Pathobiology of Foliar blights of Wheat in Punjab and NWFP Areas of Pakistan

By

SHAHZAD ASAD

Department of Microbiology
Quaid-i-Azam University
Islamabad-Pakistan
2008

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SHAHZAD ASAD

A thesis submitted to Quaid-i-Azam University, Islamabad,
in partial fulfillment of the requirements for the

Degree of

Doctor of Philosophy

In

Biological Sciences

Department of Microbiology
Quaid-i-Azam University
Islamabad-Pakistan
2008

In The Name of Allah, The Compassionate, The Merciful

Dedicated to my parents
and family for
their love and affection

DECLARATION

It is to certify that this dissertation entitled **"Pathobiology of Foliar Blights of Wheat in Punjab and NWFP Areas of Pakistan"** submitted by **Shahzad Asad** is accepted in its present form by the Department of Microbiology, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad-Pakistan in partial fulfillment of the requirement for the degree of Doctor of Philosophy in Microbiology.

Supervisor : _____
(Prof. Dr. Najma Ayub)

Co-Supervisor: _____
(Dr. Iftikhar Ahmad

External Examiners: 1) _____
(Dr. M. Afzal Akhtar

2) _____
(Dr. Tariq Mahmood)

Chairman: _____
(Prof. Dr. Abdul Hameed)

Dated: 19-11-2008

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ACKNOWLEDGEMENTS

All the praises and thanks for Almighty ALLAH who bestowed me with the potential and ability to contribute a drop of a material to the existing ocean of scientific knowledge. I offer my humblest thanks from the deepest core of my heart to the Holy Prophet Mohammad (peace be upon him) who is forever a torch of guidance and knowledge for humanity as a whole.

I am indeed grateful to the previous Chairman, Department of Biological Sciences, Quaid-i-Azam University, **Dr. Muhammad Fayyaz** and the present Chairman **Dr. Abdul Hameed** Who not only supported administratively but also morally to complete this manuscript.

I take this opportunity to record my deep sense of gratitude and in depthness to my worthy supervisor **Dr. Najma Ayub,** Professor, Department of Biological Sciences, Quaid-i-Azam University, Islamabad for her constant encouragement, inspiring guidance, keen interest, useful suggestions, sympathetic attitude throughout these investigations and help in the completion and presentation of this thesis.

Heartiest thanks are also extended to my co-supervisor **Dr. Iftikhar Ahmad,** Member, Plant Sciences, Pakistan Agricultural Research Council, and Islamabad for floating this idea for research and his valuable suggestions and critical insight to achieve this goal.

I will be failing in my duties if I do not extend my zealous thanks to my friends specially Dr. Shamim Iftikhar who supported these studies from her project and every time kind to give technical inputs. Thanks are also to Dr. Shahzia Iram and Dr. Anjum Munir who always guided in streamlining my experiments. Special thanks to Mr. Aamir Sultan and Dr. Attiq Rattu who not only accompany me during surveys but also assisted me in lab and computer work. I am also deeply thankful to friends Dr. Afzal, Dr. Athar, Dr. Hussian Shah, Mr. Fayyaz, Dr. Shahid Hameed, Mr Shahid Khanzada, Mrs. Khursheed Dr. Yasmin, Dr. Tahira, Mr Ghuman, Mr Quyyum, Mr Ishfaq and Ms. Shahzia Manan for their help and cooperation in scientific discussions and technical assistance and sincere thanks also go to Mr. Abdul Sattar, Mr. Tahir and Mr Imran for their help and cooperation in composing and compilation of this

dissertation. I would like to acknowledge my Department of National Agriculture Research Centre, for granting permission for seeking admission in the Quaid-i-Azam University and providing research facilities at Crop Diseases Research Program throughout the study period.

I do not have words at my command to express my gratitude and profound admiration to my affectionate parents, brothers and sisters, wife, kids and other relatives' affectionate and kind in-laws who always encouraged me to achieve higher ideas in life.

(Shahzad Asad)

ABSTRACT

Wheat occupies a position of paramount importance amongst worlds crop plants both in extent of area and magnitude of food production. Being a staple food, it has been ranked as the most important crop for food security in Pakistan. Foliar pathogens other than rusts and mildews are considered to contribute significantly to the low average yields of cereal crops in many developing countries. Foliar blight can often lead to the total loss and farmers are becoming more and more aware of such diseases in different agro ecological zones of the world. The foliar blight has been recorded in most growing areas of India, Bangladesh and Nepal. During a survey in spring 2000, the pathogens responsible for foliar blight were observed in different frequencies in various districts of Punjab. The fungi isolated were *Alternaria alternata*, *Stemphylium* spp., *Cladosporium* spp, *Pyrenophora tritici-repentis* and *Bipolaris sorokiniana.*

In Pakistan there are limited reports and research work on foliar blight, therefore for current studies approaches are made to analyze the situation of pathogens responsible for foliar blight in different agro ecological wheat production zones. Another purpose of the study was to observe the phenotypic characterization and aggressiveness analysis of the pathogen involved in foliar blight. Surveys in the different agro-ecological zones of wheat growing areas of Punjab and NWFP were carried out during 2004 &2005 to ascertain the prevalence, incidence and disease severity of foliar blight. Fungi were isolated from foliar samples. The phenotypic characterization of the isolates was made by measuring the size of the spores under microscope and on the basis of colour of the culture and type of growth of the fungus. One method (among five methods) for pathogenicity and aggressiveness analysis was standardized by using different protocols. Aggressiveness analysis of eighty seven isolates of *Bipolaris sorokiniana* was conducted on three commercial wheat varieties and a preliminary epidemiological study which include suitability of temperature of different isolates collected from different ecologies and hosts of the pathogen other than wheat were conducted.

The surveys conducted during 2004 and 2005 showed no wheat growing in the zones of Punjab and NWFP was free from foliar blight. In Punjab Zones, Zone 5 includes Southern Punjab, Zone 6 Central Punjab and Zone 7 Northern Punjab.

While in NWFP Zone 9 includes Foot hill areas, Zone 10 Uplands of NWFP and Zone 11 Foot hill areas. However the intensity of the disease was low at seedling stage. Maximum prevalence of foliar blight (100%) was calculated in zones 5, 6,9,10 and 11 while minimum 19% in zone7. Maximum incidence of foliar blights (59.3%) was observed in zone 5 while the minimum (1.9%) in zone 7. Maximum disease index (11.2%) was calculated in zone 5 while the minimum (0.4%) in zone 7. Maximum severity (1.5) was observed in zone 5 and 6 while the minimum (0.5) in zones 6, 9 and 10.

During 2004 low frequency of fungi was recorded at seedling stage. Total 32 isolates of five different fungi were collected with frequency of *Alternaria alternata* 46%, *Bipolaris sorokiniana* 18%, *Curvularia lunata,* 12%, *Epicoccum purpurascens,* 15% and *Drechslera spicifer*. 6% at seedling stage. At booting stage 116 isolates of similar fungi were collected with frequency of *Alternaria alternata* 67%, *Bipolaris sorokiniana* 26%, *Curvularia lunata,* .8%, *Epicoccum purpurascens,* 4% and *Drechslera spicifer* 2%. During 2005 in addition to *Alternaria alternata* and *Bipolaris sorokiniana* with frequency of 62% and 33% respectively, five more fungi were isolated with frequencies of *Drechslera rostata,*.6% *Stemphylium spp,*.6% *Cladosporium cladosporioides,*.6% *Colletotrichum graminicola* 1.6% and *Dilophospora alopecuri* 1.6%. The later two are reported to be new addition to foliar blight organisms in Pakistan. Eighty seven isolates of *Bipolaris sorokiniana,* two of *Colletotrichum graminicola* and one isolate of *Dilophospora alopecuri* were confirmed pathogenic on wheat.

Five different pathogenicity methods were tested for selection and standardization. The Test tube moist cotton swab method was found most effective method when the inoculum was placed together with the seed instead of placing after roots had initiated and later used in 'another' study for aggressiveness analysis.

The aggressiveness analysis of 87 isolates of *Bipolaris sorokiniana* has been categorized in different aggressive groups on the basis of reaction of these isolates on three commercial wheat varieties (Wafaq-2001, Inqliab-91 and Bhakkar-2002.). Among eighty seven isolates, two isolates were categorized as least aggressive, 57 as slightly aggressive, and 27 as moderately aggressive while one isolate (P2-9) exhibited aggressive reaction In Punjab only one isolate from zone 5 showed aggressiveness, nine showed moderate aggressiveness, and two were slightly aggressive. In zone 6, out of 16 isolates, 5 isolates belonged to moderately aggressive while 11 were slightly aggressive compared to zone 7 where 4 isolates were moderately aggressive and 13 were slightly aggressive. From NWFP, in zone 9 six isolates were moderately aggressive, 17 slightly aggressive while one was found least aggressive. In zone 10 two isolates were moderately aggressive while 7 were slightly aggressive. In zone 11 one isolate exhibited moderately and least aggressive reaction while 7 isolates exhibited slightly aggressive reaction.

Phenotypic characterization of eighty seven isolates of *Bipolaris sorokiniana* was carried out and correlated with aggressiveness; forty were of black colour, thirty four of grayish black, 5 of brown and 8 of albino. The black, suppressive type having more sporulation, showed maximum aggressiveness. While the albino type having low sporulation showed least aggressiveness. The measurements of the conidia collected during 2004 varied from 50-55.8µm (mean) to 16.6-19.9µm (mean) with 2 – 13 septa.while during 2005 the measurement of the conidia varied from 46.6 – 64.1 µm (mean) to 15.9- 21.6 µm having 2 – 10 numbers of septa.

As a part of epidemiological studies, the 25^0C temperature was found the most suitable one among 20^0C, 25^0C and 30^0C for the growth of *Bipolaris sorokiniana* collected from different agro ecological zones. In an other study *Brassica compestris, Glycine max, Lens culinaris*, *Vigna radiata*, *Sesamum indicum*, *Vigna mungo*, *Sorghum bicolor*, *Zea mays, Avena sativa,Hordeum vulgare* and *Panicum maximum* were found as other hosts of *Bipolaris sorokiniana* that can serve as secondary source of inoculum to cause infection on wheat. Two years studies on the assessment of the foliar blight pathogen in rice wheat cropping system indicated that *Bipolaris*

sorokiniana is prevalent in the area where conventional and zero tillage technologies are in practice as compared to bed planting.

The present study revealed that fungus had previously been reported in only a few districts of Punjab. It is now prevalent in most important wheat growing areas of both Punjab and NWFP of Pakistan

Chapter 1

INTRODUCTION

Wheat (*Triticum aestivum*) is a cereal grass of the Graminae (Poaceae) family. Over 30,000 varieties of wheat exist between the two major species, durum wheat (*T. durum*) and bread wheat (*T. aestivum*). The durum wheats are tetraploid (2n = 28) while the bread wheats are hexaploid (2n = 42) (Poehlman, 1959).

1.1 WHEAT PRODUCTION AND CULTIVATION

Wheat is a nutritious, convenient, economical source of food, rotated in annual sequence with rice, cotton, berseem (clover), soybean, sunflower and several other important crops. The per-capita availability of cereals depends upon continued productivity of wheat and needs to be explained to cope with an emergency population. Pakistan in particular has fallen short on national wheat goals necessary for self-reliance and has had to import grains to meet the requirements of population. (Khan, 2003)

In Pakistan currently the wheat crop is cultivated on an area of 8.3 million hectares with production of 21.6 m. tones (FAO Statistics., 2005). Although wheat has the largest acreage than any other crop in Pakistan and is grown on 38% of the cultivated area (Khan, 2003) the average yields are however low as compared to the other major wheat producing countries of the world (Table 1.1)

Table 1.1 Area, production and yield of leading wheat producing countries and Pakistan

Country	Area (M.h)	Yield (t/h)
Egypt	1.0	6.2
Mexico	0.6	4.8
China	22.0	3.9
USA	21.4	3.0
India	25.0	2.8
Pakistan	8.3	2.6
Canada	10.5	2.2
Australia	12.5	1.9

Source FAO Statistics 2005.

Being the major staple food crop in Pakistan, it constitutes 60% of the average daily diet of the common man. Average per capita consumption of wheat in the country is 125 kg, which works out to be 18.64 MT national requirements (Khan, 2003). If the seed, feed and wastage are added (~ 10%), the annual requirement of the country of 14.9 million people works out to 21.6 m. tons. With the increasing population trend, the country is still short of yields and work is underway to increase the yield / acre (Anonymous, 2005).

There are many reasons for the low yields. Apart from the abiotic factors like shortage of water and fertilizer, climatic factors, non-availability of quality seed etc, the biotic factors contribute a lot to the low yields. Diseases are considered as a major yield constraint in many agro-ecosystems and consequently yields are declining due to the poor emergence, weak plant vigor and a reduced number of tillers (Khan, 2003).

1.2 DISEASES OF WHEAT

The diseases of wheat are caused by fungi, bacteria, viruses and nematodes. All parts of wheat are subjected to disease attack and one or more diseases can occur on virtually every plant and in every field. In Pakistan, so far 50 diseases have been reported on wheat (Joshi *et al.*, 1978) and some of them are of economic importance. The fungal pathogens are of most significance and frequently prevalent and took a regular toll of the yield wherever wheat is sown in the country in mild to severe form depending upon the intensity of these diseases. The major fungal diseases of wheat in Pakistan which are known to occur regularly are the rusts and smuts.

1.2.1 Foliar Blight of Wheat

The blight/spot causing organisms affect the wheat crop worldwide with different intensities. There are 12 different soil borne species of *Helminthosporium* and *Alternaria* infecting wheat crop. *Helminthosporium* spp. and *Alternaria* spp. are the most devastating pathogens of wheat as foliar blight causing organisms (Prasada, 1968; Misra & Singh, 1971; Nema *et al.*, 1971; Misra and Singh, 1979; Singh *et al.*, 1998).

In Pakistan, blight diseases of wheat are considered to be of minor importance (Bajwa, 1985; Bhatti and Ilyas, 1986; Hafiz, 1986). The observational data on the diseases recorded in wheat breeding nurseries even did not included reports on the blight diseases (Bajwa, 1985; Mustafa et al., 1994). Rusts and smuts are by and far, the diseases of major concern (Bhatti and Ilyas, 1986; Hafiz, 1986; Roelfs *et al.*, 1992; Saari and Prescott, 1985; Wilcoxson and Saari, 1996). Although the information about the foliar disease is rather scanty yet it contributed significantly to low yields of cereal crops for which precise data is also not available (Bhatti and Soomro, 1996). However during 2000 a field survey in rice-wheat cropping areas in Punjab was conducted and consequently nineteen isolates of *Bipolaris sorokiniana* were isolated (Iram and Ahmed, 2004). Tan spot an important foliar disease of many wheat growing countries has first time been reported during survey conducted in the same year and it was isolated from 13 different locations. The pathogenicity was confirmed the disease was found associated with *Pyrenophora. tritici repentis.* During microscopic studies, erect, single, dark yellow, brown conidiophore with single light yellow-brown conidia of Drechslera tritici-repentis was found (Ali *et al.*, 2001). Thus the occurrence of these diseases in major wheat growing areas of Pakistan necessitated for detailed analysis and improved understanding of pathobiology of the organisms.

1.3 AIMS OF THE PROJECT

The present project is a continuation of the limited reports and research conducted in recent years on the foliar blights in Pakistan. The previous research work was conducted in only a few districts of Punjab. The present studies have been broadened to the whole of Punjab and in NWFP of different wheat growing ecologies. Therefore the ultimate aim was to establish a wide, broad base-line for the later studies with specific focus on foliar blights. The research proposed in this study aims at achieving a broad base analysis of the situation prevailing in the country as far as foliar blights of wheat are concerned, which will help the breeders and scientists involved in wheat improvement research in the country to design their research keeping in consideration the subject disease occurrence which was not of any significance in the past and showing their prevalence and/or building up of their inoculum due to one or another reason in the wheat growing agro-ecological zones of

the country. The information thus generated will ultimately contribute to the management of the disease in the areas where disease is most prevalent. The farmers in the country will get opportunities to go for the varieties having resistance against the blight diseases. In this way they will also get boost in yields/acre.

The specific objectives of this work are:

- Distribution, incidence and severity of foliar diseases of wheat in different agro-ecological zones of Punjab and NWFP.
- Phenotypic characterization of the pathogens involved in foliar blights in Pakistan.
- Aggressiveness analysis of the pathogens.
- Preliminary epidemiological studies of the pathogen.

Chapter 2

REVIEW OF LITERATURE

2.1 INTRODUCTION

Foliar blight of wheat includes rusts, smuts, powdery mildew, leaf spots and anthracnose. Rusts, smuts and powdery mildew are continued to be the major threat to successful cultivation of wheat all over the world (Saari and Prescott, 1985). Many different fungal pathogens are known to cause leaf blight worldwide, depending upon the climatic conditions, availability of inoculum's foci of a particular fungus and the cropping pattern. Therefore, in the current manuscript only the fungal pathogens related to foliar blight will be focused other than rusts smuts, powdery and downy mildews.

2.2 FOLIAR BLIGHT OF WHEAT AND THEIR CAUSAL ORGANISM

A brief account of foliar blight causing fungi (*Septoria tritici, Colletotrichum graminicola, Dilophospora alopecuri, Alternaria triticina, Alternaria alternata, Pyrenophora tritici-repentis* and *Bipolaris sorokiniana* is given below:

Septoria tritici (Roberge in Desmo, 1842) has usually been known by the anamorph (asexual), *Septoria tritici*. The teleomorph (sexual) stage was described as *Sphaerella* in 1870 by Sanderson (Sanderson, 1972; Sanderson, 1976) and the species had been transferred to *Mycosphaerella* by Schröther, about 25 years after it was described. Although the pathogen is not important wherever wheat grows, however its distribution is worldwide. It is reported to be the more damaging at higher levels of soil fertility and can be the major constraint on wheat yields in both Maritime and Mediterranean climates, nevertheless the disease tends to be more severe at low latitudes and in the regions where rainfall is higher especially during the growing season (Leath *et al.*, 1994). Although the disease is sporadic in nature but causes serious losses both on durum and bread wheat in many growing regions of the world i.e. Europe, North and South America, Africa, Asia, Australia and New Zealand (Shipton *et al.*, 1971). The total yield losses are estimated at over 9 million tones worldwide (Kolomiets, 1999). The yield losses are mainly due to poor grain filling

(Cornish *et al.*, 1990) and are determined by the severity of attack on the flag leaf and sub-flag leaf (Shaw and Royle, 1993). Different workers conducted trials worldwide in different regions and reported yield losses as 18% in Morocco (Schluter and Janati, 1976), 30-50% in Israel (Ziv and Eyal, 1978), 6-16% in former USSR (Derecha and Kostynets, 1977), 31 % in Australia (Loughman and Thomas, 1992; Brown and Paddick, 1980), 18% in New Zealand (Thomson and Gaunt, 1986) and in Europe 25% (Jorgensen *et al.*, 1996). The main affected plant part due to disease is leaf, the infected leaves show necrotic, usually grey-green to off-white patches studded with brown or black dots about 0.5 mm across (Sivanesan, 1990). *Septoria* has been reported to survive as mycelium beneath the seed coat of infected wheat heads. However its transmission from infected seeds to seedlings could not be proven (Brokenchire, 1975). The ascospores from crop residues are generally recognized as the primary source of inoculum (Shaw and Royle, 1989a; Schuh, 1990). It has been reported that adequate rainfall is generally needed for epidemics to develop (Shaw and Royle, 1993). Under drought conditions moderate amounts of disease (1.5% severity) may increase yields by increasing water use efficiency (Steinberg, 1991). However, early sowing and cool temperatures, retard plant growth, increase yield losses by allowing more time for pathogenesis and disease development (Shaw and Royle, 1993; Lovell *et al.*, 1997).

Colletotrichum graminicola (ces.) G. W. Wilson has its teleomorph, *Glomerella graminicola* (Skipp et al., 1995) which has not been found in nature (Politis and Wheeler, 1972), however only few researchers have succeeded in generating sexual progeny under laboratory conditions (Politis, 1975; Vaillancourt and Hanan, 1991-92). The primary hosts of this fungus are grasses, sorghum and maize whereas wheat, barley and oats are its secondary hosts. The disease caused by *Colletotrichum graminicola* of wheat is named Anthracnose. It occurs worldwide but the damage reports are available in isolated areas where it reduces yield as much as 25% by affecting the plant vigor and grains (Wiese, 1998). Very scanty literature is available on Anthracnose of wheat.

Very little is published about *Dilophospora alopecuri* (Sacc.) Wakler & B. Sutton and its geographic distribution is in Europe, former Yugoslavia (CAB

ABSTRACTS, 1990), India (CAB ABSTRACTS, 1988-1998), Iraq: unconfirmed record (CAB ABSTRACTS, 1989) and Australia (CAB ABSTRACTS, 1984-1998). Due to this disease the damage on wheat has been reported in the USA and Canada with minor yield losses in parts of Europe (Atanasoff, 1925). The symptoms appeared as spotted and distorted leaves. Leaf spots initially are small, elongate or spindle-shaped flecks later become tan-brown with black crusty centre. The fungus *Dilophospora* alopecuri survives as mycelium in host debris and as conidia on seed. Conidia of the fungus act as primary inoculum and those produced secondarily appear to account for later infection on upper plant parts. The conidia are dispersed by wind and splashing rain, and there appendages may function in attachment to seed, host plants and nematode vectors (Munjal *et al.*, 1961).

Alternaria triticina Prasada and Prabhu an anamorphic member of *Pleosporaceae* (Hawksworth *et al.*, 1995). The disease was first recorded from Bihar, India (Kulkarni, 1924). It is also known to occur in North Africa (Anahosur, 1978), South America (Wallar, 1981), Bangladesh (Rashid *et al.*, 1985; Ahmed *et al.* (1994), Egypt (Beshir, 1994), Italy (Casulli, 1990), the Middle East and Nigeria (Wiese, 1987), Lebanon, Greece and Portugal (Logrieco *et al.*, 1990). The disease caused serious damages in eastern and central parts of India (Nema, 1986). In severe epidemics the yield losses may exceed 60% (Prabhu and Singh, 1974; Sokhi, 1974). The symptoms appear as small, oval, discoloured lesions, irregularly scattered on leaves. As lesions enlarged they become irregularly in shape with dark brown to grey coloured scattered on leaves. A bright-yellow marginal zone is sometimes seen around these lesions. As disease progresses several lesions coalesce resulting in death of entire leaf, heavily infected fields present a burnt appearance and can be judged from a distance which is the most conspicuous phase of the disease (Prasada and Prabhu, 1962; Prabhu and Prasada, 1966; Singh, 1990). *Alternaria triticina* has been shown to be seed transmitted in wheat (Prabhu and Prasada, 1966, 1967; Kumar and Arya, 1976). The extent of infection in seeds varies from cultivar to cultivar and on an average as high as 12.2% seed infection has been reported (Kumar et al., 1974). The detection of the pathogen in wheat seeds in India and Bangladesh has been reported (Ahmed *et al.*, 1994) while from France, Greece, Italy, Portugal and Macedonia by Logrieco *et al.*, (1990). In addition to seed borne transmission, it has been reported to

be soil borne in nature also. However, the soil borne inoculum may not play a role in the perpetuation of the inoculum under hot conditions until the next planting. Thus infection is initially perpetuated by conidia on the seed surface and as mycelium inside the seed (Kumar and Arya, 1976). The initial inoculum carried with the seed may build up in the soil and with the passage of time to such an extent that the soil itself become infested (Prabhu and Prasada, 1966). The pathogen has been reported to survive in seed for 10 months (Kumar and Rao, 1979). It was found to be associated with leaf blight in warm areas in Indian sub-continent causing significant yield losses mostly on durum wheat (Mercado *et al.*, 2003)

Alternaria alternata reported to be more opportunistic or facultative in nature (Joshi *et al.*, 1986). It is encountered sporadically and is seldom important on a national scale but can cause losses in individual fields depending upon the climatic conditions, susceptible host and inoculum pressure (Joshi *et al.*, 1978; Nema, 1986). Its perfect stage does not occur in nature in South or Southeast Asia (Raemaekers, 1988).

