulphur	Syngenta Bangladesh Ltd.

3.12 Experiment 9: Evaluation of leaf extracts in contrast to *Bipolaris sorokiniana* isolates *in vitro*

Ten plants were chosen for assessing their result on colony growth of different *B. sorokiniana* isolates *in vitro*. Plants were gathered from Botanical Garden, Botany Department, University of Dhaka, Dhaka. Particulars of all these plants are given in Table 9.

3.12.1 Preparation of aqueous plant extracts

I. Each plant's leaves were carefully rinsed with water, after air dried they were utilized to make new extracts.

II. Leaf extract was made by crushing a specified weight of air dried materials in 1:1 (w/v) ratio with distilled water.

III. To eliminate particle debris, the crushed mass of leaves was pressed through required folds of cheese cloth and centrifuged at 3000 rpm for 20 minutes. The filtrate was collected in 250 ml glass conical flasks after the supernatants were filtered using No.1 filter paper.

IV. In this approach, the required quantity of each leaf extract's filtrate was combined withPDA medium to achieve concentrations of 5, 10, 15, and 20%.

3.12.2 Inoculation method

I. The medium was then placed into Petri plates that had been disinfected and allowed to harden. A 5 mm agar disc cut from the periphery of an actively developing culture of *B. sorokiniana* isolates was inoculated in the center of each Petri plate.

II. In control, a Petri plate having PDA medium in place of a leaf extract the required volume of distilled water was added, then inoculated with agar disc in the same manner as indicated above.

III. Both the experimental and control sets were three replicated. At 25 ± 1^0C, the prepared Petri plates were incubated. After 5 days of incubation, the colony growth of *B. sorokiniana* isolates was assessed.

3.12.3 Calculation

Basher and Rai (1991) used the following formula to compute the percent reduction in colony diameter of each isolate of *B. sorokiniana* when exposed to plant extracts:

$$I = \frac{C-T}{C} \times 100$$

Where, I = Percent growth inhibition

C = Growth in control

T = Growth in treatment

Table 9. Particulars of angiosperm plants used in this study

Sl. no.	Botanical name	Native name	Family	Used part	Bioactive compounds	References
1	*Allamanda cathartica* L.	Alokanand/ Harkakra	Apocynaceae	Leaf	Hydrocarbons, alcohols, esters, ethers, aldehydes, ketones, fatty acids, phospholipids, volatile compounds, phenolic compounds, flavonoids, alkaloids, steroids, terpenes, lactones.	Haron *et al.* 2013
2	*Azadirachta indica* A. Juss.	Neem	Meliaceae	Leaf	Minaral like Ca, Mg, I, P, protein, vitamin-A, crud fiber, carbohydrates, fat and alkaloids like quercetin, nimbosterol, azadirachtin, nimboflavone and nomicinol.	Gurjar *et al.* 2012
3	*Catharanthus roseus* (L.) G. Don	Nayantara (Rose Periwinkle)	Apocynaceae	Leaf	Alkaloids, carbohydrates, antioxidant-flavonoids, coumarin, quinine and phenolic compounds- 3-hydroxy-cyclooctanone, lupeol, stigmasterol.	Zahari *et al.* 2018
4	*Citrus limon* L.	Labu (Lemon)	Rutaceae	Leaf	Essential oil contains antioxidants-Limonene, Sabinene, Citronellal, Linalool, Neral, Geranial, (E)-beta-Ocimene, Myrcene, Citronellol, beta-Caryophyllene, Terpne-4-ol, Geraniol, alpha-Pinene	Ammad *et al.* 2018
5	*Lantana camara* L.	Chotra	Verbenaceae	Leaf	Essential oil β caryophyllene and δ selinene, β copaene, bicylogermacrene, β phellandrene and δ cadinene.	Bashir *et al.* 2019
6	*Lawsonia inermis* L.	Mehendi, Hena	Lythraceae	Leaf	Carbohydrates, phenolic, flavonoids, saponins, proteins, alkaloids, terpenoids, quinones, coumarins, xanthones, fat, resin and tannins. It also contained 2-hydroxy-1,4-naphthoquinone (lawsone).	Bhardwaj (2012)
7	*Moringa oleifera* Lam.	Shajna (Drum Stick tree)	Moringaceae	Leaf	Alkaloids, beta carotene, tannins, beta sitosterol, zeatin, quercetin, flavonoids, kaempferom, protein, vitamins, minerals, amino acids, phenolic acids, natural sugars, phytosterols, saponin and terpenoids.	El–Mohamedy and Abdalla 2014
8	*Polyalthia longifolia* Sonn.	Debdaru (False Ashoka, Buddha tree)	Annonaceae	Leaf	Steroids, flavonoids, clerodane diterpenes, cleroda-oic acids and alkaloids.	Bhutia *et al.* 2015
9	*Psidium guajava* L.	Payara (Guava)	Myrtaceae	Leaf	Quercetin, avicularin, apigenin, guaijaverin, kaempferol, hyperin, myricetin, gallic acid, catechin, epicatechin, chlorogenic acid, epigallocatechin gallate and caffeic acid.	Das and Goswami 2019
10	*Tagetes erecta* L.	Ganda (Marigold)	Acanthaceae	Leaf	Essential oil contains antioxidants-α tocopherol, camphor and methyl eugenol, rich in xanthophyll and carotenoids-lutein.	Mares *et al.* 2002

3.13 Experiment 10: *In vitro* evaluation of antagonistic fungi against *Bipolaris sorokiniana* isolates

Following the serial dilution approach (Koch 1883), certain soil fungus was isolated from wheat field soil. First, 1-gram soil was mixed well with 99 ml distilled water in a conical flask with a glass rod and labeled as mother suspension. Then five test tubes with 9 ml of

sterilized distilled water each were taken. The first test tube was made 10 ml by adding 1 ml of mother suspension. As a result, the mother suspension was diluted ten times in the first test tube. Then it was thoroughly blended. To make the second test tube 10 ml, 1 ml of the suspension from the first test tube was added. As a result, the mother suspension was diluted 100 times in the second test tube. This procedure was repeated for the remaining test tubes, diluting the mother suspension by 10, 100, 1000, 10000 and 100000 times, respectively.

For each dilution, 1 ml of suspension was poured into a sterilized Petri plate and then about 15 ml of sterilized melted PDA medium (about 50^0C) was added. The plate was moved gently on the laminar air flow table to get a homogenous distribution of the suspension. Five replications were maintained for each dilution. All the Petri plates were incubated in 25 ± 1^0C temperature. After 3 days, individual fungal colonies belonging to the genera *Aspergillus*, *Penicillium* and *Trichoderma* were sub cultured on PDA slants randomly, from the culture plates and stored at 4^0C in an incubator for future studies.

1 ml of suspension was placed into a sterilized Petri plate for each dilution, followed by 15 ml of sterilized melting PDA medium (about 50^0C). To achieve a uniform distribution of the suspension, the plate was gently moved on the laminar air flow table. For each dilution, five replications were kept. All of the Petri plates were incubated in in 25 ± 1^0C temperature. Individual fungal colonies from the genera *Aspergillus*, *Penicillium* and *Trichoderma* were randomly subcultured on PDA slants from the culture plates after 3 days and preserved at 4^0C in an incubator for future investigations. The standard literature was used to confirm the identification of soil fungus (Barnett and Hunter 1972, Benoit and Mathur 1970, Subramanian 1971, Booth 1971, Chidambaram *et al.* 1973, Ellis 1971, 1976, Ellis and Ellis 1997, Raper and Thom 1949, Thom and Raper 1945, Sutton 1980). After four weeks, subculturing was used to keep the cultures alive. From the isolated soil fungi, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Penicillium* sp., *Trichoderma harzianum* and *Trichoderma viride* were nominated randomly to study colony interactions with the test pathogens.

3.13.1 Colony interaction between *Bipolaris sorokiniana* isolates and antagonistic fungi

In dual cultures on PDA medium, colony interaction between *B. sorokiniana* isolates and chosen antagonist fungus was investigated. 5 mm mycelial agar discs of a *B. sorokiniana* isolate and an antagonist soil fungus were inoculated 30 mm apart in a Petri plate with 15 ml solidified PDA medium.

The discs of *B. sorokiniana* isolate and antagonist were placed at same distance from the margin of Petri plates. PDA medium inoculated with *B. sorokiniana* isolate functioned simply as a control plate. Each treatment had four replications in the experiment, which was totally randomized. The inoculated plates were incubated for 5 days at 25±1ºC. The colony development of the *B. sorokiniana* isolate was recorded on both sides, that is, from their central loci, towards and away from each other. After 5 days, the radial growth was measured. In that same time, the intermingled and inhibition zones were also assessed.

The grades used to assess colony interaction between *B. sorokiniana* isolates and antagonist soil fungi were derived using the Skidmore and Dickinson (1976) methodology (Appendix II). The following are the grades and types:

Grade 1 (Type A): Mutually intermingling growth were both fungi grew into one another without any microscopic sign of interaction.

Grade 3 (Type Bi): Intermingling growth where the fungus being observed into the opposed fungus either above or below its colony.

Grade 2 (Type Bii): Intermingling growth where the fungus under observation has ceased the growth and is being overgrown by another colony.

Grade 4 (Type C): Slight inhibition with a narrow demarcation line (12 mm).

Grade 5 (Type D): Mutual inhibition at a distance more than 2 mm.

The breadth of the inhibition zone, the intermingled zone, and the percent inhibition of radial growth were calculated to evaluate the colony interaction. The growth inhibition of the *B. sorokiniana* isolates was determined by the formula of Fokkema (1976).

$$I = \frac{r1-r2}{r1} \times 100$$

Where, I = Per cent growth inhibition

r_1 = Radial growth of *B. sorokiniana* isolates towards the opposite side

r_2= Radial growth of *B. sorokiniana* isolates towards the antagonist.

All conceivable combinations of *B. sorokiniana* isolates and chosen antagonistic fungi were tested using the same procedure.

Table 10. Particulars of the antagonistic soil fungi used in this study

Sl. No.	Name of Soil Fungi	Source	Plant name	Place
1	*Aspergillus flavus* Link.	Soil	*Triticum aestivum* L.	Wheat Field, BARI, Gazipur
2	*Aspergillus fumigatus* Frese.	Soil	*Triticum aestivum* L.	Wheat Field, BARI, Gazipur
3	*Aspergillus niger* Tiegh.	Soil	*Triticum aestivum* L.	Wheat Field, BARI, Gazipur
4	*Penicillium* sp.	Soil	*Triticum aestivum* L.	Wheat Field, BARI, Gazipur
5	*Trichoderma harzianum* Refai.	Soil	*Triticum aestivum* L.	Wheat Field, BARI, Gazipur
6	*Trichoderma viride* Pers.	Soil	*Triticum aestivum* L.	Wheat Field, BARI, Gazipur

3.13.2 The effect of volatile substances emitted by antagonist cultures on the radial growth of *Bipolaris sorokiniana* isolates

The antagonistic soil fungus and *B. sorokiniana* isolates used in this experiment were identical to the ones used in experiment 3.13.1. The process proposed by Dennis and Webster (1971b) was followed for this study. For 5 days, soil fungi were cultivated in 9 cm Petri plates on PDA media. After inoculation at 25±1ºC, the cover of each Petri plate was substituted with a bottom plate of the same size, containing 15 ml PDA media and a *B. sorokiniana* isolate in the center. The Petri plates were then closed with scotch tape so that no volatile compounds could escape from the interior. The control was made in the same way as the *B. sorokiniana* isolate, but with the *B. sorokiniana* isolate at the bottom. Three replications were maintained in each *B. sorokiniana* isolate. These sets were incubated at 25±1ºC. After the 7th day of incubation, the colony diameters of the *B. sorokiniana* isolates were recorded in all of the sets and the percent inhibition or stimulation in the colony diameter of the *B. sorokiniana* isolates was computed. Percent growth inhibition over control was estimated by the following formula:

$$I = \frac{C-T}{C} \times 100$$

Where, I = Per cent growth inhibition

C = Growth in control

T = Growth in treatment

3.13.3 Effect of non-volatile subatances of the antagonists on radial growth of the *Bipolaris sorokiniana* isolates

The antagonistic soil fungus and *B. sorokiniana* isolates used in this experiment were identical to the ones used in experiment 3.13.1. The The process proposed by Dennis and Webster (1971a) was followed for this study. Three similar size blocks of different soil

fungi were inoculated singly into the 250 ml conical flasks each containing 100 ml sterile PDA broth medium, taken from the newly developing edges of 5 days aged cultures. The culture of a soil fungus was filtered first through a Whatman no. 1 filter paper and then centrifuged at 3000 rpm for 20 minutes after 10 days of incubation at 25±1ºC.

Each soil fungus' metabolites were introduced individually to 95, 90, 85 and 80 ml sterilized PDA medium in amounts of 5, 10, 15 and 20 ml. To achieve a homogeneous dispersion of the supplemented medium, the conical flask containing the PDA media and culture filtrates was slowly pushed in various directions on the laminar air flow table. Singh and Webster discovered that this concentration is best for such research (1978). Every Petri plate included 15 ml of PDA medium and metabolites, as well as one drop (ca 0.03) of lactic acid to monitor bacterial growth. In a sterile Petri plate, 5, 10, 15, and 20 ml of enriched media were poured and allowed to harden. A 5mm agar disc was excised from the freshly developing periphery of a 5-day old culture of *B. sorokiniana* isolates and inoculated in the center of each Petri plate. In control, *B. sorokiniana* isolate was inoculated into Petri plates containing PDA medium without culture filtrates, as stated previously. Instead of culture filtrate, an equivalent volume of sterilized water was added to the PDA medium in the control set. A total of three replications of each treatment were kept and incubated at 25±1ºC. After 5 days, the radial growth of *B. sorokiniana* isolates was assessed.

The percent inhibition of mycelial growth of *B. sorokiniana* isolates was calculated by using the following formula given by Vincent (1927):

$$I = \frac{C-T}{C} \times 100$$

Where, I = Per cent growth inhibition

C = Growth in control

T = Growth in treatment

3.14 Management of leaf blight of wheat *in vivo*

3.14.1 Period of experimentation

In the Rabi seasons of 2010-11 and 2011-12, the experiment was carried out in the Plant Pathology Division of BARI, Joydebpur, Gazipur.

3.14.2 Experimental site

The experiment was took placed in the field of Plant Pathology Division, BARI, Joydebpur, Gazipur.

3.14.3 Experimental layout

The design for the first two field experiments were Randomized complete block design (RCBD) with three replications, where design for the third experiment was paired plot technique with five replications. Field layout for the three field experiments is attached in Appendix III.

3.14.4 Soil type

The texture of the soil in the experimental plot was sandy loam, belonging to Young Brahmaputra and Jamuna Flood Plain (FAO-UNDP, 1988). Land type medium high, organic matter content low to medium, fertility level medium.

3.14.5 Preparation of land

The selected field was opened by a tractor one month before sowing. Several ploughings and cross ploughings were done, followed by laddering, until the necessary tilth for sowing was reached. Weeds and stubbles were gathered and removed from the field prior to final preparation.

3.14.6 Fertilization

The field was fertilized as per recommended dose of WRC (Ahmed and Meisner 1996). In fertilizing the plot, 10 ton/ha cow dung, 220 kg/ha urea, 132 kg/ha TSP, 100 kg/ha MOP, 3.0 kg/ha Boric acid and 110 kg/ha gypsum were used. N, P, K ratio was 100:60:45. During final soil preparation, half of the nitrogen and the total amount of all other nutrients were supplied. The remaining half of the nitrogen was top dressed in two equal splits at 25 and 50 days after seeding.

3.14.7 Irrigation and weeding

The field plots were irrigated thrice: at crown root initiation (CRI), booting and grain filling stages. Weed control was repeated twice, first after 15 days and again after 30 days.

3.14.8 Collection of seed samples: BARI, Joydebpur, Gazipur, provided a seed sample of the susceptible wheat variety ‘Kanchan’.

3.14.9 Bipolaris leaf blight severity

Severity of Bipolaris leaf blight (BpLB) was documented at three stages, flowering, dough and hard dough stages. Under field condition, scoring of Bipolaris leaf blight is done according to CIMMYT’s double-digit (00–99) scale, which is basically an extention of (0–9) scale of Saari and Prescott (1975). In the double digit (00–99) scale (Eyal *et al.* 1987), the first digit (D_1) indicates relative disease height i.e., the point on the plant to which the disease has been progressed, and the second digit (D_2) representing the average

percentage of foliage infection at and below that point. The first digit of the grading is shown as follows:

0	Free from infection	=	No visible lesion present
1	Resistant	=	Few isolated lesions are found on the lower most leaves only
2	Resistant	=	Scattered lesions are found on the second set of leaves.
3	Resistant	=	Light infection on the lower third part of the plant.
4	Moderately resistant	=	Moderate infection of lower leaves with scattered to light infection extending to the leaf immediately below the middle of the plant.
5	Moderately susceptible	=	Severe infection of lower leaves; moderate to light infection extending only to the middle of the plant.
6	Moderately susceptible	=	Severe infection on the lower third of plant; moderate on middle leaves and scattered lesions beyond the middle of the plant.
7	Susceptible	=	Severe lesions on lower and middle leaves with infection extending to the leaf below the flag leaf or with trace infection on flag leaf.
8	Susceptible	=	Severe lesions on lower and middle leaves; moderate to severe infection of upper third of plant; more than a trace infection on flag leaf
9	Highly Susceptible	=	Severe infection on all leaves; spike also infected to some extent.

The second digit shows the severity as a percentage but in terms of a 0-9 scale. The scale is as follows:

0	=	no blight	5	=	41–50% coverage/area of leaf blighted
1	=	up to 10% coverage/area of leaf blighted`	6	=	51–60% coverage/area of leaf blighted
2	=	11–20% coverage /area of leaf blighted	7	=	61–70% coverage/area of leaf blighted
3	=	21–30% coverage/area of leaf blighted	8	=	71–80% coverage /area of leaf blighted
4	=	31–40% coverage/area of leaf blighted	9	=	>81% coverage/area of leaf blighted

Disease severity was scored as percent diseased leaf area (%DLA) on flag leaves and the lines and varieties were graded for their reaction using modified Ragiba *et al.*'s scale as follows: R = less than 10% DLA, MR = 11–30% DLA, MS = 31–50% DLA, S = 51–70% DLA and HS = above 70% DLA. The blight record at the dough stage was the most distinctive among the three phases in terms of providing a clear compare between resistant and susceptible stages, hence data from the dough stage was utilized for final classification of test entry resistance. For example, the score 53 indicates infection up to

midpoint of the plant with an average of 30% leaf area covered. The score may be converted to percent disease severity using the simple formula (Sharma and Duveiller 2003):

% disease severity = $D_1/9 \times D_2/9 \times 100$.

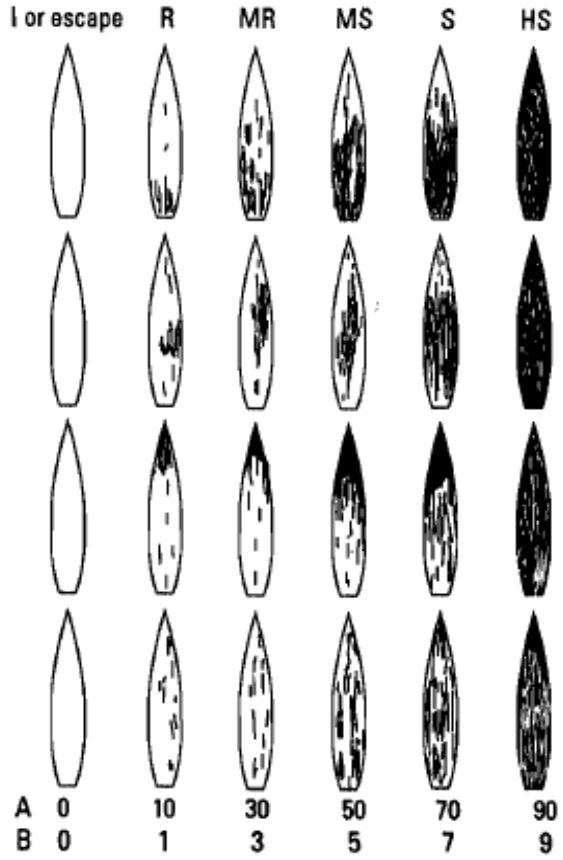


Fig.1. Field key for scoring leaf blight severity- A=Percentage of the leaf surface that is visible occupied by lesions B=Numerical score I=Immune, R=Resistant, MR=Moderately Resistant, MS=Moderately susceptible, S=Susceptible, HS=Highly susceptible

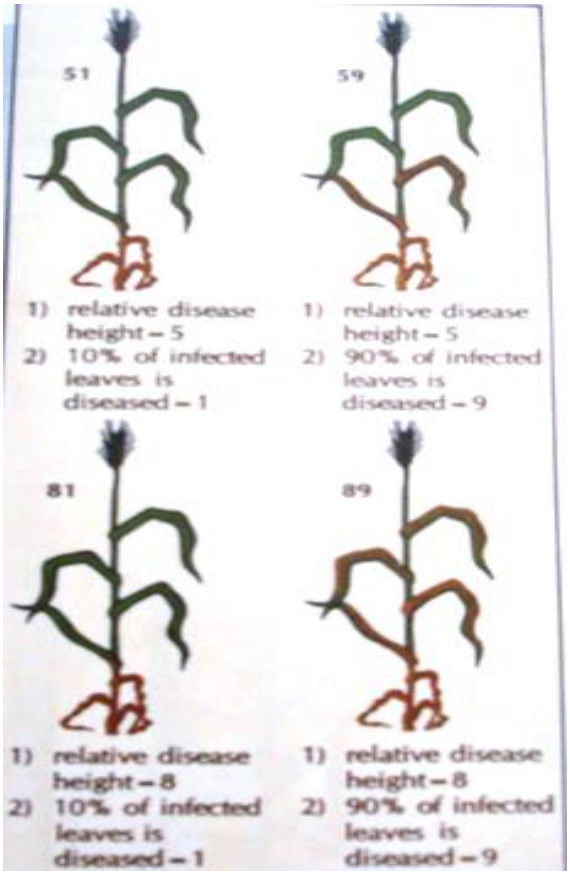


Fig. 2. CIMMYT's double digit (00–99) scales representing the vertical disease progress (first digit) and severity estimate (second digit) based on Saari and Prescott 1975

3.14.10 Disease and yield contributing parameters recorded were as follows:

a) Disease severity
b) % diseased leaf area (% DLA)
c) Number of spike/sq.m
d) Grain number/spike
e) Seed weight/spike (g)
f) 1000 grain weight (g)
g) Grain yield/sq. m (kg)
h) Grain yield plot (kg)
i) Grain yield (kg/ha)
j) Percentage of apparently healthy seeds, black point seeds and shriveled & undersized seeds
k) Grading of seeds (0–5 scale) following CIMMYT method

3.14.11 Experiment 11: Determination of number of fungicide sprays to minimize Bipolaris leaf blight of wheat

So far a good number of fungicide, have been registered in the country to manage wheat leaf blight. In general fungicides are applied three times which is expensive. Number of spray could be minimizing if it is applied in proper time, in proper dose and following proper method of application. Hence the study has been proposed with a view to reduce the spray number for maximizing higher yield and minimizing disease severity. Spray will be done based on crop age.

3.14.12 Experiment 12: Standardization of doses of Folicur and Tilt against Bipolaris leaf blight of wheat

Wheat leaf blight (Bipolaris leaf blight) is a serious disease, causes yield reduction to a great extent. A number of fungicides with specified dose have been registered against the disease but its doses have not been standardized in our climatic condition. So, the experiment has been proposed to select the optimum dose for incurring higher benefit both in terms of disease and yield.

Table 11. Experimental detail on determination of number of fungicide sprays to minimize Bipolaris leaf blight of wheat

Experimental Detail	
Seed: Kanchan (Susceptible wheat variety) Fungicides: 02, [Tilt 250 EC (0.05%) and Folicur 250 EC (0.05%)] Design: RCBD (Randomized complete block design) Replications: 03 (R_1, R_2, R_3) Total plot: 48 (2×8×3) Area per plot: 6 square meter (3×2 meter) Replication to replication spacing: 1 meter Plot to plot spacing: 0.5 meter Line to line spacing: 25 centimeter Seed to seed spacing: 5 centimeter Each block contains: 8 lines Seed sowing method: Continuous line sawing Seed rate: 120 kg/hectare (Treated with seed dressing fungicide Vitavax)	Total land area: Width × Length = (3+3+3+1+1) meter × (2×16 + 0.5×15) meter = 11-meter × 39.5-meter = 434.5 square meter Treatments: 08 (T_1, T_2, T_3, T_4, T_5, T_6, T_7, T_8) T_1 = (35-55-75) days T_2 = (40-60) days T_3 = (45-65) days T_4 = (55-70) days T_5 = (55-75) days T_6 = (65-80) days T_7 = (75) days T_8 = Control

Table 12. Experimental detail on standardization of doses of Folicur and Tilt against Bipolaris leaf blight of wheat

Experimental Detail	
Seed: Kanchan (Susceptible wheat variety) Fungicides: 02, (Tilt 250 EC and Folicur 250 EC) Design: RCBD (Randomized complete block design) Replications: 03 (R_1, R_2, R_3) Total plot: 24 (2×3×4) Area per plot: 6 square meter (3×2 meter) Replication to replication spacing: 1 meter Plot to plot spacing: 0.5 meter Line to line spacing: 25 centimeter Seed to seed spacing: 5 centimeter Each block contains: 8 lines Seed sowing method: Continuous line sawing	Seed rate: 120 kg/hectare (Treated with seed dressing fungicide Vitavax) Total land area: Width × Length = (3+3+3+1+1+0.5+0.5) meter × (2×8+0.5×7+0.5+0.5) meter = 12-meter × 20.5-meter = 246 square meter Treatments: 04 (D_1, D_2, D_3, D_4) D_1 = 0.05% D_2 = 0.075% D_3 = 0.1% D_4 = Control (without fungicide) Date of spray: Standard period = (35-55-75) days after sowing

3.14.13 Experiment 13: Estimation of avoidable yield loss of wheat due to Bipolaris leaf blight

A field experiment was set up in Paired plot technique design with five replications to analyze the crop losses caused by Bipolaris leaf blight of wheat. The plot size was 3m × 2m and the susceptible cultivar Kanchan was sowed in the field of Plant Pathology Division, BARI, Joydebpur, Gazipur, between Rabi season 2010-11 and 2011-12. The trial was conducted in paired blocks with sprayed and unsprayed treatments. However, the protected plots were sprayed 4 times with 0.1 per cent propiconazole at a gap of 15 days beginning from 35 days onwards (35-45-55-65). The observations in terms of terminal disease severity were recorded at dough stage as mentioned in 3.10.9. Disease severity was measured three times using Saari and Prescott's (1975) double-digit scale (00–99) and translated to percent diseased leaf area (% DLA).

Finally, the plants in different plots were harvested, threshed and cleaned seeds were weighed on an Avery balance to record plot yield. Observations on 1000 grain weight and grain yield were recorded from both sprayed and unsprayed plots. Wheat grains in 5 lots were sorted out randomly from all the plots and then one thousand grains from each lot were counted and their weight was recorded by using an electronic balance.

Reduction in various yield components was estimated as follows:

a = b - c

Where, a = the estimate of reduction in a particular yield component,

b = estimate of the component obtained in protected plot and

c = estimate obtained in unprotected plot

The per cent reduction of each component was ascertained as follows:

Reduction in yield component (%) = $\frac{Estimate\ of\ reduction}{Estimate\ in\ protected\ plot}$ x 100

3.14.14 Experiment 14: Screening of germplasm against Bipolaris leaf blight of wheat

In mid-December 2011, under irrigated conditions, 123 genotypes from various sources were grown in 2m long 2 row-plots with 20 cm spacing between rows and 30 cm between entries under field conditions of disease development. The experiment was place on the BARI campus at Gazipur's agricultural field.

3.14.15 Experiment 15: Quality analysis of seeds obtained from field experiments

After harvesting and threshing the seeds were cleaned. The cleaned seeds were stored in paper bags covered with polythene and kept in the refrigerator at 5–7ºC for future studies. One hundred seeds from every plot were separated for analysis of healthy and diseased seeds.

Table 13. Experimental detail on estimation of avoidable yield loss of wheat due to Bipolaris leaf blight

Experimental Detail	
Seed: Kanchan (Susceptible wheat variety) Fungicides: 01, (Tilt 250 EC) Design: Paired plot technique Replications: 05 (R_1, R_2, R_3, R_4, R_5) Total plot: 10 (2×5) Area per plot: 6 square meter (3×2 meter) Replication to replication spacing: 1 meter Plot to plot spacing: 0.5 meter Line to line spacing: 25 centimeter Seed to seed spacing: 5 centimeter Each block contains: 8 lines Seed sowing method: Continuous line sawing	Seed rate: 120 kg/hectare (Treated with seed dressing fungicide Vitavax) Total land area: Width × Length = (3+3+1) meter × (2×5+0.5×4) meter =7-meter × 12-meter = 84 square meter Treatments: 02 (Sprayed and Unsprayed) Dose of fungicide: Tilt 250 EC (propiconazole) (0.1%) Date of spray: 4 times at an interval of 15 days starting from 35 days onwards = (35-45-55-65) days after sowing.

3.15 Experiment 16: Seed Health Test of different wheat varieties

3.15.1 Collection of seed Samples

Wheat seeds of variety–BARI Gom-25, BARI Gom-26, BARI Gom-27, BARI Gom-28, BARI Gom-29, BARI Gom-30 and Kanchan were obtained from Bangladesh Agriculture Research Institute (BARI) in the year 2015.

3.15.2 Preservation of the seed Samples

Each seed sample weighed 200 grams (approx.). In refrigerator at 5–7°C the seeds were then placed in paper bags with polythene covers and preserved until they were needed for further research.

3.15.3 Categorization of seeds

The well-kept seed samples were sorted out into 3 categories such as pure seeds/apparently healthy seeds, black point seeds and shriveled & undersized seeds. Four hundred seeds from each sample were taken randomly for seed quality/seed health test.

3.15.3.1 Apparently healthy seeds

Apparently healthy seeds are those that are larger than 2.25 mm in diameter and have a normal embryo, consistent genuine bright color, seed hair and a smooth surface. The outer coating of these seeds is still intact.

Purity Percentage of seed (%) = $\frac{Weight\ of\ pure\ seeds\ (g)}{Total\ Weight\ of\ seeds\ (g)} \times 100$

3.15.3.2 Black point seeds

The seeds consisting the black discoloration generally surrounding the embryonic area and in most cases the black discoloration was found to extend outside the germ end to the ventral side or sulcus of the seeds are known as black point seeds. *Black point seeds were sorted from the collected seed samples manually by eye estimation.*

$$\textit{Percent black point incidence} = \frac{\text{Total no.of black point seeds}}{\text{Total number of seeds examined}} \times 100$$

3.15.3.3 Shriveled & undersized seeds

Seeds of various types and sizes typically malformed and discolored in the texture and structure with wrinkly and rough surface are named as shriveled seeds. The sulcus of the seeds is larger than typical. Seed hair is discovered scatteredly present with a blackish hue in the embryonic sections, which are practically malformed. Typically, the seeds are tiny, thin and light in weight (Monsura 2011).

3.15.4 Grading of seeds

Gilchrist (1985) recommended using a 0–5 rating system for grading, which was used.

Grade-0=Discolouration free seeds (apparently healthy seeds),

Grade-1=Embryo tip black to brown only,

Grade-2=Whole embryo discoloured,

Grade-3=$^1/_4$ of the seed with embryo discoloured,

Grade-4=$^1/_2$ of the seed with embryo discoloured,

Grade-5=More than $^1/_2$ of the seed with embryo discoloured and shriveled.

3.15.5 Isolatoin, Purification & identification of fungi

The International Seed Testing Association (ISTA) is a globally known organization that offers standardized seed quality testing methodologies. Seed health testing (ISTA-SHT) can be done in a variety of ways, depending on the pathogen and the kind of seed. The ISTA-recommended standard blotter method (Limonard 1966) and PDA method (Malone and Muskett 1964) were employed to detect seed-borne mycoflora.

3.15.5.1 Blotter method

Four hundred seeds were randomly taken from each of the seed samples and sorted into healthy and black pointed seeds. Twenty-five healthy seeds (but 10 seeds in case of black point) are placed on three layer of water soaked filter papers contained in each of 9 cm Petri plate. The Petri plates were incubated usually for 7 days under 12th alternating cycles of light and darkness. After incubation, fungi developed on seeds are examined under different magnification of a stereomicroscope and identified. The identification of

the fungi is based on the way they grow on the seeds and on the morphological characters of fruiting bodies, spores/conidia observed under a compound microscope. Seeds were surface sterilized in 10% Clorox for three minutes and then rinsed for two minutes each in three changes of sterile distilled water prior to plating.

3.15.5.2 Tissue planting/agar plate method

The agar plate method, is another popular method in which ten seeds (both healthy and black point seeds) were plated in each Petri plate containing 15 ml of potato dextrose agar (PDA) medium and incubated for 5–7 days at 25±2°C under 12th alternating cycles of light and darkness. At the end of the incubation period, fungi growing out from seeds on the medium are examined and identified. Identification is based on colony characters and morphology of sporulating structures under a compound microscope. Photomicrographs of fungal colonies and spores had taken. The generic and species identity of each colony was recorded and identification was determined following standard literature (Barnett and Hunter 1972, Benoit and Mathur 1970, Booth 1971, Chidambaram *et al.* 1973, Ellis 1971, 1976, Ellis and Ellis 1997, Raper and Thom 1949, Thom and Raper 1945, Subramanian 1971, Sutton 1980).

Percentage frequency of the occurrence of the fungal isolates was calculated by adopting the following formula (Spurr and Welty 1972) -

$$\text{Frequency (\%)} = \frac{\text{No.of inocula from which fungal isolates were raised}}{\text{total number of inocula culture}} \times 100$$

3.15.6 Germination test /Seedling emergence

In each plastic tray filled with sterilized sandy soil, fifty seeds were planted for the germination test. For 14 days, special efforts were made to ensure that the soil in the tray received adequate sunlight and moisture. Then, according to the International Seed Testing Association Rules, statistics on seed germination % and mortality percentage were collected (ISTA 2001). Seeds that produced plumule and radical were classified as germinated. The formula was used to count germinated seeds and convert them to a measure of seed viability (Sv).

Seed viability, $Sv = \frac{n}{N} \times 100$

Where Sv = % seed viability

n = Total number of seeds germinated from each normal or abnormal seed type and

N = Total number of seeds plated.

3.15.7 Seedling growth and vigour index

Data on seedling mortality, plant stand, root and shoot weight, vigour index were recorded. **3.16.7.1 Mortality percentage**

Seedling mortality was counted after 10 days of incubation following the formula (Anon. 2014).

$$\text{Mortality percentage of seeds (\%)} = \frac{\text{number of living seedlings}}{\text{total number of seedlings planted}} \times 100$$

3.16.7.2 Measurement of plant shoots and root growth and weight

The dirt in the tray was first moistened to make it wet enough for the plant to uproot easily. The plant and root were then removed from the tray and plunged into a pail of water. Each seedling's root was meticulously washed and cleansed under flowing tap water. With a sharp knife, the root section was separated from the shoot portion. Ten seedlings were chosen at random from each tray and their individual shoot lengths were measured using a mm scale from the base of the stem to the growth point of the youngest leaf. Similarly, the length of the root was measured from the root's beginning point to the greatest accessible lateral root apex, as well as the fresh weight of the shoot and root was also determined using digital balance.

3.15.7.3 Vigour index (VI)

Seed vigor, an essential indicator of seed quality, influences the likelihood of plants emerging quickly and uniformly. Planting low-vigor seed resulted in shorter plants, delayed panicle exertion and anthesis, reduced tillering ability and lower yield. According to Abdul-Baki and Anderson (1973), the seedlings' vigour index (VI) can be calculated as follows:

Vigor Index (VI) = (mean root length + mean shoot length) × percent emergence

$$VI = (RL+SL) \times GP,$$

where RL= Root length (cm),

SL= Shoot length (cm) and

GP= Germination percentage.

CHAPTER FOUR
RESULTS & DISCUSSION

CHAPTER FOUR

RESULTS & DISCUSSION

4.1 Fungal Flora associated with Bipolaris Leaf Blight (BpLB) infected wheat leaves

Thirty-five fungal species, representing 20 genera were found to be associated with BpLB infected leaves of twenty-one wheat varieties, collected from eight districts (Dhaka, Gazipur, Dinajpur, Joypurhat, Pabna, Sirajganj, Kushtia and Chuadanga) of Bangladesh. The isolated fungi were *Alternaria alternata* Keissler, *A. triticina* Prasada & Prabhu, *Arthrinium* Kunze ex Fr., *Aspergillus flavus* Link, *A. fumigatus* Fresen., *A. niger* Tiegh., *A. terreus* Thom., *Aspergillus* sp. Link, *Bipolaris cynodontis* (Marig.) Shoem., *B. oryzae* (Breda De Haan) Shoem., *B. sorokiniana* (Sacc.) Shoem., *B. tetramera* (Mckinney) Shoem., *B. victoriae* (Meehan & Murphy) Shoem., *Bispora antenata* (Pers.) Mason, *Cheatomium globosum* Kunze ex Fr., *Chaetophoma* Cooke, *Cladosporium cladosporioides* (Fresen.) de Vries, *Coniothyrium* sp. Corda, *Curvularia affinis* Boedijn, *C. lunata* Boedijn, C. *pallescens* Boedijn, *Drechslera dematioidea* (Bub. & Wrob.) Subram. & Jain, *D. hawaiiensis* (Bugnicourt) ex M.B. Ellis; Subram. & Jain, *Epicoccum purpurascens* Ehrneb. ex Schlecht, *Eurotium* sp., *Fusarium moniliforme* Sheldon, *F. nivale* Ces., *F. semitectum* Berk. & Rav., *Nigrospora oryzae* (Berk. & Br.) Petch., *N. sacchari* (Speg.) Mason, *Penicillium digitatum* (Fr.) Sacc., *Pestalotiopsis guepinii* (Desm.) Stay., *Rhizopus stolonifer* (Ehrenb.: Fr.) Vuill, *Syncephalastrum racemosum* Cohn ex J. Schröt. and *Trichoderma viride* Pers.

4.2 Taxonomic enumeration of fungi

Alternaria alternata (Fr.) Keissler 1912. Beih. Bot. Zbl. **29**: 433. **(Fig. 1A)**

Colony usually black or olivaceous black, sometimes grey. Conidiophores golden brown, smooth, up to 50×3–6 µm. Conidia formed in long, often branched chains, obclavate, obpyriform, ovoid or ellipsoidal, often with a short conidial or cylindrical beak, pale to mid golden brown, smooth or verruculose, with up to 8 transverse and usually several longitudinal or oblique septa, 21.6–52.4×9.2–17.1, beak pale, 2–5 µm thick.

Specimen examined: BpLB infected leaves of Wheat (*Triticum aestivum* L.), variety-Kalyansona, vill.-WRC, dist.-Dinajpur, S Momtaz 116, 25 February 2011.

Alternaria triticina Prasada & Prabhu 1963. Indian Phytopath. **15**: 292-293. **(Fig. 1B)**

Colony discrete or effuse, dark blackish brown to black. Conidiophores up to 30×3–6 µm, golden or olivaceous brown, occasionally branched. Conidia solitary, obclavate, rostrate, golden brown, smooth, 27.2–76.3×10.2–25.4 µm, body usually with 4–7 transverse and

several longitudinal or oblique septa, beak shorter than or the same length as the body, cylindrical, 3–5 µm thick.

Specimen examined: BpLB infected leaves of Wheat (*Triticum aestivum* L.), variety-Balaka, vill.-WRC, dist.-Dinajpur, S Momtaz 124, 25 March 2011.

Arthirinium Kunze ex Fr. 1821. Kunze in Kunze & Schmidt. **(Fig. 1C)**

Colonies effused, blackish brown. Conidia solitary, circular to subcircular, lateral and sometimes also terminal, frequently flattened and with a hyaline rim or germ slit, brown or dark brown, smooth, 0-septate, 9.3–10.2 µm.

Specimen examined: BpLB infected leaves of Wheat (*Triticum aestivum* L.), variety-Kanchan, vill.-BARI, dist.-Gazipur, S Momtaz 111, 13 March 2011

Aspergillus flavus Link 1809. Magazin der Gesellschaft Naturforschenden Freunde Berlin **3**(1): 16. **(Fig. 1D)**

Colonies effuse greenish. Mycelia well developed, septate, hyaline and profusely branched. Conidiophores 300–600 µm long. Vasicles 10–35 µm in diameter. Sterigmata 8–14×3–5 µm. Conidia greenish, catenulate, globose or pyriform, smooth, 3–5 µm in diameter.

Specimen examined: BpLB infected leaves of Wheat (*Triticum aestivum* L.), variety-Sonalika, vill.-BARI, dist.-Gazipur, S Momtaz, 29, 3 April 2010.

Aspergillus fumigatus Fresenius 1863. Beitrage zur Mykologie. **3**: 81. **(Fig. 1E)**

Colonies flat, olivaceous green. Conidiophores long, often with a foot cell, straight or flexuous, swollen at the apex into a spherical vesicle. Surface of vesicle covered by closely packed more or less clavate branches. Conidia catenulate, dry, usually globose, echinulate and smooth. Colonies of the fungus produced thousands of minute pale green conidia (2–3 µm).

Specimen examined: BpLB infected leaves of Wheat (*Triticum aestivum* L.), variety-Inia-66, vill.-BARI, dist.-Gazipur, S Momtaz 22, 3 April 2010.

Aspergillus niger Van Tieghem 1867. Ann. Sci. Nat. Bot. Ser. **8**(5): 240. **(Fig. 1F)**

Colonies effuse, black. Conidiophores brown 200–400×7–10 µm. Vesicles globose or sub globose, thick walled, commonly 20–50 µm, occasionally up to 100 µm in diameter. Foot cell present. Sterigmata 20–30×6–8 µm. Conidia dark brown, one celled, globose, 2–4 (5) µm in diameter.

Specimen examined: BpLB infected leaves of Wheat (*Triticum aestivum* L.), variety-Ciano-79, vill.-WRC, dist.-Dinajpur, S Momtaz 212, 23 March, 2011.

Aspergillus terreus Thom. 1918. American J. Bot. **5**(2): 85. **(Fig. 1G)**

Colonies pure brown or ochraceous brown, brighter at maturity. Reverse dull yellow. Conidiophores smooth, flexuous, commonly 100–265×4–6 µm. Vasicles dome shaped, 9–17 µm in diameter. Sterigmata 4–7×2–5 µm. Conidia pale brown, globose to slightly ellipsoidal, catenulate, 2–3 µm.

Specimen examined: BpLB infected leaves of Wheat (*Triticum aestivum* L.), variety-Sonora, vill.-BARI, dist.-Gazipur, S Momtaz 49, 3 April 2010.

Aspergillus sp. Link **(Fig. 1H)**

Colonies flat, yellowish. Mycelium well-developed, septate, brown. Conidiophores very long, often with a foot cell, straight, 100–465×5–6 µm, terminating in a globose head. Vasicles dome shaped, 8–16 µm. Surface of vesicle covered by closely packed more or less clavate sterigmata 5–8×2–5 µm. Conidia hyaline, one celled, globose, echinulate, 3–5 µm.

Specimen examined: BpLB infected leaves of Wheat (*Triticum aestivum* L.), variety-Shatabdi, vill.-Farakpur, dist.-Kushtia, S Momtaz 296, 7 March, 2012.

Bipolaris cynodontis (Marignoni) Shoemaker 1959. Canadian J. Bot. **37**(5): 883. **(Fig. 1I)**

Colonies on PDA medium dark ash to black, cottony, reverse black and mycelium profusely branched, septate, brown. Conidiophores pale to mid brown, arising singly or in groups of 2 or 3, short, straight or slightly bent. The first conidium borne at a short distance from the base of the conidiophore, 4 to 9 conidia borne acropleurogenously at the geniculated tip. Conidia light to olivaceous brown, ellipsoid to ovate, broader in the middle with rounded ends, straight or slightly curved, uniform in colour, smooth, thin walled. 3–9 (usually 7–8) pseudoseptate, 35.6–59.5×8.5–13.6 µm.

Specimen examined: BpLB infected leaves of Wheat (*Triticum aestivum* L.), variety-Saurab, vill.-Vutiapara, dist.-Joypurhat, S Momtaz 425, 4 April 2013.

Assocaition of *Bipolaris cynodontis* with wheat is a new record in Bangladesh.

Bipolaris oryzae (Breda De Haan) Shoemaker 1959. Canadian J. Bot. **37**(5): 883. **(Fig. 1J)**

On agar plate colonies spreading, ash grey (mouse gray) to dark greenish grey, mycelium fluffy, aerial, cottony. On reverse view colony light olivaceous grey with wavy margin. Conidiophores solitary or in small groups, straight or flexuous, pale to mid brown, bearing

dark brown conidia acropleurogenously. Conidia fuliginous to olivaceous brown, curved, widest in the middle or just above middle, tapering to rounded ends, base more rounded, not flat or definite, 89.1–126.9×18.9–21.6 (17) µm, 8–13 pseudoseptate.

Specimen examined: BpLB infected leaves of Wheat (*Triticum aestivum* L.), variety-Shatabdi, vill.-BARI, dist.-Gazipur, S Momtaz 11, 10 March 2010.

Bipolaris sorokiniana (Sacc.) Shoemaker 1959. Canadian J. Bot. **37**(5): 883. **(Fig. 1K)**

Colonies on PDA medium olivaceous brown to very dark becoming generally lighter towards the periphery, margin mostly smooth, sometimes wavy with easily recognizable dark band, large number of conidia usually present in the centre, sometimes entire colony covered by black shiny conidia making the colony black and shiny, rarely colonies pinkish white with almost no conidia. On reverse view colonies black to deep olivaceous brown, roughly circular with concentric rings, margin smooth or irregular, rarely light pinkish white. Conidiophores brown, short, erect, in most cases single, bearing 1-6 conidia. Conidia ellipsoid, dark brown, mostly straight or slightly curved, wall thick but less so towards the ends, broadest in the middle, ends rounded, scar clear within the basal cell. Terminal portion of the end cells subhyaline, 4–9 pseudoseptate, 48.0–88.6×17.2–25.8 µm.

Specimen examined: BpLB infected leaves of Wheat (*Triticum aestivum* L.), variety-Shatabdi, vill. - Doripara, dist.-Joypurhat, S Momtaz 362, 4 April 2013.

Bipolaris tetramera (Mckinney) Shoemaker 1959. Canadian J. Bot. **37**(5): 883. **(Fig. 1L)**

Colonies on PDA brown to olivaceous brown, mat, reverse brown, remarkable colony margin with brown band also noticed. Conidiophores brownish, single or in clusters of 2 to 3, conidia almost in cluster. Brown, ellipsoid, mostly cylindric, straight with broadly rounded ends, lighter towards the terminal cells, 3 pseudoseptate, 22.6–35.6×10.2–12.8 µm.

Specimen examined: BpLB infected leaves of Wheat (*Triticum aestivum* L.), variety- Kanchan, vill. - BARI, dist.- Gazipur, S Momtaz 109, 12 February 2011.

Bipolaris victoriae (Meehan & Murphy) Shoemaker 1959.Canadian J. Bot. **37**(5): 883. **(Fig. 2A)**

Colony color and growth characters on PDA very similar to those of *B. sorokiniana*, except in this case the conidia little lighter in color, slender and slightly curved. Conidiophores solitary or in small groups, straight or flexuous, pale to mid brown. Conidia long, ellipsoid, straight or slightly curved, thin walled, pale or mid-pale golden brown, 8–10 pseudoseptate, 46.8–70.5×12.6–18 µm.

Specimen examined: BpLB infected leaves of Wheat (*Triticum aestivum* L.), variety-Kheri, vill.-BARI, dist.-Gazipur, S Momtaz 531, 10 April 2013.

Bispora antenata (Pers.) Mason 1953. Can. J. Bot. **31**: 582. **(Fig. 2B)**

Colony effuse, black. Mycelium dark, conidiophores dark brown, short, simple or sparingly branched, 5-30×2-5 µm. Conidia brown or dark brown, oblong, 2-celled or less often 3-celled, with thick black band at the septa, catenulate, produced acropetally, 14.1–17.8×7.2–8.3 µm. Saprophytic on leaves, woods.

Specimen examined: BpLB infected leaves of Wheat (*Triticum aestivum* L.), variety-Aghrani, vill.-WRC, dist.-Dinajpur, S Momtaz 192, 24 March 2011.

The fungus is new record for Bangladesh.

Chaetomium globosum Kunze ex Fr. 1829. Systema Mycologicum. **3**: 255. **(Fig. 2C)**

Colonies punctiform. Perithecia dark brown to black and clothed, asci soon disappearing. The lemon shaped, brown ascospores free from their asci through the ostiole, 5.2–6×2.8–4 µm.

Specimen examined: BpLB infected leaves of Wheat (*Triticum aestivum* L.), variety-Seri-82, vill.-Sadar, dist.-Chuadanga, S Momtaz 318, 17 March 2013.

Chaetophoma Cooke 1878. Grevillea. **7**(41): 25. **(Fig. 2D)**

Colonies greish black, cottony. Reverse grayish black. Pycnidia dark, superficial, without ostiole, in dense or loose clusters, seated on a subiculum of interwoven hyphae, very small, globose to irregular, 52.2–66.6×41.4–61.2 µm. Conidia ovate or elliptic, hyaline, 1-celled, very small 3-5 µm. Saprophytic on plant material.

Specimen examined: BpLB infected leaves of Wheat (*Triticum aestivum* L.), variety-Saurav, vill.-Kazirhat, dist.-Pabna, S Momtaz 326, 5 April 2013.

Cladosporium cladosporioides (Fresen.) GA de Vries 1952. Contribution to the knowledge of the genus *Cladosporium*: 57. **(Fig. 2E)**

Colonies effuse, olive green, or olivaceous brown, velvety. Conidiophores sometimes up to 350 µm long, olivaceous brown, smooth or verruculose. Ramo-conidia 0-1 septate, up to 30 µm long, 2–5 µm thick, smooth. Conidia formed in long branched chains, pale olivaceous brown, mostly 0-septate, ellipsoidal or lemoniform, smooth, 4–10×2–4 µm.

Specimen examined: BpLB infected leaves of Wheat (*Triticum aestivum* L.), variety-Balaka, vill.-BARI, dist.-Gazipur, S Momtaz 62, 10 March 2010.

Cladosporium cladosporioides (Fresen.) GA de Vries 1952. Contribution to the knowledge of the genus *Cladosporium*: 57. **(Fig. 2E)**

Colonies effuse, olive green or olivaceous brown, velvety. Conidiophores sometimes up to 350 µm long, olivaceous brown, smooth or verruculose. Ramo-conidia 0-1 septate, up to 30 µm long, 2-5 µm thick, smooth. Conidia formed in long branched chains, pale olivaceous brown, mostly 0-septate, ellipsoidal or lemoniform, smooth, 4–10×2–4 µm.

Specimen examined: BpLB infected leaves of Wheat (*Triticum aestivum* L.), variety-Balaka, vill.-BARI, dist.-Gazipur, S Momtaz 62, 10 March 2010.

Coniothyrium Corda 1840. Icones fungorum hucusque cognitorum. **4**: 38. **(Fig. 2F)**

Colonies greish black, cottony. Reverse grayish black. Pycnidia globose, ostiolate, 62.4–84×58.8–84 µm conidiophores reduced, simple, conidia small, ellipsoid, dark,1-celled, 13.5×6.3–8.1 µm. Parasitic or saprophytic.

Specimen examined: BpLB infected leaves of Wheat (*Triticum aestivum* L.), variety-Ananda, vill.-WRC, dist.-Dinajpur, S Momtaz 138, 23 February, 2011.

Curvularia affinis Boedijn 1933. Bull. Jard. Bot. Buitenz. **13**(1): 130. **(Fig. 2G)**

Colonies cottony, effuse, grayish black. Conidiophores solitary, unbranched, straight. Conidia, pale brown, curved, 25–39.7×9.1–12.8 µm.

Specimen examined: BpLB infected leaves of Wheat (*Triticum aestivum* L.), variety-Barkat, vill.-BARI, dist.-Gazipur, S Momtaz 95, 10 February 2010.

Curvularia lunata (Wakker) Boedijn 1933. Bull. Jard. Bot. Buitenz. **13**(1): 123. **(Fig. 2H)**

Colonies effuse grayish black or black, hairy, cottony or velvety. Conidiophores solitary, unbranched, straight or slightly undulating, geniculate, mid brown, septate, 37–64×9.2–14.4. Conidia dark brown, mostly 3-septate, mostly curved, third cell from the base is broader and darker than others, broader cells mid brown, other cells paler, smooth, 22.5–31.2×9.3–12.6 µm.

Specimen examined: BpLB infected leaves of Wheat (*Triticum aestivum* L.), variety-Protiva, vill.-BARI, dist.-Gazipur, S Momtaz 69, 10 February 2010.

Curvularia pallescens Boedijn 1933. Bull. Jard. Bot. Buitenz. **13**(1):123. **(Fig. 2I)**

Colony effuse, gray, becoming black at maturity. Conidiophores solitary, mostly unbranched, straight, broader at the apex, up to 90.2 µm long and 4.9–7.5 µm thick. Conidia mostly 4-septate, slightly curved, smooth, 18.8–28×7.8–9.2 µm.

Specimen examined: BpLB infected leaves of Wheat (*Triticum aestivum* L.), variety-Akber, vill.-BARI, dist.-Gazipur, S Momtaz 94, 10 February 2010.

Drechslera dematioidea (Bub. & Wrob.) Subram. & Jain. 1966. Curr. Sci. 35: 354. (**Fig. 2J)**

On PDA medium colony blackish ash to black, reverse black, mycelium fluffy. Conidiophores light brown, short, straight or flexuous, sometimes geniculate and slender, arising singly or in small group. Conidia yellowish brown or golden brown to dark brown, cylindrical to clavate, broader at the tip, tapering towards the base, ending in a wide dark scar. The narrowest part is the point of attachment. Basal cell lighter in colour, smooth, thick walled, 3–5 pseudoseptate, 27.0–40.6×14.1–16 µm.

Specimen examined: BpLB infected leaves of Wheat (*Triticum aestivum* L.), variety-Saurav, vill.-Doripara, dist.-Joypurhat, S Momtaz 410, 4 April 2014.

The fungus is new record for Bangladesh.

Drechslera hawaiiensis (Bugnicourt) ex MB Ellis; Subram. & Jain.1966. Curr. Sci. 35: 354. **(Fig. 2K)**

Colony effuse, grey, dark blackish brown or grayish black or black, reverse black. Conidiophores are solitary and brown in colour. Conidia pale to mid brown, slender, borne in clusters towards the apex, pointing out in different directions. Conidia oblong or cylindrical, rounded at the ends, hilum within the contour of the basal cell, 4–7 (5 septa common) septate, 16.2–28.9×6.3–9 µm.

Specimen examined: BpLB infected leaves of Wheat (*Triticum aestivum* L.), variety-Seri-82, vill.-BARI, dist.-Gazipur, S Momtaz 32, 3 April 2010.

The association of this fungus with wheat is a new record in Bangladesh.

Epicoccum purpurascens (*E. nigrum* Link) Ehrenb. ex Schlecht. 1824. Synop. Pl. crypt. :136 **(Fig. 2L)**

Colonies olivaceous green. Sporodochia globose, dark, up to 2 mm. Conidiophores very short, unbranched, straight or flexuous, colourless to pale brown, smooth, 5–15×3–6 µm. Conidia solitary, subspherical or pyriform, dark golden brown, often with a pale protuberant basal stalk cell, most commonly 15–25 µm. But smaller and much larger (up to 50 µm) conidia are formed sometimes.

Specimen examined: BpLB infected leaves of Wheat (*Triticum aestivum* L.), variety-Gaurab, vill. - WRC, dist. - Dinajpur, S Momtaz 180, 24 March 2011.

Eurotium sp. 1809. Magazin der Gsellschaft Naturforschenden Freunde Berlin. **3**(1): 31. **(Fig. 3A)**

Colonies on PDA medium light yellowish, cottony, reverse orange red. Hyphae pale to mid brown, smooth, septate, branched, 1–4 µm diameters. Cleistothecia superficial, yellow to light brown, globose to subglobose, 93.6–146.4 µm, asci 8 spored, 9.2–10.8 µm, ascospores 4.4–5.6×5.2–6 µm.

Specimen examined: BpLB infected leaves of Wheat (*Triticum aestivum* L.), variety-Kanchan, vill.-BARI, dist.-Gazipur, S Momtaz 114, 13 March, 2011.

Fusarium moniliforme Sheldon 1904. Rep. Neb. Agric. Exp. Stn. **17**:23-32. **(Fig. 3B)**

Mycelium extensive and cottony, white, often with some tinge of pink. Reverse pinkish yellow. Mycelia hyaline, profusely branched, septate. Conidiophores hyaline 0-2 septate. Phialides hyaline, 16–20×3–4 µm. Conidia hyaline, variable, principally of two kinds. Microconidia 1-celled, ovoid or oblong, borne singly or in chains, 5–15×2–3 µm. Macroconidia several-celled, slightly curved or bent at the pointed ends, 3–4 septate, conidia 25–35×3–4 µm, 5–6 septate conidia 30–50× 3–5 µm.

Specimen examined: BpLB infected leaves of Wheat (*Triticum aestivum* L.), variety-Kheri, vill. - BARI, dist. - Gazipur, S Momtaz 01, 10 March, 2010.

Fusarium nivale (Fr.) Ces. 1839. Rabenh. Herb. Myc. Ed. 1, No. 1439. **(Fig. 3C)**

Colonies white to pale peach to apricot. Mycelium sparse to densely floccose or felted, individual hyphae irregular. Conidia hyaline, variable, principally of two kinds, curved, broadly falcate with a pointed apex and flattened, wedge shape base, 3-septate, 14.3–28.4×2.4–4.4, macroconidia several-celled, slightly curved or bent at the pointed ends, typically canoe-shaped, microconidia 1-celled, ovoid or oblong, borne singly or in chains, some conidia intermediate, 2 or 3 celled, oblong or slightly curved, parasitic or saprophytic.

Specimen examined: Isolated from BpLB infected leaves of Wheat (*Triticum aestivum* L.), variety-Seri-82, vill.-BARI, dist.-Gazipur, S Momtaz 32, 3 April 2010.

Fusarium semitectum Berk. & Rav. 1875. In Berkeley, Gravillea. 3: 98. **(Fig. 3D)**

Cultures at first white peach tinge and peach couloured from below. Macroconidia formed in aerial mycelium from loosely branched conidiophores. Macroconidia varied from 3–5 septate, curved with a wedge-shaped but not pedicellate basal cell and pointed apex. 3 septate conidia 19.8–26.8×2.7–3.6 µm and 5 septate conidia 22–40×3.7–4 µm. Microconidia aseptate pyriform to obovate, 10–12×2.5–3.5 µm.

Specimen examined: BpLB infected leaves of Wheat (*Triticum aestivum* L.), variety-Inia-66, vill.-BARI, dist.-Gazipur, S Momtaz 55, 20 March 2011.

Nigrospora oryzae (Berk. & Broome) Petch. 1924. J. Indian Bot. Soc.: 24. (**Fig. 3E)**

Colonies at first white, effuse with small shining black conidia, later brown or black when sporulation abundant. Mycelium immersed. Conidiophores solitary, ampulliform or subspherical, colorless. Conidia solitary, simple, spherical or broadly ellipsoidal, compressed dorsiventrally, black, shining, smooth, 0-septate. Conidiophores 3–7 µm thick. Conidiogenous cells 6–9 µm diam. Conidia 10–17 (mostly 14–15 µm) diam.

Specimen examined: BpLB infected leaves of Wheat (*Triticum aestivum* L.), variety-Saurav, vill.-WRC, dist.-Dinajpur, S Momtaz 112, 24 March 2011.

Nigrospora sacchari (Speg.) Mason 1927. Trans. Br. Mycol. Soc. **12**(2-3): 152-165. **(Fig. 3F)**

Colonies at first white with small shining black conidia, later brown or black, sporulation abundant. Mycelium partly superficial. Conidiophores ampulliform or subspherical, colorless. Conidia solitary, simple, spherical or broadly ellipsoidal, compressed dorsiventrally, black, shining, smooth, 0-septate. Conidiophores 4–7 µm thick. Conidiogenous cells 7–9 µm diam. Conidia 17–24 (mostly 20–22) µm diam.

Specimen examined: BpLB infected leaves of Wheat (*Triticum aestivum* L.), variety-Ananda, vill.-WRC, dist.-Dinajpur, S Momtaz 140, 24 March 2011.

Penicillium digitatum (Fr.) Sacc. 1910. Bur. Anim. Ind., Bul. 118: 31-33. **(Fig. 3G)**

Colony small, cottony, greenish, reverse creamy. Hyphae creeping, septate, branched, hyaline. Conidiophores erect, apically irregularly verticillate-penicillately branched, 15–28×3.5–5.0 µm. Conidia catenulate, spherical or elliptical, smooth, white in mass, commonly 3.5–5.0 µm.

Specimen examined: BpLB infected leaves of Wheat (*Triticum aestivum* L.), variety-Kalyansona, vill.-BARI, dist.-Gazipur, S Momtaz 17, 10 March 2010.

Pestalotiopsis guepinii (Desm.) Stay. Bulletin du Jardin Botanique de IEtat a Bruxelles. **19**(3): 312. **(Fig. 3H)**

Colonies white, cottony. Mycelia hyaline, septate, profusely branched, fruiting structure black, shining, conspicuous, condiomata 200 µm. Conidiophores short, hyaline, 10–15×1–2 µm, mostly aseptate with 1–2 proliferation. Conidia blackish brown, mostly three septate

with 2–5 hyaline appendages at the apex and short hyaline appendage at the base, apical appendages 16–33 µm long and basal appendage 4–12 µm long.

Specimen examined: BpLB infected leaves of Wheat (*Triticum aestivum* L.), variety-Balaka, vill.-WRC, dist.-Dinajpur, S Momtaz 130, 24 March 2011.

Pestalotiopsis guepinii associated with wheat is a new record in Bangladesh.

Rhizopus stolonifer (Ehrenb.: Fr.) Vuillemin 1902. Toney Bot. Clup. **69**: 592-616. **(Fig. 3I)**

Grayish, fluffy colony, light gray reverse. Coenocytic, well-developed, branching, fluffy mycelium. Numerous aerial stolons with rhizoids grow at various places. One or more long sporangiophores generated directly above the rhizoids, 300–700×3–4 µm. At the apex each sporangiophore becomes swollen as a sporangium. There is also columella, sporangium produces nonmotile, brownish sporangiospores, 4–6 µm in diam.

Specimen examined: BpLB infected leaves of Wheat (*Triticum aestivum* L.), variety-Shatabdi, vill. -Farmpara, dist.-Chuadanga, S Momtaz 324, 13 March 2013.

Syncephalastrum racemosum Cohn ex J. Schröt. 1886. Kryptogamen-Flora von Schlesien 3-1(2): 217. **(Fig. 3J)**

Transparent, fluffy colony, reverse light or yellowish-brown, rapid growing, filling the Petri dish with PDA media after 48 hours. Mycelium densely branched. Sporangiophores short and often branching. They end up in a vesicle (85 µm in diameter). Around this vesicle are the merosporangia (4–6×9–60 µm), which are filled with linear series (chains) of sporangiospores. Each merosporangium contains a single row of 3–18 merosporangiospores. Merosporangiospores (3–8 µm, may rarely reach 11 µm in diameter) are one-celled and spherical to cylindrical in shape.

Specimen examined: BpLB infected leaves of Wheat (*Triticum aestivum* L.), variety-Shatabdi, vill.-Vutiapara, dist.-Joypurhat, S Momtaz 100, 13 February 2013.

Trichoderma viride Pers. 1794. Neues Magazin fur die Botanik **1**: 92. **(Fig. 3K)**

Colony effuse, light green, hyphae elongate. Conidiophores hyaline, upright, much branched, bearing phialides single or in groups. Conidia hyaline, powdery mass, globose, 1-celled, ovoid, borne in small terminal clusters 3.5–5 µm, usually easily recognized by its rapid growth and green patches or cushions of conidia, saprophytic in soil or on wood, very common, some species reported as parasites on other fungi.

Specimen examined: Isolated from BpLB infected leaves of Wheat (*Triticum aestivum* L.), variety-Kanchan, vill.-BARI, dist.-Gazipur, S Momtaz 324, 3 March 2012.

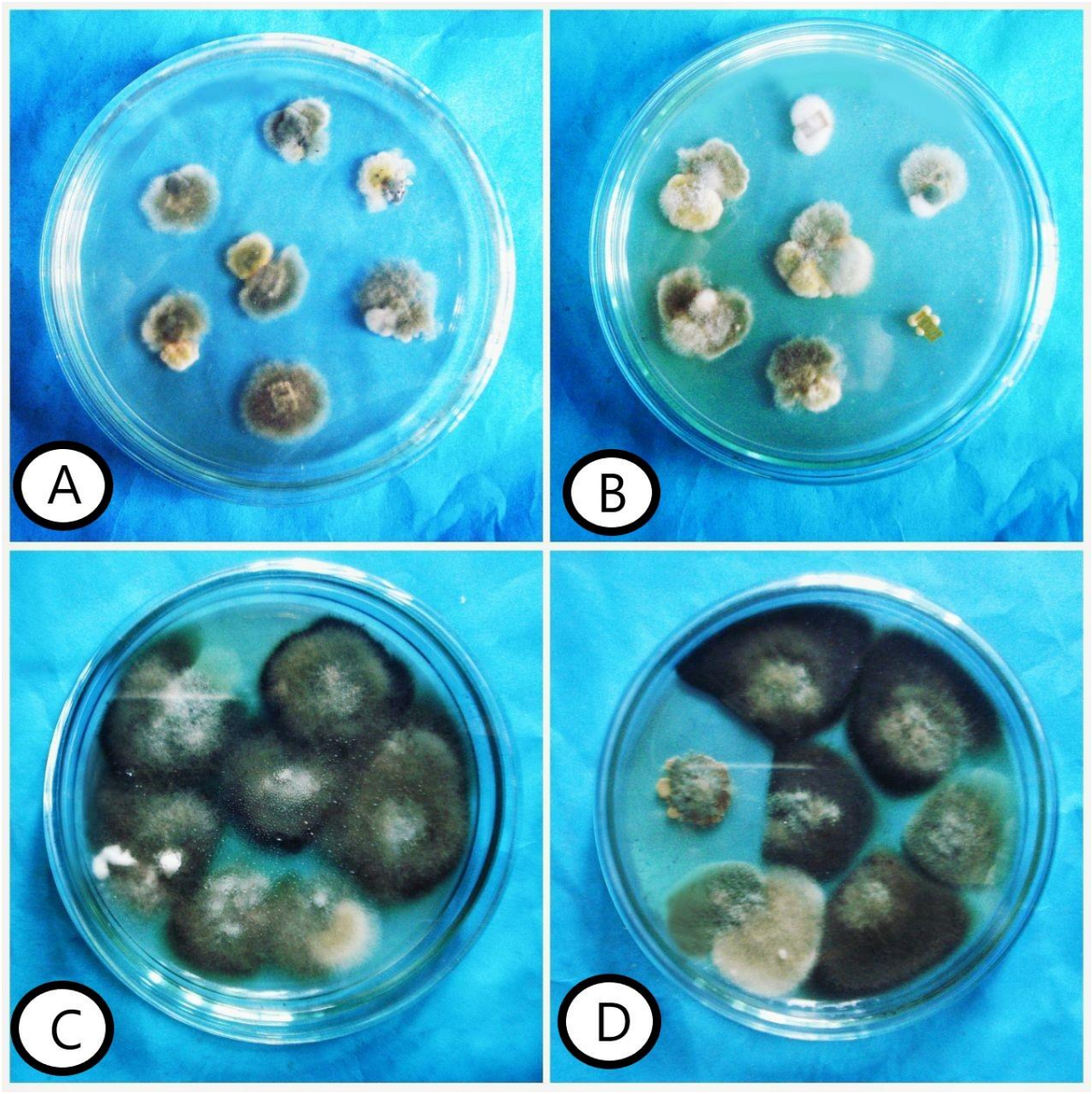


Plate 1. Isolation of fungi on tissue planting/agar plate method from wheat leaves

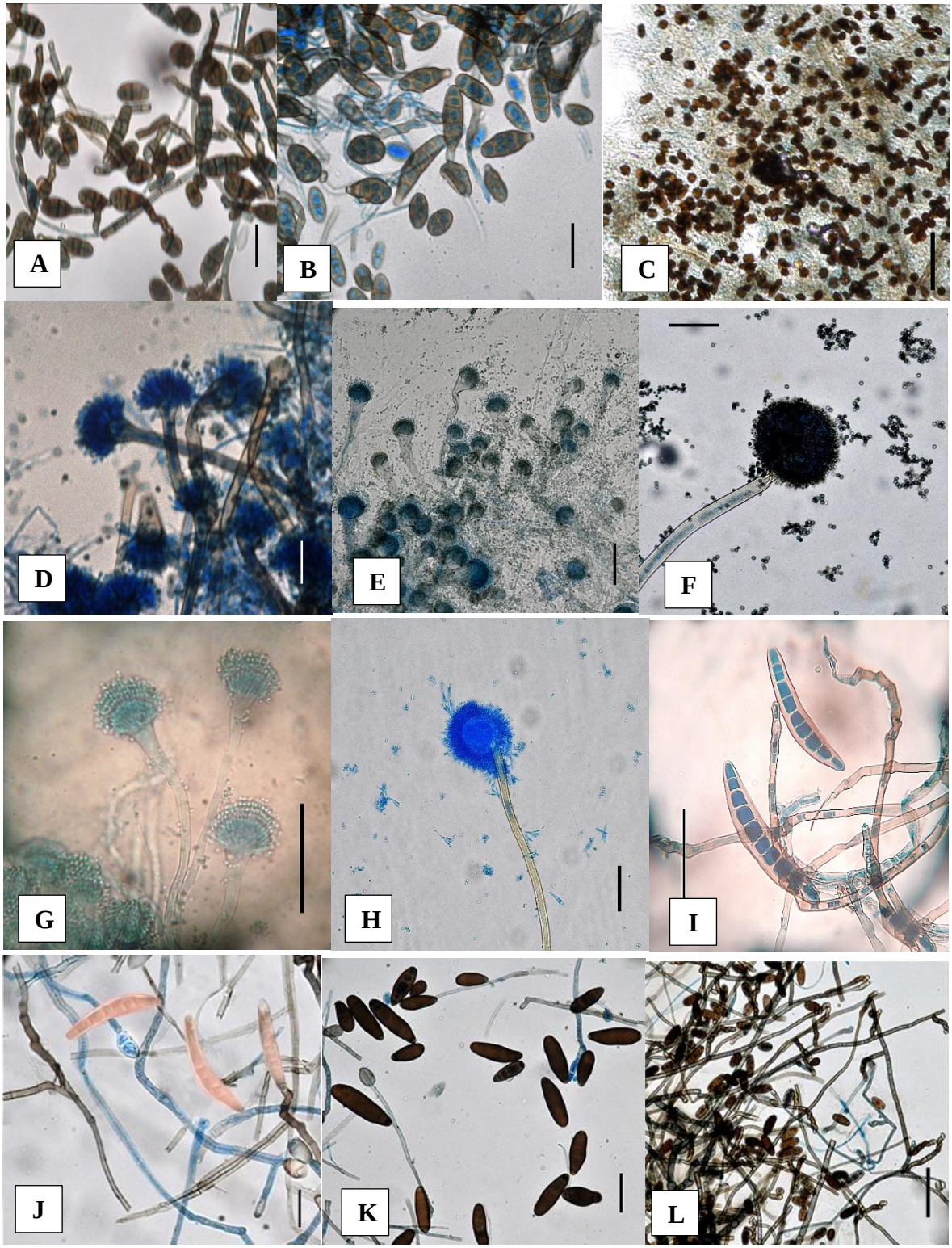


Plate 2. Microphotograph showing conidiophores and conidia of A. *Alternaria alternata*, B. *Alternaria triticina*, C. *Arthinium* sp., D. *Aspergillus flavus*, E. *Aspergillus fumigatus*, F. *Aspergillus niger*, G. *Aspergillus terrous*, H. *Aspergillus* sp., I. *Bipolaris cynodontis*; J. *Bipolaris oryzae*; K. *Bipolaris sorokiniana* and L. *Bipolaris tetramera* (Bar = 50 µm).

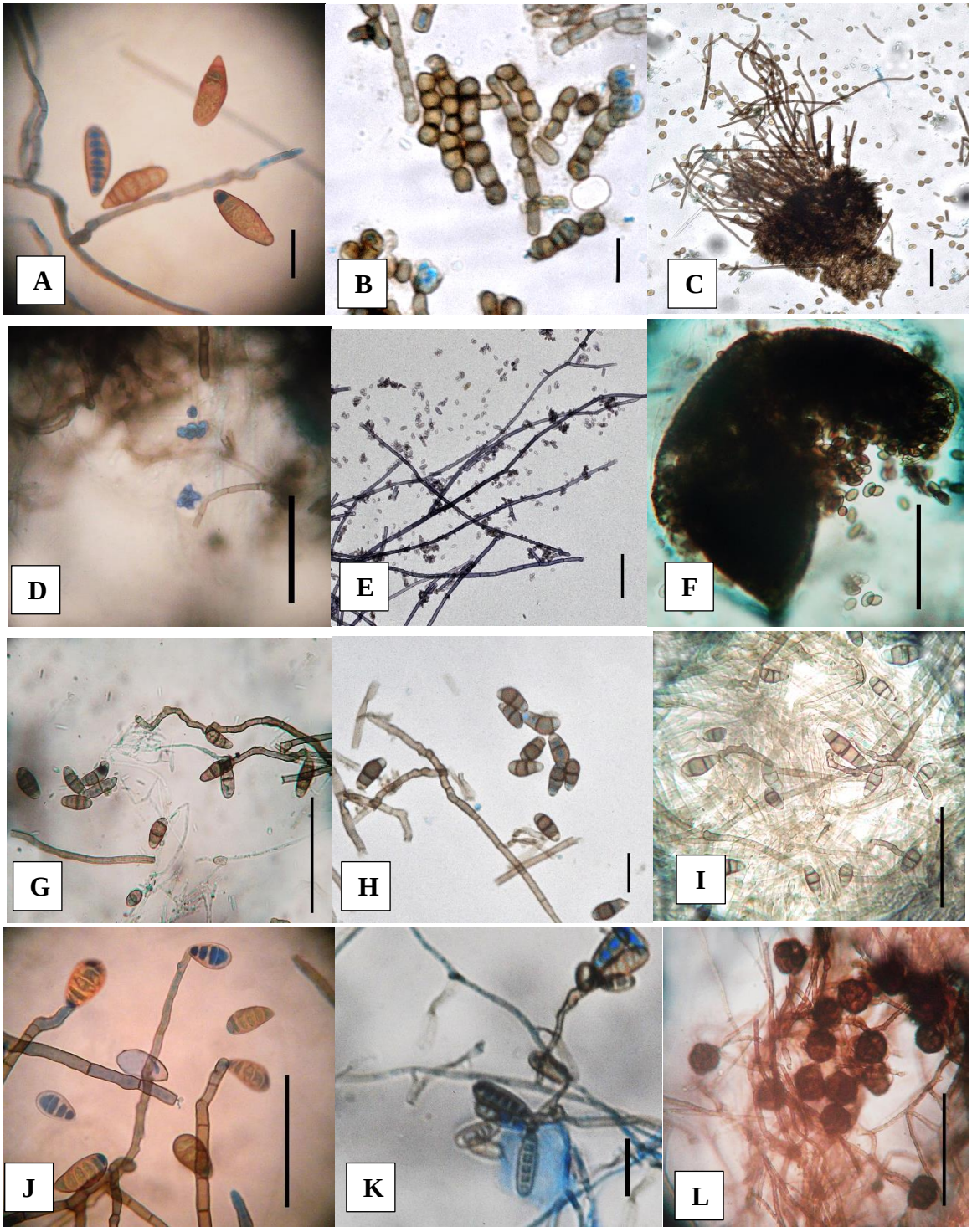


Plate 3. Microphotograph showing conidiophores and conidia of A. *Bipolaris victoriae*, B. *Bispora antenata* C. Perithecium and ascospores of *Chaetomium globosum* D. Conidia of *Chaetophoma* sp. E. Conidiophores and conidia of *Cladosporium cladosporoides*, F. Pycnidia and conidia of *Conoithyium* sp., conidiophores and conidia of G. *Curvularia affines*, H. *Curvularia lunata*, I. *Curvularia pallescens*, J. *Drechslera dematioidea*, K. *Drechslera hawaiiensis*, L. *Epicoccum purpurascens* (Bar = 50 µm).