Pyrenophora tritici-repentis (Died.) Drechsler. (Ptr). Earlier had anamorph name and described as *Helminthosporium gramineum* later raised to species rank as *Helminthosporium tritci-repentis* and finally place as *Drechslera tritici-repentis* which is a preferred name for anamorph. The preferred name for teleomorph is *Pyrenophora tritici-repentis* (Lamari *et al.*, 1995). The disease is named as tan spot and has a world wide distribution (Anon, 1980). It was first recorded in Australia (Valder and Shaw, 1952). The grain yield loss up to 29% in field trials has been reported (Rees and Platz, 1978). Tan spot is known to occur in Canada (Tekauz, 1976) with 14.8% losses in common wheat (Tekauz, 1982), in USA (Hosford, 1971a) with yield losses ranging from 4% to 28 % in North Dakota later the disease was recorded in different states(Hosford, 1971b), with variable losses. In Montana losses recorded were up to 19.7% (Sharp *et al.*, 1976), in Mississippi 65% yield loss with the incidence of 90% was recorded (Hirrell *et al.*, 1990). An epidemic was recorded in Nebraska in 1977 (Watkins *et al.*, 1978) and now tan spot had become the most prominent leaf spotting disease of wheat in Nebraska (Watkins *et al.*, 1982). It also occurs in several countries of central and South America, including Mexico, Argentina, Bolivia, Brazil, Chile,

Paraguay and Uruguay (Kohli *et al.*, 1992). The disease became economically threatening in the mid 80's resulted in severe epidemics in 1990 in Paraguay and Argentina in 1991 with estimated yield losses in farmer's field was between 27 and 70% (Kohli *et al.*, 1992). Similarly in Europe the significant effect on the yield and economic impact of this disease has been reported. A severe outbreak of *Pyrenophora tritici-repentis* was observed during 1987 (Schmitz and Grossman, 1987) and yield losses up to 23-53% was recorded (Obst, 1988). In Hungary a countrywide survey during 1989 and 1990 revealed that *Pyrenophora tritici-repentis* prevalence was 70% and 51% respectively. Its occurrence has also been observed in several wheat fields of Wales in England (Cook and Yarham, 1989). *Pyrenophora tritici-repentis* is also found to be the most prevalent pathogen in Africa with average disease severity of 47% during 1989-1990 (El-Harrak *et al.*, 1998), in Morocco, the yield losses ranging from 12% to 18% (Nsarellah and Mergoum, 1998). Consecutive surveys in Morocco during three years 1997, 1998, 1999 at the anthesis to physiological maturity stage revealed the tan spot as the most prevalent disease (Ramadani *et al.*, 2000). Similarly the pathogen has also been reported in Eastern Algeria (Lamari *et al.*, 1995b) and in Tunisia (Cherif *et al.*, 1994). However the economic losses in both the countries are not available (Hosford, 1972). The disease also appeared in mild to severe form in Asia and has been reported in many Asian countries including Japan, India, China, Thailand, Georgia, Afghanistan, Iran and Nepal (Hosford, 1982). Little is known about the economic importance of tan spot in most of these countries. The tan spot is considered to be of moderate levels in South Asia (Saari, 1998). However it is considered to be one of the main wheat diseases in Central Asia, in parts of Uzbekistan, Tajikistan and Southern and Northern Kazakhstan where disease intensities varies from 25% to 50% (Postnikova and Khazanov, 1998). A decade ago, it was generally accepted that *Bipolaris sorokiniana* prevalent mostly in warm low lands of Nepal contrary to the Gangetic plains whereas *Pyrenophora tritici-repentis* was found in the cooler climate of the hills and that both pathogens could be equally identified and characterized in a transition zone of mid hills and river basin. However in early 1990s *Pyrenophora tritici-repentis* was occasionally isolated in the warm and humid lowland of Nepal, whereas *Bipolaris sorokiniana* typically dominates, suggesting that tan spot was probably overlooked. In a study the infected leaf samples

were collected during 1998-2001. The occurrence of both the pathogens was highly variable. Out of 267, 690, 614 and 475 infected leaf samples of four surveys, *Bipolaris sorokiniana* conidia were observed in 51, 69, 28 and 11% of leaf samples respectively, suggesting a decreasing trend in incidence over the years, compared to *Pyrenophora tritici-repentis* frequency which was observed 63, 68, 84 and 82% of the samples (Sharma *et al.*, 2003). Out of the 1879 and 2790 leaf samples collected from 13-18stations of Hungary in March, April, May and June 2000 and 2002, *Drechslera tritici-repentis* was found to be the most prominent pathogen (Csosz, 2005).

The tan spot of wheat consists of two distinct symptoms necrosis (tan) and chlorosis (Lamari and Bernier, 1989). The first symptoms are well defined yellow, elliptical spots that soon become tan or grey in the centre (tan spot), followed by leaf necrosis progressing from the tip inward. An elongate chlorotic spot occurs on certain varieties with certain strains and on seeds causes pink grain (Klein, 1987) or red smudge (Fernandez *et al.*, 1994). The fungus over winters on culms, sheath and leaves and is capable of extensive saprophytic growth on plant debris. The use of minimum tillage that leaves crop debris on the soil surface seems to favor the disease (Hosford, 1971a). The initial infection of wheat plants is reported to be by ascospores. Two or three weeks later conidia are produced, dispersed by wind and cause secondary infection (Wolf and Hoffman, 1994). Studies on the biological aspects of infection of wheat by ascospores and conidia of this fungus revealed that ascospores are responsible for infection in the lower canopy while conidia spread the disease at upper levels (Wright and Sutton, 1990). The wheat seed is reported to be infected through glumes, lemma and palea by this fungus within 3 days of inoculation and is susceptible throughout most stages of development but mainly at the milking stage or in field experiments at dough stage (Schilder and Bergstorm, 1994). The conidial germination, appressorium formation, infection pegs, intracellular vesicles and growth of intracellular hyphae in the host epidermis occurred with in 24 hours. After 2 days intercellular mycelium grew in the mesophyll (Loughman and Deverall, 1986). The optimum temperature for disease development occurred between 18 and 28°C depending on cultivar (Luz and Bergstram, 1986). While the optimum relative humidity is reported to be greater than 95% (Wolf and Hoffman, 1993).

Cochliobolus sativus (S. Ito & Kurib) Drechsler ex Dastur (Teleomorph). The anamorphic name *Bipolaris sorokiniana* of the fungus has widely been adopted (Sacc.) Shoem. (Shoemaker, 1959).Synonymous names used for this fungus include *Cochliobolus sativus* (Ito and Kurib) Drechsler ex. Dastur.*Drechslera sorokiniana* (Sacc.) Subram. & B. L. Jain (Anamorph) *Helminthosporium sativum* Pammel, L. M. King & Blakke (Anamorph).

Cochliobolus sativus the (ascigerous state (teleomorph) state =*Ophiobolus sativus*) has only rarely been observed to occur in nature which was recently reported from Zambia. It is characterized by globose ascomata usually with a long cylindrical neck, obclavate-cylindric asci and helically coiled filiform ascospores (Raemackers, 1991). The anamorph state of the fungus is most abundantly found in nature all over the world. Conidiophores are solitary or in small groups, straight to flexuous, sometimes geniculate, pale to mid-dark-brown, up to 220 µm long and 6-10 µm wide. Conidiogenous cells are polytretic, integrated, terminal, sympodial, cylindrical and cicatrized. Conidia are straight to curved, fusiform to broadly ellipsoidal, 3-12 distoseptate, and olive-brown (Sivanesan and Holliday, 1981). Chlamydospores may develop on the hyphae (Valim Labres *et al.*, 1998).

The disease caused by this fungus is known as spot blotch of wheat and it occurs throughout the world, but severe losses occur in Bangladesh, Bolivia, Brazil, Paraguay and Zambia. The disease affects all plant parts and has caused yield losses up to 100% (Mehta, 1993). There are three primary phases of symptoms that occur depending on the environmental conditions, root rot, leaf blight (spot blotch) and black point of seed (Duczek and Jones-flory, 1993). The early symptoms of infection are brown lesions produced on coleoptiles, sub crown internodes, roots and culms of seedlings. Infection that progresses up the basal stem is most damaging. Early infection does not show symptoms but resulted in reduced leaf area at the 2-leaf growth stage. The leaf symptoms are most pronounced after heading and frequent on lower leaves, appeared as distinct, elongate, brown-black lesions that rarely exceed 1 cm in diameter (Duczek, 1997; Bello *et al.*, 2003). The symptoms may also vary regionally depending upon the weather conditions common root rot is difficult to diagnose. The most diagnostic symptom is a dark brown or blackened sub crown

internodes. Browning or blackening of primary or secondary root systems may be evident upon careful examination of washed roots (Chang and Wu, 1997). The organism causes root and foot rots, leaf spots, kernel infections and seedling blights in cereals. The primary source of inoculum is thick-walled conidia surviving on crop debris or in the soil and the origin of soil borne inoculum has been found from sporulation of *Cochliobolus sativus* on aerial parts of barley, oat and wheat during plant development to stubble of these crops up to the time for resowing. The sporulation started when lower leaves become necrotic, so the conidia produced on aerial parts of the plants contribute substantially to the soil borne inoculum (Reis and Santos, 1987). In Argentina *Cochliobolus sativus* was frequently isolated from roots and leaves of weed species, contributing to the inoculum levels in the soil (Carmona *et al.,* 1999). Both incidence and intensities of disease at tillering, flowering and firm dough stages of wheat development are closely related to the inoculum density of *Cochliobolus sativus* in the soil (Tinplate *et al.*, 1988).

Various other factors can affect conidial population in the soil. In Australia amoebae, which cause large perforations in conidia affect soil borne population of *Cochliobolus sativus* (Duckzek and Wildermuth, 1991), whereas in Canada the inoculum density of *Cochliobolus sativus* in the top 8 cm of soil was less under zero tillage compared to the conventional tillage (Tinlane and Spurr, 1991). But in USA, the majority of spores were found in the top 10 cm of soil regardless of tillage method. Although significantly more spores were found with conventional tillage than in the zero-tillage system (Mathieson *et al.*, 1990).

Cochliobolus sativus is reported to be seed borne in several other host plants that include *Agropyron* spp. (Meehan, 1947), *Avena sativa* (Hampton and Matthews, 1978), *Hordeum vulgare* (Benada, 1985), *Lolium* spp. (Lam, 1982), *Triticum aestivum* (Chinn, 1978) and *triticale* (Sinha and Singh, 1979). Its seed transmission has been proven when lesions on the sub crown internodes of seedlings were grown in the glasshouse from infected seed in autoclaved sand (Reis, 1982). The evidence for seed transmission under field conditions was indicated by the symptoms and the damage that were observed on seedlings and primary leaves when naturally infected seeds were planted and by the finding that seed treatment reduced disease incidence on

young plants at early developmental stages (Weisig and Fehrmann, 1993). *Cochliobolus sativus* can also be airborne, causing secondary infection, that results in severe foliar disease and yield loss. The secondary infection highly depends on environmental conditions, which requires high relative humidity. The high disease frequencies were observed in the years with high daily minimum temperatures (i.e. warm nights) (Gilbert *et al.*, 1998).

The yield losses due to spot blotch vary in different regions and cereals. In Canada the yield losses in barley were greater than in wheat (Tinlane and Ledingham, 1979). It is reported that 11-29% losses occur due to this pathogen in winter wheat compared to 27-62% in winter barley (Frank, 1985), whereas in Mexico spot blotch is reported as a more prevalent disease than in other regions of North America (Villareal *et al.*, 1995). In Europe, in Poland, (Lacicowa and Picta, 1994), Germany (Presser, 1991), Finland (Kurppa, 1985) and Russia (Krutiva, 1981), the disease causes economically important yield losses on barley rather than on wheat. However, in Brazil 19% yield losses were estimated in field trials (Diehl *et al.*, 1983). In Bolivia, crop losses up to 57% have been observed due to spot blotch infection (Toledo and Guzman, 1998). In Australia yield losses in susceptible cultivars varied between 14 and 24% whereas resistant cultivars had losses ranging from 7-14% (Wildermuth *et al.*, 1992). The wheat researchers from tropical and sub-tropical countries such as Indonesia, Bangladesh, Vietnam, Thailand, Philippines, China, Nepal, India, Zambia, Zimbabwe and Tanzania indicated that the most economically important foliar pathogen was *Cochliobolus sativus* on the basis of surveys of wheat. The yield losses estimated 5-20% on an annual basis, based on field observations (Dubin and Ginkel, 1991). The losses due to spot blotch ranged from 10-25% in Nepal, Bangladesh and India (Malaker *et al.*, 1994; Karwasra *et al.*, 1998; Ruckstuhl, 1998; Singh *et al.*, 1998) whereas in China it was considerably larger up to 60% (Chang and Yousan, 1998). Apart from grain yield, there were considerable losses in grain weight up to 50% (Karwasra *et al.*, 1998). The *Bipolaris sorokiniana* is also found to be associated with root rot in conjunction with *Fusarium* in Syria (El-Naeb *et al.*, 2002) and with black point disease on kernels of durum and bread wheat in conjunction with *Alternaria alternata, Fusarium* spp., *Cladosporium herbarum* and *Curvularia* spp. further intensify the problem (El-Khalifeh *et al.*, 2002). Even this organism has been

reported in West Siberia on spring wheat giving a climatic diversity in its distribution (Teplyakov and Teplyakov, 2003). The environmental influence on grain yield and its relationship with leaf spot disease has been evaluated during 1994-2001 in Brazil where the climatic conditions were favorable to the development of leaf spot, its occurrence was generalized. Rainfall showed the best correlation with the occurrence of leaf spots caused by *Bipolaris sorokiniana* (Felicio *et al.*, 2004). Foliar disease of wheat and barley in southeastern Idaho, USA have been surveyed in 2001 and 2002 to assess the severity and to identify the pathogens and found *Bipolaris sorokiniana* and *Fusarium culmorum* were the most frequent pathogens and also the most virulent in greenhouse pathogenicity tests (Strausbaugh *et al.*, 2004).

2.3 FOLIAR BLIGHT OF WHEAT IN SOUTH EAST ASIA AND SUB-CONTINENT

In the previous sections the different foliar blights related to fungal pathogen on wheat has been narrated in world perspective. In the following section we will focus only on foliar blight organisms which are of economic importance to South and Southeast Asia and Sub-continent.

There are numerous reports on foliar blight of wheat (Zilkinksky 1983; Prescott *et al.*, 1986; Wiese, 1987; Mahtur and Cunfer, 1993). Many of these are found in South and Southeast Asia (Joshi *et al.*, 1978; Lapis, 1985; Saari, 1985, 1986; Bhatti and Ilyas, 1986; Hafiz, 1986; Kulkarni and Naragund, 1986; Nema, 1986; Saunders, 1988; Dubin and van Ginkel, 1991; Minh, 1991; Alam *et al.*, 1994; Karki and Karki, 1996; Sharma, 1996; Sharma *et al.*, 1996; Singh and Srivastava, 1997). In a survey of foliar blight conducted during 2000, 2001 and 2002 wheat growing seasons in India, Nepal and Bangladesh; a total 198 leaf samples were collected in farmer fields and observed that *Bipolaris sorokiniana* was predominant in India and Bangladesh while *Pyrenophora tritici repentis* was mainly isolated from samples, collected from Nepal (Mercado *et al.*, 2003). In these regions, the leaf blights are caused by fungi. The leaf blight's disease represents a complex and is collectively referred as Helminthosporium leaf blight (HLB). Two of the most common diseases were spot blotch and tan spot, while the other leaf blight pathogens appear to be not important on a national scale (Joshi *et al.*, 1986). Among HLB blights, the tan spot disease has not been reported to

occur frequently in Southeast Asia (Hosford, 1982; Schilder and Bergstrom, 1993). While spot blotch is considered to be the most common and widespread disease (Fischer, 1985). Keeping in view the wide spread nature and importance of the spot blotch disease of wheat more detailed review is included in this part so as to see the work done on the organism by different researchers from this part of the world.

An account of available information documented and work done regarding *Bipolaris sorokiniana* in the region is reviewed below:

Bipolaris sorokiniana is the most frequently associated with poor germination and abnormal seedlings of wheat. The foliar disease poses varying levels of threats to the production of wheat in different agro-ecological conditions. (Ammara *et al.*, 2001). Earlier studies on foliar blights inciting pathogens in the rice-wheat cropping system of Northern Punjab, Pakistan revealed that *Bipolaris sorokiniana, Drechslera tetramera, Pyrenophora tritici-repentis, Alternaria alternata* and *Stemphylium* sp. are foliar pathogens of wheat ((Iram and Ahmed, 2004).

2.3.1 Occurrence/ Incidence

Spot blotch of wheat is the most severe constraint to the wheat production in the countries of Southeast Asia such as India, Bangladesh and Thailand, where climates are warm and moist. Several epidemics of the disease have been reported in northeastern, northwestern and southern China (Yousan *et al.*, 1957; HuanGuichas *et al.*, 1957; Ouyang Xiao, 1962; Qixiang and He Jiabi , 1987 and Wu Baichai and Le Yugan, 1983). The spot blotch is found most frequent occurring pathogen in Bangladesh and currently becoming the major limitation for wheat cultivation in the country (Alam *et al.*, 1994). In Nepal *Bipolaris sorokiniana* has also been found widely distributed throughout the country and has extended from the lower belt of the Terai region to the hilly regions (Anonymous, 1979). Incidence of *Bipolaris sorokiniana* was calculated as 86.1 – 91.2% from 504 plants collected from wheat fields in different areas of Heilong-jiang province of China (Zhang Jing Chum, 1988). Occurrence/spread of disease has also been reported from other provinces of China, Henan Province (He Jiabi, 1964), Shandong province (Jiang Zehai *et al.*, 1978) and Guangdong province (Wu Baicahi *et al.*, 1983). The organism was found frequently

associated with leaf blight or spot blotch in various agro climatic regions of India (Mahto *et al.*, 2002; Singh and Pankaj, 2005). In north eastern and north western plains of India the yield losses ranged from 27% to 56.6% during 1998-99, incited due to the leaf blight caused by *Bipolaris sorokiniana* (Satvinder *et al.*, 2002; Singh *et al.*, 2002). A severe leaf blight epidemic was observed in Indian Punjab, the dominant pathogen in the blight complex was *Drechslera sorokiniana* followed by *Fusarium* spp. The seed germination and seedling emergence significantly decreases with increasing quantities of black pointed seeds caused by *Bipolaris sorokiniana* in studies conducted in Bangladesh (Hossain and Hossain, 2001) and China (Song *et al.*, 2001). Earlier while studying the parasitic fungi associated with wheat seeds in East Hebei Province of China, found that *Cochliobolus* sativus was the second predominant species isolated from seeds of different wheat cultivars following *Alternaria alternata* (Wenlan *et al.*, 2001). According to an extensive survey report during wheat cropping season of 1997-98 in 8 locations around Bihar, India the occurrence of *Helminthosporium* disease of wheat had an incidence ranging from 35 to 61.5 % (Arvind-Kumar *et al.*, 2000).

2.3.2 Pathogen Mechanism of Infection

Aggarwal *et al.* (2000) studied the infection process of *Drechslera sorokiniana* by scanning electron microscope. The studies on spore morphology revealed that conidia are smooth– walled with polar germ pores. On coming in contact with susceptible host they germinate from the polar cells and infection hyphae penetrated the host through stomata by forming appressoria like–structures. The host responses in spot blotch disease consisted of successive growth phases characterized by cuticle and cell wall penetration followed by the development of hyphae within the invaded, living epidermal host cells, hyphae invasion into the mesophyll layer accompanied by epidermal and mesophyll cell death. Rarely the pathogen penetrates via stomata (Jagdish *et al.*, 2002). Forty-one isolates of *Drechslera sorokiniana* were found pathogenic under artificial inoculation test on wheat. The isolate from Pantnagar was found most virulent, giving the highest average infection index, whereas low infection index was recorded for isolates from Jammu and Rajasthan (Mahto *et al.*, 2002). In one of the experiments, pathogenicity of the identified fungi collected during survey

from Bihar, India was investigated by both injured and uninjured leaves by inoculating artificially with mycelium/spore suspension of both *Helminthosporium* spp. (*H. sativum, H. tritici-repentis*) separately. The two pathogens produced similar symptoms as observed in the field survey. The percentage infection was higher on injured than on uninjured leaves (Kumar *et al.*, 2000)

2.3.3 Pathogenic Variability

The proof of pathogenicity is the first step towards studies on virulence (Nishimura *et al.,* 1982 and Regmi *et al.*, 2001). The assessment of the aggressiveness of plants is the frontier at which host and pathogen interact. It is the site of pathogen's initial contact with the host's defense systems, successful circumvention of host defenses may lead to colonization, infection and invasion, pathogen replication and transmission to another host. There is an obvious benefit in developing a better understanding of the host pathogen relationship (Jain and Prabhu, 1976).

The aggressiveness of *Bipolaris sorokiniana* in sprouting wheat seeds has been evaluated by the blotter method. The seed plated on moist blotter in a Petri dish were incubated at room temperature. The pathogen grew very fast and vigorously and affected the germinating seeds only after 3 days of inoculation. The shriveled seeds showed highest deterioration (87.47%) with very fast and aggressive growth of the pathogen, suggesting the aggressive type of *Bipolaris sorokiniana* that perpetuates and remains potentially viable in the shriveled seeds (Rashid, 2005). The leaf samples of different wheat cultivars showing blight symptoms, collected from Jammu and Kashmir, Himachal Pradesh, Punjab and Bihar, India in 2000-02 resulted in 29 isolates of *Bipolaris sorokiniana* out of 88 samples and all were found pathogenic in pathogenicity test (Singh, 2004). The viable conidia collected from eastern India were multiplied and inoculated on to susceptible varieties under controlled conditions, and the typical symptoms to spot blotch were observed. (Pandey *et al.*, 2005)

The extent of variability in natural populations of *Bipolaris sorokiniana* of wheat has been investigated by many workers. The pathogen was isolated from the infected seeds and leaves of wheat from various locations in India and purified by monoconidial isolation. The use of fluorescent stains has been reported for the study

of nucleus of mycelial cell of isolates. Based on colony morphology, the isolates were classified into five groups. The majority of the isolates (44.63%) in the natural populations were black in colour, suppressed type. The group was most aggressive, causing severe epidemic. The lowest frequency of the isolates (4.96%) was categorized as the white coloured producing very few conidia, while the mean radial growth of isolates, on the 8th day ranged from 4.77 to 8.27 cm (Chand *et al.*, 2003). Duveiller and Altamirano (2000) conducted the pathogenicity of 27 isolates of *Bipolaris sorokiniana* collected at a site in Mexico and their results revealed no clear differences between groups of isolates, based on lesion density. No difference was found between individual isolates when pathogenicity was assessed. The results obtained confirmed that infection by *Bipolaris sorokiniana* is highly variable and sensitive to environmental conditions no physiological specialization was observed rather the fungus appeared as a continuum of isolates differing in aggressiveness. However this work was conducted with isolates from a single site. In another experiment nine mono-conidial isolates of *Helminthosporium sativum* were tested for their variability. The maximum linear growth (96.5 mm) was recorded in case of isolate PH 51 followed by 5, 4, 2, 9, 6, 8 and 3 grown on PDA for 10 days. The shape of the colony of different isolates also varies from circular with moderate aerial mycelium to circular with abundant aerial mycelium. Pathogenic variability was also studied for these isolates by using detached leaf test, which revealed significant differences in incubation period, lesion length and sporulation. It was found that the incubation period ranged from 2.2 to 3.4 days, lesion length 2.4-11.5mm and sporulation of 12.2 and 17.3 spores/mm2 of diseased leaf area in the least virulent and highly virulent isolates respectively (Akram *et al.*, 2001).