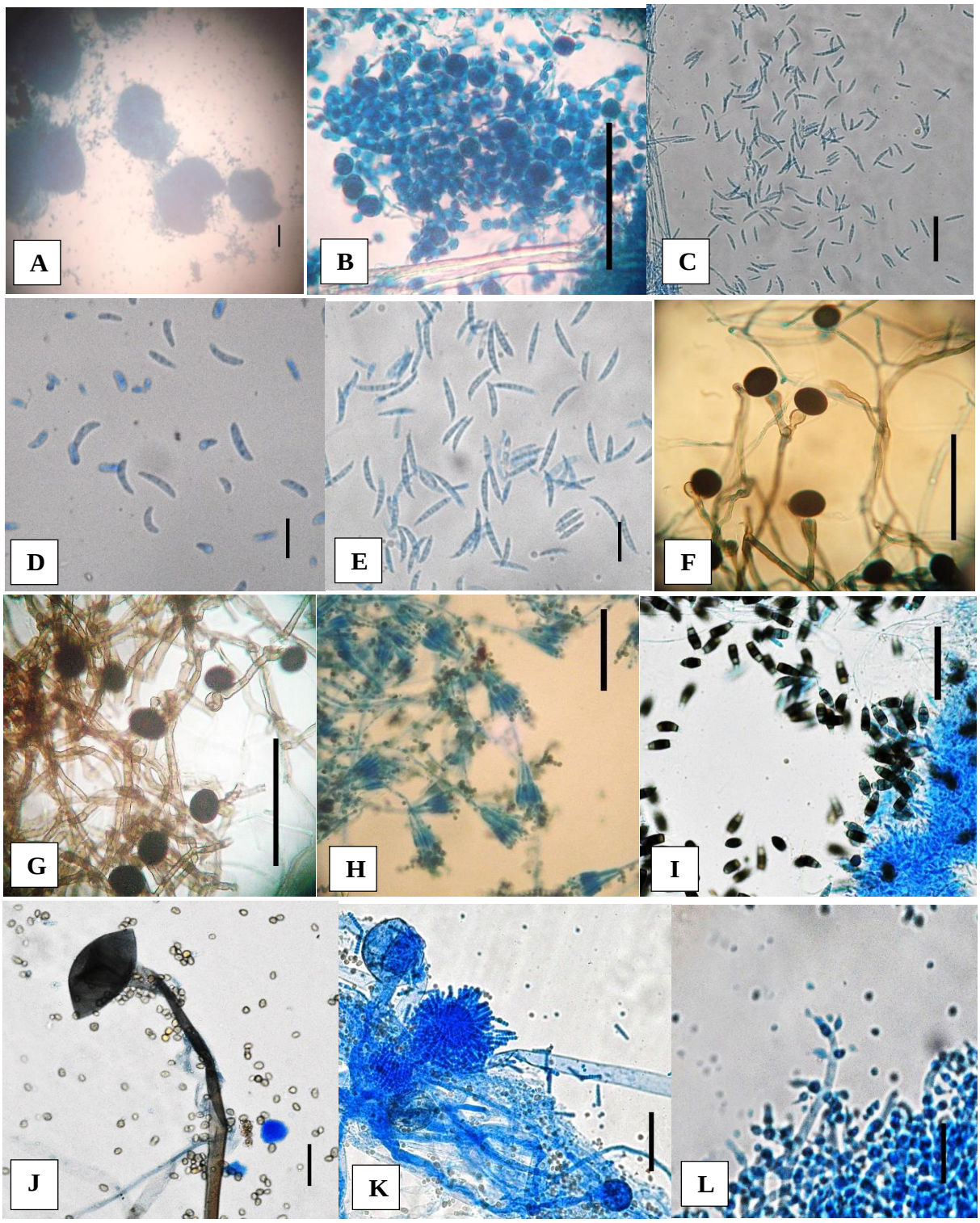


Plate 4. A.-B. Cleistothecia, asci and ascospores of *Eurotium* sp., Conidiophores and conidia of C. *Fusarium moniliforme*, D. *Fusarium nivale*, E. *Fusarium semitectum*, F. *Nigrospora oryzae*, G. *Nigrospora sacchari*, H. *Penicillium digitatum*, I. *Pestalotiopsis guepinii*, J. *Rhizopus stolonifer*; K. *Syncephalastrum* sp. and L. *Trichoderma viride* (Bar = 50 µm).

4.3 Prevalence of fungi associated with BpLB infected wheat leaves

From Bipolaris Leaf Blight (BpLB) diseased wheat leaves, 35 fungal species spanning 20 genera were identified (Table 22). Among them, seven fungi were obtained from almost eight districts. These were–*Alternaria alternata*, *Aspergillus flavus*, *A. niger*, *Bipolaris sorokiniana*, *Cladosporium cladosporioides*, *Curvularia lunata* and *Fusarium semitectum*. Also there were some fungi, which prevail in only one districts. Such as, *Aspergillus* sp., *Bipolaris oryzae*, *B. victoriae* and *Eurotium* sp. from Gazipur district, *Pestalotiopsis guepinii*, *Bispora antenata* and *Coniothyrium* sp. from Dinajpur district and *Drechslera dematioidea* and *Syncephalastrum* sp. from Joypurhat district, *Chaetophoma* sp. from Pabna district and *Cheatomium globosum* from Chuadanga district (Table 22). Among these fungi, three fungul species (*Bipolaris cynodontis*, *Drechslera hawaiiensis* and *Pestalotiopsis guepinii*) associated with wheat is new record for Bangladesh. Earlier data revealed that those fungi were obtaied from rice, rose etc. but with wheat were new in Bangladesh. The presence of two new fungi, *Bispora antenata* and *Drechslera dematioidea* also first recorded in Bangladesh.

Total 120 samples under twenty-one wheat cultivars were collected from diverse sites of eight districts–Dhaka, Gazipur, Dinajpur, Joypurhat, Pabna, Sirajganj, Kushtia and Chuadanga districts. Name of the wheat varieties were shown in Table 1. Leaf samples were collected from the BARI research station as well as farmer fields between the months of January 2011 and March 2014. These fungi have also been linked to wheat leaf blight in several investigations (Ahmed 2001). The frequency percentage of *B. sorokiniana* ranged from the lowest to the highest 6.67–85.71% based on wheat varieties. Among eight districts, mean frequency percentage was 32.5%, the maximum in Joypurhat district (53.55%) and the minimum in Kushtia (6.67%) district. During 2011 to 2014, yearly mean frequency percentage was 30.62% with the highest in the year 2014 (40.83%) and the lowest in 2012 (18.92%).

4.3.1 Incidence of fungi with BpLB infected wheat leaves in Dhaka district

From Dhaka district (Table 14) seven fungal species were isolated, the frequency percentage of *B. sorokiniana* was highest (43.13%) and the frequency percentage of *Alternaria triticina* was lowest (2.78%).

4.3.2 Incidence of fungi with BpLB infected wheat leaves in Gazipur district

From Gazipur district (Table 15) the highest number of fungi (23) were isolated, frequency percentage of *B. sorokiniana* was the highest (51.49%) and the frequency percentage of *Trichoderma viride* was the lowest (2.24%), respectively.

4.3.3 Incidence of fungi with BpLB infected wheat leaves in Dinajpur district

Twenty-two fungi were isolated from Dinajpur district (Table 16), the frequency percentage of *Alternaria alternata* was the highest 44.93% and the lowest frequency percentage of *Fusarium moniliforme* was 2.77. Frequency percentage of *B. sorokiniana* was 25.49.

4.3.4 Incidence of fungi with BpLB infected wheat leaves in Joypurhat district

From Joypurhat district (Table 17) total 13 fungi were isolated, frequency percentage of *Bipolaris sorokiniana* was the highest (53.55%, highest among eight districts) and frequency percentage of *Alternaria triticina* was the lowest (1.32%).

4.3.5 Incidence of fungi with BpLB infected wheat leaves in Pabna district

Fourteen fungal species were isolated from Pabna district (Table 18), the lowest frequency percentage of *Curvularia pallescens* was 1.77 and the highest frequency percentage of *B. sorokiniana* was 36.52, respectively.

4.3.6 Incidence of fungi with BpLB infected wheat leaves in Sirajganj district

From Sirajgonj district (Table 19), total isolated fungi were nine. The highest frequency percentage of *B. sorokiniana* was 35.37 and the lowest frequency percentage of *Aspergillus niger* was 5.26.

4.3.7 Incidence of fungi with BpLB infected wheat leaves in Kushtia district

From Kushtia district (Table 20) fourteen fungi were isolated, the highest frequency percentages of *Aspergillus flavus*, *A. fumigatus* and *Penicillium digitatum* was 33.34% and the lowest frequency percentage of *Alternaria triticina* was 2.32%. Here percentage of *B. sorokiniana* was 6.67%, the lowest percentage among the districts.

4.3.8 Incidence of fungi with BpLB infected wheat leaves in Chuadanga district

From Chuadanga district (Table 21), total seventeen fungi were isolated of BpLB symptom leaves of wheat varieties. The highest frequency percentage of *Curvularia lunata* was 24.99% and the lowest frequency percentage of *Nigrospora sacchari* was 3.15%. Frequency percentage of *B. sorokiniana* was 7.78%.

Table 22 presents, the most predominant fungi, in order of prevalence, were *Alternaria alternata, Bipolaris sorokiniana, Cladosporium cladosporioides, Curvularia lunata* and *Fusarium semitectum*. The fungi varied in prevalence with respect to location, cultivar and year. Among eight districts, frequency percentage of *A. alternata* was highest in Dinajpur district (44.93%) and lowest in Joypurhat district (5.71%), *B. sorokiniana* was the highest in Joypurhat district (53.55%) and the lowest in Kushtia district (6.67%), *C. cladosporioides* was the highest in Gazipur district (35.05%) and the lowest in Dinajpur district (7.5%), *C. lunata* was the highest in Gazipur district (32.98%) and the lowest in Kushtia district (5.0%) and *F. semitectum* was the highest in Gazipur district (25.41%) and the lowest in Dhaka district (12.08%).

Table 23 exhibits the frequency percentages of thirty-five fungal species during the year 2011 to 2014. In year 2011, total twenty fungi were obtained; among them the highest frequency percentage was *C. lunata* (36.67%) and the lowest was *T. viride* (2.24%). Total twenty-three fungi were isolated in the year 2012; the highest and the lowest frequency percentage were *A. alternata* (40.56%) and *F. moniliforme* (2.77%). In year 2013, total twenty-two fungi were obtained, among them the highest frequency percentage was *B. sorokiniana* (28.32%) and lowest *C. pallescens* (2.55%). Total twenty-one fungi were isolated in the year 2014; the highest and the lowest frequency percentage were *B. sorokiniana* (40.83%) and *A. triticina* (1.32%).

Frequency percentage of five predominant fungi, *A. alternata, B. sorokiniana, C. cladosporioides, C. lunata* and *F. semitectum* were shown in the year 2011 to 2014. Frequency percentage of *A. alternata* was the highest in the year 2012 (40.56%) and the lowest in the year 2014 (18.37%), *B. sorokiniana* was the maximum in the year 2014 (40.83%) and the minimum in the year 2012 (18.92%), *C. cladosporioides* was the highest in the year 2011 (35.05%) and lowest in the year 2012 (7.5%), *C. lunata* was the highest in the year 2011 (36.67%) and the lowest in the year 2014 (10.46%) and *F. semitectum* was the highest in the year 2011 (28.04%) and the lowest in the year 2013 (7.11%) (Table 23 and Fig. 4).

Besides the above mentioned fungi, *Aspergillus flavus* and *A. niger* were isolated from all the eight districts and all the four year. Among thirty-five fungi species the highest frequency percentage was noticed in *B. sorokiniana* in Joypurhat district (53.55%) and the lowest was *D. dematioidea* in Joypurhat district (1.43%) (Table 22).

Total twenty-one wheat varieties or genotypes were subjected for isolation of fungi collected from wheat research station of Gazipur and Dinajpur districts and also from farmers' fields of eight districts. Main focus was on *B. sorokiniana,* so frequency percentage on different varieties was shown in Fig. 5. The highest frequency percentage was noticed in variety Saurav (50.65%) and the lowest was in variety Protiva (5.56%).

Reports on mycoflora associated with wheat seeds are available and different research work have done in different countries. But mycoflora associated with BpLB infected wheat leaves have little reported. Bipolaris leaf blight is a complex syndrome due to involvement of a number of pathogens (Joshi *et al*. 1978, Singh and Srivastava 1997, Ruckstuhl 1998, Singh *et al*. 1997). *Drechslera sorokiniana*; *D. tritici-repentis*; *D. tetramera*; *Alternaria triticina* and *A. alternata* (Joshi *et al*. 1974); *H. spiciferum* (Brain) and *Curvularia* species are associated with foliar blight complex.

Mahto *et al.* (2002) studied 152 leaf blight and spot blotch samples from different agroecological regions in India and recorded *Alternaria alternata*, *A. triticina*, *Chaetomium* spp., *Fusarium moniliforme* [*Gibberella fujikuroi*], *Epicoccum purpurascens* [*E. nigrum*], *Paecilomyces variotii*, and *Penicillium* spp. with *Drechslera sorokiniana* [*Cochliobolus sativus*]. Singh *et al.* (2004) also reported *Bipolaris sorokiniana*, *Alternaria triticina*, *Alternaria alternata*, *Chaetomium* spp., *Fusarium moniliforme* [*Gibberella moniliformis*], *Epicoccum purpurascens* [*E. nigrum*], *Pestalotiopsis disseminata*, *Aspergillus flavus*, *Acremonium strictum*, *Curvularia lunata* [*Cochliobolus lunatus*], *Paecilomyces variotii* and *Penicillium* spp. were associated with blighted leaves of wheat. Reports of present investigation slightly differ from the previous report on fungal association of leaf blight infected wheat varieties might be due to change of location and cultivars. In this report, *B. sorokiniana* was the main pathogen among different *Bipolaris* and *Drechslera* species, prevalence of *A. triticina* was low than *A. alternata*, *C. cladosporioides*, *F. semitectum, C. lunata* and different species of *Aspergillus* were frequently isolated.

Table 14. Prevalence of fungi with BpLB infected wheat leaves in Dhaka district of Bangladesh

No	Species name	Name of variety				Mean
		BARI Gom-25	BARI Gom-26	BARI Gom-27	BARI Gom-28	
1	*Alternaria alternata*	20.34	7.76	31.33	7.83	12.90
2	*Alternaria triticina*	2.22	3.34	-	5.56	2.78
3	*Arthirinium* sp.	-	-	-	-	-
4	*Aspergillus flavus*	15.75	10.88	5.78	11.51	10.98
5	*Aspergillus fumigatus*	-	-	-	-	
6	*Aspergillus niger*	6.15	18.47	10.1	9.2	10.98
7	*Aspergillus terrous*	-	-	-	-	-
8	*Aspergillus* sp.	-	-	-	-	-
9	*Bipolaris cynodontis*	-	-	-	-	-
10	*Bipolaris oryzae*	-	-	-	-	-
11	*Bipolaris sorokiniana*	57.51	57.51	23.0	34.5	43.13
12	*Bipolaris tetramera*	-	-	-	-	-
13	*Bipolaris victoriae*	-	-	-	-	-
14	*Bispora antenata*	-	-	-	-	-
15	*Coniothyrium* sp.	-	-	-	-	-
16	*Chaetomium globosum*	-	-	-	-	-
17	*Chaetophoma* sp.	-	-	-	-	-
18	*Cladosporium cladosporioides*	8.75	10.25	10.25	13.43	10.67
19	*Curvularia lunata*	12.55	16.72	16.72	12.21	14.55
20	*Curvularia pallescens*	-	-	-	-	-
21	*Curvularia affinis*	-	-	-	-	-
22	*Drechslera dematioidea*	-	-	-	-	-
23	*Drechslera hawaiiensis*	-	-	-	-	-
24	*Epicoccum purpurascens*	-	-	-	-	-
25	*Fusarium semitectum*	-	-	-	-	-
26	*Fusarium* sp_1	-	-	-	-	-
27	*Fusarium* sp_2	-	-	-	-	-
28	*Nigrospora oryzae*	-	-	-	-	-
29	*Nigrospora sacchari*	-	-	-	-	-
30	*Penicillium digitatum*	-	-	-	-	-
31	*Pestalotiopsis guepinii*	-	-	-	-	-
32	*Eurotium* sp.	-	-	-	-	-
33	*Rhizopus stolonifer*	-	-	-	-	-
34	*Syncephalastrum*	-	-	-	-	-
35	*Trichoderma viride*	-	-	-	-	-
36	Sterile mycellium	-	-	-	-	-

Table 15. Prevalence of fungi with BpLB infected wheat leaves in Gazipur district of Bangladesh

No	Species name	Name of variety							Mean
		Kalyansona	Kanchan	Kheri	Seri-82	Sonora	En-45	EN-113	
1	*Alternaria alternata*	50.45	20.88	25.25	22.88	32.56	6.9	20.0	25.56
2	*Alternaria triticina*	-	10.25	6.56	5.45	3.5	8.96	-	4.96
3	*Arthirinium* sp.	-	-	12.48	-	16.64	16.64	7.06	7.54
4	*Aspergillus flavus*	10.33	30.66	-	20.65	20.6	18.0	-	14.32
5	*Aspergillus fumigatus*	13.17	26.35	-	13.17	13.17	13.17	-	11.29
6	*Aspergillus niger*	9.8	18.0	21.34	19.8	23.55	30.1	16.08	19.81
7	*Aspergillus terrous*	15.5	-	7.75	15.5	23.25	-	8.0	10.0
8	*Aspergillus* sp.	-	-	23.38	-	-	23.38	-	6.67
9	*Bipolaris cynodontis*	-	-	-	16.66	-	-	16.66	4.76
10	*Bipolaris oryzae*	-	-	20.02	-	-	-	-	2.86
11	*Bipolaris sorokiniana*	67.32	50.49	74.32	50.49	67.32	33.66	16.83	51.49
12	*Bipolaris tetramera*	15.38	7.69	-	15.38	15.38	-	-	7.69
13	*Bipolaris victoriae*	-	-	23.38	-	-	-	-	3.34
14	*Bispora antenata*	-	-	-	-	-	-	-	-
15	*Coniothyrium* sp.	-	-	-	-	-	-	-	-
16	*Chaetomium globosum*	-	-	-	-	-	-	-	-
17	*Chaetophoma* sp.	-	-	-	-	-	-	-	-
18	*Cladosporium cladosporioides*	17.52	52.56	35.04	52.56	35.05	17.52	35.1	35.05
19	*Curvularia lunata*	16.49	8.25	65.96	32.98	49.47	65.96	8.25	32.98
20	*Curvularia pallescens*	14.56	-	-	-	-	-	14.56	4.16
21	*Curvularia affinis*	-	-	-	-	-	-	-	-
22	*Drechslera dematioidea*	-	-	-	-	-	-	-	-
23	*Drechslera hawaiiensis*	-	29.72	29.72	-	44.58	-	-	14.86
24	*Epicoccum purpurascens*	-	-	-	-	-	-	-	-
25	*Fusarium semitectum*	50.8	12.7	25.4	-	38.1	25.41	25.45	25.41
26	*Fusarium* sp_1	-	-	-	-	-	-	-	-
27	*Fusarium* sp_2	-	-	-	23.38	-	-	-	3.34
28	*Nigrospora oryzae*	33.33	11.11	-	-	22.22	-	11.11	11.11
29	*Nigrospora sacchari*	-	-	-	-	-	-	-	-
30	*Penicillium digitatum*	-	-	11.66	5.83	5.83	5.83	11.66	5.83
31	*Pestalotiopsis guepinii*	-	-	-	-	-	-	-	-
32	*Eurotium* sp.	-	23.34	-	-	-	-	23.34	6.67
33	*Rhizopus stolonifer*	-	-	-	-	-	-	-	-
34	*Syncephalastrum*	-	-	-	-	-	-	-	-
35	*Trichoderma viride*	7.84	-	-	-	7.84	-	-	2.24
36	Sterile mycellium	-	30.84	15.42	-	30.84	15.42	15.42	15.42

Table 16. Prevalence of fungi with BpLB infected wheat leaves in Dinajpur district of Bangladesh

No	Species name	Name of variety											Mean
		Aghrani	Akber	Ananda	Balaka	Barkat	Bijoy	Cianno-79	Kanchan	Prodip	Shatabdi	Sonalika	
1	*Alternaria alternata*	14.98	74.90	29.96	59.92	29.96	44.94	14.98	74.90	59.92	44.94	44.83	44.93
2	*Alternaria triticina*	25.7	-	10.34	5.14	15.39	15.39	20.56	-	5.14	10.28	5.14	10.28
3	*Arthirinium* sp.	-	-	-	-	-	-	-	-	-	-	-	-
4	*Aspergillus flavus*	17.04	4.26	8.52	12.78	29.82	-	-	17.04	17.04	4.26	29.82	12.78
5	*Aspergillus fumigatus*	5.56	13.9	8.34	-	2.78	16.68	13.9	11.12	13.9	5.56	-	8.34
6	*Aspergillus niger*	7.22	10.83	3.61	28.88	-	21.66	14.44	3.61	-	14.44	14.44	10.83
7	*Aspergillus terrous*	-	-	26.68	22.25	4.45	26.68	22.25	4.34	31.15	-	8.9	13.34
8	*Aspergillus* sp.	-	-	-	-	-	-	-	-	-	-	-	-
9	*Bipolaris cynodontis*	-	-	-	-	-	-	-	-	-	-	-	-
10	*Bipolaris oryzae*	-	-	-	-	-	-	-	-	-	-	-	-
11	*Bipolaris sorokiniana*	8.5	51.0	6.67	20.01	6.67	33.35	66.67	26.68	20.01	33.35	7.48	25.49
12	*Bipolaris tetramera*	10.88	-	16.32	8.16	16.32	-	-	-	8.16	-	-	5.44
13	*Bipolaris victoriae*	-	-	-	-	-	-	-	-	-	-	-	-
14	*Bispora antenata*	-	-	-	-	-	-	-	-	-	-	-	2.86
15	*Coniothyrium* sp.	-	-	26.68	-	23.35	-	-	-	-	-	23.34	6.67
16	*Chaetomium globosum*	-	-	-	-	-	-	-	-	-	-	-	-
17	*Chaetophoma* sp.	-	-	-	-	-	-	-	-	-	-	-	-
18	*Cladosporium cladosporioides*	3.75	11.25	3.75	15	3.75	-	18.75	7.5	11.25	3.75	3.75	7.5
19	*Curvularia lunata*	53.44	13.36	20.04	26.72	53.44	46.76	46.76	-	13.36	13.36	6.68	26.72
20	*Curvularia pallescens*	-	-	-	-	-	-	-	-	-	-	-	-
21	*Curvularia affinis*	-	5.92	-	-	-	-	-	14.8	2.96	-	8.88	2.96
22	*Drechslera dematioidea*	-	-	-	-	-	-	-	-	-	-	-	-
23	*Drechslera hawaiiensis*	36.82	-	42.08	-	-	36.82	15.78	42.08	-	-	-	15.78
24	*Epicoccum purpurascens*	-	3.33	-	33.35	-	-	26.64	6.67	-	-	3.38	6.67
25	*Fusarium semitectum*	16.04	5.35	10.7	26.75	10.7	53.5	16.04	-	42.8	48.15	5.35	21.39
26	*Fusarium* sp_1	-	11.08	-	-	8.31	-	-	11.08	-	-	-	2.77
27	*Fusarium* sp_2	-	-	-	-	-	-	-	-	-	-	-	-
28	*Nigrospora oryzae*	19.36	-	24.2	4.84	14.52	-	29.04	33.88	9.68	-	24.2	14.52
29	*Nigrospora sacchari*	-	8.88	-	-	-	14.8	-	-	-	8.88	-	2.96
30	*Penicillium digitatum*	26.64	-	6.67	-	26.64	-	-	13.42	-	-	-	6.67
31	*Pestalotiopsis guepinii*	-	48.88	-	61.1	-	-	54.99	-	-	-	36.66	18.33
32	*Eurotium* sp.	-	-	-	-	-	-	-	-	-	-	-	-
33	*Rhizopus stolonifer*	-	-	-	-	-	-	-	-	-	-	-	-
34	*Syncephalastrum*	-	-	-	-	-	-	-	-	-	-	-	-
35	*Trichoderma viride*	-	-	-	-	-	-	-	-	-	-	-	-
36	Sterile mycellium	26.67	-	4.44	-	-	4.44	-	26.67	8.88	17.76	8.96	8.89

Table 17. Prevalence of fungi with BpLB infected wheat leaves in Joypurhat district of Bangladesh

No	Species name	Name of variety				Mean
		Bijoy	Prodip	Saurav	Shatabdi	
1	*Alternaria alternata*	8.55	2.85	8.55	2.85	5.71
2	*Alternaria triticina*	-	2.64	2.64	-	1.32
3	*Arthirinium* sp.	10.0	6.67	3.34	6.67	6.67
4	*Aspergillus flavus*	4.76	19.05	28.56	4.76	14.29
5	*Aspergillus fumigatus*	28.56	-	7.15	21.43	14.29
6	*Aspergillus niger*	5.24	31.42	15.71	10.48	15.71
7	*Aspergillus terrous*	-	-	-	-	-
8	*Aspergillus* sp.	-	-	-	-	-
9	*Bipolaris cynodontis*	13.34	2.66	-	5.33	5.33
10	*Bipolaris oryzae*	-	-	-	-	-
11	*Bipolaris sorokiniana*	85.71	48.18	26.77	53.55	53.55
12	*Bipolaris tetramera*	3.39	6.78	16.95	-	6.78
13	*Bipolaris victoriae*	-	-	-	-	-
14	*Bispora antenata*	-	-	-	-	-
15	*Coniothyrium* sp.	-	-	-	-	-
16	*Chaetomium globosum*	-	-	-	-	-
17	*Chaetophoma* sp.	-	-	-	-	-
18	*Cladosporium cladosporioides*	16.12	12.88	3.22	6.46	9.67
19	*Curvularia lunata*	4.05	12.15	16.16	-	8.09
20	*Curvularia pallescens*	-	-	-	-	-
21	*Curvularia affinis*	-	-	-	-	-
22	*Drechslera dematioidea*	-	-	-	-	-
23	*Drechslera hawaiiensis*	-	-	-	-	-
24	*Epicoccum purpurascens*	-	-	-	-	-
25	*Fusarium semitectum*	23.8	19.04	4.76	9.52	14.28
26	*Fusarium* sp_1	-	-	-	-	-
27	*Fusarium* sp_2	-	-	-	-	-
28	*Nigrospora oryzae*	-	-	-	-	-
29	*Nigrospora sacchari*	-	-	-	-	-
30	*Penicillium digitatum*	-	-	-	-	-
31	*Pestalotiopsis guepinii*	-	-	-	-	-
32	*Eurotium* sp.	-	-	-	-	-
33	*Rhizopus stolonifer*	1.43	-	8.58	1.43	2.86
34	*Syncephalastrum*	-	-	-	-	-
35	*Trichoderma viride*	-	-	-	-	-
36	Sterile mycellium	8.56	5.71	2.85	5.71	5.71

Table 18. Prevalence of fungi with BpLB infected wheat leaves in Pabna district of Bangladesh

No	Species name	Name of variety		Mean
		Bijoy	Shatabdi	
1	*Alternaria alternata*	17.25	34.49	25.87
2	*Alternaria triticina*	2.67	8.01	5.34
3	*Arthirinium* sp.	-	-	-
4	*Aspergillus flavus*	20.24	20.24	20.24
5	*Aspergillus fumigatus*	22.22	44.44	33.33
6	*Aspergillus niger*	21.02	4.2	12.61
7	*Aspergillus terrous*	-	-	-
8	*Aspergillus* sp.	-	-	-
9	*Bipolaris cynodontis*	-	-	-
10	*Bipolaris oryzae*	-	-	-
11	*Bipolaris sorokiniana*	45.56	27.39	36.52
12	*Bipolaris tetramera*	-	-	-
13	*Bipolaris victoriae*	-	-	-
14	*Bispora antenata*	-	-	-
15	*Coniothyrium* sp.	-	-	-
16	*Chaetomium globosum*	-	-	-
17	*Chaetophoma* sp.	-	6.68	3.34
18	*Cladosporium cladosporioides*	10.0	10.0	10.0
19	*Curvularia lunata*	20.11	10.05	15.08
20	*Curvularia pallescens*	-	3.54	1.77
21	*Curvularia affinis*	-	-	-
22	*Drechslera dematioidea*	-	-	-
23	*Drechslera hawaiiensis*	-	-	-
24	*Epicoccum purpurascens*	-	-	-
25	*Fusarium semitectum*	21.27	10.63	15.95
26	*Fusarium* sp_1	-	-	-
27	*Fusarium* sp_2	-	10.9	5.45
28	*Nigrospora oryzae*	10.71	3.57	7.14
29	*Nigrospora sacchari*	-	-	-
30	*Penicillium digitatum*	-	-	-
31	*Pestalotiopsis guepinii*	-	-	-
32	*Eurotium* sp.	28.57	28.57	28.57
33	*Rhizopus stolonifer*	-	-	-
34	*Syncephalastrum*	-	-	-
35	*Trichoderma viride*	-	-	-
36	Sterile mycellium	-	-	-

Table 19. Prevalence of fungi with BpLB infected wheat leaves in Sirajgonj district of Bangladesh

No	Species name	Name of variety				Mean
		Bijoy	Prodip	Shatabdi	Saurav	
1	*Alternaria alternata*	5.56	27.81	5.56	5.56	11.12
2	*Alternaria triticina*	-	-	-	-	-
3	*Arthirinium* sp.	-	-	-	-	-
4	*Aspergillus flavus*	12.28	4.09	2.05	14.34	8.19
5	*Aspergillus fumigatus*	-	-	-	-	-
6	*Aspergillus niger*	-	5.26	13.15	2.63	5.26
7	*Aspergillus terrous*	-	-	-	-	-
8	*Aspergillus* sp.	-	-	-	-	-
9	*Bipolaris cynodontis*	-	-	-	-	-
10	*Bipolaris oryzae*	-	-	-	-	-
11	*Bipolaris sorokiniana*	14.15	28.3	63.65	35.38	35.37
12	*Bipolaris tetramera*	3.29	6.6	3.29	13.18	6.59
13	*Bipolaris victoriae*	-	-	-	-	-
14	*Bispora antenata*	-	-	-	-	-
15	*Coniothyrium* sp.	-	-	-	-	-
16	*Chaetomium globosum*	-	-	-	-	-
17	*Chaetophoma* sp.	-	-	-	-	-
18	*Cladosporium cladosporioides*	25.55	5.11	20.44	10.22	15.33
19	*Curvularia lunata*	2.7	13.55	2.7	2.7	5.41
20	*Curvularia pallescens*	-	-	-	-	-
21	*Curvularia affinis*	-	-	-	-	-
22	*Drechslera dematioidea*	-	-	-	-	-
23	*Drechslera hawaiiensis*	-	-	-	-	-
24	*Epicoccum purpurascens*	-	-	-	-	-
25	*Fusarium semitectum*	11.2	5.6	28.0	22.4	16.8
26	*Fusarium* sp_1	-	-	-	-	-
27	*Fusarium* sp_2	-	-	-	-	-
28	*Nigrospora oryzae*	-	-	-	-	-
29	*Nigrospora sacchari*	-	-	-	-	-
30	*Penicillium digitatum*	16.68	8.34	4.17	4.17	8.34
31	*Pestalotiopsis guepinii*	-	-	-	-	-
32	*Eurotium* sp.	-	-	-	-	-
33	*Rhizopus stolonifer*	-	-	-	-	-
34	*Syncephalastrum*	-	-	-	-	-
35	*Trichoderma viride*	-	-	-	-	-
36	Sterile mycellium	-	-	-	-	-

Table 20. Prevalence of fungi with BpLB infected wheat leaves in Kushtia district of Bangladesh

No	Species name	Name of variety			Mean
		Bijoy	Prodip	Shatabdi	
1	*Alternaria alternata*	12.24	30.6	12.18	18.34
2	*Alternaria triticina*	2.32	-	4.64	2.32
3	*Arthirinium* sp.	-	-	-	-
4	*Aspergillus flavus*	41.67	50.04	8.31	33.34
5	*Aspergillus fumigatus*	22.22	22.22	55.55	33.34
6	*Aspergillus niger*	15.56	38.9	15.56	23.34
7	*Aspergillus terrous*	33.34	20.0	26.67	26.67
8	*Aspergillus* sp.	-	-	-	-
9	*Bipolaris cynodontis*	-	-	-	-
10	*Bipolaris oryzae*	-	-	-	-
11	*Bipolaris sorokiniana*	6.67	6.67	6.67	6.67
12	*Bipolaris tetramera*	4.58	11.4	4.58	6.85
13	*Bipolaris victoriae*	-	-	-	-
14	*Bispora antenata*	-	-	-	-
15	*Coniothyrium* sp.	-	-	-	-
16	*Chaetomium globosum*	-	-	-	-
17	*Chaetophoma* sp.	-	-	-	-
18	*Cladosporium cladosporioides*	15.96	5.33	2.68	7.99
19	*Curvularia lunata*	2.5	7.5	5.0	5.0
20	*Curvularia pallescens*	3.34	6.68	-	3.34
21	*Curvularia affinis*	-	-	-	-
22	*Drechslera dematioidea*	-	-	-	-
23	*Drechslera hawaiiensis*	6.25	6.25	15.64	9.38
24	*Epicoccum purpurascens*	-	-	-	-
25	*Fusarium semitectum*	-	-	-	-
26	*Fusarium* sp_1	-	-	-	-
27	*Fusarium* sp_2	-	-	-	-
28	*Nigrospora oryzae*	-	-	-	-
29	*Nigrospora sacchari*	-	-	-	-
30	*Penicillium digitatum*	44.45	33.34	22.23	33.34
31	*Pestalotiopsis guepinii*	-	-	-	-
32	*Eurotium* sp.	-	-	-	-
33	*Rhizopus stolonifer*	-	-	-	-
34	*Syncephalastrum*	-	-	-	-
35	*Trichoderma viride*	8.56	5.7	2.87	5.71
36	Sterile mycellium	-	-	-	-

Table 21. Prevalence of fungi with BpLB infected wheat leaves in Chuadanga district of Bangladesh

No	Species name	Name of variety		Mean
		Bijoy	Shatabdi	
1	*Alternaria alternata*	11.12	22.2	16.66
2	*Alternaria triticina*	6.68	-	3.34
3	*Arthirinium* sp.	-	-	-
4	*Aspergillus flavus*	4.16	20.82	12.49
5	*Aspergillus fumigatus*	24.99	8.33	16.66
6	*Aspergillus niger*	2.67	10.7	5.35
7	*Aspergillus terrous*	-	-	-
8	*Aspergillus* sp.	-	-	-
9	*Bipolaris cynodontis*	-	-	-
10	*Bipolaris oryzae*	-	-	-
11	*Bipolaris sorokiniana*	7.78	7.78	7.78
12	*Bipolaris tetramera*	-	-	-
13	*Bipolaris victoriae*	-	-	-
14	*Bispora antenata*	-	-	-
15	*Coniothyrium* sp.	-	-	-
16	*Chaetomium globosum*	-	7.14	3.57
17	*Chaetophoma* sp.	-	-	-
18	*Cladosporium cladosporioides*	17.19	8.59	12.89
19	*Curvularia lunata*	37.48	12.49	24.99
20	*Curvularia pallescens*	-	-	-
21	*Curvularia affinis*	3.26	3.26	3.26
22	*Drechslera dematioidea*	-	-	-
23	*Drechslera hawaiiensis*	-	-	-
24	*Epicoccum purpurascens*	16.06	5.35	10.71
25	*Fusarium semitectum*	11.43	22.85	17.14
26	*Fusarium* sp_1	6.34	-	3.17
27	*Fusarium* sp_2	-	-	-
28	*Nigrospora oryzae*	8.98	2.99	5.99
29	*Nigrospora sacchari*	-	6.3	3.15
30	*Penicillium digitatum*	5.35	16.07	10.71
31	*Pestalotiopsis guepinii*	-	-	-
32	*Eurotium* sp.	-	-	-
33	*Rhizopus stolonifer*	23.8	4.76	14.28
34	*Syncephalastrum*	-	-	-
35	*Trichoderma viride*	-	-	-
36	Sterile mycellium	-	-	-

Table 22. Prevalence of fungi with BpLB infected wheat leaves in eight districts of Bangladesh

No	Species name	Dhaka	Gazipur	Dinajpur	Joypurhat	Pabna	Sirajganj	Kushtia	Chuadanga	Mean
1	*Alternaria alternata*	12.90	25.56	44.93	5.71	25.87	11.12	18.34	16.66	20.14
2	*Alternaria triticina*	2.78	4.96	10.28	1.32	5.34	-	2.32	3.34	4.33
3	*Arthirinium* sp.	-	7.50	-	6.67	-	-	-	-	7.08
4	*Aspergillus flavus*	10.98	14.32	12.78	14.29	20.24	8.19	33.34	12.49	15.83
5	*Aspergillus fumigatus*	-	11.29	8.34	14.29	33.33	-	33.34	16.66	19.54
6	*Aspergillus niger*	10.98	19.81	10.83	15.71	12.61	5.26	23.34	5.35	12.99
7	*Aspergillus terrous*	-	10.0	13.34	-	-	-	26.67	-	16.67
8	*Aspergillus* sp.	-	6.67	-	-	-	-	-	-	6.67
9	*Bipolaris cynodontis*	-	4.76	-	5.33	-	-	-	-	5.04
10	*Bipolaris oryzae*	-	2.86	-	-	-	-	-	-	2.86
11	*Bipolaris sorokiniana*	43.13	51.49	25.49	53.55	36.52	35.37	6.67	7.78	32.5
12	*Bipolaris tetramera*	-	7.69	5.44	6.78	-	6.59	6.85	-	6.67
13	*Bipolaris victoriae*	-	3.34	-	-	-	-	-	-	3.34
14	*Bispora antenata*	-	-	2.86	-	-	-	-	-	2.86
15	*Coniothyrium* sp.	-	-	6.67	-	-	-	-	-	6.67
16	*Chaetomium globosum*	-	-	-	-	-	-	-	3.57	3.57
17	*Chaetophoma* sp.	-	-	-	-	3.34	-	-	-	3.34
18	*Cladosporium cladosporioides*	10.67	35.05	7.50	9.67	10.0	15.33	7.99	12.89	13.64
19	*Curvularia lunata*	14.55	32.98	26.72	8.09	15.08	5.41	5.00	24.99	16.6
20	*Curvularia pallescens*	-	4.16	-	-	1.77	-	3.34	-	3.09
21	*Curvularia affinis*	-	-	2.96	-	-	-	-	3.26	3.11
22	*Drechslera dematioidea*	-	-	-	1.43	-	-	-	-	1.43
23	*Drechslera hawaiiensis*	-	14.86	15.78	-	-	-	9.38	-	13.34
24	*Epicoccum purpurascens*	-	-	6.67	-	-	-	-	10.71	8.69
25	*Fusarium semitectum*	-	25.41	21.39	14.28	15.95	16.8	-	17.14	18.49
26	*Fusarium* sp_1	-	-	2.77	-	-	-	-	3.17	2.97
27	*Fusarium* sp_2	-	3.34	-	-	5.45	-	-	-	4.39
28	*Nigrospora oryzae*	-	11.11	14.52	-	7.14	-	-	5.99	9.69
29	*Nigrospora sacchari*	-	-	2.96	-	-	-	-	3.15	3.05
30	*Penicillium digitatum*	-	5.83	6.67	-	-	8.34	33.34	10.71	12.98
31	*Pestalotiopsis guepinii*	-	-	18.33	-	-	-	-	-	18.33
32	*Eurotium* sp.	-	6.67	-	-	-	-	-	-	6.67
33	*Rhizopus stolonifer*	-	-	-	2.86	28.57	-	-	14.28	15.24
34	*Syncephalastrum*	-	-	-	3.57	-	-	-	-	3.57
35	*Trichoderma viride*	-	2.24	-	-	-	-	5.71	-	3.97
36	Sterile mycellium	-	15.42	8.89	5.71	-	-	-	-	10.0

Table 23. Prevalence of fungi from BpLB infected wheat leaves in Year 2011 to 2014 in Bangladesh

Sl. No	Species name	Year 2011	Year 2012	Year 2013	Year 2014	Mean
1	*Alternaria alternata*	26.42	40.56	18.43	18.37	25.94
2	*Alternaria triticina*	4.96	6.53	3.67	1.32	4.12
3	*Arthirinium* sp.	-	8.34	-	6.67	7.5
4	*Aspergillus flavus*	15.09	10.83	16.66	14.28	14.21
5	*Aspergillus fumigatus*	13.33	7.28	27.14	16.02	15.94
6	*Aspergillus niger*	20.44	12	10.07	11.02	13.38
7	*Aspergillus terrous*	10	13.34	26.67	4.17	13.54
8	*Aspergillus* sp.	6.67	-	-	-	6.67
9	*Bipolaris cynodontis*	4.76	-	-	5.33	5.04
10	*Bipolaris oryzae*	2.86	-	-	-	2.86
11	*Bipolaris sorokiniana*	34.44	18.92	28.32	40.83	30.62
12	*Bipolaris tetramera*	6.67	5.12	5.78	4.78	5.58
13	*Bipolaris victoriae*	-	-	-	3.33	3.33
14	*Bispora antenata*	-	2.86	-	-	2.86
15	*Coniothyrium* sp.	-	6.67	-	-	6.67
16	*Chaetomium globosum*	-	-	3.57	-	3.57
17	*Chaetophoma* sp.	-	-	3.34	-	3.34
18	*Cladosporium cladosporioides*	35.05	7.5	14.28	11.42	17.06
19	*Curvularia lunata*	36.67	25.21	12.98	10.46	21.33
20	*Curvularia pallescens*	4.16	-	2.55	-	3.35
21	*Curvularia affinis*	-	2.96	3.26	-	3.11
22	*Drechslera dematioidea*	-	-	1.43	-	1.43
23	*Drechslera hawaiiensis*	14.86	15.78	-	9.38	13.34
24	*Epicoccum purpurascens*	-	6.67	10.71	-	8.69
25	*Fusarium semitectum*	28.04	18.35	7.11	17.19	17.67
26	*Fusarium* sp_1	-	2.77	-	3.17	2.97
27	*Fusarium* sp_2	3.34		5.45	-	4.39
28	*Nigrospora oryzae*	13.33	14.52	7.14	6.67	10.41
29	*Nigrospora sacchari*	-	2.96	3.15	-	3.05
30	*Penicillium digitatum*	6.67	6.11	15.18	10	9.49
31	*Pestalotiopsis guepinii*	-	18.33	-	-	18.33
32	*Eurotium* sp.	-	-	-	6.67	6.67
33	*Rhizopus stolonifer*	-	-	21.42	2.86	12.14
34	*Syncephalastrum* sp.	-	-	3.57	-	3.57
35	*Trichoderma viride*	2.24	-	5.71	-	3.97
36	Sterile mycellium	6.67	16.11	-	15.36	12.71

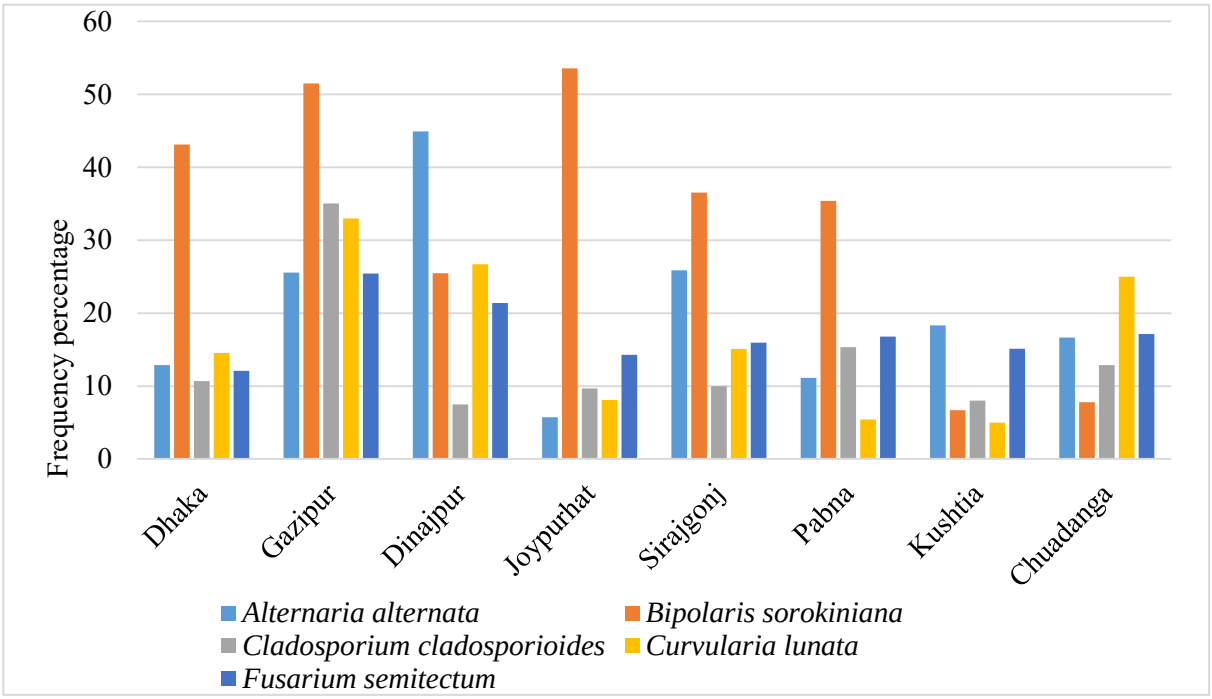


Fig. 3. Prevalence of *Alternaria alternata, Bipolaris sorokiniana, Cladosporium cladosporioides, Curvularia lunata* and *Fusarium semitectum* in eight districts of Bangladesh

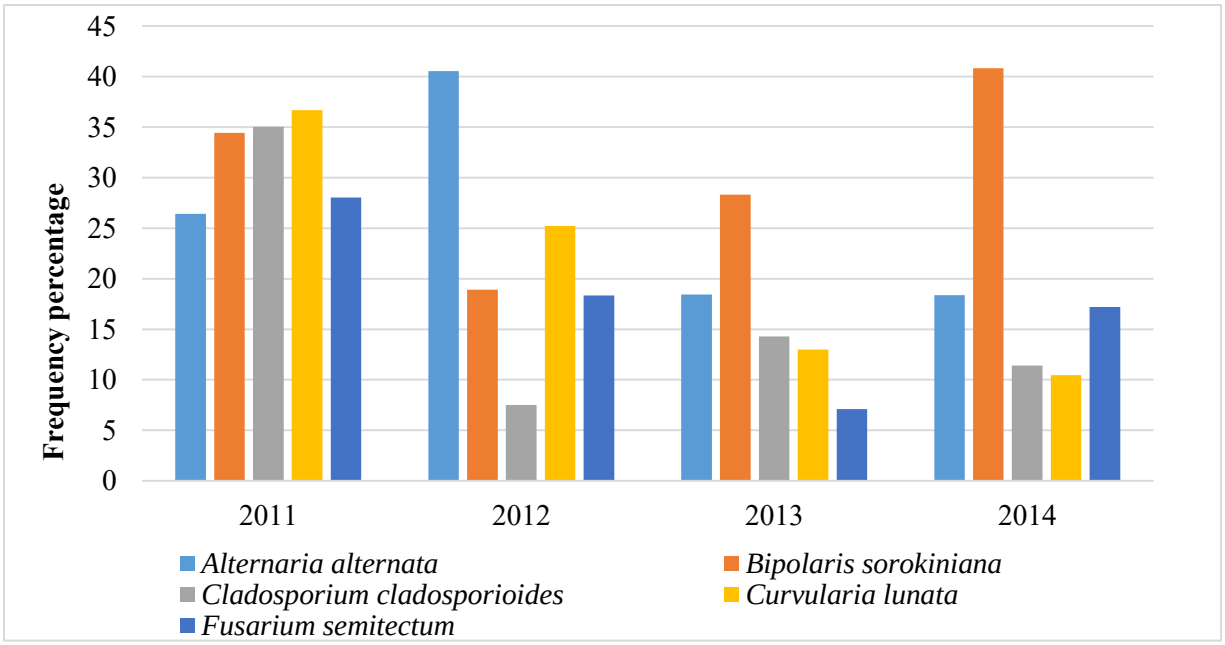


Fig. 4. Prevalence of *Alternaria alternata, Bipolaris sorokiniana, Cladosporium cladosporioides, Curvularia lunata* and *Fusarium smitectum* in Year 2011 to 2014 from BpLB infected wheat leaves in Bangladesh

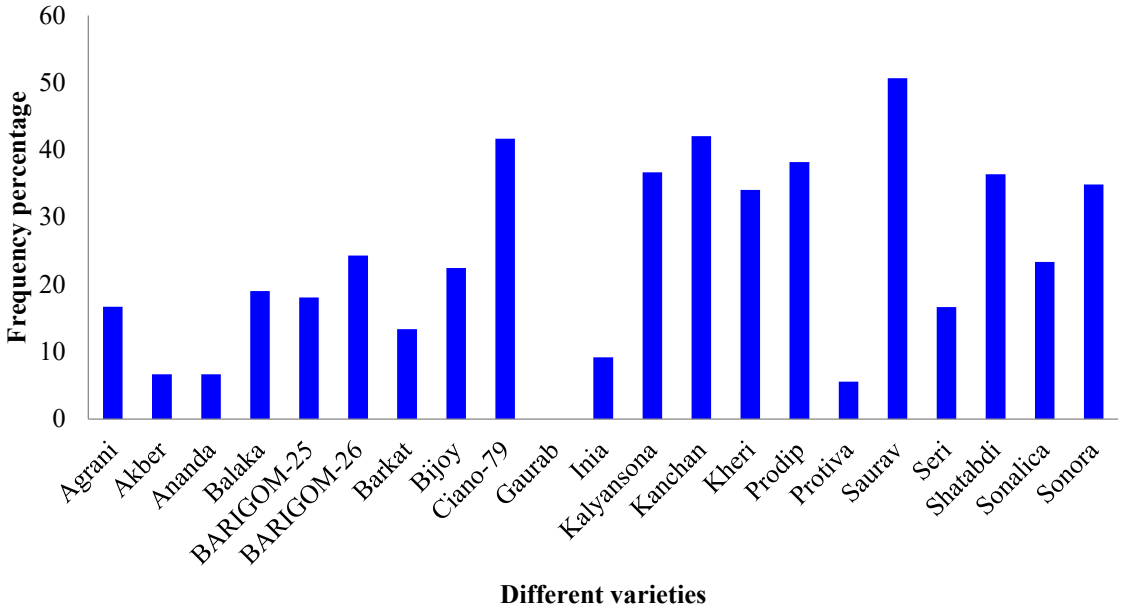


Fig. 5. Prevalence of *Bipolaris sorokiniana* with BpLB infected leaves of twenty-one wheat genotypes

Table 24. District wise distribution of *Bipolaris sorokiniana* isolates from different locations and varieties

Division	District	Upazilla	Village/Area	No. of isolates	Source	Variety
Dhaka	**Dhaka**	DU	Carzon Hall	15	Leaf	BARI GOM-25, BARI GOM-26, BARI GOM-27, BARI GOM-28
	Gazipur	Joydebpur	BARI	17	Leaf	Kalyansona, Kanchan, Kheri, Seri-82, Sonora-64, EN-45, EN-113
				22	Seed	BARI GOM-25, BARI GOM-26, BARI GOM-27, BARI GOM-28, BARI GOM-29, BARI GOM-30, Kanchan
Rangpur	**Dinajpur**	BARI	Nashipur	12	Leaf	Ananda, Akber, Aghrani, Balaka, Barkat, Bijoy, Ciano-79, Kanchan, Prodip, Shatabdi, Shonalika
	Joypurhat	Sadar	Vutiapara	29	Leaf	Bijoy, Prodip, Saurav, Shatabdi,
			Doripara	47		
Rajshahi	**Pabna**	Bera	Kazir Hat	2	Leaf	Bijoy, Shatabdi
			Kashinathpur	1		
		Sujanagar	Matighara	1		
			Vatikaya	1		
	Sirajgonj	Sadar	Binoypur	16	Leaf	Bijoy, Prodip, Saurav, Shatabdi
			Vennabari	7		
Khulna	**Kushtia**	Veramara	Farakpur	1	Leaf	Bijoy, Shatabdi
			Khemirdiar	1	Leaf	Bijoy, Prodip
		Mirpur	Kodalipara	1	Leaf	Prodip
	Chuadanga	Sadar	Farmpara	2	Leaf	Shatabdi
4	**8**	**10**	**15**	**174**	**02**	**22+07=29**

Table 25. Yearly distribution of *Bipolaris sorokiniana* isolates from different locations and varieties

Year	District	Total no. of samples examined	*B. sorokiniana* obtained from no of sample	Total no. of isolates	Varieties	Source	No. of isolates of respective variety
2011	**Gazipur**	20	05	10	Kalyansona	Leaf	03
					Kanchan	Leaf	01
					Kheri	Leaf	04
					Seri-82	Leaf	01
	Dinajpur	17	11	12	Sonora	Leaf	01
					Aghrani	Leaf	01
					Akber	Leaf	01
					Ananda	Leaf	01
					Balaka	Leaf	01
					Barkat	Leaf	01
					Bijoy	Leaf	02
					Ciano-79	Leaf	01
					Kanchan	Leaf	01
					Prodip	Leaf	01
2012					Shatabdi	Leaf	01
					Sonalika	Leaf	01
	Gazipur	08	03	04	Kanchan	Leaf	01
					En-113	Leaf	02
					En-45	Leaf	01
	Kushtia	08	03	03	Bijoy	Leaf	01
					Shatabdi	Leaf	01
					Prodip	Leaf	01
	Chuadanga	06	02	02	Shatabdi	Leaf	01
					Bijoy	Leaf	01
	Pabna	10	04	05	Shatabdi	Leaf	02
					Bijoy	Leaf	03
	Sirajgonj	10	06	22	Bijoy	Leaf	05
					Prodip	Leaf	02
					Shatabdi	Leaf	07
					Saurav	Leaf	08
	Dhaka	12	04	15	BARI Gom-25	Leaf	05
					BARI Gom-26	Leaf	05
					BARI Gom-27	Leaf	02
2013					BARI Gom-28	Leaf	03
					Kanchan	Seed	02
	Joypurhat	16	10	47	Shatabdi	Leaf	10
					Bijoy	Leaf	06
					Prodip	Leaf	15
					Saurav	Leaf	16
	Gazipur	06	02	03	Kanchan	Leaf	02
					EN-113	Leaf	01
	Joypurhat	10	06	29	Shatabdi	Leaf	04
					Bijoy	Leaf	03
					Prodip	Leaf	14
					Saurav	Leaf	08
2014					BARI Gom-25	Seed	02
					BARI Gom-26	Seed	02
	Gazipur	07	06	22	BARI Gom-28	Seed	06
					BARI Gom-29	Seed	05
					BARI Gom-30	Seed	02
					Kanchan	Seed	03
Total	**08**	**130**	**62**	**174**	**29**	**02**	**174**

4.4 Variability study of *Bipolaris sorokiniana*

The pathogen is culturally, morphologically, physiologically, pathologically and molecularly diverse. Because *Bipolaris sorokiniana* reproduces asexually, parasexual recombination is the source of genetic variation. Variable rearranging of 1-6 nuclei per cell is another proposed reason of diversity in this disease. Researchers from all around the world have undertaken extensive study on the variability of the pathogen *B. sorokiniana* in terms of cultural, physiological and pathogenic specialization. Earlier research has also revealed that the *B. sorokiniana* pathogen has a high amount of morphological diversity (Chand *et al.* 2003, Nelson 1960, Oliveira *et al.* 2002, Misra *et al.* 1981).

Understanding the heterogeneity within the *B. sorokiniana* population, in addition to other parameters, is critical for creating location-specific disease control strategies. In this work, 150 isolates of *B. sorokiniana* were examined for cultural and morphological parameters such as colony characteristics, spore size and spore shape. The results showed a lot of diversity amongst the isolates. The colony color ranged from black to ash to brown to green to white, with effusive (fluffy) to suppressed (mat) mycelial development and regular to irregular edges.

4.5 Cultural Variability of *Bipolaris sorokiniana* isolates

The colony colour and texture of *Bipolaris sorokiniana* isolates obtained from foliar and seed samples of wheat in eight districts of Bangladesh between 2011 and 2014 were characterized. The total number of isolates was 174. Among them detail studies were done on 150 isolates. They were grouped in eight cultural groups/types. They were-Black Mat (B-M), Black Fluffy (B-F), Blackish Ash Mat (BlA-M), Brownish Ash Fluffy (BrA-F), Ash Mat (A-M), Whitish Ash Mat (WA-M), Greenish Ash Fluffy (GA-F), Pinkish White Mat (PW-M). Eight different combination of colours and textures of mycelial growth of isolates of *B. sorokiniana* were found on PDA medium. Twenty-five isolates had black colony color, with eighteen Black Mat type isolates and seven Black Fluffy type isolates. Thirty-eight isolates were detected in the Blackish Ash group, ten isolates were in the Brownish Ash group, twenty and twenty-one isolates were placed in the Ash and Whitish Ash group, thirty-two isolates in the Greenish Ash group and only four isolates had Albino colour colony or Pinkish White colony. Brownish Ash and Greenish Ash cultural groups had fluffy mycelial development. On the medium, more than half of

the isolates exhibited suppressed/mat type colonies, while the remainder displayed fluffy type development. Blackish Ash was the most common colour, followed by Greenish Ash, Black, Ash, Whitish Ash and Pinkish White. Out of 150 isolates, 38 had Blackish Ash Mat (BlA-M) colony, 32 had a Greenish Ash Fluffy (GA-F) colony, 21 had Whitish Ash Mat (WA-M), 20 had Ash Mat (A-M), 18 had a Black Mat (B-M), 10 had Brownish Ash Fluffy (BrA-F), 7 had Black Fluffy (B-F) and only 4 exhibited Pinkish White Mat (PW-M) colony. 25.34, 21.34, 14.0, 13.34, 12.0, 6.67, 4.67 and 2.67% correspondingly, were the percentages of above mentioned categories.

The colony edge and colony growth patterns both showed variation among the isolates. Colony development patterns of both regular and irregular types were detected in the isolates. The majority of fluffy type colonies grew in a regular pattern, while the majority of mat type colonies grew in an irregular pattern. There were, however, certain exceptions. The isolates have two different types of colony margins. The colony margins were smooth and wavy. There were several exceptions to the rule that the fluffiest type colonies had smooth colony margins and most mat type colonies had wavy colony margins. The Blackish Ash Mat (BlA-M) type colony had wavy colony edges, whereas the Black Mat (B-M) type colony had smooth colony margins. Another notable feature of the colony was the existence of white sectors. There were no white sectors in black-colored colonies, which suggests isolates of the Black Mat (B-M) and Black Fluffy (B-F) types. Brownish Ash Fluffy (BrA-F) and Greenish Ash Fluffy (GA-F) colonies, as well as Pinkish White Mat (PW-M) isolates, all had no white sectors. White sectors appeared on the surface of the colonies in three types of isolates. Ash Mat (A-M), Blackish Ash Mat (BlA-M) and Whitish Ash Mat (WA-M) were the three types. However, the number of sectors, as well as their form and size, varied. The number of sectors could be counted in certain cases, but not in others. In some circumstances, white sectors were quite small in size, with a circular or ellipsoidal shape, making countdown relatively simple; nevertheless, in other cases, sectors were huge, subsequently intermingled with one other, making countdown impossible. As a result, these traits were recorded as morphological variability for individual isolates, along with additional parameters such as conidial length-breadth, conidial abundance, conidiophore size and so on.

The findings of Ahmed *et al.* (1997) and Aminuzzaman *et al.* (2001) are supported by the current study (2010). Based on colony form and colony colour, Aminuzzaman divided 86 isolates into nine cultural groupings. With a frequency of 39.53 percent, the maximum

number of 34 isolates created effuse black regular colony, whereas 29 isolates generated effuse black irregular colony with a frequency of 33.72 percent of collected isolates. Ahmed (2001) collected 262 *B. sorokiniana* isolates from 16 main wheat-growing regions in Bangladesh and classified them into 13 physiological groups based on cultural features. Debnath (1997) investigated the presence of chromogenic variants in *B. sorokiniana*, classifying pigment-producing and non-pigment-producing isolates as chromogenic and non-chromogenic, correspondingly. Non-chromogenic isolates generated brown to black compact colonies with more or less regular margins and shorter spores than chromogenic isolates, whereas chromogenic isolates produced fluffy cottony white colonies with often irregular margins and larger spores. Alam *et al.* (1997) agree with the findings of the current investigation. Among 27 isolates of *B. sorokiniana*, they discovered seven morphological and physiological differences. Valim *et al.* (1997) found pathogenic and morphologic variability among 10 isolates of *B. sorokiniana* obtained from four wheat-growing areas in Brazil in another investigation.

Poloni *et al.* (2009) investigated 35 isolates of *B. sorokiniana* and classified them into five morphological categories based on their development patterns: black fluffy growth with white sectors, black fluffy growth, gray cottony growth, white cottony growth and white suppressed growth. Various researchers have documented *B. sorokiniana* as a variable fungus with many morphological and physiological variations due to heterokaryosis and parasexual mechanisms (Christensen 1925, Tinline 1958, Chand *et al.* 2003).

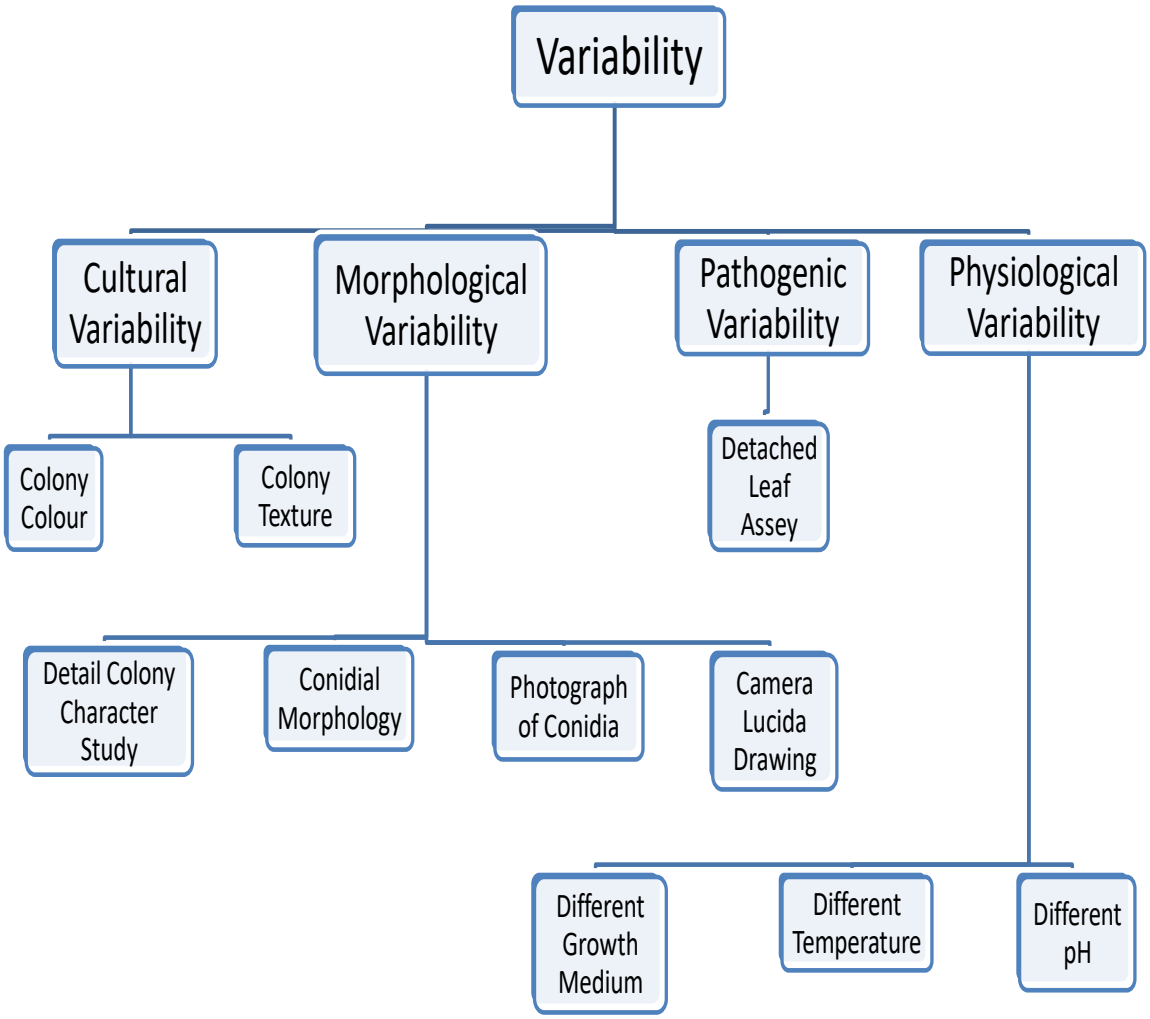


Fig. 6. Different level of variability studied among *Bipolaris sorokiniana* isolates

Table 26. Cultural variations among the *Bipolaris sorokiniana* isolates

SL No.	Cultural Group	Detail Description	Code of *B. sorokiniana* isolates	No	Percentage
1	Black Mat (B-M)	Upper side black, reverse side black, mat, no white dots, growth rate medium to fast, growth pattern regular.	GJBKhL-08, GJBEnL-01, DiWRSlL-01, KMKPdL-01, JSDStL-07, JSDStL-10, JSDSvL-01, JSDSvL-18, JSVBjL-08, JSVPdL-01, GJB28S-04, GJB28S-05, GJB28S-06, GJB29S-02, GJB29S-04, GJB29S-05, GJB29S-06, GJB29S-07	18	12.0 %
2	Black Fluffy (B-F)	Upper side black with ash center and periphery is shiny black, make it velvety, reverse side black, fluffy, no white dots, growth rate medium, growth pattern regular or irregular.	GJBKhL-16, GJBKnL-01, KVKBjL-01, PBKStL-01, JSDStL-11, JSDPdL-01, JSVPdL-08,	7	4.67 %
3	Ash Mat (A-M)	Upper side ash, reverse side comparatively light ash, mat, many white sectors, growth medium, growth pattern irregular.	GJBKhL-01, GJBKlL-01, GJBSnL-01, DiWR25L-01, SSBStL-04, SSBStL-06, DUC27L-02, DUC28L-02, CSFStL-01, JSDSvL-20, JSDSvL-22, JSDSvL-24, JSDSvL-25, JSDSvL-28, JSVPdL-05, JSVPdL-06, GJBKnL-02, GJB26S-03, GJB28S-08, GJB28S-09	20	13.34 %
4	Brownish Ash Fluffy (BrA-F)	Upper side brownish ash, reverse side brownish ash with black centre, fluffy, no white sectors, growth rate slow to medium, growth pattern regular.	GJBSrL-01, DiWRStL-01, DiWRBjL-01, DiWRBjL-03, JSDStL-02, JSDStL-03, JSDStL-05, JSDBjL-03, JSVPdL-16, JSVSvL-01	10	6.67 %
5	Blackish Ash Mat (BlA-M)	Upper side blackish ash, reverse side blackish ash, mat, white sectors present in few isolates, growth slow to medium, growth pattern regular or irregular.	DiWRBlL-01, DiWRPdL-02, DiWR26L-02, DiWRCL-10, DiWRBkL-02, GJBEnL-02, PSVStL-02, JSDPdL-02, JSDPdL-03, JSDPdL-04, JSDPdL-06, JSDPdL-12, JSDPdL-13, JSDPdL-14, JSDPdL-16, JSDSvL-02, JSDSvL-03, JSDSvL-21, JSVBjL-01, JSVBjL-02, JSVBjL-09, JSVBjL-10, JSVPdL-02, JSVPdL-03, JSVPdL-09, JSVPdL-10, JSVPdL-11, JSVPdL-12, JSVPdL-13, JSVPdL-14, JSVPdL-15, JSVPdL-17, GJB25S-01, GJB26S-02, GJB28S-01, GJB29S-01, GJB29S-03, GJB30S-01	38	25.34 %
6	Whitish Ash Mat (WA-M)	Upper side whitish ash, reverse side ash, mat/fluffy, many white sectors, growth medium, growth pattern irregular.	GJBKlL-09, GJBKlL-12, GJBEnL-03, SSBStL-03, SSBStL-05, SSVSvL-01, SSVSvL-06, SSVSvL-08, SSVPdL-01, KVFStL-01, JSDStL-06, JSDBjL-02, JSDSvL-04, JSDSvL-19, JSDSvL-23, JSDSvL-26, JSDSvL-27, JSVPdL-04, GJB25S-02, GJB28S-02, GJB28S-03	21	14.0 %
7	Greenish Ash Fluffy (GA-F)	Upper side greenish ash, center to periphery colour dark to light, reverse side ash with black center and ray pattern, mainly fluffy, no white sectors, growth medium to fast, growth pattern regular.	GJBKhL-18, DiWRAnL-01, DiWRKnL-01, DiWRAgL-01, GJBEnL-04, SSBStL-01, SSBStL-02, SSBStL-07, SSVSvL-02, SSVSvL-03, SSVSvL-04, SSVSvL-05, SSVSvL-07, SSVPdL-02, SSBBjL-01, SSBBjL-02, SSBBjL-03, SSBBjL-04, SSBBjL-05, DUC25L-01, DUC25L-02, DUC25L-03, DUC25L-04, DUC26L-01, DUC26L-05, PBKBjL-02, JSDStL-04, JSDSvL-05, JSVBjL-07, JSVPdL-07,GJBKnL-03, GJB30S-02	32	21.34 %
8	Pinkish White Mat (PW-M)	Upper side pinkish white, reverse side same, mat, no white sectors, growth medium to fast, growth pattern regular to irregular.	CSFBjL-01, PBCBjL-03, JSDStL-01, JSDSvL-09	04	2.67 %
			Total	150	100%

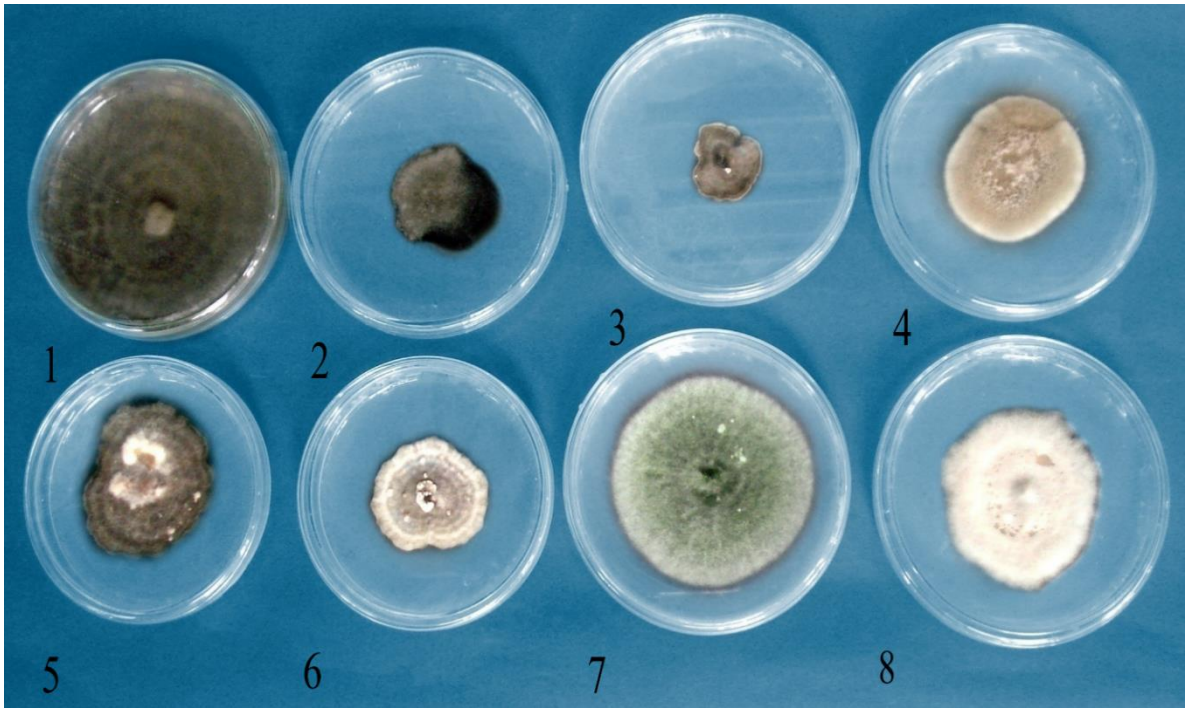


Plate 5. Cultural variation among 8 cultural groups of *Bipolaris sorokiniana* based on colony colour & texture – 1. Black Mat (B-M) 2. Black Fluffy (B-F) 3. Ash Mat (A-M) 4. Brownish Ash Fluffy (BrA-F) 5. Blackish Ash Mat (BlA-M) 6. Whitish Ash Mat (WA-M), 7. Greenish Ash Fluffy (GA-F), 8. Pinkish White Mat (PW-M)

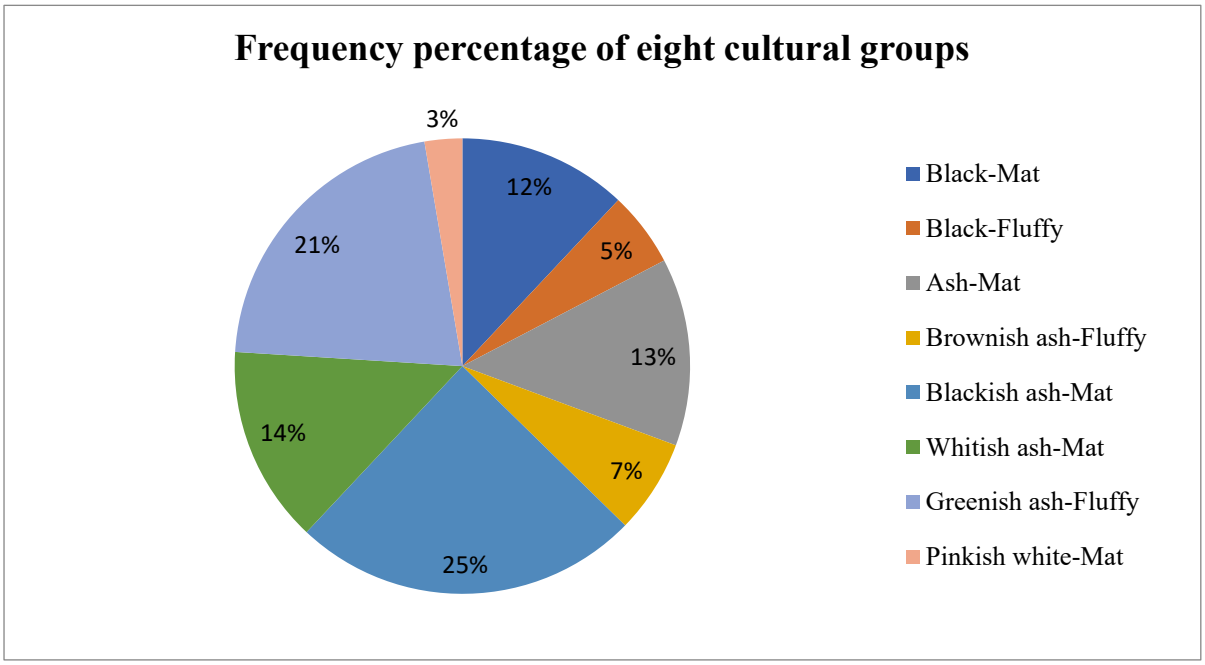


Fig. 7. Pie chart shows the percentage of eight cultural groups of *Bipolaris sorokiniana* based on colony colour & texture

4.6 Morphological variability of *Bipolaris sorokiniana* isolates

According to variation in culture features or colour and texture of colony, as well as conidial morphology, 150 *B. sorokiniana* isolates were morphologically diverse. As a result, the research was separated into two sections: variation in colony characters/features and conidial variability.

4.6.1 Variation in colony characters of *Bipolaris sorokiniana*

Under colony characters' observations were made on colony colour (upper view and reverse view), colony texture, colony margin, growth rate, growth pattern and radial mycelial growth measurement. Growth measurement among 150 isolates was done on 3rd, 5th, 7th and 10th days of colony growth. Then growth rate/day was measured. Table 27 shows the growth rate/day ranged from 1.67–9.00 mm/day. That mean some isolates were very slow in growth, some were very fast growing, while most isolates were moderate growing. More than 50% of total isolates growth ranged from 4.0–5.0 mm/day. From this range number of isolates in both fast growing and slow growing gradually decreases. So, the lowest growth rate showing isolate was only 1(JSVPdL-05) and the highest growth rate showing isolate were only 2 (JSDSvL-01 and JSDSvL-18). On the basis of colony texture cultural variations had already shown in Table 26. They were 2 types–mat (suppressed) and fluffy (effuse). Colony margin were smooth and wavy. Growth rate was classified by slow growing, moderate growing and fast growing. Isolates showed regular and irregular growth pattern. The majority of the suppressed colonies had a wavy edge and an irregular growth pattern.

The results of the current research back up Ahmed *et al.* (1997) conclusions. They discovered physiological and morphological variability among 27 isolates of *B. sorokiniana* collected from 14 districts in Bangladesh's wheat-growing areas. Colonies were ash brown, olive green, light green or dark green in colour, with regular or wavy edges, fluffy, spread or velvety texture and a sector or not. Depending on the isolate, the number of cells per conidium ranged from 3 to 10, while the length and breadth of conidia ranged from 35–270 µm and 15–65 µm, respectively. This study shows similarities with Aminuzzaman *et al.* (2010). In 18 districts of Bangladesh, they found 86 isolates of *B. sorokiniana* from leaves and seeds of 17 wheat cultivars. The rate of radial mycelial growth ranged from 2.77 to 9.1 mm/day.

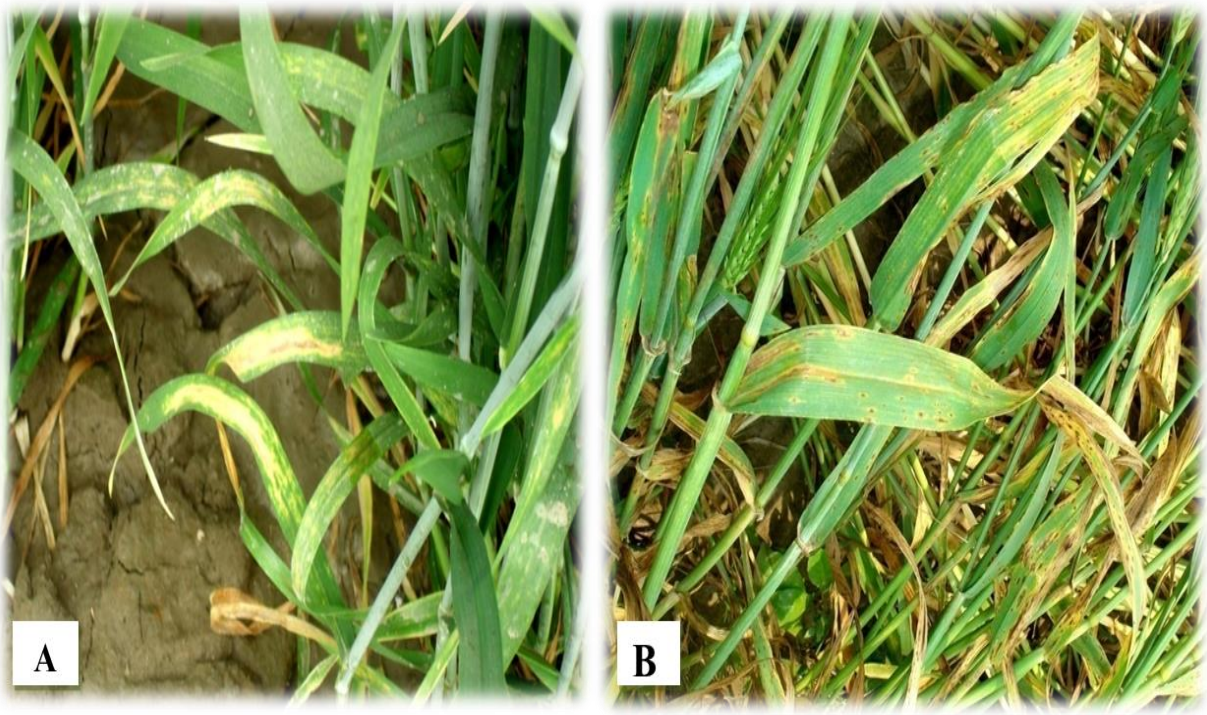


Plate 6. Leaf blight disease of wheat. A. Early stage and B. Grain filling stage

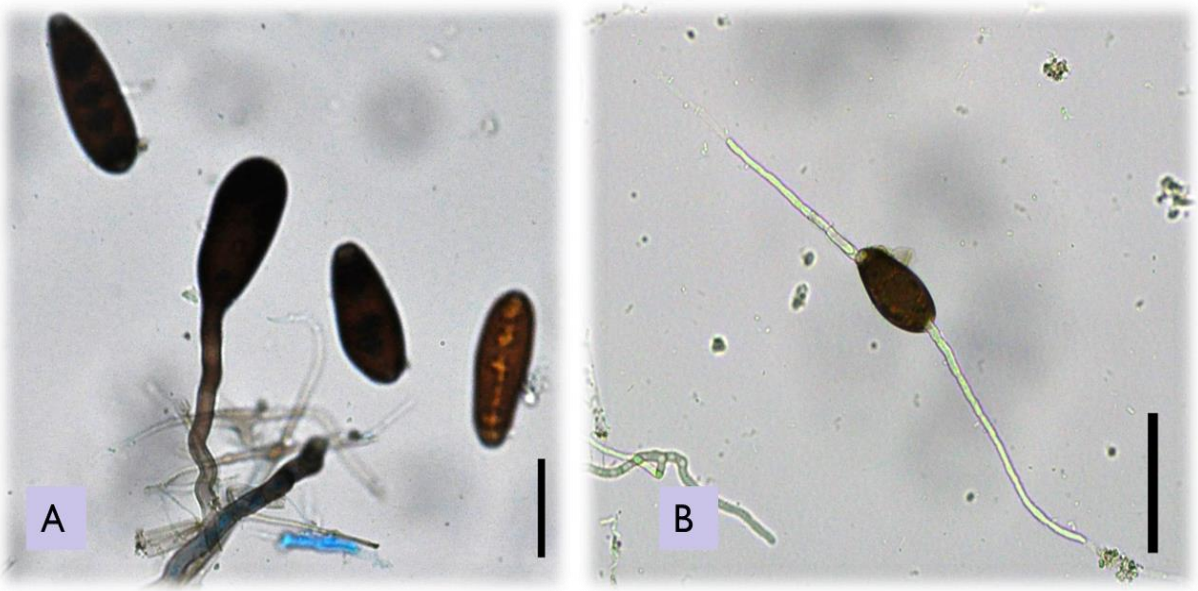


Plate 7. Conidiophores and conidia: A. *Bipolaris sorokiniana* and B. Bipolar germination of conidia

Table 27. Colony characters of different *Bipolaris sorokiniana* isolates

Sl. No.	Isolates	Colony colour		Colony Texture	Colony Margin	Growth rate	Growth Pattern	Growth Measurement				
		Upper view	Reverse view					3rd Day	5th Day	7th Day	10th Day	Per Day
1	**GJBKhL-01**	Ash	Ash	Mat	W	S	IR	15.5	23.5	29.75	30.0	3.0
2	**GJBKhL-08**	Black	Black	Mat	W	M	IR	11.25	23.5	28.0	33.75	3.3
3	**GJBKhL-16**	Black	Black	Fluffy	S	M	R	15.5	30.25	37.75	46.5	4.6
4	**GJBKhL-18**	G Ash	Ash	Fluffy	S	M	R	19.5	32.75	45.75	56.0	5.6
5	**GJBSnL-01**	Ash	Ash	Mat	S	M	IR	13.25	21.75	33.5	45.5	4.5
6	**GJBKlL-01**	Ash	Ash	Mat	S	S	IR	9.75	20.0	22.75	25.5	2.5
7	**GJBKlL-09**	W Ash**	Ash	Mat	W	M	IR	15.5	29.75	40.75	49.5	4.9
8	**GJBKlL-12**	W Ash**	Ash	Mat	W	S	IR	13.5	25.75	28.25	30	3.0
9	**GJBSrL-01**	Br Ash	Ash	Fluffy	S	M	R	16.5	28.75	39.0	51.5	5.1
10	**GJBKnL-01**	Black	Black	Fluffy	S	M	IR	16.5	30.25	42.75	50.0	5.0
11	**DiWRSlL-01**	Black	Black	Mat	S	M	R	12.25	24.25	30.5	37.75	3.7
12	**DiWRBlL-01**	Bl Ash**	Ash	Mat	W	M	IR	15.5	27.75	39.0	52.5	5.2
13	**DiWRAnL-01**	G Ash	G Ash	Fluffy	S	M	R	17.0	28.0	40.5	51.75	5.1
14	**DiWRStL-01**	Br Ash	Br Ash	Fluffy	S	M	R	16.0	30.0	35.0	45.5	4.5
15	**DiWRBjL-01**	Br Ash	Br Ash	Fluffy	S	M	R	18.25	36.75	41.75	50.0	5.0
16	**DiWRBjL-03**	Br Ash	Br Ash	Fluffy	S	F	R	31.25	47.25	56.0	62.25	6.2
17	**DiWRKnL-01**	G Ash	W Ash	Fluffy	S	M	R	20.0	31.5	42.75	55.5	5.5
18	**DiWRPdL-02**	Bl Ash	Black	Mat	S	M	R	11.5	24.75	36.5	40.5	4.0
19	**DiWRAgL-01**	G Ash	Ash	Mat	S	F	R	20.25	37.25	42.75	61.25	6.1
20	**DiWR25L-01**	Ash**	Ash	Mat	S	M	IR	13.75	23.0	33.5	42.5	4.2
21	**DiWR26L-02**	Bl Ash*	Bl Ash	Mat	S	M	IR	12.75	24.25	36.0	49.75	4.9
22	**DiWRCL-10**	Bl Ash**	Bl Ash	Mat	S	F	R	17.75	30.0	46.5	62,75	6.2
23	**GJBEnL-01**	Black	Black	Mat	S	M	IR	18.5	32.25	45.5	58.75	5.8
24	**GJBEnL-02**	Bl Ash**	Bl Ash	Mat	S	M	IR	11.5	22.0	35.5	42.75	4.2
25	**GJBEnL-03**	W Ash**	Ash	Mat	W	F	R	24.25	35.5	38.25	63.25	6.3
26	**GJBEnL-04**	G Ash	Ash	Fluffy	S	F	R	18.5	31.25	49.75	63.25	6.3
27	**SSBStL-01**	G Ash	Ash	Fluffy	S	F	R	19.0	33.5	45.75	64.25	6.4
28	**SSBStL-02**	G Ash	Ash	Fluffy	W	F	IR	23.25	33.5	38.25	66.25	6.6
29	**SSBStL-03**	W Ash**	W Ash	Mat	W	M	IR	16.5	26.5	36	51.75	5.2
30	**SSBStL-04**	Ash*	W Ash	Mat	S	M	R	15.5	25.25	36.5	47.0	4.7
31	**SSBStL-05**	W Ash	Ash	Mat	S	M	IR	21.5	36.0	39.0	41.0	4.1
32	**SSBStL-06**	Ash	Bl Ash	Mat	S	M	R	19.75	24.0	28.0	37.25	3.7
33	**SSBStL-07**	G Ash	G Ash	Fluffy	S	M	R	24.5	41.0	47.0	57.75	5.7
34	**SSVSvL-01**	W Ash	Ash	Fluffy	S	F	R	24.0	42.25	58.0	73.0	7.3
35	**SSVSvL-02**	G Ash	G Ash	Fluffy	S	F	R	20.5	31.75	49.5	68.5	6.8
36	**SSVSvL-03**	G Ash	G Ash	Fluffy	S	S	R	18.0	20.0	24.25	27.75	2.7
37	**SSVSvL-04**	G Ash	G Ash	Fluffy	S	M	R	18.75	29.5	31.75	33.5	3.3
38	**SSVSvL-05**	G Ash	G Ash	Fluffy	S	M	R	20.75	33.5	34.25	35.25	3.5
39	**SSVSvL-06**	W Ash	W Ash	Fluffy	S	S	R	8.5	16.0	19.75	22.75	2.2
40	**SSVSvL-07**	G Ash	W Ash	Fluffy	S	M	R	18.25	26.25	47.0	58.5	5.8
41	**SSVSvL-08**	W Ash	W Ash	Fluffy	S	M	R	26.25	38.25	50.25	57.0	5.7
42	**SSVPdL-01**	W Ash	Bl Ash	Fluffy	S	S	R	11.0	18.5	27.0	28.25	2.8
43	**SSVPdL-02**	G Ash	Bl Ash	Fluffy	S	M	R	22.5	34.5	44.25	51.75	5.1
44	**SSBBjL-01**	G Ash	G Ash	Fluffy	S	M	R	22.0	29.75	35.25	44.5	4.4
45	**SSBBjL-02**	G Ash	G Ash	Fluffy	S	M	R	22.25	33.75	35.25	37.0	3.7
46	**SSBBjL-03**	G Ash	G Ash	Fluffy	S	M	R	21.0	30.0	37.5	51.0	5.1
47	**SSBBjL-04**	G Ash	G Ash	Fluffy	S	F	R	14.0	26.5	42.5	85.25	8.5
48	**SSBBjL-05**	G Ash	G Ash	Fluffy	S	M	R	12.75	23.5	29.5	34.75	3.5
49	**DUC25L-01**	G Ash	G Ash	Fluffy	S	M	IR	13.75	27.25	35.0	46.75	4.7
50	**DUC25L-02**	G Ash	G Ash	Fluffy	S	M	IR	14.75	26.25	37.25	46.5	4.6
51	**DUC25L-03**	G Ash	G Ash	Fluffy	S	F	R	24.25	34.0	49.75	63.25	6.3
52	**DUC25L-04**	G ash	G Ash	Fluffy	S	M	R	18.25	33.75	44.25	49.75	4.9
53	**DUC26L-01**	G Ash	G Ash	Fluffy	S	M	R	14.75	25.25	33.25	44.5	4.4
54	**DUC26L-05**	G Ash	G Ash	Fluffy	S	M	R	16.75	22.5	36.5	55.75	5.5

Sl. No.	Isolates	Colony colour		Colony Texture	Colony Margin	Growth rate	Growth Pattern	Growth Measurement				
		Upper view	Reverse view					3rd Day	5th Day	7th Day	10th Day	Per Day
55	**DUC27L-02**	Ash	Ash	Mat	W	M	IR	14.75	18.5	23.25	31.25	3.1
56	**DUC28L-02**	Ash	Ash	Mat	W	M	IR	15.25	23.5	29.25	35.75	3.6
57	**KVFStL-01**	W Ash	W Ash	Mat	S	M	R	21.25	34.25	46.25	56.0	5.6
58	**KVKBjL-01**	Black	Black	Fluffy	S	M	IR	12.25	23.75	36.75	51.0	5.1
59	**KMKPdL-01**	Black	Black	Mat	S	M	IR	13.5	24.75	37.5	49.75	4.9
60	**CSFStL-01**	Ash	Ash	Mat	S	M	R	17.0	24.25	30.0	37.5	3.7
61	**CSFBjL-01**	P White	P White	Mat	S	M	IR	16.5	27.0	35.5	46.5	4.6
62	**PBKStL-01**	Black	Black	Fluffy	S	M	IR	14.5	25.5	35.5	50.5	5.0
63	**PBKBjL-02**	G Ash	G Ash	Fluffy	S	F	R	22.25	51.25	70.0	88.25	8.8
64	**PSVStL-02**	Bl Ash	Bl Ash	Mat	S	M	IR	11.5	22.75	33.5	42.75	4.3
65	**PBCBjL-03**	P White	P White	Mat	S	M	IR	15.5	24.75	37.5	45.75	4.5
66	**JSDStL-01**	P White	P White	Mat	S	F	R	24.75	53.75	63.0	72.5	7.2
67	**JSDStL-02**	Br Ash	Br Ash	Fluffy	S	M	R	18.25	36.75	41.75	50.0	5.0
68	**JSDStL-03**	Br Ash	Br Ash	Fluffy	S	F	R	31.25	47.25	56.0	62.25	6.2
69	**JSDStL-04**	G Ash	Ash	Fluffy	S	F	R	29.25	47.0	65.75	85.0	8.5
70	**JSDStL-05**	Br Ash	Br Ash	Fluffy	S	S	R	11.0	22.5	23.0	27.0	2.7
71	**JSDStL-06**	W Ash	W Ash	Fluffy	S	M	R	17.5	38.5	43.75	51.0	5.1
72	**JSDStL-07**	Black	Black	Mat	W	M	IR	9.25	21.25	22.25	36.5	3.6
73	**JSDStL-10**	Black	Black	Mat	W	M	IR	13.25	28.0	29.0	33.0	3.3
74	**JSDStL-11**	Black	Black	Fluffy	S	M	R	15.5	35.25	38.75	44.5	4.4
75	**JSDBjL-02**	W Ash**	Ash	Mat	S	M	R	17.5	40.25	49.0	54.25	5.4
76	**JSDBjL-03**	Br Ash	Brown	Fluffy	S	M	R	23.5	33.2	34.75	38.0	3.8
77	**JSDPdL-01**	Black	Black	Fluffy	S	F	R	19.25	41.0	53.5	67.0	6.7
78	**JSDPdL-02**	Bl Ash	Black	Mat	W	M	IR	19.0	39.75	49.5	59.5	5.9
79	**JSDPdL-03**	Bl Ash*	Black	Mat	W	M	IR	20.25	28.0	41.0	48.75	4.9
80	**JSDPdL-04**	Bl Ash	Black	Mat	S	M	R	10.0	20.25	27.0	31.75	3.2
81	**JSDPdL-06**	Bl Ash**	Black	Mat	S	M	IR	17.75	29.5	47.75	56.75	5.7
82	**JSDPdL-12**	Bl Ash	Black	Mat	W	M	IR	13.5	24.0	34.5	41.5	4.1
83	**JSDPdL-13**	Bl Ash**	Black	Mat	W	M	IR	19.25	33.75	48.25	59.75	5.9
84	**JSDPdL-14**	Bl Ash*	Black	Mat	S	M	IR	20.0	37.75	48.25	56.75	5.7
85	**JSDPdL-16**	Bl Ash*	Black	Mat	S	F	R	21.5	32.0	42.0	64.5	6.4
86	**JSDSvL-01**	Black	Black	Mat	S	F	R	42.0	83.25	90.0	90.0	9.0
87	**JSDSvL-02**	Bl Ash	Bl Ash	Mat	W	M	IR	21.5	35.5	40.0	45.5	4.5
88	**JSDSvL-03**	Bl Ash**	Black	Mat	S	S	IR	12.25	20.75	29.5	30.25	3.0
89	**JSDSvL-04**	W Ash*	Ash	Mat	S	M	IR	18.5	37.5	39.5	44.5	4.4
90	**JSDSvL-05**	G Ash	G Ash	Fluffy	S	M	R	17.25	23.75	35.5	44.75	4.5
91	**JSDSvL-09**	P White	P White	Mat	S	M	R	21.0	40.0	46.0	55.25	5.5
92	**JSDSvL-18**	Black	Black	Mat	S	F	R	39.0	81.25	90.0	90.0	9.0
93	**JSDSvL-19**	W Ash**	Ash	Mat	S	M	R	15.0	29.25	31.25	35.0	3.5
94	**JSDSvL-20**	Ash	Ash	Mat	W	S	IR	14.75	25.0	26.25	30.25	3.0
95	**JSDSvL-21**	Bl Ash*	Ash	Mat	W	M	R	24.5	51.0	54.25	56.5	5.6
96	**JSDSvL-22**	Ash	Ash	Mat	S	S	R	8.5	16.75	16.75	25.75	2.6
97	**JSDSvL-23**	W Ash	Ash	Mat	S	M	R	16.75	35.0	44.75	45.5	4.5
98	**JSDSvL-24**	Ash	Ash	Mat	W	S	IR	10.5	21.5	22.5	24.25	2.4
99	**JSDSvL-25**	Ash	Ash	Mat	S	S	R	13.0	19.25	20.0	23.5	2.3
100	**JSDSvL-26**	W Ash	Ash	Mat	W	F	IR	27.75	57.0	68.0	73.75	7.4
101	**JSDSvL-27**	W Ash	Ash	Mat	S	M	R	17.75	35.75	41.5	43.75	4.4
102	**JSDSvL-28**	Ash	Ash	Mat	W	M	IR	18.25	36.5	44.25	49.75	5.0
103	**JSVStL-02**	Black	Black	Fluffy	W	M	R	15.25	33.75	41.0	52.75	5.3
104	**JSVBjL-01**	Bl Ash*	Black	Mat	W	S	IR	9.25	15.25	21.0	25.5	2.5
105	**JSVBjL-02**	Bl Ash**	Black	Mat	S	M	R	20.0	30.5	41.0	50.5	5.0
106	**JSVBjL-07**	G Ash	Ash	Fluffy	S	F	R	30.75	54.25	68.75	78.0	7.8
107	**JSVBjL-08**	Black	Black	Mat	S	M	R	12.5	23.75	33.25	43.0	4.3
108	**JSVBjL-09**	Bl Ash**	Black	Mat	W	M	IR	18.25	31.75	40.5	47.5	4.7
109	**JSVBjL-10**	Bl Ash*	Black	Mat	W	M	IR	17.25	33.25	46.25	60.25	6.0
110	**JSVPdL-01**	Black **	Black	Mat	S	M	R	19.0	35.5	46.25	54.0	5.4

Sl. No.	Isolates	Colony colour		Colony Texture	Colony Margin	Growth rate	Growth Pattern	Growth Measurement				
		Upper view	Reverse view					3rd Day	5th Day	7th Day	10th Day	Per Day
111	**JSVPdL-02**	Bl Ash	Ash	Mat	W	M	IR	18.75	37.5	43.75	48.25	4.8
112	**JSVPdL-03**	Bl Ash*	Black	Mat	W	M	IR	14.5	26.5	42.25	51.5	5.1
113	**JSVPdL-04**	W Ash*	Ash	Mat	S	M	IR	11.5	23.75	33.25	42.0	4.2
114	**JSVPdL-05**	Ash**	Ash	Mat	S	S	IR	9.75	16.75	16.75	16.75	1.7
115	**JSVPdL-06**	Ash*	Ash	Mat	S	M	IR	14.5	22.5	32.25	40.5	4.0
116	**JSVPdL-07**	G Ash*	Ash	Mat	S	M	IR	18.0	22.5	36.5	41.5	4.1
117	**JSVPdL-08**	Black *	Black	Fluffy	S	M	R	9.75	18.5	27.25	34.5	3.4
118	**JSVPdL-09**	Bl Ash	Black	Mat	S	M	IR	17.75	35.0	36.0	40.0	4.0
119	**JSVPdL-10**	Bl Ash**	Ash	Mat	W	M	IR	26.75	50.75	55.75	60.5	6.0
120	**JSVPdL-11**	Bl Ash	Black	Mat	S	M	IR	20.5	28.75	40.25	50.0	5.0
121	**JSVPdL-12**	Bl Ash	Black	Mat	S	F	IR	22.25	41.5	56.0	62.75	6.3
122	**JSVPdL-13**	Bl Ash	Black	Mat	S	F	R	25.25	45.5	59.5	65.5	6.5
123	**JSVPdL-14**	Bl Ash**	Black	Mat	W	M	IR	15.5	35.0	43.25	53.0	5.3
124	**JSVPdL-15**	Bl Ash	Black	Mat	S	M	IR	12.75	26.0	34.75	44.5	4.4
125	**JSVPdL-16**	Br Ash	Br Ash	Fluffy	S	F	R	23.0	39.5	47.75	63.0	6.3
126	**JSVPdL-17**	Bl Ash	Black	Mat	S	M	R	13.0	27.0	39.75	48.75	4.9
127	**JSVSvL-01**	Br Ash	Br Ash	Fluffy	S	M	R	19.0	31.0	43.75	59.0	5.9
128	**GJBKnL-01**	Ash	Black	Mat	W	F	IR	33.75	55.25	60.25	71.25	7.1
129	**GJBKnL-03**	G Ash	W Ash	Fluffy	S	F	R	18.25	34.25	38.75	64.25	6.4
130	**GJB25S-01**	Bl Ash	Black	Mat	S	M	R	11.25	18.0	25.75	34.25	3.4
131	**GJB25S-02**	W Ash	Ash	Mat	S	M	R	13.75	24.0	32.25	39.75	3.9
132	**GJB26S-02**	Bl Ash	Black	Mat	S	F	IR	20.0	36.0	54.0	69.5	6.9
133	**GJB26S-03**	Ash	Black	Mat	S	F	R	22.0	48.0	70.0	87.0	8.7
134	**GJB28S-01**	Bl Ash**	Black	Mat	S	F	R	20.75	39.75	58.25	73.0	7.3
135	**GJB28S-02**	W Ash	Black	Mat	W	M	IR	14.75	25.75	39.5	50.5	5.0
136	**GJB28S-03**	W Ash	Black	Mat	S	M	IR	13.25	24.75	37.75	47.75	4.8
137	**GJB28S-04**	Black	Black	Mat	S	F	R	22.0	39.5	63.25	82.75	8.3
138	**GJB28S-05**	Black*	Black	Mat	S	F	IR	18.25	39.5	55.0	66.75	6.7
139	**GJB28S-06**	Black	Black	Mat	S	M	IR	18.75	36.5	42.5	48.25	4.8
140	**GJB28S-08**	Ash	Bl Ash	Mat	S	F	R	18.5	34.25	57.5	81.25	8.1
141	**GJB28S-09**	Ash	Black	Mat	S	M	R	15.75	33.75	48.25	57.25	5.7
142	**GJB29S-01**	Bl Ash	Black	Mat	S	M	IR	15.5	30.5	38.75	44.0	4.4
143	**GJB29S-02**	Black	Black	Mat	W	M	IR	22.0	32.25	46.25	59.5	5.9
144	**GJB29S-03**	Bl Ash	Black	Mat	S	M	R	13.75	24.5	34.25	43.75	4.4
145	**GJB29S-04**	Black**	Black	Mat	S	F	R	20.5	39.25	52.5	65.5	6.5
146	**GJB29S-05**	Black**	Black	Mat	W	M	IR	18.75	34.5	46.5	57.0	5.7
147	**GJB29S-06**	Black	Black	Mat	S	M	R	13.25	21.25	27.75	36.75	3.7
148	**GJB29S-07**	Black**	Black	Mat	W	M	IR	15.0	26.5	34.75	43.0	4.3
149	**GJB30S-01**	Bl Ash**	Black	Mat	W	M	IR	12.75	20.75	31.5	40.25	4.0
150	**GJB30S-02**	G Ash	Br Ash	Fluffy	S	M	R	13.5	21.5	27.5	35.75	3.6

S= Smooth, W= Wavy, F= Fast growing, M= Moderate growing, S= Slow growing
R= Regular, IR= Irregular, *= Presence of white sectors (Few), **= Presence of white sectors (Many)

4.6.2 Conidial variability of *Bipolaris sorokiniana*

Conidial variability observations were made on length & breadth of conidia, septation, colour, shape, spore abundance and length & breadth of conidiophores of 150 isolates of *B. sorokiniana*. Conidia ellipsoid, briown to dark brown, mostly straight or slightly curved, wall thick but less so towards the ends, broadest in the middle, ends rounded, scar clear within the basal cell. Terminal portion of the end cells subhyaline. Conidiophores brown, short, erect, in most cases single, bearing 1–6 conidia. The length of conidia ranged from the lowest to the highest 23.4–98.1 µm. The conidial breadth ranged from the lowest to the highest 13.3–27.9 µm. Most of the isolates mean conidial length and breadth ranged from 40–60 µm and 17–20 µm. The lowest conidial length was noticed in GJBEnL-03 and the highest conidial length was noticed in GJB29S-05. The lowest and the highest conidial breadth were noticed in DiWRPdL-02 and GJB29S-05. Some isolates had long, slender conidia, while others had conidia that were uniformly straight and cylindrical. Number of pseudo-septation in conidia ranged from 3–12, but in most cases it was 5–7. The highest 12 septation was found in GJB26S-02 isolated from seed of BARI Gom-26. Except for three isolates, GJBKlL-09, JSDSvL-18 and GJB29S-03, which generated both straight and slightly curved shaped conidia in culture, all isolates produced straight shaped conidia. Conidia range in colour from light brown to dark brown. The maximum number of isolates with brown coloured conidia was 82, with light brown, light brown to brown, brown to deep brown and deep brown coloured conidia being 03, 29, 16 and 20, respectively. Conidial abundance was also measured by less sporulated (+), medium sporulated (++) and profusely sporulated (+++). Most of the isolates were profusely sporulated, which was also noticed by blackish shiny colony colour and velvety colony texture. Another feature was observed that most of the isolates obtained from seed were larger than the isolates from leaf. Source also effect the pathogen to show conidial diversity.

The present study is supported by Burnett and Hunter (1999). They reported brown several celled, elliptical, straight or curved conidia. But Mathur and Kongsdal (2000) described conidia as ellipsoid, dark brown to black, smooth and mainly straight or slightly curved, with a diameter of 40–120×17–28 µm. The conidia of four biologic types of *H. sativum* differed somewhat in septation and diameter, as per Christensen (1922). According to Shahzad Asad *et al.* (2009), black cultures sporulate abundantly and expand in a repressed manner. Others were grey to brownish in hue and a few were albino

(whitish) in appearance and less sporulated. According to Aminuzzaman *et al.* (2010), conidial length from the lowest to the highest is 33.40 to 75.86 µm and mean conidial breadth ranged from 10.52–22.86 µm.

Table 28. Conidial characters of different *Bipolaris sorokiniana* isolates

Sl. No.	Isolates	Conidiophore		Size of conidia				Mean Septation	Shape	Color	Spore Abundance
		Mean length (µm)	Mean Breadth (µm)	Length (Lowest-Highest) (µm)	Mean length (µm)	Breadth (Lowest-Highest) (µm)	Mean Breadth (µm)				
1	**GJBKhL-01**	67.5	9.9	41.4–61.2	52.92	17.1–22.5	19.62	6.0	St	Br–DBr	+++
2	**GJBKhL-08**	75.6	8.1	49.5–63	57.78	18–19.8	19.44	5.42	St	Br–DBr	+++
3	**GJBKhL-16**	65.7	7.2	44.1–62.1	52.2	15.3–18.9	16.58	4.71	St	Br	+++
4	**GJBKhL-18**	60.3	8.1	43.2–60.3	52.2	18.9–20.7	20.16	4.62	St	Br	++
5	**GJBSnL-01**	81.0	9.9	50.4–71.1	60.17	16.2–20.7	17.74	5.43	St	LBr–Br	+++
6	**GJBKlL-01**	72.0	8.1	45.0–63.0	54.13	24.3–26.1	24.94	4.28	St	DBr	+++
7	**GJBKlL-09**	64.8	8.1	39.6–57.6	49.72	15.3–21.6	17.89	5.37	St–FC	Br–DBr	+++
8	**GJBKlL-12**	85.5	9.0	45.0–58.5	50.14	16.2–20.7	19.28	3.86	St	Br–DBr	+++
9	**GJBSrL-01**	103.5	10.0	59.4–72	64.98	18–21.6	20.52	6.2	St	Br–DBr	+++
10	**GJBKnL-01**	63.0	6.3	40.5–51.3	44.64	13.5–18	15.48	5.22	St	Br	++
11	**DiWRSlL-01**	90.0	9.9	54.0–63.0	59.66	16.2–19.8	18.51	5.57	St	Br	+++
12	**DiWRBlL-01**	81.0	8.1	41.4–65.7	55.28	16.2–21.6	18.38	4.71	St	Br	++
13	**DiWRAnL-01**	85.5	8.1	55.8–72.9	64.67	19.8–22.5	20.7	4.71	St	LBr	+++
14	**DiWRStL-01**	103.5	9.9	53.1–72.9	61.71	16.2–23.4	19.16	6.28	St	LBr–Br	+++
15	**DiWRBjL-01**	74.7	7.2	51.3–66.6	58.32	17.1–21.6	19.8	6.7	St	Br	+++
16	**DiWRBjL-03**	72.0	8.1	55.8–67.5	61.02	18–21.6	20.16	5.6	St	Br	+++
17	**DiWRKnL-01**	90.0	9.9	44.1–61.2	53.74	18–20.7	19.67	4.0	St	Br	++
18	**DiWRPdL-02**	49.5	7.2	37.8–53.1	44.36	13.3–18	15.81	6.0	St	Br	+++
19	**DiWRAgL-01**	76.5	8.1	47.7–61.2	54.6	17.1–27	21.9	5.83	St	Br	++
20	**DiWR25L-01**	148.5	9.9	52.2–90.9	71.87	19.8–23.4	22.37	7.71	St	Br	++
21	**DiWR26L-02**	85.5	8.1	46.8–60.3	52.42	17.1–22.5	20.59	5.78	St	Br	+++
22	**DiWRCL-10**	52.2	7.2	45.9–52.2	48.0	16.2–18.9	17.25	5.17	St	Br	++
23	**GJBEnL-01**	65.7	8.1	41.4–54.0	47.96	16.2–18	17.48	4.28	St	Br	+++
24	**GJBEnL-02**	75.6	9.9	45–69.3	57.6	17.1–21.6	19.46	6.0	St	Br	+++
25	**GJBEnL-03**	38.7	6.3	23.4–38.7	30.26	14.4–19.8	17.32	4.5	St	Br	+++
26	**GJBEnL-04**	104.4	9.9	47.7–67.5	58.5	16.2–22.5	18.77	5.14	St	Br	+++
27	**SSBStL-01**	130.5	8.1	37.8–76.5	52.74	19.8–22.5	21.42	5.0	St	Br	+++
28	**SSBStL-02**	72.0	9.0	42.3–58.5	50.14	18–25.2	20.06	4.14	St	DBr	+++
29	**SSBStL-03**	124.2	9.9	57.6–76.5	63.77	17.1–22.5	19.41	5.0	St	Br	+++
30	**SSBStL-04**	54.0	7.2	41.4–59.4	45.72	17.1–21.6	19.62	5.21	St	LBr–Br	++
31	**SSBStL-05**	111.6	7.2	58.5–65.7	61.58	18–20.7	19.28	6.28	St	Br	++
32	**SSBStL-06**	51.3	6.3	37.8–49.5	43.07	14.4–19.8	17.74	4.00	St	Br	+++
33	**SSBStL-07**	42.3	5.4	34.2–45	38.16	16.2–22.5	18.9	3.6	St	Br	++
34	**SSVSvL-01**	63.0	7.2	42.3–54.9	48.78	18.9–24.3	22.5	5.8	St	Br	++
35	**SSVSvL-02**	121.5	8.1	50.4–73.8	59.91	18–22.5	19.67	6.14	St	LBr–Br	++
36	**SSVSvL-03**	58.5	8.1	36.0–55.8	45.0	17.1–21.6	18.9	4.62	St	DBr	+++
37	**SSVSvL-04**	96.3	8.1	55.8–65.7	62.36	16.2–19.8	17.61	7.14	St	Br	+++
38	**SSVSvL-05**	63.0	7.2	37.8–54.9	49.24	14.4–20.7	16.71	4.86	St	LBr–Br	+++
39	**SSVSvL-06**	109.8	9.9	54.9–74.7	64.16	16.2–21.6	18.9	7.43	St	Br	+++
40	**SSVSvL-07**	60.3	7.2	36.0–46.8	43.31	19.8–25.2	22.5	4.37	St	Br	++
41	**SSVSvL-08**	82.8	8.1	40.5–66.6	53.85	16.2–20.7	17.7	4.84	St	LBr–Br	+++
42	**SSVPdL-01**	72.0	7.2	45.0–68.4	56.1	17.1–19.8	18.15	5.67	St	LBr–Br	++
43	**SSVPdL-02**	46.8	6.3	31.5–42.3	38.34	16.2–22.5	17.64	3.85	St	Br	++
44	**SSBBjL-01**	44.1	6.3	36.0–49.5	40.84	17.1–23.4	20.25	4.25	St	DBr	+++
45	**SSBBjL-02**	54.0	6.3	37.8–52.2	44.43	18.9–24.3	22.58	6.27	St	Br	+++
46	**SSBBjL-03**	135.0	9.9	49.5–58.5	54.13	16.2–19.8	18.51	7.71	St	Br	+++
47	**SSBBjL-04**	45.0	5.4	30.6–43.2	37.65	18.0–20.7	19.5	4.5	St	Br	+++
48	**SSBBjL-05**	58.5	7.2	39.6–50.4	43.54	19.8–27	23.74	5.12	St	Br	++
49	**DUC25L-01**	108.0	8.1	56.7–76.5	70.56	18.0–22.5	20.7	7.2	St	Br	+++
50	**DUC25L-02**	103.5	8.1	68.4–75.6	73.2	18.0–20.7	19.5	7.34	St	LBr–Br	+++
51	**DUC25L-03**	54.0	7.2	36.9–49.5	43.5	11.7–16.2	13.5	5.5	St	Br	++
52	**DUC25L-04**	99.0	9.9	48.6–77.4	60.0	16.2–23.4	19.35	6.0	St	LBr–Br	+++
53	**DUC26L-01**	103.5	8.1	40.5–69.3	57.3	15.3–20.7	18.15	5.84	St	Br	++

Sl. No.	Isolates	Conidiophore		Size of conidia				Mean Septation	Shape	Color	Spore Abundance
		Mean length (µm)	Mean Breadth (µm)	Length (Lowest-Highest) (µm)	Mean length (µm)	Breadth (Lowest-Highest) (µm)	Mean Breadth (µm)				
54	**DUC26L-05**	75.6	8.1	42.3–54	48.06	16.2–18	16.74	4.6	St	Br	++
55	**DUC27L-02**	47.7	6.3	31.5–37.8	34.2	13.5–15.3	14.76	3.4	St	Br	++
56	**DUC28L-02**	177.3	9.9	54.0–81.0	64.95	18.7–22.5	20.25	6.0	St	LBr–Br	+++
57	**KVFStL-01**	45.0	5.4	34.2–41.4	37.26	14.4–18	16.02	4.6	St	Br–DBr	+++
58	**KVKBjL-01**	74.7	7.2	43.2–70.2	54.6	16.2–18.8	17.1	6.5	St	LBr–Br	+++
59	**KMKPdL-01**	108.0	9.9	52.2–70.2	60.17	15.3–20.7	18.3	6.86	St	LBr–Br	++
60	**CSFStL-01**	90.0	9.9	45.9–58.5	52.02	14.4–18	16.2	5.2	St	Br–DBr	+++
61	**CSFBjL-01**	80.5	7.0	45–58.5	53.23	15.3–17.1	16.07	5.65	St	Br	+++
62	**PBKStL-01**	153.0	9.9	41.4–54.0	50.14	14.4–17.1	16.07	5.71	St	Br	++
63	**PBKBjL-02**	85.5	8.1	52.2–67.5	58.14	17.1–22.5	18.0	6.0	St	Br–DBr	+++
64	**PSVStL-02**	67.5	6.3	42.3–54	48.26	15.3–18	16.54	5.75	St	Br	+++
65	**PBCBjL-03**	93.5	9.6	54.9–72	63.77	18.9–20.7	19.8	6.4	St	Br	+++
66	**JSDStL-01**	73.8	7.2	33.3–52.2	45.54	14.4–18	16.56	4.6	St	LBr	+
67	**JSDStL-02**	93.6	9.9	54.9–67.5	59.53	12.6–17.1	15.68	6.86	St	LBr–Br	+++
68	**JSDStL-03**	79.2	8.1	43.2–67.5	54.77	15.3–20.7	17.87	5.86	St	Br	+++
69	**JSDStL-04**	63.0	7.2	39.2–49.5	45.18	14.4–18	16.02	5.4	St	Br	+
70	**JSDStL-05**	59.4	7.2	41.4–54.0	49.37	18–24.3	20.96	5.71	St	DBr	++
71	**JSDStL-06**	50.4	6.3	36.0–52.2	42.56	16.2–20.7	18.38	5.43	St	DBr	+++
72	**JSDStL-07**	64.8	5.4	40.5–60.3	52.7	17.1–19.8	18.3	6.56	St	Br	+++
73	**JSDStL-10**	61.2	7.2	40.5–54.0	46.93	15.3–20.7	17.61	5.28	St	DBr	++
74	**JSDStL-11**	136.8	9.9	59.4–86.4	70.65	17.1–25.2	20.81	5.75	St	Br	+++
75	**JSDBjL-02**	51.3	7.2	30.6–54.9	42.75	14.4–21.6	17.64	5.0	St	Br	+++
76	**JSDBjL-03**	45.0	6.3	36.0–46.8	41.4	16.2–18.9	17.36	4.0	St	DBr	+++
77	**JSDPdL-01**	54.0	7.2	36–51.3	43.65	17.1–27.0	20.92	4.0	St	DBr	+++
78	**JSDPdL-02**	52.2	7.2	40.5–46.8	43.2	15.3–18.9	17.74	4.6	St	Br	++
79	**JSDPdL-03**	81.0	8.1	43.2–68.4	54.36	17.1–22.5	19.26	5.8	St	Br	+++
80	**JSDPdL-04**	99.0	9.9	44.1–60.3	53.01	13.5–19.8	16.65	4.2	St	DBr	+++
81	**JSDPdL-06**	181.8	9.9	69.3–84.6	76.5	17.1–18.9	18.38	8.0	St	Br	+++
82	**JSDPdL-12**	126.0	8.1	38.7–67.5	55.12	15.3–24.3	19.35	6.37	St	LBr–Br	+++
83	**JSDPdL-13**	94.5	9.9	46.8–67.5	56.57	15.3–19.8	17.23	6.57	St	LBr–Br	++
84	**JSDPdL-14**	177.3	9.9	67.5–83.7	74.31	18.0–24.3	20.83	7.43	St	Br	+++
85	**JSDPdL-16**	166.5	8.1	48.6–75.6	58.76	16.2–18.9	17.87	6.14	St	LBr–Br	++
86	**JSDSvL-01**	42.3	5.4	28.8–33.3	32.85	11.7–15.3	13.2	3.34	St	Br	+++
87	**JSDSvL-02**	36.0	5.4	27.0–44.1	34.8	14.4–21.6	18.1	3.56	St	DBr	+++
88	**JSDSvL-03**	58.5	6.3	36.0–47.7	42.2	15.3–20.7	17.0	5.11	St	Br	+++
89	**JSDSvL-04**	108.0	8.1	46.8–63.0	53.9	17.1–21.6	19.8	5.8	St	Br	+++
90	**JSDSvL-05**	121.5	7.2	50.4–67.5	58.14	16.2 –18	17.1	7.0	St	LBr–Br	++
91	**JSDSvL-09**	103.5	8.1	45.0–60.3	53.1	14.4–18	16.46	6.43	St	LBr	+
92	**JSDSvL-18**	47.7	6.3	31.5–42.3	36.36	15.3–18.9	16.92	4.2	St	Br	+++
93	**JSDSvL-19**	117.0	9.9	46.8–57.6	53.1	17.1–18.9	18.51	4.71	St	Br	+++
94	**JSDSvL-20**	78.3	8.1	57.6–67.5	61.71	19.8–21.6	20.57	5.71	St	LBr–Br	+++
95	**JSDSvL-21**	74.7	7.2	32.4–45.9	39.86	15.3–22.5	20.18	4.4	St	Br–DBr	++
96	**JSDSvL-22**	81.0	8.1	49.5–68.4	59.17	16.2–20.7	18.34	6.62	St	LBr–Br	+++
97	**JSDSvL-23**	76.5	7.2	44.1–72.9	56.5	19.8–24.3	21.4	5.44	St	DBr	+++
98	**JSDSvL-24**	160.0	8.1	60.3–92.7	66.73	18.9–24.3	21.47	6.28	St	LBr–Br	++
99	**JSDSvL-25**	85.5	8.1	47.7–59.4	53.65	18–22.5	19.9	6.34	St	Br	+++
100	**JSDSvL-26**	72.0	7.2	39.6–48.6	43.92	17.1–20.7	18.9	5.6	St	Br	++
101	**JSDSvL-27**	58.5	6.3	31.5–54.9	45.36	14.4–18.9	17.1	5.4	St	Br	+++
102	**JSDSvL-28**	58.5	8.1	33.3–47.7	38.4	13.5–18.9	16.3	5.67	St	LBr–Br	+
103	**JSVStL-02**	109.8	9.9	47.7–76.5	60.75	17.1–27.0	20.02	7.37	St	LBr–Br	++
104	**JSVBjL-01**	111.6	7.2	36.0–54.0	46.54	17.1–22.5	19.28	6.28	St–FC	LBr–Br	+++
105	**JSVBjL-02**	124.2	8.1	51.3–68.4	58.5	17.1–19.8	18.9	6.12	St	Br	+++
106	**JSVBjL-07**	108.0	8.1	51.3–58.5	55.54	18.9–27	22.24	4.86	St	Br	+++
107	**JSVBjL-08**	99.0	9.9	45.9–54.9	51.43	15.3–19.8	16.58	6.71	St	LBr–Br	++
108	**JSVBjL-09**	74.7	7.2	35.1–54.9	43.5	14.4–20.7	17.8	4.11	St	DBr	+++
109	**JSVBjL-10**	49.5	6.3	42.3–50.4	46.2	18–22.5	20.7	5.6	St	Br	+
110	**JSVPdL-01**	99.0	9.9	36.9–52.2	46.2	16.2–18.9	17.7	4.44	St	DBr	+++

Sl. No.	Isolates	Conidiophore		Size of conidia				Mean Septation	Shape	Color	Spore Abundance
		Mean length (µm)	Mean Breadth (µm)	Length (Lowest-Highest) (µm)	Mean length (µm)	Breadth (Lowest-Highest) (µm)	Mean Breadth (µm)				
111	**JSVPdL-02**	85.5	8.1	34.2–47.7	42.94	15.3–20.7	17.74	4.57	St	DBr	++
112	**JSVPdL-03**	63.0	6.3	41.4–52.2	46.69	16.2–19.8	17.89	5.25	St	DBr	+++
113	**JSVPdL-04**	67.5	7.2	25.2–45.9	37.98	16.2–22.5	19.08	5.4	St	Br	++
114	**JSVPdL-05**	103.5	8.1	38.7–56.7	46.69	16.2–21.6	19.01	5.62	St	Br	+++
115	**JSVPdL-06**	111.6	9.9	40.5–59.4	49.11	18–26.1	21.34	6.14	St	Br	+++
116	**JSVPdL-07**	144.0	9.9	46.8–75.6	60.98	19.8–22.5	21.04	5.37	St	Br	+++
117	**JSVPdL-08**	121.5	7.2	49.5–63.9	57.32	17.1–24.3	20.14	4.75	St	Br	+++
118	**JSVPdL-09**	126.0	9.9	53.1–67.5	58.37	17.1–21.6	18.51	5.0	St	DBr	+++
119	**JSVPdL-10**	159.3	9.9	59.4–69.3	63.9	15.3–20.7	18.9	6.43	St	Br	+
120	**JSVPdL-11**	94.5	9.9	39.6–61.2	47.7	16.2–18.9	17.55	5.17	St	Br–DBr	+++
121	**JSVPdL-12**	129.6	9.9	44.1–68.4	54.77	15.3–23.4	18.13	6.43	St	Br	+++
122	**JSVPdL-13**	109.8	8.1	53.1–67.5	58.37	16.2–20.7	19.03	5.86	St	LBr–Br	++
123	**JSVPdL-14**	117.0	7.2	44.1–56.7	50.66	14.4–18	16.07	6.43	St	LBr–Br	+++
124	**JSVPdL-15**	85.5	8.1	43.2–52.2	47.7	13.5–16.2	14.53	6.71	St	LBr–Br	++
125	**JSVPdL-16**	63.0	6.3	27.9–43.2	37.91	15.3–18.9	16.65	5.0	St	Br	+++
126	**JSVPdL-17**	77.4	7.2	33.3–51.3	41.96	13.5–18.9	16.87	5.25	St	Br	+++
127	**JSVSvL-01**	86.4	8.1	38.7–60.3	50.66	15.3–16.2	15.56	7.0	St	LBr–Br	++
128	**GJBKnL-02**	88.2	8.2	45.0–58.5	53.23	15.3–17.1	16.07	5.86	St	Br	+++
129	**GJBKnL-03**	144.0	9.9	54.9–72.0	63.77	18.9–20.7	19.8	7.28	St	LBr–Br	+++
130	**GJB25S-01**	126.0	8.1	49.5–66.6	60.3	18–24.3	20.06	5.57	St	Br	+++
131	**GJB25S-02**	165.5	9.9	76.5–91.8	83.7	16.2–22.5	20.96	9.0	St	Br	+++
132	**GJB26S-02**	141.3	9.9	67.5–81.9	74.7	19.8–26.1	22.16	8.75	St	Br	+++
133	**GJB26S-03**	70.2	7.2	36.9–63	48.78	17.1–20.7	19.08	5.8	St–FC	Br	+++
134	**GJB28S-01**	82.8	9.9	45.9–55.8	50.91	16.2–22.5	19.54	5.86	St	Br–DBr	+++
135	**GJB28S-02**	74.7	7.2	36.0–46.8	42.56	17.1–19.8	18.26	4.57	St	DBr	+++
136	**GJB28S-03**	100.8	9.9	41.4–49.5	44.87	14.4–15.3	15.17	4.57	St	DBr	+++
137	**GJB28S-04**	99.0	9.9	45.9–60.3	52.92	16.2–21.6	18.9	5.83	St	Br–DBr	+++
138	**GJB28S-05**	144.0	9.9	55.8–79.2	66.73	18–23.5	19.67	6.71	St	Br	+++
139	**GJB28S-06**	158.4	9.9	78.3–90	85.11	19.8–23.4	21.73	8.0	St	Br	+++
140	**GJB28S-08**	122.4	8.1	36.9–59.4	48.47	15.3–18.9	17.1	5.86	St	Br	+++
141	**GJB28S-09**	99.0	9.9	42.3–61.2	54.26	16.2–20.7	19.03	4.28	St	Br–DBr	+++
142	**GJB29S-01**	75.6	7.2	43.2–54.9	49.5	15.3–19.8	17.66	5.37	St	DBr	+++
143	**GJB29S-02**	130.5	9.9	63.9–86.4	75.86	18–25.2	21.47	6.57	St	Br	+++
144	**GJB29S-03**	108.0	8.1	43.2–79.2	63.9	18–23.4	20.02	7.0	St–FC	Br	+++
145	**GJB29S-04**	136.8	9.9	63–88.2	78.3	21.6–27	24.41	7.87	St	Br	+++
146	**GJB29S-05**	179.1	9.9	65.7–98.1	83.55	18–27.9	25.35	6.83	St	Br	+++
147	**GJB29S-06**	65.7	8.1	48.6–64.8	56.7	17.1–24.3	19.05	5.83	St	Br	++
148	**GJB29S-07**	173.7	9.9	71.1–83.7	78.3	17.1–19.8	18.64	7.57	St	Br–DBr	+++
149	**GJB30S-01**	124.2	9.9	57.6–76.5	67.32	16.2–23.4	19.44	6.14	St	Br–DBr	+++
150	**GJB30S-02**	103.5	8.1	52.2–74.7	61.07	13.5–20.7	17.74	6.86	St	Br–DBr	+++

St= Straight, FC= Few Curved, Br= Brown, DBr= Dark Brown, LBr= Light Brown

Presence of spore (+++ = sporulation abundent, ++ = sporulation moderate, + = sporulation scanty)

4.7 Pathogenic variability of *Bipolaris sorokiniana* isolates

The pathogenic potential of *B. sorokiniana* isolates from eight different cultural groups was examined on healthy mature leaves of the susceptible wheat cultivar Kanchan. In terms of virulence, isolates from various cultural groups show a wide range of degrees of pathogenicity (Table 25). Mean lesion coverage on leaf (%) ranged from 21.25–70%, where the highest was observed in isolate JSVPdL-01 and the lowest was in JSDSvL-09. Considering mean lesion coverage on detached leaf the highest aggressiveness (4.2) was recorded in cultural group Black Mat and the lowest aggressiveness (3.0) was recorded in cultural group Pinkish White Mat. In respect of isolates most show mean lesion coverage ranged from 40.0–60.0%, so the aggressiveness was 4.0. Only two isolates found with 5.0 aggressiveness, while mean lesion coverage ranged from 62.5–70%. Other 20 isolates show 3.0 aggressiveness. The aggressiveness of pathogens was strongly linked to the colonies, that are dark and black coloured. The results of this study are consistent with those of Aminuzzaman *et al.* (2010). In the pathogenicity test, all of the isolates produced lesions on detached leaves, although their total reaction under a culture group varied in terms of aggressiveness. In another experiment, selected two isolates under each cultural group were tested through seed inoculation technique. All isolates showed symptoms on leaf or leaf sheath as black coloured spot or blighted area. So, data were taken as positive or negative to fulfill Koch's postulate (Table 26).

Hetzler *et al.* (1991) observed a lot of variation in *B. sorokiniana* and recognized 15 pathotypes based on response patterns on a differentiated set of 12 members. Bakonyi *et al.* (1993) also observed the variation among the isolates of *B. sorokiniana* in respect of pathogenicity on artificial inoculation experiment. These findings are also supported by Valjavex-Gratain and Steffenson (1997). They found variation in virulence of 33 isolates of *C. sativus* on three differential barley genotypes. Adhekary (2000) investigated the differences between 122 *B. sorokiniana* isolates. However, he discovered eight virulent cluster II isolates and 78 the least virulent cluster IV isolates, whereas Ahmed *et al.* (2003) observed no significant variation among six pathotypes of *B. sorokiniana* in healthy kernel development of wheat and grain yield of Kanchan variety in Bangladesh.

Maraite *et al.* (1997) made the same discovery when investigating 27 *B. sorokiniana* isolates and discovered that the colonies on basic media ranged in color from white to pale pink to dark green. The darker colony displayed a solid correlation with virulence of the pathogen. Chand *et al.* (2003) studied the variability in natural populations of the leaf

blight pathogen (*B. sorokiniana*) and classified the isolates into five groups based on colony morphology. He discovered that the majority (44.63 percent) of the isolates of the black suppressed type in the natural population were of the most aggressive and was identified as the epidemic population as compared to white coloured isolates with the lowest frequency of 4.96% and few spore in pathogenicity test.

In terms of incubation duration, lesion length, spore formation and mean reaction value, Akram and Singh (2001) observed substantial differences among *H. sativum* isolates. Duveiller and Altamirano (2000) examined the pathogenicity of twenty-seven *B. sorokiniana* isolates obtained from spring wheat seed, root and leaf, concluding that *B. sorokiniana* infection was very diverse. *Drechslera sorokiniana* isolates obtained from Pantnagar (Uttarakhand) were similarly found to be more virulent than another 40 isolates, according to Mahto *et al.* (2002).

Table 29. Pathogenic potentiality of *Bipolaris sorokiniana* isolates following detached leaf assay

Cultural Group	Sl. No	Isolates	Lesion coverage (%)						Mean Lesion coverage (%)	Pathogenicity/ Aggressiveness (0-5) scale	Mean Pathogenicity/ Aggressiveness
			T_1	T_2	T_3	T_4	T_5	T_6			
Black Mat (B-M)	1	GJBKhL-08	C	C	80	50	40	60	57.5	4	4.2
	2	DiWRSlL-01	C	C	30	25	50	90	48.75	4	
	3	GJBKnL-04	C	C	50	40	60	80	57.5	4	
	4	JSDStL-07	C	C	50	50	60	60	55	4	
	5	JSDStL-10	C	C	80	60	35	60	58.75	4	
	6	JSDSvL-01	C	C	50	75	40	30	48.75	4	
	7	JSDSvL-18	C	C	20	30	80	35	41.25	4	
	8	JSVBjL-08	C	C	40	30	80	35	46.25	4	
	9	JSVPdL-01	C	C	70	80	40	90	70	5	
	10	GJB28S-04	C	C	60	70	50	70	62.5	5	
Black Fluffy (B-F)	01	GJBSrL-01	C	C	80	70	50	20	55	4	4.0
	02	GJBKhL-16	C	C	50	75	20	25	42.5	4	
	03	GJBKnL-01	C	C	35	40	55	40	42.5	4	
	04	JSDStL-08	C	C	20	80	40	40	45	4	
	05	JSDStL-11	C	C	50	60	50	60	55	4	
	06	JSDPdL-01	C	C	50	55	45	45	48.75	4	
	07	JSVPdL-08	C	C	30	75	45	55	51.25	4	
Blackish Ash Mat (BlA-M)	01	PSVStL-02	C	C	40	60	70	30	50	4	3.9
	02	JSDPdL-03	C	C	75	50	65	35	56.25	4	
	03	JSDPdL-04	C	C	40	25	70	35	42.5	4	
	04	JSDSvL-03	C	C	15	80	45	30	42.5	4	
	05	JSDSvL-21	C	C	55	45	70	35	51.25	4	
	06	JSVBjL-09	C	C	70	30	35	30	41.25	4	
	07	JSVBjL-10	C	C	40	25	65	30	40	3	
	08	JSVPdL-02	C	C	25	45	65	35	42.5	4	
	09	JSVPdL-03	C	C	20	75	45	30	42.5	4	
	10	JSVPdL-09	C	C	60	40	50	75	56.25	4	
Brownish Ash Fluffy (BrA-F)	01	DiWRStL-01	C	C	20	45	55	35	38.75	3	3.44
	02	DiWRBjL-01	C	C	55	20	35	50	40	3	
	03	DiWRBjL-03	C	C	35	75	30	25	41.25	4	
	04	JSDStL-02	C	C	40	60	70	35	51.25	4	
	05	JSDStL-03	C	C	40	60	70	30	50	4	
	06	JSDStL-05	C	C	35	25	45	40	36.25	3	
	07	JSDBjL-03	C	C	30	30	50	45	38.75	3	
	08	JSVPdL-16	C	C	20	30	35	60	36.26	3	
	09	GJBEnL-01	C	C	35	70	25	40	42.5	4	
Ash Mat (A-M)	01	GJBKhL-01	C	C	30	25	50	70	43.75	4	3.9
	02	DiWR25L-01	C	C	25	35	50	80	47.5	4	
	03	SSBStL-04	C	C	30	30	70	35	41.25	4	
	04	SSBStL-06	C	C	40	40	45	45	42.5	4	
	05	JSDSvL-20	C	C	45	25	50	35	38.75	3	
	06	JSDSvL-25	C	C	40	60	65	35	50	4	
	07	JSDSvL-28	C	C	50	55	45	60	52.5	4	
	08	JSVPdL-05	C	C	50	60	55	55	55	4	
	09	JSVPdL-06	C	C	40	25	70	35	42.5	4	
	10	GJBKnL-02	C	C	30	45	70	25	42.5	4	
Whitish ASh Mat (WA-M)	01	GJBKlL-09	C	C	50	45	20	35	37.5	3	3.5
	02	SSBStL-05	C	C	60	50	20	20	37.5	3	
	03	SSVSvL-01	C	C	20	80	40	40	45	4	
	04	SSVSvL-06	C	C	70	30	35	30	41.25	4	
	05	JSDStL-06	C	C	25	35	30	50	35	3	
	06	JSDSvL-04	C	C	20	30	40	60	37.5	3	
	07	JSDSvL-23	C	C	70	75	50	25	55	4	
	08	JSDSvL-26	C	C	50	70	25	25	42.5	4	
	09	JSDSvL-27	C	C	25	25	50	25	31.25	3	
	10	JSVPdL-04	C	C	40	35	40	55	42.5	4	

Cultural Group	Sl. No	Isolates	Lesion coverage (%)						Mean Lesion coverage (%)	Pathogenicity/ Aggressiveness (0-5) scale	Mean Pathogenicity/ Aggressiveness
			T_1	T_2	T_3	T_4	T_5	T_6			
Greenish Ash Fluffy (GA-F)	01	GJBKhL-18	C	C	20	25	50	10	26.25	3	3.4
	02	DiWRKnL-01	C	C	10	40	60	20	32.5	3	
	03	SSVSvL-03	C	C	15	45	60	30	37.5	3	
	04	SSVSvL-04	C	C	20	55	45	35	38.75	3	
	05	SSBBjL-03	C	C	50	50	60	45	51.25	4	
	06	SSBBjL-04	C	C	25	20	50	30	31.25	3	
	07	JSDSvL-05	C	C	40	50	50	40	45	4	
	08	JSVBjL-07	C	C	60	45	60	50	53.75	4	
	09	JSVPdL-07	C	C	50	20	25	20	28.75	3	
	10	GJBKnL-03	C	C	45	35	55	35	42.5	4	
sh White Mat (PW-	01	JSDStL-01	C	C	10	20	30	30	22.5	3	3.0
	02	JSDSvL-09	C	C	15	5	25	40	21.25	3	

C= Control

Table 30. Pathogenicity test of different isolates of *Bipolaris sorokiniana* on healthy seeds through seed inoculation technique

Sl. No.	Cultural Group	Code of Isolates	Control/ un-inoculated Seeds	Inoculated Seeds
			Disease Reaction	
1	Black Mat (B-M)	JSDSvL-01	–	+
		GJBEnL-01	–	+
2	Black Fluffy (B-F)	JSVPdL-08	–	+
		GJBKhL-01	–	+
3	Ash Mat (A-M)	JSDSvL-28	–	+
		GJBKnL-01	–	+
4	Brownish Ash Fluffy (BrA-F)	JSDBjL-03	–	+
		DiWRBjL-01	–	+
5	Blackish Ash Mat (BlA-M)	JSVPdL-10	–	+
		PSVStL-02	–	+
6	Whitish Ash Mat (WA-M)	JSDSvL-26	–	+
		JSVPdL-04	–	+
7	Greenish Ash Fluffy (GA-F)	JSDSvL-05	–	+
		GJBKnL-03	–	+
8	Pinkish White Mat (PW-M)	JSDSvL-09	–	+
		JSDStL-01	–	+

(–) =No disease symptom and (+) = Disease symptoms appeared

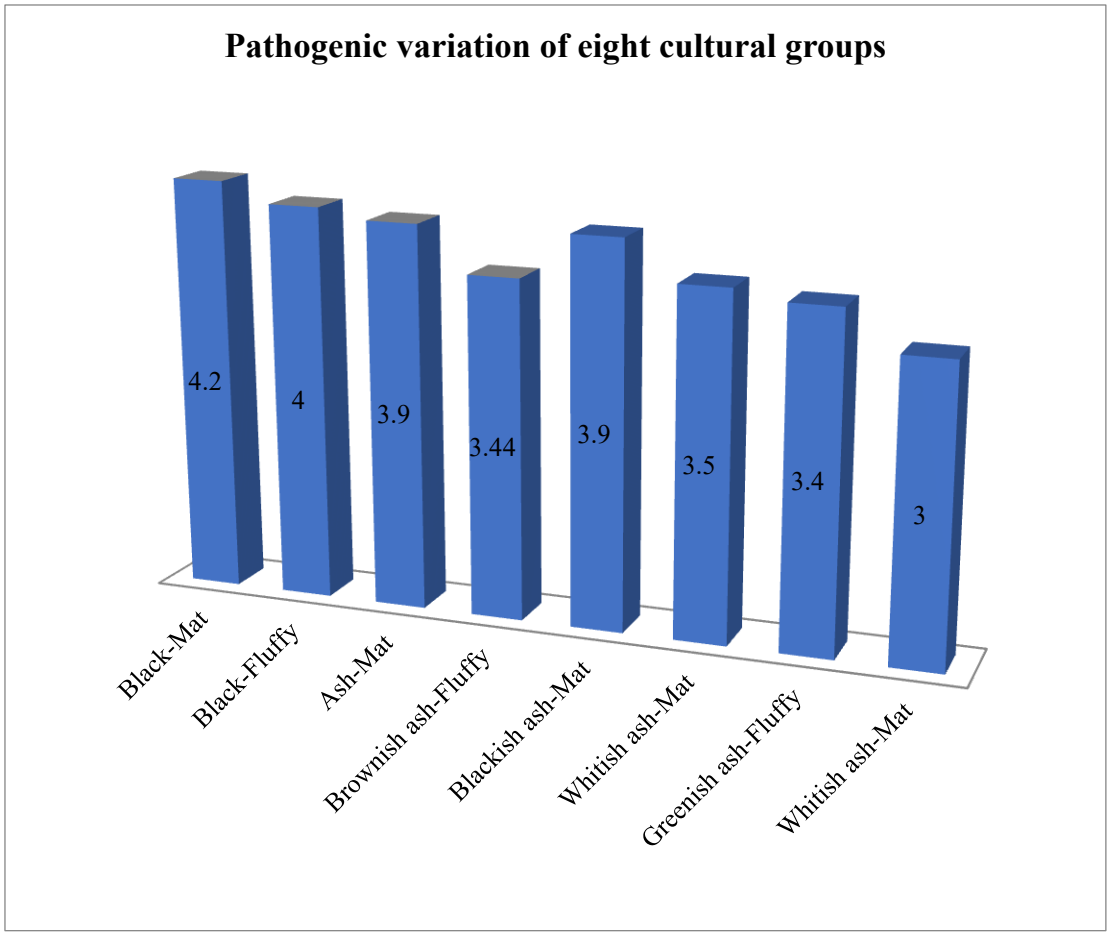


Fig. 8. Pathogenic variability among eight cultural groups of *Bipolaris sorokiniana* isolates

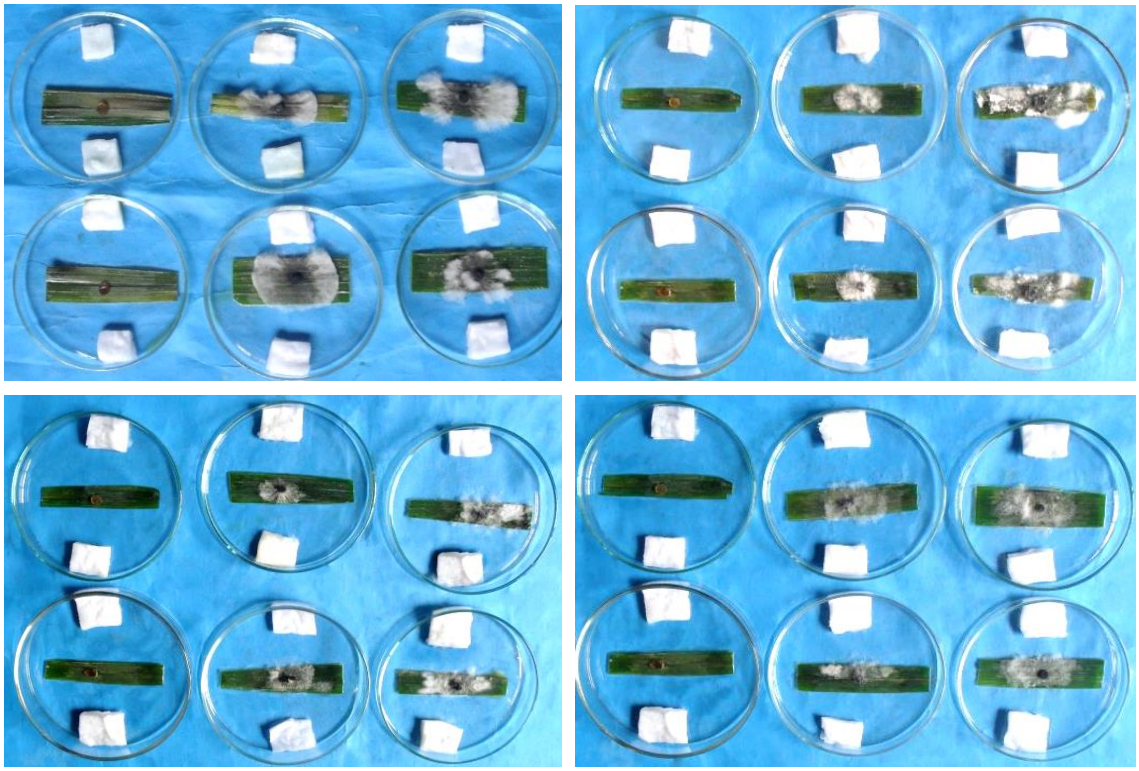


Plate 8. Pathogenic potentiality of *Bipolaris sorokiniana* isolates following detached leaf assay

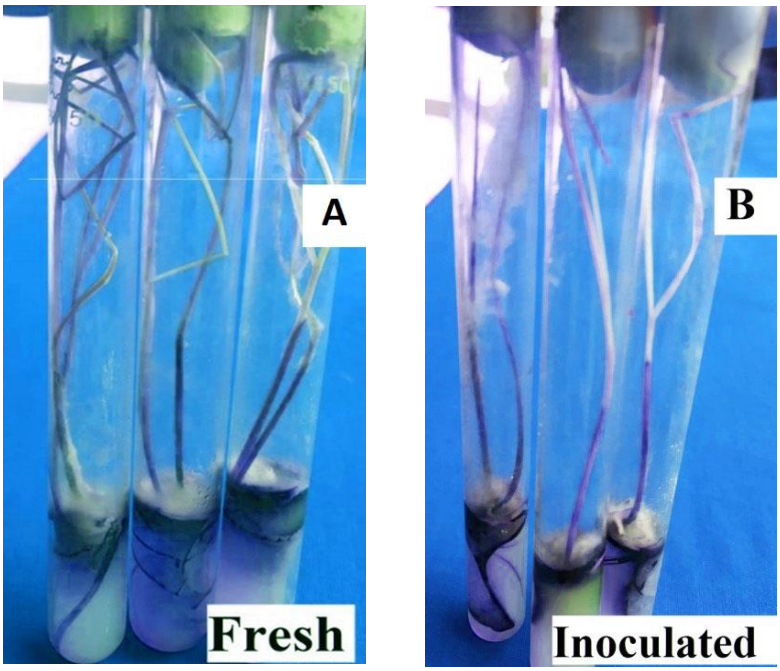


Plate 9. Pathogenicity test of *Bipolaris sorokiniana* through seed inoculation technique

4.8 Physiological variability of *Bipolaris sorokiniana* isolates

Physiological variation among *B. sorokiniana* isolates were observed on sixteen isolates belongs to eight cultural groups (Two isolates from each group). Three different experiments were arranged based on different culture medium, different temperature and different pH.

4.8.1 Physiological variability of *Bipolaris sorokiniana* isolates due to different culture medium

Isolates of *B. sorokiniana* revealed variation in the radial growth after 10 days of inoculation on five different culture medium *viz.* Potato dextrose agar (PDA), Carrot dextrose agar (CDA), Tomato dextrose agar (TDA), Host extract agar (HEA) and Water agar (WA). Data revealed that isolate JSDSvL-26 with radial growth rate (73.75 mm) was the fastest growing fungus followed by isolates JSDStL-01, GJBKnL-01 and JSDSvL-01 having growth rate of 72.5, 71.25 and 70.25 mm, respectively on PDA medium. The least growth rate (30.0 mm) was observed in GJBKhL-01. On CDA medium, maximum growth rate (68.5 mm) was observed in JSDStL-01 and least by GJBKhL-01 with radial growth (28.45 mm). GJBKnL-01 isolate exhibited the highest growth rate (64.88 mm) on TDA medium, whereas GJBKhL-01 was the lowermost (25.5 mm) growing fungus. On HEA medium, maximum growth rate (55.7 mm) was observed in JSDSvL-01 and least by GJBKhL-01 with radial growth (20.4 mm). JSDSvL-26 with radial growth rate (65.5 mm) was the fastest growing fungus on WA medium, the lowest was as usual GJBKhL-01 (24.34 mm).

The present result coincides with the finding of Poloni *et al.* (2008) who studied the morphological variability of monosporic and polysporic *B. sorokiniana* cultures grown in different media and observed high rates of morphological variability in the replicates of polysporic cultures with few differences among monosporic cultures. Raguchander *et al.* (1988) experimented on the colony characters and spore formation of *B. sorokiniana*, found oatmeal and PDA giving the superior radial colony diameter and spore formation of the pathogen among 12 media tested. Pascual and Raymundo (1995) observed cultural variability among 200 isolates of *H. sativum* when cultured on potato dextrose agar, wheat extract agar and V-8 juice agar.

4.8.2 Physiological variability of *Bipolaris sorokiniana* isolates due to different temperatures

On the basis of different temperatures *B. sorokiniana* isolates exposed variation in the radial mycelial growth after 10 days of inoculation. The temperatures were-10ºC, 15ºC, 20ºC, 25ºC and 30ºC. All the isolates of *B. sorokiniana* showed their maximum growth on PDA medium at 25ºC. For the growth of *B. sorokiniana* 25ºC was the optimum temperature. Above and below 25ºC, the *B. sorokiniana* isolates showed growth reduction. 10ºC temperature was the least temperature for their growth. At 25ºC, the highest growth rate (70.75 mm) was observed in JSDSvL-26 following by JSDStL-01 (70.5 mm), JSDSvL-01 (70 mm) and GJBKnL-01 (70 mm) and the lowest by GJBKhL-01 with radial growth (32.25 mm).

4.8.3 Physiological variability of *Bipolaris sorokiniana* isolates due to different pH

Isolates of *B. sorokiniana* revealed variation in the radial mycelial growth after 10 days of inoculation on five different pH, on PDA medium *viz.* 5.5, 6.0, 6.5, 7.0 and 7.5. All the isolates of *B. sorokiniana* showed their maximum growth at 7.0 pH on PDA medium. For the growth of *B. sorokiniana* 7.0 pH was the optimum. Above and below 7.0 pH, the radial mycelial growth of *B. sorokiniana* isolates reduced. Growth was minimum at 5.5 pH level. At 7.0 pH of PDA medium, data revealed that isolate JSDSvL-01 with radial growth rate (71.75 mm) was the fastest growing fungus followed by isolates GJBKnL-01, JSDSvL-26 and JSDStL-01 having growth rate of 70.75, 70.5 and 70.5 mm, respectively. The least growth rate (29.25 mm) was observed in GJBKhL-01. Similar results were also observed in other pH level.

Sucrose concentration and initial pH and of the medium obviously affected sporulation and conidial features specially septation in four *Bipolaris* species. *Bipolaris sorokiniana* produced spore high at all pH levels. Maximum isolates of *B. sorokiniana* made conidia huge at all sucrose levels (Harding 1975).

Table 31. Physiological variability of *Bipolaris sorokiniana* isolates due to different culture medium (radial mycelial growth after ten days)

Sl No.	Cultural group	Code of isolates	Culture medium				
			PDA	CDA	TDA	HEA	WA
1	Black Mat (B-M)	JSDSvL-01	70.25	66.64	60.25	55.7	61.34
		GJBEnL-01	58.75	55.44	50.65	45.25	48.44
2	Black Fluffy (B-F)	JSVPdL-08	34.5	30.55	31.66	25.34	30.45
		GJBKhL-01	30.0	28.45	25.5	20.4	24.34
3	Ash Mat (A-M)	JSDSvL-28	49.75	45.66	42.88	32.75	40.36
		GJBKnL-01	71.25	67.25	64.88	55.25	50.45
4	Brownish Ash Fluffy (BrA-F)	JSDBjL-03	38.0	35.5	32.75	22.86	33.66
		DiWRBjL-01	50.0	45.55	42.38	32.88	38.8
5	Blackish Ash Mat (BlA-M)	JSVPdL-10	60.5	56.44	51.98	34.34	40.55
		PSVStL-02	42.75	40.75	37.25	30.66	35.5
6	Whitish Ash Mat (WA-M)	JSDSvL-26	73.75	68.45	62.75	42.5	65.5
		JSVPdL-04	42.0	40.88	36.5	25.5	35.75
7	Greenish Ash Fluffy (GA-F)	JSDSvL-05	44.75	41.75	40.5	28.0	35.0
		GJBKnL-03	64.25	60.5	55.0	34.5	42.5
8	Pinkish White Mat (PW-M)	JSDSvL-09	55.25	50.75	46.75	30.5	40.25
		JSDStL-01	72.5	68.5	62.8	40.5	60.75

Table 32. Physiological variability of *Bipolaris sorokiniana* isolates due to different temperatures (radial mycelial growth after ten days)

Sl No.	Cultural group	Code of isolates	Temperature				
			10°C	15°C	20°C	25°C	30°C
1	Black Mat (B-M)	JSDSvL-01	42.75	50.5	65.5	70.0	53.75
		GJBEnL-01	37.75	45.75	52.5	56.75	42.5
2	Black Fluffy (B-F)	JSVPdL-08	18.75	24.5	31.75	34.0	30.25
		GJBKhL-01	20.25	22.75	30.5	32.25	28.75
3	Ash Mat (A-M)	JSDSvL-28	27.75	36.75	45.5	50.25	42.75
		GJBKnL-01	37.5	45.5	62.75	70.0	38.75
4	Brownish Ash Fluffy (Br A-F)	JSDBjL-03	20.5	26.25	33.75	36.5	30.5
		DiWRBjL-01	28.5	32.5	47.75	52.5	45.75
5	Blackish Ash Mat (BlA-M)	JSVPdL-10	24.5	38.75	52.0	58.5	50.0
		PSVStL-02	15.5	25.5	34.0	40.75	31.5
6	Whitish Ash Mat (WA-M)	JSDSvL-26	32.5	40.0	63.75	70.75	60.0
		JSVPdL-04	22.0	30.0	42.5	45.0	38.5
7	Greenish Ash Fluffy (GA-F)	JSDSvL-05	18.5	26.5	37.75	42.75	35.5
		GJBKnL-03	25.5	35.25	58.5	62.75	55.5
8	Pinkish White Mat (PW-M)	JSDSvL-09	25.5	35.0	46.5	52.5	50.5
		JSDStL-01	26.75	36.5	64.75	70.5	60.5

Table 33. Physiological variability of *Bipolaris sorokiniana* isolates due to different pH (radial mycelial growth after ten days)

Sl No.	Cultural group	Code of isolates	pH				
			5.5	6.0	6.5	7.0	7.5
1	Black Mat (B-M)	JSDSvL-01	50.75	64.5	70.5	71.75	67.25
		GJBEnL-01	40.5	48.25	52.5	56.75	50.25
2	Black Fluffy (B-F)	JSVPdL-08	22.5	28.0	32.25	35.75	30.5
		GJBKhL-01	18.0	23.5	28.5	29.25	25.0
3	Ash Mat (A-M)	JSDSvL-28	24.0	38.5	47.75	51.25	45.5
		GJBKnL-01	42.75	58.5	66.5	70.75	61.5
4	Brownish Ash Fluffy (BrA-F)	JSDBjL-03	20.5	28.5	35.5	37.75	31.75
		DiWRBjL-01	32.25	40.0	49.25	52.5	46.5
5	Blackish Ash Mat (BlA-M)	JSVPdL-10	38.25	45.5	55.5	58.25	52.0
		PSVStL-02	23.5	31.75	37.75	40.75	34.5
6	Whitish Ash Mat (WA-M)	JSDSvL-26	40.5	56.75	64.5	70.5	61.5
		JSVPdL-04	23.0	32.75	40.5	44.0	37.75
7	Greenish Ash Fluffy (GA-F)	JSDSvL-05	30.0	40.0	43.75	45.75	40.5
		GJBKnL-03	35.5	49.75	58.5	63.0	56.5
8	Pinkish White Mat (PW-M)	JSDSvL-09	36.0	45.5	50.0	54.5	52.5
		JSDStL-01	47.0	58.5	65.5	70.5	61.75

4.9 Molecular identification of *Bipolaris sorokiniana*

Bipolaris sorokiniana was identified by analyzing ITS regions sequences using the ITS1 and ITS4 as forward and reverse primers. PCR generated bands (-600bp) from samples were submitted to automated sequencing followed by BLAST analysis to identify the genomic sequence. *B. sorokiniana* strain NRRL 62873 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence. Maximum score-953, total score-953, Query coverage- 95%, E-value-0.0, Identity-99%.