2.3.4 Epidemiology

The most conducive conditions especially at the advance growth stage have been reported to be very problematic for the wheat crop. Continuous rains for 5-6 days followed by relatively higher temperatures (daily average of 20-23°C), a spot blotch epidemic develops very poor grain quality, especially in high susceptible cultivars (Mehta, 1993). The spot blotch is found to be widespread in three ecological zones of India (Fischer, 1985). The three zones were classified on the basis of coolest month

(January) mean temperature 1. Very hot > 22.5°C, 2. Hot > 17.5°C and 3. Warm 12.5°C (Dubin and Van Ginkel, 1991). Among the three zones, the spot blotch was found dominant when the high relative humidity (RH) prevails or dew is common (Dubin and Van Ginkel, 1991; Sharma, 1996; Sharma *et al.*, 1996; Singh and Srivastava, 1997). A severe leaf blight epidemic on wheat in Punjab (India) and adjoining areas in 1995 was observed due to the high moisture and temperature at terminal crop stage (Satvinder *et. al.*, 2001). The role of weather factors in the development of major foliar diseases of wheat was observed during 3rd week of February to 1st week of April for three crop seasons (1995-96, 1996-97 and 1997-98) at Pantnagar, Utter Pradesh, and India. The minimum and maximum average temperature of 11.7°C (range 10.8 –12.5°C) and 27.7°C (range 26.8 –28.5°C), 39.7 mm rain, 1.2 rainy days per week, 42.8-88.2 relative humidity and 8.2 hours bright sunshine per day were found to be the most favorable for rapid leaf blight development (Singh and Tewari, 2001). Studies on the effect of temperature on lesion expansion rate of *Bipolaris sorokiniana* revealed, nocturnal and diurnal temperatures of 23 and 30°C respectively, were the most favorable for lesion expansion (Prates and Fernandes, 2001). One of the major limiting factors to wheat production is the very hot (<22.5^{0}C) and humid climates prevailed in Vietnam, Philippine and Indonesia caused the various diseases of wheat. The most important is caused by *Bipolaris sorokiniana* (Lapis 1985; Saari, 1986; Saunders, 1988; Mann, 1992). Although the blight initiation began as early as the second fortnight in December, but its severity increased during heading and flowering stages. The temperature of 28°C and relative humidity (RH) of 92% promote maximum foliar blight development and so as rainfall (20.6mm) increased the disease intensity. The other factors assumed to increased foliar blight intensity were found to be due to minimum tillage, irrigation, late planting and inappropriate fertilizer regimes (Singh *et al.*, 1995). A 10 year survey conducted in Bangladesh during 1985/86-1995/96 revealed, spot blotch damage occurs late in the season when the crop is approaching maturity (Alam and Saha, 1991). Typically spot blotch occurs in warm and humid environments, such as the non-traditional, subtropical low land wheat growing areas in the Andean region of Latin America, including Bolivia and in the warmer regions of Brazil (Diehl *et al.*, 1983) North-East Argentina and Paraguay (Toledo and Guzman, 1998). In Africa, similar environments

are found in Tanzania and the rain fed wheat growing areas of Zambia and Madagascar. In the Indian sub-continent, similar conditions occur in the warmer parts of eastern India, most of Bangladesh, and in the Teria region of Nepal. In Southeast Asia, most small wheat growing areas in Thailand, Philippine and Indonesia, and the high rainfall and warm wheat growing areas of southern China also have similar growing conditions (Ginker and Raja ram, 1997). So in warmer seasons, it appears that *Bipolaris sorokiniana* establishes early and dominates (Nema, 1986). However more recently, spot blotch has begun to dramatically expand to more moderate and temperate regions of irrigated rice-wheat system. Such as the vast central and Northwestern regions of the Indian subcontinent (Diman *et al.*, 1994). This might be due to pathogen adaptation, changes in varietals spectrum, use of reduced tillage or changes in climates. In Nepal, increase in spot blotch was associated with above average warm temperatures in January (Dubin and van Ginkel, 1991; Dubin and Bimb, 1994). In the rice area even though the spot blotch fungus may be present in small amounts and is a basically a non-host of the disease. The rice may then serve as a "green bridge" to the subsequent wheat crop (Duveiller and Gilchrist, 1994) which is evident and one of the factor that in particular, rice-wheat rotations in which zero or minimum tillage is adopted there is an increasing trend of spot blotch pressure (Saunders, 1989). High temperature and humidity favor the outbreak of the disease particularly in South Asia's intensive irrigated wheat rice production system (Jagdish *et al.*, 2002). While studying the optimum temperature for infection, it was found that infection of wheat leaves by *Bipolaris sorokiniana*, optimum temperature was 28°C while at 22°C the development of lesions was faster (Mishra *et al.*, 2001). The effect of rainfall, temperature and relative humidity on the development of spot blotch disease was investigated during 1997/98 and 1998/99 in Taria region of India. The disease severity was high (75%) in 1997/98 when total rainfall was 37.4 mm and temperature and relative humidity ranged from 14.06 to 27.6°C and from 46.8 to 86.4 % respectively. While disease severity was low (10%) in 1998/99 where there was no rainfall, temperature and humidity ranged from 12.48 to 31.8°C and from 44.28 to 90.57% during the observation period respectively (Akram and Amerikasingh, 2003).

Preliminary epidemiological observation suggests that the combined effects of high temperature, high relative humidity and long periods (>12 h) of leaf wetness caused by rainfall or dew are conducive to foliar blight development in the Indo-Gangetic Plains where wheat is grown from November to April (Duveiller, 2004).

Chapter 3

MATERIALS AND METHODS

3.1 INTRODUCTION

The present studies were conducted to establish a wide, broad base analysis of the situation prevailing in Punjab and NWFP wheat growing areas as far as foliar blight disease other then rusts and smuts are concerned. In addition focused was made on morphological studies (phenotypic characterization) and aggressiveness analysis of fungi isolated. To conduct these studies following materials and methods were used

3.2 SURVEYS

Wheat surveys were conducted in the major wheat growing agro-ecological zones of Punjab and NWFP provinces of Pakistan. These surveys were an attempt to determine the extent of foliar blight disease of wheat, incidence, prevalence and severity during the years 2004 and 2005. Diseased samples having foliar blight symptoms were collected from farmer's fields. Disease assessment was done in the field and samples were brought in laboratory for the isolation of fungi from foliage. The laboratory work (Isolation of fungi, identification, Phenotypic characterization of the pathogens involved in foliar blight, pathogenicity studies, suitability of temperature for the radial mycelial growth of isolates of fungi collected from different ecologies and aggressiveness studies) was done in the Crop Diseases Research Programme (CDRP), Institute of Plant and Environmental Protection (IPEP), National Agricultural Research Centre (NARC), Islamabad, Pakistan.

3.2.1 Assessment of Foliar Blight Diseases and its Geographical Distribution

To initiate the project activities, a 2 stage survey was conducted in 2004 to ascertain the status of foliar blight disease of wheat in Punjab and NWFP wheat growing agro-ecological zones (Fig.3.1). The two stages included seedling and booting. The wheat growing agro-ecological zones have been prepared mainly from disease occurrence point of view specifically keeping in consideration the rusts of wheat (Tahir, 1979).

A brief of the zones constituted and surveyed in Punjab and NWFP is as fallows:

3.2.1.1 Punjab province

The Punjab province constitute zone 5, 6 and 7.

3.2.1.2 Zone 5 Southern Punjab

The main districts included in this zone are Bahawalpur, Muzaffargarh, Vehari and Multan.The major disease of this zone is leaf rust.

3.2.1.3 Zone 6 Central Punjab

The major districts are Sahiwal, Faisalabad, Jhang, Kasur, Lahore, Sheikhupura, southern parts of Mainwali and Sargodha.In these regions leaf rust disease is important.

3.2.1.4 Zone 7 Northern Punjab

The major districts are Gujranwala, Sialkot, Gujrat, Jhelum, Rawalpindi and Attock. The major diseases of this region are stripe and brown rusts, however there is also incidence of flag smut, complete bunt and karnal bunt.

3.2.1.5 NWFP Province

The NWFP province constitute zone 9, 10 and11.

3.2.1.6 Zone 9 Foot hill areas

The major districts are Peshawar, Mardan, northern Kohat.Besides leaf rust, stripe rust is a major disease in this region. In addition when the conditions are favorable karnal bunt can also become problem in the region.

3.2.1.7 Zone 10 Uplands of NWFP

The areas included in this zone are Hazara, Swat, and parts of Gilgat. Besides stripe and brown rusts the major disease is powdery mildew.

3.2.1.8 Zone 11 Foot hill areas

The areas included in this zone are part of Gilgat and Skurdu and part of AJK.The major diseases are stripe and leaf rusts.

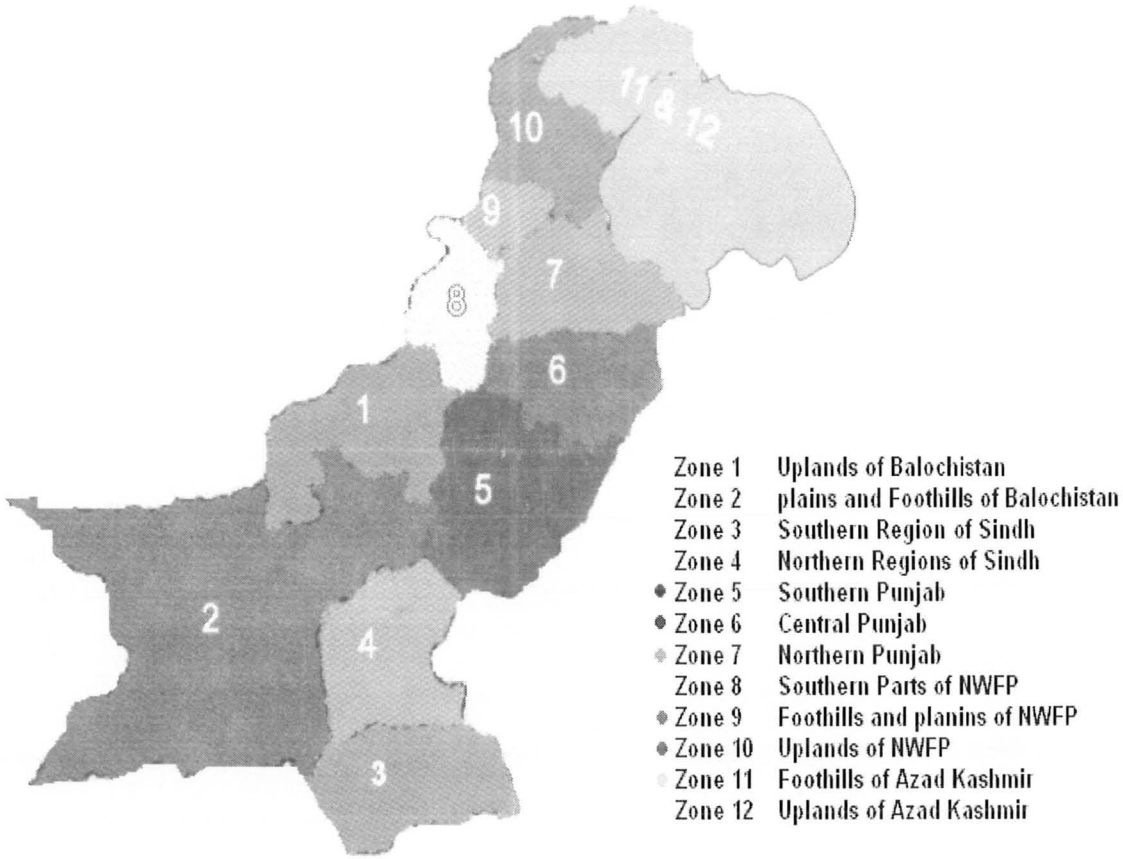


Fig.3.1. Wheat production agro-ecological zones of Pakistan.

The monthly mean temperature and the rainfall distribution in the surveying zones of Punjab and NWFP during 2004 and 2005 are shown in Fig.3.2 and 3.3.

Fig.3.2 Distribution of temperature and rainfall in different agro ecological zones of Punjab during 2004 and 2005.

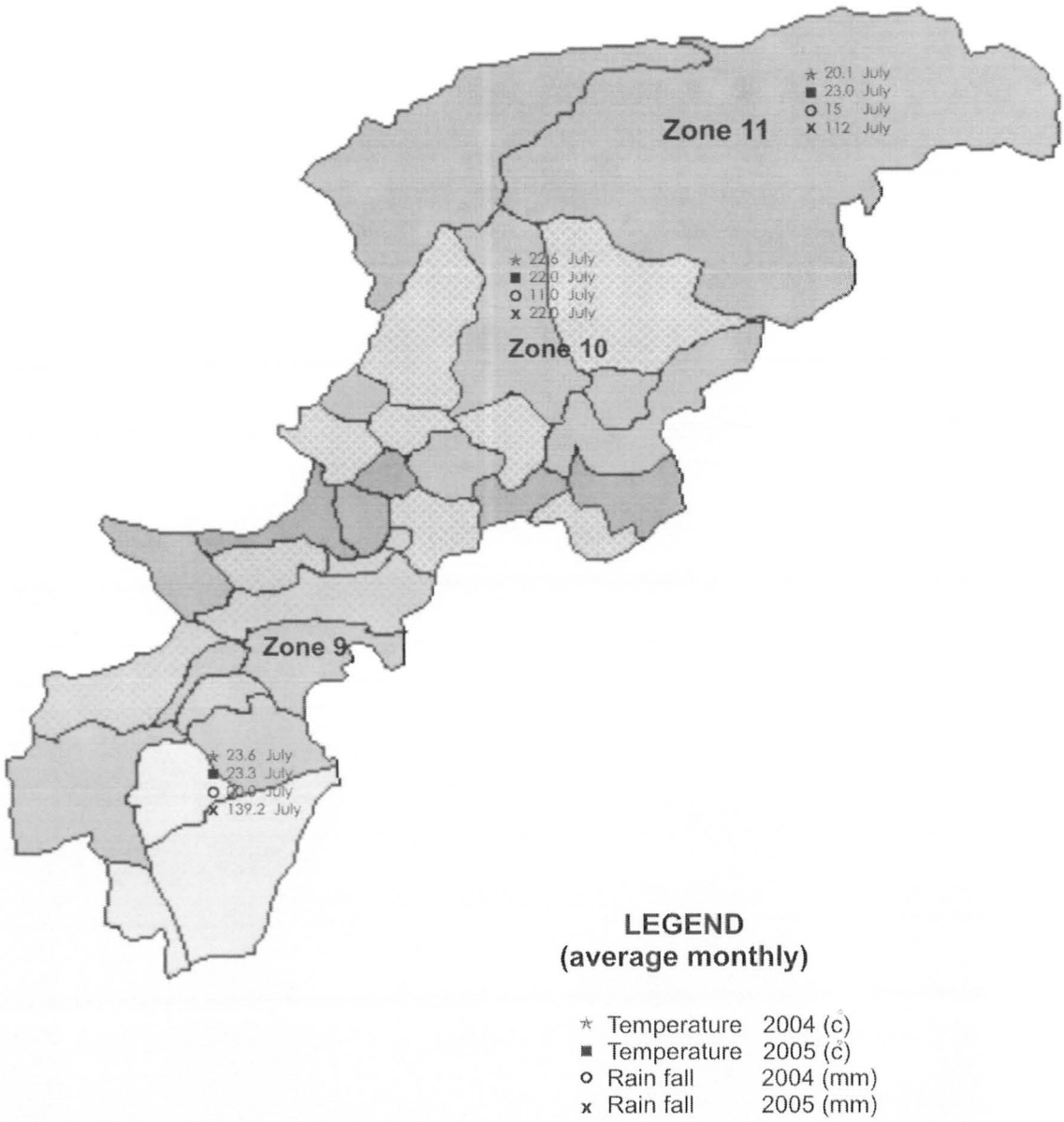
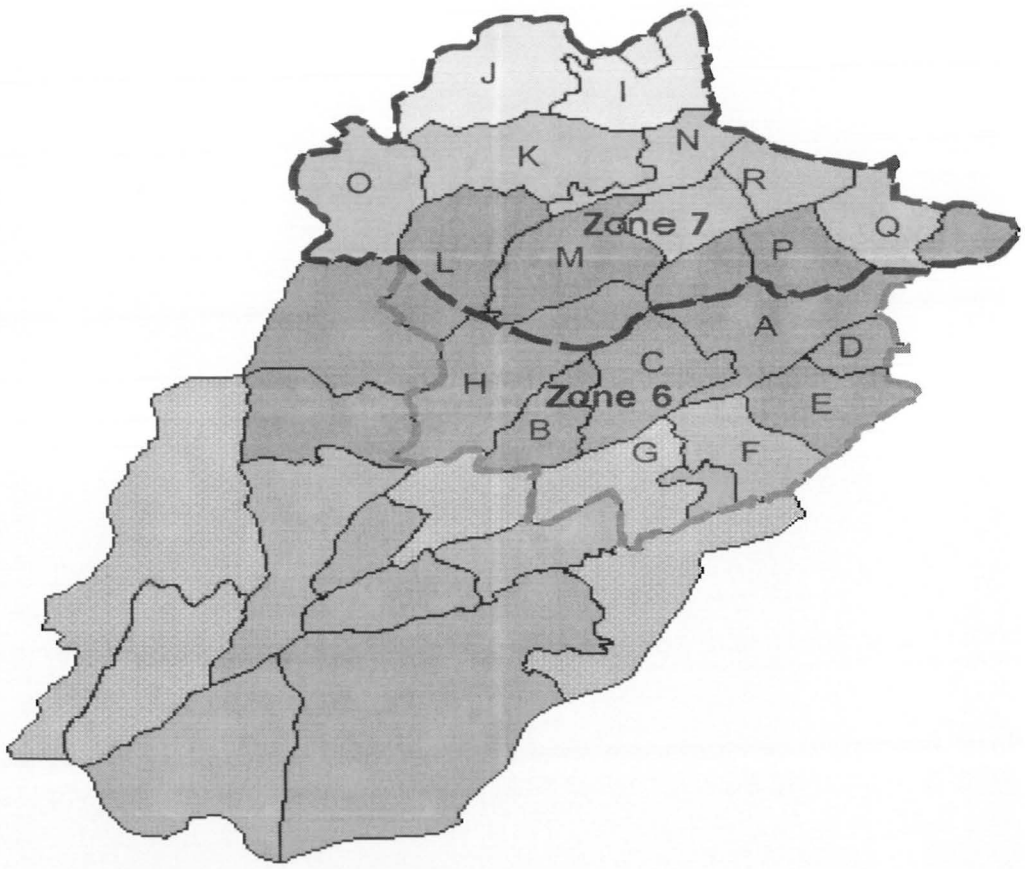


Fig.3.3 Distribution of temperature and rainfall in different agro ecological zones of NWFP during 2004 and 2005.

3.2.2 Surveys at Seedling stage during 2004

3.2.2.1 Punjab

The first survey was conducted from 8th - 11th December, 2004 in Punjab covering part of zone 7 and from twenty one fields (focusing the main rainfed areas of the zone) samples were collected and disease assessment was made. The details of locations are shown in Fig. 3.4.



A = Sheikhupura
B = Tobateksingh
C = Faisalabad
D = Lahore
E = Kasur
F = Okara
G = Sahiwal
H = Jhang
I = Rawalpindi
J = Attock
K = Chakwal
L = Khushab
M = Sargodha
N = Jehlum
O = Mianwali
P = Gujranwala
Q = Sialkot
R = Gujrat

Fig.3.4: Locations surveyed at seedling stage of wheat crop in Punjab, Pakistan Wheat growing areas during 2004

While the second survey was conducted from 19th to 25th January covering forty two fields in zone 6 and 7 of Punjab (Fig.3.4).

3.2.2.2 NWFP

The third survey was conducted in the month of February, in NWFP in zone 9 and 10 and twenty nine fields were covered. The locations are shown in Fig.3.5. During all the three surveys in addition to disease assessment, the disease leaf samples were also collected from each location.

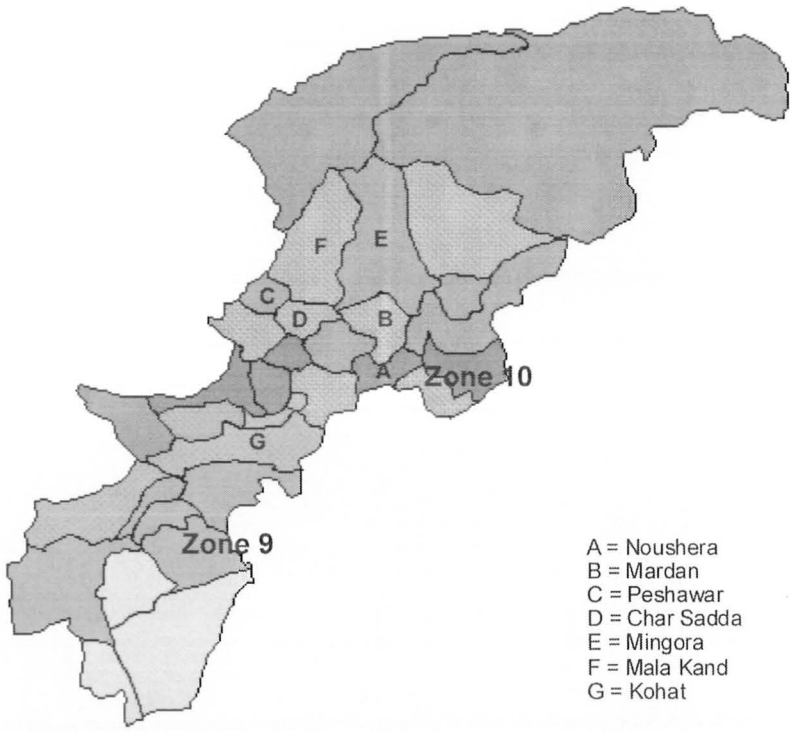


Fig.3.5: Locations surveyed at seedling stage of wheat crop in NWFP, Pakistan wheat growing areas during 2004

3.2.3 Surveys at booting stage during 2004

i) Punjab

The survey at booting stage was conducted from 10th to 16th March in zone 5, 6 and 7 of Punjab covering seventy five fields for disease assessment and samples collection. The locations are shown in Fig.3.6.

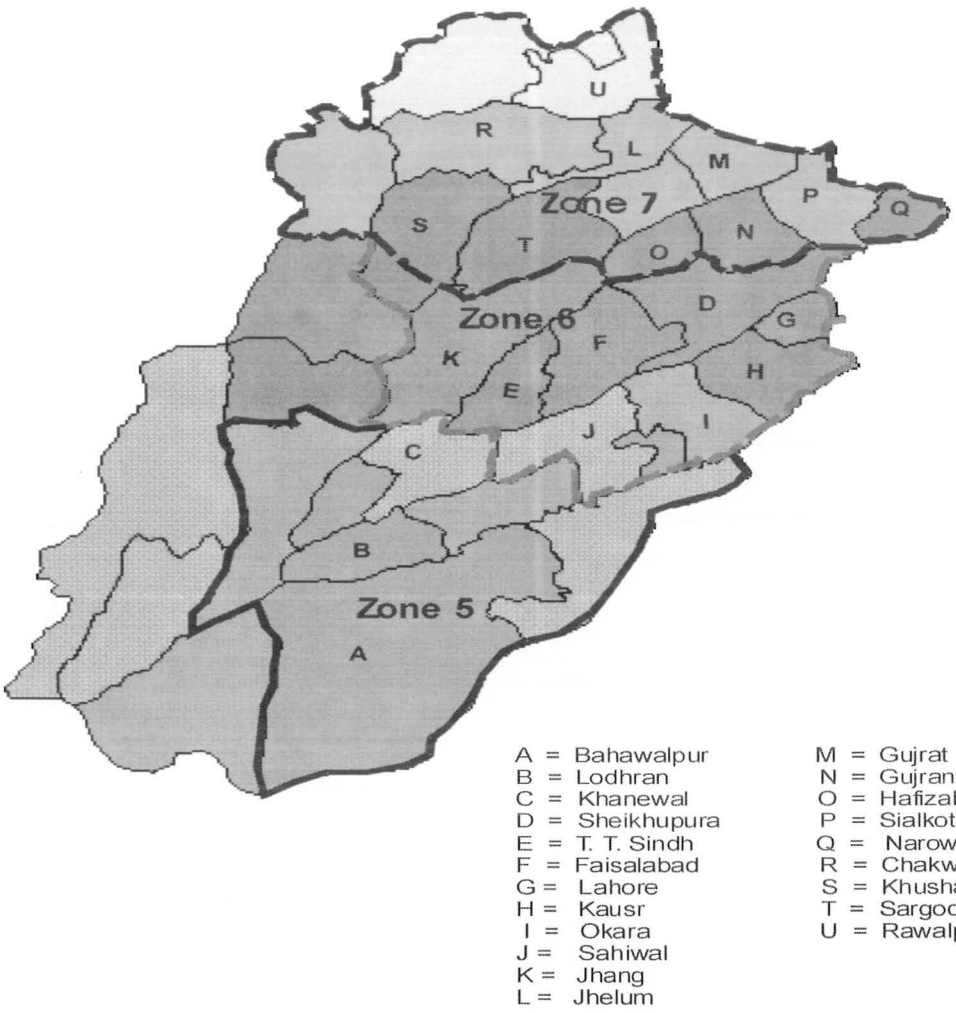


Fig.3.6: Locations surveyed at booting stage of wheat crop in Punjab, Pakistan Wheat growing areas during 2004

ii) NWFP

The surveys in zones 9, 10 and 11 of NWFP were conducted in two different months.

The first survey for the assessment of disease and sample collection was done in the month of March covering twenty five fields in zone 9 and thirty four in zone 10. While the second survey was conducted from May 31st to June 12th in zone 11 covering twenty four fields. The locations are shown in Fig.3.7.

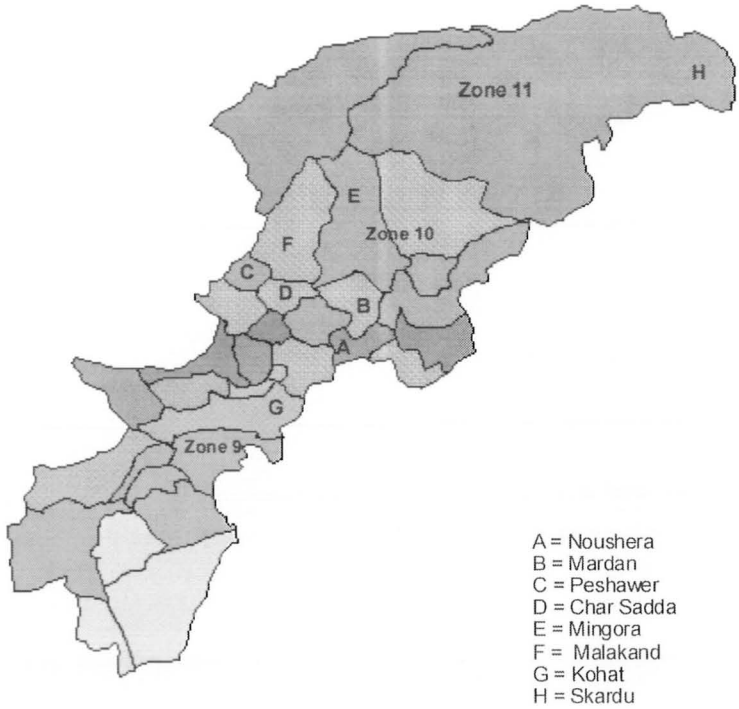


Fig. 3.7: Locations surveyed at booting stage of wheat crop in NWFP, Pakistan Wheat growing areas during 2004

3.2.4 Surveys at booting stage during 2005

During the year 2005 keeping in consideration the low incidence of foliar blight at seedling stage, the survey was conducted only at booting stage of the wheat crop.

i) Punjab

Two surveys were conducted during the month of March at booting stage to assess the disease and sample collection. First survey was conducted in zone 6 and 7 focusing on rice-wheat area and a total of twenty nine fields were visited, while the second was conducted in zone 5 and 6 and 7, other than rice areas covering seventy seven fields. The locations are shown in Fig.3.8.

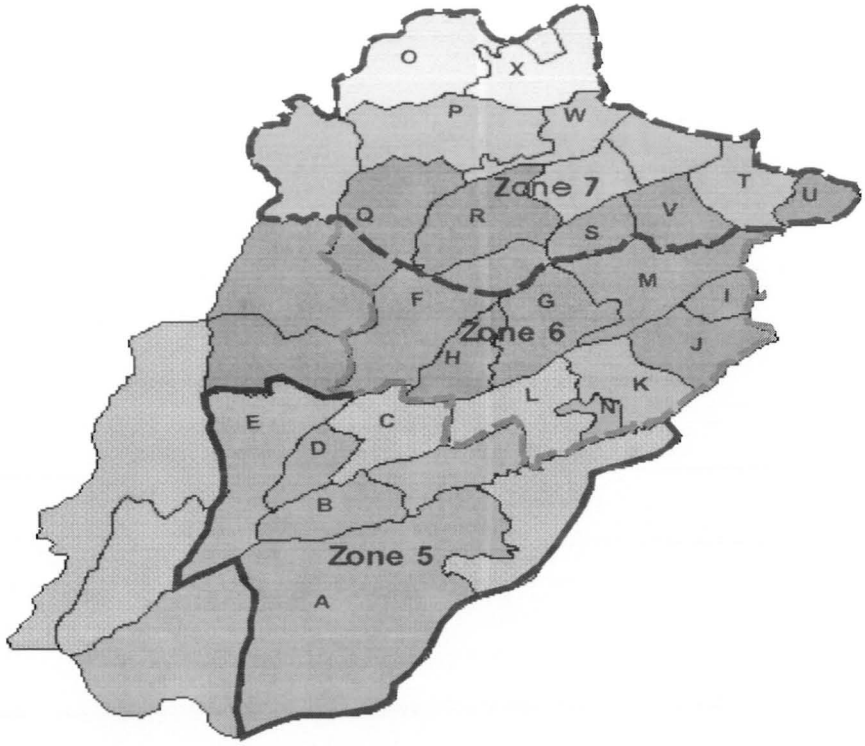


Fig.3.8: Location surveyed at booting stage of wheat crop in Punjab, Pakistan Wheat growing areas during 2005

ii) NWFP

Two surveys were conducted in NWFP zones to assess the disease and sample collection. The first survey was done in the month of March covering twenty six fields in zone 9 while the second survey was conducted in zone 10 covering 12 fields and in 11 the extreme Northern area of the province in June and a total of 8 fields were visited. The locations are shown in Fig.3.9.

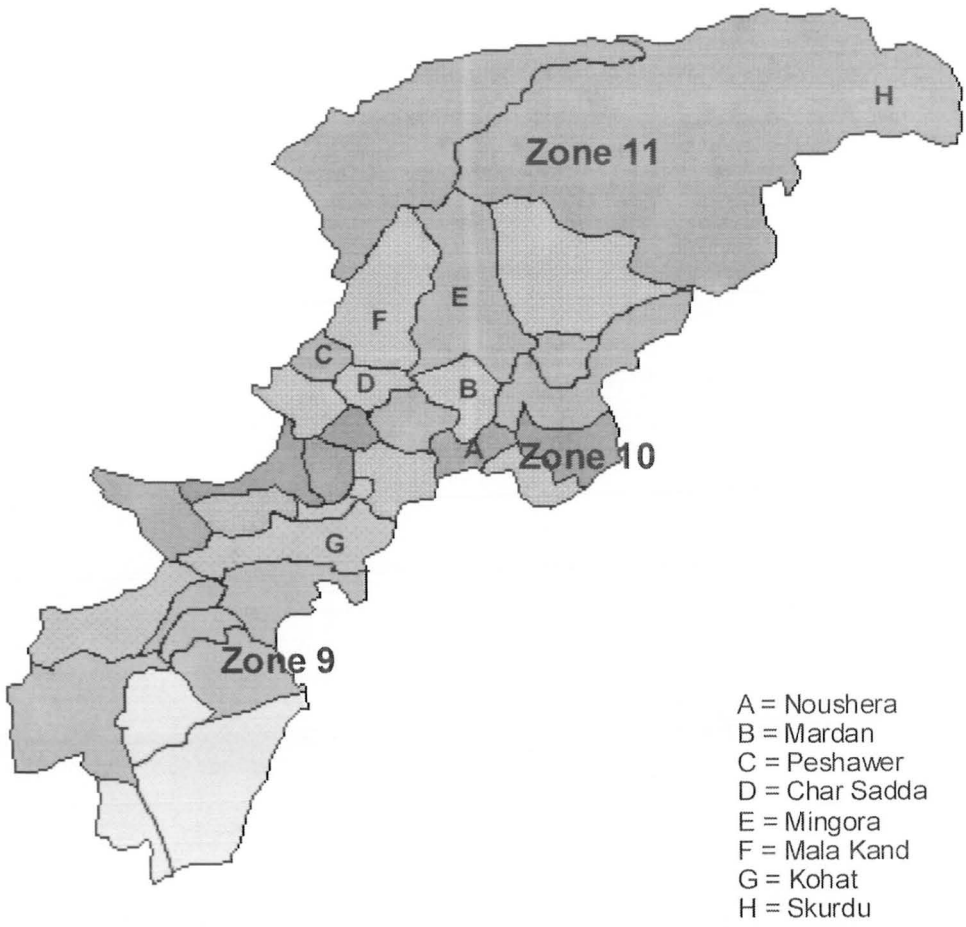


Fig.3.9: Location surveyed at booting stage of wheat crop in NWFP, Pakistan wheat growing areas during 2005

3.2.5 General protocol for foliar blight sampling in the field

The survey was started with first field after 10 km on the car-odometer and subsequently stops were made for each sample every 40 km on either side of road.

During seedling stage a quadrant (50 cm^2) was used for sampling and was dropped randomly in each field after every ten paces. The plants were observed within quadrant for blight disease.

At the booting stage of the crop the sampling was done with general protocol in the diagonal transect (Anonymous, 1996) and the samples were taken at 10 points in an individual field (IRRI, 1996).

3.2.5.1 Assessment of incidence, severity and disease index of foliar blight diseases

By entering into a field, plants were assessed for disease symptoms and samples were collected at each of 10 points. A model design for sample collection and disease assessment is given in Fig.3.10, regardless of field shape the sampling size will from 10 point in diagonal transect.

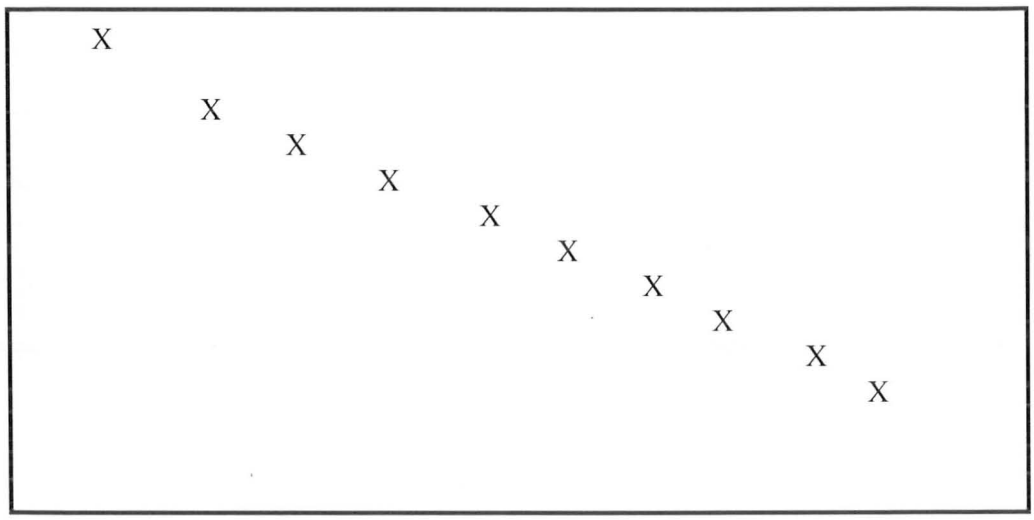


Fig.3.10. Model Design for disease assessment and sample collection in the field

In each field surveyed, overall view of the crop was obtained and general observations were recorded for the presence or absence of disease symptoms that gave an approximate percentage of occurrence. Then from ten spots, plants (10 plants/spot) were assessed for the disease incidence, severity and sample collection. The prevalence was calculated at each location. The prevalence was calculated with the help of following formula:

$$\text{Prevalence \%} = \frac{\text{Locations showing foliar blights}}{\text{Total locations}} \times 100$$

While the incidence was calculated with the help of the following formula:

$$\text{Incidence \%} = \frac{\text{Diseased plant}}{\text{Total no. of plants}} \times 100$$

To determine disease severity for foliar blights, a 0-5 scale was used (IRRI, 1996).

Where

0 = No symptoms,

1=1-5% few spots on < 50% of leaves,

2 = 5-20% spots on <50% of leaves,

3 = 5-20% spots on < 50% leaves

4 = 20-50% spots on < 50% leaves

5 = >50% spots on >50% leaves

In addition the disease index (D.I) was also calculated by putting the collected data from the field into following formula:

$$D.I\ \% = \frac{[\text{Foliar in scale 1}]+[\text{Foliar in scale 2}].....+[\text{Foliar in scale 5}]}{\text{Total foliar samples}} \times \frac{100}{5}$$

After foliar blight diseases assessment the diseased samples collected from each field were placed in paper bags, identity of location/ field and date of collection was recorded and brought to the laboratory for isolation.

3.3 ISOLATION OF FUNGI FROM FOLIAGE

The leaf samples were washed under tap water to remove dust and other materials. The identity of the samples was recorded on the dry filter paper previously placed in the bottom of each Petri plate. Enough autoclaved sterilized distilled water was added to moisten the filter paper; ensuring no excess free water in the plate. The leaf sections with disease symptoms were cut into small pieces (1cm) approximately (4-5 pieces) and were surface sterilized in 1% Clorox for 1 minute and then rinsed twice with autoclaved distilled water. The pieces were plated on the filter paper as mentioned above. These plates were placed for 24 hours at 25^0C in light and then 24 hours at 18^0C in dark, the presence of organisms on leaf sections was observed under stereomicroscope (De Wolf *et al.*, 1998).

3.3.1 Identification of Fungi

Each plate and section of diseased sample was observed under stereomicroscope and subsequently a slide of each organism was prepared and observed under microscope at 40x magnification. All identifications were based on the characteristics of fungal morphology in accordance with the literature (Gilman, 1945; Barnett, 1960 and Domsch *et al.*, 1980).

3.3.2 Single Spore Culture/Purification

The isolated fungi were purified by single spore culture technique on Potato Dextrose Agar Medium (PDA). (Patoto200gm, glocose15gm, agar15gm, distillated water 1000ml) (Usmani and Ghaffar, 1982). A single conidium was picked with the help of flamed pointed needle upon cooling and was placed on autoclaved solidified PDA already poured in Petri plates. These plates were incubated at $22^{o}C \pm 3^{0}C$ for 12 days till the full growth of the fungus was observed on the medium and were refrigerated at $4^{0}C$ to serve as mother cultures for further studies.

3.3.3 Multiplication of the Cultures

Full grown mother (fungal) cultures were cut into small pieces with the help of cork borer and placed onto PDA, incubated as mentioned in section 3.3.2 and upon full growth these cultures were used for each experiment and activity.

3.4 PHENOTYPIC CHARACTERIZATION OF PATHOGEN

3.4.1 Morphology of Culture of *Bipolaris sorokiniana*

The culture of eighty seven isolates of *Bipolaris sorokiniana* were grown on PDA and colour and growth pattern of isolates was noted.

3.4.1.1 Morphology of *Bipolaris sorokiniana*

The slides of eighty seven isolates of *Bipolaris sorokiniana* were prepared as mentioned in 2.3.1 and observed under light microscope at 40 x and100 x magnifications, the spore shape and size was noted and measured (Sivanesan and Holliday, 1981).

3.5 SELECTION OF METHODS FOR PATHOGENICITY TEST

Five different methods were tested for pathogenicity to select the best method which could be used for pathogenicity and to test the aggressiveness of the pathogen in the present studies. The two aggressive isolate of *Bipolaris sorokiniana* (proven to be most frequently isolated pathogenic fungus in our studies), one from Punjab (P2-9) and one from NWFP (NP-4)) were used on wheat variety Wafaq-2001. So rest of the experimentation was exclusively focused only on *Bipolaris sorokiniana* (87 isolates). The methodology used for each method is given below:

3.5.1 Seed and Inoculum Disk in Soil

The surface disinfected seed with Clorox were sown in the autoclaved soil media (soil mix with sand and peat moss), (1:1:1) in the plastic pots. Three seeds /pot were sown with a disk of fungal isolate, placed adjacent to seed. The pots were incubated at 22 to 25^{0}C in the growth room by placing on the racks randomly. The data was recorded on leaves for spots on 0-5 scale and afterwards roots were observed on 0-3 scale by uprooting and washing with tap water (Ledingham *et al.*, 1973).

3.5.2 Seedling Root Dip in Spore Suspension

The wheat seed were sown in autoclaved sand and watered. After 6-8 days of the sowing, the raised seedlings were removed from the sand very carefully so that roots remain intact. These seedlings were dipped in the spore suspension and were planted in small pots, already filled with autoclaved soil mix consisting of silt loam, sand and peat moss (1:1:1). The pots were placed for incubation at 22 to 25^{0}C. After four week, plants were uprooted and roots were washed with water and browning and blackening on roots were observed with the help of severity scale (0-3) (Ledingham *et al.*, 1973). However the spots appeared on foliage were recorded on 0-5 scale, (IRRI, 1996).

3.5.3 Spore Suspension Spray on Foliage at Seedling Stage

The single spore of the most aggressive isolate of *B. sorokiniana* (P2-9) was transferred on PDA (to obtain fresh mother culture) and placed in an incubator at 25^{0}C until the full growth of the fungus obtained. Then Small plugs of mother culture were

made with the help of cork borer for sub culturing and multiplication of inoculum by transferring again onto Potato Dextrose Agar (PDA) with 100 ppm of streptomycin added in PDA in Petri plates to avoid any bacterial contamination and incubated at 25^0C. Upon completion of growth of the fungus in the plates the mycelium was scraped with a bent metal rod. Conidial suspensions were prepared by flooding the plates with sterilized distilled water. A drop of tween 20 was also added per 100ml of suspension to break the surface tension of the suspension and the concentration was determined with a haemocytometer. (Lamari and Nernier, 1989)

Five seeds of wheat variety Wafaq-2001 were sown in plastic pots containing sterilized soil (as mention in 3.5.1 and 3.5.2) in a growth room. At seedling stage 2-3 leaf stage, (after planting of 17-18 days) were sprayed with a suspension of 3.2×10^4 conidia/milliliter. Approximately 15 ml of inoculum was sprayed per plant with a sprayer. The plants were covered with plastic bag for attaining high humidity and after 30 hours, plants were allowed to dry and then returned to growth chamber at 22^0C to 25^0C for incubation. The experiment was designed in Factor Randomized Block Design (FRBD). The data of disease severity was recorded upon the appearance of spots on the leaves on (0-5) scale, (IRRI, 1996).

One or two leaves were randomly excised from each replicate. These leaves were surface sterilized and then incubated on moistened filter paper in Petri plates in light and dark cycles. After 48 hours of incubation fungus was examined under stereomicroscope. These samples were plated on PDA and after 3-4 days the sporulation was observed by making slide under microscope and compared with mother culture for confirmation of Koch's postulate.

3.5.4 Pre-sowing Seed Coating with Fungal Conidia

The wheat seeds were surface sterilized with 1% Clorox and was rinsed three times with sterilized distilled water. A few drops of tween 20 were sprayed on these seeds and then were put on the actively grown culture of *B. sorokiniana* showing dark black appearance. These seeds were mixed in the culture with the help of rod so that maximum conidia may stick with the seed. Later these seeds were sown in the pots as mentioned in 3.5.1 and incubated at 22 ± 3^0C. Later the Koch's postulates were also

confirmed and the data was recorded upon the appearance of spots on the leaves on (0-5) scale, (IRRI, 1996).

3.5.5 Test Tube Moist Cotton Swab Method

Test tubes measuring (20 cm x 3 cm) were prepared by filling 1/4th of cotton in the bottom of the tube. Sterilized distilled water (20 ml) was added in each tube and lids were covered with aluminum foil and were autoclaved. The disinfected wheat seeds with inoculum were placed on the moist cotton swab in the test tube. One disk of 5 mm of fungal isolates to be tested for pathogenicity was placed adjacent to the seeds (Giri *et al.*, 2001). The tubes were arranged in randomized design (RCD) in the steel racks, after inoculation were again sealed with aluminum foil and were placed in growth room at 25^0C for incubation. The Koch's postulates were confirmed and the data was recorded upon the appearance of spots on the leaves by (0-5 scale) by the revising the (IRRI, 1996), disease rating scale as fallows:

0 = No Symptoms

1 = 1-5% spots on leaves.