Barcode based molecular technique is more accurate, rapid and reliable means of fungal identification. ITS-based molecular techniques may be a significant complement to traditional mycological detection by culture, which is becoming important in plant pathology.

4.10 Compatibility between isolates of *Bipolaris sorokiniana* under different cultural groups

Bipolaris sorokiniana is highly variable in terms of cultural, morphology, physiological, pathological, and molecular characteristics. Because the pathogen is reproduced asexually or clonally, chromosomal rearrangements may play an important role in pathogen variation and the mechanisms involved may be operative through somatic growth and parasexual recombination. (Kaufmann and Weidemann 1996). Anastomosis and nuclear migration, according to Tinline (1962), cause diversity in *B. sorokiniana*. Mycelial compatibility or the capability of two filamentous fungi to create one continuous colony via anastomosing, is equivalent with vegetative compatibility. Vegetative heterokaryons are formed when two individuals fuse, resulting in genetically distinct nuclei. In heterokaryon compatibility, a clear difference between two isolates must be maintained until it is established that the two strains not only anastomose but also form a stable heterokaryon. Vegetative compatibility has been particularly effective in intraspecific strain comparisons as a simple test for self-recognition (Saupe 2000). One confluent colony resulted after compatible pairing. Incompatible pairing causes a visible response in the interface zone and hyphal fusion cells are swiftly compartmentalized and destroyed if individuals differ in allelic specificity at heterokaryon incompatibility loci (Glass *et al.* 2000).

After inoculating and growing two distinct isolates on the same plate, it was discovered that as the colony size became larger, their mycelia came into touch. There were a total of thirty-one confrontations. Three distinct behaviors were seen in the hyphal overlapping region, allowing us to categorize the confrontations as incompatible, compatible or partially compatible (Plate 9, 10, 11). Eight of the 31 confrontations were compatible, six were partially compatible and seventeen were incompatible (Table 30). It was feasible to see hyphae overlapping in even parts of the colonies during compatible confrontations. Initial intermingling of the mycelia of two incompatible isolates occurred in partially compatible confrontations, but subsequently lysis of the mycelia of two isolates and the establishment of a clear zone were seen at the interaction site (mycelial contact). Plate 11 depicts the mycelial incompatibility response (top and bottom). The confrontations revealed that 54.84 percent of the participants were vegetatively incompatible. As a result of the research, it was discovered that the mycelial compatibility response may be utilized to identify morphologically similar isolates from the same species. In their study of

incompatibility, Slippers *et al.* (2001) found that antagonistic interactions are more prevalent among related species than between genetically or geographically distinct isolates.

Higher frequency phenotypic differences in the data suggest that *B. sorokiniana* is distributed randomly across the country. Although there is just one example of sexual reproduction in nature (Raemekers 1985), it is extremely likely that sexual reproduction occurs in *B. sorokiniana*. When monoconidial isolates of *B. sorokiniana* were coupled in culture, Tinline *et al.* (1950) discovered that perithecia developed. Tinline (1951) later discovered that the fungus had two compatibility groups (a & A) in Canada and that perithecia could only be generated in partnerships that included a member from each group. Jones (1971) discovered the same results and two *B. sorokiniana* compatibility groups (a & A) among Australian isolates. Poloni *et al.* (2009) examined 35 isolates of *B. sorokiniana* from various parts of Brazil and other countries and classified them into five morphological groups: black fluffy growth with white sectors, black fluffy growth, gray cottony growth, white cottony growth and white suppressed growth. They also looked at how they reacted to one other's compatibility and incompatibility. Eighteen of the thirty-one encounters revealed vegetative incompatibility. The findings of the vegetative compatibility/incompatibility genotypes obtained with various growing mediums showed that the kind of substratum effects these reactions. The particular environmental and nutritional needs for perithecia formation in culture have been determined (Tinline and Dickson 1958, Shoemaker 1955, Tinline 1951). The considerable variation in phenotypes across isolates, on the other hand, suggested that sexual reproduction may have happened seldom in nature. If this happens, it might lead to the development of new fungal strains that are more economically valuable to researchers than the present ones. However, the tremendous range of cultural variation suggested that mutations, heterokaryosis, parasexuality and other recombination processes may have significant role.

Table 34. Compatibility between isolates of *Bipolaris sorokiniana* belong to different cultural groups

Sl no.	Isolate code pair	Cultural group pairs	District pairs	Compatible/ Incompatible
1	JSDSvL-01:JSDSvL-18	Black Mat:Black Mat	Joypurhat:Joypurhat	Compatible
2	JSDSvL-01:JSDStL-10	Black Mat:Black Mat	Joypurhat:Joypurhat	Compatible
3	JSVPdL-10:GJBKnL-01	Blackish Ash Mat:Black Fluffy	Joypurhat:Gazipur	Incompatible
4	JSVPdL-05:JSVPdL-08	Ash Mat:Black Fluffy	Joypurhat:Joypurhat	P.compatible
5	JSVPdL-01:JSDSvL-21	Black Mat:Blackish Ash Mat	Joypurhat:Joypurhat	Compatible
6	GJBKhL-01:GJBEnL-02	Ash Mat:Blackish Ash Mat	Gazipur:Gazipur	Compatible
7	JSVPdL-15:JSDSvL-21	Blackish Ash Mat:Blackish Ash Mat	Joypurhat:Joypurhat	P. compatible
8	GJBKnL-02:PSVStL-02	Ash Mat:Blackish Ash Mat	Gazipur:Pabna	P. compatible
9	JSDSvL-28:JSDSvL-25	Ash Mat:Ash Mat	Joypurhat:Joypurhat	P. compatible
10	JSVPdL-04:JSDSvL-27	Whitish Ash Mat:Whitish Ash Mat	Joypurhat:Joypurhat	Compatible
11	JSDSvL-19:JSDSvL-23	Whitish Ash Mat:Whitish Ash Mat	Joypurhat:Joypurhat	Compatible
12	JSDSvL-25:JSDSvL-26	Ash Mat:Whitish Ash Mat	Joypurhat:Joypurhat	Compatible
13	JSDSvL-26:JSVPdL-17	Whitish Ash Mat:Blackish Ash Mat	Joypurhat:Joypurhat	P. compatible
14	JSDStL-01:DiWRCL-10	Pinkish White Mat:Blackish Ash Mat	Joypurhat:Dinajpur	Incompatible
15	JSDSvL-09:JSDStL-06	Pinkish White Mat:Whitish Ash Mat	Joypurhat:Joypurhat	Compatible
16	JSDStL-01:JSDSvL-09	Pinkish White Mat:Pinkish White Mat	Joypurhat:Joypurhat	Incompatible
17	JSDPdL-02:JSDSvL-05	Blackish Ash Mat:Greenish Ash Fluffy	Joypurhat:Joypurhat	Incompatible
18	GJBKhL-01:JSVPdL-07	Ash Mat:Greenish Ash Fluffy	Gazipur:Joypurhat	Incompatible
19	GJBKlL-09:JSVBjL-07	Whitish Ash Mat:Greenish Ash Fluffy	Gazipur:Joypurhat	Incompatible
20	JSDStL-01:GJBKnL-03	Pinkish White Mat:Greenish Ash Fluffy	Joypurhat:Gazipur	Incompatible
21	JSDSvL-09:JSDSvL-28	Pinkish White Mat:Ash Mat	Joypurhat:Joypurhat	P. compatible
22	JSDBjL-03:JSVBjL-07	Brownish Ash Fluffy:Greenish AshFluffy	Joypurhat:Joypurhat	Incompatible
23	GJBKnL-03:DiWRBjL-01	GreenishAsh Fluffy:Brownish Ash Fluffy	Joypurhat:Dinajpur	Incompatible
24	JSVSvL-01:JSDSvL-18	Brownish Ash Fluffy:Black Mat	Joypurhat:Joypurhat	Incompatible
25	JSVPdL-16:JSVPdL-08	Brownish Ash Fluffy:Black Fluffy	Joypurhat:Joypurhat	Incompatible
26	DiWRBjL-01:DiWRCL-10	Brownish Ash Fluffy:Blackish Ash Mat	Dinajpur:Dinajpur	Incompatible
27	JSVPdL-16:JSDSvL-28	Brownish Ash Fluffy:Ash Mat	Joypurhat:Joypurhat	Incompatible
28	GJBKnL-02:GJBSrL-01	Ash Mat:Brownish Ash Fluffy	Gazipur:Gazipur	Incompatible
29	GJBKnL-02:JSDStL-01	Ash Mat:Pinkish White Mat	Gazipur:Joypurhat	Incompatible
30	JSDSvL-09:GJBEnL-01	Pinkish White Mat:Black Mat	Joypurhat:Gazipur	Incompatible
31	JSDSvL-05:JSDSvL-28	Greenish Ash Fluffy:Ash Mat	Joypurhat:Joypurhat	Incompatible

P. compatible=Partially compatible

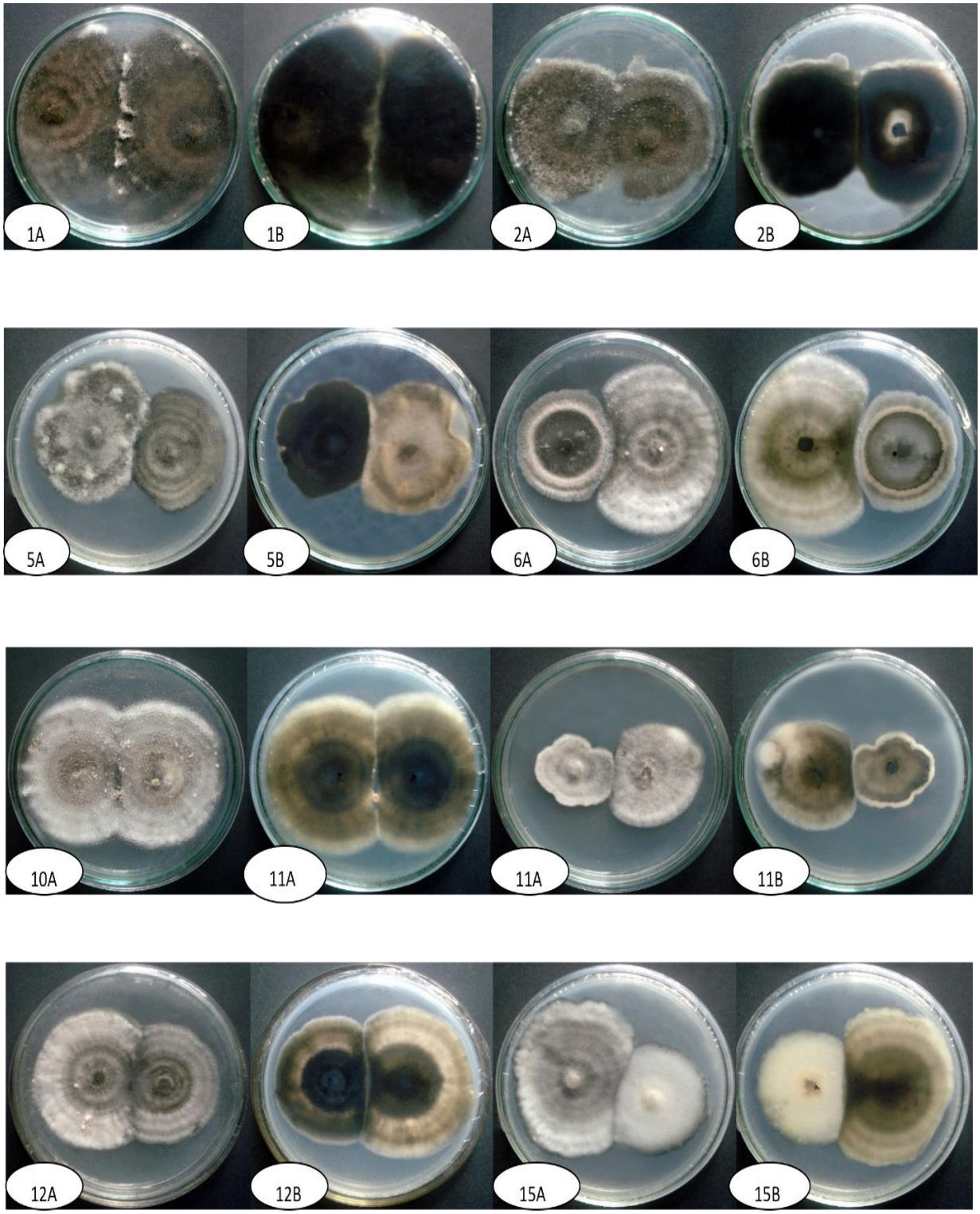


Plate 10. Compatible confrontation of eight pairs of *Bipolaris sorokiniana* isolates 1A-B, 2A-B, 5A-B, 6A-B, 10A-B, 11A-B, 12A-B and 15A-B (A shows upper view and B lower view)

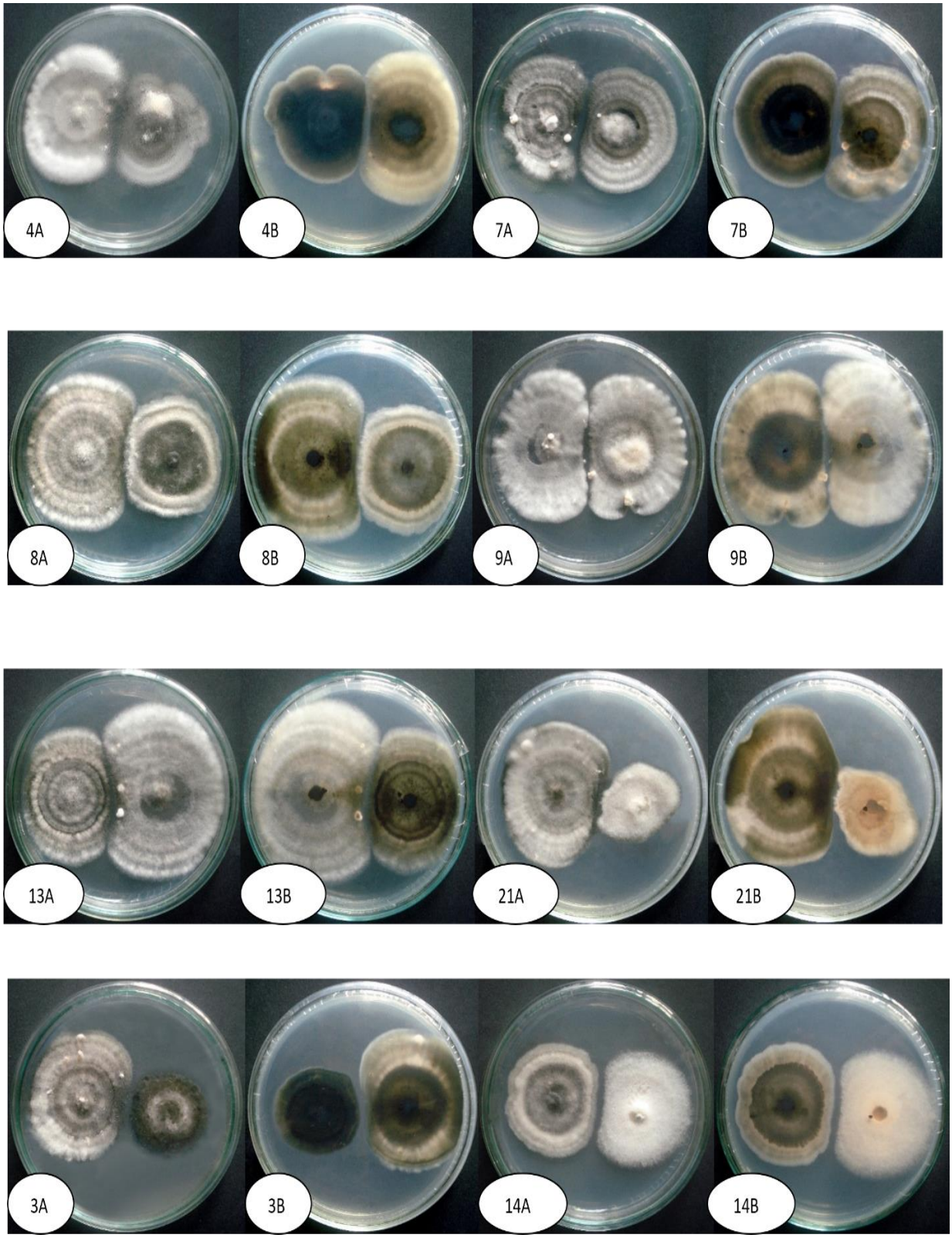


Plate 11. Partially compatible confrontation of six pairs of *Bipolaris sorokiniana* isolates 4A-B, 7A-B, 8A-B, 9A-B, 13A-B and 21A-B (A shows upper view and B lower view)

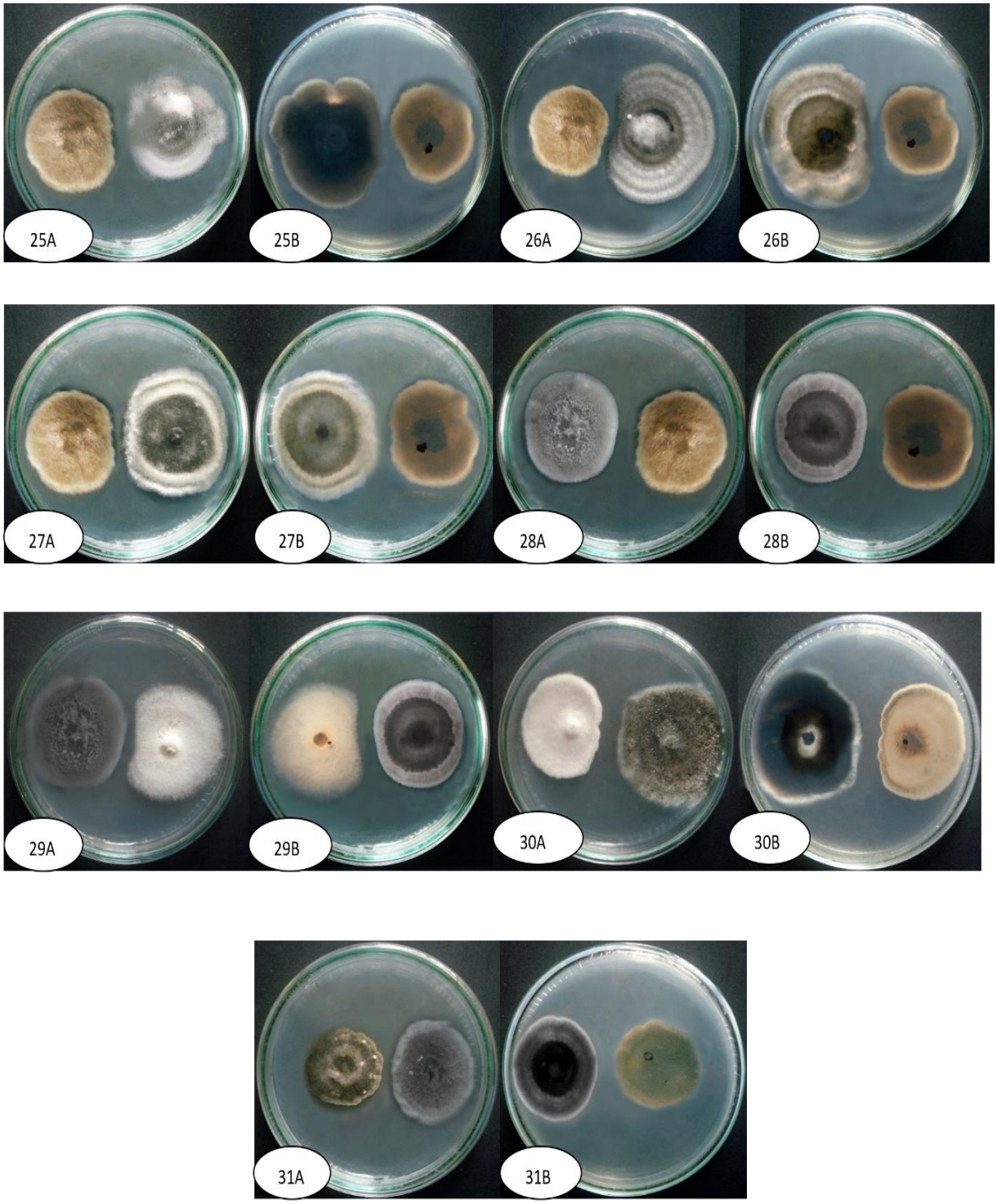


Plate 12. Incompatible confrontation of seven pairs of *Bipolaris sorokiniana* isolates 25A-B, 26A-B, 27A-B, 28A-B, 29A-B, 30A-B and 31A-B (A shows upper view and B lower view)

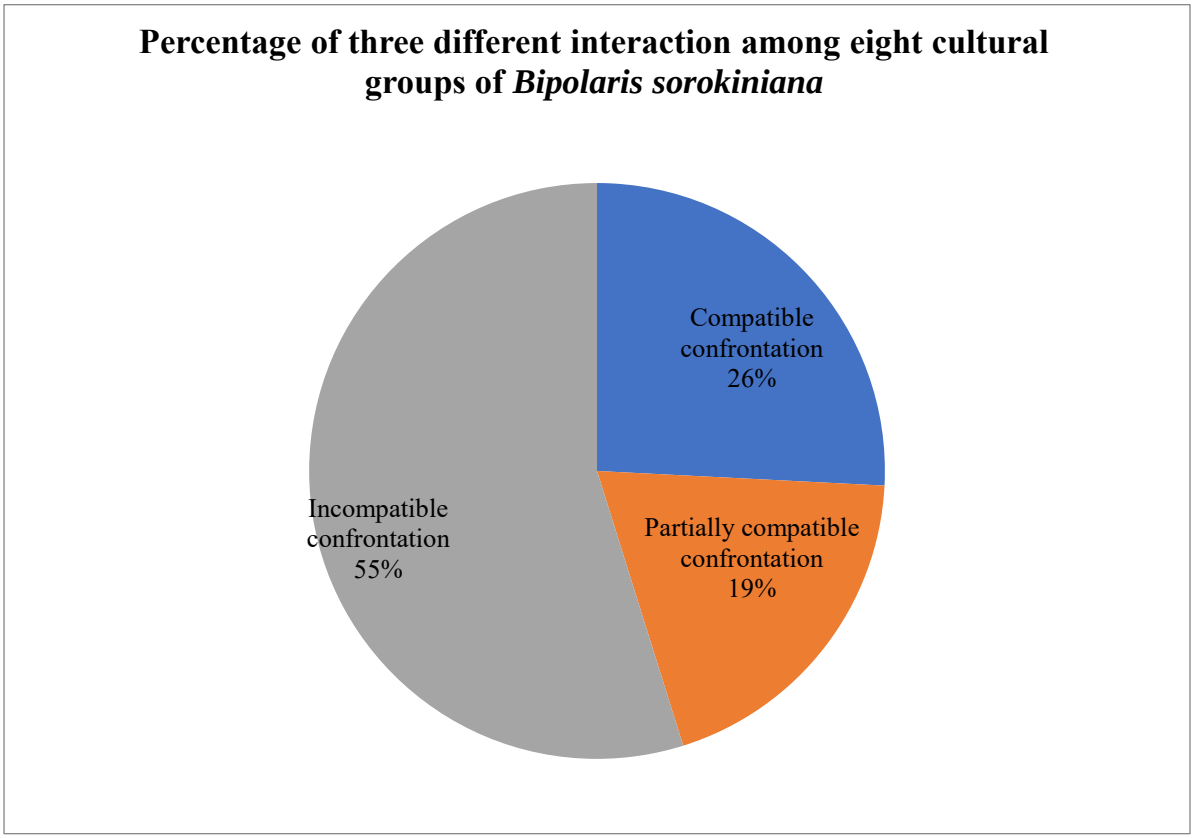


Fig. 9. Pie chart shows three different types of confrontations among *Bipolaris sorokiniana* isolates

4.11 *In vitro* evaluation of fungicides against *Bipolaris sorokiniana* isolates

Evaluation of fungicides under *in vitro* conditions is a handy tool to screen a large number of fungicides. In this study ten fungicides were used, among them eight fungicides (Contaf, Diathane, Folicur, Knowin, Ridomil, Score, Secure and Tilt) were systemic and rest of the two fungicides (Capvit and Silika) were protectant (contact) fungicides. All the fungicides were used in five concentrations (100, 200, 300, 400 and 500 ppm) against sixteen isolates of *B. sorokiniana* belongs to eight different cultural groups [Black Mat (B-M), Black Fluffy (B-F), Ash Mat (A-M), Brownish Ash Fluffy (BrA-F), Blackish Ash Mat (BlA-M), Whitish Ash Mat (WA-M), Greenish Ash Fluffy (GA-F), Pinkish White Mat (PW-M)], two isolates from each group (Table 35, 36, 37, 38, 39, 40, 41 and 42).

In the present study, the laboratory evaluation of fungicides by poison food technique revealed that all the evaluated fungicides inhibited the mycelial growth of *B. sorokiniana.* It was observed that with the increase in the concentration of fungicide, there was a significant decrease in the respective mycelial growth and accordingly more inhibition was observed at higher concentrations than at lower concentrations. A significant interaction between fungicides and concentrations of fungicides was observed. No mycelial growth was found even at the lowest concentration of Tilt (propiconazole) and Folicur (tubeconazole) after 7 days of inoculation in all isolates of *B. sorokiniana*. Tilt and Folicur were the most efficient fungicides against the pathogen (100% mycelial growth suppression at all concentrations). At 300 ppm concentrations, Contaf (hexaconazole) and Score (difenoconazole) totally suppressed *B. sorokiniana* radial mycelial growth. These two fungicides achieved about 80% and 90% inhibition at 100 and 200 ppm concentration individually. This conclusion was backed up by Hasan *et al.* (2012), who discovered that difenoconazole+propiconazole, propiconazole, hexaconazole and completely inhibited *B. sorokiniana* growth. In an *in vitro* study of fifteen fungicides against *B. sorokiniana* of barley, Kavita *et al.* (2017) discovered that propiconazole at 0.1 and 0.05 percent was the most efficient in reducing the pathogen's mycelial growth.

Three combinations of mancozeb were used in the experiment, Diathane (80% mancozeb) and Ridomil (64% mancozeb and 4% metalaxyl) and Secure (50% mancozeb and 10% fenamidon). Among them Secure was best, at 400 ppm concentration Secure completely stopped radial mycelial growth of all isolates of *B. sorokiniana*, where 50–60% inhibition was done at 100 ppm concentration. Performance of Diathane and Ridomil were more or

less same, both achieved about 50–60% inhibition at the lowest concentration and complete growth reduction at the highest concentration. 70.83% inhibition at 400 ppm concentration due to mancozeb against *B. sorokiniana* was reported by Hasan *et al.* (2012), mancozeb at 300 ppm concentration inhibited 34–62% radial growth by Samia *et al.* (2015), similar result was reported by Giri *et al.* (2001), Kavita *et al*. (2017), Angdembe *et al.* (2020) etc. Mancozeb, a dithiocarbamate, disrupts fungal metabolism by blocking either glucose oxidation or nucleic acid synthesis, as well as fatty acids breakdown. At different concentrations of mancozeb inhibition percentage varied against *B. sorokiniana*.

All the fungicide visibly inhibited mycelial growth over the control under *in vitro*. As a protectant fungicide, Capvit (copper oxychloride) showed good performance (89.01–100% inhibition at 500 ppm), only 100% inhibition was achieved at the highest concentration against isolate JSDSvL-28 and GJBKhL-01 under Ash Mat (A-M) group and isolate JSDSvL-26 and JSVPdL-04 under Whitish Ash Mat (WA-M) group, both group showed slow radial mycelial growth habitually. Sharma (2006) reported that copper oxychloride was the most effective and had successfully repressed the growth of the fungus *B. tetramera*. At 300 ppm copper oxychloride concentration, Samia *et al.* (2015) found 70–80 percent inhibition of growth in isolates of *B. sorokiniana* obtained from different regions of Bangladesh. Copper fungicides work by causing nonspecific denaturation of cellular proteins. After absorption, it causes cell damage and membrane leakage by disrupting the activity of proteins and enzymes (Husak 2015).

Among the ten fungicides the least inhibition (20.99–36.34% at 500 ppm concentration) of radial mycelial growth was seen by Knowin (carbendazim). Similar report was presented by Angdembe *et al.* (2019), Giri *et al.* (2001) and Kavita *et al.* (2017). In contradiction to our findings, Samia *et al.* (2015) found that at 300 and 200 ppm of Bavistin (carbendazim), mycelial development was inhibited by 100% and 77 percent, accordingly. As a result, there are several inconsistencies over whether the fungicide carbendazim has an effect on the pathogen or not. However, from my research it should be concluded that Tilt (propiconazole) and Folicur (tubeconazole) screened as best fungicides to control *B. sorokiniana*, wheat leaf blight pathogen.

Table 35. Fungitoxicity of fungicides against *Bipolaris sorokiniana* Black Mat (B-M) isolates at different concentrations

Name of fungicides	Percent inhibition of radial growth at different concentrations (ppm)									
	Isolate name: JSDSvL-01					Isolate name: GJBEnL-01				
	100	200	300	400	500	100	200	300	400	500
Capvit	51.41cd	65.95de	71.41bc	82.51b	89.01b	50.35cd	65.88de	71.45bc	82.69b	89.45b
Contaf	78.33b	92.52b	100a	100a	100a	78.33b	92.51b	100a	100a	100a
Diathane	55.80c	63.73de	71.98bc	82.43b	100a	55.85c	63.73de	72.18bc	82.25b	100a
Folicur	100a	100a	100a	100a	100a	100a	100a	100a	100a	100a
Knowin	10.49e	15.47i	20.38e	24.73ef	32.21d	10.76e	15.59i	20.49e	24.63ef	32.49d
Ridomil	50.38cd	62.34e	73.39bc	84.53ab	100a	50.61cd	61.60e	73.58bc	84.34ab	100a
Score	79.38b	90.16b	100a	100a	100a	79.66b	90.34b	100a	100a	100a
Secure	59.39c	74.84cd	81.49b	100a	100a	59.34c	74.44cd	80.86b	100a	100a
Silica	42.20d	47.08f	55.45cd	61.17d	74.36c	41.46d	47.32f	55.85cd	61.24d	74.19c
Tilt	100a	100a	100a	100a	100a	100a	100a	100a	100a	100a

By LSD, values in the same column with the same letter (s) do not differ substantially at the 5% level.

Table 36. Fungitoxicity of fungicides against *Bipolaris sorokiniana* Black Fluffy (B-F) isolates at different concentrations

Name of fungicides	Percent inhibition of radial growth at different concentrations (ppm)									
	Isolate name: JSVPdL-08					Isolate name: GJBKnL-01				
	100	200	300	400	500	100	200	300	400	500
Capvit	62.03c	73.25cd	81.30b	90.69b	94.10a	61.52c	72.60cd	81.95b	89.77b	95.23a
Contaf	83.48b	95.34ab	100a	100a	100a	82.79b	94.16ab	100a	100a	100a
Diathane	50.36cd	64.27de	70.42bc	80.46c	100a	50.86cd	64.78de	70.59bc	80.80c	100a
Folicur	100a	100a	100a	100a	100a	100a	100a	100a	100a	100a
Knowin	8.58e	12.81i	17.37e	20.65i	24.73c	8.59e	12.79i	17.48e	20.55i	24.82c
Ridomil	61.98c	70.28d	83.82ab	88.55b	100a	61.47c	70.45d	84.63ab	89.21b	100a
Score	79.38b	90.16b	100a	100a	100a	79.56b	90.72b	100a	100a	100a
Secure	62.77c	72.61d	81.75b	100a	100a	62.92c	72.80d	82.35b	100a	100a
Silica	45.32cd	52.63f	60.21c	66.31de	70.67b	45.89cd	52.62f	60.38c	66.22de	70.51b
Tilt	100a	100a	100a	100a	100a	100a	100a	100a	100a	100a

By LSD, values in the same column with the same letter (s) do not differ substantially at the 5% level.

Table 37. Fungitoxicity of fungicides against *Bipolaris sorokiniana* Ash Mat (A-M) isolates at different concentrations

Name of fungicides	Percent inhibition of radial growth at different concentrations (ppm)									
	Isolate name: JSDSvL-28					Isolate name: GJBKhL-01				
	100	200	300	400	500	100	200	300	400	500
Capvit	65.03de	74.35b	81.65b	92.92b	100a	65.81de	74.69b	81.73b	92.66b	100a
Contaf	85.27bc	95.31a	100a	100a	100a	85.74bc	95.34a	100a	100a	100a
Diathane	58.44e	62.44c	75.51bc	85.39bc	100a	58.28e	62.75c	75.51bc	85.66bc	100a
Folicur	100a	100a	100a	100a	100a	100a	100a	100a	100a	100a
Knowin	15.75h	19.61e	25.76e	30.51h	35.60c	15.91h	19.55e	25.69e	30.75h	35.58c
Ridomil	52.57f	61.21c	76.42bc	83.90c	100a	52.67f	61.46c	76.36bc	84.37c	100a
Score	90.65b	95.33a	100a	100a	100a	90.15b	95.60a	100a	100a	100a
Secure	62.40e	75.62b	85.28ab	100a	100a	62.59e	75.13b	85.85ab	100a	100a
Silica	40.68g	42.31d	48.88cd	55.46de	64.40b	40.70g	42.54d	47.88cd	55.64de	64.94b
Tilt	100a	100a	100a	100a	100a	100a	100a	100a	100a	100a

By LSD, values in the same column with the same letter (s) do not differ substantially at the 5% level.

Table 38. Fungitoxicity of fungicides against *Bipolaris sorokiniana* Brownish Ash Fluffy (BrA-F) isolates at different concentrations

Name of fungicides	Percent inhibition of radial growth at different concentrations (ppm)									
	Isolate name: JSDBjL-03					Isolate name: DiWRBjL-01				
	100	200	300	400	500	100	200	300	400	500
Capvit	42.63d	56.64ef	66.98bc	82.71b	91.23b	42.75d	56.78ef	66.59bc	83.15b	90.43b
Contaf	81.64b	91.05b	100a	100a	100a	81.70b	91.32b	100a	100a	100a
Diathane	47.05cd	59.30e	69.85bc	79.54b	100a	47.34cd	59.51e	69.64bc	79.61b	100a
Folicur	100a	100a	100a	100a	100a	100a	100a	100a	100a	100a
Knowin	13.67e	17.51i	22.18e	25.44d	31.60d	13.83e	17.68i	22.49e	25.70d	31.59d
Ridomil	63.99c	73.44d	80.75b	86.33b	100a	64.66c	72.82d	81.41b	85.82b	100a
Score	80.78b	91.55b	100a	100a	100a	81.32b	91.56b	100a	100a	100a
Secure	50.95cd	70.22d	81.15b	100a	100a	51.45cd	70.81d	81.33b	100a	100a
Silica	40.56d	47.26fg	53.65cd	61.46c	65.40c	41.44d	47.28fg	53.17cd	61.33c	66.34c
Tilt	100a	100a	100a	100a	100a	100a	100a	100a	100a	100a

By LSD, values in the same column with the same letter (s) do not differ substantially at the 5% level.

Table 39. Fungitoxicity of fungicides against *Bipolaris sorokiniana* Blackish Ash Mat (BlA-M) isolates at different concentrations

Name of fungicides	Percent inhibition of radial growth at different concentrations (ppm)									
	Isolate name: JSVPdL-10					Isolate name: PSVStL-02				
	100	200	300	400	500	100	200	300	400	500
Capvit	50.88cd	64.17de	73.73bc	85.99ab	93.79ab	51.10cd	63.65de	73.60bc	86.34ab	93.67ab
Contaf	82.64b	91.71b	100a	100a	100a	81.84 b	91.49b	100a	100a	100a
Diathane	51.19cd	55.55ef	67.48bc	78.24b	100a	51.27cd	55.82ef	67.41bc	78.53b	100a
Folicur	100a	100a	100a	100a	100a	100a	100a	100a	100a	100a
Knowin	10.66e	14.15i	17.73e	21.83d	24.71d	10.64e	14.62i	17.98e	21.70d	25.06d
Ridomil	49.43cd	61.11e	71.58bc	81.55b	100a	48.75cd	61.26e	71.43bc	81.11b	100a
Score	84.15ab	90.76b	100a	100a	100a	84.19ab	90.81b	100a	100a	100a
Secure	52.29cd	67.94d	82.49b	100a	100a	51.46cd	67.72d	82.48b	100a	100a
Silica	40.85d	44.06fg	50.39cd	54.24c	61.63c	40.51d	44.59fg	50.66cd	54.62c	61.49c
Tilt	100a	100a	100a	100a	100a	100a	100a	100a	100a	100a

By LSD, values in the same column with the same letter (s) do not differ substantially at the 5% level.

Table 40. Fungitoxicity of fungicides against *Bipolaris sorokiniana* Whitish Ash Mat (WA-M) isolates at different concentrations

Name of fungicides	Percent inhibition of radial growth at different concentrations (ppm)									
	Isolate name: JSDSvL-26					Isolate name: JSVPdL-04				
	100	200	300	400	500	100	200	300	400	500
Capvit	56.86ef	76.94bc	84.25b	90.37b	100a	56.58ef	76.65bc	84.54b	90.80b	100a
Contaf	85.25bc	95.41a	100a	100a	100a	85.30bc	95.49a	100a	100a	100a
Diathane	53.19 ef	61.00c	73.25c	81.45c	100a	53.34ef	61.56c	73.55c	82.79c	100a
Folicur	100 a	100a	100a	100a	100a	100a	100a	100a	100a	100a
Knowin	15.32i	20.94f	24.43h	30.37h	35.70c	15.63i	21.56f	24.60f	30.69g	36.33c
Ridomil	53.73ef	64.06d	75.19bc	82.49c	100a	53.67ef	63.71d	74.56c	82.40c	100a
Score	90.82b	95.52a	100a	100a	100a	90.63b	95.33a	100a	100a	100a
Secure	59.62ef	73.59bc	85.02b	100a	100a	60.42ef	73.67bc	85.74b	100a	100a
Silica	38.84g	41.58e	45.78e	51.45f	62.49b	37.82g	41.43e	46.05e	51.56f	62.81b
Tilt	100a	100a	100a	100a	100a	100a	100a	100a	100a	100a

By LSD, values in the same column with the same letter (s) do not differ substantially at the 5% level.

Table 41. Fungitoxicity of fungicides against *Bipolaris sorokiniana* Greenish Ash Fluffy (GA-F) isolates at different concentrations

Name of fungicides	Percent inhibition of radial growth at different concentrations (ppm)									
	Isolate name: JSDSvL-05					Isolate name: GJBKnL-03				
	100	200	300	400	500	100	200	300	400	500
Capvit	46.55fg	62.57e	75.60bc	86.80b	91.48b	46.35fg	62.76e	75.46bc	86.66b	91.38b
Contaf	78.47b	91.09b	100a	100a	100a	78.49b	91.48b	100a	100a	100a
Diathane	57.33ef	65.28de	78.47b	86.56b	100a	56.79ef	65.19de	78.70b	87.33b	100a
Folicur	100a	100a	100a	100a	100a	100a	100a	100a	100a	100a
Knowin	12.92i	16.78i	21.72e	26.45e	30.90d	13.37i	16.57i	21.88e	26.35e	31.18d
Ridomil	62.30e	69.46d	73.21bc	84.24b	100a	62.65e	69.40d	73.60bc	84.52b	100a
Score	80.86b	92.88b	100a	100a	100a	81.42b	92.44b	100a	100a	100a
Secure	58.47ef	72.21d	82.15b	100a	100a	57.41ef	72.47d	82.76b	100a	100a
Silica	50.76f	56.73ef	62.05c	65.03cd	71.30c	51.55f	56.77ef	62.66c	65.14cd	72.03c
Tilt	100a	100a	100a	100a	100a	100a	100a	100a	100a	100a

By LSD, values in the same column with the same letter (s) do not differ substantially at the 5% level.

Table 42. Fungitoxicity of fungicides against *Bipolaris sorokiniana* Pinkish White Mat (PW-M) isolates at different concentrations

Name of fungicides	At different dosages, the percent inhibition of radial mycelial growth (ppm)									
	Isolate name: JSDSvL-09					Isolate name: JSDStL-01				
	100	200	300	400	500	100	200	300	400	500
Capvit	52.29 cd	71.47d	81.55b	85.56ab	94.33a	52.53cd	71.53d	81.74b	85.40ab	93.50a
Contaf	83.18 ab	94.21ab	100a	100a	100a	82.76b	94.99ab	100a	100a	100a
Diathane	49.00 cd	61.68e	70.50bc	80.42b	100a	48.60cd	62.55e	70.37bc	81.54b	100a
Folicur	100a	100a	100a	100a	100a	100a	100a	100a	100a	100a
Knowin	8.64e	11.86i	14.52e	17.48e	20.99c	8.86e	12.10i	14.29e	17.78e	21.18c
Ridomil	66.22c	72.41d	84.46ab	89.62ab	100a	66.38c	72.48d	84.32ab	90.04ab	100a
Score	84.26ab	90.79b	100a	100a	100a	84.31ab	91.37b	100a	100a	100a
Secure	52.95cd	74.10cd	84.25ab	100a	100a	52.02cd	73.75cd	85.12ab	100a	100a
Silica	45.43cd	51.22f	54.49c	61.21c	64.55b	45.34cd	51.66f	54.87c	60.48c	64.51b
Tilt	100a	100a	100a	100a	100a	100a	100a	100a	100a	100a

By LSD, values in the same column with the same letter (s) do not differ substantially at the 5% level.

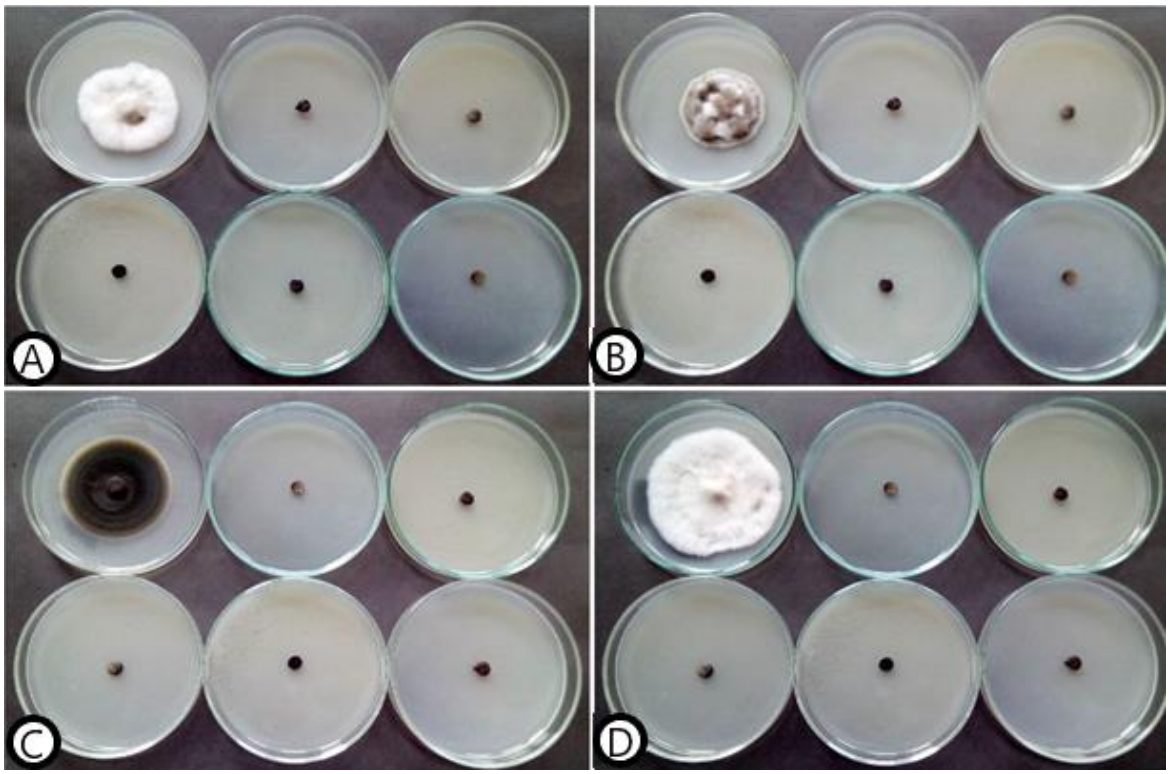


Plate 13. Per cent inhibition of radial growth of *Bipolaris sorokiniana* isolates at 100, 200, 300, 400 and 500 ppm concentrations A) JSDSvL-26 B) JSVPdL-10 C) JSDSvL-01 and D) JSDSvL-09 against Tilt 250 EC

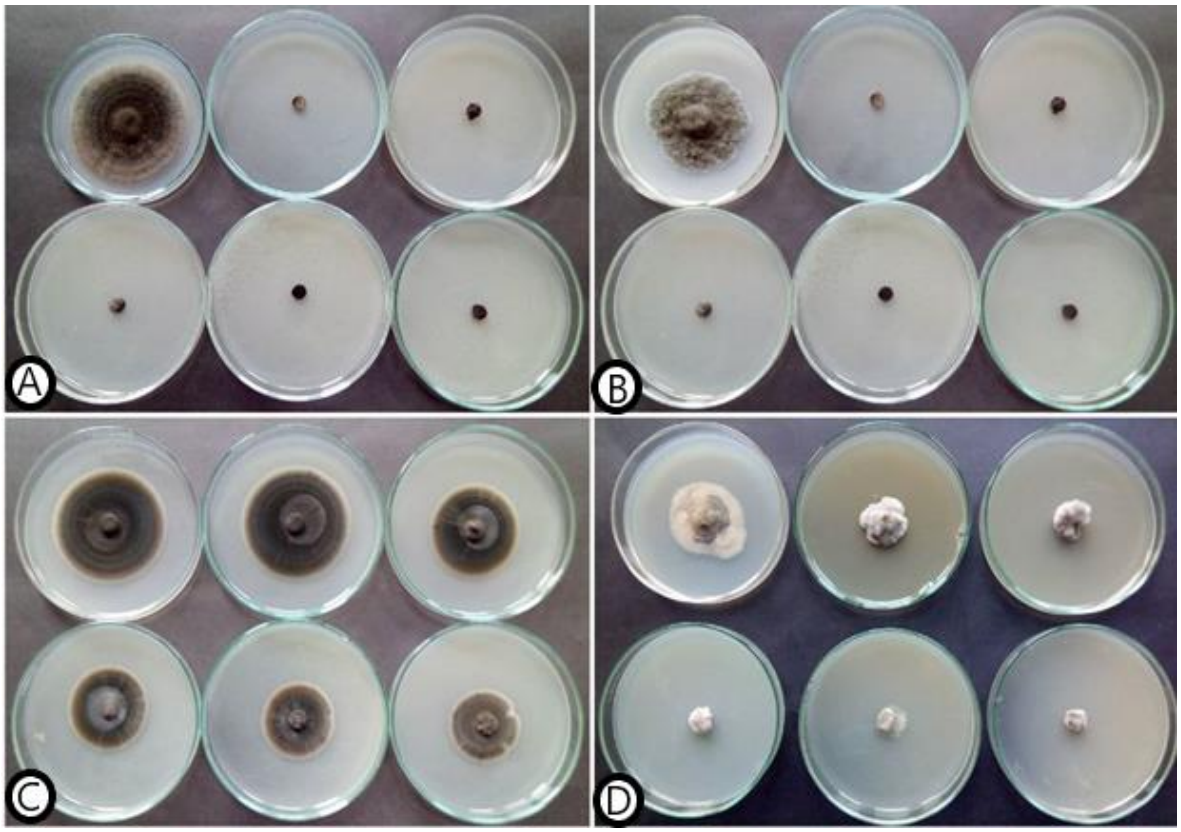


Plate 14. Per cent inhibition of radial growth of *Bipolaris sorokiniana* isolates at 100, 200, 300, 400 and 500 ppm concentrations A) JSDSvL-01 B) GJBKnL-03 against Folicur 250 EC) JSDSvL-01 against Knowin and D) JSVPdL-04 against Diathane

4.12 *In vitro* evaluation of leaf extracts against *B. sorokiniana* isolates

Antifungal properties of ethanol extracts of leaf of ten angiospermic plants belongs to different families (*Allamanda cathartica* L., *Azadirachta indica* A. Juss., *Catharanthus roseus* (L.) G. Don, *Citrus limon* L., *Lantana camara* L., *Lawsonia inermis* L., *Moringa oleifera* Lam., *Polyalthia longifolia* Sonn., *Psidium guajava* L. and *Tagetes erecta* L) were evaluated in four concentrations (5%, 10%, 15% and 20%) against sixteen isolates of *B. sorokiniana* belongs to eight different cultural groups [Black Mat (B-M), Black Fluffy (B-F), Ash Mat (A-M), Brownish Ash Fluffy (BrA-F), Blackish Ash Mat (BA-M), Whitish Ash Mat (WA-M), Greenish Ash Fluffy (GA-F), Pinkish White Mat (PW-M)], two isolates from each group.

Two isolates of *B. sorokiniana* belonging to Black Mat (B-M) group, percent reduction of radial mycelial growth was highest owing to *Azadirachta indica* (75.11–84.75%) followed by *Citrus limon* (71.83–75.14%), *Allamanda cathartica* (69.43–73.21%) and *Catharanthus roseus* (62.35–70.52%) and the lowest inhibition due to *Lawsonia inermis* (39.3–40.95%) followed by *Lantana camara* (44.76–44.99%). *Azadirachta indica* proved best against all isolates of *B. sorokiniana*, percent inhibition range was 74.56 to 84.88% followed by *Citrus limon* (71.73–75.99%), then *Allamanda cathartica* (69.43–75.16%) and *Catharanthus roseus* (61.58–72.49%). There was significant variation in percent inhibition among the ten plants used in the experiment. Variation in percent inhibition due to isolates of *B. sorokiniana* was not analyzed, but range of inhibition percentage owing to a selected plant also remarkable. However, among the ten angiospermic plants minimum inhibition was seen in *Lawsonia inermis* (37.55–42.31%) followed by *Lantana camara* (44.29–45.92%). *In vitro*, all of the leaf extracts evaluated had fungicidal activity and significantly suppressed *B. sorokiniana* colony growth as compared to that of control. Also, basis of the findings, it can be stated that the proportion of mycelial growth inhibition increases as the concentrations of all leaf extracts increase.

Because plant diseases have developed resistance to many fungicides, a novel and alternative fungi-toxic chemical derived from many sources, including leaf extracts, must be researched. The results showed that when compared to the control, several plant extracts suppressed *B. sorokiniana* mycelia growth. The efficiency of leaf extracts in suppressing *B. sorokiniana* mycelial growth increased as the concentration of leaf extracts rose. Several researchers have shown similar antifungal activities of several plant extracts (Hasan *et al.* 2012, Yasmin 2019). Many studies have discovered that garlic bulb extracts

are beneficial against *B. sorokiniana* (Miah *et al.* 1990, Khan and Fakir 1995, Rahman *et al.* 1999, Ahmed *et al.* 2000, Hasan *et al.* 2005, Hoassain *et al.* 2005). But in my experiment, care was taken not to use economically important or agriculturally produced commodities, plants species were selected according to references. *Azadirachta indica* extracts (leaf, root, bark, seed) are effective against protozoa, bacteria and various kinds of fungi, it is well established (Gurjar *et al.* 2012). Haron *et al.* (2013) found that leaf extracts of *Allamanda cathartica* significantly reduced anthracnose disease incidence, severity caused by *Colletotrichum gloeosporioides* in papaya. According to Zahari *et al.* (2018), an antifungal agent derived from *Catharanthus roseus* proved efficient in reducing root rot rubber illnesses caused by *Rigidoporus microporus*, *Ganoderma philippii* and *Phellinus noxius*. Bashir *et al.* (2019) described that methanolic fruit extract (5%) of *Lantana camara* significantly reduced the biomass of *Colletotrichum gloeosporioides* up to 66%. Ammad *et al.* (2018) stated that essential oil of *Citrus limon* significantly inhibited three plant pathogenic fungi of grapevine wood (*Eutypa* sp., *Botryosphaeria dothidea* and *Fomitiporia mediterranea*).

There were similarities between the plant species selection and result with Ganguly (1994). *C. roseus* extract inhibited mycelial development and spore germination of fungi the most, followed by *P. longifolia* and *A. indica* extracts. But in my experiment *A. indica* was best followed by *C. limon*, *A. cathartica* then *C. roseus*. *P. longifolia* showed moderate inhibition. My findings were also backed up by the report of Rahman *et al.* (1999), Hossain *et al.* (2005), Hasan *et al.* (2005), Akhter *et al.* (2006) and Yadav *et al.* (2015).

This discovery will need to be confirmed *in vivo* to see whether there is any phytotoxic influence on the host plant. At last, the result of my investigation is *Azadirachta indica*, *Allamanda cathartica*, *Catharanthus roseus* and *Citrus limon* should be prescribed as botanical fungicides after evaluating them *in vivo* condition.

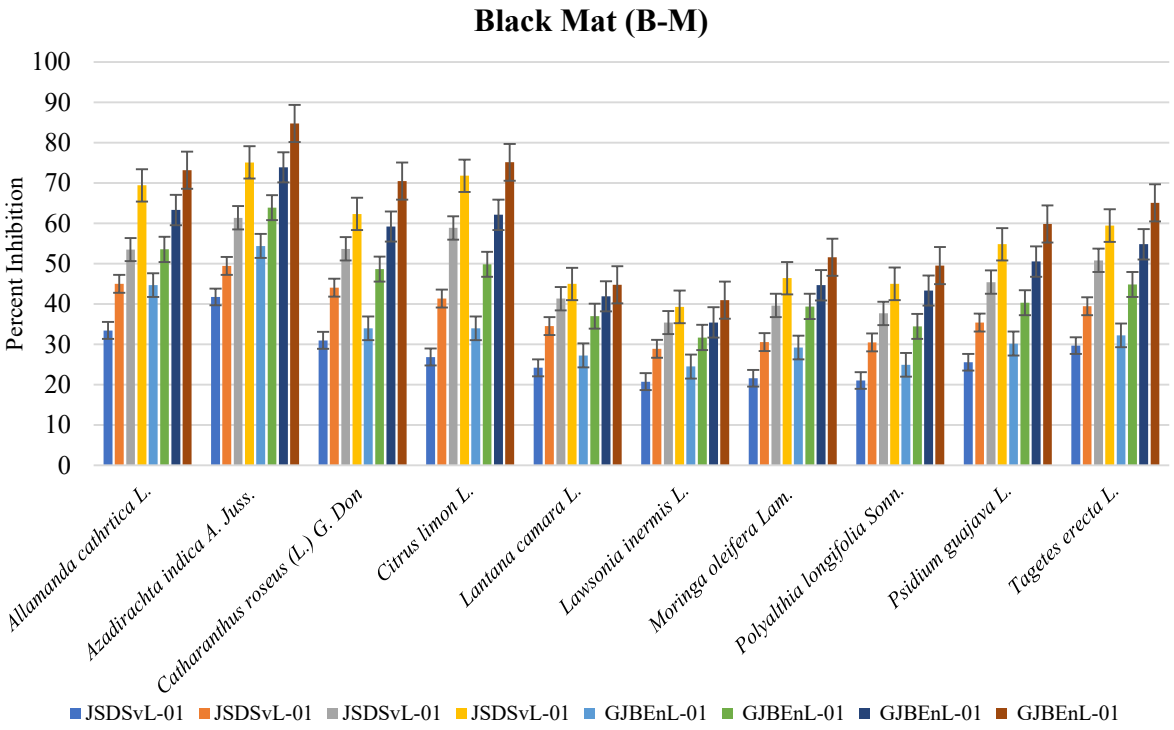


Fig. 10. Percent inhibition owing to botanicals on radial growth of *Bipolaris sorokiniana* isolates of Black Mat (B-M) group

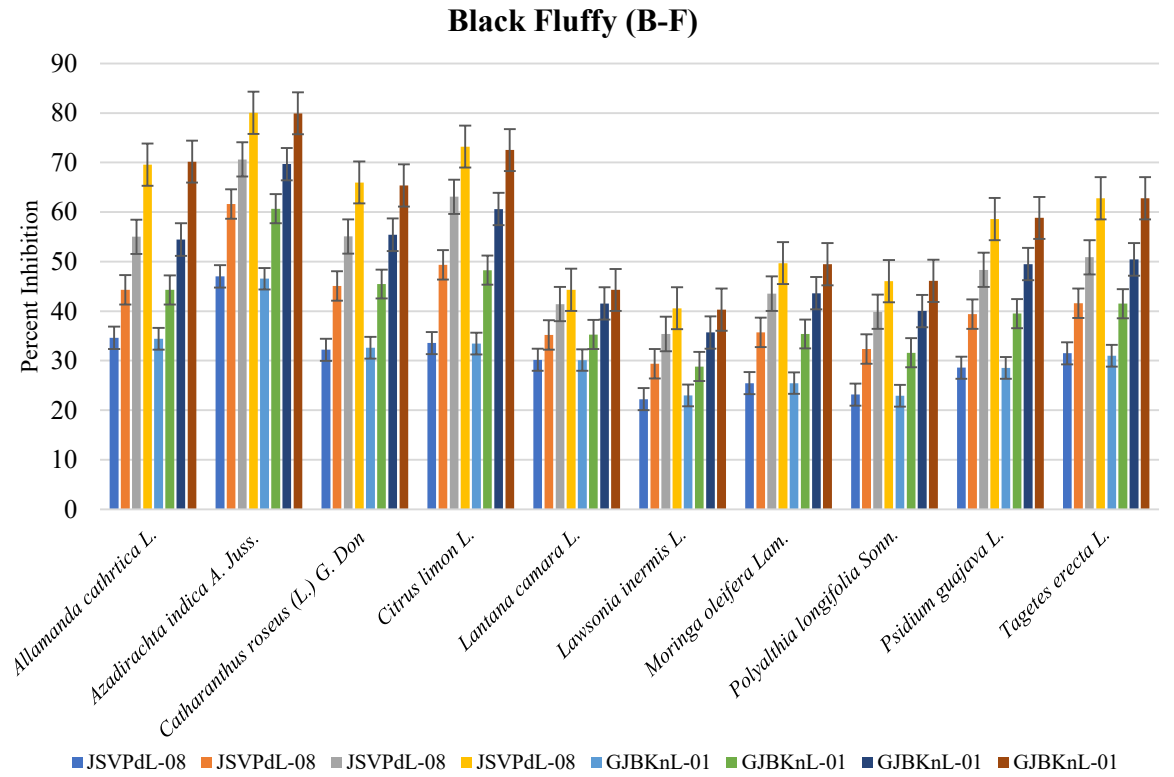


Fig. 11. Percent inhibition owing to botanicals on radial growth of *Bipolaris sorokiniana* isolates of Black Fluffy (B-F) group

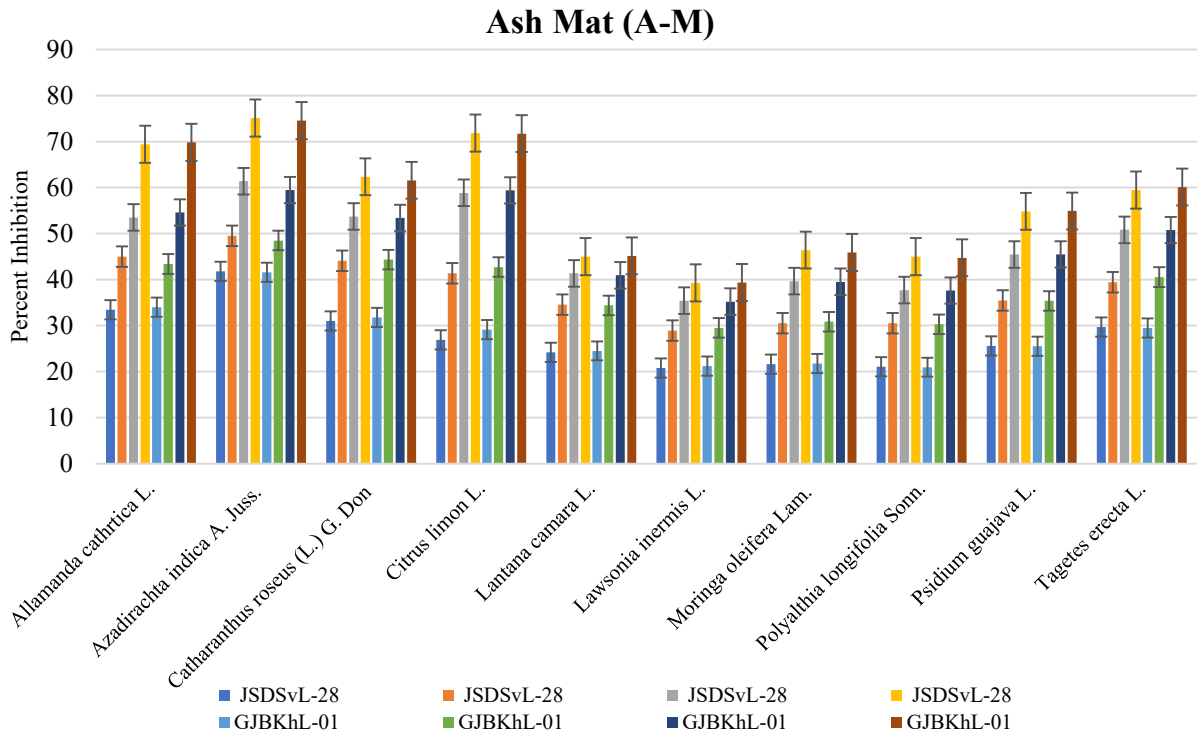


Fig. 12. Percent inhibition owing to botanicals on radial growth of *Bipolaris sorokiniana* isolates of Ash Mat (A-M) group

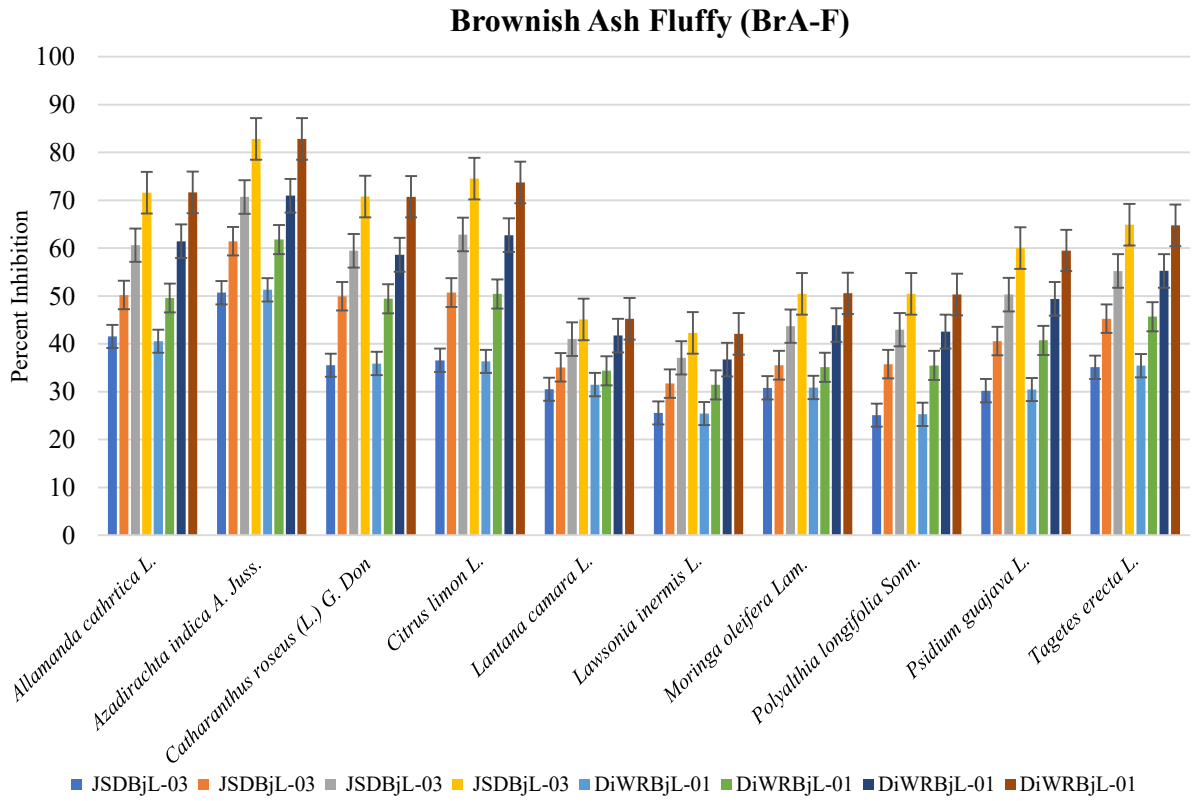


Fig. 13. Percent inhibition owing to botanicals on radial growth of *Bipolaris sorokiniana* isolates of Brownish Ash Fluffy (BrA-F) group

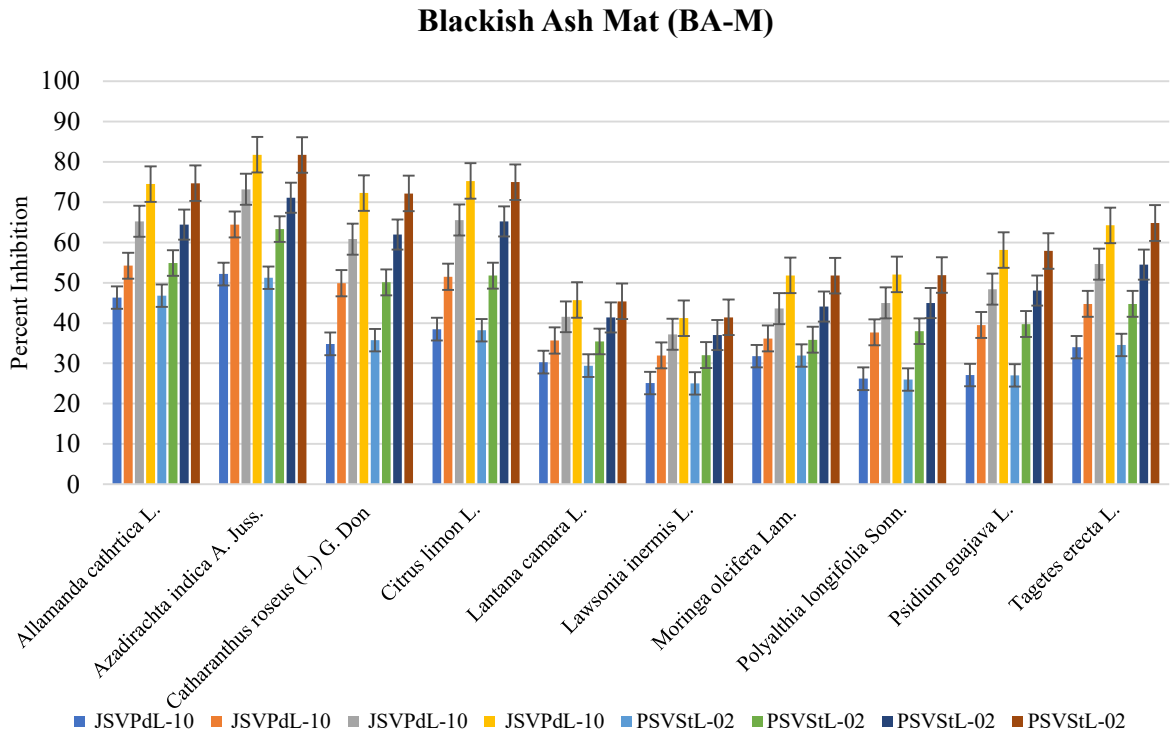


Fig. 14. Percent inhibition owing to botanicals on radial growth of *Bipolaris sorokiniana* isolates of Blackish Ash Mat (BA-M)

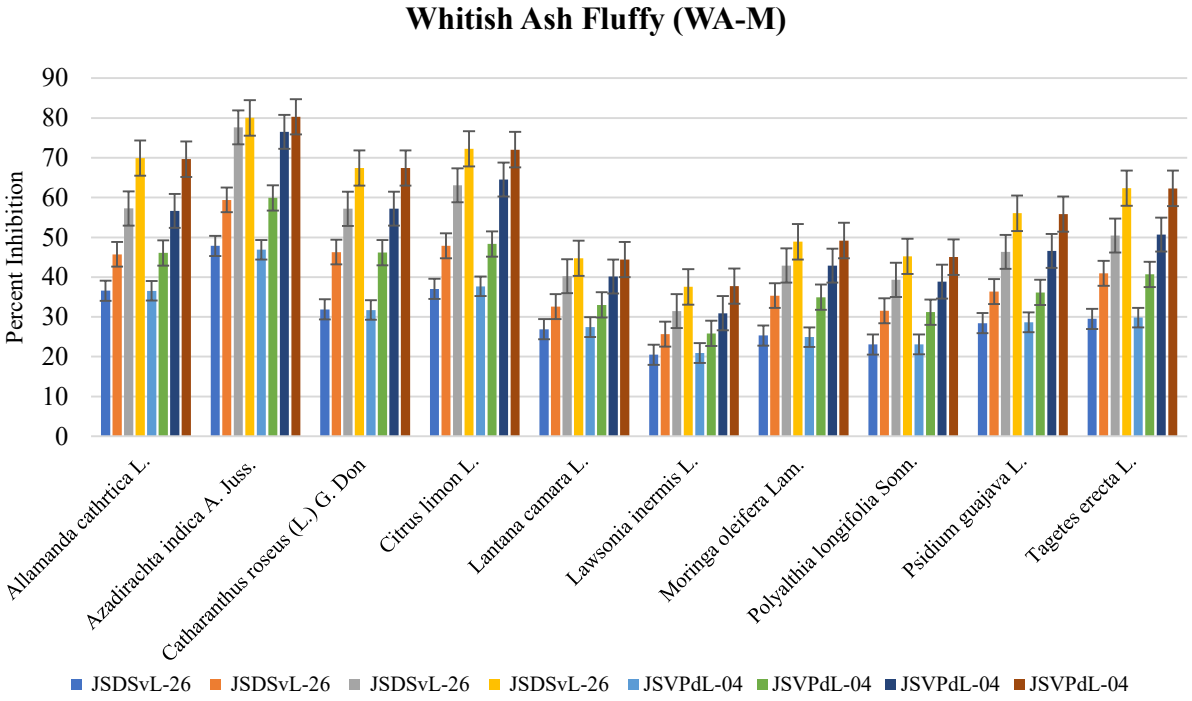


Fig. 15. Percent inhibition owing to botanicals on radial growth of *Bipolaris sorokiniana* isolates of Whitish Ash Mat (WA-M) group

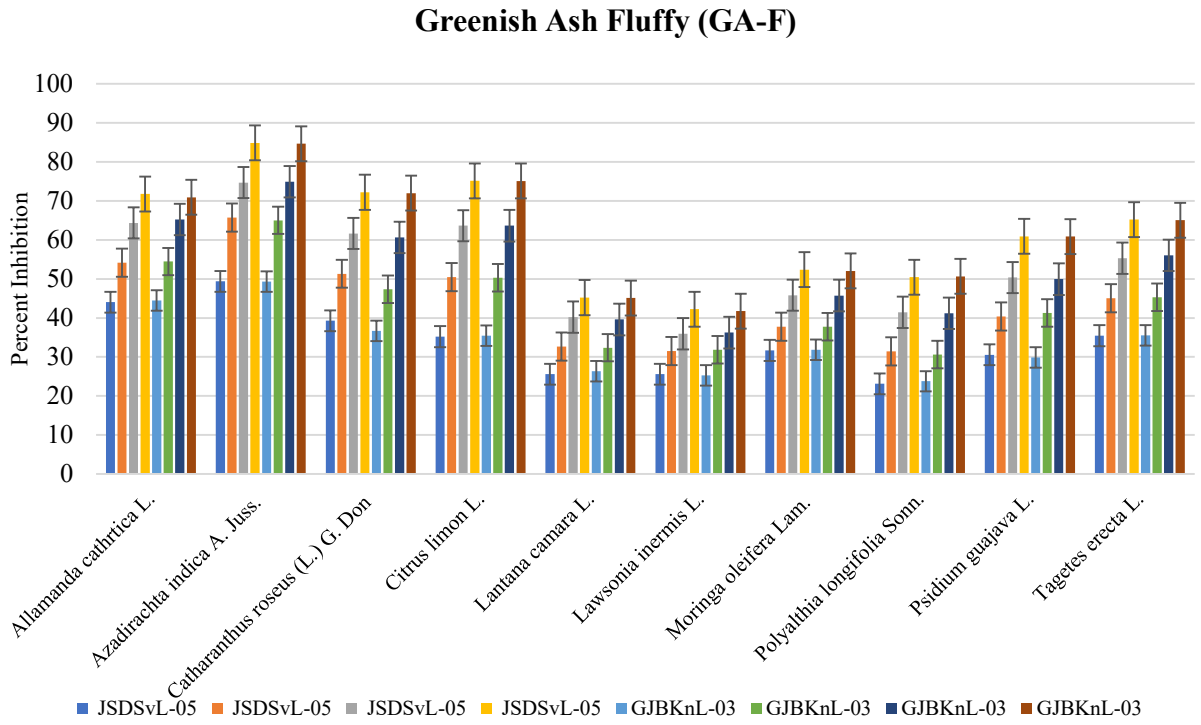


Fig. 16. Percent inhibition owing to botanicals on radial growth of *Bipolaris sorokiniana* isolates of Greenish Ash Fluffy (GA-F) group

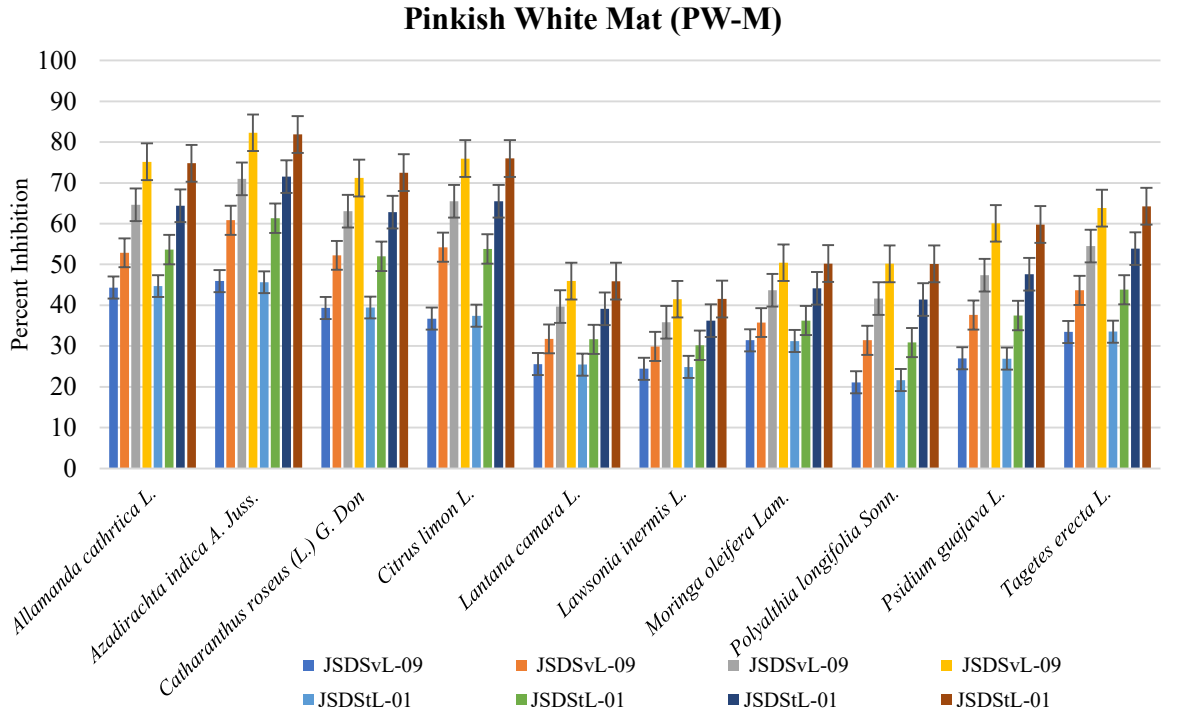


Fig. 17. Percent inhibition owing to botanicals on radial growth of *Bipolaris sorokiniana* isolates of Pinkish White Mat (PW-M) group

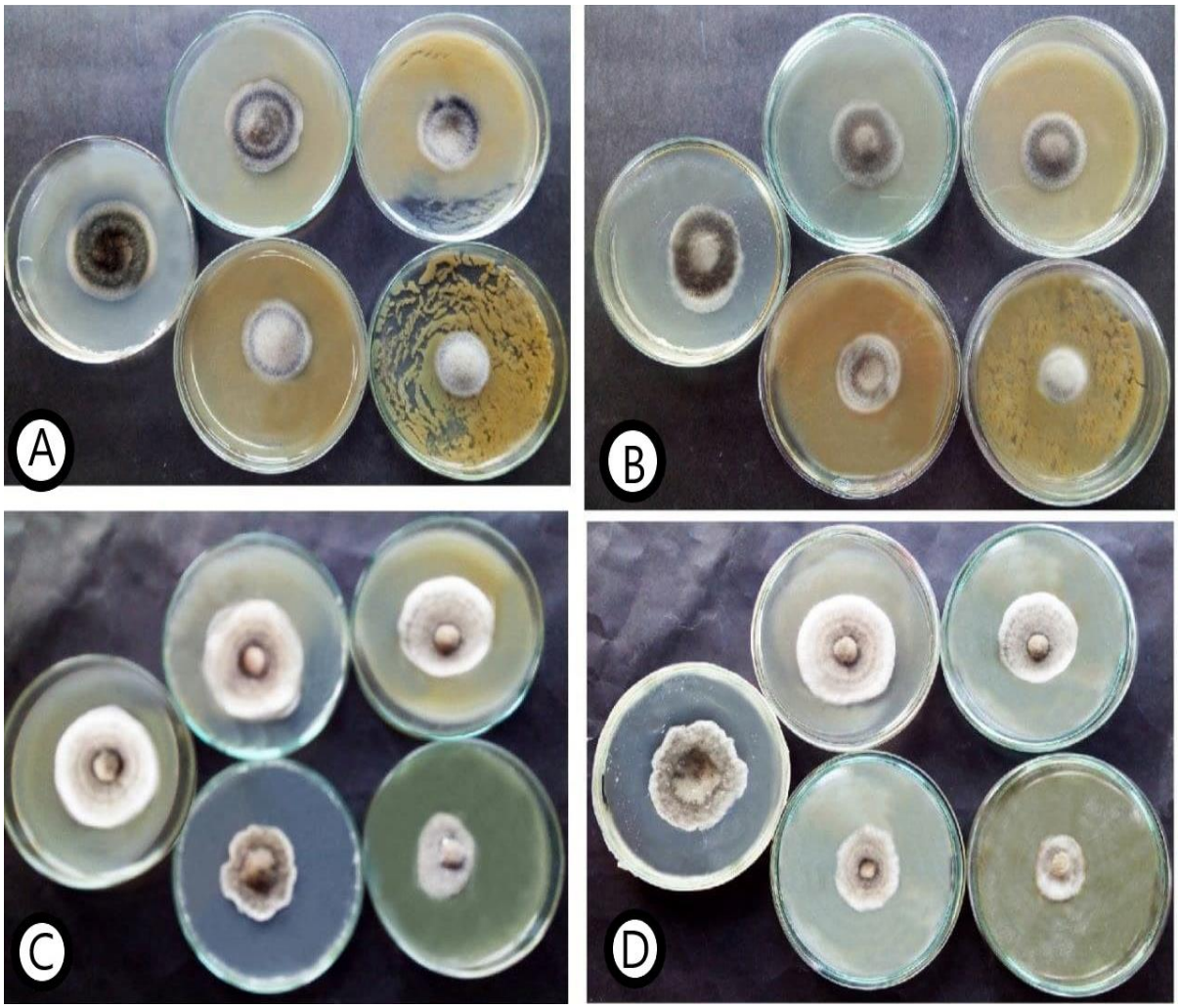


Plate 15. Per cent inhibition of radial growth of *Bipolaris sorokiniana* isolates A) JSVPdL-04 B) JSVPdL-10 at 5, 10, 15 and 20 % concentration of *Azadirakhta indica* and C) JSDSvL-26 D) JSVSvL-05 at 5, 10, 15 and 20 % concentrations of *Allamanda cathartica* and *Citrus limon*

4.13 *In vitro* evaluation of some antagonistic soil fungi against *Bipolaris sorokiniana*

The antagonistic potential of the six soil fungus was evaluated towards sixteen *B. sorokiniana* isolates belongs to eight distinct cultural groups [Black Mat (B-M), Black Fluffy (B-F), Ash Mat (A-M), Brownish Ash Fluffy (BrA-F), Blackish Ash Mat (BA-M), Whitish Ash Mat (WA-M), Greenish Ash Fluffy (GA-F), Pinkish White Mat (PW-M)], two isolates from each group (Table 39, 40).