2 = 6-20% spots on leaves

3 = 21-40% spots on leaves.

4 = 41-60% spots on leaves.

5 = 61% and above spots on leaves.

3.5.5.1 Standardization of test tube moist cotton swab method

The method mentioned above in section above was standardized by using two treatments as follows:

a) Application of inoculum on seed

The prescribed method was followed (Giri *et al.*, 2001) as mentioned in above section 3.5.5.

b) Application of inoculum at root initiation

The wheat seed were prepared and placed in the test tubes in the same manner as mentioned above and it was allowed to germinate. When the roots appeared and seedling became 5 days old, the inoculum disc was placed on the roots. The rest of the protocol was followed as mentioned in section.3.5.5. Data for both the procedures followed in section 3.5.5.1a and 3.5.5.1bwas subjected to analysis of variance and mean interaction by computer package MSTAT-C.

3.6 PATHOGENICITY TEST

The pathogenicity of all the isolates of fungi obtained from samples collected during surveys 2004 & 2005 was done. The fungi *Alternaria alternata, Bipolaris sorokiniana, Colletotrichum graminicola, Cladosporium cladosporioides, Curvularia lunata, Dilophospora alopecuri, Drechslera specifer, Drechslera rostata, Epicoccum purpurascens* and *Stemphylium* sp were tested for pathongenicity. Pathogenicity of the organisms isolated from barley and oat samples was also tested on wheat. The pathogenicity was conducted by the selected in vitro method in section (3.5.5.1b.) Data was recorded upon the appearance of spots on the leaves in the test tube on (0-5) scale (IRRI, 1996).

3.7 AGGRESSIVENESS STUDIES

Eighty-seven isolates of , *Bipolaris sorokiniana*, collected during 2004-2005 were evaluated for their aggressiveness and classified into different severity classes with the help of severity scales (0-5) scale (IRRI, 1996). For the evaluation of aggressiveness, experiment was conducted by using test tube moist cotton swab method in section (3.5.5.1b) under controlled conditions and the commercial varieties of wheat were tested.

3.7.1 Test Varieties

Out of ten commercial varieties of wheat, three varieties (Wafaq-2001, Inqilab-91 and Bhakkar-2001) were selected on the basis of its pathogenic reaction to *Bipolaris sorokiniana.*Later eighty seven isolates were tested for aggressiveness reaction to these three varieties.

3.7.2 Data Analysis

All isolates of the fungus *Bipolaris sorokiniana* were selected as slightly, moderately and highly aggressive isolates on the basis of pathogenic behaviour. Data were analyzed by using two computer statistical softwares i.e. MSTATC and Minitab Programmes. Disease severity means of all isolates were subjected to analysis of variance using MSTAT-C package (Steel and Torrie, 1980) while the Cluster analysis was done by computer software Statistika CLUSTER procedure in minitab.

3.8 EPIDEMIOLOGICAL STUDIES

3.8.1 Effect of Different Temperatures on the Radial Mycelial Growth of *Bipolaris sorokiniana*

For this study the isolates of *Bipolaris sorokiniana* {aggressive (01), moderately aggressive (15), slightly aggressive (29) and least aggressive (01)} were selected from the previous experiment of aggressiveness analysis. The pure mother culture of each isolate of *Bipolaris sorokiniana* was obtained by single spore culture technique. Three temperature regimes viz., 20^0C, 25^0C and 30^0C were used. The plates poured with autoclaved and solidified Potato Dextrose Agar Medium were inoculated in triplicate by taking a disc of each isolate with the help of cork borer in the centre. Later these plates were placed in three incubators having 20^0C, 25^0C and 30^0C temperatures. Data on measurement of radial myeclial growth was recorded at 4, 8 and 12 days interval after inoculation (Abou Heilah *et al.*, 1985). Later the data was subjected to analysis of variance and means interaction by using computer statistical software MSTAT-C (Steel and Torrie, 1980) while the graphics were prepared using Microsoft Excel programme.

3.8.2 Hosts other than Wheat

The hosts of *Bipolaris sorokiniana* other than wheat were also studied (Table 3.1). In addition the plants of oat and barley showing the foliar blight symptoms in the wheat fields or adjacent fields were also collected and after isolation and purification of all isolates of the pathogen, inoculum was prepared and was tested for pathogenicity on host plant and than on wheat.

Table 3.1 List of host plants determined for host of *Bipolaris sorokiniana* other than wheat

S.No.	Crop
1.	*Avena sativa*
2.	*Hordeum vulgare*
3.	*Brassica compestris*
4.	*Glycine max*
5.	*Lens culinaris*
6.	*Vigna radiata*
7.	*Oryza sativa*
8.	*Halianthus annus*
9.	*Sesamum indicum*
10.	*Arachis hypogea*
11.	*Vigna mungo*
12.	*Sorghum bicolour*
13.	*Zea maize*
14.	*Cicer arientenum*
15.	*Panicum maximum*

3.8.3 Assessment of foliar blight in different production technologies followed in rice-wheat cropping system

Surveys were conducted during 2004 and 2005 in rice wheat cropping areas to ascertain the prevalence of foliar blight of wheat. Samples were collected from 17 fields during 2004 having 4 fields in zone 6 where bed planting was followed and 13 fields in zone 7 where at 6 fields bed and at 7 fields conventional methods were followed (Fig.3.6). Total 17 fields were visited during 2005 having 5 fields in zone 6 /where at one field bed, 2 conventional and at two fields zero tillage was followed.

In zone 7 twelve fields were visited whereas at 9 fields conventional and at 3 fields zero tillage was followed. Samples were brought to lab and were plated under controlled conditions and identified by the same procedure as mentioned in section 3.3.

Chapter 4

RESULTS

Survey on wheat crop was conducted to ascertain the prevalence, incidence and severity of foliar blight of wheat in two consecutive years, 2003-2004 and 2004-2005 during wheat cropping seasons in different wheat growing agro-ecological zones of Punjab and NWFP provinces of Pakistan. The first survey was conducted during 2004 at two growth stages of wheat in both the provinces i.e., seedling stage and booting stage. Different fungi viz *Alternaria alternata, Bipolaris sorokiniana, Curvularia lunata, Epicoccum purepurasecns and Drechslera spicifer* were isolated. While during 2005, survey was conducted only at booting stage. In addition to *Alternaria alternata* and, *Bipolaris sorokinaina,* the other fungi isolated from foliage samples of wheat were *Colletotrichum graminicola, Cladosporium cladosporioides, Dilophospora alopecuri, Drechslera rostata,* and *Stemphylium* sp. These fungi were studied for the pathogenicity test at first and upon the confirmation of Koch's postulates further studies were planned.

4.1 PREVALENCE, INCIDENCE, SEVERITY AND DISEASE INDEX OF FOLIAR BLIGHT OF WHEAT CROP SURVEYS DURING 2004 AND 2005

The survey of wheat growing areas in Punjab and NWFP during 2004 was conducted at two growth stages of wheat during viz., seedling stage and booting stage. The total numbers of fields surveyed during 2004 at seedling stage (zone 6, 7, 9 and 10) and booting stage (zone 5, 6, 7, 9, 10 and 11) were 92 and 158 respectively from where a total of 2500 foliage samples were collected (Appendix 1, 2, 3 and 4). In comparison, during 2005, the survey for foliar blights of wheat was conducted in same six zones of Punjab and NWFP and a total of 1520 samples were collected from 152 fields. This survey was conducted at booting stage only (Appendix 5 and 6).

4.1.1 Seedling Stage Survey 2004

4.1.1.1 Prevalence

The data in Fig.4.1 represents the single value of prevalence of foliar blights in each ecological zone according to their ecological categorization.

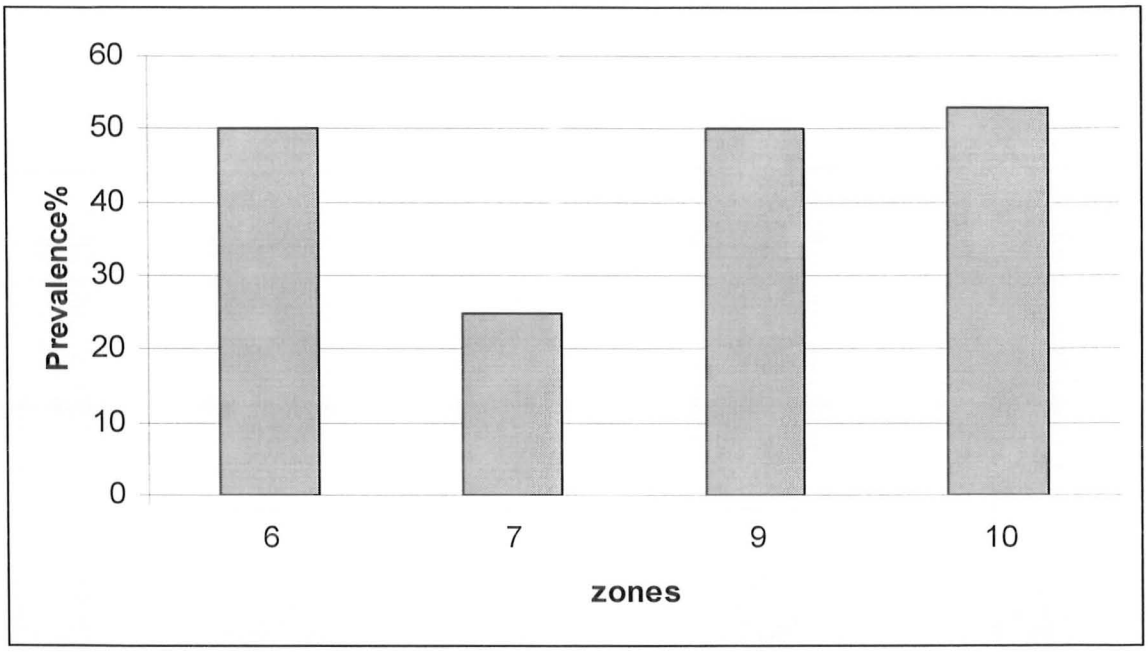


Fig. 4.1 Prevalence of foliar blight of wheat in different wheat growing agro-ecological zones of Punjab and NWFP at seedling stage during 2004

Punjab

Maximum prevalence (50%) of foliar blight at seedling stage was calculated in zone 6 and minimum prevalence of 25% at zone 7 (Fig.4.1). The distribution of foliar blight prevalence in different zones of Punjab at seedling stage during 2004 is shown in Fig.4.2.

NWFP

Maximum prevalence (53%) of foliar blights was calculated in zone 10 while minimum (50%) in zone 9 (Fig. 4.1). The distribution of foliar blight prevalence in different zones of NWFP at seedling stage during 2004 is shown in Fig.4.3.

4.1.1.2 Incidence

Punjab

Maximum incidence (5%) was calculated in zone 6 whereas minimum (1.9%) in zone 7 (Table 4.1). The distribution of foliar blights incidence at different locations at seedling stage in Punjab is given in Fig 4.4.

NWFP

Maximum incidence (5.3%) was calculated from zone 10 closely followed by (5 %), minimum in zone 9 (Table 4.1). The distribution of foliar blights incidence at different locations seedling stage in NWFP is given in Fig 4.5.

4.1.1.3 Severity

Punjab

Maximum severity was recorded in zone 6 i.e. 0.5 while minimum in zone 7 (0.1) (Table 4.1). The distribution of foliar blights severity at different locations seedling stage in Punjab is given in Fig 4.4.

NWFP

In both the zones 9 and 10 the severity recorded was same i.e.0.5 (Table 4.1). The distribution of foliar blights severity different locations at seedling stage in NWFP is given in Fig 4.5.

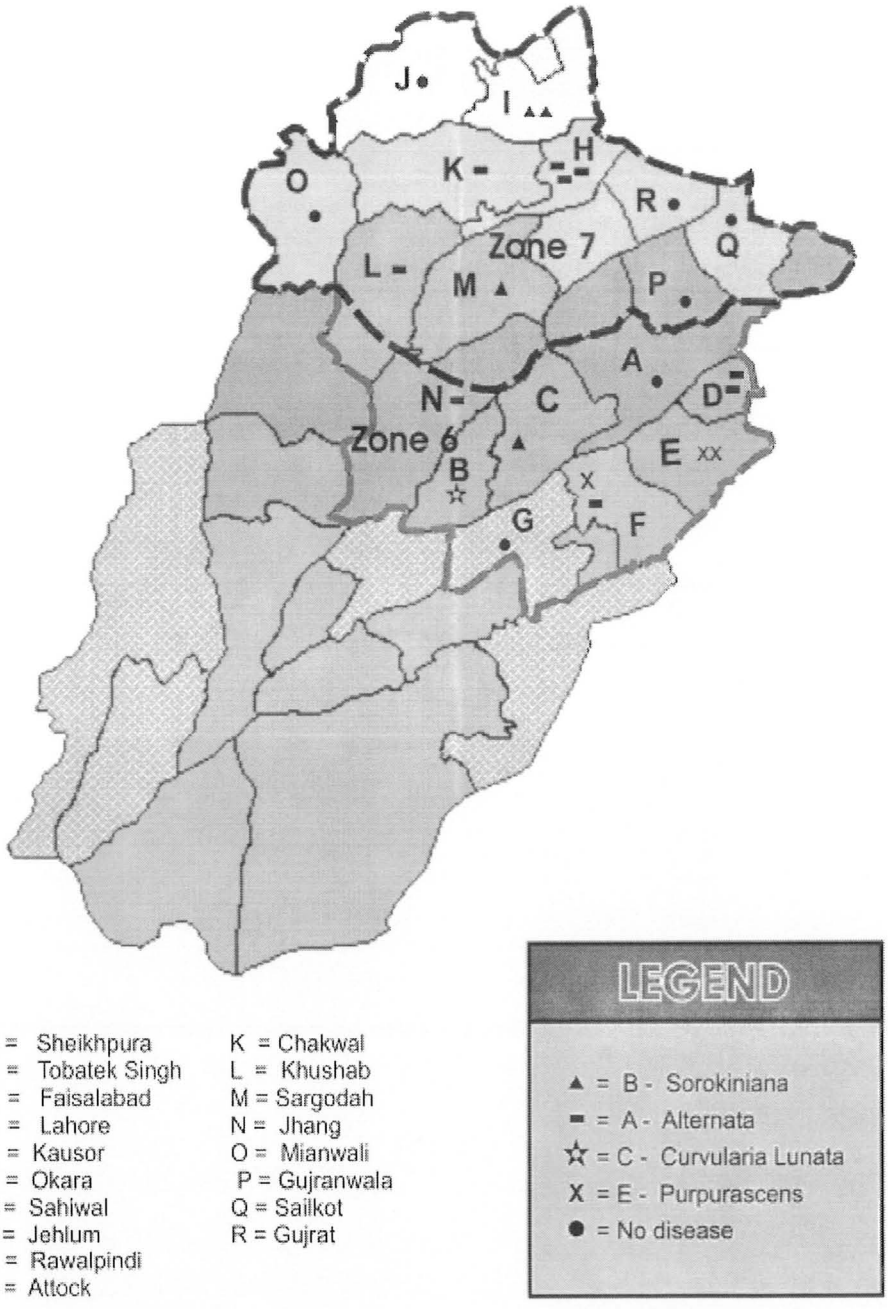


Fig.4.2 Distribution of Foliar blight Prevalence in Punjab Zones at seedling stage during 2004.

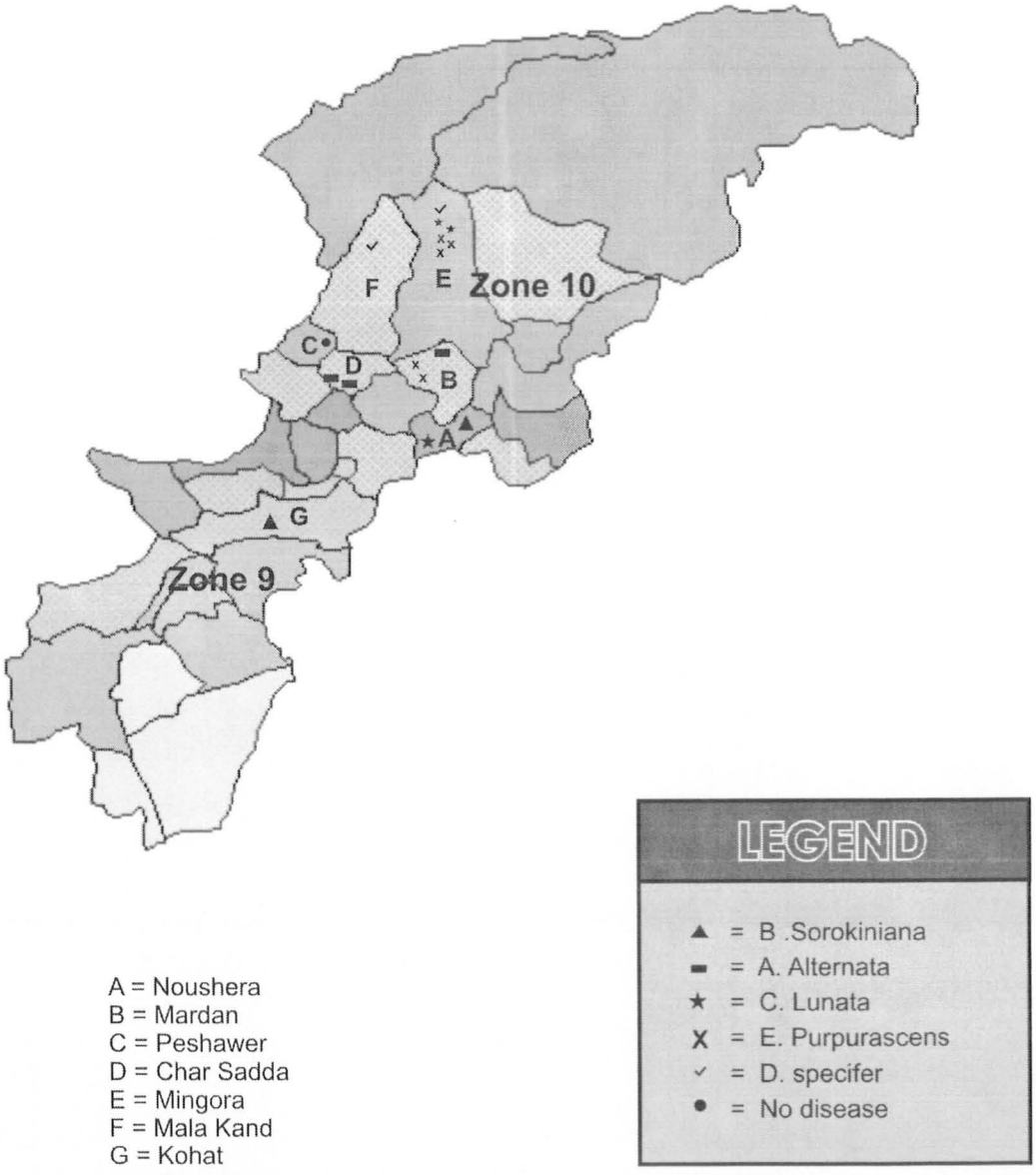
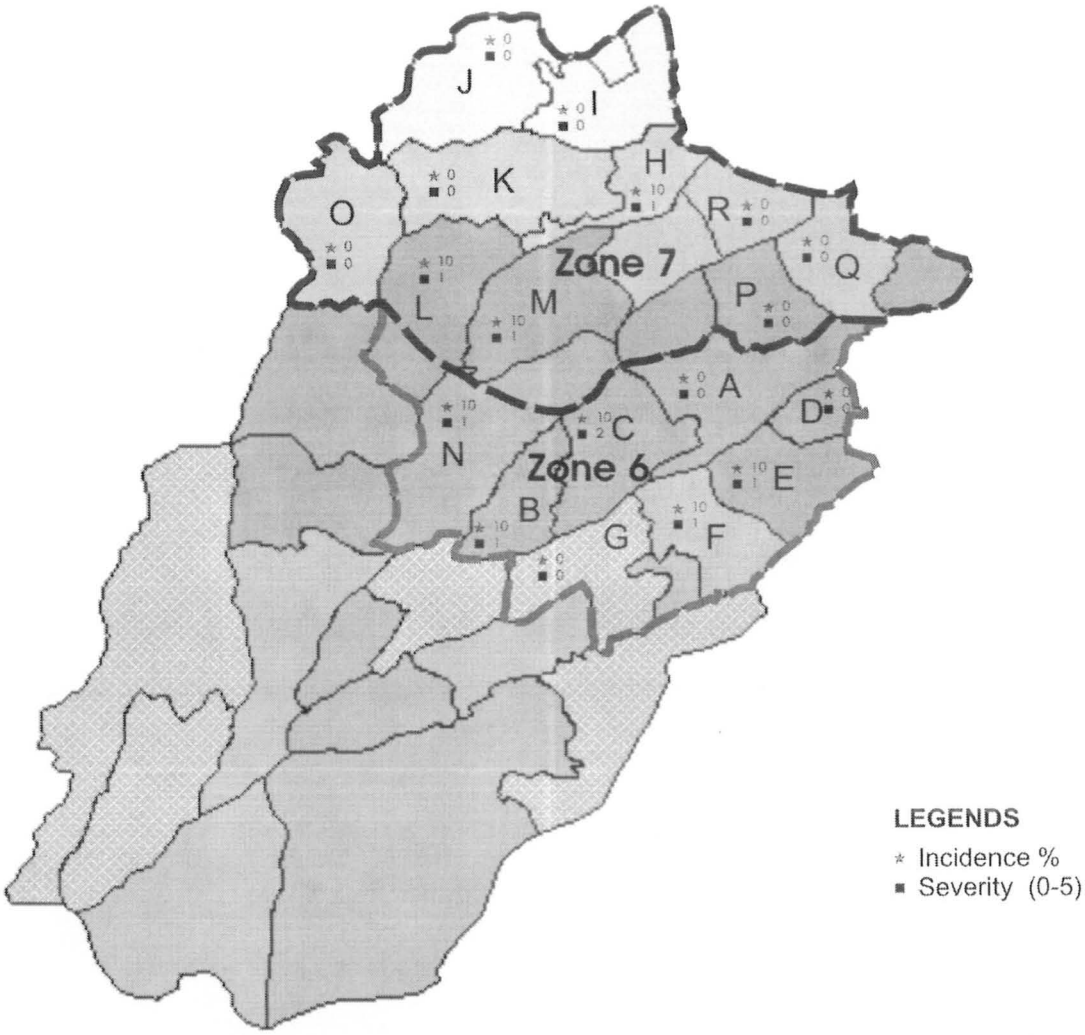
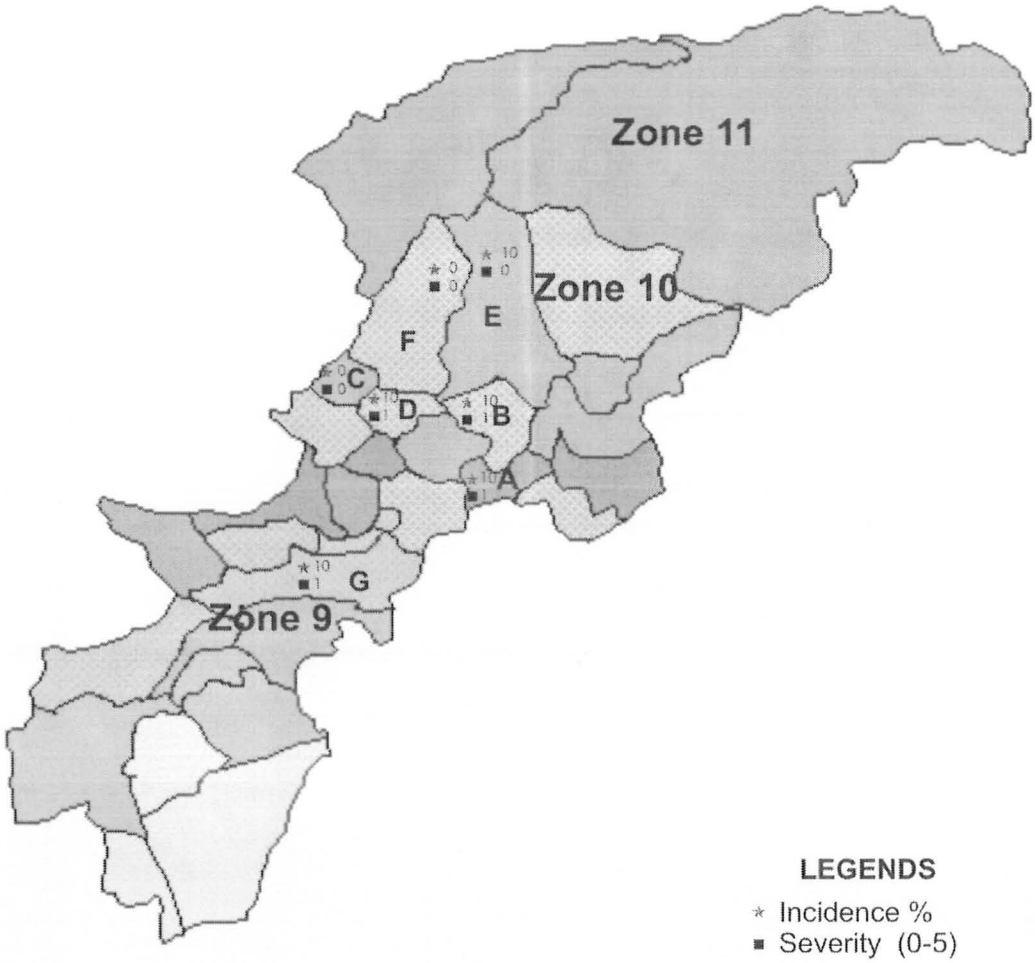


Fig.4.3 Distribution of Foliar blight Prevalence in NWFP Zones at seedling stage during 2004.



A = Sheikhupura
B = Tobateksingh
C = Faisalabad
D = Lahore
E = Kasur
F = Okara
G = Sahiwal
H = Jehlum
I = Rawalpindi
J = Attock
K = Chakwal
L = Khushab
M = Sargodha
N = Jhang
O = Mianwali
P = Gujranwala
Q = Sialkot
R = Gujrat

Fig.4.4 Distribution of foliar blights incidence and severity at different locations at seedling stage in Punjab during 2004.



A = Noushera
B = Mardan
C = Peshawer
D = Char Sadda
E = Mingora
F = Mala Kand
G = Kohat

Fig.4.5 Distribution of foliar blights incidence and severity at different locations at seedling stage in NWFP during 2004.

4.1.1.4 Disease index

Punjab

Maximum disease index (1.2%) was calculated in zone 6 while minimum (0.4%) in zone 7 (Table 4.1).

NWFP

In both the zones 9 and 10 the disease index calculated was same i.e.1 (Table 4.1).

Table 4.1 Incidence, severity and disease index (Mean values) of foliar blights of wheat in different wheat growing agro-ecological zones at seedling stage during 2004.

Zones	Incidence (%)	Severity (0-5)	Dis. index (%)
6	5 ±5.16	0.5 ± .07	1.2 ±1.4
7	1.9 ±3.9	0.1 ±0.4	0.4 ±0.9
9	5 ±5.1	0.5 ±.05	1 ±1.0
10	5.3 ± 5	0.5 ±.05	1 ±1.0

The values represent means ±SD.

4.1.2 Booting Stage Survey 2004 and 2005

4.1.2.1 Prevalence

The data in Fig.4.6 represents the single value of prevalence of foliar blights in each ecological zone according to their ecological categorization.

Punjab

The results of survey conducted during booting stage (2004) revealed highest prevalence (88%) of foliar blights in zone 5 while it was calculated 80% in zone 6 and minimum (43%) in zone 7 (Fig.4.6) The distribution of foliar blight prevalence in different zones of Punjab at booting stages during 2004 is shown in Fig.4.7.

Whereas during 2005, prevalence of foliar blight was calculated 100% in zones 5 and 6 while it was (91%) in zone 7 (Fig.4.6). The distribution of foliar blight prevalence in different zones of Punjab at booting stage during 2005 is shown in Fig.4.8.

NWFP

During 2004, maximum prevalence (100%) was calculated in zone 11 while minimum (84%) in zone 9 followed by 79% in zone 10. The distribution of foliar blight prevalence in different zones of NWFP at booting stage during 2004 is shown in Fig.4.9.

During 2005 the three zones (9, 10 and 11) had 100% prevalence of foliar blight (Fig. 4.6). The distribution of foliar blight prevalence in different zones of Punjab at booting stage during 2005 is shown in Fig.4.10.

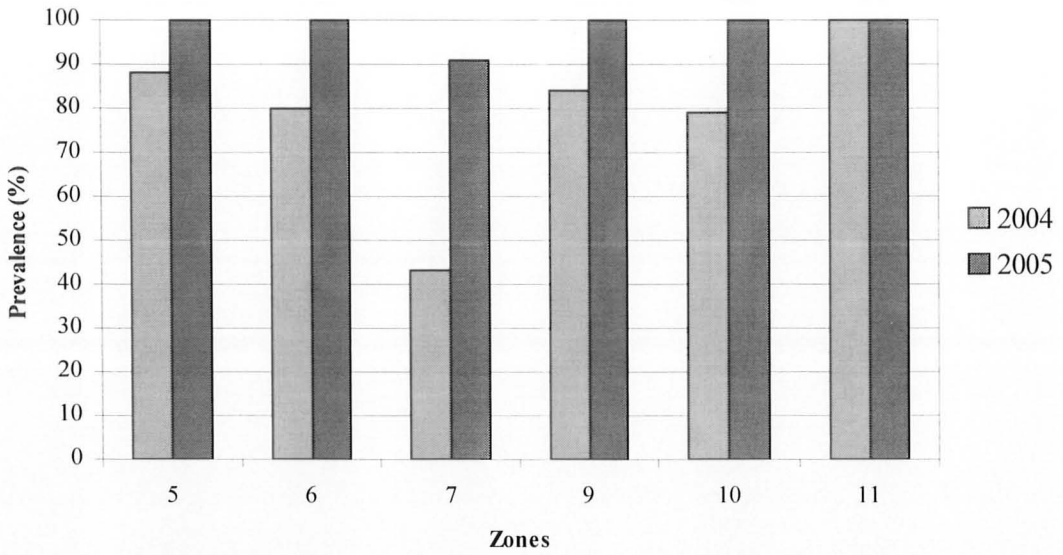
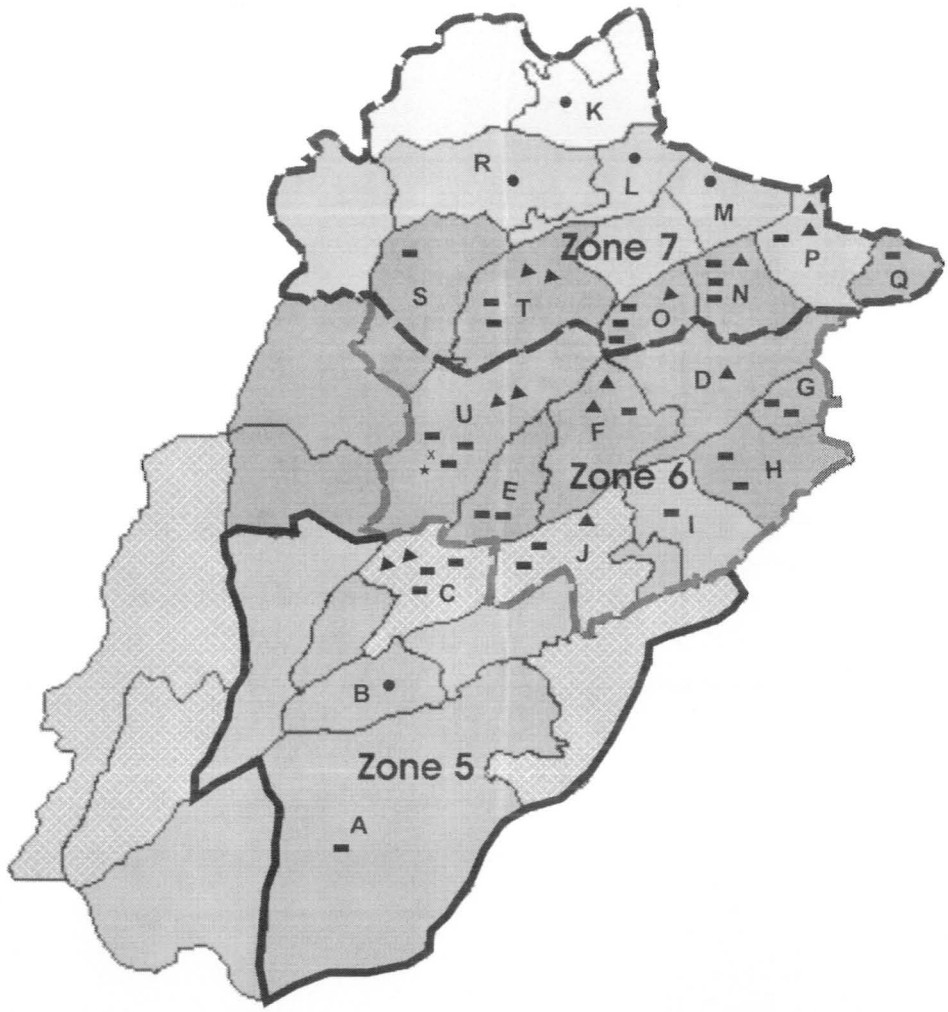


Fig 4.6 Prevalence of foliar blights of wheat in different wheat growing agro-ecological zones of Punjab and NWFP at booting stage during 2004 and 2005



A = Bahawalpur
B = Lodhran
C = Khanewal
D = Sheikhupura
E = T. T. Sindh
F = Faisalabad
G = Lahore
H = Kausr
I = Okara
J = Sahiwal
K = Rawalpindi
L = Jhelum
M = Gujrat
N = Gujranwala
O = Hafizabad
P = Sialkot
Q = Narowal
R = Chakwal
S = Khushab
T = Sargodha
J = Jhang

LEGEND

▲ = B .sorokiniana
▬ = A. alternata
★ = D. specifer
X = E. purpurascens
● = No disease

Fig.4.7 Distribution of foliar blight Prevalence in Punjab zones at booting stage during 2004.

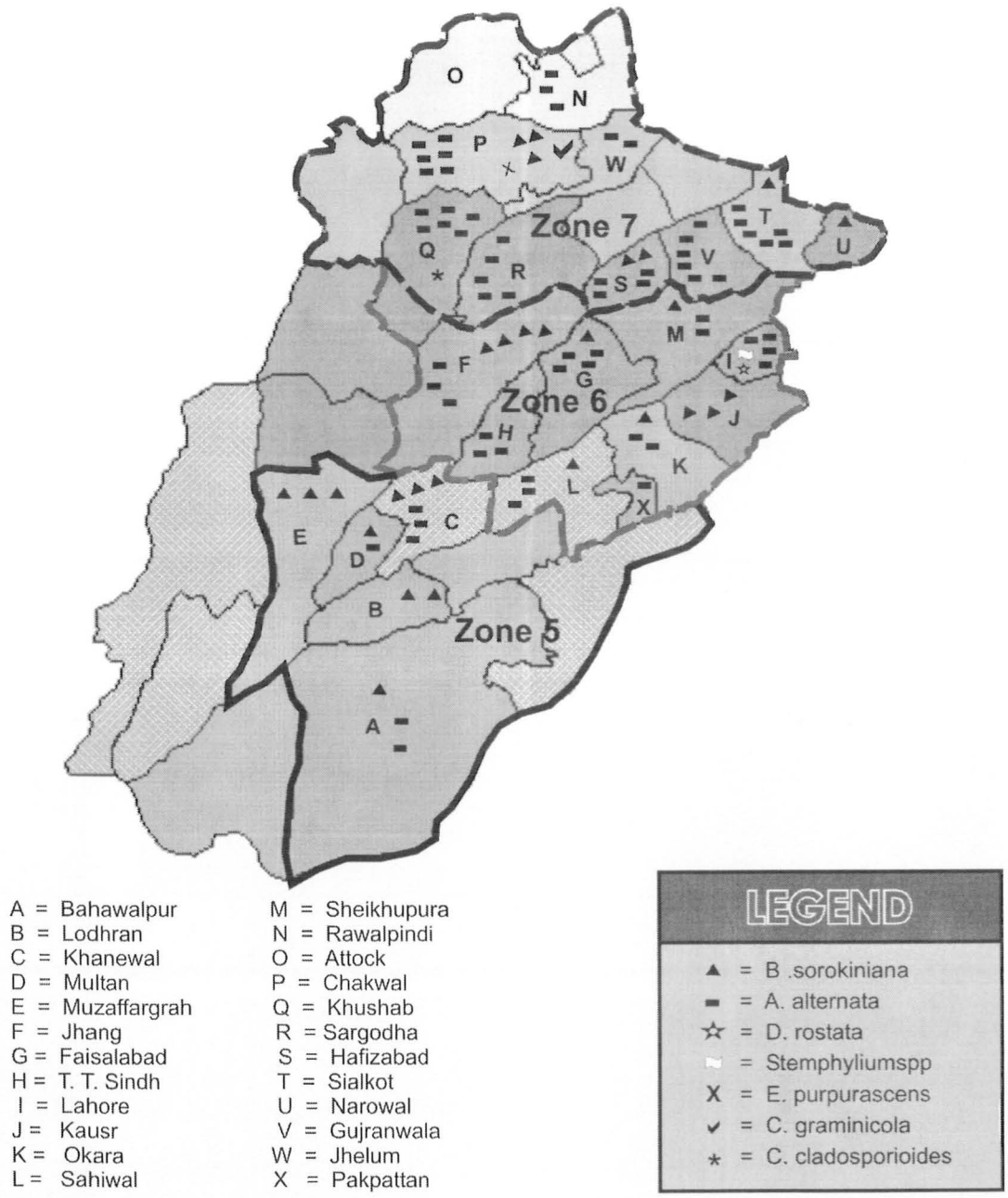
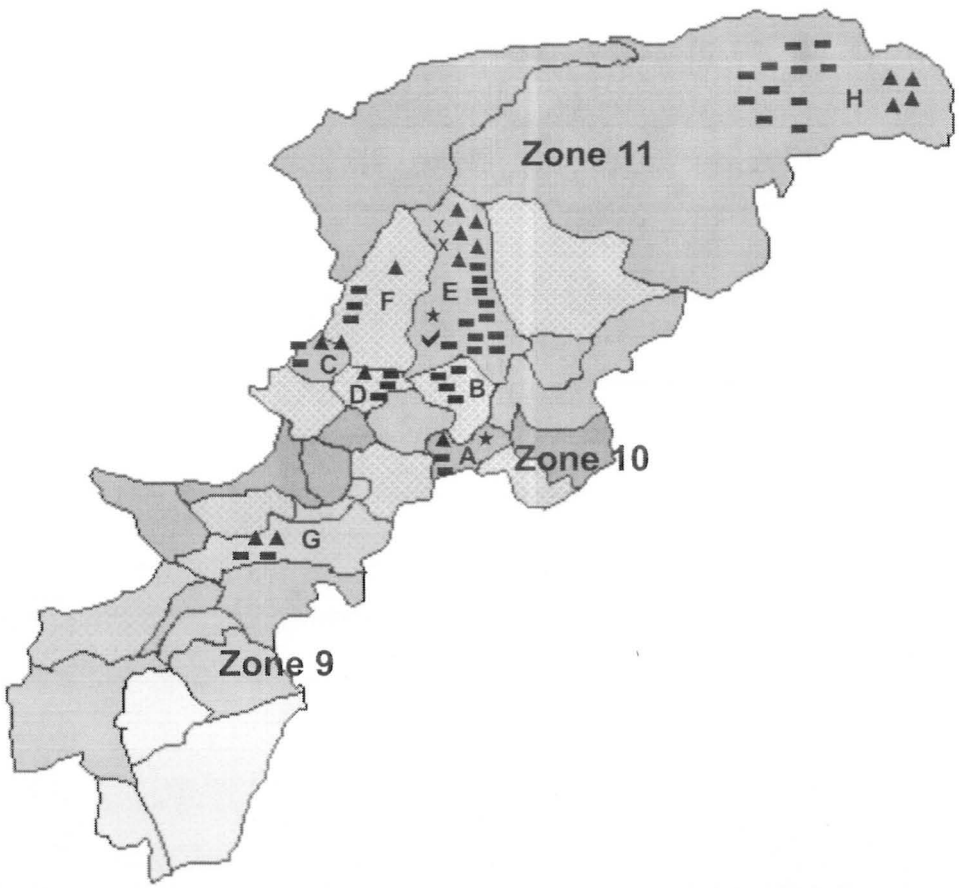


Fig. 4.8 Distribution of foliar blight Prevalence in Punjab zones at booting stage during 2005



A = Noushera
B = Mardan
C = Peshawer
D = Char Sadda
E = Mingora
F = Mala Kand
G = Kohat
H = Skurdu

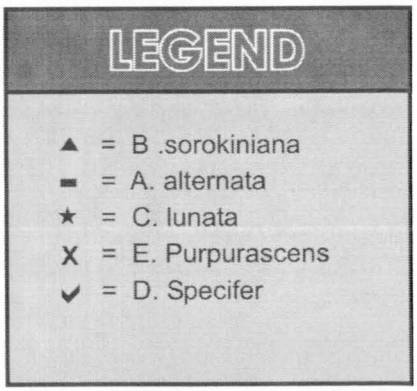


Fig. 4.9 Distribution of foliar blight Prevalence in NWFP zones at booting stage at booting stage during 2004.

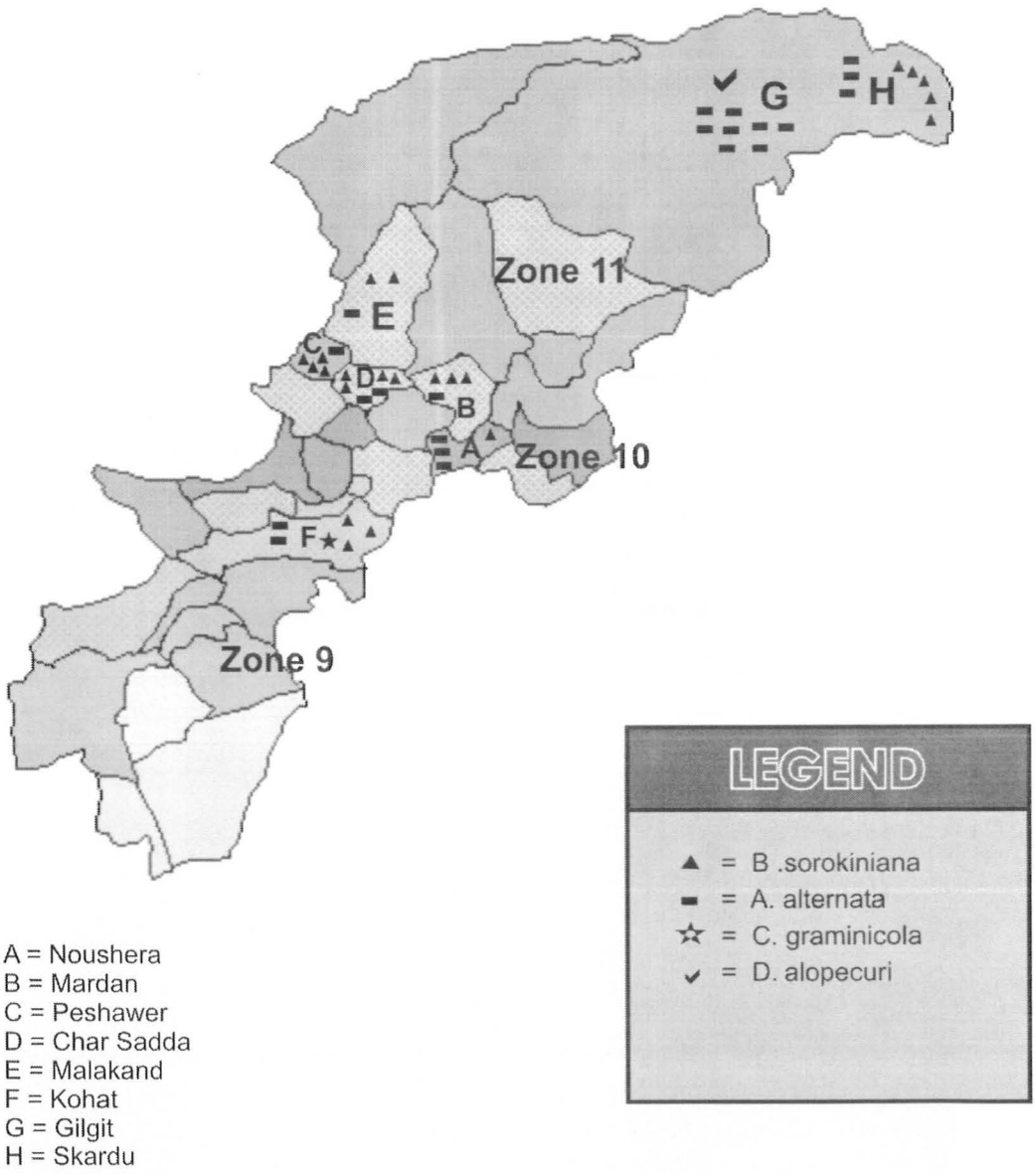


Fig. 4.10 Distribution of foliar blight Prevalence in NWFP zones at booting stage during 2005.

4.1.2.2 Incidence

Punjab

During 2004 maximum incidence (18.8%) of foliar blights was calculated in zone 5 while minimum (5.1%) in zone 7, whereas (13.6%) was calculated in zone 6. The distribution of foliar blights incidence at different locations at booting stage during 2004 in Punjab is given in Fig 4.11 During 2005 maximum incidence (59.3%) was calculated in zone 5 while minimum (24.8%) was in zone7, whereas 40% was calculated in zone 6 (Table 4.2). The distribution of foliar blights incidence during 2005 is given in Fig.4.12.