Although the mechanisms by which antagonistic microorganisms actually impact pathogen populations are often not clear, they are usually attributed to one of three effects: (1) direct parasitism or lysis and death of the pathogen, (2) direct toxic effects on the pathogen by antibiotic substances released by the antagonist, or (3) indirect toxic effects on the pathogen by volatile compounds released by the antagonist's metabolic activities. (Dennis and Webster 1971 a, b, c). The result of the present investigation reveals the above mention mechanisms.

4.13.1 Colony interaction between *Bipolaris sorokiniana* isolates and antagonistic fungi

Table 39 shows the antagonistic potential of the selected six soil fungus against the sixteen isolates of *B. sorokiniana*. Antagonistic relationships in the research ranged from grade 2 to 4. However, grade 2 was found to be the most regularly encountered sort of confrontation, with 59 out of 96 encounters falling into this category, followed by grade 3 (23 out of 96) and grade 4 (rarely) (14 out of 96). Among 6 soil fungi *Aspergillus niger*, *Trichoderma harzianum* and *T. viride* showed grade 4 interaction against some isolates. Prince *et al.* (2011) noticed grade 4 interaction between *T. harzianum* and *Colletotrichum falcatum*. P. Chowdhury (2020) noticed grade 4 interaction between *T. harzianum* and 8 pathogenic fungi of rice.

All antagonists inhibited *B. sorokiniana* growth in colony interaction (37.91–79.02%), having *T. harzianum* the highest inhibition effect against isolate GJBKhL-01, followed by JSVPdL-08 (78.61%) under Black Fluffy (B-F) group. The range of per cent radial growth inhibition of 16 isolates of *B. sorokiniana* against *Aspergillus flavus* was 51.49–56.92%, *A. fumigatus* was 46.08–52.09%, *A. niger* was 55.38–65.63%, *Penicillium* sp. was 37.91–46.73%, *T. harzianum* was 61.69–79.02% and *T. viride* was 62.55–72.32% (Plate 1).

Yassin *et al.* 2022 reported *B. sorokiniana*, black point pathogen in duel culture 62.65% and 59.04% inhibition was done by *T. viride* and *T. harzianum*. Salehpour *et al.* (2005) investigated biological control of wheat common root rot. *T. harzianum* and *T. viride* were used towards two isolates of *B. sorokiniana*. In duel culture, inhibition varied from 29.56 to 69.82%, which is comparable to my investigation. In a dual culture test, *Trichoderma* spp. were shown to limit the growth of *D. sorokiniana* (Prasad *et al.* 1978).

Trichoderma spp. significant antifungal effect may be due to their quick growth rate, high colonization rate, rapid sporulation and synthesis of several secondary metabolites, resulting in suppression of the competitive microorganisms. The hyphae of pathogens substantially collapsed between the contact zones, as seen in the dual culture experiment, suggesting that *Trichoderma* spp. may have a suppressive function in the development of *B. sorokiniana*. The development of a clear interface region of hypha elimination of harmful pathogenic fungus due to the use of secondary bioactive compounds such as gliotoxin generated by *Trichoderma* spp, which was supposed to play a significant role in the advancement of antibiosis (Dennis and Webster 1971c, Khan *et al.* 2020).

Table 43. Antagonistic potential of soil fungi against *Bipolaris sorokiniana* isolates

Sl. No.	Group	Name of fungi	Name of the antagonist											
			Aspergillus flavus		*Aspergillus fumigatus*		*Aspergillus niger*		*Penicillium* sp.		*Trichoderma herzianum*		*Trichoderma viride*	
			Grades & Type	% Inhibition	Grades & Type	% Inhibition	Grades & Type	% Inhibition	Grades & Type	% Inhibition	Grades & Type	% Inhibition	Grades & Type	% Inhibition
1	Black Mat (B-M)	**JSDSvL-01**	2 Bii	52.12 efg	2 Bii	46.43 d	2 Bii	56.08 def	3 Bi	37.91 e	2 Bii	74.76 cd	4 C	67.77 b
		GJBEnL-01	2 Bii	51.97 efg	2 Bii	46.91 d	2 Bii	55.38 f	3 Bi	38.2 e	2 Bii	74.68 cd	4 C	67.6 b
2	Black Fluffy (B-F)	**JSVPdL-08**	2 Bii	54.48 b	2 Bii	47.77 bcd	2 Bii	60.87 b	3 Bi	41.22 cd	2 Bii	78.61 ab	2 Bii	67.73 b
		GJBKhL-01	2 Bii	54.27 bc	2 Bii	47.55 bcd	2 Bii	60.64 b	3 Bi	41.63 bcd	2 Bii	79.02 a	2 Bii	67.2 bc
3	Ash Mat (A-M)	**JSDSvL-28**	3 Bi	53.32 bcde	2 Bii	47.34 bcd	4 C	57.87 cd	3 Bi	41.35 cd	2 Bii	72.98 de	2 Bii	62.55 e
		GJBKnL-01	3 Bi	53.24 bcdef	2 Bii	47.24 cd	4 C	57.67 cde	3 Bi	41.5 bcd	2 Bii	72.98 de	2 Bii	62.98 de
4	Brownish Ash Fluffy (BrA-F)	**JSDBjL-03**	3 Bi	56.87 a	3 Bi	51.74 a	2 Bii	65.48 a	2 Bii	46.68 a	4 C	76.44 bc	2 Bii	71.81 a
		DiWRBjL-01	3 Bi	56.92 a	2 Bii	52.09 a	2 Bii	65.63 a	2 Bii	46.73 a	4 C	76.83 abc	2 Bii	71.54 a
5	Blackish Ash Mat (BlA-M)	**JSVPdL-10**	2 Bii	51.6 fg	3 Bi	46.08 d	3 Bi	55.78 ef	2 Bii	41.03 d	2 Bii	61.78 g	2 Bii	66.07 bc
		PSVStL-02	2 Bii	51.49 g	3 Bi	46.44 d	3 Bi	56.14 def	2 Bii	41.21 cd	2 Bii	61.69 g	2 Bii	65.83 bc
6	Whitish Ash Mat (WA-M)	**JSDSvL-26**	2 Bii	52.45 defg	2 Bii	49.28 b	4 c	56.83 cdef	3 Bi	42.87 bcd	4 C	72.19 e	2 Bii	65.09 cd
		JSVPdL-04	2 Bii	52.73 cdefg	2 Bii	49.3 b	4 C	56.96 cdef	3 Bi	43.31 bc	4 C	72.88 de	2 Bii	67.02 bc
7	Greenish Ash Fluffy (GA-F)	**JSDSvL-05**	2 Bii	51.77 efg	2 Bii	47.21 cd	2 Bii	56.16 def	3 Bi	38.08 e	2 Bii	67.29 f	4 C	63.05 de
		GJBKnL-03	2 Bii	51.7 efg	2 Bii	47.06 cd	2 Bii	57.07 cdef	3 Bi	38.15 e	2 Bii	67.15 f	4 C	63.06 de
8	Pinkish White Mat (PW-M)	**JSDSvL-09**	3 BI	54.04 bcd	2 Bii	48.99 bc	4 C	58.21 c	3 Bi	43.27 bc	2 Bii	62.46 g	2 Bii	71.56 a
		JSDStL-01	3 Bi	54.22 bc	2 Bii	49 bc	4 C	58.24 c	3 Bi	43.59 b	2 Bii	62.33 g	2 Bii	72.32 a

By LSD, values in the same column with the same letter (s) do not differ substantially at the 5% level. Grades from 1 (mutually intermingling growth) to 5 (mutual inhibition at a distance), as proposed by Skidmore and Dickinson (1976) are as follows: Grade 1 = Mutual intermingling without any microscopic sights of interaction. Grade 2 = Mutual intermingling growth where the growth of the fungus is ceased and being overgrowth by the opposed fungus. Grade 3 = Intermingling growth where the fungus under observation is growing into the opposed fungus either above (or) below. Grade 4 = Sight inhibition of both the interacting fungi with narrow demarcation line (1-2 mm). Grade 5 = Mutual inhibition of growth at a distance of > 2 mm.

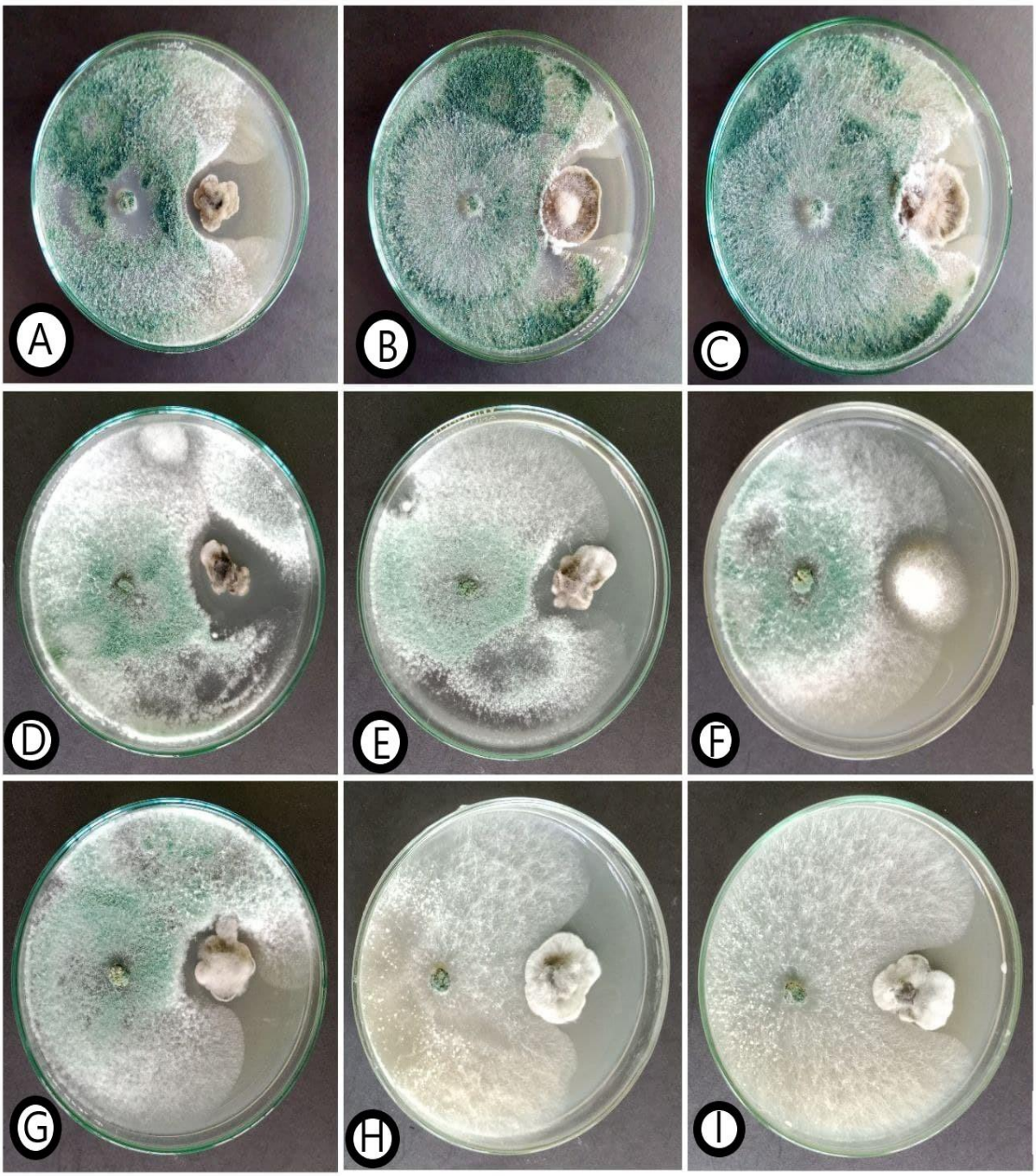


Plate 16. Colony interaction between: A) JSDSvL-28, B) JSDBjL-03, C) GJBKnL-01 D) JSVPdL-10 E) JSDSvL-26 F) JSDSvL-09 G) GJBKnL-03 and *Trichoderma viride*, H) JSVPdL-10 and I) GJBKhL-01 and *Trichoderma harzianum*

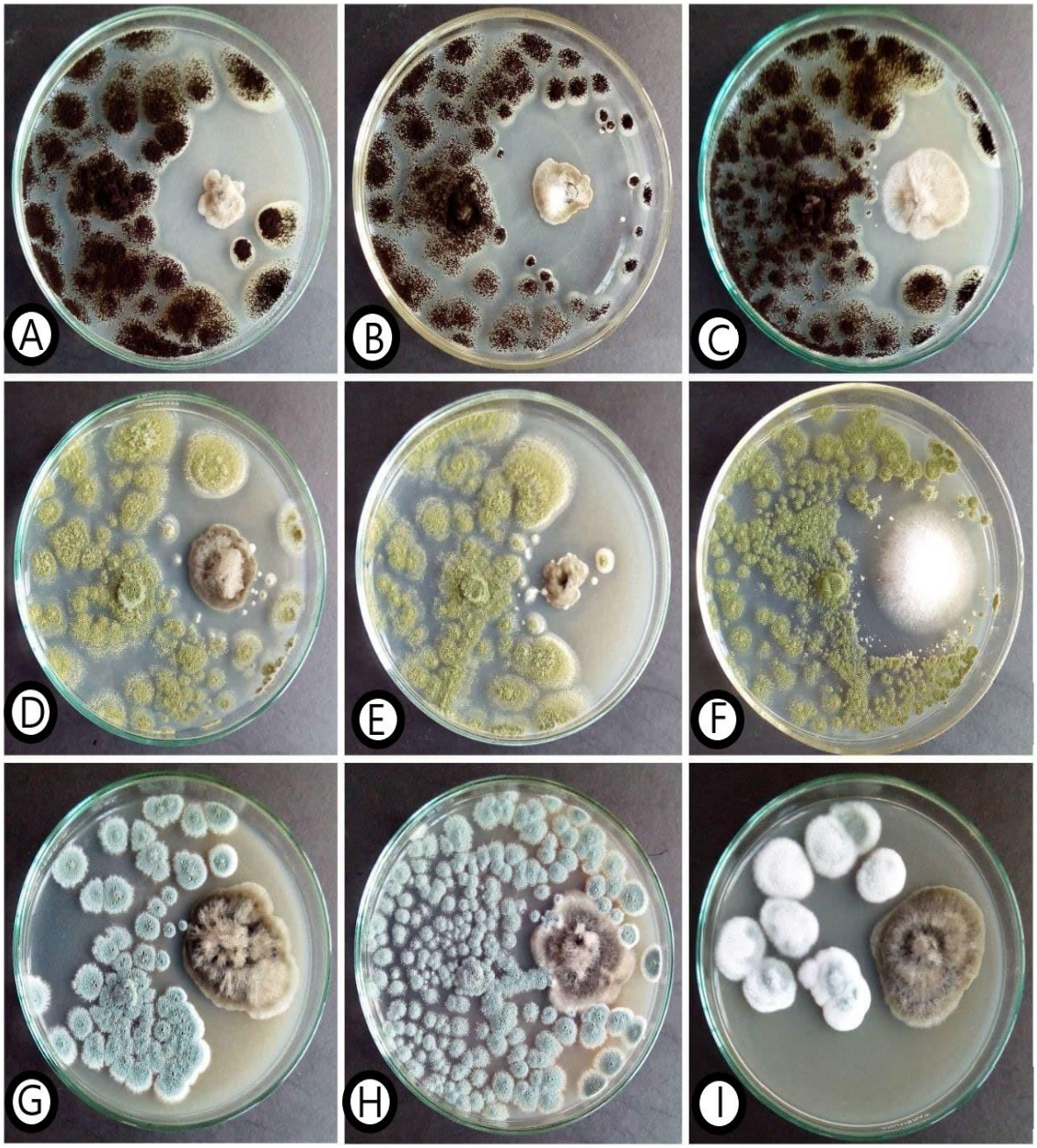


Plate 17. Colony interaction between: A) JSDSvL-28, B) JSDBjL-03, C) GJBKnL-01 and *Aspergillus niger* D) JSVPdL-10 E) JSDSvL-26 F) JSDSvL-09 and *Aspergillus flavus* G) GJBKnL-01, H) JSVPdL-10 and *Aspergillus fumigatus* I) GJBKnL-01 and *Penicillium* sp.

4.13.2 Effect of volatile substances of antagonistic fungi towards *Bipolaris sorokiniana* isolates

Table 40 displays the effects of volatile substances or compounds on six antagonistic soil fungi. The results show that volatile compounds emitted by *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *Penicillium* sp., *Trichoderma harzianum* and *T. viride* inhibited the radial growth of the isolates of *B. sorokiniana* to wide-ranging degrees (7.87–61.34%).

The maximum inhibition (61.34%) was found owing to volatile metabolites of *T. harzianum* to isolate GJBEnL-01 following DiWRBjL-01 (61.12%), JSDBjL-03 (56%), PSVStL-02 (55.54%) and JSVPdL-08 (53.98%) due to *T. viride*. *Aspergillus niger* reduced the radial colony growth of 16 isolates of *B. sorokiniana* was 29.33–43.6%, in case of *A. flavus* inhibition range was 17.36–36%, due to *A. fumigatus* radial growth reduction was 7.87–27.45%, owing to *Penicillium* sp. inhibition range was 9.56–17.62%, the maximum inhibition record for *T. harzianum* was 44.14–61.34% and last of all *T. viride* inhibition range was 37.34–53.98% (Plate 17).

Salehpour *et al.* 2005 achieved greater per cent inhibition due to volatile metabolites of *T. harzianum* towards two isolates of *B. sorokiniana* 66.66 to 98.25%, where present investigation suggested the highest inhibition per cent was only 61.34% against 16 isolates. Suppression of radial mycelial development of different plant pathogenic fungi was achieved by *T. harzianum* owing to volatile metabolites was recorded by Akter *et al.* (2014), Yasmin and Shamsi (2019). The reduction of mycelial growth of plant pathogenic fungi could be accredited by the existence of growth inhibiting compounds in the metabolites of antagonistic fungi (Bilai 1966, Dick and Hutchinson 1966, Marshall and Hutchinson 1970).

Antibiotic synthesis may be partly linked to the biocontrol activity of antagonist fungi and bacteria (Faull *et al.* 1994, Etebarian *et al.*2000). *T. harzianum* acts as an antagonist by the production of isonitrin, homothallin II, melanoxadin (Faull *et al.* 1994, Lee *et al.* 1995 a, b). Different isolates of *T. viride* produced trichodermin, ergokonin, viridin, viridiofungin A, B and C which acts in biological control (Godtfredsen and Vangedal 1965, Grove *et al.* 1965, Harris *et al.* 1993, Kumeda *et al.* 1994). The overall impact might be influenced by the contact of two fungi and their volatile components, which could result in a chemical reaction. However, these compounds could not be extracted from the *T. harzianum* and *T. viride* utilized in my experiment. *T. harzianum* and *T. viride*

generated metabolites that provided biocontrol in this experiment that are likely comparable to metabolites produced by the other isolates evaluated by the other scientists. To identify the metabolites and their functions further research is needed. According to the findings, there were qualitative and quantitative variations in percent inhibition attributable to the amount of volatile compounds generated by distinct soil fungus or changes in the organisms participating in the interaction. Several Trichoderma spp. generated volatile antibiotics that inhibited the development of Rhizoctonia solani, Pythium ultimum, and Fusarium oxysporum, according to Dennis and Webster (1971 b). These authors reported no mortality to any of the test fungus, and no full chemical analysis of the volatile components of fungal cultures was done, however acetaldehyde was indicated as one of the volatiles. Taxol is one of the defensive chemicals identified from endophytes (plant alkaloid, anti-cancer chemotherapy drug), oocudin A (isolated from a strain of *Serratia marcescens*), cryptocin, ambuic acid and jesteron (Li *et al.* 2000, Li and Strobel 2001). Hutchinson (1973) used quantitative measurement of these volatiles to provide direct proof. Fries (1973) described the mechanism of action of volatile chemicals in following manner:

i) By the activation of enzymes

ii) By removal or neutralization of the inhibitors

iii) By influence of nutrient uptake from the medium

iv) By stimulation of a limiting factor in intermediary metabolites.

Dennis and Webster (1971 b) found acetaldehyde, n-propanol, propionaldehyde, isobutanol, n-butyraldehyde, ethyl acetate, isobutyl acetate and acetone in the volatile portion of fungi culture filtrates. *Muscodor albus*, a unique endophytic fungus produced alcohols, esters, ketones including 1-butanol, 3-methyl acetate, styrene, methyl isobutyl ketone, naphthalene and butylatedhydroxytoluene (Strobel *et al.* 2001).

Table 44. Percent inhibition owing to volatile substances against *Bipolaris sorokiniana* isolates

Sl. No.	Group	Name of Isolates	Name of the antagonist					
			Aspergillus flavus	*Aspergillus fumigatus*	*Aspergillus niger*	*Penicillium* sp.	*Trichoderma herzianum*	*Trichoderma viride*
			% Inhibition	% Inhibition	% Inhibition	% Inhibition	% Inhibition	% Inhibition
1	Black Mat (B-M)	JSDSvL-01	20.17 de	19.32 def	43.18 a	17.39 a	46.34 efg	45.6 b
		GJBEnL-01	35.63 a	8.04 h	30.29 d	16.54 a	61.34 a	41.85 bcd
2	Black Fluffy (B-F)	JSVPdL-08	29.08 b	23.97 bc	40.77 ab	17.39 a	51.16 bcde	53.98 a
		GJBKhL-01	22.27 cd	26.54 ab	39.26 abc	16.22 a	53.16 bcd	41.81 bcd
3	Ash Mat (A-M)	JSDSvL-28	26.61 bc	27.45 a	38.72 abc	16.97 a	50.86 cde	44.88 bc
		GJBKnL-01	19.99 de	19.24 def	43.6 a	17.28 a	46.41 efg	45.66 b
4	Brownish Ash Fluffy (BrA-F)	JSDBjL-03	17.36 e	12.3 g	43.5 a	9.65 b	56 b	46.28 b
		DIWRBjL-01	36 a	7.87 h	29.33 d	17.02 a	61.12 a	41.21 bcd
5	Blackish Ash Mat (BlA-M)	JSVPdL-10	26.94 bc	27.34 a	38.8 abc	17 a	51.13 bcde	44.41 bcd
		PSVStL-02	17.57 e	12.68 g	43.56 a	9.29 b	55.54 bc	46.12 b
6	Whitish Ash Fluffy (WA-F)	JSDSvL-26	28.59 b	22.08 cd	34.13 bcd	13.75 ab	44.14 g	37.34 d
		JSVPdL-04	22.54 cd	26.97 ab	40.49 ab	17.62 a	53.66 bcd	41.58 bcd
7	Greenish ash Fluffy (GA-F)	JSDSvL-05	29.11 b	21.77 cde	32.48 cd	13.99 ab	45.52 fg	38.3 cd
		GJBKnL-03	28.77 b	24 bc	40.38 ab	16.71 a	51.78 bcd	53.81 a
8	Pinkish White Mat (PW-M)	JSDSvL-09	22.83 cd	18.68 f	39.02 abc	15.94 a	50.58 cde	43.9 bcd
		JSDStL-01	22.67 cd	18.88 ef	39.06 abc	16.27 a	50.34 def	44.05 bcd

By LSD, values in the same column with the same letter (s) do not differ substantially at the 5% level.

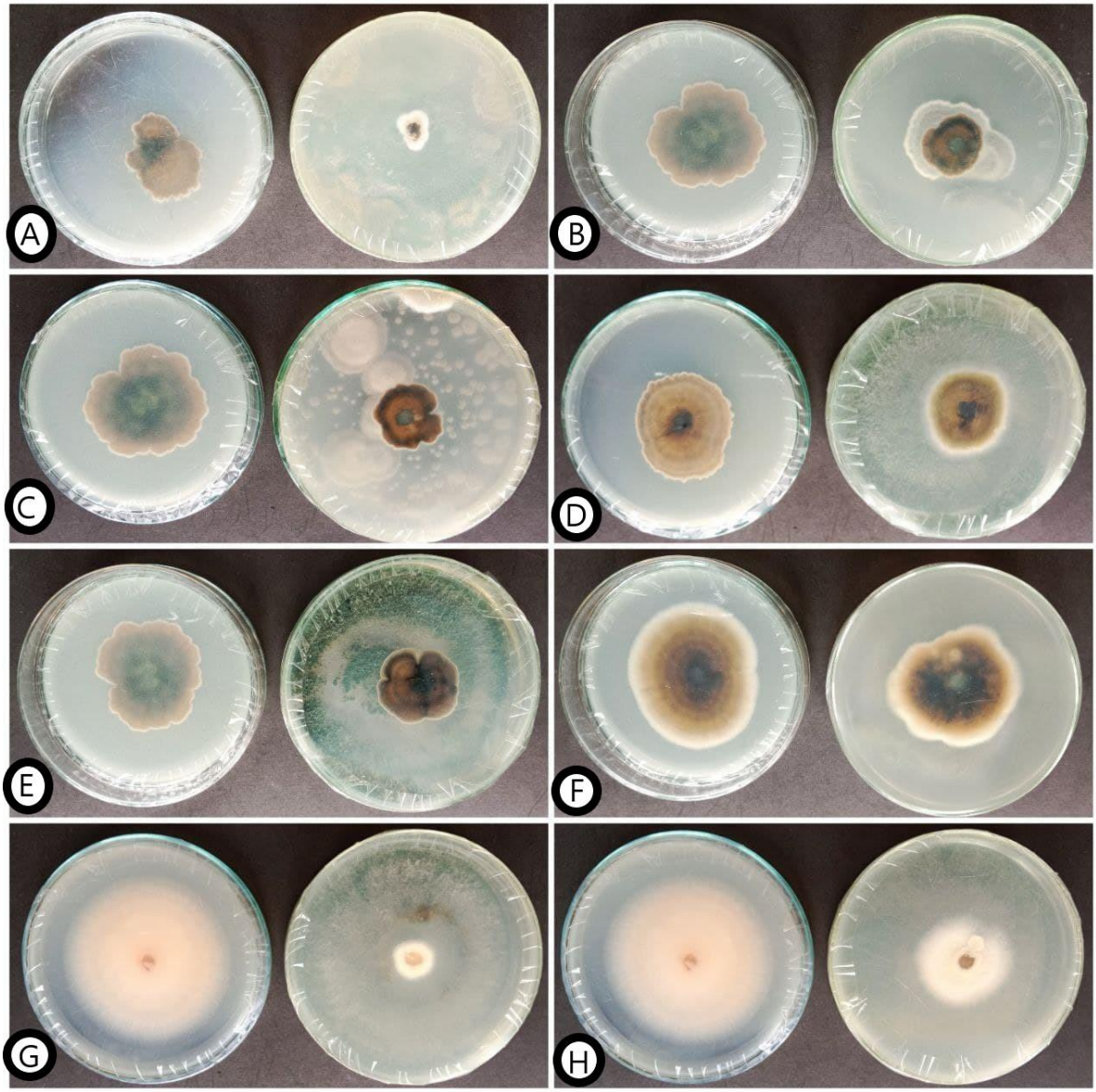


Plate 18. Percent inhibition owing to volatile substances between A) JSDSvL-28 C) and G GJBKnL-01 with *Trichoderma harzianum* B) GJBKnL-01 and D) JSDBjL-03 with *Aspergillus niger* and E) GJBKnL-01 F) GJBKnL-03 and H) GJBKnL-01 with *Trichoderma viride*

4.13.3 Effect of non-volatile substances of antagonistic fungi against *Bipolaris sorokiniana* isolates

The effects of non-volatile compounds or metabolites of six antagonistic soil fungi towards 16 isolates of *B. sorokiniana* are demonstrated in Fig. 18-23. Almost all antagonistic fungi repressed radial growth of *B. sorokiniana* isolates from 13.99–68.14%, in concentration 20%. The best inhibition was achieved due to culture filtrate at 20% concentration by *T. harzianum* towards isolate JSDSvL-09, followed by JSDSvL-01 (67.83%), JSDStL-01 (67.5%). The lowest per cent inhibition owing to *T. harzianum* was shown against isolate JSVPdL-04 (47.28%) at 20% concentration. The inhibition ranges owing to non-volatile metabolites at 20% concentration of *A. flavus* was 19.93 to 43.41%, *A. fumigatus* was 10.23 to 35.42%, *A. niger* was 31.99 to 56.95%, *Penicillium* sp. was 13.99 to 35.4% and second highest *T. viride* was 30.32 to 59.57%.

From my investigation, inhibition proportion owing to non-volatile metabolites was higher than volatile substances. This might be due to antibiotic or hazardous chemical synthesis in the culture filtrates, nutrient depletion, or a change in the pH of the culture medium as a result of staling growth products (Brain 1945, Gottlieb and Shaw 1970, Dennis and Webster 1971 a, Skidmore and Dickinson 1976) attribute the inhibition of radial growth of plant pathogen. Antibiotic or inhibitory substances production varies in properties, qualities and quantities depending on comparing organisms.

Despite the fact that no antibiotics or mycolytic enzymes were recovered, it is likely that the antagonists and their culture filtrates contain some kind of antibiotics or enzymes that cause suppression of mycelial growth of *B. sorokiniana*. *Trichoderma* spp. developed trichodermin which completely prevented *Helminthosporium* and *Fusarium* rots in wheat (Krivoshchekova and Mischenk 1990). *Trichoderma* spp. produced a wide spectrum of secondary metabolites (volatile, non-volatile, diffusible) that were important for insect protection, nutritional support, mineral solubilization and pharmacological activity in plants. To attack significant agricultural pests, *Trichoderma* used mycoparasitism, antibiosis and competition techniques. The utilization of integrated control techniques is critical for the biocontrol of plant pathogenic fungus in the future. The present investigation established the powerful efficacy of *T. harzianum* and *T. viride* against *B. sorokiniana* causing leaf blight disease of wheat. The proved antagonistic efficacies of *T. viride* and *T. harzianum* against *B. sorokiniana* were coincident with the report by Yassin *et al.* 2022.

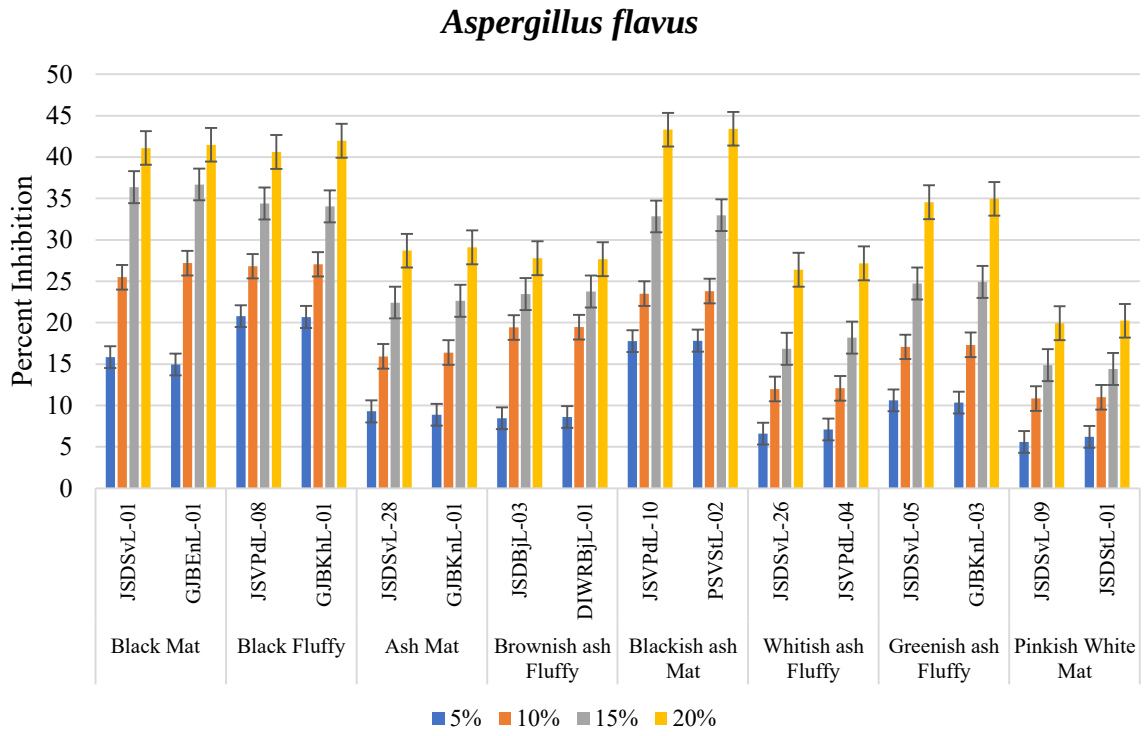


Fig. 18. Percent inhibition owing to nonvolatile substances of *Aspergillus flavus* on radial growth of *Bipolaris sorokiniana* isolates

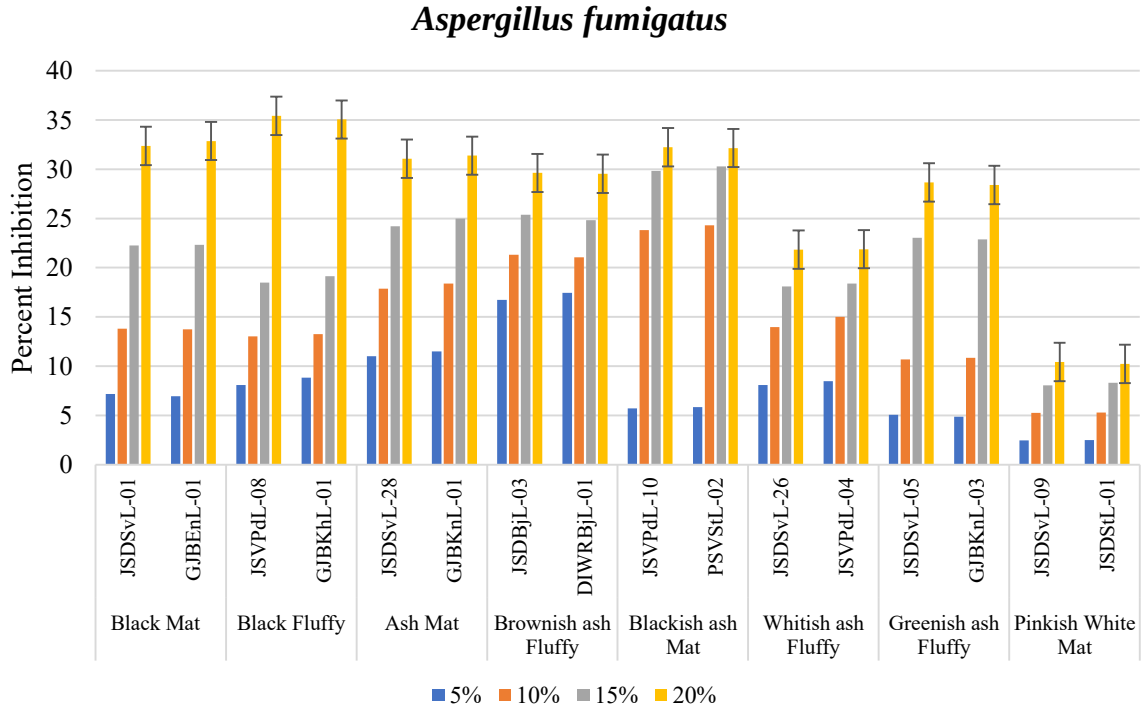


Fig. 19. Percent inhibition owing to nonvolatile substances of *Aspergillus fumigatus* on radial growth of *Bipolaris sorokiniana* isolates

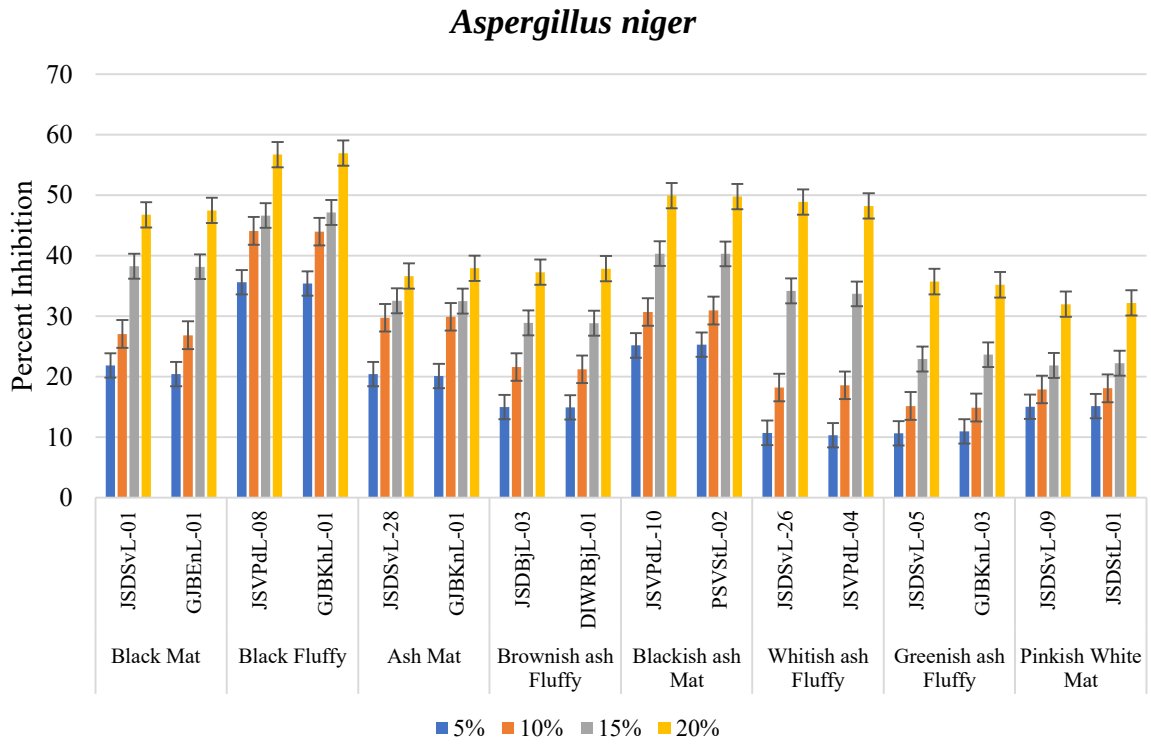


Fig. 20. Percent inhibition owing to nonvolatile substances of *Aspergillus niger* on radial growth of *Bipolaris sorokiniana* isolates

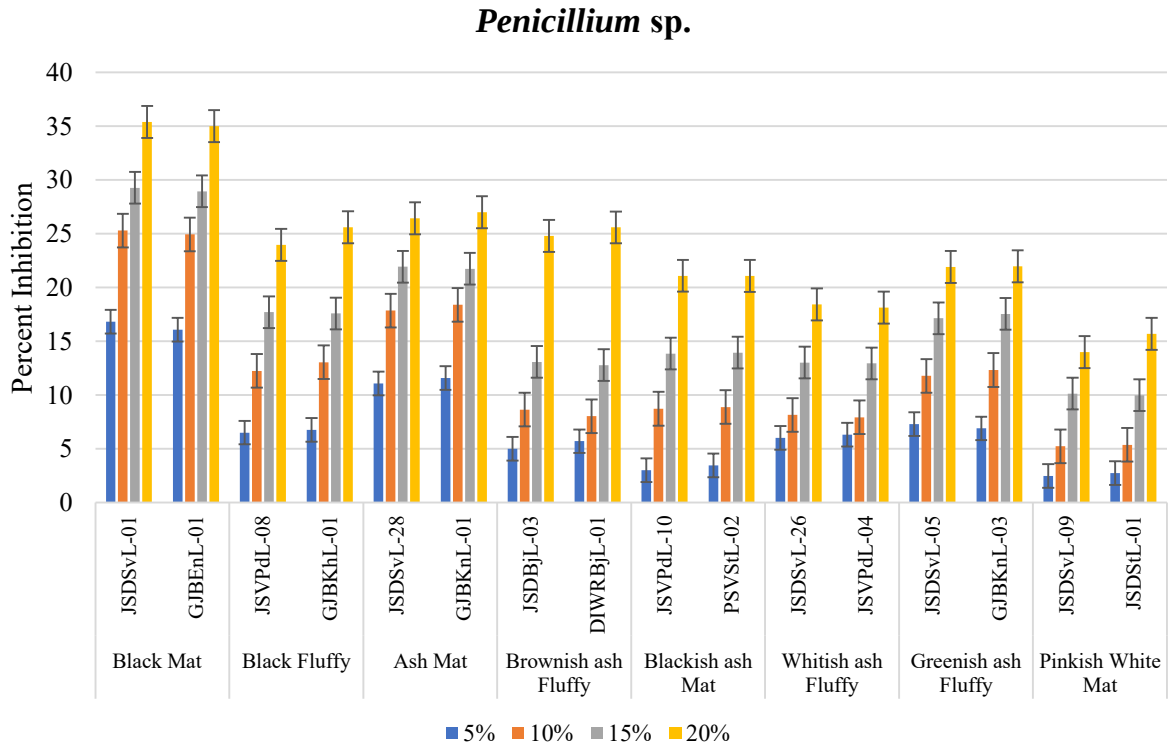


Fig. 21. Percent inhibition owing to nonvolatile substances of *Penicillium* sp. on radial growth of *Bipolaris sorokiniana* isolates

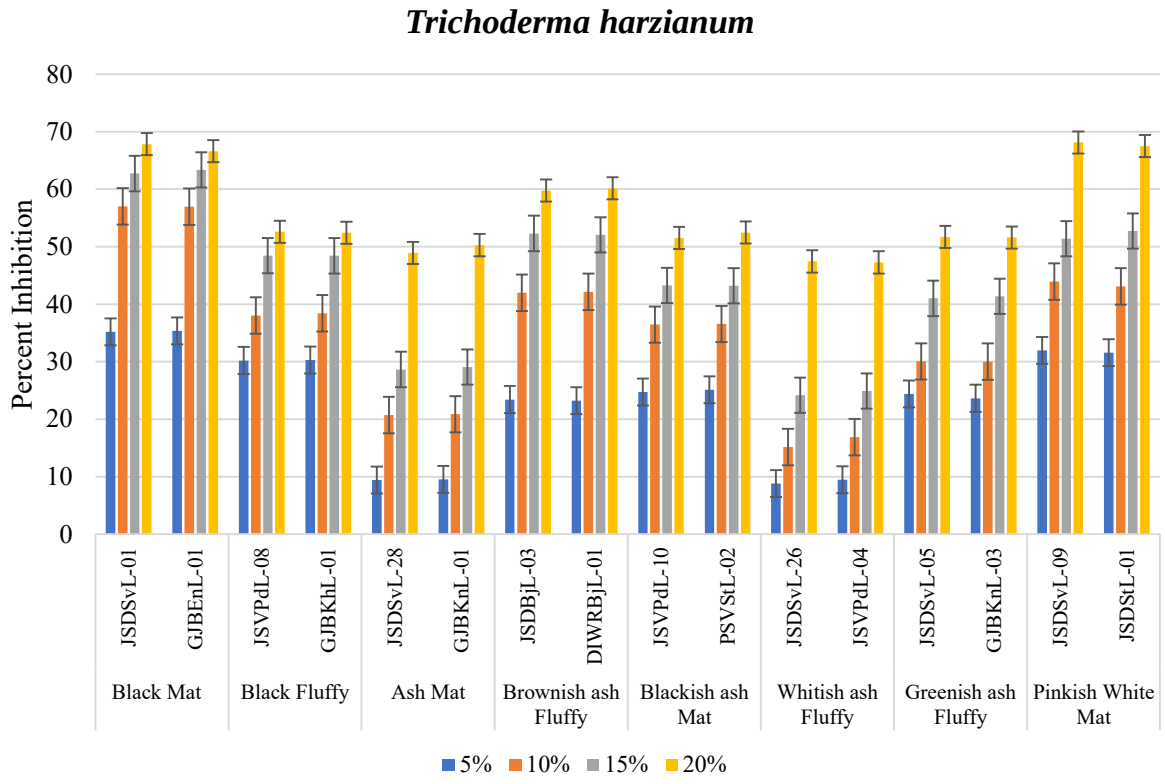


Fig. 22. Percent inhibition owing to non volatile substances of *Trichoderma harzianum* on radial growth of *Bipolaris sorokiniana* isolates

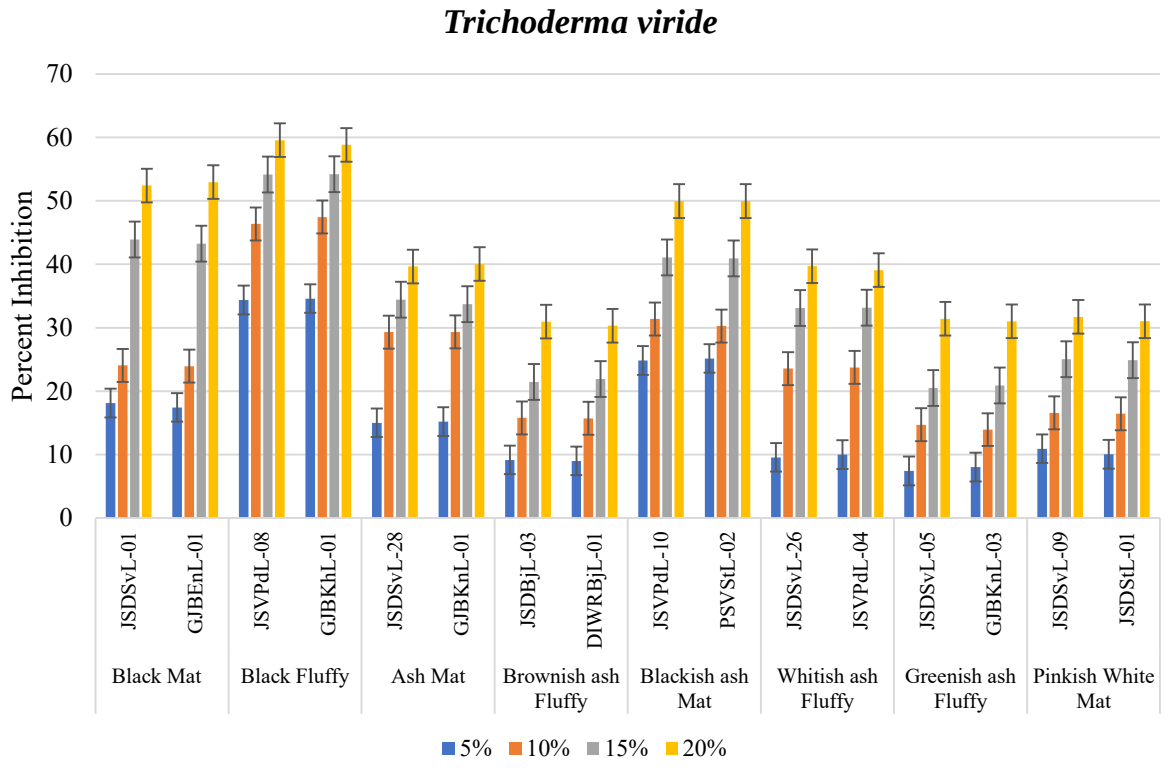


Fig. 23. Percent inhibition owing to non-volatile substances of *Trichoderma viride* on radial growth of *Bipolaris sorokiniana* isolate

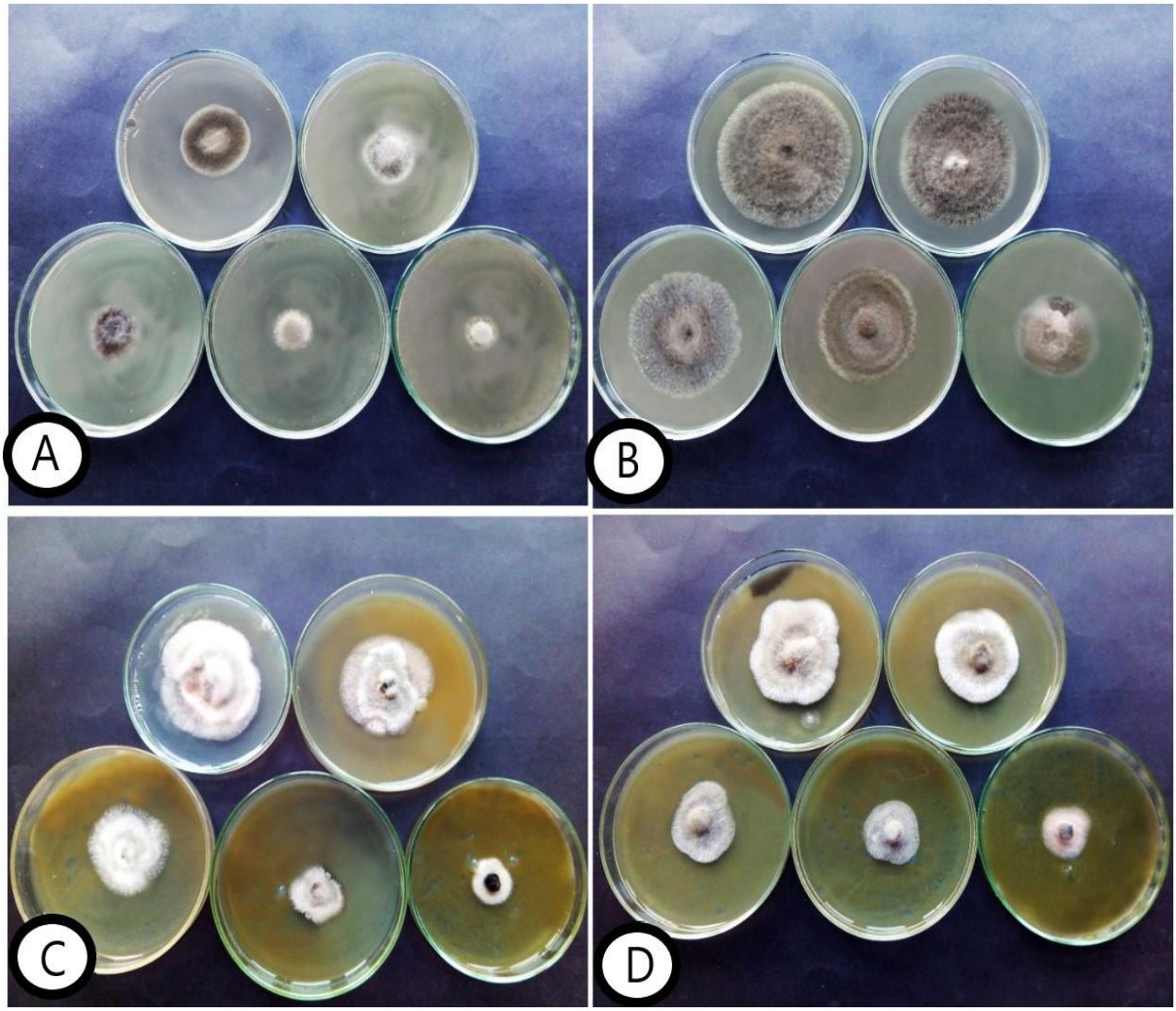


Plate 19. Percent inhibition owing to non-volatile substances of *Trichoderma harzianum* (Left-A, C) and *Trichoderma viride* (Right-C, D) on radial growth of *Bipolaris sorokiniana* isolates at 5, 10, 15, 20% concentration

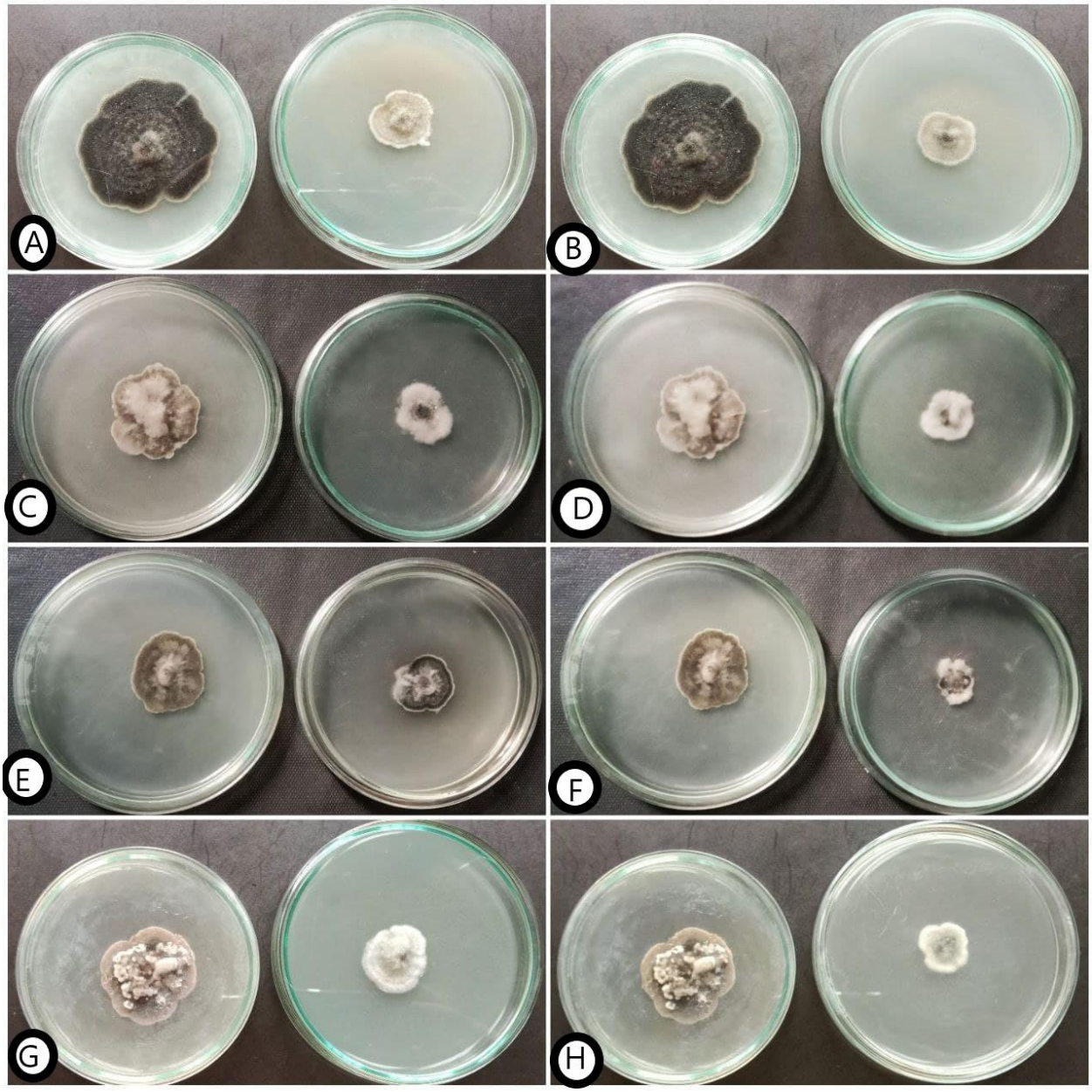


Plate 20. Percent inhibition owing to non-volatile substances of *Trichoderma viride* (Left-A, C, E, G) and *Trichoderma harzianum* (Right-B, D, F, H) on radial growth of *Bipolaris sorokiniana* isolates at 20% concentration

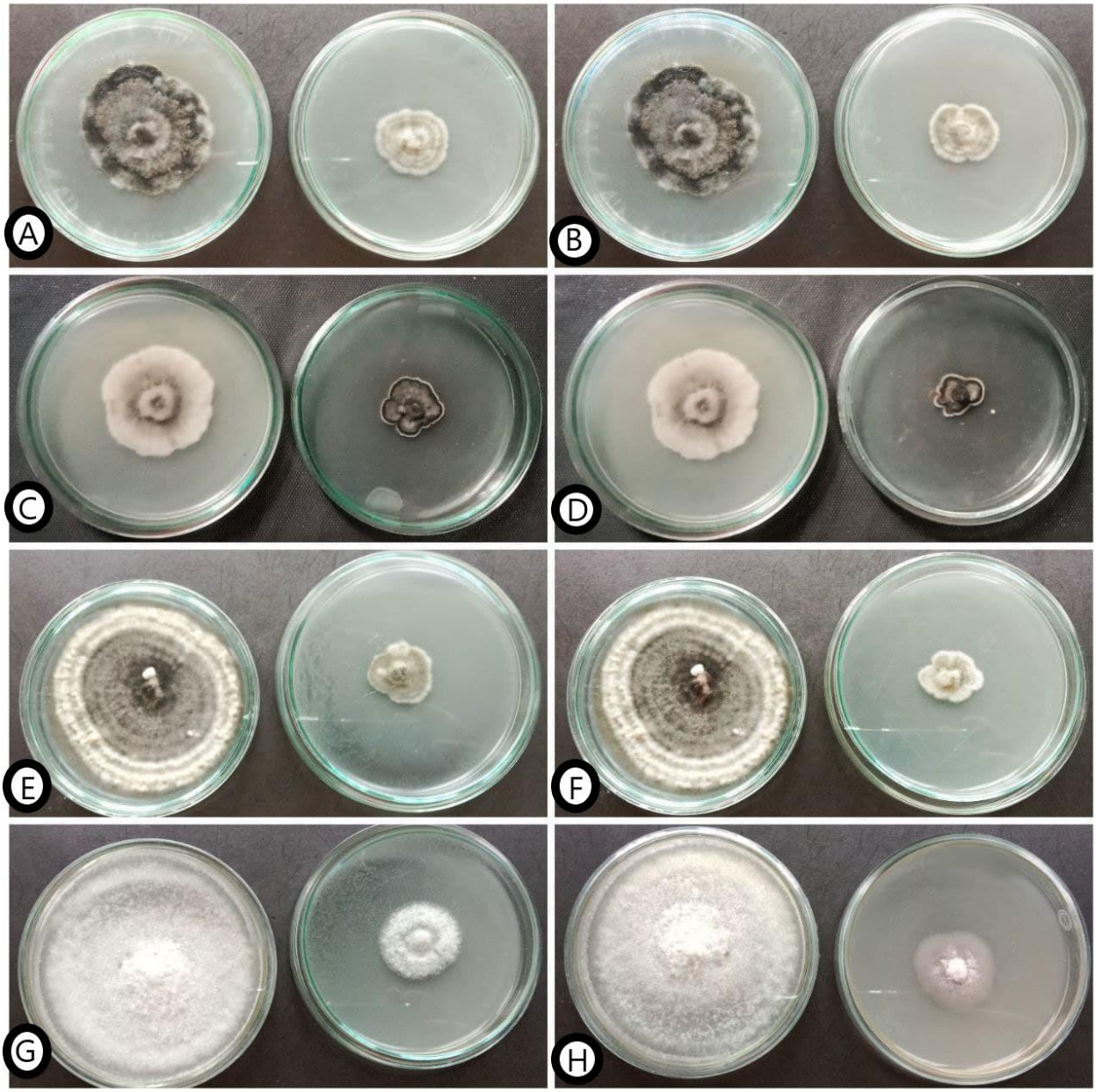


Plate 21. Percent inhibition owing to non-volatile substances of *Trichoderma viride* (Left-A, C, E, G) and *Trichoderma harzianum* (Right-B, D, F, H) on radial growth of *Bipolaris sorokiniana* isolates at 20% concentration

4.14 Management of leaf blight disease of wheat in field condition

Four distinct field trials were done at the Plant Pathology Division, Bangladesh Agriculture Research Institute (BARI), Joydebpur, Gazipur, for the control of wheat leaf blight disease (BpLB) caused by *Bipolaris sorokiniana*, using susceptible wheat variety Kanchan, during two successive years 2010-11 and 2011-12. Title of the experiments were-

i) Determination of number of fungicide sprays to minimize Bipolaris leaf blight (BpLB) disease of wheat.

ii) Standardization of doses of fungicide named Tilt and Folicure against Bipolaris leaf blight (BpLB) disease of wheat.

iii) Estimation of yield loss of wheat due to Bipolaris leaf blight (BpLB).

iv) Screening of wheat germplasms against Bipolaris leaf blight (BpLB).

4.14.1 Determination of disease severity of leaf blight of wheat

Initial BpLB signs appeared on older leaves near to the ground in all fields at the end of January, but the disease progressed slowly throughout February. At the end of February, the affected flag leaf area averaged less than 1%. BpLB lesions reached 10-60% of the flag leaves just two weeks later, while in most fields, only 15-25% of the flag leaf area was damaged by the disease in the early dough stage (GS 83). After two days of unusual spring rain, periods of fast disease development occurred. This happened on two separate occasions. After March 10, daily maximum temperatures exceeded 30ºC, resulting in a quick loss of green leaf area and early ripening, therefore the ultimate spread of the blight symptoms was not fully recorded. Just before the leaves were destroyed by heat and moisture stress, a last disease evaluation was done.

So far a good number of fungicide, have been registered in the country to manage Bipolaris leaf blight (BpLB) of wheat. In general fungicides are applied three times which is expensive. Number of spray could be minimizing if it is applied in proper time, in proper dose and following proper method of application with effective fungicides. Hence the study has been proposed with a view to reduce the spray number for maximizing higher yield and minimizing disease severity. Spray were done based on crop age, which were called as treatments. Total eight treatments belong to each fungicide [Tilt 250 EC (0.05%) and Folicur 250 EC (0.05%)] were evaluated using RCBD design with three

replications (Field layout, Appendix III). Treatments were- T_1 (35-55-75) days, T_2 (40-60) days, T_3 (45-65) days, T_4 (55-70) days, T_5 (55-75) days, T_6 (65-80) days, T_7 (75) days and T_8 Control.

Table 41 presents the disease parameters of different treatments during 2011 and 2012. Disease severity was maximum in control F_1T_8 (78.15) followed by F_2T_8 (78.13), F_2T_2 (69.1) and F_1T_2 (68.8) and percent leaf area diseased (%DLA) was also highest in control F_1T_8 (70.43) followed by F_2T_8 (68.85), F_2T_7 (50.62), F_1T_7 (45.68). On the contrary, the lowest disease severity was logged in F_1T_6 (43.17) and F_2T_6 (43.17) followed by F_2T_5 (43.68), F_1T_5 (46.72). Reduction of percent leaf area diseased (%DLA) over control was achieved highest in F_1T_6 (77.79%) followed by F_2T_6 (77.28%), F_2T_5 (77.08%) and F_2T_1 (76.73%) in 2011.

In 2012, disease severity was highest in F_2T_8 (77.69) followed by F_1T_8 (77.62), F_2T_2 (67.07) and F_1T_2 (66.8). The least disease severity was shown in F_2T_6 (42.34) after that F_1T_6 (42.82), F_2T_5 (43.68) and F_1T_5 (44.62). Percent leaf area diseased (%DLA) was also highest in control F_2T_8 (66.49) followed by F_1T_8 (65.85), F_2T_2 (52.35), F_1T_2 (50.37). The lowest percent DLA was recorded in F_2T_6 (11.56) followed by F_1T_6 (13.91), F_2T_5 (18.19) and F_1T_1 (21.26). Comparatively better result was shown in 2012, over control percent leaf area diseased (%DLA) was reduced, the highest in F_2T_6 (82.61) followed by F_1T_6 (78.88), F_2T_5 (72.64) and F_1T_1 (67.71) in 2012.

In Table 42 shows agronomic characters or yield contributing characters of different treatments. Number of spikes per square metre was the highest in F_1T_6 (388.67) followed by F_2T_6 (386.33), F_2T_5 (382) and F_1T_5 (379), the lowest was in control F_2T_8 (251.67) followed by F_1T_8 (261), F_2T_7 (293.33) and F_1T_2 (296.67). Number of grain per spike was maximum in the treatment T_6 for both the fungicides (40.67) and lowest in control F_1T_8 (26) and F_2T_8 (27). Seed weight per spike (g) was best in F_1T_6 (2.29 g) followed by F_2T_6 (2.27 g), F_1T_5 (2.26 g) and F_2T_5 (2.25 g), in control least seed weight was recorded 1.94 g. 1000 grain weight (g) was the highest in F_1T_6 (48.93 g) followed by F_2T_6 (48.63 g), F_1T_5 (48.3 g) and F_2T_5 (48.13) and the lowest was noted in F_2T_8 (41.55 g) followed by F_1T_8 (41.76 g). Grain yield per square metre (kg) was maximum in both T_6 treatment (0.44 kg) followed by T_5 (0.43 kg) and minimum was in control both T_8 (0.3 kg). Grain yield per plot (kg) was best in F_1T_6 (2.64 kg) followed by F_2T_6 (2.63 kg), F_2T_5 (2.56 kg) and F_1T_5 (2.55 kg) and the least was F_2T_8 (1.77 kg) followed by F_1T_8 (1.8 kg), F_2T_2 (2.1 kg) and F_1T_2 (2.13 kg). The most important parameter grain yield (kg/ha), the highest was usually

for F_1T_6 (4405.64 kg/ha) followed by F_2T_6 (4384.25 kg/ha), F_2T_5 (4266.34 kg/ha) and F_1T_5 (4256.5 kg/ha) and the lowest was F_2T_8 (2954.23 kg/ha) followed by F_1T_8 (3008.22 kg/ha), F_2T_2 (3508.25 kg/ha) and F_1T_2 (3542.38 kg/ha). The last column of the table exhibits yield increase over control, the best result shows F_2T_6 (48.41%) followed by F_1T_6 (46.45%), F_2T_5 (44.41%) and F_1T_5 (41.5%). So, disease parameters are negatively correlated with yield parameters. The next year, more or less comparable results were discovered (Table 43). T_6 or treatment-6 was best according to table, that means fungicides two spray at 65 days and 80 days after sowing will be more effective than the standard three spray 35 days, 55 days and 75 days. As a fungicide Tilt 250 EC (0.05%) and Folicur 250 EC (0.05%) more or less same compare to yield, during 2011 there was a difference, but in 2012 difference was not significant. Figure 23 presents bar diagram of grain yield (kg/ha) of 2011 and 2012.

Tilt (propiconazole) was effective for control of leaf blight disease of wheat, reported by many researchers in home and abroad (Anon. 1992, Anon. 1993, Malaker *et al.* 1994, Mahto and Bimb 1996, Singh *et al.* 1995, Singh and Chauhan 1995, Khan and Ilyas 1996, Picinini *et al*. 1996, Rahman 1998, Mahto 1999, Pandey and Tewari 2001, Bazlur Rashid *et al.* 2001, Malaker and Mian 2009, Singh *et al.* 2011, Yadav *et al*. 2015, Selvakumar *et al*. 2015). Many of the above-mentioned studies employed Tilt and Folicur and the results were comparable to the current study.

4.14.3 Quality analysis of seeds obtained from the experiment

Table 44 presents percentage of three categories of seeds, these were- apparently healthy seeds, black point seeds and shriveled & undersized seeds. During 2011, the highest 91.75 percent apparently healthy seeds were from F_1T_6 followed by F_2T_6 (91.5%), F_2T_5 (90.75%) and F_1T_5 (90.35%). The lowest 82.25 percent apparently healthy seeds were from F_1T_8 followed by F_2T_8 (83%). Percentage of black point seeds varied from 4.25 to 8%, the greatest percentage was noticed in F_2T_8 (8%) following F_1T_8 (7.75%), again the lowest black point seed percentage (4.25%) was in three treatments- F_1T_4, F_1T_6 and F_2T_5. The percentage of shriveled & undersized seeds differed from 4 to 10%, where the maximum percentage was shown in F_1T_8 following F_2T_8 (9%). The next year, it almost comparable results were discovered.

Table 45 and 46 shows the grading of seeds (G_0–G_5) based on the severity of black point incidence, obtained from field experiment during 2011 and 2012. According to Gilchrist

(1985) wheat seeds of different treatments were categorized G_0–G_5 grade on the basis of percentage. G_0 presents percentage of apparently healthy seeds, G_1 to G_4 shows percentage of different types of black point seeds and G_5 shows percentage of shriveled & undersized seeds. G_0 = Grains free from discolouration (apparently healthy), G_1 = Only tip of the embryo brown to blackish, G_2 = Discolouration covering the whole embryo, G_3 = Embryo with $^1/_4$ of the grain discoloured, G_4 = Embryo with $^1/_2$ of the grain discoloured, G_5 = Embryo with more than $^1/_2$ of the grain discoloured and shriveled.

According to Table 45, in G_1 class the highest (3.25%) seeds was in F_2T_7 and the lowest (0.78%) seeds was in F_1T_7, in G_2 class the highest (2.02%) seeds was in F_1T_8 and the lowest (0.5%) seeds in F_2T_5, in G_3 class F_1T_4 treatment had the highest percentage (1.85%) seeds and F_2T_5 treatment had the lowest percentage (0.5%), in G_4 class the highest 2.48% seeds were in F_1T_8 and the lowest 0.5% was in F_1T_5 treatment. Under G_5 grading shriveled and undersized seeds were presented, so, this was discussed earlier according to Table 44. Data on the grading of seeds in next year were more or less similar, as mentioned earlier.

Table 45. Determination of number of fungicide (Tilt & Folicur) sprays to minimize Bipolaris leaf blight (BpLB) on disease parameters of wheat during 2011 & 2012

SL No.	Treatment	2011			2012		
		Disease severity	% diseased leaf area (%DLA)	Diseased leaf area reduced over control (%)	Disease severity	% diseased leaf area (%DLA)	Diseased leaf area reduced over control (%)
1	**F_1 T_1** (35-55-75)	62.4 d	17.78 c	74.76	62.87 c	21.26 cde	67.71
2	**F_1 T_2** (40-60)	68.8 bc	40.95 abc	41.86	66.8 bc	50.37 abc	23.51
3	**F_1 T_3** (45-65)	64.63 bcd	34.32 bc	51.27	63.45 bc	25.56 bcde	61.18
4	**F_1 T_4** (55-70)	63.73 cd	27.65 c	60.74	64.01 bc	29.7 bcde	54.90
5	**F_1 T_5** (55-75)	46.72 e	17.33 c	75.39	44.62 d	22.81 bcde	65.36
6	**F_1 T_6** (65-80)	43.17 e	15.64 c	77.79	42.82 d	13.91 de	78.88
7	**F_1 T_7** (75)	66.17 bcd	45.68 abc	35.14	65.8 bc	42.96 abcd	34.76
8	**F_1 T_8** (control)	78.15 a	70.43 a	-	77.62 a	65.85 a	-
9	**F_2 T_1** (35-55-75)	61.83 d	16.02 c	76.73	63.07 bc	22.72 bcde	65.83
10	**F_2 T_2** (40-60)	69.1 b	44.6 abc	35.22	67.07 b	52.35 ab	21.27
11	**F_2 T_3** (45-65)	64.23 bcd	32.35 c	53.01	64.77 bc	35.31 bcde	46.89
12	**F_2 T_4** (55-70)	63.29 d	21.63 c	68.58	63.13 bc	23.19 bcde	65.12
13	**F_2 T_5** (55-75)	43.68 e	15.78 c	77.08	43.68 d	18.19 de	72.64
14	**F_2 T_6** (65-80)	43.17 e	15.64 c	77.28	42.34 d	11.56 e	82.61
15	**F_2 T_7** (75)	66.13 bcd	50.62 abc	26.48	65 bc	37.04 abcde	44.29
16	**F_2 T_8** (control)	78.13 a	68.85 ab	-	77.69 a	66.49 a	-

By LSD, values in the same column with the same letter (s) do not differ substantially at the 5% level.