NWFP

During 2004, maximum incidence (31%) was calculated in zone 9 whereas Minimum (11.2%) in zone 11 and 12.5% in zone 10. (Table 4.2). The distribution of foliar blights incidence at different locations at booting stage during 2004 in NWFP is given in Fig 4.13. During 2005, maximum incidence (16.8%) was calculated in zone 9 and minimum (10.5%) in zone 10. While the zone 11 had 11.2 % disease incidence (Table 4.2). The distribution of foliar blights incidence during 2005 in NWFP is given in Fig.4.14.

4.1.2.3 Severity

Punjab

During 2004, maximum severity of foliar blights (1) was observed in zone 6 while the minimum 0.5 in zone 7, whereas it was scored 0.8 in zone 5. (Table 4.2). The distribution of foliar blights severity at different locations at booting stage during 2004 in Punjab is given in Fig 4.11 During 2005, maximum severity (1.5) of foliar blights was observed in zone 5 and 6 whereas 1 in zone 7 (Table 4.2). The distribution of foliar blights severity at different locations booting stage in Punjab in 2005 is given in Fig 4.12.

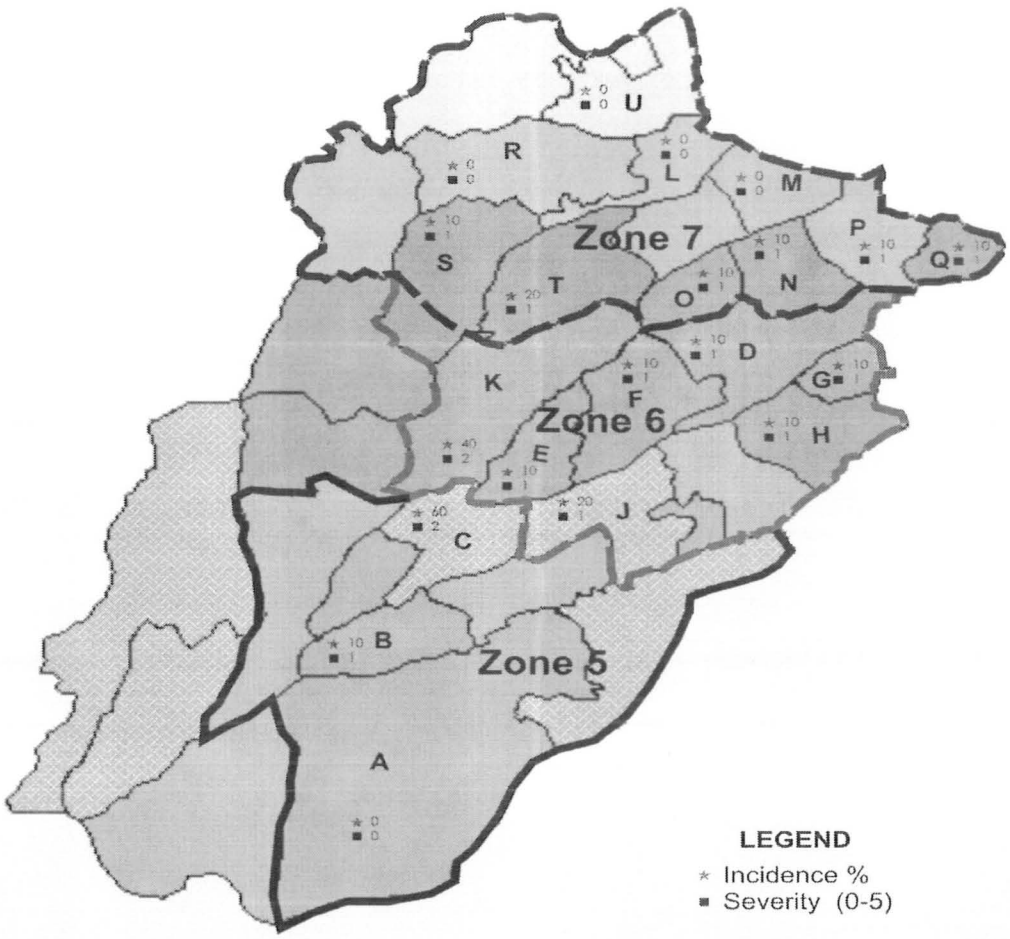


Fig.4.11. Distribution of foliar blights incidence and severity at different locations at booting stage in Punjab during 2004.

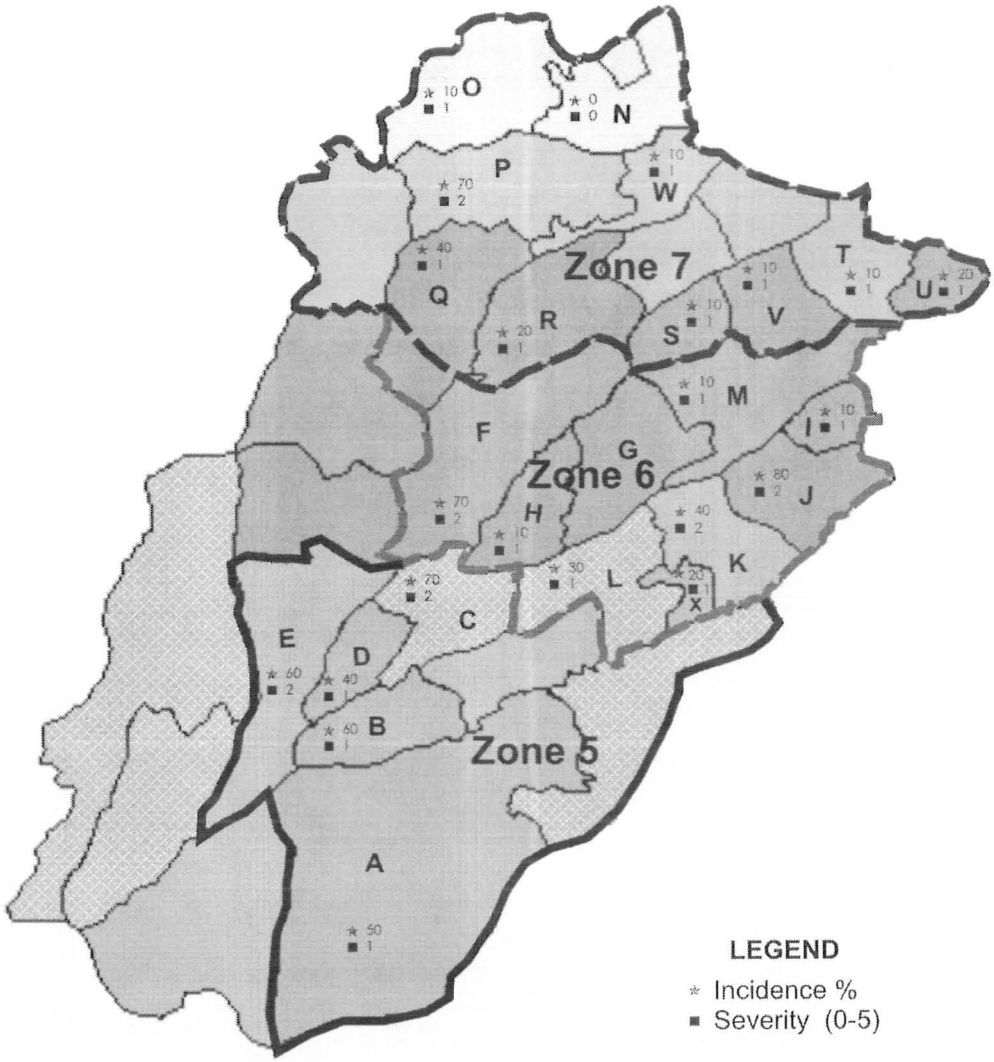


Fig.4.12. Distribution of foliar blights incidence and severity at different locations at booting stage in Punjab during 2005

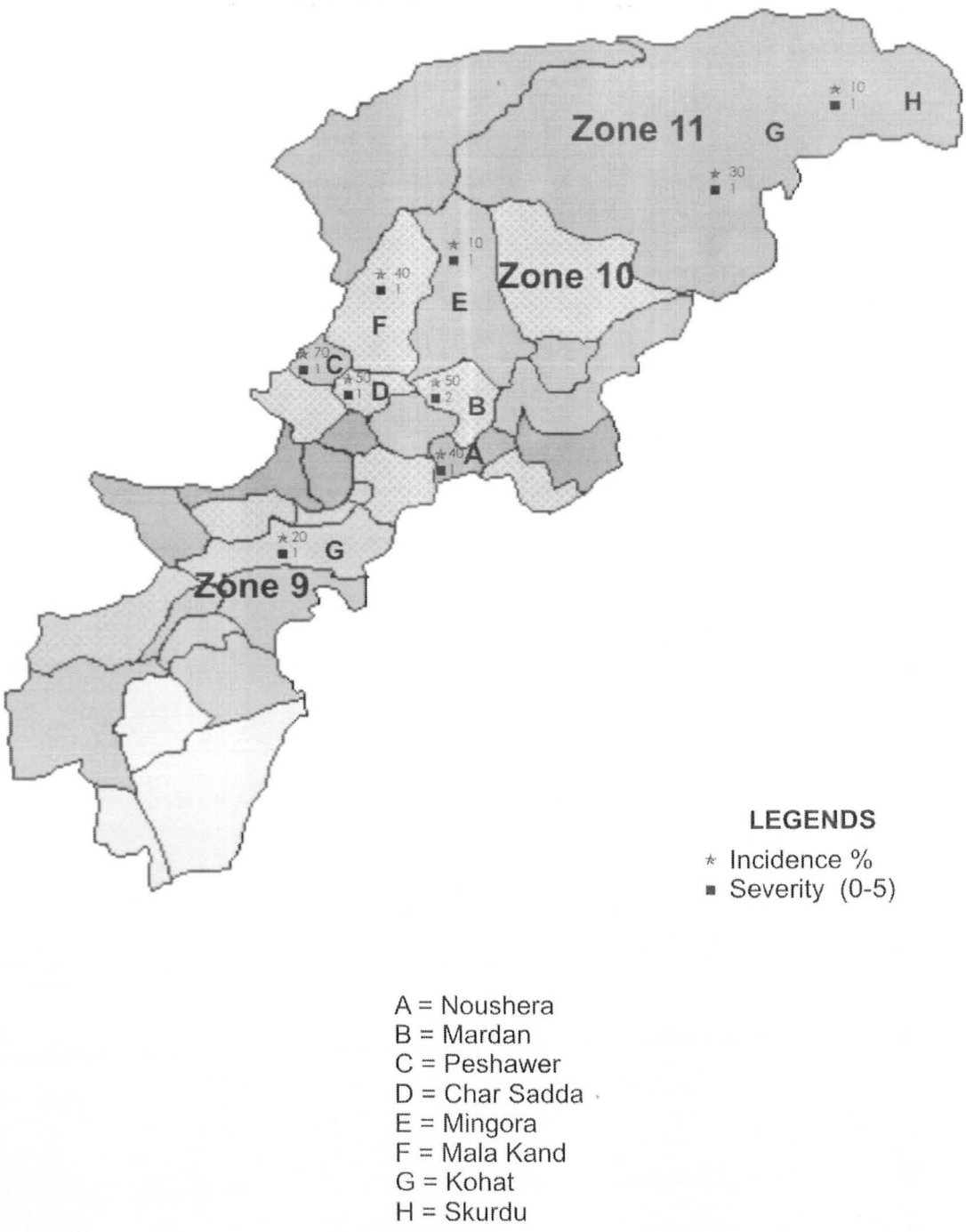
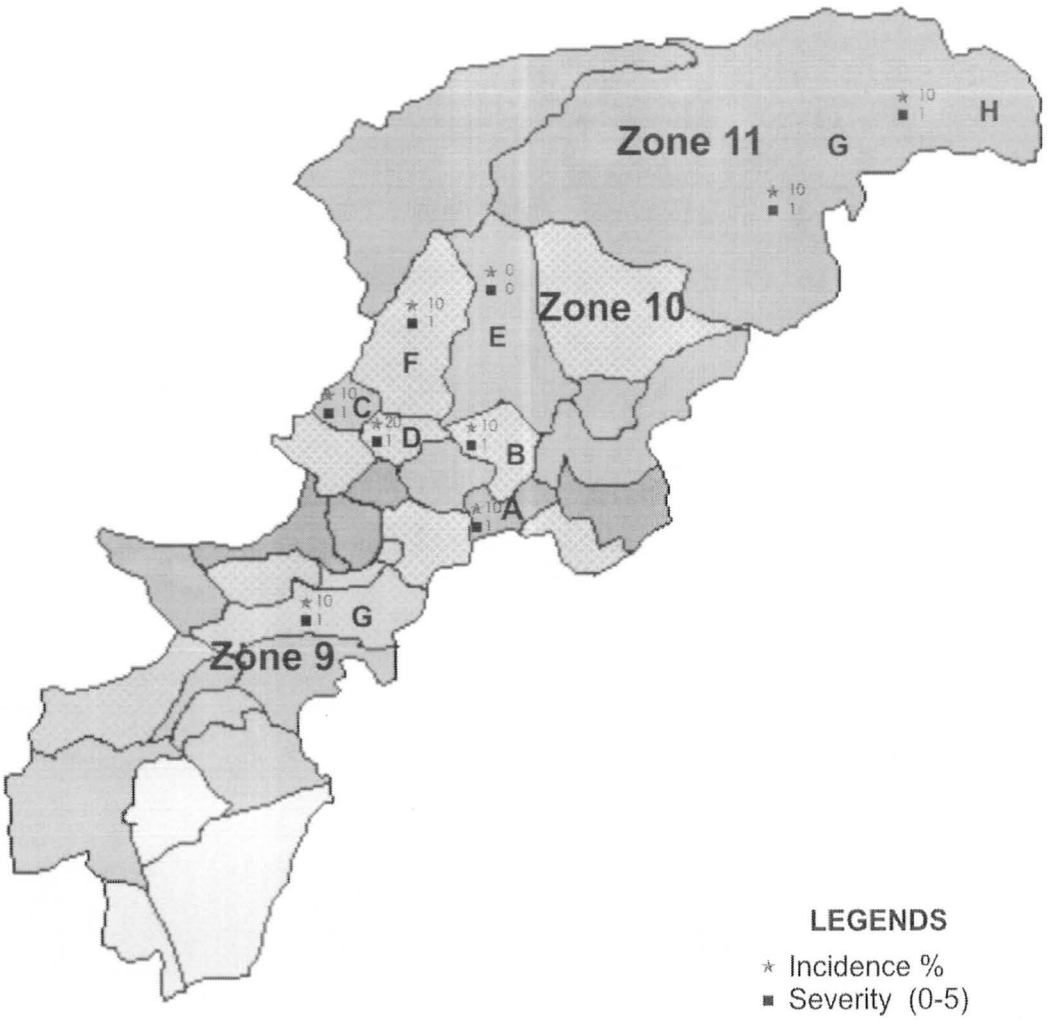


Fig.4.13. Distribution of foliar blights incidence and severity at different locations at booting stage in NWFP during 2004.



A = Noushera
B = Mardan
C = Peshawer
D = Char Sadda
E = Mingora
F = Mala Kand
G = Kohat
H = Skurdu

Fig.4.14. Distribution of foliar blights incidence and severity at different locations at booting stage in NWFP during 2005.

NWFP

During 2004, maximum severity (1.1) was recorded in zone 9 while minimum 0.8 was recorded in zone10 whereas severity rated to 1 was observed in zone 11. (Table 4.2). The distribution of foliar blights severity at different locations at booting stage during 2004 in NWFP is given in Fig 4.13. During 2005, maximum severity (1.2) was recorded in zone 9 while it was rated 1 in both the zones 10 and 11 (Table 4.2). The distribution of foliar blights severity at different locations booting stage in NWFP during 2005 is given in Fig 4.14.

4.1.2.4 Disease Index

Punjab

During 2004, maximum D.I (4.7 %) was calculated in zone 6 while minimum (1%) was calculated in zone 7 and 3.3% in zone 5. During 2005 maximum disease index (11.2% and 11.1%) was calculated in zone 5 and 6 while minimum (4.7%) in zone 7 (Table 4.2).

NWFP

During 2004, maximum disease index (7.1%) was calculated in zone 9 whereas minimum (2.2 and 2.3%) was calculated in zone 10 and 11 respectively. During 2005, maximum disease index (4.3%) was calculated in zone while minimum (2.3 and 2.5%) was calculated in zone 10 and 11 respectively (Table 4.2).

Table 4.2 Incidence, severity and disease index (Mean values) of foliar blights of wheat in different wheat growing agro-ecological zones at booting stage during 2004 and 2005

Zones	Incidence (%)		Severity (0-5)		Disease index (%)	
	2004	2005	2004	2005	2004	2005
5	18.8 ± 20.2	59.3 ± 17.6	0.8 ± .05	1.5 ± .05	3.3 ± 2.9	11.2 ± 8.0
6	13.6 ± 12.4	40.0 ± 32.4	1.0 ± 0.7	1.5 ± .07	4.7 ± 3.5	11.1 ± 15.3
7	5.1 ± 6.8	24.8 ± 25.0	0.5 ± .06	1.0 ± 0.5	1 ± 1.2	4.7 ± 5.7
9	31.0 ± 29.9	16.8 ± 7.0	1.1 ± 0.8	1.2 ± 0.3	7.1 ± 7.7	4.3 ± 2.6
10	12.5 ± 18.6	10.5 ± 2.8	0.8 ± 0.4	1.0 ± 0.0	2.2 ± 2.1	2.3 ± 0.5
11	11.2 ± 3.3	11.2 ± 3.5	1.0 ± 0	1.0 ± 0	2.3 ± 0.7	2.5 ± 0.9

The values represent means ± SD

4.2 ISOLATION/IDENTIFICATION OF FUNGI FROM FOLIAGE OF WHEAT CROP FROM DIFFERENT WHEAT GROWING ZONES OF PUNJAB AND NWFP DURING 2004 AND 2005

All of the fungi *Alternaria alternata*, (Plate 4.1) *Bipolaris sorokiniana* (Plate 4.2) *Curvularia lunata,* (Plate 4.3) *Drechslera spicifer* (Plate 4.4) and *Epicoccum purpurascens* isolated from the samples (foliage of wheat crop) collected in the survey at both seedling and booting stages during 2004 were identified to species level. While eight different fungi were isolated during 2005 surveys and out of these, seven fungi were identified at species level on the basis of cultural characteristics and literature, with the exception of one fungus, *Stemphylium* sp. (Plate 4.5) that could not be identified to species level. The fungi, *Alternaria alternata, Bipolaris sorokiniana, Cladosporium cladosporioides, Colletotrichum graminicola, Dilophospora alopecuri, Drechslera rostata,* (Plate 4.6) *Epicoccum purpurascens* and *Stemphylium* sp were found associated with diseased samples.

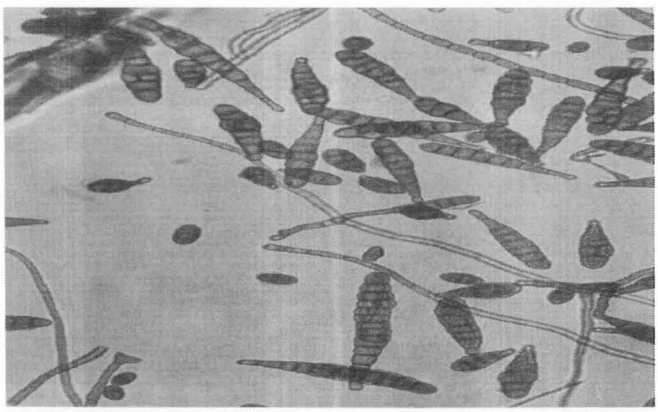


Plate 4.1. Conidia of *Alternaria alternata*

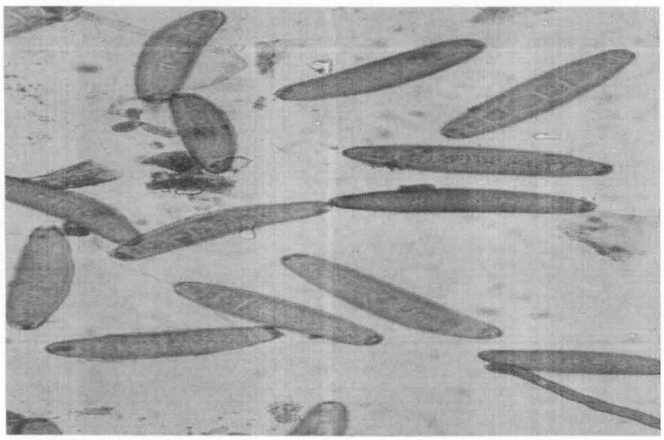


Plate 4.2. Conidia of *Bipolaris sorokiniana*

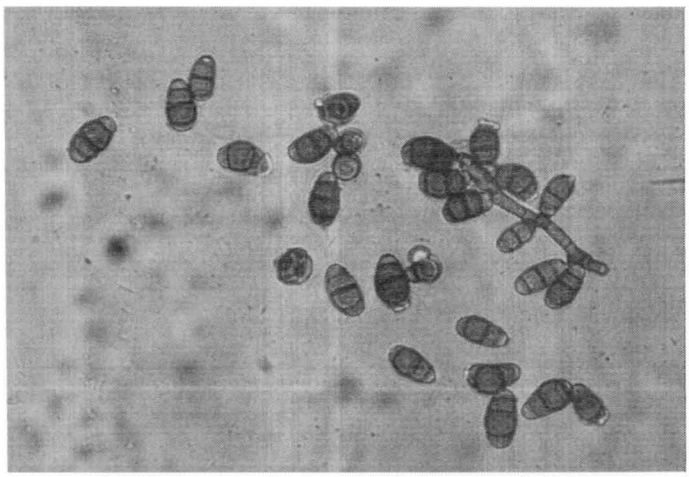


Plate 4.3. Conidia of *Curvularia lunata*

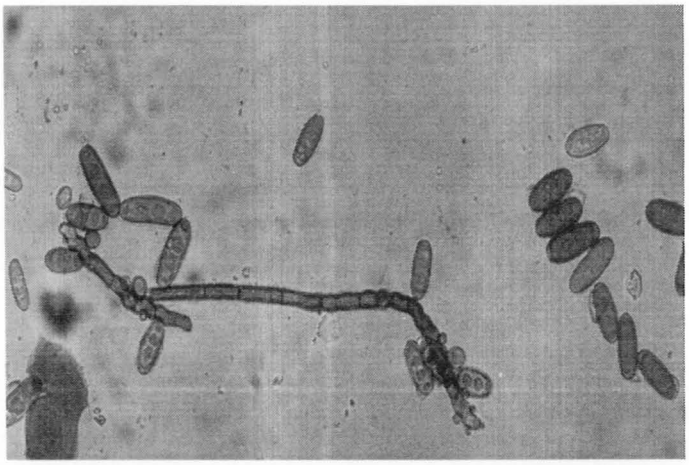


Plate 4.4. Conidia of *Drechslera spicifer*

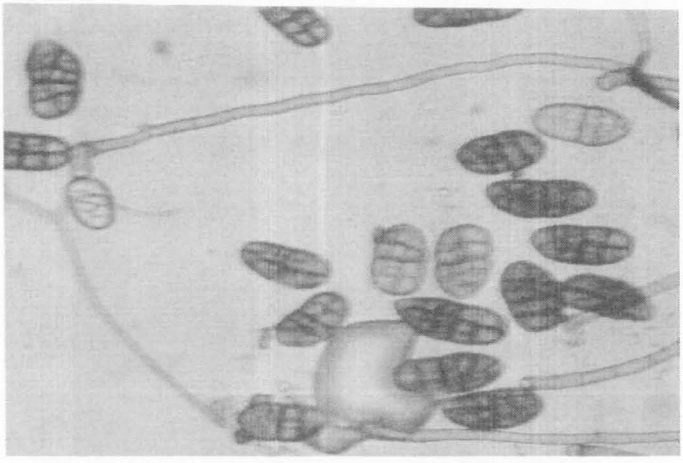


Plate 4.5. Conidia of *Stemphylium spp.*

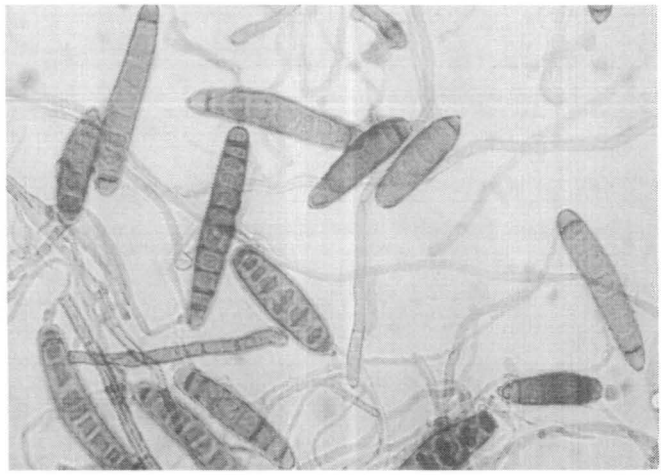


Plate 4.6. *Conidia of Drechslera rostata*

4.2.1 Frequency of fungi isolated at seedling stage during 2004

At seedling stage 32 fungal colonies were isolated from 920 samples collected. *Alternaria alternata* was the most frequently occurring fungus and 12 samples were positive, followed by *Epicoccum purpurascens* (8), *Curvularia lunata* (4), *Bipolaris sorokiniana* (6) and *Drechslera spicifer* (2). The maximum number of fungi (4) from both the zones 6 and 9 while minimum (2) were isolated from zone 7. Whereas 3 fungi were isolated from zone 10 of NWFP (Fig.4.15).