Table 46. Determination of number of fungicide (Tilt & Folicur) sprays to minimize Bipolaris leaf blight (BpLB) on yield parameters of wheat during 2011

SL No.	Treatment	No. of spike/ sq.m	Grain no./spike	Seed weight/ spike (g)	1000 grain weight (g)	Grain yield/sq. m (kg)	Grain yield/plot (kg)	Grain yield (kg/ha)	Yield increased over control (%)
1	F_1 T_1 (35-55-75)	376.33a	39.33ab	2.23abc	47.62abc	0.41abc	2.45abc	4081.46ab	35.68
2	F_1 T_2 (40-60)	296.67def	34d	2.07defg	44.49e	0.35de	2.13de	3542.38abc	17.76
3	F_1 T_3 (45-65)	354.67abc	36.33abcd	2.09cdef	45.41de	0.38bcd	2.27bcd	3775.33abc	25.50
4	F_1 T_4 (55-70)	358.67ab	37.33abcd	2.2 abcde	46.6bcd	0.41abc	2.45 abc	4077.25ab	35.54
5	F_1 T_5 (55-75)	379a	39.67a	2.26ab	48.3ab	0.43ab	2.55abc	4256.5ab	41.50
6	F_1 T_6 (65-80)	388.67a	40.67a	2.29a	48.93a	0.44a	2.64a	4405.64a	46.45
7	F_1 T_7 (75)	306.67cde	34.67cd	2.06defg	45.09e	0.37cd	2.21cde	3676.34abc	22.21
8	F_1 T_8 (control)	261ef	26e	1.94g	41.76f	0.3e	1.8fg	3008.22c	-
9	F_2 T_1 (35-55-75)	374.33a	39abc	2.23abc	47.55abc	0.41abc	2.45abcd	4076.66ab	37.99
10	F_2 T_2 (40-60)	313.33bcd	33.67d	2.01fg	44.65e	0.35de	2.1efg	3508.25bc	18.75
11	F_2 T_3 (45-65)	354abc	36.33abcd	2.11bcd	45.56cde	0.37cd	2.24bcde	3727.33abc	26.17
12	F_2 T_4 (55-70)	363.67ab	38abcd	2.21abcd	46.62bcd	0.4abc	2.4abcd	3957.34ab	33.96
13	F_2 T_5 (55-75)	382a	39abc	2.25ab	48.13ab	0.43ab	2.56ab	4266.34ab	44.41
14	F_2 T_6 (65-80)	386.33a	40.67a	2.27a	48.63ab	0.44a	2.63a	4384.25ab	48.41
15	F_2 T_7 (75)	293.33def	35bcd	2.05efg	45.03e	0.36cd	2.19de	3642.34abc	23.29
16	F_2 T_8 (control)	251.67f	27e	1.94g	41.55f	0.3e	1.77g	2954.23c	-

By LSD, values in the same column with the same letter (s) do not differ substantially at the 5% level.

Table 47. Determination of number of fungicide (Tilt & Folicur) sprays to minimize Bipolaris leaf blight (BpLB) on yield parameters of wheat during 2012

SL No.	Treatment	No. of spike/ sq.m	Grain no./spike	Seed weight/ spike (g)	1000 grain weight (g)	Grain yield/ sq. m (kg)	Grain yield/plot (kg)	Grain yield (kg/ha)	Yield increased over control (%)
1	F_1 T_1 (35-55-75)	360a	38.67ab	2.24abc	47.42abc	0.4a	2.43abcd	4047.23a	37.36
2	F_1 T_2 (40-60)	330ab	33.67d	2.11d	44.14efg	0.37ab	2.19bcde	3653.33abc	23.99
3	F_1 T_3 (45-65)	352.33a	37abcd	2.18bcd	44.78de	0.38ab	2.26abcd	3944.7ab	33.88
4	F_1 T_4 (55-70)	361a	37.67abcd	2.19abcd	46.21cde	0.41a	2.43abcd	4057.77a	37.72
5	F_1 T_5 (55-75)	362.67a	39.33ab	2.27ab	48.45ab	0.42a	2.53abc	4216.4a	43.1
6	F_1 T_6 (65-80)	368.33a	40.67a	2.28a	48.82a	0.44a	2.63a	4381.13a	48.69
7	F_1 T_7 (75)	331.67a	36bcd	2.13d	44.38def	0.36ab	2.19bcde	3643.37abc	23.65
8	F_1 T_8 (control)	245.33bc	26.33e	1.93e	42.16fg	0.29b	1.77ef	2946.4bc	-
9	F_2 T_1 (35-55-75)	355.33a	38.33abc	2.24abc	47.67abc	0.41a	2.43abcd	4057.5a	38.9
10	F_2 T_2 (40-60)	325.67abc	34cd	2.1d	44.07efg	0.35ab	2.09def	3483.03abc	19.24
11	F_2 T_3 (45-65)	331a	36.33abcd	2.15cd	44.8de	0.37ab	2.23abcd	3712.5abc	27.09
12	F_2 T_4 (55-70)	351a	37.33abcd	2.19abcd	46.55bcd	0.39a	2.34abcd	3902.23abc	33.59
13	F_2 T_5 (55-75)	354.67a	39ab	2.27ab	48.25abc	0.42a	2.53abc	4218.07a	44.4
14	F_2 T_6 (65-80)	360.33a	39.67ab	2.28a	48.77ab	0.43a	2.61ab	4343.9a	48.71
15	F_2 T_7 (75)	329.67ab	35.67bcd	2.12d	44.17ef	0.36ab	2.15cdef	3581.7abc	22.61
16	F_2 T_8 (control)	243.33c	26.67e	1.93e	41.94g	0.29b	1.75f	2921.1c	-

By LSD, values in the same column with the same letter (s) do not differ substantially at the 5% level.

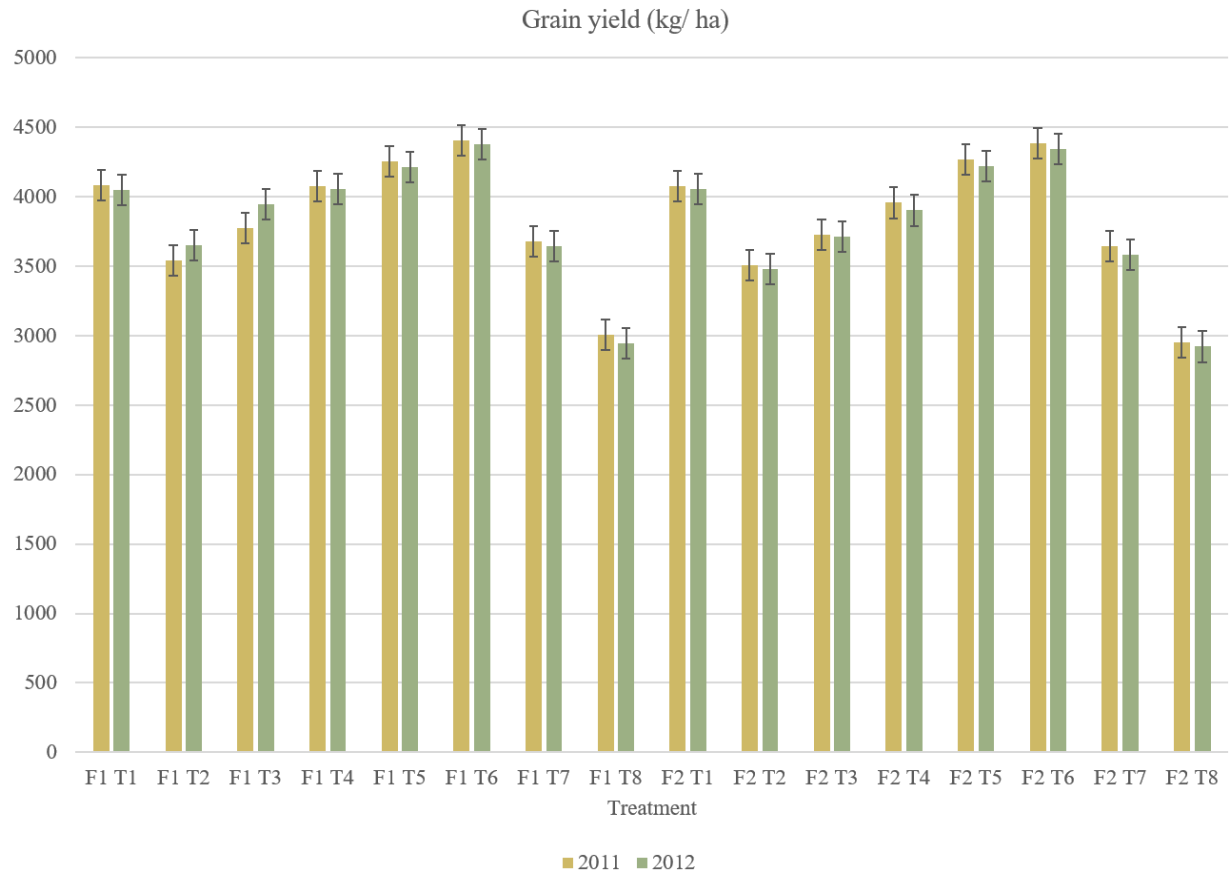


Fig. 24. Determination of number of fungicide (Tilt & Folicur) sprays to minimize Bipolaris leaf blight (BpLB) on grain yield (kg/ha) of wheat during 2011 & 2012

Table 48. Determination of number of fungicide (Tilt & Folicur) sprays to minimize Bipolaris leaf blight (BpLB) on black point grains of wheat during 2011 and 2012

SL No.	Treatment	2011			2012		
		Apparently healthy seeds (%)	Black point seeds (%)	Shriveled & undersized seeds (%)	Apparently healthy seeds (%)	Black point seeds (%)	Shriveled & undersized seeds (%)
1	F_1 T_1 (35-55-75)	89.75	5.25	5.0	89.55	6.75	3.7
2	F_1 T_2 (40-60)	87.75	6.75	5.5	87.75	5.25	7.0
3	F_1 T_3 (45-65)	88.45	5.55	6.0	88.25	6.0	5.75
4	F_1 T_4 (55-70)	89.58	4.25	6.17	89.58	7.72	2.7
5	F_1 T_5 (55-75)	90.35	5.5	4.15	90.56	8.36	1.08
6	F_1 T_6 (65-80)	91.75	4.25	4.0	91.05	4.75	4.2
7	F_1 T_7 (75)	86.0	7.5	6.5	86.26	6.5	7.25
8	F_1 T_8 (control)	82.25	7.75	10.0	82.0	9.0	9.0
1	F_2 T_1 (35-55-75)	89.05	5.5	5.45	89.83	5.17	5.0
2	F_2 T_2 (40-60)	87.55	6.25	6.2	87.92	4.58	7.5
3	F_2 T_3 (45-65)	88.75	6.25	5.0	88.75	5.6	5.65
4	F_2 T_4 (55-70)	88.95	6.05	5.0	89.42	5.13	5.45
5	F_2 T_5 (55-75)	90.75	4.25	5.0	91.0	4.75	4.25
6	F_2 T_6 (65-80)	91.5	4.5	4.0	91.5	4.25	4.75
7	F_2 T_7 (75)	85.5	6.5	8.0	85.42	6.45	8.13
8	F_2 T_8 (control)	83.0	8.0	9.0	82.5	8.5	9.0

Table 49. Determination of number of fungicide (Tilt & Folicur) sprays to minimize Bipolaris leaf blight (BpLB) on grading of wheat seeds (G_0–G_5 scale) during 2011

Sl. No.	Name of treatment	Grades of wheat grain (Percentage-%)					
		G_0	G_1	G_2	G_3	G_4	G_5
1	F_1 T_1 (35-55-75)	89.75	1.35	1.4	1.75	0.75	5.0
2	F_1 T_2 (40-60)	87.75	2.75	0.75	1.5	1.75	5.5
3	F_1 T_3 (45-65)	88.45	3.05	1.0	0.75	0.75	6.0
4	F_1 T_4 (55-70)	89.58	2.1	1.65	1.85	1.35	6.17
5	F_1 T_5 (55-75)	90.35	3.0	1.25	0.75	0.5	4.15
6	F_1 T_6 (65-80)	91.75	1.17	1.5	1.0	1.25	4.0
7	F_1 T_7 (75)	86.0	0.78	0.93	1.16	1.16	6.5
8	F_1 T_8 (control)	82.25	1.5	2.02	1.84	2.48	10
9	F_2 T_1 (35-55-75)	89.05	1.75	1.5	1.0	1.25	5.45
10	F_2 T_2 (40-60)	87.55	2.75	1.5	1.0	1.0	6.2
11	F_2 T_3 (45-65)	88.75	3.0	1.25	1.25	0.75	5.0
12	F_2 T_4 (55-70)	88.95	2.65	1.55	1.0	0.85	5.0
13	F_2 T_5 (55-75)	90.75	1.75	0.5	0.5	1.5	5.0
14	F_2 T_6 (65-80)	91.5	2.0	1.25	1.25	-	4.0
15	F_2 T_7 (75)	85.5	3.25	1.0	1.0	1.25	8.0
16	F_2 T_8 (control)	83.0	3.0	2.0	1.0	2.0	9.0

G_0 = Grains free from discolouration (apparently healthy), G_1 = Only tip of the embryo brown to blackish, G_2 = Discolouration covering the whole embryo, G_3 = Embryo with $^1/_4$ of the grain discoloured, G_4 = Embryo with $^1/_2$ of the grain discoloured, G_5 = Embryo with more than $^1/_2$ of the grain discoloured and shriveled

Table 50. Determination of number of fungicide (Tilt & Folicur) sprays to minimize Bipolaris leaf blight (BpLB) on grading of wheat seeds (G_0–G_5 scale) during 2012

Sl. No.	Name of wheat Varieties	Grades of wheat grain (Percentage-%)					
		G_0	G_1	G_2	G_3	G_4	G_5
1	F_1 T_1 (35-55-75)	89.55	2.5	0.85	1.9	1.5	3.7
2	F_1 T_2 (40-60)	87.75	2.25	1.25	1.0	0.75	7.0
3	F_1 T_3 (45-65)	88.25	2.55	1.15	0.9	1.4	5.75
4	F_1 T_4 (55-70)	89.58	4.5	-	2.25	0.97	2.7
5	F_1 T_5 (55-75)	90.56	3.5	1.15	2.71	1.0	1.08
6	F_1 T_6 (65-80)	91.05	1.0	1.5	1.0	1.25	4.2
7	F_1 T_7 (75)	86.26	2.85	1.0	1.25	1.4	7.25
8	F_1 T_8 (control)	82.0	4.0	2.25	1.75	1.0	9.0
9	F_2 T_1 (35-55-75)	89.83	2.25	1.15	0.90	0.87	5.0
10	F_2 T_2 (40-60)	87.92	1.75	1.75	-	1.08	7.5
11	F_2 T_3 (45-65)	88.75	2.2	1.0	1.25	1.15	5.65
12	F_2 T_4 (55-70)	89.42	2.0	1.05	1.13	0.95	5.45
13	F_2 T_5 (55-75)	91.0	1.75	1.25	0.75	1.0	4.25
14	F_2 T_6 (65-80)	91.5	1.0	1.1	1.05	1.1	4.75
15	F_2 T_7 (75)	85.42	2.25	1.5	1.25	1.45	8.13
16	F_2 T_8 (control)	82.5	3.25	1.75	2.0	1.5	9.0

G_0 = Grains free from discolouration (apparently healthy), G_1 = Only tip of the embryo brown to blackish, G_2 = Discolouration covering the whole embryo, G_3 = Embryo with $^1/_4$ of the grain discoloured, G_4 = Embryo with $^1/_2$ of the grain discoloured, G_5 = Embryo with more than $^1/_2$ of the grain discoloured and shriveled

4.14.4 Standardization of doses of fungicide named Tilt and Folicur against Bipolaris leaf blight (BpLB) disease of wheat

In the experiment effort with a view to reduce the dose for maximizing higher yield and minimizing disease severity. Maintaining standard fungicide spray schedule [(35-55-75) days after sowing], spray with standard dose (D_3 = 0.1%), lower than standard dose (D_1 = 0.05% and D_2 = 0.075%) and without dose (D_4 = Control) were done, which were called as treatments. Total four treatments belong to each fungicide [Tilt 250 EC and Folicur 250 EC] were evaluated using RCBD design with three replications (Field layout, Appendix III).

Table 47 presents the disease parameters of different treatments during 2011 and 2012. Disease severity was maximum in control F_2D_4 (75.3) followed by F_1D_4 (74.3) and percent leaf area diseased (%DLA) was also the highest in control F_2D_4 (45.8) followed by F_1D_4 (37.16). On the contrary, the lowest disease severity was logged in F_1D_3 (38.66) followed by F_2D_3 (42.49) and percent leaf area diseased (%DLA) was also the lowest in F_1D_3 (8.48) followed by F_2D_3 (12.31). Reduction of percent leaf area diseased (%DLA) over control was achieved the highest in F_1D_3 (77.18%) followed by F_2D_3 (73.12%), in 2011. In the same way, during 2012, disease severity was maximum in control F_1D_4 (75.1) followed by F_2D_4 (74.5), the lowest disease severity was logged in F_1D_3 (39.5) followed by F_2D_3 (39.83). Percent leaf area diseased (%DLA) was also the highest in control F_1D_4 (44.07) followed by F_2D_4 (38.89), the the lowest percent leaf area diseased (%DLA) was logged in F_1D_3 (12.02) followed by F_2D_3 (13.07). In 2012, reduction of percent leaf area diseased (%DLA) over control was attained the highest in F_1D_3 (72.72%) followed by F_2D_3 (66.39%). So, the conclusion of the experiment will be-disease control was not satisfactory by reducing doses of fungicides than the standard dose, which usually used in farmer's fields. Between two fungicides (Tilt and Folicur) performance was non-significant in case of disease severity but a little significant difference was noticed in percent leaf area diseased.

In Table 48 shows agronomic characters or yield contributing characters of different treatments during 2011. Number of spikes per square metre was the highest in F_2D_3 (382) followed by F_1D_3 (381.67), the lowest was in control F_2D_4 (262.45) followed by F_1D_4 (267.33). Number of grain per spike was maximum in the treatment T_3 for both the fungicides (40.67) and lowest in control F_2D_4 (26.86) followed by F_1D_4 (29.25). Seed weight per spike (g) was best in treatment T_3 for both the fungicides (2.29 g) and was the

lowest in control F_2D_4 (1.93 g). 1000 grain weight (g) was the highest in F_1D_3 (48.9 g) followed by F_2D_3 (48.85 g) and the lowest was noted in F_2D_4 (41.98 g) followed by F_1D_4 (42.08 g). Grain yield per square metre (kg) was maximum (0.43 kg) in three different treatments–F_1D_2, F_1D_3 and F_2D_3 and minimum was in control F_2D_3 (0.29 kg) following F_1D_4 (0.31 kg). Grain yield per plot (kg) was the best (2.6 kg) in F_1D_3 and F_2D_3. The least grain yield per plot was in control F_2D_4 (1.78 kg) followed by F_1D_4 (1.79 kg). The most important parameter grain yield (kg/ha), the highest was usually for F_1D_3 (4340.28 kg/ha) followed by F_2D_3 (4332.93 kg/ha) and the lowest was F_2D_4 (2875.64 kg/ha) followed by F_1D_4 (2954.3 kg/ha). The last column of the table exhibits yield increase over control, the best result shows F_2D_3 (50.68%) followed by F_1D_3 (46.91%) and F_2D_2 (45.94%). So, disease parameters are negatively correlated with yield parameters. The next year, more or less comparable results were discovered (Table 49). D_3 or dose-3 was the best according to table, that means fungicides sprays as per standard dose (0.1%) will be more effective than the different lower doses. As a fungicide Tilt 250 EC and Folicur 250 EC more or less were same compare to yield, there was a no significant difference. Figure 23 presents bar diagram of grain yield (kg/ha) of 2011 and 2012.

4.14.5 Quality analysis of seeds obtained from the second experiment

Table 50 presents, percentage of three categories of seeds, these were-apparently healthy seeds, black point seeds and shriveled & undersized seeds. During 2011, the highest 90.25 percent apparently healthy seeds were from F_1D_3 followed by F_2D_3 (90.0%). The lowest 80.75 percent apparently healthy seeds were from control F_1D_4 followed by F_2D_4 (82.75%). Percentage of black point seeds varied from 4.0 to 8.25%, the greatest percentage was noticed in control F_1D_4 following F_2D_1 (6.75%), again the lowest black point seed percentage (4.0%) was in F_2D_3 followed by F_1D_3 (4.15%). The percentage of shriveled & undersized seeds differed from 5.6 to 12%, where the maximum percentage was shown in F_2D_4 following F_1D_4 (11%). The next year, virtually identical findings were discovered.

Table 51 and 52 shows the grading of seeds (G_0–G_5) based on the severity of black point incidence, obtained from field experiment during 2011 and 2012. According to Gilchrist (1985) wheat seeds from the field experiment of different doses of two selected fungicides were categorized G_0–G_5 grade on the basis of percentage. G_0 presents percentage of apparently healthy seeds, G_1 to G_4 shows percentage of different types of black point seeds and G_5 shows percentage of shriveled & undersized seeds. G_0 = Grains

free from discolouration (apparently healthy), G_1 = Only tip of the embryo brown to blackish, G_2 = Discolouration covering the whole embryo, G_3 = Embryo with $^1/_4$ of the grain discoloured, G_4 = Embryo with $^1/_2$ of the grain discoloured, G_5 = Embryo with more than $^1/_2$ of the grain discoloured and shriveled.

According to Table 51, G_0 represents pure seeds or apparently healthy seeds, so data was same as the earlier table (Table 50). In G_1 class the highest (2.0%) seeds were in control F_1D_4 and the lowest (0.75%) seeds were in F_1D_2 and F_2D_2, in G_2 class the highest (2.5%) seeds were in control F_1D_4 and the lowest (0.8%) seeds in F_1D_3, in G_3 class F_1D_4 and F_2D_1 dose had the highest percentage (2.25%) seeds and F_2D_3 dose had the lowest percentage (0.95%), in G_4 class the highest 2.65% seeds were in F_2D_1 dose and the lowest 0.9% was in F_2D_4 treatment. Under G_5 grading shriveled and undersized seeds were presented, so, this was discussed earlier according to Table 50. Data on the grading of seeds in next year (Table 52) were more or less similar, as mentioned earlier.

Table 51. Standardization of doses of Tilt and Folicur against Bipolaris leaf blight (BpLB) on disease parameters of wheat during 2011 & 2012

SL No.	Treatment	2011			2012		
		Disease severity	% diseased leaf area (%DLA)	Diseased leaf area reduced over control (%)	Disease severity	% diseased leaf area (%DLA)	Diseased leaf area reduced over control (%)
1	$F_1 D_1$ (0.05%)	62.91 b	21.58 b	41.93	62.97 bc	21.98 cd	50.12
2	$F_1 D_2$ (0.075%)	52.9 c	17.9 c	51.83	52.5 d	15.43 de	64.99
3	$F_1 D_3$ (0.1%)	38.66 d	8.48 d	77.18	39.5e	12.02 e	72.72
4	$F_1 D_4$ (Control)	74.3 a	37.16a	-	75.1 a	44.07 a	-
5	$F_2 D_1$ (0.05%)	63.09 b	22.86 b	50.09	63.93 bc	24.43 cd	37.18
6	$F_2 D_2$ (0.075%)	52.97 c	18.36 bc	59.91	61.27 c	23.39 cd	39.86
7	$F_2 D_3$ (0.1%)	42.49 d	12.31 c	73.12	39.83 e	13.07 de	66.39
8	$F_2 D_4$ (Control)	75.3 a	45.8 a	-	74.5 a	38.89 ab	-

By LSD, values in the same column with the same letter (s) do not differ substantially at the 5% level.

Table 52. Standardization of doses of Tilt and Folicur against Bipolaris leaf blight (BpLB) on yield parameters of wheat during 2011

SL No.	Treatment	No. of spike/sq.m	Grain no./spike	Seed weight/ spike (g)	1000 grain weight (g)	Grain yield/sq. m (kg)	Grain yield/ plot (kg)	Grain yield (kg/ha)	Yield increased over lowest (%)
1	$F_1 D_1$ (0.05%)	377.33 a	39.33 a	2.23 a	47.82 a	0.42 a	2.51 a	4175 a	41.32
2	$F_1 D_2$ (0.075%)	378.33 a	39.67 a	2.27 a	48.15 a	0.43 a	2.55 a	4251.38 a	43.9
3	$F_1 D_3$ (0.1%)	381.67 a	40.67 a	2.29 a	48.9 a	0.43 a	2.6 a	4340.28 a	46.91
4	$F_1 D_4$ (Control)	267.33 b	29.25 b	1.96 b	42.08 b	0.31 b	1.79 b	2954.3 b	-
5	$F_2 D_1$ (0.05%)	375.67 a	39 a	2.23 a	47.72 a	0.4 a	2.41 a	4015.9 a	39.65
6	$F_2 D_2$ (0.075%)	377 a	39.67 a	2.27 a	48.08 a	0.42 a	2.52 a	4196.67 a	45.94
7	$F_2 D_3$ (0.1%)	382 a	40.67 a	2.29 a	48.85 a	0.43 a	2.6 a	4332.93 a	50.68
8	$F_2 D_4$ (Control)	262.45 b	26.86 b	1.93 b	41.98 b	0.29 b	1.78 b	2875.64 b	-

By LSD, values in the same column with the same letter (s) do not differ substantially at the 5% level.

Table 53. Standardization of doses of Tilt and Folicur against Bipolaris leaf blight (BpLB) on yield parameters of wheat during 2012

SL No.	Treatment	No. of spike/ sq.m	Grain no./spike	Seed weight/ spike (g)	1000 grain weight (g)	Grain yield/sq. m (kg)	Grain yield/plot (kg)	Grain yield (kg/ ha)	Yield increased over lowest (%)
1	F_1 D_1 (0.05%)	376 a	39 a	2.23 a	47.87 a	0.3033 b	1.85 b	4089.17 a	40.63
2	F_1 D_2 (0.075%)	376.67 a	39.67 a	2.28 a	48.12 a	0.3533 a	2.13 a	4270.7 a	46.87
3	F_1 D_3 (0.1%)	381.33 a	40.67 a	2.29 a	48.76 a	0.37 a	2.16 a	4307.21 a	48.13
4	F_1 D_4 (Control)	265.88 b	29.34 b	1.94 b	42.11 b	0.28 b	1.73 b	2907.77 b	-
5	F_2 D_1 (0.05%)	375.33 a	38.67 a	2.23 a	47.7 a	0.3167 b	1.89 b	3998.83 a	38.06
6	F_2 D_2 (0.075%)	376.67 a	40 a	2.28 a	48.17 a	0.34 a	2.01 a	4205.35 a	45.19
7	F_2 D_3 (0.1%)	381 a	40.33 a	2.29 a	48.75 a	0.3467 a	2.45 a	4322.6 a	49.24
8	F_2 D_4 (Control)	270.11b	28.15 b	1.95 b	42.18 b	0.29 b	1.75 b	2896.4 b	-

By LSD, values in the same column with the same letter (s) do not differ substantially at the 5% level.

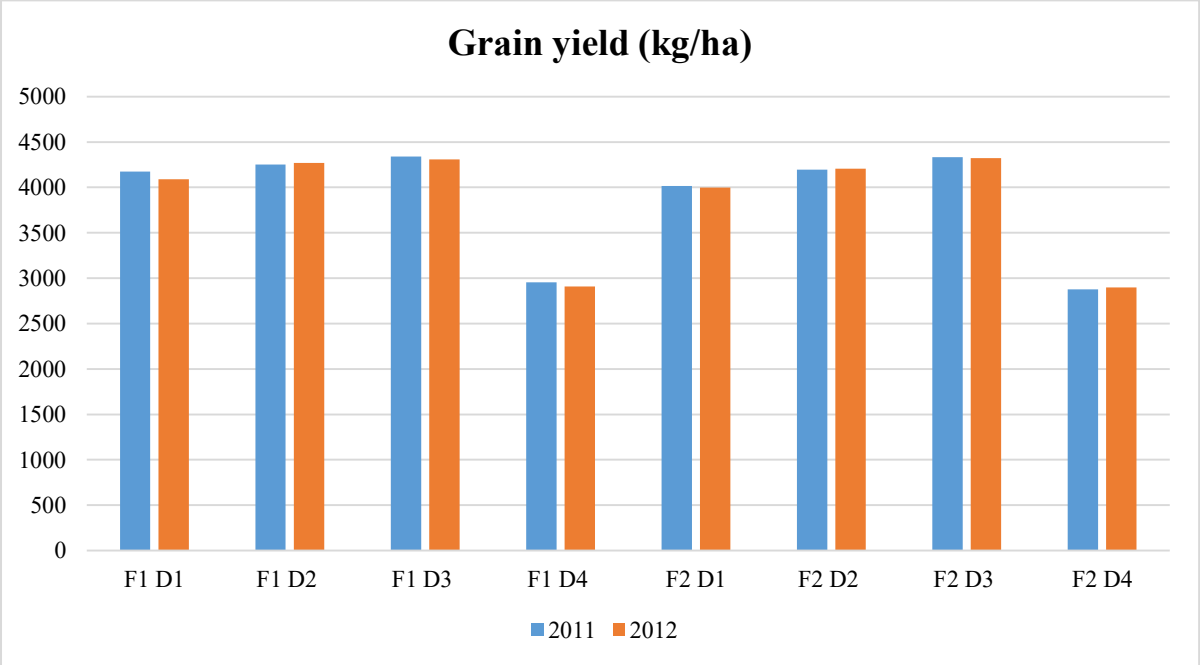


Fig. 25. Standardization of doses of Tilt and Folicur against Bipolaris leaf blight (BpLB) on grain yield (kg/ha) of wheat during 2011 & 2012

Table 54. Standardization of doses of Tilt and Folicur against Bipolaris leaf blight (BpLB) on black point grains of wheat during 2011 and 2012

SL No.	Treatment	2011			2012		
		Apparently healthy seeds (%)	Black point seeds (%)	Shriveled & undersized seeds (%)	Apparently healthy seeds (%)	Black point seeds (%)	Shriveled & undersized seeds (%)
1	**F_1 D_1** (0.05%)	85.65	6.5	7.85	86.25	6.25	7.5
2	**F_1 D_2** (0.075%)	88.55	5.85	5.6	87.75	6.75	5.5
3	**F_1 D_3** (0.1%)	90.25	4.15	5.6	91.5	4.5	4.0
4	**F_1 D_4** (Control)	80.75	8.25	11.0	81.0	8.55	10.45
5	**F_2 D_1** (0.05%)	86.45	6.75	6.8	85.15	5.75	9.1
6	**F_2 D_2** (0.075%)	87.75	5.25	7.0	88.25	4.95	6.8
7	**F_2 D_3** (0.1%)	90.0	4.0	6.0	91.75	4.75	3.5
8	**F_2 D_4** (Control)	82.75	5.25	12.0	81.65	6.5	11.85

Table 55. Standardization of doses of Tilt and Folicur against Bipolaris leaf blight (BpLB) on grading of wheat seeds (G_0–G_5 scale) during 2011

Sl. No.	Name of wheat varieties	Grades of wheat grain (Percentage-%)					
		G_0	G_1	G_2	G_3	G_4	G_5
1	**F_1 D_1** (0.05%)	85.65	1.25	1.75	2.0	1.5	7.85
2	**F_1 D_2** (0.75%)	88.55	0.75	1.1	1.75	2.25	5.6
3	**F_1 D_3** (0.1%)	90.25	1.0	0.8	1.2	1.15	5.6
4	**F_1 D_4** (Control)	80.75	2.0	2.5	2.25	1.5	11.0
5	**F_2 D_1** (0.05%)	86.45	0.8	1.05	2.25	2.65	6.8
6	**F_2 D_2** (0.75%)	87.75	0.75	1.25	1.0	2.25	7.0
7	**F_2 D_3** (0.1%)	90.0	1.0	0.75	0.95	1.3	6.0
8	**F_2 D_4** (Control)	82.75	1.25	1.55	1.55	0.9	12.0

G_0 = Grains free from discoloration (apparently healthy), G_1 = Only tip of the embryo brown to blackish, G_2 = Discoloration covering the whole embryo, G_3 = Embryo with $^1/_4$ of the grain discolored, G_4 = Embryo with $^1/_2$ of the grain discolored, G_5 = Embryo with more than $^1/_2$ of the grain discolored and shriveled

Table 56. Standardization of doses of Tilt and Folicur against Bipolaris leaf blight (BpLB) on grading of wheat seeds (G_0–G_5 scale) during 2012

Sl. No.	Name of wheat varieties	Grades of wheat grain (Percentage-%)					
		G_0	G_1	G_2	G_3	G_4	G_5
1	**F_1 D_1** (0.05%)	86.25	1.5	1.2	1.55	2.0	7.5
2	**F_1 D_2** (0.75%)	87.75	1.75	2.05	1.5	1.45	5.5
3	**F_1 D_3** (0.1%)	91.5	0.5	1.05	1.55	1.4	4.0
4	**F_1 D_4** (Control)	81.0	1.75	2.05	1.35	3.4	10.45
5	**F_2 D_1** (0.05%)	85.15	1.25	1.5	1.0	2.0	9.1
6	**F_2 D_2** (0.75%)	88.25	1.0	1.75	1.25	0.95	6.8
7	**F_2 D_3** (0.1%)	91.75	1.0	1.0	1.25	1.5	3.5
8	**F_2 D_4** (Control)	81.65	1.5	1.75	1.75	1.5	11.85

G_0=Grains free from discoloration (apparently healthy), G_1=Only tip of the embryo brown to blackish, G_2=Discoloration covering the whole embryo, G_3=Embryo with $^1/_4$ of the grain discolored, G_4=Embryo with $^1/_2$ of the grain discolored, G_5=Embryo with more than $^1/_2$ of the grain discolored and shriveled.

4.14.6 Estimation of yield loss of wheat due to Bipolaris leaf blight (BpLB)

The effect of *B. sorokiniana* on grain filling of wheat is enormous and dangerous. According to Raemaekers (1988), yield loss estimations calculated under very favorable conditions were 85% and yearly yield loss vary from 30-80% respectively. That most economic varieties are either moderately resistant or susceptible to the disease, according to Duveiller and Gilchrist (1994a). During 1991-92, a yield loss of up to 29% was estimated. The disease leaf blight is considered a hazard to wheat farming worldwide. Saari (1998) summarized a variety of regional research from South Asia and concluded that foliar blights caused a 20% output decline on average. In this experiment, the unsprayed plot had much higher disease severity than the sprayed plot. Fungicide application decreased disease severity, resulting in a better yield when compared to an unprotected crop.

In Bangladesh, Razzaque and Hossain (1990) found that the disease reduced grain production by 4-21 percent in commercial cultivars such as Sonalica (20%), Akber (14%), Kanchan (8%), and Aghrani (4%). According to Bazlur Rashid *et al.* (1994), yield loss was greatest in Kalyansona when assessed in terms of seed number/ear, seed weight/ear, and 1000 seed weight (81.68%).

Starting from the initial emergence of disease symptoms, Tilt 100 EC @ 1.25% was sprayed at a 15-day interval, at three wheat locations and reduced the leaf blight disease and loss in grain yield. The average yield loss was 24%. The 1000 grain weight of non-sprayed and sprayed plots varied from 39.6 to 42.59g and 43.3 to 47.39, respectively (Anonymous 1993).

Malaker *et al.* (1994) observed severity of disease at four locations under conditions of natural infection by *Helminthosporium sativum* in each location there were two treatments sprayed with Tilt 100 EC @ 0.125% and unsprayed (control). Tilt 100 EC was effective against Helminthosporium leaf blight (HLB) and disease severity were significantly different 3.04 and 3.44 for unsprayed and sprayed plots respectively. In compared to unsprayed plots, the use of Tilt 25 EC as a foliar spray resulted in higher grain number per plot, 1000 grain weight and big seeds, resulting in a better grain yield. Alam *et al.* (1995) assessed the wheat production loss due to Bipolaris leaf blight at farmer's field at four locations–Dinajpur, Jessore, Jamalpur and Ishwardi. Propiconazole was applied at 0.04% that decreased significantly the infection. They reported the actual

loss of wheat owing to leaf blight was 14.97% across the sites. Leaf blight is a disease that can cause significant output losses, ranging from 2.7 to 36.2%.

At the greatest disease incidence, Rashid and Fakir (1998) assessed a yield drop of 57.6% and 64.5% in cultivars Kanchan and Sonalika, correspondingly, owing to *B. sorokiniana*. Worldwide many researchers in different agronomical and weather conditions, in different varieties estimated yield loss due to Bipolaris leaf blight, such as Zhang *et al.* (1990) at Shaanxi province, China, yield loss was 1.95-13.5%, in India Parashar and Chohan (1967) reported 41.07% losses in the field, Nema and Joshi (1971) assessed 3–20 percent losses in yield in cultivars Sonora-64 and NP-884, Prabhu and Singh (1974) recorded 60% yield reduction in the cultivar NP 830, Pandey and Tiwari (2001) observed the highest yield drop (15.5%) in UP 2338, Villareal *et al.* (1995) at Poza rica, Mexico reported yield reduction were 43.2%, in Nepal Mahto (1999) reported yield loss 16.19 and 28.99% and the 1000 kernel weights were lowered by 6.97 and 15.78% in 1994-95, correspondingly.

The findings of yield loss assessment of wheat owing to leaf blight disease caused by *B. sorokiniana* on susceptible wheat variety Kanchan during 2011 and 2012, was remarkable and well measured. Disease severity was higher (71.56 and 69.92) in unsprayed plot compare to sprayed plot (44.89 and 42.16). Percent leaf area diseased was also higher in unprotected plots (35.65 and 32.68) than to protected plots (16.49 and 19.19). But all the agronomic characters related to yield were estimated in the experiment, were upper in protected plots compare to unprotected plots. No of spike/sq.m was bigger (380.6 and 370.6) in sprayed plot, whereas 277 and 266 were in unsprayed plot, so loss was estimated 27.17% and 28.19%. In case of, grain number per spike were 38 and 28, again 38 and 28.4 in sprayed and unsprayed plots during 2011 and 2012, respectively. Here reduction was calculated 26.27 and 25.17 percent, separately. Again yield loss was measured 15% and 14.36%, in seed weight/spike (g), where sprayed plot had 2.28 and 2.27g and unsprayed plot had 1.94g weight. 1000 grain weight (g) was 49.56g and 49.37g in protected plot and 41.89g and 41.85g was in unprotected plots in 2011 and 2012 crop season, correspondingly. Loss was calculated 15.47 and 15.19 percent. The highest reduction was estimated 33.56% and 36.27% in grain weight per square metre (kg). Here protecyed plots showed 0.44 kg yield in successive two year and unprotected plots yielded 0.29 kg and 0.28 kg. Grain yield per plot (kg) was 2.65 kg and 1.8 kg in 2011 and 2.85 kg and 2.31 kg in 2012, in sprayed and unsprayed plots, respectively. Yield loss was

32.16% and 19%. Last of all, grain yield (kg/ha) was 4281.66 and 3049.13 were in 2011 and 4293.6 and 3038.77 were in 2012 in sprayed and unsprayed plots, respectively. Yield loss was 28.68% and 29.19%. So, fungicide can minimize the disease and increase the yield significantly.

Table 57. Yield loss assessment of wheat in sprayed and unsprayed treatments of variety Kanchan

Sl. No.	Characters	2011			2012		
		Sprayed	Un sprayed	Loss	Sprayed	Un sprayed	Loss
1	Disease Severity	44.89	71.56		42.16	69.92	
2	%Diseased leaf area (DLA)	16.49	35.65		19.19	32.68	
3	No. of spike/sq.m	380.60	277.00	27.17	370.60	266.00	28.19
4	Grain no./spike	38.00	28.00	26.27	38.00	28.40	25.17
5	Seed weight/spike (g)	2.28	1.94	15.00	2.27	1.94	14.36
6	1000 grain weight (g)	49.56	41.89	15.47	49.37	41.85	15.19
7	Grain weight/sq. m (kg)	0.44	0.29	33.56	0.44	0.28	36.27
8	Grain yield/plot (kg)	2.65	1.80	32.16	2.85	2.31	19.00
9	Grain yield (kg/ha)	4281.66	3049.13	28.68	4293.60	3038.77	29.19

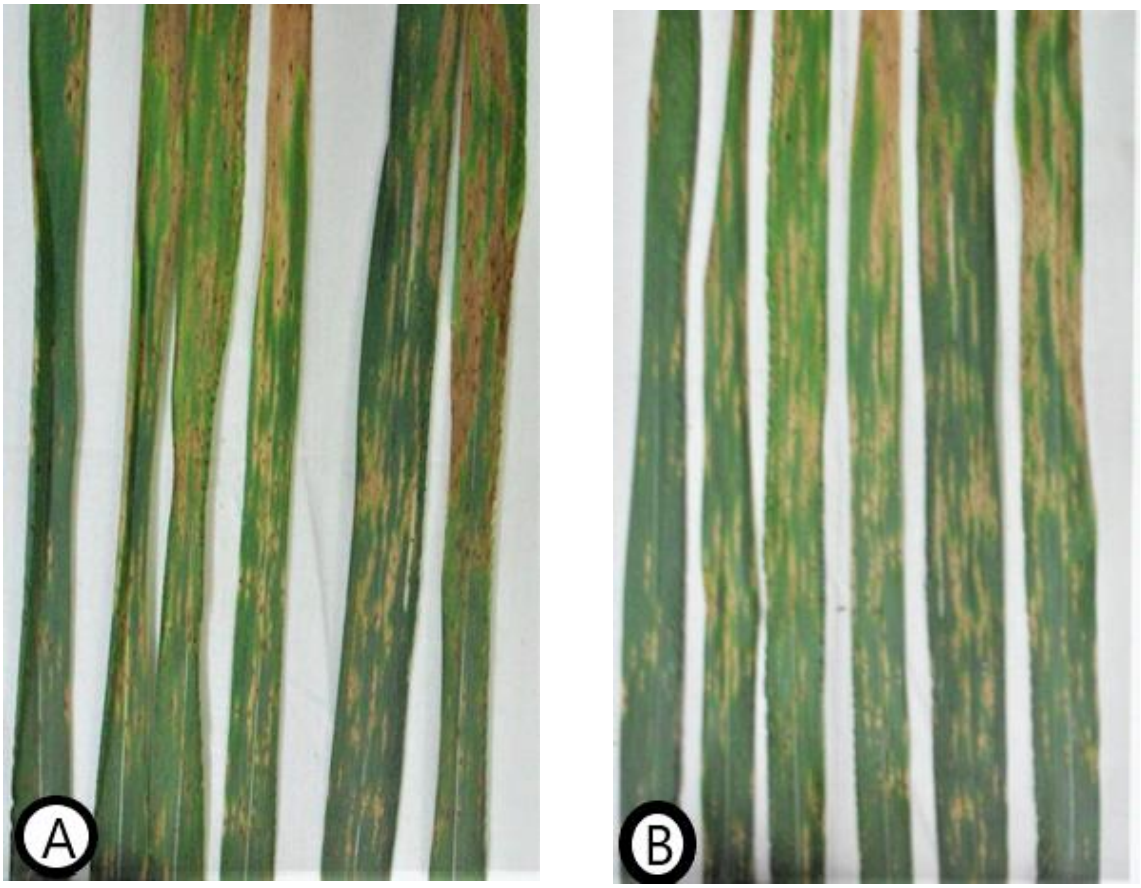


Plate 22 A. Percent diseased leaf area (%DLA) measurement on wheat leaves-A. Lesion on ventral surface B. Lesion on dorsal surface

Plate 23. Field experiment on standardization of doses of Tilt and Folicur against Bipolaris leaf blight (BpLB) of wheat at research field, Pathology Department, BARI, Joydebpur, Gazipur

Plate 24. Field experiment on estimation of yield loss of wheat due to Bipolaris leaf blight (BpLB) at research field, Pathology Department, BARI, Joydebpur, Gazipur

4.14.7 Germplasm evaluation of wheat against Bipolaris leaf blight

For sustainable disease control, the development of resistant cultivars is the most effective, long-term and ecologically friendly strategy. A number of sources have been recognized in wheat resistant to leaf blight (Duveiller and Gilchrist 1994). Researchers has detected the resistance, like *Aegilops squarrosa* crosses has shown remarkable resistance to leaf spot in Mexico (Ginkel and Rajaram 1997). At hot spot of Poza Rica, Mexico (Mujeeb-Kazi *et al*. 1996) five resistant germplasm lines have been identified. To identify the level of resistance an attempt was taken in the study which will be helpful in controlling the disease and finally provides low cost, environment kindly and tranquil way of increasing the productivity.

A total of 123 genotypes from various sources were tested against BpLB in the field under disease development conditions. The experiment was place on agricultural field in BARI campus, Gazipur. In mid-December 2011, under irrigated conditions, the materials were planted in 2 m long 2 row-plots with 20 cm spacing between rows and 30 cm between entries. Spreader rows made up of a variety of susceptible cultivars encircled the nursery. The experiment used an Alpha Lattice design with two replications. For proper crop development, recommended fertilizers and cultural methods were used. From the water ripe through early dough stage, the severity of leaf blight was graded three times on a double digit scale (00-99). Table 54 shows how disease data was converted to percent diseased leaf area (%DLA).

On flag leaves, disease severity was measured as a percentage of diseased leaf area (%DLA) and the genotypes were graded for their reaction using modified Ragiba *et al.* (2001)'s scale as follows: R=less than 10% DLA, MR=1-30 %DLA, MS=31-50 %DLA, S=51-70 %DLA and HS=above 70 %DLA. From my study only 02 genotypes (no. 19 and 34) were scored under 10, so they were selected as resistant genotypes (R) and the percentage of resistant genotypes was 1.63%, total 48 genotypes were score between 11-30 range, so they showed moderately resistant (MR) reaction and the percentage was 39.02% and total 67 genotypes were scored between 31-50, so they were moderately susceptible (MS) and the percentsge was 54.47%. Last of all total 06 genotypes (no. 67, 70, 84, 88, 89 and 111) were score above 50, so they were selected as susceptible (S) genotypes and their percentage was 4.88%. More than 50% genotypes showed moderately susceptible (MS) reaction. According to highest to lowest the reaction percentage was MS (54.47), MR (39.02), S (4.88) and R (1.63). There was no genotype as

highly susceptible (HS). This result is authenticated by several earlier investigators because Bipolaris disease reaction not only depends on genetical makeup of the genotypes, but also on the weather condition prevail on the particular grain filling condition of wheat, especially sudden spring rain fall and increase of temperature.

World wide a lot of work has done in search of resistance against *B. sorokiniana*, pathogen of wheat leaf blight. Hossain (1991) screened 533 wheat germplasm in field and found none was free from leaf blight infection. Hossain and Khan (1993) tested 37 materials, few were selected as resistant, Singh *et al*. (1995) found only 15 out of 257 genotypes resistant, Ragiba *et al*. (2001) screened 567 wheat entries where 57 were resistant, Molan *et al*. (2001) studied 18 genotypes and 2 cultivars of wheat, none was resistant, Akram and Singh (2003) informed that not a single genotype was immune but 26 genotypes were resistant (1-10% area diseased on the flag leaf) out of 148 genotypes. Reza *et al.* (2004) looked over 30 genotypes and found that number of different genotypes-resistant, moderately resistant, moderately susceptible, susceptible and highly susceptible were 4, 3, 6, 12 and 5, respectively to the disease. Findings of the experiment is quite similar with others.

Table 58. Screening of genotypes against Bipolaris leaf blight of wheat

Sl. No.	Genotypes	Disease severity	% Diseased Leaf Area	Cate gory	Sl. No.	Genotypes	Disease severity	% Diseased Leaf Area	Cate gory
1	Genotype-01	74.58	39.58	MS	63	Genotype-63	73.75	32.41	MS
2	Genotype-02	64.95	36.73	MS	64	Genotype-64	73.7	31.98	MS
3	Genotype-03	63	22.23	MR	65	Genotype-65	74.5	38.89	MS
4	Genotype-04	72.85	24.63	MR	66	Genotype-66	73	25.93	MR
5	Genotype-05	73.8	32.84	MS	67	Genotype-67	75.8	50.12	S
6	Genotype-06	54.95	30.56	MS	68	Genotype-68	75.5	47.53	MS
7	Genotype-07	74.1	35.44	MS	69	Genotype-69	74.1	35.43	MS
8	Genotype-08	65.05	36.36	MS	70	Genotype-70	76.45	55.74	S
9	Genotype-09	74.2	36.3	MS	71	Genotype-71	74.15	35.87	MS
10	Genotype-10	64.4	32.59	MR	72	Genotype-72	74.35	37.6	MS
11	Genotype-11	73.5	30.25	MS	73	Genotype-73	72.85	24.63	MR
12	Genotype-12	52.95	18.21	MR	74	Genotype-74	64.1	35.43	MS
13	Genotype-13	73.5	30.25	MS	75	Genotype-75	63.85	27.11	MR
14	Genotype-14	66	42.23	MS	76	Genotype-76	72.55	22.04	MR
15	Genotype-15	74.15	35.87	MS	77	Genotype-77	53	18.52	MR
16	Genotype-16	63.6	25.93	MR	78	Genotype-78	73.85	33.27	MS
17	Genotype-17	74.3	37.16	MS	79	Genotype-79	74.75	41.05	MS
18	Genotype-18	64.3	31.61	MS	80	Genotype-80	74.15	35.87	MS
19	Genotype-19	51.4	9.26	R	81	Genotype-81	73.55	30.68	MS
20	Genotype-20	53.6	23.46	MR	82	Genotype-82	75.45	47.09	MS
21	Genotype-21	63.3	24.94	MR	83	Genotype-83	74.45	38.46	MS
22	Genotype-22	53.85	23.77	MR	84	Genotype-84	75.9	50.99	S
23	Genotype-23	54.9	30.25	MS	85	Genotype-85	74.55	39.32	MS
24	Genotype-24	74.15	35.87	MS	86	Genotype-86	74.35	41.92	MS
25	Genotype-25	73.6	30.25	MS	87	Genotype-87	71.5	12.97	MR
26	Genotype-26	62.45	18.95	MR	88	Genotype-88	76.95	60.06	S
27	Genotype-27	62.6	19.01	MR	89	Genotype-89	76.45	55.74	S
28	Genotype-28	75	43.21	MS	90	Genotype-90	72.6	22.47	MR
29	Genotype-29	74.8	41.48	MS	91	Genotype-91	73	25.92	MR
30	Genotype-30	53.05	18.83	MR	92	Genotype-92	74.5	38.89	MS
31	Genotype-31	73.5	30.25	MS	93	Genotype-93	73	26.36	MR
32	Genotype-32	74.65	40.19	MS	94	Genotype-94	72.55	22.04	MR
33	Genotype-33	63	22.22	MR	95	Genotype-95	73.6	31.12	MS
34	Genotype-34	51.1	6.79	R	96	Genotype-96	73.75	32.41	MS
35	Genotype-35	72.85	24.63	MR	97	Genotype-97	53.4	20.99	MR
36	Genotype-36	53.5	21.61	MR	98	Genotype-98	73.65	31.55	MS
37	Genotype-37	72.95	25.49	MR	99	Genotype-99	74.2	36.3	MS
38	Genotype-38	54	24.69	MR	100	Genotype-100	73.1	26.79	MR
39	Genotype-39	43.45	15.25	MR	101	Genotype-101	74.15	35.86	MS
40	Genotype-40	54.9	30.25	MS	102	Genotype-102	63.95	27.96	MR
41	Genotype-41	74.25	37.16	MS	103	Genotype-103	74.95	42.78	MS
42	Genotype-42	64.5	33.34	MS	104	Genotype-104	74.25	36.73	MS
43	Genotype-43	64.8	35.56	MS	105	Genotype-105	74.4	38.03	MS
44	Genotype-44	64.3	31.48	MS	106	Genotype-106	75.45	41.91	MS
45	Genotype-45	74.15	35.87	M	107	Genotype-107	53	18.52	MR
46	Genotype-46	73.7	31.97	MS	108	Genotype-108	74.25	37.16	MS
47	Genotype-47	54.3	26.86	MR	109	Genotype-109	73.9	33.7	MS
48	Genotype-48	62.8	20.5	MR	110	Genotype-110	72.25	19.45	MR
49	Genotype-49	75.05	43.64	MS	111	Genotype-111	77.1	61.36	S
50	Genotype-50	73.5	30.25	MS	112	Genotype-112	56.2	35.8	MS
51	Genotype-51	53.55	21.92	MR	113	Genotype-113	74.75	41.05	MS
52	Genotype-52	63.5	26.06	MR	114	Genotype-114	73.8	32.84	MS
53	Genotype-53	72.6	22.47	MR	115	Genotype-115	74.1	35.43	MS
54	Genotype-54	72.7	23.34	MR	116	Genotype-116	72.5	21.6	MR
55	Genotype-55	73.15	27.23	MR	117	Genotype-117	72.95	25.5	MR
56	Genotype-56	73.1	26.79	MR	118	Genotype-118	75	43.21	MS
57	Genotype-57	74.15	35.87	MS	119	Genotype-119	54.05	23.77	MR
58	Genotype-58	73.15	27.23	MR	120	Genotype-120	73.6	31.12	MS
59	Genotype-59	52.5	16.05	MR	121	Genotype-121	44.15	19.32	MR
60	Genotype-60	73.2	27.65	MR	122	Genotype-122	74.4	38.02	MS
61	Genotype-61	53.55	21.91	MR	123	Genotype-123	75	43.21	MS
62	Genotype-62	72.5	21.61	MR					

R= Resistant, MR= Moderately resistant, MS= Moderately susceptible, S= Susceptible

4.15 Seed health test of different wheat varieties

Over the year's seeds are considered as very effective means for conveying pathogens. Seed-borne infections not only influence the development and growth of agricultural plants, but they are also responsible for seed quality degradation during storage. Using high-yielding cultivars and minimizing crop failures can help to ensure production. So, germination, purity and health status are the pre-requisite of better quality seed. Every year, about 20% of wheat loss is reported due to disease which would be offered for food and feed (Fakir 1999). A brown black discoloration of the embryos is characterized as black point seed. Seedlings from black point seeds show reduced vigour. Malakar (2003) discovered that when black pointed seeds were sowed, seedling emergence, seedling vigour, plant development, and grain output were lowered, whereas post-emergence mortality, *B. sorokiniana* disease severity, and black pointed seed incidence were all increased. Above 10% incidence of black point seeds also decrease the quality of flour. In light of the above, the current study was conducted with the goals of determining seed quality, as well as the presence of seed-borne fungus in wheat and their impact on germinating seed and seedlings.

4.15.1 Seed quality analysis of wheat

Freshly harvested eight seed lots consisted one from each six varieties (BARI Gom-30, BARI Gom-28, BARI Gom-27, BARI Gom-26, BARI Gom-29, BARI Gom-25) from BARI and two from variety Kanchan (each from BARI and DU campus) were studied. Seed quality analysis was used to establish the quality condition of seven wheat varieties. Seeds were classified into three classes- apparently healthy seeds, black point seeds and shriveled & undersized seeds (Plate 22). Moisture contents of the seed samples varied from 13.4–13.8.

Wheat seed has a moisture level of 12.00%, which is the national standard. However, moisture content data gathered in this investigation revealed that all seed samples had moisture content higher than the national standard. The health of stored seeds is jeopardized by high moisture content in seed prior to storage. Because wheat seeds with a greater moisture content are more susceptible to storage fungi and stored grain insects, which can result in significant losses due to reduced germination.

4.15.2 Apparently healthy seeds of wheat

Quality analysis presented that apparently healthy seeds percentage varies from 85.02–93.06% (Table 55). The greatest percentage of apparently healthy seeds was noted in BARI Gom-30 followed by BARI Gom-25 (90.71%), BARI Gom-29 (90.56%) and BARI Gom-27 (90.06%). The lowermost percentage of apparently healthy seeds was found in Kanchan (DU) variety.

4.15.3 Black point seeds of wheat

Black point seeds and its frequency of occurrence in different varieties are shown in Table 55. The highest amount of black point seeds (8.72%) were logged in BARI Gom-28 following BARI Gom-29 (8.36%), Kanchan (DU) (7.84%) and BARI Gom-26 (7.46%) and the least (4.04%) found in Kanchan (BARI) variety. During 1987-88 and 1988-89, Dey *et al.* (1992) inspected 232 and 150 seed samples of wheat collected from 12 districts of Bangladesh. The average black point infection of the varieties/lines during 1987-88 and 1988-89 ranged from 5.0 to 20.50% and 8.0 to 55.0%, respectively.

4.15.4 Shriveled & undersized seeds of wheat

The percentage of shriveled & undersized seeds varies from 1.08-8.7% (Table 55). The highest occurrence of shriveled & undersized seeds (8.7%) was recorded in Kanchan (BARI) variety followed by Kanchan (DU) (7.14%), BARI Gom-27 (5.71%) and BARI Gom-26 (3.34%). The lowest percentage of shriveled & undersized seeds was recorded in BARI Gom-29.

Seed malformation caused by seed-borne fungus is quite widespread, according to Varshney (1990) and responsible for a substantial percentage of yield loss. According to Owolade *et al.* (2001), the kind and degree of seed abnormalities may be influenced by the associated fungi's type and pathogenic potential, as well as the meteorological circumstances. Despite the fact that a variety of fungi linked with wheat seed abnormalities have been identified (Prescott *et al.*1986, Wiese *et al.* 1987, Varshney 1990), further research is needed to discover the consequences of fungus related with wheat seed abnormalities.

4.15.5 Grading of wheat seeds of wheat

According to Gilchrist (1985) wheat seeds of seven varieties were categorized G_0–G_5 grade on the basis of percentage (Table 56 and Plate 23). G_0 means grains free from

discoloration that was apparently healthy seeds, so the data was same presented in table 55, G_1 means only tip of the embryo brown to blackish, the highest percentage of G_1 grade seeds was recorded in BARI Gom-28 (2.56%) followed by BARI Gom-26 (1.97%) and the lowest was Kanchan (BARI) (0.78%). G_2 means discoloration covering the whole embryo, percentage range of this grade was 0.85–2.74%, the highest percentage of G_2 grade seeds was recorded in BARI Gom-28 (2.74%) followed by BARI Gom-29 (2.17%) and the lowest was BARI Gom-27 (0.85%). G_3 means embryo with $^1/_4$ of the grain discolored, percentage range of this grade was 0.92-2.71%, the highest percentage of G_3 grade seeds was recorded in BARI Gom-29 (2.71%) followed by BARI Gom-28 (2.31%) and the lowest was BARI Gom-27 (0.92%). G_4 means embryo with $^1/_2$ of the grain discolored, percentage range of this grade was 1.12-2.48%, the highest percentage of G_4 grade seeds was recorded in Kanchan (DU) (2.48%) followed by BARI Gom-26 (1.98%) and the lowest was BARI Gom-28 (1.12%). G_5 means embryo with more than $^1/_2$ of the grain discolored and shriveled, so the data was same presented in table 55.

4.15.6 Purity percentage of wheat seeds

Purity percentage was determined based on weight. Purity percentage of wheat seeds varied from 87.83 - 94.05% (Table 57). The highest purity percentage was in variety BARI Gom-27 followed by BARI Gom-30 (93.34%), BARI Gom-25 (92.25%) and the lowest was in Kanchan (DU). Another weight parameter was presented in Fig. 26, which shows weight differences among three different types of seeds. The maximum weight of 10 apparently healthy seeds was BARI Gom-30 (0.63 g) followed by BARI Gom-28 (0.62 g) and minimum was variety Kanchan (DU) (0.45 g). The maximum differences between the weight of apparently healthy seeds and black point seeds was noticed in variety BARI Gom-29 (0.57 to 0.42 g) and BARI Gom-30 (0.63 to 0.49 g). From the measurement, we can understand how yield loss varied among the varieties due to Bipolaris leaf blight. This depends on the percentage of black point and shriveled & undersized seeds and also their weight reduction compares to healthy seeds.

4.15.7 Determination of germination of wheat seeds

It appears from the Fig. 27 that about 90% of germination was recorded for the seven wheat varieties after 7 days. The highest germination of healthy seed (95%) was logged in cultivar Kanchan (BARI) followed by BARI Gom-27 (94.67%) and BARI Gom-25 (93.67%). Differences of germination percentage between apparently healthy seeds and

black point seeds were up to 10%. Percentage of seedling emergence varied in apparently healthy seeds 75–91%, where in case of black point seeds the range was 62.34–72%. As a result, mortality percentage was bigger in black point seeds (10.48–21.09%) compare to apparently healthy seeds (3.37–16.73%). The maximum seedling emergence percentage in apparently healthy seeds was achieved by BARI Gom-27 followed by Kanchan (BARI) (90%). Though variety Kanchan was used in this experiment as a susceptible check, but germination and seedling emergence quality was better than other advanced variety. Differences in germination percentages might be attributed to the variation in genetic composition or the presence of seed-borne pathogens. Low germination is also due to the incidence of seed-borne infection (Fakir 1998 and Islam *et al.* 2015). Reduced germination percentage, on the other hand, was linked to the severity of black point infection. Many studies have found that poor germination of wheat seeds is linked to the severity of black point infection, which is consistent with my findings (Chowdhury 2008, Hossain 2000). According to Hossain (2000), black point disease had a significant impact on wheat seed germination and seedling emergence and the percentage reduction in germination increased as the degree of black point seed increased. Seeds infected with seed-borne diseases may not germinate because the infections assault and destroy the seedling. Crop plants' development and yield have been proven to be affected by seed-borne illnesses.

4.15.8 Determination of vigour index of wheat seeds

Vigour index was related with shoot length, root length and germination percentage. In apparently healthy seeds and black point seeds the highest shoot length was recorded in BARI Gom-25 (25.56 and 20.2 cm) followed by BARI Gom-27 (24.82 and 16.8 cm) and the lowest shoot length was recorded in Kanchan (DU) (15.13 and 8.46 cm) followed by Kanchan (BARI) (15.62 and 8.62 cm), respectively (Table 58). In case of root length, the apparently healthy seeds and black point seeds showed the maximum root length in BARI Gom-27 (30.8 and 20.8 cm) followed by BARI Gom-29 (18.06 and 14.02 cm) and minimum was in variety Kanchan (DU) (9.96 cm in apparently healthy seeds) and Kanchan (BARI) (7.72 cm in black point seeds). So, in both cases vigour index was best in BARI Gom-27 (5265.54 and 3083.2) followed by BARI Gom-25 (3836.72 and 2461.35) and the least vigour index was Kanchan (DU) (2333.37 and 1372.56) followed by Kanchan (BARI) (2441.5 and 1372.56). Again fresh shoot weight and fresh root weight (g) also measured in apparently healthy seeds and black point seeds, differences

were noticed not only between apparently healthy seeds and black point seeds, but also susceptible variety Kanchan with other high yielding wheat varieties. Seed vigour and seed germinability are not the same thing. ISTA defines vigour as the sum of those seed qualities that affect the potential level of activity and performance of a non-dormant seed. As a result, germination tests done under ideal conditions in the laboratory can only suggest the ability of a seed lot to established seedling. A vigour test evaluates the physiological and physical foundation of projected seed lot performance, either directly or indirectly and gives a more sensitive discrimination among seed lots than a typical germination test. Seed vigour tests are widely utilized in the seed business and two-thirds of ISTA seed testing laboratories currently provide them.

4.15.9 Detection of seed borne fungi associated with wheat seeds

4.15.9.1 Detection of seed borne fungi by blotter method

Results of present study revealed that the wheat seeds are quite frequently infected by fungi. In the present study a total of twelve genera i.e. *Alternaria* spp., *Aspergillus* spp., *Bipolaris* spp., *Curvularia* spp., *Chaetomium* sp., *Cladosporium* sp., *Drechslera* sp., *Epicoccum* sp., *Fusarium* spp., *Nigrospora* sp., *Penicillium* sp. and *Rhizopus* sp. were isolated from seven wheat varieties (Table 59). *A. alternata* showed the highest (26.1%) of fungal infections on Kanchan (DU) followed by BARI Gom-27 (18.18%) and BARI Gom-29 (18.18%). *A. triticina* was also highest in Kanchan (DU) (13.05%) followed by BARI Gom-27 (9.09%). *B. sorokiniana* was highly (30.45%) in Kanchan (DU) followed by Kanchan (BARI) (26.3%) and BARI Gom-27 (22.73%). Frequency percentage range of *B. sorokiniana* was 4.54-30.45%. Variation in association of *Aspergillus* spp. with different wheat varieties were noticed. According to Sulaiman and Hussain (1984), *A. flavus* inhibited wheat seed germination by 90% when compared to healthy seeds. *B. oryzae* was obtained from only BARI Gom-30 in blotter method, but *B. tetramera* isolated frequently. The highest (26.09%) *C. lunata* and (21.74%) *Fusarium* spp. was found in Kanchan (DU) variety.

The percentage of fungal infection of wheat varieties ranged from 2.08-30.45%. The species *A. alternata*, *A. flavus*, *A. niger*, *B. sorokiniana*, *C. lunata* and *Fusarium* spp. were isolated from seven varieties of wheat seeds. Incidences of *A. fumigatus*, *A. terrous*,

Cheatomium sp., *Cladosporium* sp., *C. pallescens*, *Nigrospora* sp., *Penicillium* sp., were also remarkable from several varieties of wheat seeds.

In total more than twenty species belong to twelve genera of fungi were indentified, the most pre-dominant fungi were *A. niger*, *A. flavus*, *A. alternata*, *B. sorokiniana*, *C. lunata* and *Fusarium* spp. In a seed health test using the Blotter technique, Naznine *et al.* (2016) discovered eight distinct seed-borne fungus belonging to six genera, *viz. B. sorokiniana* (0.5–30.5%), *A. tenuis* (0.5–25%), *F. moniliforme* (0.0–33.5%), *F. oxysporum* (2.7–53%), *C. lunata* (0.0–5.5%), *A. niger* (0.0–18.5%) and *Penicillium* sp. (0.0–1.5%).

Naznine *et al.* (2016) conducted a seed health test by Blotter method resulted eight different seed borne fungi belonging to six genera Out of 11samples tested germination of seeds ranged from 98.0 to 73.5%. Their findings are almost similar to my result.

4.15.9.2 Detection of seed borne fungi by agar plate method

By agar plate method a total of thirteen genera i.e. *Alternaria* spp., *Aspergillus* spp., *Bipolaris* spp., *Curvularia* spp., *Chaetomium* sp., *Cladosporium* sp., *Drechslera* sp., *Epicoccum* sp., *Fusarium* spp., *Nigrospora* sp., *Penicillium* sp., *Rhizopus* sp. and *Trichoderma* spp. were recorded (Table 60). *Alternaria alternata* showed the highest (27.24%) of fungal infections on Kanchan (DU) followed by BARI Gom-27 (21.05%) on black point seeds. But *A. triticina* was highest in variety Kanchan (BARI) (23.8%) followed by BARI Gom-29 (13.64%) on black point seeds. *B. sorokiniana* was isolated from all the varieties, *B. sorokiniana* highly (45.45%) found in BARI Gom-29 and then found (42.86%) in BARI Gom-28 on black point seeds. Frequency percentage range of *B. sorokiniana* was 4.45-45.4%. Frequency percentage was highest of *A. fumigatus* on BARI Gom-28 (28.57%) followed by Kanchan (DU) (27.24%) on black point seeds. The highest (11.76%) *C. lunata* was found in BARI Gom-26 variety and *Fusarium* spp. was highest on Kanchan (DU) (13.62%) on black point seeds.

The frequency percentage of fungal infection on different wheat varieties ranged from 2.38%-45.45%. The species *A. alternata*, *B. sorokiniana*, *C. lunata* and *Fusarium* spp. were isolated from seven varieties of wheat seeds. Incidences of *A. triticina*, *A. flavus*, *A. fumigatus*, *A. niger* and *C. lunata* was notable. In total twenty-one species of fungi were indentified, the most pre-dominant fungi were species of *Alternaria* spp., *Aspergillus* spp., *B. sorokiniana*, *Curvularia* spp. and *Fusarium* spp.

Many researchers have discovered a large number of seed-borne fungal pathogens belonging to the genera *Bipolaris*, *Alternaria*, *Curvularia*, *Fusarium*, *Penicillium* and *Aspergillus* in wheat seeds (Ashrafuzzaman and Hossain 1992, Hossain and Schlosser 1993). A higher number of *Bipolaris* infected seeds results in a higher number of diseased fully-grown plants (Bazlur Rashid 1994, Malaker 2003). The disease leaf spot/leaf blight is caused by *B. sorokiniana*, seed to plant and plant to seed transmited (Nema and Joshi 1974, Hossain 2000).

Seed quality is defined by a number of characteristics, including genetic and physical purity, excellent germination and vigour index, and the absence of disease and insects. Seed quality is regulated by crop management strategies and storage conditions under favourable conditions. The storage state, which is linked to the assault of storage fungi under the impacts of temperature, relative humidity and seed wetness, is the most critical of these elements. Variable storage conditions not only cause severe seed degeneration, but also render them useless for planting in subsequent seasons.

Table 59. Percentage of pure seeds/apparently healthy seeds, black point seeds and shriveled & undersized seeds of seven wheat varieties with moisture contents

Sl. No.	Name of wheat varieties	Pure seeds/ apparently healthy seeds (%)	Black point seeds (%)	Shriveled & undersized seeds (%)	Moisture contents (%)
1	**BARI Gom-25**	90.71	6.56	2.74	13.5
2	**BARI Gom-26**	89.19	7.46	3.34	13.4
3	**BARI Gom-27**	90.06	4.23	5.71	13.6
4	**BARI Gom-28**	89.74	8.72	1.54	13.4
5	**BARI Gom-29**	90.56	8.36	1.08	13.5
6	**BARI Gom-30**	93.06	4.93	2.0	13.3
7	**Kanchan (BARI)**	87.26	4.04	8.7	13.5
8	**Kanchan (DU)**	85.02	7.84	7.14	13.8

Table 60. Percentage of G_0–G_5 grades of wheat seeds on the basis of severity of black point infection

Sl. No.	Name of wheat varieties	Grades of wheat grain (Percentage %)					
		G_0	G_1	G_2	G_3	G_4	G_5
1	**BARI Gom-25**	90.71	1.66	1.49	1.92	1.5	2.74
2	**BARI Gom-26**	89.19	1.97	1.72	1.89	1.98	3.34
3	**BARI Gom-27**	90.06	1.06	0.85	0.92	1.41	5.71
4	**BARI Gom-28**	89.74	2.56	2.74	2.31	1.12	1.54
5	**BARI Gom-29**	90.56	1.7	2.17	2.71	1.78	1.08
6	**BARI Gom-30**	93.06	1.17	1.5	1.0	1.25	2.0
7	**Kanchan (BARI)**	87.26	0.78	0.93	1.16	1.16	8.7
8	**Kanchan (DU)**	85.02	1.5	2.02	1.84	2.48	7.14

G_0=Grains free from discolouration (apparently healthy), G_1=Only tip of the embryo brown to blackish, G_2=Discolouration covering the whole embryo, G_3=Embryo with $^1/_4$ of the grain discoloured, G_4=Embryo with $^1/_2$ of the grain discoloured, G_5=Embryo with more than $^1/_2$ of the grain discoloured and shriveled

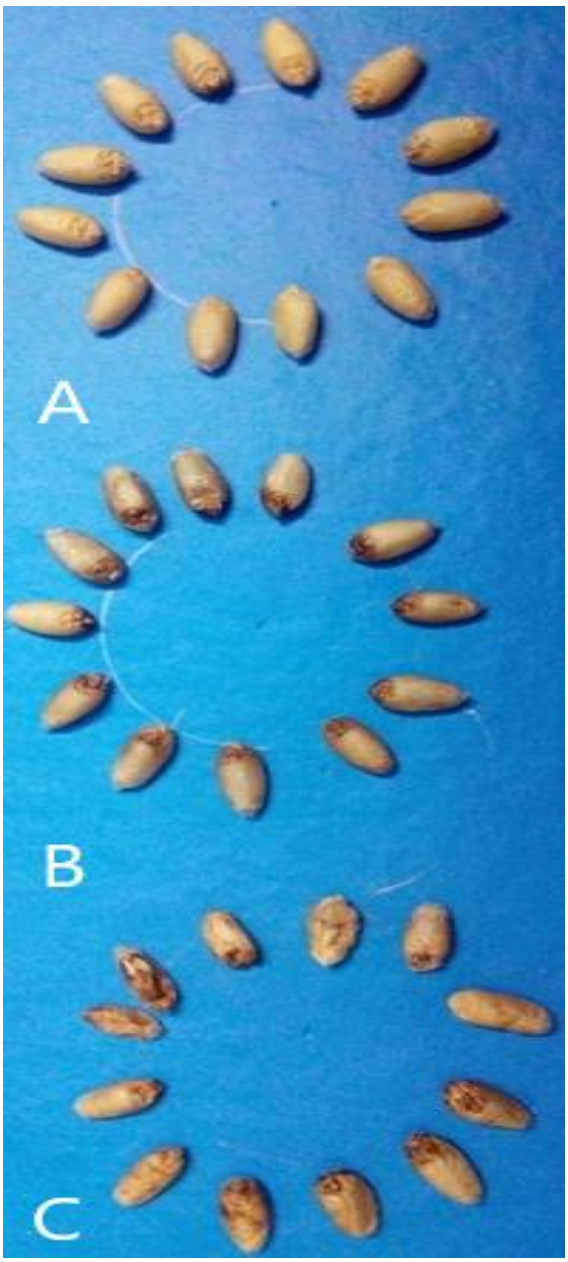


Plate 25. A. Pure seeds/apparently healthy seeds B. Black point seeds C. Shriveled & undersized seeds

Plate 26. G_0=Grains free from discolouration (apparently healthy), G_1=Only tip of the embryo brown to blackish, G_2=Discolouration covering the whole embryo, G_3=Embryo with $^1/_4$ of the grain discoloured, G_4=Embryo with $^1/_2$ of the grain discoloured, G_5=Embryo with more than $^1/_2$ of the grain discoloured and shriveled

Table 61. Weight of apparently healthy seeds, black point seeds, shriveled & undersized seeds and inert matters of seven wheat varieties (100 g) with purity percentage

Sl. No.	Name of wheat varieties	Pure seeds/ Apparently healthy seeds (g)	Black point seeds (g)	Shriveled & undersized seeds (g)	Inert matters (g)	Purity Percentage (%)
1	**BARI Gom-25**	91.54	6.24	1.45	0.77	92.25
2	**BARI Gom-26**	89.86	7.72	1.93	0.49	90.3
3	**BARI Gom-27**	93.71	4.97	0.96	0.36	94.05
4	**BARI Gom-28**	89.71	8.49	1.24	0.56	90.21
5	**BARI Gom-29**	91.08	7.78	0.43	0.71	91.73
6	**BARI Gom-30**	93.27	5.23	1.43	0.07	93.33
7	**Kanchan (BARI)**	86.79	4.29	5.93	2.99	89.46
8	**Kanchan (DU)**	85.13	7.77	4.02	3.08	87.83

Table 62. Effect of apparently healthy seeds and black point seeds on seedling growth with vigour index

Sl. No.	Wheat variety	Shoot length (cm)		Root length (cm)		Fresh shoot weight (g)		Fresh root weight (g)		Vigour Index	
		HS	BPS	HS	BPS	HS	BPS	HS	BPS	HS	BPS
1	**BARI Gom-25**	25.56	20.2	15.4	10.06	0.17	0.1	0.19	0.11	3836.72	2461.35
2	**BARI Gom-26**	21.56	15.32	12.92	9.44	0.17	0.11	0.48	0.31	3103.2	1956.04
3	**BARI Gom-27**	24.82	16.8	30.8	20.8	0.18	0.12	0.65	0.46	5265.54	3083.2
4	**BARI Gom-28**	21.32	12.96	10.06	9.2	0.16	0.1	0.16	0.1	2792.82	1691.69
5	**BARI Gom-29**	20	13.7	18.06	14.02	0.18	0.12	0.18	0.11	3463.46	2227.02
6	**BARI Gom-30**	20.36	15.2	12.62	9.04	0.17	0.11	0.30	0.21	3034.16	1971.68
7	**Kanchan (BARI)**	15.62	8.62	10.08	7.72	0.06	0.04	0.07	0.05	2441.5	1372.56
8	**Kanchan (DU)**	15.13	8.46	9.96	7.92	0.06	0.04	0.07	0.05	2333.37	1343.16

HS=Healthy seed, BPS=Black Point seed

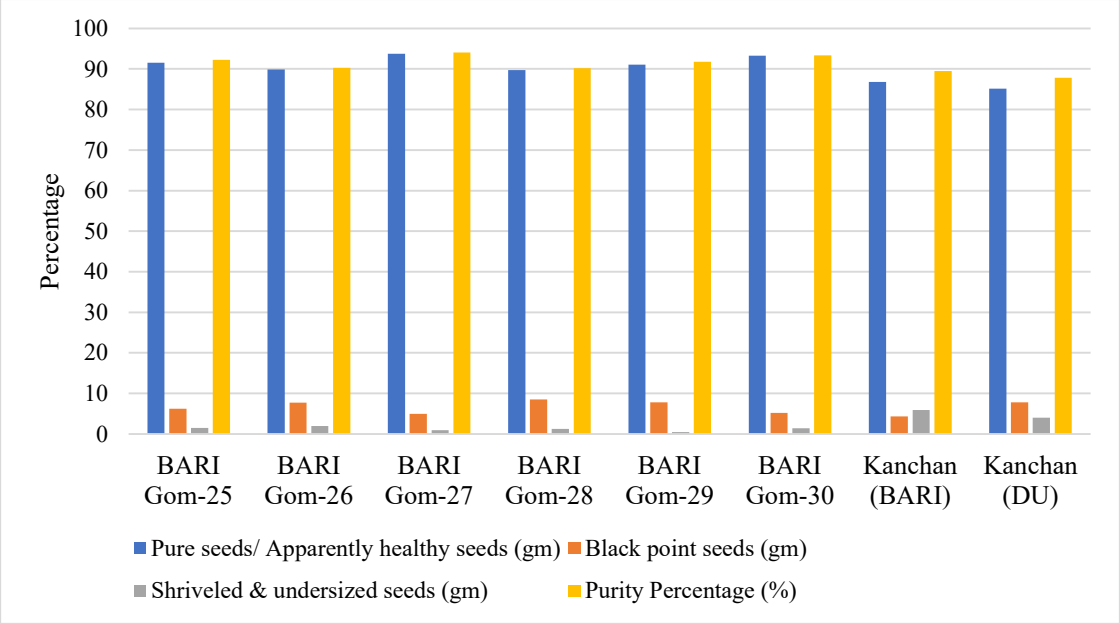


Fig. 26. Weight of pure seeds/apparently healthy seeds, black point seeds and shriveled & undersized seeds of seven wheat varieties (100 g) with purity percentage

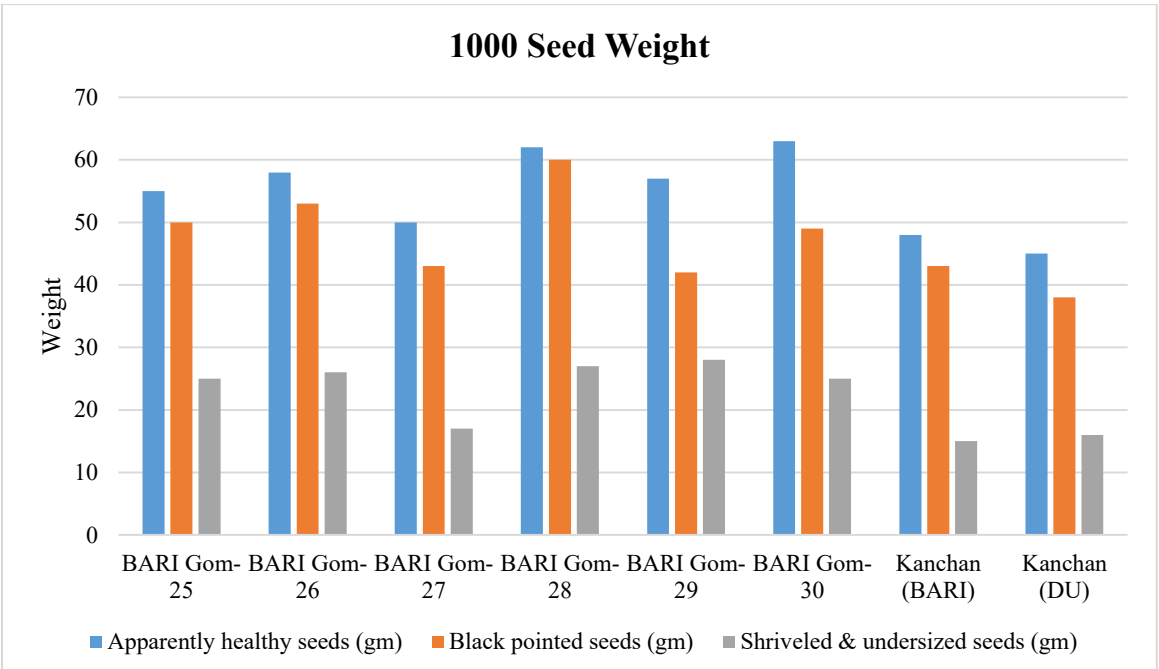


Fig. 27. 1000 seed weight of apparently healthy seeds, black point seeds and shriveled & undersized seeds of seven wheat varieties

Plate 27. A. Ten shoot from apparently healthy seeds and B. Ten shoot from black point seeds

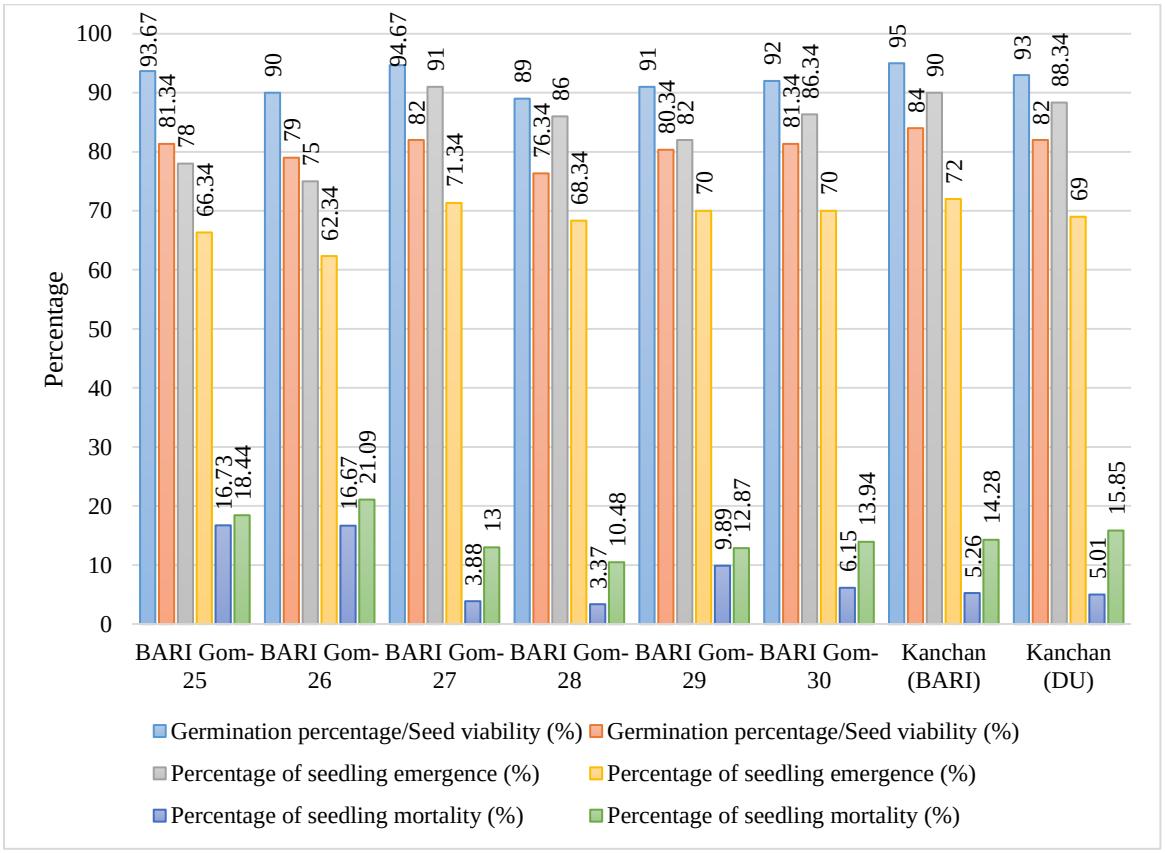


Fig. 28. Effect of apparently healthy seeds and black point seeds on seed viability, seedling emergence and mortality percentage

Table 63. Frequency percentage of fungi from apparently healthy seeds and black point seeds of wheat detected by blotter method

Sl. No.	Name of Fungi	BARI Gom-25		BARI Gom-26		BARI Gom-27		BARI Gom-28		BARI Gom-29		BARI Gom-30		Kanchan (BARI)		Kanchan (DU)	
		HS	BPS	HS	BPS	HS	BPS	HS	BPS	HS	BPS	HS	BPS	HS	BPS	HS	BPS
1	*Alternaria alternata*	6.25	14.58	5.4	13.51	4.54	18.18	4.16	16.67	9.09	18.18	9.52	19.05	-	10.53	4.35	26.1
2	*Alternaria triticina*	2.08	6.25	-	5.4	-	9.09	-	-	-	4.54	-	-	5.26	5.26	-	13.05
3	*Aspergillus flavus*	4.17	8.34	-	2.7	4.53	13.64	-	8.33	9.09	22.73	4.76	14.28	-	10.53	4.35	8.69
4	*Aspergillus fumigatus*	6.25	6.25	2.7	8.12	4.54	18.18	8.33	16.67	9.09	22.73	9.52	28.57	-	-	-	-
5	*Aspergillus niger*	16.7	33.34	5.4	21.62	-	4.54	-	8.33	-	9.09	-	4.76	10.5	10.53	-	8.69
6	*Aspergillus terrous*	10.4	22.92	8.12	8.12	-	-	-	-	-	-	-	-	-	5.26	-	-
7	*Bipolaris oryzae*	-	-	-	-	-	-	-	-	-	-	-	4.76	-	-	-	-
8	*Bipolaris sorokiniana*	-	8.34	5.4	10.81	9.09	22.73	-	12.5	4.54	13.64	4.76	19.05	10.5	26.3	8.69	30.45
9	*Bipolaris tetramera*	2.08	6.25	2.7	13.51	-	4.54	-	8.33	-	-	-	9.52	10.5	21.05	8.69	17.39
10	*Chaetomium* sp.	10.4	12.5	-	8.12	-	4.54	-	-	9.09	9.09	-	9.52	-	-	-	-
11	*Cladosporium* sp.	-	-	-	-	-	-	8.33	-	-	-	-	-	-	-	4.35	-
12	*Curvularia lunata*	8.34	8.34	2.7	13.51	4.54	9.09	-	12.5	-	9.09	4.46	9.52	5.26	15.79	13	26.09
13	*Curvularia pallescens*	-	2.08	-	8.12	-	-	-	-	-	-	-	4.46	-	-	4.35	8.69
14	*Curvularia* sp.	-	-	-	-	-	4.54	-	4.16	-	-	-	-	-	-	-	-
15	*Drechslera* sp.	-	-	-	-	-	-	12.5	-	-	-	-	-	-	-	4.35	-
16	*Epicoccum* sp.	-	4.17	-	-	-	-	-	-	-	2.27	-	-	-	-	-	-
17	*Fusarium* sp.	2.08	8.34	-	8.12	-	9.09	4.16	4.16	9.09	9.09	4.46	9.52	10.5	26.31	13	21.74
18	*Nigrospora* sp.	-	2.08	-	-	-	-	-	-	4.54	9.09	-	-	-	-	-	4.35
19	*Penicillum* sp.	-	-	-	8.12	-	-	-	8.33	-	-	4.46	4.46	-	-	4.35	-
20	*Rhizopus* sp.	-	2.08	-	-	-	9.09	-	-	-	-	-	9.52	-	-	-	-
21	*Trichoderma* sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

HS = Healthy seed, BPS = Black point seed

Table 64. Frequency percentage of fungi from apparently healthy seeds and black point seeds of wheat detected by agar plate method

Sl. No.	Name of Fungi	BARI Gom-25		BARI Gom-26		BARI Gom-27		BARI Gom-28		BARI Gom-29		BARI Gom-30		Kanchan (BARI)		Kanchan (DU)	
		HS	BPS	HS	BPS	HS	BPS	HS	BPS	HS	BPS	HS	BPS	HS	BPS	HS	BPS
1	*Alternaria alternata*	6.25	18.75	5.88	11.76	5.26	21.05	4.76	14.28	9.09	13.64	5.88	11.76	4.76	14.28	9.09	27.24
2	*Alternaria triticina*	-	-	-	5.88	-	-	-	-	4.54	13.64	-	5.88	4.76	23.8	4.54	9.09
3	*Aspergillus flavus*	-	18.75	-	5.88	5.26	-	4.76	-	-	-	5.88	-	9.52	-	-	9.09
4	*Aspergillus fumigates*	-	25	5.88	23.53	-	-	4.76	28.57	9.09	22.73	-	11.76	-	23.8	-	27.24
5	*Aspergillus niger*	6.25	-	-	5.88	-	10.53	-	9.52	-	4.54	-	-	-	-	4.54	13.62
6	*Aspergillus terrous*	-	-	-	-	5.26	-	-	-	4.54	-	-	-	-	-	-	4.54
7	*Bipolaris oryzae*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	*Bipolaris sorokiniana*	6.25	18.75	5.88	17.65		5.26	9.52	42.86	9.09	45.45	11.76	11.76	9.52	33.32	4.45	22.25
9	*Bipolaris tetramera*	-	-	-	-	5.26	-	-	-	-	-	-	11.76	-	4.76	-	9.09
10	*Chaetomium* sp.	-	-	-	-	-	-	-	-	-	-	-	11.76	-	-	-	-
11	*Cladosporium* sp.	6.25	-	-	-	-	-	-	-	-	-	-	-	9.52	-	-	-
12	*Curvularia lunata*	-	6.25	-	11.76	-	5.25	-	9.52	4.54	-	5.88	17.65	9.52	9.52	-	9.09
13	*Curvularia pallescens*	-	-	-	-	-	5.26	-	-	-	9.09	5.88	5.88	-	-	-	-
14	*Curvularia* sp.	-	-	5.88	-	-	-	4.76	-	-	-	-	-	-	-	-	-
15	*Drechslera* sp.	-	-	-	-	-	21.05	-	-	-	-	-	-	-	-	14.28	-
16	*Epicoccum* sp.	-	6.25	-	-	-	-	-	-	-	-	-	2.94	2.38	-	-	-
17	*Fusarium* sp.	6.25	12.5	-	5.88	5.26	5.26	4.76	-	4.54	9.09	-	-	9.52	-	-	13.62
18	*Nigrospora* sp.	-	6.25	-	-	-	-	-	-	-	9.09	-	-	-	9.52	-	-
19	*Penicillum* sp.	6.25	-	-	-	-	5.26	-	-	-	-	-	-	-	-	-	-
20	*Rhizopus* sp.	-	-	-	5.88	-	-	-	-	-	-	-	-	4.76	-	-	-
21	*Trichoderma* sp.	-	-	5.88	-	-	-	-	-	-	-	-	-	-	-	-	4.76

HS = Healthy seed, BPS = Black point seed

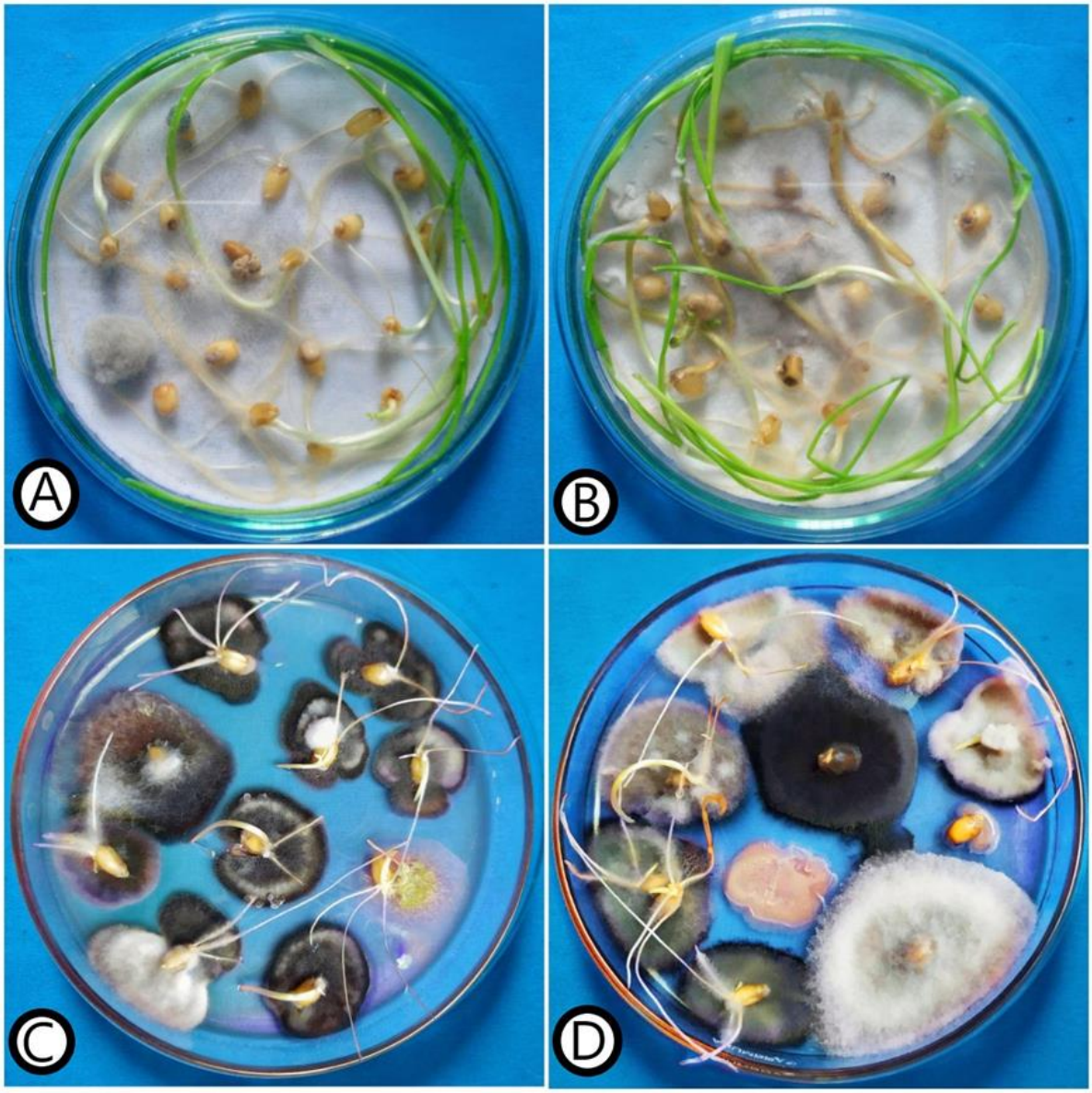


Plate 28. A and B. Fungi isolation on blotter method, C and D. Fungi isolation on tissue planting/agar plate method from wheat seeds

CHAPTER FIVE
SUMMARY

CHAPTER FIVE

SUMMARY

Wheat (*Triticum aestivum* L.) is a dominant cereal crop and staple food of millions of people in the world. Wheat, the second grain crop in Bangladesh, is mainly grown in north-western part of the country as a winter crop. Bipolaris leaf blight (BpLB) caused by *Bipolaris sorokiniana* (Sacc.) Shoemaker, is the most prevalent disease of wheat in Bangladesh. The present studies were conducted to find out the variability of *B. sorokiniana* and management of leaf blight of wheat. Investigation on variability was done on the basis of cultural, morphological, pathological and physiological variation among *B. sorokiniana* isolates and compatibility test. Attempts were also made to develop different control measure strategies using the most effective fungicides in different doses and spray schedules, searching resistance sources and estimate the yield loss caused by the disease. *In vitro* evaluation of angiospermic plant leaf extracts and bio-control agents was tried against *B. sorokiniana* to minimize the mycelial growth of the fungus.

Thirty-five fungal species, representing 20 genera were found to be associated with BpLB infected leaves of twenty-one wheat varieties, collected from eight districts (Dhaka, Gazipur, Dinajpur, Joypurhat, Pabna, Sirajganj, Kushtia and Chuadanga) of Bangladesh. The isolated fungi were *Alternaria alternata* Keissler, *A. triticina* Prasada & Prabhu, *Arthrinium* Kunze ex Fr., *Aspergillus flavus* Link, *A. fumigatus* Fresen., *A. niger* Tiegh., *A. terreus* Thom., *Aspergillus* sp. Link, *Bipolaris cynodontis* (Marig.) Shoem., *B. oryzae* (Breda De Haan) Shoem., *B. sorokiniana* (Sacc.) Shoem., *B. tetramera* (Mckinney) Shoem., *B. victoriae* (Meehan & Murphy) Shoem., *Bispora antenata* (Pers.) Mason, *Cheatomium globosum* Kunze ex Fr., *Chaetophoma* Cooke, *Cladosporium cladosporioides* (Fresen.) de Vries, *Coniothyrium* sp. Corda, *Curvularia affinis* Boedijn, *C. lunata* Boedijn, C. *pallescens* Boedijn, *Drechslera dematioidea* (Bub. & Wrob.) Subram. & Jain, *D. hawaiiensis* (Bugnicourt) ex M.B. Ellis; Subram. & Jain, *Epicoccum purpurascens* Ehrneb. ex Schlecht, *Eurotium* sp., *Fusarium moniliforme* Sheldon, *F. nivale* Ces., *F. semitectum* Berk. & Rav., *Nigrospora oryzae* (Berk. & Br.) Petch., *N. sacchari* (Speg.) Mason, *Penicillium digitatum* (Fr.) Sacc., *Pestalotiopsis guepinii* (Desm.) Stay., *Rhizopus stolonifer* (Ehrenb.: Fr.) Vuill, *Syncephalastrum racemosum* Cohn ex J. Schröt. and *Trichoderma viride* Pers. The frequency percentage of *B. sorokiniana* ranged from 6.67–85.71% owing to different wheat varieties. The average incidence of *B. sorokiniana* ranged from 6.67–53.55% from eight

districts, with mean frequency percentage of 32.5% and 30.62% based on districts data and yearly data, respectively. It was the maximum in Joypurhat district and minimum in Kushtia district. Among these fungi, three fungul species (*Bipolaris cynodontis*, *Drechslera hawaiiensis* and *Pestalotiopsis guepinii*) associated with wheat is new record for Bangladesh. The presence of two new fungi, *Bispora antenata* and *Drechslera dematioidea* also new record in Bangladesh. The major fungi associated with *B. sorokiniana* were *Alternaria alternata, Cladosporium cladosporioides, Curvularia lunata* and *Fusarium semitectum.*

A total number of 174 isolates of *B. sorokiniana* were obtained from 120 samples of leaf blight infected wheat leaves and seeds collected during 2011 to 2014. Among them detail morphological studies were done on 150 isolates. Based on colony colours & textures of different isolates on PDA medium, they were categorized in eight different cultural groups/types. They were–Black Mat (B-M), Black Fluffy (B-F), Blackish Ash Mat (BlA-M), Brownish Ash Fluffy (BrA-F), Ash Mat (A-M), Whitish Ash Mat (WA-M), Greenish Ash Fluffy (GA-F), Pinkish White Mat (PW-M). Number of isolates belongs to different groups varied with the percentages of 12.0, 4.67, 13.34, 6.67, 25.34, 14.0, 21.34 and 2.67%, correspondingly. Detail colony characters' observations were made on colony colour (upper view and reverse view), colony texture, colony margin, growth rate, growth pattern and radial mycelial growth measurement. Though most of the isolates showed growth rate between 4.0–5.0 mm/day, but minimum to maximum growth rate/day ranged from 1.67–9.00 mm/day. That mean some isolates were very slow in growth, some were very fast growing, while most isolates were moderate growing. Conidial variability observations were made on length & breadth of conidia, septation, colour, shape, spore abundance and length & breadth of conidiophores of 150 isolates of *B. sorokiniana*. The length of conidia ranged from the lowest to the highest 23.4–98.1 µm. The conidial breadth ranged from the lowest to the highest 13.3–27.9 µm. Most of the isolates mean conidial length and breadth ranged from 40–60 µm and 17–20 µm. The lowest conidial length was noticed in GJBEnL-03 and the highest conidial length was noticed in GJB29S-05. The lowest and the highest conidial breadth were noticed in DiWRPdL-02 and GJB29S-05. Number of pseudo-septation in conidia ranged from 3–12, but in most cases it was 5–7. The highest 12 septation was found in GJB26S-02 isolated from seed of BARI Gom-26. Except for three isolates, GJBKlL-09, JSDSvL-18 and GJB29S-03, which generated both straight and slightly curved shaped conidia in culture, all isolates produced straight shaped conidia. Conidia range in colour from light brown to dark brown. The maximum number of isolates with brown coloured conidia was 82, with light

brown, light brown to brown, brown to deep brown and deep brown coloured conidia being 03, 29, 16 and 20, respectively. Conidial abundance was also measured by less sporulated (+), medium sporulated (++) and profusely sporulated (+++). Most of the isolates were profusely sporulated, which was also noticed by blackish shiny colony colour and velvety colony texture. Another feature was observed that most of the isolates obtained from seed were larger than the isolates from leaf. Source also effect the pathogen to show conidial diversity.

According to detached leaf assey, the pathogenic variability of *B. sorokiniana* isolates from eight different cultural groups was examined on healthy mature leaves of the susceptible wheat cultivar Kanchan. Mean lesion coverage on leaf (%) ranged from 21.25–70%, where the highest was observed in isolate JSVPdL-01 and the lowest was in JSDSvL-09. Considering mean lesion coverage on detached leaf the highest aggressiveness (4.2) was recorded in cultural group Black Mat and the lowest aggressiveness (3.0) was recorded in cultural group Pinkish White Mat. In respect of isolates most show mean lesion coverage ranged from 40.0–60.0%, so the aggressiveness was 4.0. The aggressiveness of pathogens was strongly linked to the colonies, that are dark and black coloured. To discover the variation among morphologically similar isolates from same or different group of *B. sorokiniana* isolates, mycelial compatibility response was utilized. Three distinct behaviors were seen in the hyphal overlapping region, allowing us to categorize the confrontations as incompatible, compatible or partially compatible. Eight of the 31 confrontations were compatible, six were partially compatible and seventeen were incompatible. The confrontations revealed that 54.84 percent of the participants were vegetatively incompatible, that means diversity prevails in the population.

Eight systemic fungicides (Contaf, Diathane, Folicur, Knowin, Ridomil, Score, Secure and Tilt) and two protectant fungicides (Capvit and Silika) were evaluated *in vitro* against sixteen isolates of *B. sorokiniana* belongs to eight different cultural groups (two from each group). All the fungicides were used in five concentrations (100, 200, 300, 400 and 500 ppm). All the fungicide visibly inhibited mycelial growth over the control but Tilt (propiconazole) and Folicur (tubeconazole) were the most efficient fungicides against the pathogen (100% mycelial growth suppression at the lowest concentrations). Contaf (hexaconazole) and Score (difenoconazole) may be recommended as second choice and mancozeb fungicides and the combinations as third option.

Antifungal properties of ethanol extracts of leaf of ten angiospermic plants belongs to different families (*Allamanda cathartica* L., *Azadirachta indica* A. Juss., *Catharanthus roseus* (L.) G. Don, *Citrus limon* L., *Lantana camara* L., *Lawsonia inermis* L., *Moringa oleifera* Lam., *Polyalthia longifolia* Sonn., *Psidium guajava* L. and *Tagetes erecta* L) were evaluated in four concentrations (5, 10, 15 and 20%) against sixteen isolates of *B. sorokiniana* belongs to eight different cultural groups. *Azadirachta indica* proved best against all isolates of *B. sorokiniana*, percent inhibition range was 74.56 to 84.88% followed by *Citrus limon* (71.73–75.99%), then *Allamanda cathartica* (69.43–75.16%) and *Catharanthus roseus* (61.58–72.49%). The antagonistic potential of the six soil fungi (*Aspergillus flavus*, *A. fumigatus*, *A. niger*, *Penicillium* sp., *Trichoderma harzianum* and *T. viride*) was evaluated towards sixteen *B. sorokiniana* isolates belongs to eight distinct cultural groups. All antagonists inhibited *B. sorokiniana* growth in colony interaction (37.91–79.02%), having *T. harzianum* the highest inhibition effect, 61.69–79.02% and *T. viride* second highest inhibition effect 62.55–72.32%. The maximum inhibition was found owing to volatile metabolites of *T. harzianum* 44.14–61.34% and *T. viride* inhibition range was 37.34–53.98%. The best inhibition was achieved due to culture filtrate (non-volatile substances) at 20% concentration by *T. harzianum* 47.28–68.14% and second highest *T. viride* was 30.32 to 59.57%. The present investigation established the powerful efficacy of *T. harzianum* and *T. viride* against *B. sorokiniana* causing leaf blight disease of wheat.

Four distinct field trials were done at the experimentan fied, Plant Pathology Division, Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur, for the control of wheat leaf blight disease (BpLB) caused by *Bipolaris sorokiniana*, using susceptible wheat variety Kanchan. Title of the experiments were–i) Determination of number of fungicide sprays to minimize Bipolaris leaf blight (BpLB) disease of wheat ii) Standardization of doses of fungicide named Tilt and Folicure against Bipolaris leaf blight (BpLB) disease of wheat iii) Estimation of yield loss of wheat due to Bipolaris leaf blight (BpLB) and iv) Screening of wheat germplasms against Bipolaris leaf blight (BpLB). For experiment 1, total eight treatments belong to two fungicides [Tilt 250 EC (0.05%) and Folicur 250 EC (0.05%)] were evaluated using RCBD design with three replications, T_1 (35-55-75) days, T_2 (40-60) days, T_3 (45-65) days, T_4 (55-70) days, T_5 (55-75) days, T_6 (65-80) days, T_7 (75) days and T_8 Control. According to results, T_6 or treatment-6 was the best, that means fungicides two spray at 65 days and 80 days after sowing will be more effective than the standard three spray 35 days, 55 days and 75 days. As a fungicide Tilt 250 EC (0.05%) and Folicur 250 EC (0.05%) more

or less same compare to yield and disease severity. Maintaining the standard fungicide spray schedule [(35-55-75) days after sowing], spray with standard dose (D_3 = 0.1%), lower than standard dose (D_1 = 0.05% and D_2 = 0.075%) and without dose (D_4 = Control) were done, which were called as treatments. Total four treatments belong to each fungicide [Tilt 250 EC and Folicur 250 EC] were evaluated using RCBD design with three replications. D_3 or dose-3 was the best according to calculations, that means fungicides sprays as per standard dose (0.1%) will be more effective than the different lower doses. There was no significant difference as a fungicide Tilt 250 EC and Folicur 250 EC. For experiment 3, starting from the initial emergence of disease symptoms, Tilt 250 EC @ 0.1% was sprayed at a 15-day interval, to reduced the leaf blight disease and loss in grain yield. The average yield loss was 28.68–29.19%, with 1000 grain weight more than 15%, respectively. For experiment 4, a total of 123 genotypes from various sources were tested against BpLB in the field under disease development conditions using an Alpha Lattice design with two replications. From my study only 02 genotypes (1.63%) were selected as resistant genotypes (R), 48 genotypes (39.02%) were moderately resistant (MR), 67 genotypes (54.47%) were moderately susceptible (MS) and 06 genotypes (4.88%) were susceptible (S) genotypes. There was no genotype as highly susceptible (HS).

Freshly harvested eight seed lots consisted one from each six varieties (BARI Gom-25, BARI Gom-26, BARI Gom-27, BARI Gom-28, BARI Gom-29, BARI Gom-30) from BARI and two from variety Kanchan (each from BARI and DU campus) were studied. Seeds were classified into three classes–apparently healthy seeds, black point seeds and shriveled & undersized seeds. According to Gilchrist (1985) seeds were also categorized G_0–G_5 grade. Purity percentage of wheat seeds varied from 87.83–94.05%. Differences of germination percentage between apparently healthy seeds and black point seeds were up to 10%. Percentage of seedling emergence varied in apparently healthy seeds 75–91%, where in case of black point seeds the range was 62.34–72%. Vigour index was best in BARI Gom-27 (5265.54 in apparently healthy seeds and 3083.2 in black point seeds) followed by BARI Gom-25 (3836.72 and 2461.35 in apparently healthy and black point seeds, respectively). In total more than twenty species belong to twelve genera of fungi were indentified, the most pre-dominant fungi were *A. niger*, *A. flavus*, *A. alternata*, *B. sorokiniana*, *C. lunata* and *Fusarium* spp. Frequency percentage range of *B. sorokiniana* was 4.54–30.45% in blotter method and 4.45–45.4% in agar plate method. *B. sorokiniana* was isolated from all the seven varieties with higher percentage in agar plate method and also from black point seeds.

CHAPTER SIX
CONCLUSION
&
RECOMMENDATIONS

CHAPTER SIX

CONCLUSION & RECOMMENDATIONS

Based on the findings of the present investigation the following conclusions are drown:

- A total of 35 fungal species belongs to 20 genera were isolated from BpLB infected wheat leaves of twenty-one varieties from eight districts of Bangladesh.
- Association of *Bipolaris cynodontis*, *Drechslera hawaiiensis* and *Pestalosiopsis guepinii* with wheat are new record. *Bispora antenata* and *Drechslera dematioadea* are new record for Bangladesh.
- The frequency percentage of *B. sorokiniana* ranged from 6.67–85.71% owing to different wheat varieties. It was maximum in Joypurhat district and minimum in Kushtia district.
- Total 174 isolates of *B. sorokiniana* were obtained and 150 isolates under 8 different cultural groups were showed morphological, physiological and pathogenic variability at different levels among themselves.
- Based on colony colours & textures of different isolates on PDA medium, they were categorized in eight different cultural groups/types. They were-Black Mat (B-M), Black Fluffy (B-F), Blackish Ash Mat (BlA-M), Brownish Ash Fluffy (BrA-F), Ash Mat (A-M), Whitish Ash Mat (WA-M), Greenish Ash Fluffy (GA-F), Pinkish White Mat (PW-M). Number of isolates belongs to different groups varied with the percentages of 12.0, 4.67, 13.34, 6.67, 25.34, 14.0, 21.34 and 2.67%, correspondingly.
- Conidia ellipsoid, briown to dark brown, mostly straight or slightly curved, wall thick but less so towards the ends, broadest in the middle, ends rounded, scar clear within the basal cell. The length and breadth of conidia ranged from 23.4–98.1 µm and 13.3–27.9 µm. Most of the isolates mean conidial length and breadth ranged from 40–60 µm and 17–20 µm.
- In pathogenic variability, mean lesion coverage on leaf (%) ranged from 21.25–70% and aggressiveness range was 3.0–4.2.
- Compatibility test reveals that 53.34% confrontations were incompatible. So, there must be variation among *B. sorokiniana* population.

- Ten fungicides were evaluated against *B. sorokiniana* isolates, Tilt 250 EC and Folicur 250 EC were identified as the complete inhibiting chemical fungicides.
- Out of 10 plant extracts, *Azadirachta indica, allamanda cathrtica* and *Citrus limon* showed best inhibition on *B. sorokiniana* isolates.
- The present investigation suggests that *Trichoderma harzianum* and *T. viride* showed promising inhibitory effect on *B. sorokiniana* isolates.
- *In vivo* experiment-1, determination of number of fungicide sprays to minimize Bipolaris leaf blight (BpLB) disease of wheat, out of 8 treatments, T_6 (65-80) showed lowest disease severity and highest yield performance. Effective fungicides two spray at 65 days and 80 days after sowing will be more fruitful than the standard three spray schedule.
- *In vivo* experiment-2, standardization of doses of fungicide named Tilt and Folicure against Bipolaris leaf blight (BpLB) disease of wheat, out of 4 treatments, D_3 (0.1%) showed lowest disease severity and highest yield performance. Effective fungicides spray as per standard dose (0.1%) will be more operative.
- *In vivo* experiment-3, estimation of yield loss of wheat due to Bipolaris leaf blight (BpLB), the average yield loss was 28.68–29.19%, with 1000 grain weight more than 15%, respectively.
- *In vivo* experiment-4, screening of wheat germplasms against Bipolaris leaf blight (BpLB), only 02 genotypes (1.63%) were selected as resistant genotypes (R), 48 genotypes (39.02%) were moderately resistant (MR), 67 genotypes (54.47%) were moderately susceptible (MS) and 06 genotypes (4.88%) were susceptible (S) genotypes. There was no genotype as highly susceptible (HS).
- Seed health test reveals that all seed samples were infected by *B. sorokiniana* in various degrees and black point seeds show lower seed viability, germination and emergence percentage, vigour index etc.

Recommendations

- *Bipolaris sorokiniana* is a serious pathogen, not only because it results in significant yield losses, but also because it can attack most wheat organs, including roots, crown area, stems, leaves and kernels.
- This means that management strategies should not only focus on limiting the presence of the fungus in the aerial parts of the plants, but attention should be given to *B. sorokiniana* inoculum present in seeds and also in soil.
- In addition, it is important to develop an integrated disease management programmes for managing *B. sorokiniana* using cultural practices, biological control and chemical fungicides.
- Since the search for biocontrol agents has been given more attention during recent years, it is important to find antagonistic strains that can complement cultural and chemical practices in the field.
- In small scale, *Azadirachta indica* and *Citrus limon* can be used for controlling diseases and production of healthy grains.
- Findings of this research work will be helpful for designing a proper management of leaf blight of wheat.

REFERENCES

REFERENCES

Abdul-Baki AA and Anderson JD 1973. Vigour determination in soybean seed by multiple criteria. Crop Sci. **13**: 630-633.

Adlakha KL, Wilcoxson RD and Ray Chaudhari SP 1984. Resistance of wheat to leaf spot caused by *Bipolaris sorokiniana.* Plant Disease. **68**(4): 320-321.

Adhikary SK 2000. Study on the variability in isolates of *Bipolaris sorokiniana* causing spot blotch of wheat. PhD Thesis. Department of Plant Pathology, BSMRAU, Gazipur, Bangladesh.

Aggarwal PK, Talukdar KK and Mall RK 2000. Potential yields of rice-wheat system in the Indo-Gangetic Plains of India. Consortium paper series 10. Rice-wheat consortium for the Indo-Gangetic Plains. New Delhi, India. 16 pp.

OECD. Environment Directorate Development Co-operation Directorate. *In:* Agrawala S, Ota T, Ahmed AU, Smith J, Aalst MV (Eds.) 2008. Development and climate changes in Bangladesh: focus on coastal flooding and the Sundarbans pp.1-70.

Ahmed AU 1989. Resistance in spring wheat to the various diseases caused by *Cochliobolus sativus*. PhD Thesis. Department of Plant Pathology, Montana State University, USA.

Ahmed F 2001. Studies on pathogenicity of *Bipolaris sorokiniana* and its effect on grain filling of wheat in Bangladesh. PhD Thesis. Department of Plant Pathology, Bangladesh Agriculture University, Mymensingh.

Ahmed F 2007. Reaction of wheat genotypes to leaf blight caused by *Bipolaris sorokiniana*. Bangladesh J. Pl. Pathol. **23** (1/2): 75-78.

Ahmed HU 1986. Prevailing Wheat Diseases in Bangladesh. *In:* Anonymous (ed.), Third National Wheat Training Workshop. Wheat Research Centre, BARI, Joydebpur, Gazipur. pp 124-134.

Ahmed HU and Hossain MM 1985. Crop diseases survey and establishment of an herbarium at BARI. Plant Pathology Division, BARI, Joydebpur, Gazipur. pp 107.

Ahmed SM and Meisner C 1996. Wheat research and development in Bangladesh. Bangladesh Australia wheat improvement project and CIMMYT-Bangladesh. Published by Bangladesh Australia wheat improvement project and CIMMYT-Bangladesh.

Ahmed AU, Rahman MZ, Bhuiyan KA and Mian IH 1997. Variation in the isolates of *Bipolaris sorokiniana* from wheat. Bangladesh J. Pl. Pathol. **13** (1&2): 29-36.

Ahmed MF and Islam MT 2000. Efficacy of some fungicides and plant extracts against *Bipolaris oryzae*. MS Thesis. Dept. of Plant Pathology. Bangladesh Agriculture University, Mymensingh.

Ahmed F, Hossain I and Aminuzzaman FM 2003. Effect of different pathotypes of *Bipolaris sorokiniana* on leaf blight severity and yield contributing characters of wheat cv. Kanchan inoculated at maximum tillering stage. Pak. J. Biol. Sci. **6**(7): 693-696.

Akram M and Singh A 2001. Variability and characterization of host response to *Helminthosporium sativum*, incitant of spot blotch of wheat. Indian Phytopath. **54**(4): 429-434.

Akram M and Singh A 2003. Screening and host response characterization of wheat against *Bipolaris sorokiniana*. Indian Phytopath. **56**(2): 201-204.

Akram M and Singh A 2003. Determination of wheat varieties tolerant to spot blotch disease, *Bipolaris sorokiniana* (Sacc.) Shoem. Annals Pl. Prot. Sci. **11**(1): 75-78.

Akhter N, Begum MF, Alam S and Alam MS 2006. Inhabitory effect of different plant extracts, cow dung and cow urine on conidial germination of *Bipolaris sorokiniana*. J. bio-sci. **14**: 87-92.

Akter MT, Hossain KS and Bashar MA 2014. Antagonistic potentiality of rhizosphere fungi against leaf spot and fruit rot pathogens of brinjal. Bangladesh J. Bot. **43**(2): 213-217.

Alam KB and Saha NK 1991. Helminthosporium leaf blight of wheat-a new problem of Bangladesh. A paper presented at the CIMMYT regional workshop on Helminthosporium leaf Blight, December 2-3, Kathmundu, Nepal. pp.12.

Alam KB, Shaheed MA, Ahmed AU and Malaker PK 1994. Bipolaris leaf blight (spot blotch) of wheat in Bangladesh. *In*: Wheat is heat stressed environments: irrigated, dry Areas and rice-wheat farming system. Saunders DA and Hettel GP (Eds.), CIMMYT, El Batán, Mexico. pp. 339-342.

Alam KB, Malaker PK, Shaheed MA, Ahmed MU, Ahmed F and Haque MS 1995. Yield loss assessment of wheat due to Bipolaris leaf blight in Bangladesh. Bangladesh J. Pl. Pathol. **11** (1&2): 35-38.

Alam KB, Banu SP and Shaheed MA 1997. Occurrence and significance of spot blotch in Bangladesh. *In*: Helminthosporium blights of wheat: spot blotch and tan spot. Duveiller E, Dubin HJ, Reeves J and McNab A (Eds.), Proc. International workshop, 9-14 February. CIMMYT, El Batán, Mexico. pp. 63-66.

Alamgir M 1980. Famine in South Asia: political economy of mass starvation. **18**: 420. Cambridge MA: Oelgeschlager. Gunn & Hain.

Alcorn JL 1988. The taxonomy of "*Helminthosporium*" species. Ann. Rev. Phytopathol. **26:** 37-56.

Aminuzzaman FM and Hossain I 2005. Pathotype variation of *Bipolaris sorokiniana* on wheat. Bangladesh J. Pl. Pathol. **21**(1&2): 81-88.

Aminuzzaman FM, Hossain I and Ahmed F 2010. Cultural variation and pathogenicity of *Bipolaris sorokiniana* on wheat in Bangladesh. Int. J. Agriculture, Enviornment and biotechnology. **3**(1): 93-98.

Ammad F, Moumen O, Gasem AB, Othmane S, Hisashi KN, Zebib B and Merah O 2018. The potency of lemon (*Citrus limon*) essential oil to control some fungal diseases of grapevine wood. Comptes Rendus Biologies. **341**(2): 97-101.

Ainsworth G 1961. Ainsworth's and Bisby's Dictionary of The Fungi. 5th ed. London. Commonwealth Agricultural Bureau. pp: 1-547.

Angdembe B, Dhakal N, SGC, Pant KR and Manandhar HK 2019. *In vitro* evaluation of commercially available fungicides against *Bipolaris sorokiniana*, the cause of spot blotch of barley. J. Agriculture and Forestry University. **3**: 105-111.

Anonymous 1989. Chemical control of Bipolaris leaf blight of wheat. Ann. Rept. BARI, Joydebpur, Gazipur. 28 pp.

Anonymous 1992a. Assessment of yield loss due to foliar pathogens. Ann. Rept. for the year 1991-92. BARI, Joydebpur, Gazipur. 21 pp.

Anonymous 1992b. Assessment of yield loss of wheat due to leaf blight disease at farmer's field. Ann. Rept. for the year 1991-92. BARI, Joydebpur, Gazipur. pp 45-46.

Anonymous 1993. Assessment of yield loss of wheat due to Bipolaris leaf blight at farmer's field. Ann. Rept. for the year 1991-92. BARI, Joydebpur, Gazipur. 46 pp.

Anonymous 1994. Assessment of yield loss of wheat due to Bipolaris leaf blight at farmer's field. Ann. Rept. BARI, Joydebpur, Gazipur. 48 pp.

Anonymous 2013. Ann. Germplasm evaluation of wheat against Bipolaris leaf blight under field condition. Rept. BARI, Joydebpur, Gazipur. 16 pp.

Anonymous 1995. Report of co-ordinated experiment, 1994-95. Crop Protection (Pathology), Parasher M, Nagarajan S, Goel LB and Kumar J (Eds.). AICWIP, Directorate of Wheat Research, Karnal, India. **1**: 43-54.

Anonymous 1996. Standard evaluation system for rice. 4th Edition. International Rice Research Institute, Philippines. 56 pp.

Anonymous 1999. Indian Agriculture. Indian Economic Data Research Centre, New Delhi, India, pp. 72-83.

Anonymous 2008. Statistical year book of Bangladesh-2006. Bangladesh Bureau of Statistics, Planning Division, Ministry of Planning, Government of the People's Republic of Bangladesh.

Asad S, Iftikhar S, Munir A and Ahmad I 2009. Characterization of *Bipolaris sorokiniana* isolated from different agro-ecological zones of wheat production in Pakistan. Pak. J. Bot. **41**(1): 301-308.

Ashok M, Patel NM, Patel DB, Patel JR and Jadon BS 1989. Economical spray schedule for the management of leaf blight/blotch of wheat. Pestology. **13**(8): 22-25.

Ashworth LJ Jr, Lahr KA and Collons 1960. Pathogenic specialization of *Helminthosporium sativum*. Phytopathol. **50**: 627(Abstr.).

Ashrafuzzaman H and Hossain I 1992. Antifungal activity of crude extracts of plants against *Rhizoctonia solani* and *Bipolaris sorokiniana*. Proc. BAU Res. Prog. **6**: 188-192.

Bahadar K, Munir A and Asad S 2016. Management of *Bipolaris sorokiniana* the causal pathogen of spot blotch of wheat by eucalyptus extracts. J. Plant Pathol. Microbiol. **7**(1): 326.

Baker R 1988. *Trichoderma* spp. as plant growth stimulants. CRC Crit. Rev. Biotechnol. **7**: 97-106.

Baker KF and Cook RJ 1983. The nature and practice of biological control of plant pathogens. St. Paul, MN, USA: American Phytopathological Society.

Bakonyi J, Fischl G and Falusi J 1993. Traits of infection of *Bipolaris sorokiniana* (Sacc.) Shoem. and *Drechslera tritici-repentis* (Died.) Shoem. seedling leaves of winter wheat. Novenytermeles Hungary. **42**(2): 127-134.

Bakonyi J, Aponyi I and Fischl G 1997. Diseases caused by *Bipolaris sorokiniana* and *Drechslera tritici-repentis* in Hungary. *In:* Helminthosporium blights of wheat: spot

blotch and tan spot. Duveiller E, Dubin HJ, Reeves J and McNab A (Eds.), CIMMYT, El Batán, Mexico. pp. 80-85.

Banglapedia 2006. BANGLAPEDIA, national encyclopedia of Bangladesh.

BARI (Bangladesh Agricultural Research Institute) 2010. Wheat production in Bangladesh: a success story.

BARI (Bangladesh Agricultural Research Institute) 2012b. Year of release and average yield of Bangladesh wheat varieties developed since 1974.

Barnett HL and Hunter BB 1972. Illustrated genera of imperfect fungi. 3rd. edition. Burgess Press. The American Phytopathological society, St. Paul, Minnesota. 241pp.

Baturo A 2006. Effects of thermotherapy, grain treatment and leaf spraying with biological control agents on spring barley health in organic system. Phytopathol. Pol. **41**: 15-26.

Bashar MA and Rai B 1994. Antagonistic potential of root-region microflora of chickpea against *Fusarium oxysporum* f. sp. *ciceri*. Bangladesh J. Bot. **23**(1): 13-19.

Bashar MA and Chakma 2014. *In vitro* control of *Fusarium solani* and *F. oxysporum* the causative agent of brinjal wilt. Dhaka Univ. J. Biol. Sci. **23**(1): 53-60.

Bashir S, Jabeen K, Iqbal S and Javed S 2019. *Lantana camara*: Phytochemical analysis and antifungal prospective. Planta Daninha. 37(3).

Bazlur Rashid AQM, Meah MB, Fakir GA 1992. Importance and distribution of seed-borne *Bipolaris sorokiniana* in wheat in Bangladesh. Bangladesh J. Plant Pathol. **8**: 5-7.

Bazlur Rashid AQM, Fakir GA and Hossain I 1983. Studies on leaf blight of wheat in Bangladesh. Bangladesh J. Agric. Sci. **10**(1): 49-57.

Bazlur Rashid AQM, Lahiri BP and Islam T 1994. Effect of *Bipolaris sorokiniana* leaf blight on some yield components and seed quality of wheat. Bangladesh J. Agril. Sci. **21**(1): 185-192.

Bazlur Rashid AQM, Fakir GA, Hossain I, Kulshrestha DD 1997. Association of *Bipolaris sorokiniana* with wheat seeds and its transmission from seed to plant. Bangladesh J. Plant Pathol. **13**: 17-20.

Bazlur Rashid AQM 1998. Effect of seed transmitted *Bipolaris sorokiniana* on growth and survival of wheat seedlings. Indian Phytopathol. **51**: 329-333.

Bazlur Rashid AQM and Fakir GA 1998. Seed-borne nature and transmission of *Bipolaris sorokiniana* in wheat. Proceedings of the 1st national workshop on seed pathology. Progress and prospect of seed pathological research in Bangladesh. Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh. 10 pp.

Bazlur Rashid AQM, Sarker K and Khalequzzaman KM 2001. Control of Bipolaris leaf blight of wheat with foliar spray of Tilt 250 EC. Bangladesh J. Pl. Pathol. **17**(1&2): 45-47.

Bazlur Rashid AQMB, Dhar RC and Khalequzzaman KM 2004. Association of *Bipolaris sorokiniana* in wheat seed and its effect on subsequent plant infection at different growth stages. J. Agric. Rural Devel., Gazipur. **2**(1): 67-72.

BBS 2019. Bangladesh Bureau of Statistics (BBS). Statistical Year Book of Bangladesh. Statistics and Informatics Division, Ministry of Planning, Government of the People's Republic of Bangladesh, Dhaka.

Benoit MA and Mathur SB 1970. Identification of species *Curvularia* on rice seed. Proc. Int. Seed Testing Ass. Proc. **35**(1): 1-23.

Bhardwaj SK 2012. Evaluation of plant extracts as antifungal agents against *Fusarium solani* (Mart.) Sacc. World J. Agricultural Sciences. **8**(4): 385-388.

Bhutia DD, Zhimo Y, Kole R and Saha J 2016. Antifungal activity of plant extracts against *Colletotrichum musae*, the post-harvest anthracnose of banana cv. Martaman. Nutrition and Food Science. **46**(1): 2-15.

Bhanupriya B, Das NH, Satyanarayana H, Mukherjee S and Sarker KK 2014. Genetic diversity of wheat genotypes based on principal component analysis in gangetic alluvial soil of west Bengal. J. Crop and Weed. **10**(2): 104-107.

Bilai VI 1966. Volatile antibiotics in fungi of the genus *Trichoderma*. Microbiologia. **25**: 458-465.

Bimb HP and Mahto BN 1992. Wheat disease report. Paper presented at winters crops technology workshop held at WRP. Bhairahwa, Nepal.

Booth C 1971. The Genus Fusarium. Commonwealth Mycological Institute, Kew, Surrey, England. 273 pp.

Brian PW, McGowan JC 1945. Viridin: a highly fungistatic substance produced by *Trichoderma viride*. Nature. **156**: 144-145.

CAB (Commonwealth Agricultural Bureau) 1968. Plant Pathologist's Pocket Book. 1st edition. The Commonwealth Mycological Institute, England. 267 pp.

Chand R, Pandey SP, Singh HV, Sundeep K and Joshi AK 2003. Variability and its probable cause in natural populations of spot blotch pathogen *Bipolaris sorokiniana* of wheat (*T. aestivum* L.) in India. J. Plant diseases and Protection. **110**(1): 27-35.

Chang N and Wu Y 1998. Incidence and current management of spot blotch of wheat in China. *In:* Helminthosporium diseases of wheat: spot blotch and tan spot. Duveiller E, Dubin HJ, Reeves J and McNab A, (Eds.), CIMMYT, El Batán, Mexico. pp. 119-125.

Chauhan PK, Singh DP and Karwasra SS 2017. Morphological and pathogenic variability in *Bipolaris sorokiniana* causing spot blotch in wheat (*Triticum aestivum*, *T. durum*, *T. dicoccum*) in India. Int. J. Curr. Microbiol. App. Sci. **6**(11): 3499-3520.

Chidambaram P, Mathur SB and Neergaard P 1973. Identification of seed-borne *Drechslera* species. Friesia. **10**(3): 165-207.

Chin SHF, Lidingham RJ and Sallans BJ 1960. Population and variability studies of *Helminthosporium* sativum in field soils. Can. J. Bot. **31**: 718-724.

Chowdhury AK, Garain PK, Mukherjee S, Dutta S, Bhattacharya PM, Singh DP and Singh G 2005. Zonate eyespot of wheat. A new report. J. Mycopathological Research. **43**(1): 139-140.

Chowdhury AK, Singh G, Tyagi BS, Ojha A, Dhar T and Battacharya MP 2013. Spot blotch disease of wheat-a new thrust area for sustainable productivity. J. wheat research. **5**: 1-11.

Chowdhury P 2020. Pathogenic mycoflora of rice and its management. PhD Thesis. Department of Botany, University of Dhaka, Dhaka, Bangladesh.

Christensen JJ 1922. Studies on the parasitism of *Helminthosporium sativum.* Minn. Agr. Expt. Sta. Tech. Bull. **11**: 38.

Christensen JJ 1925. Physiological specialization and mutation in *Helminthosporium sativum.* Phytopathology. **15**: 785-795.

Christensen JJ 1926. Physiological specialization and parasitism of *Helminthosporium sativum*. Minn. Agr. Expt. Sta. Tech. Bull. **37**: 101.

Conner RL and Atkinson TG 1989. Influence of continuous cropping on severity of common root rot in wheat and barley. Can. J. Pl. Pathol. **11**: 127-132.

Dal Bello GM, Sisterna MN and Monaco CI 2003. Antagonistic effect of soil rhizosphere microorganisms on *Bipolaris sorokiniana*, the causal agent of wheat seedling blight. Int. J. Pest-Management. **49**(4): 313-317.

Dal Bello GM, Monaco CI, Sisterna MN and Nico AI 2008. Relationship between an *in vitro* and greenhouse assay for biological control of *Bipolaris sorokiniana*-induced seedling blight of wheat. Biological Agriculture & Horticulture: An Int. J. Sustainable Production Systems. **26**(2): 103-119.

Das M and Goswami S 2019. Antifungal and antibacterial property of guava (*Psidium guajava*) leaf extract: role of phytochemicals. Int. J. Health Sci. Res. **9**(2): 39-45.

Dastur JF 1942. Notes on some fungi isolated from 'black point' affected kernels in the central provinces. Int. J. Agric. Sci. **12**: 731-742.

Dey TK, Chowdhury N, Ayub A and Goswami BK 1992. Black point of wheat: occurrence, effect of fungicidal seed treatment on germination and quality characters. Bangladesh J. Bot. **21**(1): 27-32.

Debnath MK 1997. Occurrence of chromogenic variant in *Bipolaris sorokiniana*. MS Thesis. Department of Plant Pathology, Bangladesh Agriculture University, Mymensingh, Bangladesh.

Dehne HW and Oerke EC 1985. Investigations on the occurrence of *Cochliobolus sativus* on barley and wheat-infection, colonization and damage to stem and leaves. J. Plant Dis. Protection. **92**(2): 606-617.

Dennis C and Webster J 1971a. Antagonistic properties of species groups of *Trichoderma*. I. Production of non-volatile antibiotics. Trans. Brit. Mycol. Soc. **57**: 25-29.

Dennis C and Webster J 1971b. Antagonistic properties of species groups of *Trichoderma*. II. Production of volatile antibiotics. Trans. Brit. Mycol. Soc. **57**: 41-48.

Dennis C and Webster J 1971 c. Antagonistic properties of species groups of *Trichoderma*. III. Hyphal interactions. Trans. Brit. Mycol. Soc. **57**: 363-369.

Dick CM and Hutchinson SA 1966. Biological activity of volatile fungal metabolites. Nature. **211**: 868.

Dickson JG 1956. Diseases of Field Crops. 2nd Ed. McGraw Hill. New York, USA. 517 pp.

Domiciano GP, Rodrigues FA, Vale F, Filha M, Moreira W, Andrade C and Pereira S 2009. Wheat resistance to spot blotch potentiated by Silicon. J. Phytopathology. **158**(5): 334-343.

Drechsler C 1923. Some graminicolous species of *Helminthosporium*. Int. J. Agricultural Research. **24**: 641-740.

Dubey RC 1991. Fungicidal effect of essential oils of three higher plants on sclerotia of *Macrophomina phaseolina*. Indian Phytopath. **44**: 241-243.

Dubin HJ and van Ginkel M 1991. The status of wheat diseases and disease research in warm areas. *In*: Saunders DA (Ed.), Wheat for the non-traditional warm areas: A proceedings of the international conference. July 29-August 3, 1990. Foz do Iguacu, Brazil. pp. 125-145.

Dubin HJ and Bimb HP 1991. Effects of soil and foliar treatments on yields and diseases of wheat in lowland Nepal. *In*: Saunders DA and Hettel GP (Eds.), Wheat in heat stressed environments: irrigated, dry areas and rice-wheat farming systems. pp. 484-485. Mexico DF: CIMMYT.

Dubin HJ and Bimb HP 1994. Soil-borne diseases of wheat in warmer areas of South Asia: an update. *In*: Saunders DA and Hettel GP (Eds.), Wheat in heat stressed environments: Irrigated, dry areas and rice-wheat farming systems. pp. 353-359. Mexico DF: CIMMYT.

Dubin HJ and Rajaram S 1996. Breeding disease-resistant wheats for tropical highlands and lowlands. Annu. Rev. Phytopathology. **34**: 503-526.

Duczek LJ and Jones-Flory LL 1994. Relationship between common root rot, tillering and yield loss in spring wheat and barley. Can. J. Plant Pathol. **15**: 153-158.

Duveiller E and Gilchrist L 1994. Production constraints due to *Bipolaris sorokiniana* in wheat: current situation and future prospects. *In*: Wheat in warm area, rice-wheat farming systems. Dinajpur (Bangladesh). 13-15 Feb 1993.

Duveiller E, Dubin HJ, Reeves J, McNab A 1998. Proc. Int. workshop Helminthosporium diseases of wheat: spot blotch and tan spot (9-14 Feb. 1997). CYMMYT; El Batan, Mexico: 1998a.

Duveiller E and Garcia Altamirano I 2000. Pathogenicity of *Bipolaris sorokiniana* isolates from wheat roots, leaves and grains in Mexico. Plant Pathol. **49**: 235-242.

Duveiller E, Kandel YR, Sharma RC and Shrestha SM 2005. Epidemiology of foliar blights (spot blotch and tan spot) of wheat in the plains bordering the Himalayas. Phytopathology **95**(3): 248-256.

Duveiller E and Sharma RC 2009. Genetic improvement and crop management strategies to minimize yield losses in warm non-traditional wheat growing areas due to spot blotch pathogen *Cochliobolus sativus*. J. Phytopathology. **157**(9): 521-534.

El-Mohamedy RSR1 and Abdalla AM 2014. Evaluation of antifungal activity of *Moringa oleifera* extracts as natural fungicide against some plant pathogenic fungi *in vitro*. Int. J. Agric. Techno. **10**(4): 963-982.

El-Nashaar HM and Stack RW 1989. Effect of long-term continuous cropping of spring wheat on aggressiveness of *Cochliobolus sativus*. Can. J. Plant Sci. **69**: 395-400.

Ellis MB 1971. Dematiaceous Hyphomycetes. Commonwealth Mycological Institute, Kew, Surrey, England. pp. 608.

Ellis MB 1976. More Dematiaceous Hyphomycetes. Commonwealth Mycological Institute, Kew, Surrey, England. pp. 507.

Ellis MB and Ellis JP 1997. Micro Fungi on Land Plants: An Identification Handbook. 2nd (New Enlarged) Edn. The Richmond Publishing Co. Ltd. pp. 868.

Etebarian HR, Scott ES and Wicks TJ 2000. *Trichoderma harzianum* T39 and *T. virens* DAR 74290 as potential biological control agents for *Phytophathora erythroseptica*. Eur. J. Plant Pathol. **106**: 329-337.

Etebarian HR and Mohammadifar M 2009. Evaluation of some *Trichoderma* isolates for biological control of crown and root rot of wheat by *Bipolaris specifera* (Bainier) Subram. J. Seed and Plant. **23**(3): 343-356.

Fakir GA, Khan AL, Neergaard P and Mathur SB 1977. Transmission of *Drechslera* spp. through wheat seed in Bangladesh. Bangladesh J. Agril. **1**: 113-118.

Fakir GA 1986. Impact of diseases on crop production in Bangladesh. In: Information Bulletin. Department of Plant Pathology, Bangladesh Agriculture University, Mymensingh. 1: 1-12.

Fakir GA 1999. Seed health-an indispensable agro-technology for crop production. Lecture note for course on agro-technology and environment management for the CARITAS offers at GTI, Bangladesh Agricultural University, Mymensingh. 1-4.

Fakir GA 1988. Report on investigation into black point disease of wheat in Bangladesh. Seed Pathology Laboratory. Department of Plant Pathology, Bangladesh Agriculture University, Mymensingh.

Fakir GA 1998. Comments on symposium on "Vision for agricultural research and development''. Symposium held in BARC on 16.04.1998 in honour of Nobel Louret Dr. Norman E. Borlaug.

FAOSTAT 2009.World food and agriculture in review. Food and Agriculture Organization of the United States, Rome, Italy.

FAOSTAT 2021. Global primary crop production. Food and Agriculture Organization of the United States, Rome, Italy.

FAOSTAT 2021. Cereals trade. Food and Agriculture Organization of the United States, Rome, Italy.

Farias CR, Del Ponte J, Lucca EM, Filho AO and Pierobom CR 2005. Fungos causadores de helmintosporiose associados a`s sementes de aveia-preta (Avena strigosa, Schreb). Revista Brasileira de Sementes. **11**: 57-61.

Faull JL, Craene-Cook KA and Pilkington B 1994. Production of an isonitrin antibiotic by a UV induced mutant of *Trichoderma harzianum*. Phytochemistry. **36**: 1273-1276.

Fetch TG and Steffenson BJ 1994. Identification of *Cochliobolus sativus* isolates expressing differential virulence on two-row barley genotypes from North Dakota. Can. J. Plant Pathol. **16**: 202-206.

Fokkema NJ 1976. Antagonism between fungal saprophytes and pathogens on aerial plant surfaces. *In*: Microbiology of Aerial Plant Surfaces (Dickinson CH and Preece TF Eds.), Academic Press, London. pp. 487-505.

Fries N 1973. The growth promoting activity of terpenoids on wood decomposing fungi. Eur. J. For. Pathol. **3**: 169-180.

Giri GK, Gade RM and Patil CU 2001. Seed borne *Bipolaris sorokiniana* in wheat and its chemical control. J. Soils and Crops. Association of Soils and Crops Research Scientists. Nagpur, India. **11**(1): 109-112.

Ganguly LK 1994. Fungitoxic effect of certain plant extracts against rice blast and brown spot pathogen. Enviro. and Ecol. **12**(3): 731-733.

Gilchrist LI 1985. CIMMYT methods for screening wheat for *Helminthosporium sativum* resistance. pp. 149-151. *In:* Wheat for more tropical environments: A proceeding of the international symposium, September 24-28, 1984. Mexico, D.F.: CIMMYT.

Glass NL, Jacobson DJ, Shiu PK 2000. The genetics of hyphal fusion and vegetative incompatibility in filamentous ascomycete fungi. Annu. Rev. Genet. **34**: 165-186.

Godtfredsen WO and Vangedal S 1965. Trichodermin a new sequiterpene antibiotic. Acta Chem. Scand. **19**: 1088-1102.

Goel L, Nagarajan S, Singh R, Sinha V and Kumar J 1999. Foliar blights of wheat: current status in India and identification of donor lines for resistance through multilocational evaluation. Indian Phytopathology. **52**: 398-402.

Gottlieb D and Shaw PD 1970. Mechanism of action of antifungal antibiotics. Ann. Rev. Phytopathol. **8**: 371-402.

Gray SF 1821. A natural arrangement of British plants. Vol. 1. Baldwin, Cradock and Joy, London, UK.

Grove JF, Moffatt JS and Vischer EB 1965. Viridin. Part I. Isolation and characterization. J. Chem. Soc. **1965**: 3803-3811.

Gurjar MS, Ali S, Akhter M, Singh KS 2012. Efficacy of plant extracts in plant disease management. Agricultural Sciences. **3**(3): 425-433.

Hasan MM, Chowdhury SP, Alam S, Hossain B and Alam MS 2005. Antifungal effect of plant extracts on seed-borne fungi of wheat seed regarding seed germination, seedling health and vigour index. Pakistan J. Biol. Sci. **8**(9): 1284-1289.

Hasan MM, Ahmed F, Islam MR and Murad KFI 2008. *In vitro* effect of botanical extracts and fungicides against *Bipolaris sorokiniana*, causal agent of leaf blotch of barley. J. Agroforestry and Environ. **2**(2): 9-13.

Hasan MM 2013. Antifungal effects of plant extracts on seed-borne fungi of wheat. Bangladesh J. Prog. Sci. Techn. **11**(1): 13-20.

Hasan MM 2013. Biological control of wheat diseases caused by *Bipolaris sorokiniana*, *Fusarium graminearum* and *Aspergillus flavus* with antagonists of *Trichoderma* spp. Persian Gulf Crop Protection. **2**: 1-9.

Harding H 1975. Effect of pH and sucrose concentration on conidium size and septation in four *Bipolaris* species. Can. J. Bot. **53**(15): 1457-1464.

Harding H 1984. Spore color mutants of *Bipolaris sorokiniana*. Canadian J. Pl. Pathol. **6**(4): 273-279.

Haron FF, Sijam K, Omar D and Rahmani M 2013. Chemical composition and screening for antifungal activity of *Allamanda* spp. (Apocynaceae) crude extracts against *Colletotrichum gloeosporioides*, causal agent of anthracnose in papaya. Australian J. Basic and Applied Sciences**. 7**(1): 88-96.

Harris GH, Jones ETT, Meinz MS, Nallin-Omstead M and Bills GL 1993. Isolation and structure elucidation of viridiofungins A, B and C. Tetrahedron Lett. **34**: 5235-5238.

Hawksworth DL 1986. Fungal genera in urgent need of taxonomic work. Microbiological Sciences. **3**: 58.

Hawksworth DL, Kirk PM, Sutton BC and Pegler DN 1995. Ainsworth and Bisby's dictionary of the fungi. International Mycological Institute, CAB, Wallingford, UK.

Hetzler J, Eyal J, Mehta YR, Campos LA, Fehrmann H, Kushnir U, Zakaria-Oren J and Cohen L 1991. Interactions between *Cochliobolus sativus* and wheat cultivars. *In*: Saunders DA (Ed.), Wheat for the non-traditional warmer areas. A proceedings of the international symposium. Pp. 266-283. Mexico, DF: CIMMYT.

Hetzler J 1992. Host-pathogen interactions in populations of *Bipolaris sorokiniana* in the warm non-traditional areas. PhD Thesis. Georg-August University, Gottingen. Germany. 132 pp.

Hirsmath SV, Kulkarni S, Hegde RK, Pall BS, Bhatt MN, Naik KS, Raguchander T and Hirsmath PC 1991. New host record of fungi. Current Research**. 20**: 93-94.

Hollomon DW 2015b. Fungicide resistance: facing the challenge. Plant Protect. Sci. **51**: 70-176.

Hossain I 1991. Status of leaf blight of wheat in Bangladesh and isolation, growth study and preservation of its causal organism. Proc. BAU Res. Progr. **5**: 209-213.

Hossain I and Azad AK 1992. Reaction of wheat to *Helminthosporium sativum* in Bangladesh. Hereditas. Sweden. **116**: 203-205.

Hossain I and Azad AK 1994. *Bipolaris sorokiniana*: its reaction and effect on grain yield of wheat. Prog. Agril. **5**(2): 63-69.

Hossain I and Khan AA 1993. An investigation into the source of resistance in germplasms of wheat and *Aegilops* species against leaf spot disease. BAU. Res. Progr. **7**: 270-273.

Hossain I and Schlosser E 1993. Control of *Bipolaris sorokiniana* in wheat with neem extract. Bangladesh J. Microbial. **10**(1): 39-42.

Hossain I, Bazlur Rashid AQM, Fakir GA and Meah MB 1998. Leaf blight of wheat: its status and impact on grain formation. Proceedings of the 1st national workshop on seed pathology. Progress and prospect of seed pathological research in Bangladesh.

Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh. pp 9-10.

Hossain MM 2000. Effect of different level of black pointed seed on germination, seedling vigor, plant stand and seed quality of wheat. MS Thesis. Department of Plant Pathology. BAU, Mymensingh.

Hossain MM, Khalequzzaman KM, Aminuzzaman FM, Mollah MRA and Rahman GMM 2005. Effect of plant extracts on the incidence of seed-borne fungi of wheat. J. Agriculture and Rural Development. **3**(1&2): 39-43.

Hossain A and Teixeira da Silva JA 2013. Wheat production in Bangladesh: its future in the light of global warming. AoB plants. **5**.

Hugo G 1984. Famine as a geographical phenomenon. Boston: Reidel. 7-31.

Huguelet JE, Kiesling RL 1973. Influence of inoculum composition on the black point disease of durum wheat. Phytopathology. 63: 1220-1225.

Husak VV 2015. Copper and copper-containing pesticides: metabolism, toxicity and oxidative stress. J. Vasyl Stefanyk Precarpathian National University. **2**(1): 39-51.

Hutchinson SA 1973. Biological activity of volatile fungal metabolites. Ann. Rev. Phytopathol. **11**: 223-246.

Ibeagha AE, Huchelhoven R, Schafer P, Singh DP and Kogel KH 2005. Model wheat genotypes as tool to uncover effective defense mechanism against the hemobiotophic fungus *Bipolaris sorokiniana*. Phytopathology. **95**: 528-532.

Iftikhar S, Shahzad A, Munir A, Iftikhar A and Amir S 2006. Prevalence and distribution of foliar blights pathogens of wheat in different agro-ecological zones of Pakistan with special reference to *Bipolaris sorokiniana*. Pak. J. Bot. **38**(1): 205-210.

Iftikhar S, Shahzad A, Munir A and Iftikhar A 2009. Characterization of *Bipolaris sorokiniana* isolated from different agro-ecological zones of wheat production in Pakistan. Pak. J. Bot. **41**(1): 301-308.

Iftikhar S, Asad S, Munir A, Sultan A and Ahmad I 2009. Hosts of *Bipolaris sorokiniana,* the major pathogen of spot blotch of wheat in Pakistan. Pakistan J. Bot. **41**: 1433-1436.

Iram S and Ahmad I 2004. Diversity of *Bipolaris sorokiniana* isolates from wheat growing areas under rice-wheat cropping system in Punjab, Pakistan. Pak. J. Bot. **36**(2): 439-444.

Islam MA, Aminuzzaman FM, Islam MR and Zamal MS 2006. Seed treatment with plant extract and Vitavax-200 in controlling leaf spot (*Bipolaris sorokiniana*) with increasing grain yield of wheat. Int. J. Sustain. Agril. Tech. **2**(8): 15-20.

Islam MS, Sarker MNI and Ali MA 2015. Effect of seed borne fungi on germinating wheat seed and their treatment with chemicals. Int. J. Natural and Social Sciences. **2**(1): 28-32.

ISTA (International Seed Testing Association). 1996. International rules of seed testing association. In. Proc. Int. Seed Test. Assoc., pp. 19-41.

Ito S 1930. On some new ascigerous stages of the species of *Helminthosporium* parasitic on cereals. Proc. Imp. Acad., Tokyo. **6**: 352-355.

Jahan QSA, Hossain I and Momin A 1998. Effect of date of sowing on leaf blotch (*Bipolaris sorokiniana*) of wheat and its interaction on grain formation. Bangladesh J. Seed Science & Technology. **2**(1&2): 69-76.

Jaiswal SK, Sweta, Prasad LC, Sharma S, Kumar S, Prasad R, Pandey SP, Chand R and Joshi AK 2007. Identification of molecular marker and aggressiveness for different groups of *Bipolaris sorokiniana* isolates causing spot blotch disease in wheat (*Triticum aestivum* L.). Current Microbiology. **55**(2): 135-141.

Jones LC 1971. Studies of compatibility in Australian isolates of *Bipolaris sorokiniana*. Aust. J. boil. Sci. **24**: 51-55.

Joshi LM, Gera SD, Adlakha KL, Srivastava KD, Ram B and Palmer LT 1974. Some foliar diseases of wheat during 1969-70 crop season. Indian Phytopathology. **27**: 178-181.

Joshi LM, Srivastava KD, Singh DV, Goel LB and Nagarajan S 1978. Annotated compendium of wheat diseases in India. ICAR, New Delhi, India, pp. 332.

Kabir MS 1997. Integrated control of leaf blotch of wheat caused by *Bipolaris sorokiniana*. MS Thesis. Department of Plant Pathology, Bangladesh Agriculture University, Mymensingh. pp 31-50.

Kabir MS and Hossain I 2000. Integrated management of Bipolaris leaf blight of wheat. Bangladesh J. Plant Pathol. **16**(1/2): 9-12.

Karki CB 1982. Tan spot and other foliar diseases of wheat in Nepal. *In:* Proceedings of the tan spot of wheat and related diseases workshop. Hosford Jr. RM (Ed.), July 14-15, 1981, North Dakota State University, Fargo, North Dakota, USA. pp. 116.

Katooli N, Maghsodlo R and Razavi SE 2014. Evaluation of eucalyptus essential oil against some plant pathogenic fungi. Int. J. Agroforestry Silviculture. **1**(2): 16-17.

Kaufmann PJ and Weidemann GJ 1996. Isozyme analysis of *Colletotrichum gloeosporioides* from five host genera. Plant Dis. **80**: 1289-1293.

Kaur S and Aulakh KS 1988. Incidence of *Drechslera sorokiniana* in wheat seed and its effect on seed germination. Seed Research. **16**(1): 128-130.

Kavita, Pande SK, Yadav JK, Dalbeer 2017. *In vitro* evaluation of fungicides against *Bipolaris sorokiniana* causing spot blotch of barley (*Hordeum vulgare* L.). Int. J. Current Microbiology and Applied Sciences. **6**(10): 4734-4739.

Karim MR, Awal MA and Akter M 2010. Forecasting of wheat production in Bangladesh. Bangladesh J. Agril. Res. **35**(1): 17-28.

Khaleduzzaman SM 1996. Control of seed-borne prevalence by seed treatment in wheat (*Triticum aestivum* L.). MS Thesis, Department of Plant Pathology, Bangladesh Agriculture University, Mymensingh. 64 pp.

Khan AZM Nowsher A and Shamsi S 1983. *Cercosporae* from Bangladesh. I. Bangladesh J. Bot. **12**(1): 66-80.

Khan MQ and Bhutta AR 1994. Seed-borne fungi of wheat cultivars in Pakistan. Pakistan J. Scientific and Industrial Research. **37**(9): 397-398.

Khan MA and Ilyas MB 1996. Effect of foliar applied fungicides on wheat varieties infected by *Puccinia recondita* f. sp. *tritici* and *Drechslera sorokiniana*. Sultania **1**(1): 7-11.

Khan H and Chowdhury H 2011. Identification of resistance source in wheat germplasm against spot blotch disease caused by *Bipolaris sorokiniana*. Archives of Phytopathology and Plant Protection. **44**: 840-844.

Khan RAA, Najeeb S, Hussain S 2020. Bioactive secondary metabolites from *Trichoderma* spp. against phytopathogenic fungi. Microorganisms. **8**(6): 817.

Khair A, Ara I, Joarder GK and Begum F 1995. Effect of clove oil and Citrus on toxin producing fungal flora of poultry feed in Bangladesh. J. Sci. Ind. Res. **30**: 191-195.

Klatt AR 1988. Wheat production constraints in tropical environments. Mexico DF: CIMMYT.

Knight NL, Platz GJ and Lehmensiek A and Sutherland MW 2010. An investigation of genetic variation among Australian isolates of *Bipolaris sorokiniana* from different cereal tissues and comparison of their abilities to cause spot blotch on barley. Australasian Plant Pathol. **39**(3): 207-216.

Koch R 1883. Uber die neuen Untersuchungsmethoden zum Nachweis der Mikrokosmen in Boden, Luft und Wasser. Vortrag auf dem XI. Deutschen Arztetag in Berlin, Vereinsblatt fur Deutschland, Komnussions-Verlag von FCW Vogel, Leipzig. pp. 284.

Krishnendu A, Dutta AK and Pradhan P 2011. *Bipolaris sorokiniana* (Sacc.) Shoem: the most destructive wheat fungal pathogen in the warmer areas. Australian J. Crop Sciences. **5**: 1064-1071.

Krivoshchekova TG and Mishchenk VS 1990. The effectiveness of trichodermin. Zashchita Rastenii (Moskova). **11**: 22.

Kumar J, Schafer P, Huckelhoven R, Lungen G, Baltruschat H, Stein E, Nagarajan S and kogel KH 2002. *Bipolaris sorokiniana*, a cereal pathogen of global concern: cytological and molecular approaches towards better control. Molecular Plant Pathol. **3**(4): 185-195.

Kumeda Y, Asao T, Lida A, Wada S, Futami S and Fuijita T 1994. Effects of ergokonin produced by *Trichoderma viride* on the growth and morphological development of fungi. Bokin Bobai. **22**: 663-670.

Kuribayashi K 1929. The ascigerous stages of *Helminthosporium sativum*. Trans. Sapporo Nat. Hist. Soc. **10**: 138-145. [Abstract in Rev. appl. Mycol. **8**: 438-439 (1929).]

Kwasna H 1995. Ecology, taxonomy and nomenclature of *Helminthosporia*-history and actual situation. *In: Helminthosporia*-metabolites, biology and plant diseases: *Bipolaris, Drechslera*, *Exserohilum*. (Chelkowski J Ed.). Institute of Plant Genetics, Polish Academy of Science. Poznan, Poland. pp. 27-60.

Link JHF 1824. Species Hyphomycetum et Gymnomycetum. *In:* Species Plantarum (Linne C Ed.). GC Nauk, Germany: 1-33.

Lapis DB 1985. Insect pests and diseases of wheat in the Philippens. *In*: Wheat for more tropical environments. A proceedings of the international symposium. Villareal RL and Klatt AR (Eds), CIMMYT, El Batán, Mexico. pp. 152-157.

Lee CH, Chang MC, Lee HJ, Kho YH and Koshiso H 1995. MR-93A, A new oxazole from *Trichoderma harzianum* KCTC 0114BP. J. Natl. Prod. **58**: 1605-1607.

Lee CH, Koshino H, Chang MC, Lee HJ and Kho YH 1995. MR-304A, a new melanin synthesis inhibitor from the fungus *Trichoderma harzianum*. J. Antibiot. **48:** 1168-1170.

Leyva MG, Villasenor ME and Rodriguez CE 1992. Incidence and control of wheat (*Triticum aestivum* L) head blight (*Fusarium graminearum* Schw.). Revista Mexicana de Fitopatologia (Mexico). **10**(1): 75-77.

Li JY, Strobel GA, Harper J, Lobkovsky E and Clardy J 2000. Crytocin, a potent tetramic acid antimycotic from the endophytic fungus *Cryptosporiopsis cf. quercina*. Org. Lett. **2**: 767-770.

Li JY and Strobel GA. 2001. Jesterone and hydroxyl-jesteroneantioomycetecyclohexenone epoxides from the endophytic fungus *Pestalotiopsis jester*. Phytochemistry. **57**: 261-265.

Loladze A, Campbell KG and Chen X 2005. Development of a detached leaf assay for stripe rust resistance screening. Department of Agriculture, Washington State University, Washington.

Luttrell ES 1955. A taxonomic revision of *Helminothsporium sativum* and related species. Pak. J. Bot. **33**: 338-351.

Magar PB, Baidya S, Koju R and Adhikary S 2020. *In vitro* evaluation of botanicals and fungicides against *Bipolaris sorokiniana*, causing spot blotch of wheat. J. Agric. and Natural Resources. **3**(2): 296-305.

Mahto BN and Bimb HP 1993. Foliar blight of wheat in Nepal. Paper presented in Plant Pathological Research Review Meeting held at Plant Pathological Division, NARC, May 29- 30, Khumaltar, Kathmandu.

Mahto BN 1999. Management of Helminthosporium leaf blight of wheat in Nepal. Indian Phytopath. **52**(4): 408-413.

Mahto BN, Singh DV, Srivastava KD and Aggarwal R 2002. Mycoflora associated with leaf blight of wheat and pathogenic behaviour of spot blotch pathogen. Indian Phytopathology.

Majumder M 1991. Crops of Eastern India. West Bengal Text Book Board. Arg. Manson, C/A Raja Subodh Mallik Square, Calcutta. 85 pp.

Malaker PK, Saha NK, Rahman MM, Hossain ABS, Huq MI and Kabir KH 1994. Yield loss assessment of wheat due to Helminthosporium leaf blight of farmer's field. Bangladesh J. Sci. and Ind. Res. **29**(4): 49-57.

Malakar PK 2003. Studies on black point (*Bipolaris sorokiniana*) of wheat and its management. PhD Thesis. Department of plant pathology, BSMMU, Gazipur, Bangladesh. Pp: 13-21.

Malaker PK, Ahmed MU, Reza MMA, 2007. Research on cereal disease management at Bangladesh Agricultural Research Institute. Page 3- 20. *In:* Bakr MA, Ahmed HU, Wadud Mian MA (eds.) proceedings of the national workshop on "Strategic intervention on Plant Pathological Research in Bangladesh" 11-12 February 2007, BARI, Joydebpur, Gazipur.

Malaker PK and Miah I 2009. Effect of seed treatment and foliar spray with fungicides in controlling black point disease of wheat. Bangladesh J. Agricultural Research. **34**(3): 425-434.

Mandal S, Srivastava KD, Aggarwal R and Singh DV 1999. Mycoparasitic action of some fungi on spot blotch pathogen (*Drechslera sorokiniana*) of wheat. Indian Phytopath. **52**(1): 39-43.

Maraite H, Di Zinno T, Longrée H, Daumerie V and Duveiller E 1998. Fungi associated with foliar blight of wheat in warmer areas. *In*: Duveiller E, Dubin HJ, Reeves J and McNab A (eds.), Proceedings of the international workshop on Helminthosporium diseases of wheat: spot blotch and tan spot. pp. 293-300. February 9-14, 1997. El Batan, Mexico DF: CIMMYT.

Mares D, Tosi B, Romagnoli C and Poli F 2002. Antifungal activity of *Tagetes patula* extracts. Pharm. Biol. **40**: 400-404.

Mathur SB, and Cunfer BM 1993. Seed borne diseases and seed health testing of wheat. Danish Government Institute of Seed Pathology for Developing Countries, Copenhagen, Denmark. 168 pp.

Mathur SB and Kongsdal O 2003. Common laboratory seed health testing methods for detecting fungi. Danish Government Institute of Seed Pathology for Developing Countries, Copenhagen, Denmark. 425 pp.

Marshal AM and Hutchinson SA 1970. Biological activity of volatile metabolites from the culture of *Fomes scutellatus*. Trans. Br. Mycol. Soc. **55**: 239-251.

Mehta YR 1981a. Conidial production, sporulation period and extension of lesion *Helminthosporium sativum* of flag leaves of wheat. Pesquisa Agropecuaria Brasiliera (Brasilia). **16**: 79-99.

Mehta YR 1981b. Identification of races of *Helminthosporium sativum* of wheat in Brazil. Pesquisa Agropecuaria Brasiliera (Brasilia). **16**: 331-336.

Mehta YR, Riede CR, Campos LAC and Kohli MM 1992. Integrated management of major diseases in Brazil: an example for the southern cone region of Latin America. Crop. Protection. **11**:517-524.

Mehta YR 1993. Spot blotch (*Bipolaris sorokiniana*). *In*: Seed-borne diseases and seed health testing of wheat (Mathur SB and Cunfer BM eds.), pp 105-112. Copenhagen, Denmark: Institute of seed Pathology for developing countries. 168 pp.

Mehta YR 1998. Constraints on the integrated management of spot blotch of wheat. Germplasm improvement and future challenges for sustainable, high yielding wheat production. *In:* Helminthosporium blights of wheat: spot blotch and tan spot. Duveiller E, Dubin HJ, Reeves J and McNab A (eds.) Mexico DF, Mexico. CIMMYT. pp. 18-27.

Misra AP, Singha SP and Sharma SM 1969. Seed borne fungi in certain durum and aestivum wheat, their pathogenicity and chemical control. J. Applied Science, India. **1**(1): 5-9.

Misra AP and Singh RA 1972. Comparative pathogenicity of different species of Helminthosporium on wheat in India. Proc. 59th Indian Sci. Congr. pp 567.

Misra AP 1973. *Helminthosporium* species occurring on cereals and other Gramineae. Final report on PL 480 project, Tirhut College of Agriculture, Dholi, Muzaffarpur, Bihar, India. India. Catholic Press. Ranchi, Bihar. pp 289.

Misra AP 1979. Variability, physiologic specialization and genetics of pathogenicity in graminicolous Helminthosporia affecting cereal crops. Indian Phytopathology. **32**: 1-22.

Misra AP, Pandey SP and Misra AK 1981. Pathogenic difference in isolates of *Bipolaris sorokiniana* Shoemaker (*Helminthosporium sativum* Pam King & Bakke) from wheat, barley and triticales from different geographic region. (Abstr.) 3rd Int. Symp. Pl. Path. IARI. New Delhi. pp. 131-132.

Mikhalova LA, Gogoleva SG and Gultyaeva EI 2004. Sources and donors of resistance to wheat spot blotch caused by *Bipolaris sorokiniana* Shoem. (*Cochliobolus sativus* Drechs. *ex* Dastur). Archives of Phytopathology and Plant Protection. Abingdon, UK: Taylor & Francis Ltd. **37**(3): 161-167.

Mohammadi K, Rahimian H, Etebarian HR and Ghalandar M 2005. Biological control of common root rot of wheat by antagonistic bacteria isolated from wheat rhizosphere. Iranian J. Pl. Pathol. Iranian Phytopathological Society. Tehran, Iran. **41**(3): 383-402.

Molan Y, Al-Doss AA and El-Hussieni S 2001. Evaluation of wheat genotypes for susceptibility to common root rot and foot rot diseases cause by *Bipolaris sorokiniana* and *Fusarium graminearum*. Assiut J. Agri. Sci. **32**(5): 121-132.

Momtaz S, S Shamsi and T K Dey 2018. Prevalence of fungi associated with Bipolaris leaf blight (BpLB) of different wheat varieties in Bangladesh. Bioresearch Communications. **4**(2): 530-540.

Momtaz S, S Shamsi and T K Dey 2019. Mycoflora associated with Bipolaris leaf blight of different wheat varieties in Bangladesh. Dhaka University J. of Biol. Sci. **28**(1): 21-35.

Momtaz S, S Shamsi and T K Dey 2019. Association of *Bipolaris* and *Drechslera* species with Bipolaris leaf blight (BpLB) infected wheat leaves. J. Bangladesh Acad. Sci. **43**(1): 11-16.

Momtaz S, S Shamsi and T K Dey 2019. Mycelial growth variation and compatibility of the selected isolates of *Bipolaris sorokiniana* (Sacc.) Shoemaker. Dhaka Univ. J. of Biol. Sci. **28**(2): 195-209.

Mondal NA, Asaduzzaman SM, Malaker PK, Rouf MA and Huq MI 1994. Evaluation of fungicides against Bipolaris leaf blight of wheat (*Triticum aestivum* L.). Ann. Bangladesh Agric. **4**(1): 37-40.

Morris ML, Chowdhury N, Meisner C (eds.) 1997. IFPRI (International Food Policy Research Institute). Wheat production in Bangladesh: technological, economic, and policy issues. Research report-106. pp.95.

Mujeeb-Kazi A, Gul A, Ahmad I, Farooq M, Rizwan S, Bux H, Iftikhar S, Asad S and Delgado R 2007. *Aegilops squarrosa*, as a spot blotch (*Cochliobolus sativus*) resistance source for bread wheat improvement. Pak. J. Bot. **39**(4): 1207-1216.

Mustarin KE, Roy KK, Rahman ME 2021. Surveillance and monitoring of some major diseases of wheat in Bangladesh with special emphasis on wheat blast-a new disease in Bangladesh. J. Pl. Pathol. **103:** 473-481.

Nagarajan S and Kumar J 1998. Foliar blights of wheat in India: germplasm improvement and future challenges for sustainable, high yielding wheat production. *In*: Helminthosporium blights of wheat: spot blotch and tan spot. Duveiller E, Dubin HJ, Reeves J and McNab A (eds.). CIMMYT, Mexico DF. pp. 52-58.

Naitao C and Yousan W 1997. Incidence and current management of spot blotch of wheat in china. *In*: Helminthosporium Blights of Wheat: Spot Blotch and Tan Spot. Duveiller E, Dubin HJ, Reeves J and McNab A (eds). CIMMYT, Mexico DF. pp. 119-125.

Naznine F, Hossain I and Akter MA 2016. Investigation on quality and management of wheat seed in Bogra and Naogaon. Progressive Agriculture. **27**(2): 101-109.

Nelson RR 1960. Evolution of sexuality and pathogenicity. I. Interspecific crosses in the genus *Helminthosporium*. Phytopathology **50**: 357-377.

Nelson RR 1961. Evidence of gene pools for pathogenicity in species of Helminthosporium. Phytopathology. **51**: 736-737.

Nelson RR and Klyne DM 1962. Interspecific variation in the pathogenicity in the genus *Helminthosporium* to gramineous species. Phytopathology. **52**: 1045-1049.

Nelson RR and Kline DM 1966. The Pathogenicity of 91 morphologically similar isolates of *Helminthosporium* to 30 gramineous species. Pl. Disease Reptr. **50**: 382-384.

Nene YL and Thapliyal PN 1993. Fungicides in plant disease control. 3rd ed. Oxford and IBH Publishing Co. Pvt. Ltd. New Delhi. pp. 525.

Nema KG and Joshi LM 1973. Spot blotch disease of wheat in relation to host age, temperature and moisture. Indian Phytopathology. **26**: 41-48.

Neergaard P 1979. Seed Pathology. Vol. I and II. A Halsted Presss Book. Macmillan Press, London. (I) xxiv +839 p. + 4 pl.; (II) vii + 841-1187 p.; Vol. I ill.; Vol. II with cumulative index.

Nisikado Y 1928. Studies on the *Helminthosporium* diseases of Gramineae in Japan. Ann Phytopath Soc, Japan. 2: 96. Special report, Ohara Institute Agricultural Research 4 iv, 384, 11.

OECD-FAO 2009. Agricultural outlook 2009-2018.Org. 4 November 2012.

Owolade BF, Fawole B and Osinkanlu YOK 2001. Fungi associated with maize seed discolouration and abnormalities in South Western Nigeria. African Crop Science J. **9**: 693-697.

Ole Becker J and Schwinn FJ 1993. Control of soil-borne pathogens with living bacteria and fungi: status and outlook. Pesticide Sci. **37**: 355-363.

Oliveira AMR, Matsumura ATS, Prestes AM and Van der Sand ST 2002. Intraspecific variability of *Bipolaris sorokiniana* isolates determined by random-amplified polymorphic DNA (RAPD). Genetics and Molecular Research. **1**(4): 350-358.

Özer N 2005. Determination of the fungi responsible for black point in bread wheat and effects of disease on emergence and seedling vigour. Trakya Univ. J. Sci. **6**: 35-40.

Kilic-Ekici O and Yuen GY 2003. Induced resistance as a mechanism of biological control by *Lysobacter enzymogenes* strain C3. Phytopathology. **93**: 1103-1110.

Padmanabhan, SY 1973. The Great Bengal famine. Annual Review of Phytopathology. **11**: 11-26.

Pammel LH, King CM and Baker AL 1910. Two barley blights, with comparison of species of *Helminthosporium* upon cereals. Bull. Iwoa Agric. Exp. Stn. **116**: 178-190.

Pandey S and Tewari AN 2001. Losses due to leaf blight in two cultivars of wheat. Pl. Dis. Res. **16**(1): 113-115.

Pandey SP, Kumar S, Kumar U, Chand R and Joshi AK 2005. Sources of inoculum and re-appearance of spot blotch of wheat in rice-wheat cropping system in eastern India. European J. Pl. Pathol. **111**(1): 47-55.

Pandey SP, Sharma S, Chand R, Pandey P and Joshi A 2008. Clonal variability and its relevance in generation of new pathotypes in the spot blotch pathogen *Bipolaris sorokiniana.* Curr. Microbiol. **56**(1): 33-41.

Parashar RD and Chohan JS 1967. Effect of black point, both *Alternaria* sp. and *Helminthosporium sativum* on seed germination under laboratory and field conditions and on yield. J. Research in Ludhiana. **4**: 73-75.

Pascual CB and Raymundo AD 1995. Variability of *Helminthosporium sativum* isolates causing leaf spot in wheat. Philippines J. Crop Sci**. 16(**1): 38.

Pathak N and Zaidi RK 2013. Studies on seed-borne fungi of wheat in seed health testing programme. Archives of Phytopathology and plant protection. **46**(4).

Perry DA 1972. Seed vigor and field establishment. Hort. Abstr. **42**: 334-342.

Picinini EC, Fernandes JMC, Ignaczak JC and Ambrosi I 1996. Economic impact due to propiconazole use on wheat. Fitopatologia Brasileira. **21**(3): 362-368.

Poloni A, Muller MVG, Pessi IS and Van der Sand ST 2008. Analysis of morphological and growth rate variability of polyconidial and monoconidial cultures of *Bipolaris sorokiniana.* Biociencias **16**(1): 52-63.

Poloni A, Pessi IS, Frazzon APG and Van der Sand ST 2009a. Morphology, Physiology and virulence of *Bipolaris sorskiniana* isolates. Curr. Microbiol. **59**(3): 267-273.

Poloni A, Pessi IS, Frazzon APG and Van der Sand ST 2009b. Vegetative incompatibility among monoconidial isolates of *Bipolaris sorokiniana.* Curr. Microbiol. **58**(2): 153-158.

Prabhu AS and Singh A 1974. Appraisal of yield loss in wheat due to foliage disease caused by *Alternaria triticina* and *Helminthosporium sativum*. Indian Phytopath. **27**(4): 632-634.

Prasad KSK, Kulkarni S and Siddrmaiah AL 1978. Antagonistic action of *Trichoderma* spp. and *Streptomyces* spp. on *Drechslera sativum* (*Helminthosporium sativum* Pam, King & Bakke) Subram & Jain. Curr. Res. **12**: 202-203.

Prescott JM, Burnett PA, Saari EE, Ransom JK, Bowman JD, Milliano WDe, Singh RP and Bekele G 1986. Wheat Diseases and Pests: A Guide for Field Identification. CIMMYT, Mexico. 135 pp.

Purseglove JW 1975. Tropical crops. Monocotyledons band 1and 2, Longman, London.

Prince L, Raja A and Prabakaran P 2011. Antagonistic potentiality of some soil mycoflora against *Colletotrichum falcatum*. World J. Sci. Technol. **1**(4): 39-42.

Raemaekers RH 1985. Breeding wheats with resistance to *Helminthosporium sativum* in Zambia. *In:* Wheats for more tropical environments. A proceedings of the International symposium. Mexico DF: CIMMYT. pp. 145-148.

Raemaekers RH 1988. *Helminthosporium sativum*: disease complex on wheat and sources of resistance in Zambia. *In:* Wheat production constraints in tropical environments. Klatt AR (Ed.). Mexico DF: CIMMYT. pp. 175-185.

Raemaekers RH 1991. Contribution to the epidemiology of *Bipolaris sorokiniana* disease and the development of rainfed wheat, a new crop in Zambia. Dissertationes de Agricultura, Faculteit der Landbouwwetenschappen, Katholieke Universiteit te Leuven, Belgium.

Ragiba M, Prabhu KV and Singh RB 2001. Characterisation of wheat germplasm against Helminthosporium leaf blight. Indian Phytopath. **54**(3):316-318.

Raguchander T, Kulkarni S, Hegde RK 1988. Cultural characteristics of *Bipolaris sorokiniana*- a causal agent of leaf blight of triticale. Pl. Path. Newsletter. **6**: 43-44.

Rahman MH, Fakir GA, Rahman GMM and Islam MR 1988. Survey on the prevalence of black point of wheat in Bangladesh. Bangladesh J. Plant Pathol. **4**(1&2): 35-42.

Rahman ML, Rahman MZ, Miah IH and Alam KB 1996. Effect of different levels of temperature and pH on growth of *Bipolaris sorskiniana* and method of its inoculations into wheat. Bangladesh J. Plant Pathol. **12**(1&2): 51-53.

Rahman MA 1998. Study on the effect of seed treatments and foliar spray in controlling Bipolaris leaf blight of wheat. MS Thesis. Department of Plant Pathology, Bangladesh Agriculture University, Mymensingh.

Rahman GMM and Islam MR 1998. Effect of black point of wheat on some qualitative characters of its grain and seed vigour. Bangladesh J. Agril. Res. **23**(2): 283-287.

Rahman GMM, Islam MR and Wadud MA 1999. Seed treatment with plant extracts and hot water: a potential biophysical method of controlling seed-borne infection of wheat. Bangladesh J. Training and Development. **12**(1&2): 185-190.

Rahman MA, Rashid AQMB and Islam MA 1999. Effect of seed treatment and foliar spray on the yield of wheat as affected by Bipolaris leaf blight. Bangladesh J. Plant Pathol. **15**(1&2): 17-19.

Rahman MA, Rashid AQMB, Islam MA 2001. Control of Bipolaris leaf blight of wheat through seed dressing chemical and garlic sp. Seed-Research. Indian Society of Seed Technology. **29**(1): 121-123.

Raper KB and Thom C 1949. A manual of the *Penicillia*. CAB International, UK. pp. 608.

Razzaque A 1982. Wheat in Bangladesh. Review on ten years of modern wheat research in Bangladesh. Mexico DF, Mexico. CIMMYT. **15**: 60-84.

Razzaque MA, Hossain ABS 1990. The wheat development programme in Bangladesh. Wheat for the non-traditional warm areas (Saunders DA Ed.). A Proc. Intl. Conf. CIMMYT. Mexico. pp 44-54

Razzaque MA, Hossain ABS, Ahmed SM, Sarkar AKD, Rahman M, Barma NCD, Amin R, Saunders DA and Hettel GP. 1994. Wheat breeding strategies in the rice farming system of Wheat in heat-stressed environments: irrigated, dry areas and rice-wheat farming systems Bangladesh. Proceedings of the International Conferences Wheat in Hot, Dry, Irrigated Environments, Wad Medani, Sudan, 1-4 February 1993, and Wheat in Warm Area, Rice-Wheat Farming Systems, Wheat Research Center, Dinajpur, Bangladesh, 13-15 February 1993, organized by CIMMYT. Mexico DF. pp. 415.

Reis EM, Argenta JA and Velloso Deo JAR 1985. Multiplication of *Helminthosporium* species in senescent tissues of graminaceous weeds. Fitopatologia Brasileira. **10**: 634-648.

Reis EM, Medeiros CA and Casa TA 1998. Control of leaf blight of wheat by elimination of the inoculum source. *In*: Helminthosporium blights of wheat: spot blotch and tan spot. Duveiller E, Dubin HJ, Reeves J and McNab A (Eds). Mexico DF, Mexico. CIMMYT. pp 327-332.

Reza MMA, Malaker PK, Shaheed MA and Barma NCD 2004. Evaluation of wheat genotypes against leaf blight caused by *Bipolaris sorskiniana*. Bangladesh J. Plant Pathol. **20** (1/2): 35-41.

Reza MMA, Khalequzzaman KM and Bazlur Rashid AQMB 2006. Effect of different levels of black pointed seed and plant infection by *Bipolaris sorokiniana* on wheat. Bangladesh J. Agric. Res. **31**(2): 241-248.

Riker AJ and Riker RS 1936. Introduction to Research on Plant Diseases. Laboratory Manual. John'S Swift Co., St. Louis, Chicago, New York and Indianapolis, USA. pp. 117

Ruckstuhl M 1998. Population structure and epidemiology of *Bipolaris sorokiniana* in the rice-wheat cropping pattern of Nepal. *In:* Helminthosporium blights of wheat: spot blotch and tan spot. Duveiller E, Dubin HJ, Reeves J and McNab A (Eds.). Mexico DF, Mexico. CIMMYT. pp. 88-106.

Saari EE and Wilcoxson RD 1974. Plant disease situation of high yielding dwarf wheats in Asia and Africa. Ann. Rev. Phytopathology. **12**: 49-68.

Saari EE 1985. Distribution and importance of root rot disease of wheat, barley and triticale in South and Southeast Asia. *In:* wheat for more tropical environments. A Proc. Int. Symp., CIMMYT. pp.189-195.

Saari EE 1998. Leaf blight disease and associated soil-borne fungal pathogens of wheat in South and South-east Asia. *In:* Helminthosporium blights of wheat: spot blotch and tan spot. Duveiller E, Dubin HJ, Reeves J and McNab A (Eds.), CIMMYT, Mexico DF. pp 37-51.

Sadat MA and Choi J 2016. Wheat Blast: a new fungal inhabitant to Bangladesh, threatening world wheat production. Bangladesh J. Pl. Pathol. **33**(2): 103-108.

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

Saupe SJ 2000. Molecular genetics of heterokaryon in-compatibility in filamentous ascomycetes. Microbial Mol. Boil. Rev. **64**: 489-502.

Shabeer A and Bockus WW 1988. Tan spot effects on yield and yield components relative to growth stage in winter wheat. Plant Dis. **72**: 599-602.

Schafer MR and Kohn LM 2006. An optimized method for mycelial compatibility testing in Sclerotinia sclerotiorum. Mycologia. **98**(4): 593-597.

Selvakumar R, MS Saharan, S Kumar and I Sharma. 2015. Efficacy of fungicides as seed treatment and foliar application for managing leaf blight (*Bipolaris sorokiniana*) on wheat (*Triticum aestivum* L.). J. Wheat Research. **7**(2): 14-18.

Sen A 1982. Poverty and famines: an essay and entitlement and deprivation. Oxford: Clarendon.

Shahzad A, Iftikhar S, Munir A and Ahmad I 2009. Characterization of *Bipolaris sorokiniana* isolated from different agro-ecological zones of wheat production in Pakistan. Pak. J. Bot., **41**(1): 301-308.

Sharma SC, Randhawa HS and Sharma HL 1983. Seed infection in relation to the susceptibility of wheat to *Alternaria triticina* and *Cochliobolus sativus.* Indian phytopathol. **36**: 372-374.

Sharma RC and Duveiller E 2003. Effect of stress on Helminthosporium leaf blight in wheat. *In:* Proceedings of the fourth international wheat tan spot and spot blotch workshop (Rasmusseen JB, Friesen TL and Ali S Eds.). North Dakota State University, Fargo, USA. pp. 140-144.

Sharma RC, Shrestha SM and Duveiller E 2003. Incidence of *Bipolaris sorokiniana* and *Pyrenophora tritici-repentis* on wheat in the lowlands of Nepal. *In:* Proceedings of the fourth international wheat tan spot and spot blotch workshop (Rasmusseen JB, Friesen TL and Ali S Eds.). North Dakota State University, Fargo, USA. pp. 122-127.

Sharma V 2006. Chemical control of an unrecorded rot of *Coccinia indica* caused by *Bipolaris tetramera*. Indian Phytopath. **59**: 161-163.

Shazia I and Iftikhar A 2005. Prevalence and disease incidence of foliar blight of wheat in rice- wheat cropping system of Punjab. Pakistan J. Bot. **37**(4): 973-980.

Shoemaker RA 1955. Biology, cytology and taxonomy of *Cochliobolus sativus*. Can. J. Bot. **33**: 562-576.

Shoemaker RA 1959. Nomenclature of *Drechslera* and *Bipolaris*, grass parasites segregated from *Helminthosporium*. Can. J. Bot. **37**: 879-887.

Shoemaker RA 1962. *Drechslera* Ito. Can. J. Bot. **40**:809-836.

Siddique MAKS, Bazlur Rashid AQM, Hossain I, Khalequzzaman KM, Uddin MK 2002. Reaction of some wheat varieties to seed borne *Bipolaris sorokiniana* and *Fusarium moniliforme*. Pak. J. Biol. Sci., 5: 1211- 1213.

Singh DV and Singh VK 1971. Control of leaf blight of wheat with fungicides. Indian Phytopathology. **24**: 694-697.

Siddique AB, Hossain MH, Duveiller E and Sharma RC 2006. Progress in wheat resistance to spot blotch in Bangladesh. J. Phytopath. **154**(1): 16-22.

Singh N and Webster J 1978. Antagonism between *Stibella erythrocephala* and other coprophilous fungi. Trans. Brit. Mycol. Soc. **61**: 487-495.

Singh DV, Srivastava KD, Joshi LM and Nagarajan S 1980. Note on the occurrence of foliar pathogens of wheat in India. Indian J. Agricultural Sciences. **50**: 633-634.

Singh BP, Singh SP and Mohammad A 1990. Economic efficacy of different fungicides for the control of leaf spot of cauliflower. Indian Phytopath. **43**: 207-209.

Singh RV, Singh AK, Singh D, Singh SP and Chaudhary VP 1995. Management of foliar blight of wheat through chemicals. Indian J. Mycol. Plant Pathol. **25**(1&2): 113.

Singh VA and Chauhan SKS 1995. Efficacy of fungicide *in vitro* and *in vivo* against leaf blight of wheat. Indian J. Mycol. Plant Pathol. **25**: 113.

Singh DV and Srivastava KD 1997. Foliar blights and Fusarium scab of wheat: Present status and strategies for management. *In:* Management of threatening plant diseases of national importance. Malhotra Publishing House, New Delhi, India. pp. 1-16.

Singh RV, Singh AK and Singh SP 1997. Distribution of pathogens causing foliar blight of wheat in India and neighbouring countries. *In:* Helminthosporium blight of wheat: spot blotch and tan spot (Duveiller E, Dubin HJ, Reeves J and McNab A Eds.). 9-14 February, CIMMYT, Mexico. pp. 59-62.

Singh RV, Singh AK and Singh SP 1998. Distribution of pathogens causing foliar blights of wheat in India and neighboring countries. *In*: Helminthosporium blights of wheat: spot blotch and tan spot (Duveiller E, Dubin HJ, Reeves J and McNab A Eds.). CIMMYT, Mexico. pp. 59-62.

Singh RV, Singh AK, Singh SP and Singh BN 2001. Prevalence and distribution of foliar blight pathogens attacking wheat in India. Indian Phytopathol. **54**(2): 175-178.

Singh DP, Sharma AK, Singh A, Singh RV, Tewari AN, Singh AK, Singh RN, Singh SP, Khanna BM, Dodan DS, Bagga PS and Kalappanavar TK 2002. Losses caused due to leaf blight in wheat in different agro climatic zones of India. Plant Diseases Research. **17**(2): 313-317.

Singh RN, Prasad D, Singh V and Singh AK 2005. Prevalence and management of spot blotch (*Cochliobolus sativus*) of wheat (*Triticum aestivum*) under late sown conditions of eastern Uttar Pradesh. Indian J. Agric. Sciences. **77**(2): 129-131.

Singh V 2006. Comparative morphology and pathogenicity of *Bipolaris sorokiniana* (Sacc.) Shoem. Isolates from wheat, barley and *Phalaris minor*. Retz. Farm Science J. **15**(1): 41-44.

Singh DP, Shoran J and Kumar P 2007. Leaf blight (*Bipolaris sorokiniana*) resistant wheat genetic stock (registered). Indian Phytopath. **60**(1): 118-120.

Singh SK, Srivastava KD, Aggarwal R and Singh VB 2011. Control of spot blotch (*Bipolaris sorokiniana*) of wheat using systemic fungicides. Indian Phytopath. **64**(1): 82-84.

Singh R, Sengar RMS and Singh S 2012. Incidence of foliar blight pathogens of wheat (*Triticum aestivum* L.) in Agra Region. Indian J. L. Sci. **1**(2): 39-41.

Singh SK, Singh M, Razdan VK, Singh VB, Singh AK, Gupta S, Singh R, Gupta A, Shankar U, Singh AK, Pandey MK and Sharma R 2018. Prevalence of spot blotch (*Bipolaris sorokiniana*) of wheat and its management through host resistance. Int. J. Curr. Microbiol. App. Sci. **7**(02): 686-694.

Singh M, Singh SK, Singh VB, Singh AK, Gupta S, Singh R, Razdan VK, Gupta A, Shankar U and Singh AK 2018. Variability in *Bipolaris sorokiniana* causing spot blotch in wheat (*Triticum aestivum* L.) in Jammu sub-tropics. Int. J. Curr. Microbiol. App. Sci. 7(02): 355-366.

Sivanesan A and Holliday P 1981. CMI Descriptions of Pathogenic Fungi and Bacteria. Sheet-No. 701-710. Kew, CAB-Commonwealth Mycological Institute. Kew, Surrey, UK.

Sivanesan A 1987. Graminicolous species of *Bipolaris*, *Curvularia*, *Drechslera*, *Exserohilum* and their teleomorphs. Mycological Papers. Sheet-No 158. CAB-International Mycological Institute. Wallingford, UK.

Sivanesan A 1990. Lists of sets, index of species, and list of accepted names for some obsolete species names in CMI descriptions of pathogenic fungi and bacteria, sets 1-100, issued January 1964-March 1990. Mycopathol. **111**: 91-108.

Skidmore AM and Dickinson CH 1976. Colony interaction and hyphal interference between *Septoria nodorum* and phylloplane fungi. Trans. Brit. Mycol. Soc. **66**(1): 57-64.

Slippers B, Wingfield MJ, Coutinho TA and Wingfield BD 2001. Population structure and possible origin of *Amylostereum areolatum* in South Africa. Plant Pathol. **50**: 206-210.

Sobhan R 1979. Politics of food and famine in Bangladesh. 1973-1980. Economic and Political Weekly **14**:1973-1980.

Spurr HW Jr. and Welty RE 1972. Incidence of tobacco leaf microflora in relation to brown spot disease and fungicidal treatment. Phytopathology. **62**: 916-920.

Srinivas P, Aggarwal R, Singh VB, Gurjar MS and Gupta S 2011. Intraspecific variations in Indian isolates of *Bipolaris sorokiniana* infecting wheat based on morphological, pathogenic and molecular characters. Indian phytopathol. **62**(4).

Strobel GA, Dirske E, Sears J and Markworth C 2001. Volatile antimicrobials from *Muscodor albus*, a novel endophytic fungus. Microbiol. **147**: 2943-2950.

Subramanian CV and Jain BL 1966. A revision of some graminicolous *Helminthosporia*. Current Sciences. **35**: 350-355.

Subramanian CV 1971. *Bipolaris* Shoemaker. *In:* Hyphomycetes: An account of Indian species, except *Cercosporae* (Subramanian CV Ed.). Indian Council of Agricultural Research. New Delhi. India. pp. 930+xi.

Sutton BC 1980. The Coelomycetes. Fungi Imperfecti with Pycnidia, Acervuli and Stromata. Commonwealth Mycological Institute, Kew, Surrey, England. 696 pp.

Talukdar MJ 1974. Plant diseases in Bangladesh. Bangladesh J. Agril. Res. **1(**1): 61-86.

Thom C and Raper KB 1945. A manual of the Aspergilli. Soil Science. **60**(4): 333-341.

Tinline RD, Sallans BJ and Simmonds PM 1950. The perithecial stage of *Helminthosporium sativum*. Proc. Can. Phytopath. Soc. **17**:16 [Abstr.]

Tinline RD 1951. Studies on the perfect stage of *Helminthosporium sativum*. Can. J. Bot. **29**: 467-478.

Tinline RD, Diehl JA and Spurr DT 1994. Assessment of methods for evaluating common root rot in spring wheat and infection of subterranean plant parts by the causal fungus (*Cochliobolus sativus*). Can. J. plant pathol. **16**(3): 207-214.

Tinline RD and Dixon JG 1958. *Cochliobolus sativus* I Perithecial development and the inheritance of spore color and mating type. Mycologia. **50**: 697-706.

Tinline RD 1962. *Cochliobolus sativus*. V. Heterocariosis and parasexuality. Can. J. Bot. **40**: 425-437.

Tuite J 1969. Plant pathological methods. Fungi and bacteria. Department of Botany and Plant Pathology, Purdue University, Lafayette, Indiana. Burgess Publishing Company, Minneapolis, Minnesota, USA. pp. v+239 pp.

USDA 2006. National Nutrient Database for Standard. Reference, Release 19. US Department of Agriculture.

Valim LME, Pretes AM, Saud SV der, Matsumra ATZ and Sand SV der 1997. Variation in cultural characteristics, morphology and virulence among isolates of *Bipolaris sorokiniana*. Fitopatologia-Brasileira. **22**(4): 483-487.

Valjavec-Gratian M and Steffenson BJ 1997a. Genetics of virulence in *Cochliobolus sativus* and resistance in barley. Phytopathology. **87**: 1140-1143.

Valjavec-Gratian M and Steffenson BJ 1997b. Pathotypes of *Cochliobolus sativus* on barley in North Dakota. Plant Dis. **81**: 1275-1278.

Van Ginkel M and Rajaram S 1998. Breeding for resistance to spot blotch in wheat: Global perspective. *In*: Helminthosporium Blights of Wheat: Spot Blotch and Tan Spot (Duveiller E, Dubin HJ, Reeves J and McNab A eds.).

Varshney JL 1990. Seed-borne diseases of wheat- Their impact in relation to production and productivity. Int. J. Tropical Plant Diseases. **8**: 173-192.

Verma PR, Morrall RAA and Tinline RD 1976. The effect of common root rot on components of grain yield in Manitou wheat. Can. J. Bot. **54**: 24.

Verma PN, Singh BN, Singh G, Singh MK and Setter TL 2014. Genetic diversity analysis for yield and other agronomic traits in bread wheat under water logged sodic soil condition. J. Wheat Research. **6**(1): 51-58.

Villareal R L, Mujeeb KA, Gilchrist LI and Del Toro E 1995. Yield loss to spot blotch in spring bread wheat in warm non-traditional wheat production areas. Pl. Dis. **79**: 893-897.

Vincent JM 1947. Distortion of fungal hyphae in the presence of certain inhibitors. Nature. 150: 850.

Vir D 1974. Study of some problems associated with post-harvest fungal spoilage of seeds and grains. *In*: Current Trends in Plant Pathology. pp. 221-226.

Wegulo SN, Breathnach JA and Baenziger PS 2009. Effect of growth stage on the relationship between tan spot and spot blotch severity and yield in winter wheat. Crop Protection. **28** (8): 696-702.

Wildermuth GB, Tinline RD and McNamara RB 1992. Assessment of yield loss caused by common root rot in wheat cultivars in Queensland. Aust. J. Agric. Res. **43**: 43-58.

Wiese MV 1987. Compendium of Wheat Diseases. (2nd Edn.). American Phytopathological Society. St. Paul, Minnesota, USA. APS Press. 112 pp.

Wood LS 1962. Relation of variation in *Helminthosporium sativum* to seedling blight of small grains. Phytopathology. **52**: 493-497.

Wu WS 1977. Antibiotic and mycoparasitic effects of several fungi against seed and soilborne pathogens associated with wheat and oats. Botanical Bulletin of Academia Sinica. **18**: 25-31.

Yadav B, Singh R and Kumar A 2015. Management of spot blotch of wheat using fungicides, bio-agents and botanicals. African J. agricultural research. **10**(25): 2494-2500.

Yasmin Z and Shamsi S 2019. Antagonistic potential of soil fungi against *Colletotrichum gloeosporiodes* (Penz.) Sacc., the causal agent of anthracnose of *Rauwolfia serpentine* (L.) Benth. Ex Kurz. Dhaka Univ. J. Biol. Sci. **28**(2): 219-226.

Yassin MT, Mostafa AAF and Al-Asker AA 2022. *In vitro* antagonistic activity of *Trichoderma* spp. against fungal pathogens causing black point disease of wheat. J. Taibah Univ. Sci. **16**(1): 57-65.

Zahari R, Halimoon N, Ahmed MF and Ling SK 2018. Antifungal compound isolated from *Catharanthus roseus* L. (Pink) for biological control of root rot rubber diseases. Int. J. Analytical Chemistry. V-2018, pp 6.

Zhang TY, Wang HL and Xu FL 1990. Effects of grain black point disease of wheat and the pathogenic fungi. Acta Phytophylacica Sinica. **17**(4): 313-316.

Zhang W and Pfender WF 1993. Effect of wetting-period duration on ascocarp suppression by selected antagonistic fungi in wheat straw infested with *Pyrenophora tritici-repentis*. Phytopathology. **83**(12): 1288-1293.

Zhang Z and Yuen GY 1999. Biological control of *Bipolaris sorokiniana* on Tall Fescue by *Stenotrophomonas maltophilia* Strain C3. The American Phytopathological Society. Phytopathology. **89**(9): 817-822.

Zillinsky FJ 1983. Common Diseases of Small Grain Cereals: A Guide to Identification. CIMMYT, Mexico DF, Mexico. CIMMYT. pp.141.

APPENDICES

APPENDICES
Appendix-1

a. Preparation of Lacto phenol

Composition of Lacto phenol solution, used as mounting medium (Anisworth 1963):

Substances	Amount
Phenol crystals	20 g
Lactic Acid	20 ml
Glycerol	40 ml
Distilled water	20 ml

After weighting, the constituents were taken in a conical flask to which distilled water was added. The flask was shaken well till a homogenous solution was obtained.

b. Preparation of Cotton Blue Stain

One gram of cotton blue was added to 100 ml of Lacto phenol and shaken well till it was dissolved. The solution of Lacto phenol and cotton blue was stored in cool and dark place. Generally, it is stored in an amber coloured bottle.

c. Medium used for isolation of fungi

i. Potato Dextrose Agar (PDA) medium

Substances	Amount
Peeled and sliced potatoes	200 g
Dextrose	20 g
Agar (Powder)	15 g
Distilled water	1000 ml
P^H	6.0

ii. Carrot Dextrose Agar (CDA) Medium

Substances	Amount
Carrot	200 g
Dextrose	20 g
Agar (Powder)	15 g
Distilled water	1000 ml
P^H	6.0

iii. Tomato Dextrose Agar (TDA) Medium

Substances	Amount
Tomato	200 g
Dextrose	20 g
Agar (Powder)	15 g
Distilled water	1000 ml
P^H	6.0

iv. Host Extract Agar (HEA) Medium

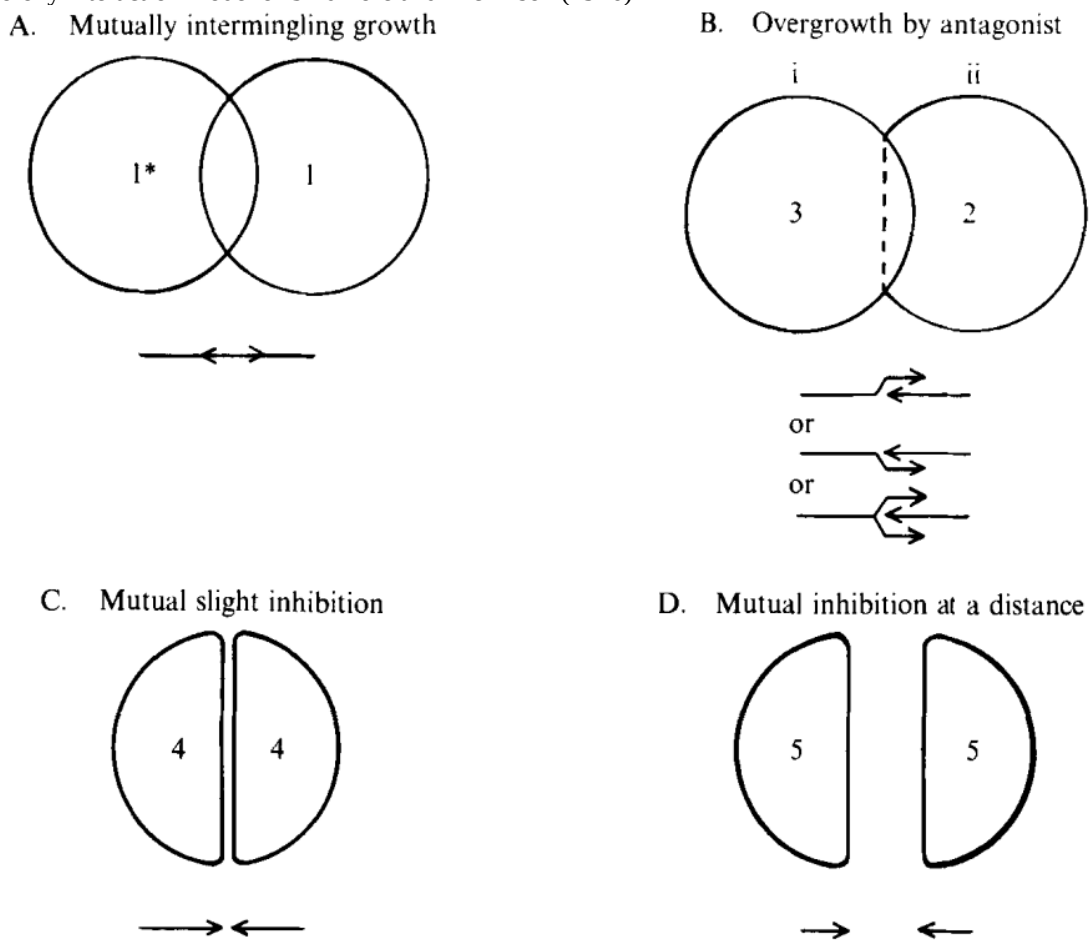
Substances	Amount
Wheat leaf	200 g
Dextrose	20 g
Agar (Powder)	15 g
Distilled water	1000 ml
P^H	6.0

v. Water Agar (WA) Medium

Substances	Amount
Agar (Powder)	15 g
Distilled water	1000 ml
P^H	6.0

Appendix-II

The colony interaction model of Skidmore and Dickinson (1976)



* = Grades from 1 (mutually intermingling growth) to 5 (mutual inhibition at a distance)

**** A=** Mutually intermingling growth were both fungi grew into one another without any microscopic sign of interaction (1).

Bi = Intermingling growth where the fungus being observed into the opposed fungus either above or below its colony (3).

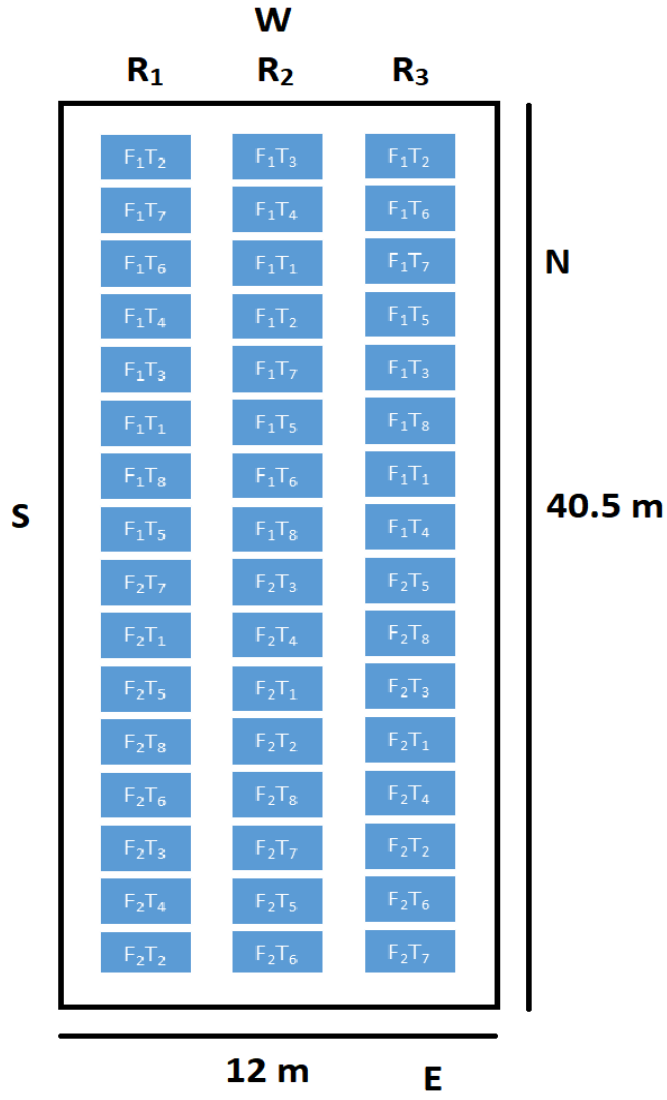
Bii = Intermingling growth where the fungus under observation has ceased the growth and is being overgrown by another colony (2).

C = Slight inhibition with a narrow demarcation line (1-2mm) (4).

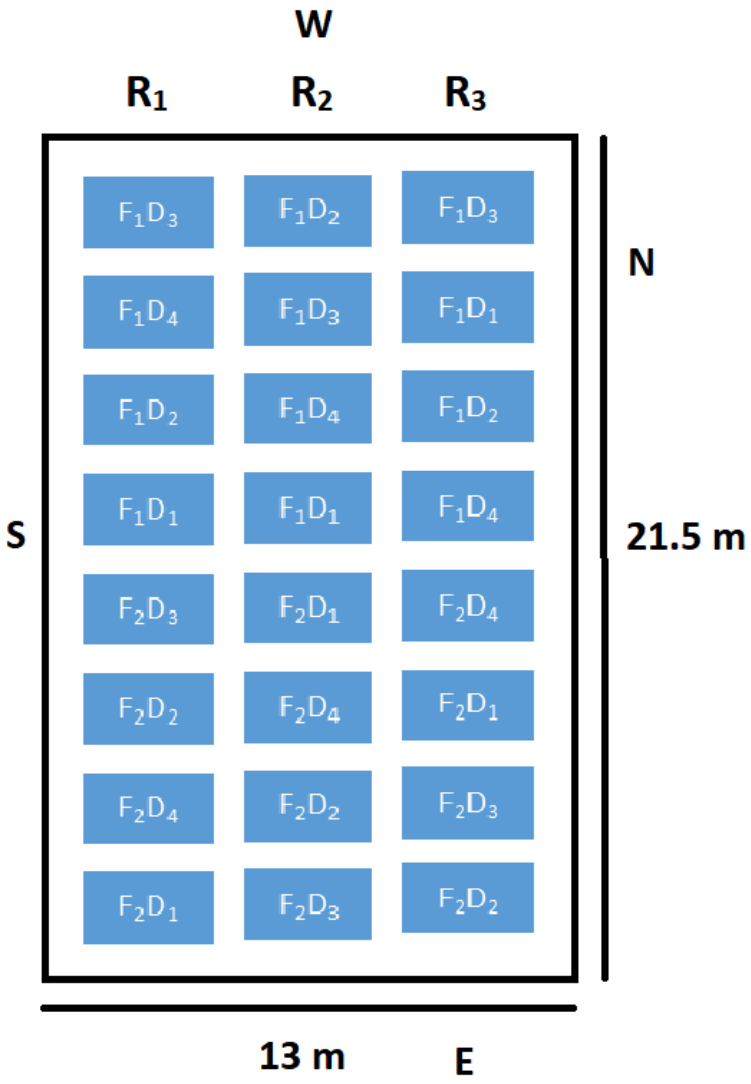
D = Mutual inhibition at a distance more than 2 mm (5).

Appendix-III

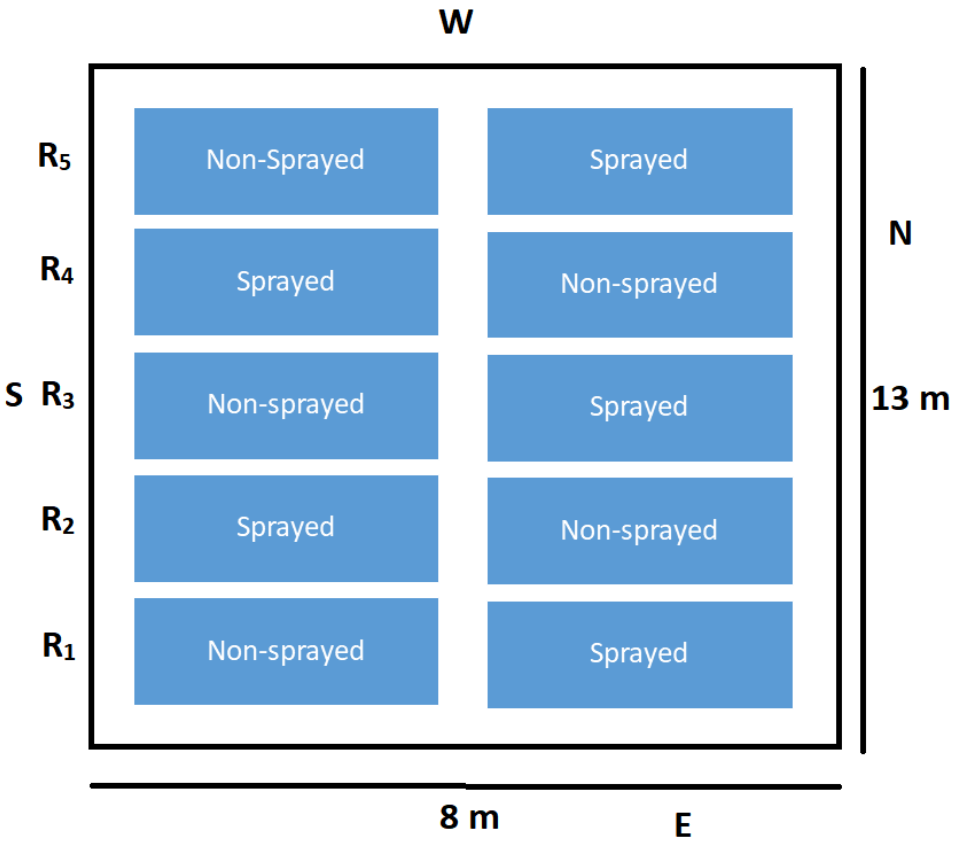
Experiment 1: Determination of number of fungicide sprays to minimize Bipolaris leaf blight (BpLB) disease of wheat.



Experiment 2: Standardization of doses of fungicide named Tilt and Folicure against Bipolaris leaf blight (BpLB) disease of wheat.



Experiment 3: Estimation of yield loss of wheat due to Bipolaris leaf blight (BpLB).



Appendix-IV
ABBREVIATIONS AND UNITS

Some commonly used Abbreviation and Symbols

Abbreviation	Full word
%	Per cent
@	At the rate
ºC	Degree Celsius
a.i./ha	amount of active ingredient per hectare
Agri.	Agricultural
lbs	Pound Unit
BARI	Bangladesh Agricultural Research Institute
BBS	Bangladesh Bureau of Statistics
BpLB	Bipolaris leaf blight
Bot.	Botany
CAB	Commonwealth Agricultural Bureau
CDA	Carrot Dextrose Agar
CIMMYT	International Maize and Wheat Improvement Center
cm	centimetre
Co.	Company
CRR	Common root rot
Curr.	Current
cv	coefficient of variation
cv.	Cultivar
Dept.	Department
DU	Dhaka University
Ed.	Editor
Eds.	Editors
Edn.	Edition
et al.	*et alibi* (and others).
etc.	et cetera (and so on)
e.g.	Latin *exempli gratia* (for example)
i.e.	Latin *id est* (that is)
g	gram
ha	Hectare
HEA	Host Extract Agar
ISTA	International Seed Testing Association
IFPRI	International Food Policy Research Institute
J.	Journal
Int.	International
kg	Kilo gram
l	Litre
Ltd.	Limited
m	Metre
m^2	square metre

min	Minute
ml	millilitre
mm	millimetre
Microbiol.	Microbiology
Mycol.	Mycology
No.	Number
Pathol.	Pathology
PDA	Potato Dextrose Agar
Pl.	Plant
ppm	parts per million
Prot.	Protection
psi	per square inch
Pvt.	
RCBD	randomized complete block design
Res.	Research
rpm	revolutions per minute
Sci.	Science
Symp.	Symposium
TDA	Tomato Dextrose Agar
Technol.	Technology
Univ.	University
UK	
Viz.	Latin *Videlicet* (namely)
WA	Water Agar

Appendix-V

SUPPORTING PUBLICATION

1. Momtaz S, S Shamsi and T K Dey 2018. Prevalence of fungi associated with Bipolaris leaf blight (BpLB) of different wheat varieties in Bangladesh. Bioresearch Communications. **4**(2): 530-540.
2. Momtaz S, S Shamsi and T K Dey 2019. Mycoflora associated with Bipolaris leaf blight of different wheat varieties in Bangladesh. Dhaka University J. of Biol. Sci. **28**(1): 21-35.
3. Momtaz S, S Shamsi and T K Dey 2019. Association of *Bipolaris* and *Drechslera* species with Bipolaris leaf blight (BpLB) infected wheat leaves. J. Bangladesh Acad. Sci. **43**(1): 11-16.
4. Momtaz S, S Shamsi and T K Dey 2019. Mycelial growth variation and compatibility of the selected isolates of *Bipolaris sorokiniana* (Sacc.) Shoemaker. Dhaka Univ. J. of Biol. Sci. **28**(2): 195-209.
5. Shamsi S, Islam MN, Hosen S, Mamun MA, Chowdhury P, Momtaz S, Naher N, Yeasmin Z, Sultana S, Khatun A, Islam AA and Bashar MA 2019. Morphological and molecular identification of ten plant pathogenic fungi. Bangladesh J. Plant Taxon. **26**(2): 169-177.
6. Association of *Pestalotiopsis guepinii* (Desm.) Stay. with Bipolaris leaf blight (BpLB) infected wheat leaves - a new record. 2016. Journal of Bangladesh Academy of Sciences. 40(1): 87-90.
7. *Drechslera Dematioidea* (Bubak & Wroblewski) Subram. & Jain - A new fungal record from Bangladesh. 2018. Bangladesh Journal of Plant Taxon. 25(1): 119-121.

Appendix-VI
STATISTICAL ANALYSIS

Field experiment 1. Determination of number of fungicide (Tilt & Folicur) sprays to minimize Bipolaris leaf blight (BpLB) on disease parameters of wheat during 2011

Table 1 A. Disease severity

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Replication	2	7.7415	3.8708	1.30	0.2872
Treatment	15	5828.3986	388.5599	130.58	0.0000
Error	30	89.2663	2.9755		
Total	47	5925.4064			

CV(%) 2.81

Table 1 B. Diseased leaf area (DLA)

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Replication	2	853.5158	426.7579	3.08	0.0607
Treatment	15	15530.3993	1035.3600	7.47	0.0000
Error	30	4156.8320	138.5611		
Total	47	20540.7471			

CV(%) 35.19

Table 1 C. No of spike/sq.m

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Replication	2	103.1667	51.5833	0.19	0.8316
Treatment	15	94620.9792	6308.0653	22.69	0.0000
Error	30	8338.8333	277.9611		
Total	47	103062.9792			

CV(%) 4.9

Table 1 D. Grain no./spike

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Replication	2	3.2917	1.6458	0.79	0.4642
Treatment	15	855.9167	57.0611	27.30	0.0000
Error	30	62.7083	2.0903		
Total	47	921.9167			

CV (%) 4.01

Table 1 E. Seed weight/ spike (g)

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Replication	2	0.0082	0.0041	1.59	0.2214
Treatment	15	0.6381	0.0425	16.55	0.0000
Error	30	0.0771	0.0026		
Total	47	0.7233			

CV(%) 2.37

Table 1 F. 1000 grain weight (g)

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Replication	2	1.0302	0.5151	0.98	0.3864
Treatment	15	226.0428	15.0695	28.72	0.0000
Error	30	15.7430	0.5248		
Total	47	242.8159			

CV(%) 1.57

Table 1 G. Grain yield/sq.m (kg)

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Replication	2	0.0038	0.0019	4.85	0.0150
Treatment	15	0.0906	0.0060	15.57	0.0000
Error	30	0.0116	0.0004		
Total	47	0.1060			

CV(%) 5.12

Table 1 H. Grain yield/plot (kg)

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Replication	2	0.0320	0.0160	1.28	0.2932
Treatment	15	3.1695	0.2113	16.90	0.0000
Error	30	0.3750	0.0125		
Total	47	3.5765			

CV(%) 4.86

Table 1 I. Grain yield (kg/ha)

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Replication	2	771886.5211	385943.2605	4.56	0.0187
Treatment	15	8738866.8116	582591.1208	6.88	0.0000
Error	30	2540786.4584	84692.8819		
Total	47	12051539.7911			

CV(%) 7.59

Field experiment 2. Determination of number of fungicide (Tilt & Folicur) sprays to minimize Bipolaris leaf blight (BpLB) on disease parameters of wheat during 2012

Table 2 A. Disease severity

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Replication	2	2.9768	1.4884	0.80	0.4575
Treatment	15	5858.4954	390.5664	210.61	0.0000
Error	30	55.6330	1.8544		
Total	47	5917.1052			

CV(%) 2.24

Table 2 B. Diseased leaf area (DLA)

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Replication	2	222.0269	111.0134	1.15	0.3301
Treatment	15	13629.3571	908.6238	9.42	0.0000
Error	30	2895.1858	96.5062		
Total	47	16746.5697			

CV(%) 29.15

Table 2 C. No of spike/sq.m

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Replication	2	9580.2917	4790.1458	6.09	0.0060
Treatment	15	65358.6458	4357.2431	5.54	0.0000
Error	30	23613.0417	787.1014		
Total	47	98551.9792			

CV(%) 8.37

Table 2 D. Grain no./spike

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Replication	2	0.7917	0.3958	0.17	0.8432
Treatment	15	790.9792	52.7319	22.86	0.0000
Error	30	69.2083	2.3069		
Total	47	860.9792			

CV(%) 4.22

Table 2 E. Seed weight/ spike (g)

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Replication	2	0.0070	0.0035	3.07	0.0611
Treatment	15	0.5476	0.0365	31.92	0.0000
Error	30	0.0343	0.0011		
Total	47	0.5889			

CV(%) 1.56

Table 2 F. 1000 grain weight (g)

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Replication	2	0.2966	0.1483	0.28	0.7602
Treatment	15	235.4005	15.6934	29.29	0.0000
Error	30	16.0728	0.5358		
Total	47	251.7698			

CV(%) 1.6

Table 2 G. Grain yield/sq.m (kg)

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Replication	2	0.0012	0.0006	0.65	0.5303
Treatment	15	0.0891	0.0059	6.64	0.0000
Error	30	0.0268	0.0009		
Total	47	0.1171			

CV(%) 7.86

Table 2 H. Grain yield/plot (kg)

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Replication	2	0.0053	0.0027	0.14	0.8714
Treatment	15	3.1206	0.2080	10.78	0.0000
Error	30	0.5789	0.0193		
Total	47	3.7048			

CV(%) 6.08

Table 2 I. Grain yield (kg/ha)

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Replication	2	433219.7204	216609.8602	2.00	0.1528
Treatment	15	8711632.9565	580775.5304	5.37	0.0000
Error	30	3246676.2329	108222.5411		
Total	47	12391528.9098			

CV(%) 8.61

Field experiment 3. Standerdization of doses of tilt and Folicur against Bipolaris leaf blight (BpLB) on yield parameters of wheat during 2011

Table 3 A. Disease severity

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Replication	2	1.1007	0.5504	1.55	0.2599
Treatment	5	1313.9838	262.7968	738.54	0.0000
Error	10	3.5583	0.3558		
Total	17	1318.6429			
CV(%) 1.13					

Table 3 B. Diseased leaf area (DLA)

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Replication	2	41.2231	20.6115	1.49	0.2713
Treatment	5	473.5445	94.7089	6.85	0.0051
Error	10	138.2730	13.8273		
Total	17	653.0406			
CV(%) 21.18					

Table 3 C. No of spike/sq.m

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Replication	2	7.0000	3.5000	0.21	0.8170
Treatment	5	101.3333	20.2667	1.19	0.3778
Error	10	169.6667	16.9667		
Total	17	278.0000			
CV(%) 1.09					

Table 3 D. Grain no./spike

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Replication	2	0.3333	0.1667	0.33	0.7242
Treatment	5	7.1667	1.4333	2.87	0.0735
Error	10	5.0000	0.5000		
Total	17	12.5000			
CV(%) 1.78					

Table 3 E. Seed weight/ spike (g)

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Replication	2	0.0005	0.0002	1.57	0.2554
Treatment	5	0.0118	0.0024	15.54	0.0002
Error	10	0.0015	0.0002		
Total	17	0.0138			
CV(%) 0.545					

Table 3 F. 1000 grain weight (g)

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Replication	2	0.2671	0.1336	0.26	0.7768
Treatment	5	3.8649	0.7730	1.50	0.2734
Error	10	5.1547	0.5155		
Total	17	9.2868			
CV(%) 1.49					

Table 3 G. Grain yield/sq.m (kg)

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Replication	2	0.0004	0.0002	1.67	0.2373
Treatment	5	0.0020	0.0004	3.42	0.0465
Error	10	0.0012	0.0001		
Total	17	0.0037			

CV(%) 2.6

Table 3 H. Grain yield/plot (kg)

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Replication	2	0.2732	0.1366	2.52	0.1304
Treatment	5	0.0753	0.0151	0.28	0.9154
Error	10	0.5432	0.0543		
Total	17	0.8916			

CV(%) 9.21

Table 3 I. Grain yield (kg/ha)

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Replication	2	125663.5747	62831.7874	1.44	0.2827
Treatment	5	217265.3450	43453.0690	0.99	0.4680
Error	10	437100.2237	43710.0224		
Total	17	780029.1434			

CV(%) 4.96

Field experiment 4. Standerdization of doses of tilt and Folicur against Bipolaris leaf blight (BpLB) on yield parameters of wheat during 2012

Table 4 A. Disease severity

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Replication	2	2.3008	1.1504	2.56	
Treatment	5	1061.0244	212.2049	471.43	0.0000
Error	10	4.5013	0.4501		
Total	17	1067.8266			

CV(%) 1.17

Table 4 B. Diseased leaf area (DLA)

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Replication	2	28.3953	14.1977	1.26	0.3250
Treatment	5	445.0849	89.0170	7.90	0.0030
Error	10	112.6598	11.2660		
Total	17	586.1400			

CV(%) 17.0

Table 4 C. No. of spike/sq.m

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Replication	2	19.0000	9.5000	0.52	0.6113
Treatment	5	103.8333	20.7667	1.13	0.4045
Error	10	183.6667	18.3667		
Total	17	306.5000			

CV(%) 1.13

Table 4 D. Grain no./spike

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Replication	2	1.4444	0.7222	1.38	0.2949
Treatment	5	8.9444	1.7889	3.43	0.0461
Error	10	5.2222	0.5222		
Total	17	15.6111			
CV(%) 1.82					

Table 4 F. Seed weight/spike (g)

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Replication	2	0.0016	0.0008	6.28	0.0171
Treatment	5	0.0113	0.0023	17.33	0.0001
Error	10	0.0013	0.0001		
Total	17	0.0142			
CV(%) 0.503					

Table 4 G. 1000 grain weight (g)

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Replication	2	0.4493	0.2247	1.20	0.3419
Treatment	5	2.9262	0.5852	3.12	0.0593
Error	10	1.8768	0.1877		
Total	17	5.2524			
CV(%) 0.8983					

Table 4 H. Grain.yield/sqm (Kg)

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Replication	2	0.0009	0.0005	0.18	0.8413
Treatment	5	0.0090	0.0018	0.68	0.6507
Error	10	0.0265	0.0027		
Total	17	0.0365			
CV(%) 15.22					

Table 4 I. Grain yield/plot (kg)

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Replication	2	0.4001	0.2001	1.36	0.2991
Treatment	5	0.7160	0.1432	0.98	0.4767
Error	10	1.4657	0.1466		
Total	17	2.5819			
CV(%) 18.39					

Table 4 I. Grain yield (kg/ha)

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Replication	2	111897.0165	55948.5083	1.91	0.1980
Treatment	5	252898.6004	50579.7201	1.73	0.2155
Error	10	292501.3603	29250.1360		
Total	17	657296.9772			
CV(%) 4.07					

Experiment 5. Colony interaction between Bipolaris sorokiniana and antagonistic fungi

Table 5 A. *Aspergillus niger*

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
No. of Isolates	15	609.1351	40.6090	19.86	0.0000
Error	48	98.1299	2.0444		
Total	63	707.2650			
CV(%) 2.45					

Table 5 B. *Aspergillus flavus*

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
No. of Isolates	15	179.3504	11.9567	8.31	0.0000
Error	48	69.0718	1.4390		
Total	63	248.4222			
CV(%) 2.25					

Table 5 C. *Aspergillus fumigatus*

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
No. of Isolates	15	192.8006	12.8534	6.37	0.0000
Error	48	96.8367	2.0174		
Total	63	289.6373			
CV(%) 2.95					

Table 5 D. *Penicillium*

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
No..of.Isolates	15	454.1203	30.2747	13.44	0.0000
Error	48	108.1578	2.2533		
Total	63	562.2781			
CV(%) 3.6					

Table 5 E. *Tricoderma herzinium*

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
No..of.Isolates	15	2298.5507	153.2367	57.48	0.0000
Error	48	127.9693	2.6660		
Total	63	2426.5200			
CV(%) 2.3					

Table 5 F. *Tricoderma viridae*

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
No..of.Isolates	15	669.5650	44.6377	16.60	0.0000
Error	48	129.0709	2.6890		
Total	63	798.6359			
CV(%) 2.44					

Experiment 6. Effect of volatile substances emanating from antagonistic fungi towards Bipolaris sorokiniana isolates

Table 6 A. Aspergillus niger

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
No.of Isolates	15	1276.6881	85.1125	2.82	0.0033
Error	48	1447.6833	30.1601		
Total	63	2724.3714			
CV(%) 14.25					

Table 6 B. Aspergillus.Flavus

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
No..of.Isolates	15	1936.7148	129.1143	11.87	0.0000
Error	48	522.2196	10.8796		
Total	63	2458.9345			
CV(%) 12.99					

Table 6 C. *Aspergillus fumigatus*

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
No..of.Isolates	15	2586.3205	172.4214	38.31	0.0000
Error	48	216.0418	4.5009		
Total	63	2802.3623			
CV(%) 10.70					

Table 6 D. *Penicillium*

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
No..of.Isolates	15	413.6910	27.5794	2.36	0.0125
Error	48	561.3915	11.6957		
Total	63	975.0825			
CV(%) 21.97					

Table 6 E. *Tricoderma herzinium*

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
No..of.Isolates	15	1508.9833	100.5989	8.15	0.0000
Error	48	592.5951	12.3457		
Total	63	2101.5784			
CV(%) 6.78					

Table 6 F. *Tricoderma viridae*

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
No..of.Isolates	15	1235.1997	82.3466	3.31	0.0008
Error	48	1194.6112	24.8877		
Total	63	2429.8109			
CV(%) 11.23					

Experiment 7. Effect of non-volatile substances emanating from antagonistic fungi towards Bipolaris sorokiniana isolates

Table 7 A. *Aspergillus niger*.5.percent

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
No..of.Isolates	15	5266.2063	351.0804	52.54	0.0000
Error	48	320.7343	6.6820		
Total	63	5586.9406			

CV(%) 10.99

Table 7 B. *Aspergillus niger*.10.percent

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
No..of.Isolates	15	9635.3837	642.3589	141.78	0.0000
Error	48	217.4773	4.5308		
Total	63	9852.8610			

CV(%) 5.99

Table 7 C. *Aspergillus niger*.15.percent

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
No..of.Isolates	15	9051.4479	603.4299	126.68	0.0000
Error	48	228.6356	4.7632		
Total	63	9280.0836			

CV(%) 4.94

Table 7 D. *Aspergillus niger*.20.percent

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
No..of.Isolates	15	3561.0042	237.4003	35.38	0.0000
Error	48	322.0408	6.7092		
Total	63	3883.0450			

CV(%) 4.62

Table 7 E. *Aspergillus flavus*.5.percent

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
No. of Isolates	15	1665.2087	111.0139	28.33	0.0000
Error	48	188.0970	3.9187		
Total	63	1853.3057			

CV(%) 16.71

Table 7 F. *Aspergillus flavus*.10.percent

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
No..of.Isolates	15	2117.3640	141.1576	39.05	0.0000
Error	48	173.5106	3.6148		
Total	63	2290.8745			

CV(%) 9.96

Table 7 G. *Aspergillus flavus*.15.percent

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
No..of.Isolates	15	3574.4157	238.2944	68.04	0.0000
Error	48	168.1050	3.5022		
Total	63	3742.5207			

CV(%) 7.24

Table 7 H. *Aspergillus flavus*.20.percent

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
No..of.Isolates	15	3990.9915	266.0661	80.25	0.0000
Error	48	159.1444	3.3155		
Total	63	4150.1360			

CV(%) 5.51

Table 7 I. *Aspergillus fumigatus*.5.percent

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
No..of.Isolates	15	1113.4853	74.2324	28.09	0.0000
Error	48	126.8521	2.6428		
Total	63	1240.3374			

CV(%) 19.91

Table 7 J. *Aspergillus fumigatus*.10.percent

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
No..of.Isolates	15	1986.6903	132.4460	47.97	0.0000
Error	48	132.5234	2.7609		
Total	63	2119.2137			

CV(%) 11.00

Table 7 K. *Aspergillus fumigatus*.15.percent

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
No..of.Isolates	15	2352.1384	156.8092	51.21	0.0000
Error	48	146.9672	3.0618		
Total	63	2499.1056			

CV(%) 8.22

Table 7 L. *Aspergillus fumigatus*.20.percent

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
No..of.Isolates	15	3631.2074	242.0805	64.93	0.0000
Error	48	178.9515	3.7282		
Total	63	3810.1589			

CV(%) 6.97

Table 7 M. *Penicillium*.5.percent

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
No..of.Isolates	15	1155.5576	77.0372	21.35	0.0000
Error	48	173.204	3.6085		
Total	63	1328.7669			

CV(%) 25.80

Table 7 N. *Penicillium*.10.percent

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
No..of.Isolates	15	2350.2015	156.6801	78.56	0.0000
Error	48	95.7292	1.9944		
Total	63	2445.9307			

CV(%) 11.47

Table 7 O. *Penicillium*.15.percent

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
No..of.Isolates	15	2087.6205	139.1747	62.52	0.0000
Error	48	106.8535	2.2261		
Total	63	2194.4740			

CV(%) 8.79

Table 7 P. *Penicillium*20.percent

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
No..of.Isolates	15	2112.0418	140.8028	54.23	0.0000
Error	48	124.6194	2.5962		
Total	63	2236.6612			

CV(%) 6.85

Table 7 Q. *Tricoderma herzinium*.5.percent

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
No..of.Isolates	15	4899.2961	326.6197	123.13	0.0000
Error	48	127.3262	2.6526		
Total	63	5026.6222			

CV(%) 10.07

Table 7 R. *Tricoderma herzinium*.10.percent

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
No..of.Isolates	15	6467.3137	431.1542	91.59	0.0000
Error	48	225.9496	4.7073		
Total	63	6693.2632			

CV(%) 8.63

Table 7 S. *Tricoderma herzinium*.15.percent

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
No..of.Isolates	15	7681.6050	512.1070	187.74	0.0000
Error	48	130.9339	2.7278		
Total	63	7812.5389			

CV(%) 4.83

Table 7 T. *Tricoderma herzinium*.20.percent

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
No..of.Isolates	15	6754.7332	450.3155	152.62	0.0000
Error	48	141.6262	2.9505		
Total	63	6896.3594			

CV(%) 4.11

Table 7 U. *Tricoderma viridae*.5.percent

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
No..of.Isolates	15	3910.1679	260.6779	88.95	0.0000
Error	48	140.6671	2.9306		
Total	63	4050.8350			

CV(%) 8.92

Table 7 V. *Tricoderma viridae*10.percent

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
No..of.Isolates	15	5009.9566	333.9971	97.33	0.0000
Error	48	164.7235	3.4317		
Total	63	5174.6801			

CV(%) 7.24

Table 7 W. *Tricoderma viridae*1.5.percent

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
No..of.Isolates	15	4055.8243	270.3883	114.27	0.0000
Error	48	113.5772	2.3662		
Total	63	4169.4015			

CV(%) 4.62

Table 7 X. *Tricoderma viridae*.20.percent

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
No..of.Isolates	15	4203.3051	280.2203	117.79	0.0000
Error	48	114.1944	2.3791		
Total	63	4317.4996			

CV(%) 3.58