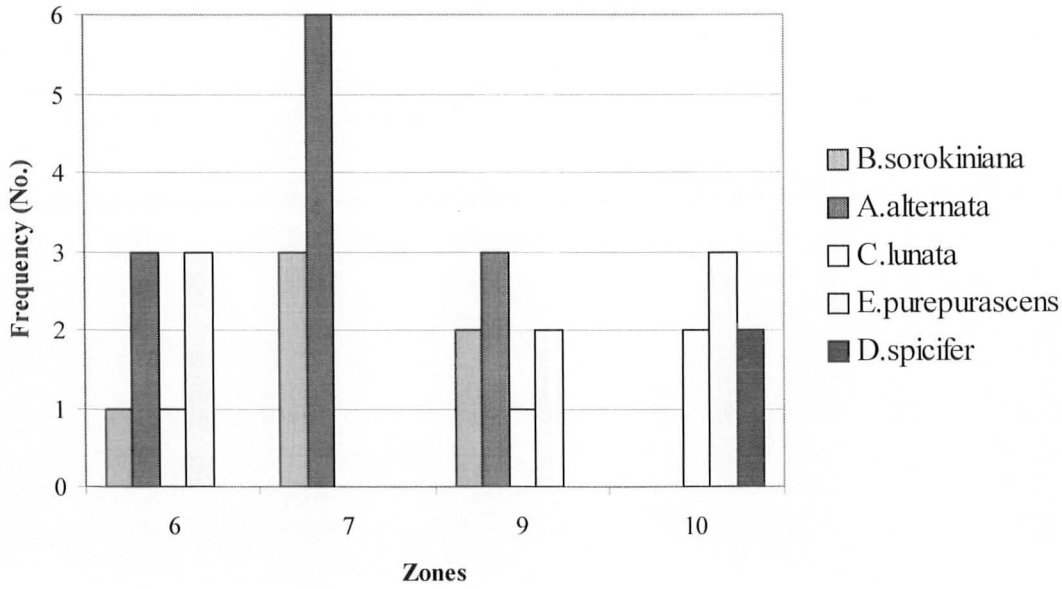


Fig.4.15. Frequency of different fungi isolated from different wheat growing agro-ecological zones of Punjab and NWFP at seedling stage during 2004

4.2.2 Frequency of fungi isolated at booting stage during 2004 and 2005

At booting stage during 2004 a total of 116 fungal colonies were isolated from 1580 samples. *Alternaria alternata* was found the predominant occurring fungus with isolation from 78 samples followed by *Bipolaris sorokiniana* (31), *Epicoccum purpurascens* (4), *Drechslera spicifer* (2) and *Curvularia lunata* (1) isolates respectively (Table.4.3). The fungal isolation during 2004 at booting stage from zones 9, 10 and 11 revealed that maximum number of samples infested were in zones 10 i.e. 28. The overall fungal isolates frequency from the samples collected during 2004 crop at booting and seedling stage was *Alternaria alternata* (90) followed by *Bipolaris sorokiniana* (37), *Epicoccum purpurascens* (12), *Curvularia lunata* (5) and *Drechslera spicifer* (4). The maximum number of isolates *Alternaria alternata* (20) were recovered from zone 11 while the maximum number of isolates of *Bipolaris sorokinaina* (7) from zone 9 closely followed by 6 number of isolates each from zone6, 7 and 10.

During 2005 the results of frequency of fungi isolated revealed, maximum number of isolates (91) of fungus *Alternaria alternata* was isolated from all the zones collectively. The distribution of the isolates frequency (6, 22, 41, 8, 11 and 3) in zone 5, 6, 7, 9, 10 and 11 was observed respectively (Table.4.3). Maximum number of isolates (16) of *Bipolaris sorokiniana* was recovered from the sample collection from zones 9 followed by 11, 10, 6, 5, and 2 from zone 6, 5, 7, 11 and 10 respectively. The overall frequency of different foliar blight fungi isolated during 2005 revealed that out of 1520 samples fungi were isolated from148 samples. The highest number of isolations were of *Alternaria alternata* (91) followed by 50 isolates of *Bipolaris sorokiniana*, 2 isolates of *Colletotrichum graminicola*, while one isolate each of *Epicoccum purpurascens, Drechslera rostata, Cladosporium cladosporioides, Stemphylium* sp and *Dilophospora alopecuri* were recovered.

Table.4.3 Frequency of different fungi isolated from different wheat growing agro-ecological zones of Punjab and NWFP at booting stage during 2004 and 2005

Fungi isolated	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005
A.alternata	6	6	13	22	10	41	11	8	18	11	20	3
B.sorokiniana	2	10	6	11	6	6	7	16	6	2	4	5
C.lunata	0	0	0	0	0	0	0	0	1	0	0	0
E.purpurascenes	0	0	2	0	0	1	0	0	2	0	0	0
D.spicifer	0	0	1	0	0	0	0	0	1	0	0	0
C.graminicola	0	0	0	0	0	1	0	0	0	1	0	0
D.alopecuri	0	0	0	0	0	0	0	0	0	1	0	0
D.rostata	0	0	0	1	0	0	0	0	0	0	0	0
Stemphylium Sp	0	0	0	1	0	0	0	0	0	0	0	0
C.cladosporioides	0	0	0	1	0	0	0	0	0	0	0	0

4.3 PHENOTYPIC CHARACTERISTICS OF PATHOGENS

The Phenotypic study was concentrated only on the fungi that were found pathogenic; these include *Bipolaris sorokiniana*, *Colletotrichum graminicola* and *Dilophospora alopecuri*.

4.3.1 Colony Colour/Growth of the Isolates of *Bipolaris sorokiniana* Isolated during 2004 and 2005.

The colony colours of thirty-seven isolates of *Bipolaris sorokiniana* were observed. Four different types of colours of isolates were found on Potato dextrose agar medium (Appendix 8). Thirteen isolates exhibited black colony colour, nineteen grayish black, 2 brownish while 3-showed albino colour colony. All of the black isolates had suppressed type of colony while the rest showed fluffy type of growth on the medium (Plate 4.7), (Plate 4.8), (Plate 4.9) and (Plate 4.10). Four coloured colonies were observed during observations of growth of different isolates from collection of year 2005 (Appendix 10). The maximum isolates exhibited black colour followed by grayish black, albino and brown (Table 4.4). Out of fifty isolates twenty seven isolates exhibited black colour, fifteen grayish black; three exhibited brownish appearance while five isolates showed albino appearance (Table 4.4).

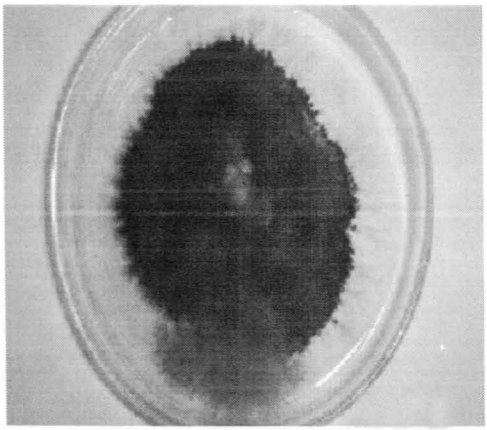


Plate 4.7. Black coloured colony of *Bipolaris sorokiniana*

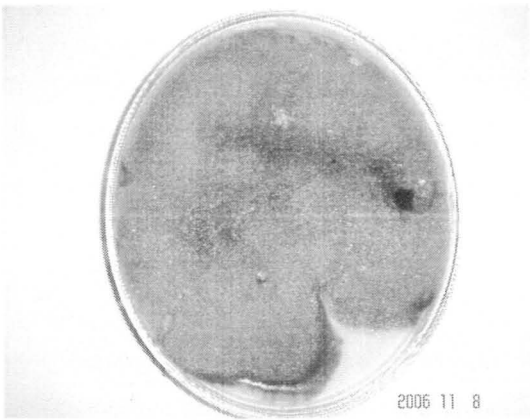


Plate 4.8. Grayish black coloured colony of *Bipolaris sorokiniana*

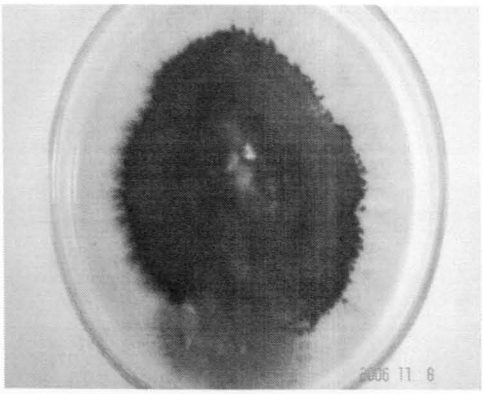


Plate 4.9. Brown coloured colony of *Bipolaris sorokiniana*

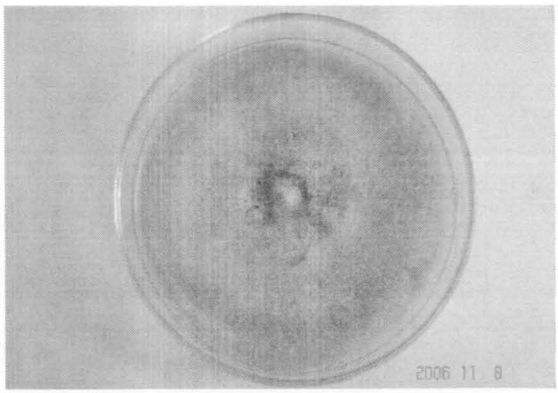


Plate 4.10. Albino coloured colony of *Bipolaris sorokiniana*

Table 4.4. Cultural Characteristics of *Bipolaris sorokiniana* Isolates collected from different wheat growing areas of Punjab and NWFP during 2004-2005 and their aggressiveness on three commercial wheat varieties.

Zone	No. Of Isolates		Colony Colour		Type of Growth		Range of Aggressiveness					
							Wafaq 2001		Inqilab 91		Bhakhar 2001	
	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005
5	1 1	9 1	B* GB	B GB	S* S	S S	5 2.6	3.1-5 3	4 4.6	2-4 3	3 3	2-4 3
6	5 1 -	7 2 1	B GB -	B GB Br	S S S	S S S	2.3-3.6 2.3-3.6 -	3-4 4 3	2.6-3.6 2-3 -	2-4 2.6 2	1-3.3 2-2.3 -	2-3.3 1 2.3
7	5 2 2 1	1 5 1 -	B GB Br Al	B GB Br -	S S S F	S S S -	3-3.4 3 2.6-3 2.3	3.3 1.6-4 3 -	2-4 3.6 1.3-2.3 2.3	4 3 1.3 -	2-3.3 2.6 2.3- 2.6 2.3	4 1-3 2 -
9	2 6 - 1	6 6 1 2	B GB - Al	B GB Br Al	S S S F	S S S F	3.3-3.4 2-3.6 - 2	3-5 3-4 2 2-2.3	3-3.6 2-3.3 - 2	2-4 1.6-3 1 2-2.3	2.3-3 2.3- 3.6 - 2.3	2-3.3 2-3 2 2-2.6
10	- 4 1	2 1 -	- GB AL	B GB -	- S F	S S -	- 2-3.3 2	3.6-4 3.6 -	- 2.3-3.3 2.3	2.3-3 2 -	- 2.3-3 2.3	3 2.3 -
11	- 5 -	2 - 3	- GB -	B - AL	- S -	S - F	- 2-3.3 -	3-3.6 - 1-2.3	- 1.6-3.3 -	2-2.6 - 2-2.3	- 3-3.6 -	3-4 - 1-2.3

B*= Black, GB=Grayish Black, Br= Brown, AL= Albino, S=Suppressed, F= Fluffy.

4.3.2 Morphology of *Bipolaris sorokiniana* isolates collected during 2004 and 2005

The measurements of the conidia collected during 2004 varied from 50-55.8µm (mean) to 16.6-19.9µm (mean) with the septa ranged from 2 - 13 (Table 4.5.). There were two isolates NP-8 and NP-11 with relatively longer conidia than others (Appendix 7). The conidia of all isolates collected during 2004 were slightly curved with brown to olivaceous brown colour (Plate 4.11). During 2005, the measurement of the conidia varied from 46.6 – 64.1 µm (mean) to 15.9- 21.6 µm having 2 – 10 numbers of septa (Table 4.5). There were few isolates (P4-2, P4-16, P4-18, P4-28) having conidia of relatively long and broad with dark brown colour, slender and slightly curved, while in most of the isolates conidia were uniformly straight and slenderical, light brown to brown in colour (Appendix 9).

Table 4.5 Dimension of conidia of *Bipolaris sorokiniana* isolated from different wheat growing agro ecological zones of Punjab and NWFP during 2004 and 2005.

Zones	No. of Isolate		Length of conidia (Mean) (µ)		Width of conidia (Mean) (µ)		No. of Septa (Mean Range)	
	2004	2005	2004	2005	2004	2005	2004	2005
5	2	10	50± 21.2	64.1±8..8	16.6± 2..3	20.1±1.2	2 – 6	2 – 10
6	6	10	54.7± 9.0	55.6±10..3	19.4±3..2	16.7±2..3	2 – 7	2 – 9
7	10	7	53.4±7..3	49.1± 9.1	19.9±3.0	16.6±2..3	2 – 7	2 – 9
9	9	15	54.4 ±6..9	48.4± 7..3	17.3± 1.4	15.6± 2.4	2 – 13	2 – 9
10	6	3	51.3± 3..2	61.1± 17.1	17.1± 1..3	21.6± 3..3	2 – 8	2 – 8
11	4	5	55.8± 10..9	46.6± 10..9	17.0±2..5	15.9±3..2	2 – 8	2 – 7

The values represent means ±SD

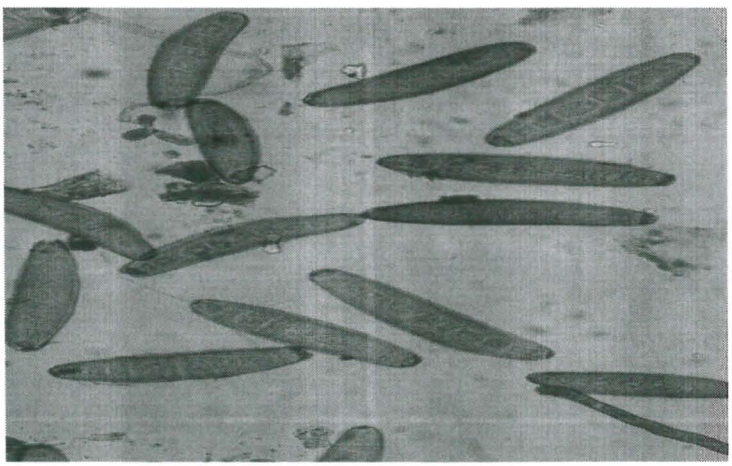


Plate 4.11 Conidia of *Bipolaris sorokiniana.*

4.3.3 Morphology of *Colletotrichum graminicola* Isolated during 2005

Two isolates of *Colletotrichum graminicola,* were isolated from zone 7 (Punjab) and zone 9 (NWFP). The ascervuli of 200µm diameter with prominent dark spines (setae) measuring 160-230 µm of length were observed at 100 x magnification (Plate 4.12). The hyaline, unicellular, sickle shaped conidia measuring 15-20 x 2-3 µm were observed at 400 x magnification. The colony black in colour showed dotted appearance with suppressive type of growth. This is the first report of *Colletotrichum graminicola* as leaf spot causing organism on wheat in Pakistan.

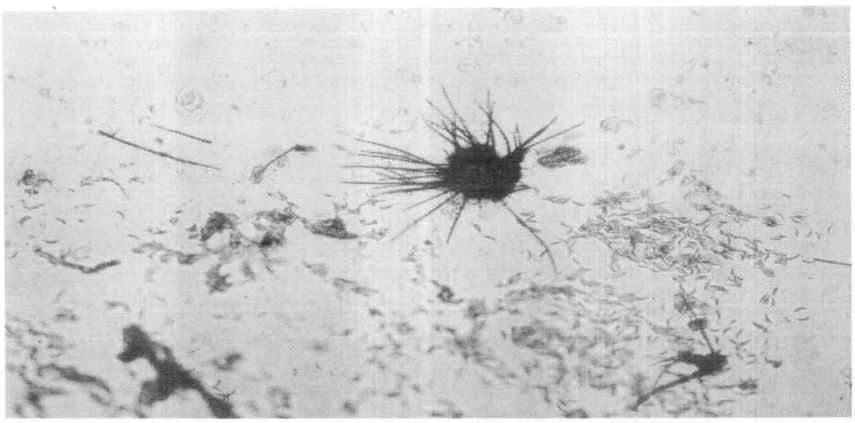


Plate 4.12 Ascervuli of *Colletotrichum graminicola* with hyaline, unicellular, sickle shaped conidia at 40x magnification

4.3.4 Morphology of *Dilophospora alopecuri* Isolated during 2005

Only one isolate of *Dilophospora alopecuri*, was isolated from zone 10 (NWFP), Gulmit Gojal, Hunza Valley of Northern areas. The black ostiolated pycnidia of this fungus were observed measuring 250 µm in diameter. The hyaline, cylindrical to ellipsoid conidia having 0-3 septa with distinctive hyaline appendages at both ends were observed at 100 x magnification (Plate 4.13). The dimension of conidia was 8-15 x 1.5 µm. This is the first report of *Dilophospora alopecuri* as leaf spot causing organism on wheat in Pakistan.

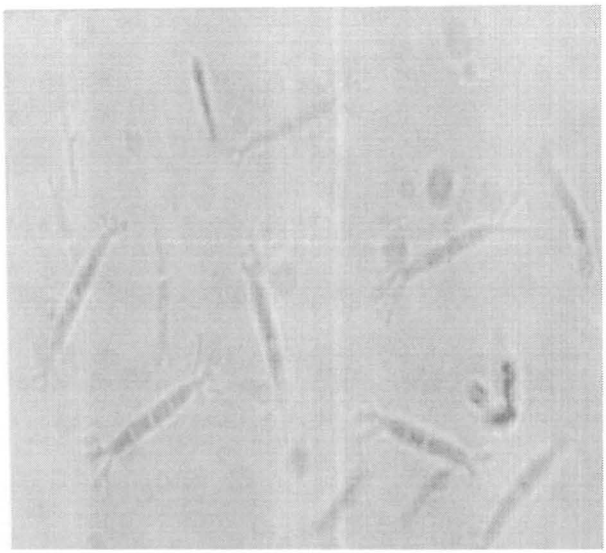


Plate 4.13. Conidia of *Dilophospora alopecuri* with appendages at 100x magnification

4.4 SELECTION AND STANDARDIZATION OF METHOD FOR PATHOGENICITY TEST/ AGGRESSIVENESS ANALYSIS

Among the five methods four were conducted in pots and one in test tube. Out of five methods, test tube moist cotton swab method was found the best one for pathogenicity test and subsequently for aggressiveness analysis. The results were analyzed for analysis of variance (ANOVA), (Table 4.6). It revealed that there was no significant effect of replications. However it was observed that highly significant effect of isolate, methods and isolate x methods interaction when tested the five methods and its efficiency.

The mean values of different methods (Table 4.7) revealed that M1 and M5 showed highly significant results as compared to one another and rest of the methods. However there was no significant difference between M2, M3 and M4 (Table 4.7).

Table 4.6 Analysis of variance of different methods applied for testing pathogenicity using two fungal isolates of *Bipolaris soroikiniana*

Sources of variation	Degree of freedom	Sum, of squares	Mean square	
			Leaf	Root
Replication	4	1.00	0.250 NS	0.200 NS
Isolates	1	5.780	5.780**	3.920**
Methods	4	65.400	16.350**	13.400**
Isolate x method	4	2.520	0.630**	0.720**
Error	36	7.800	0.217	0.078

** Mean squares of isolates, methods and isolate x methods interaction are highly significant at probability level 1.

Table 4.7: Mean value of different methods applied for testing pathogenicity using two isolates of *Bipolaris* *sorokiniana*

Methods	Mean values (0-5) scale	(0-3) scale
	Leaf	Root
M1	1.900b	2.200b
M2	1.300c	2.400b
M3	0.900c	0.000c
M4	2.200c	2.400b
M5	4.200a	3.00b
LSD Value	0.4225	0.2533

Column means followed by a common letter are not statistically different at 5 %.

The interaction means of the methods M1, M3 and M5 showed highly significant difference from one another while M2 and M3 had slightly significant difference. Whereas M1 and M4 had no significant difference. The results showed that M5 had highly difference compare to other methods, hence selected for pathogenicity and further aggressiveness analysis (Table 4.8).

Table 4.8 Mean value of interaction of different methods applied for pathogenicity test using two isolates of *Bipolaris sorokiniana*

Methods	Leaf (0-5) scale		Root (0-3) scale	
	Isolate P2-9	Isolate NP1-4	Isolate P2-9	Isolate NP-4
M1	2.600b	1.200de	2.600b	1.800c
M2	1.400bc	1.200de	2.800ab	2.00b
M3	1.000c	0.800e	0.000d	0.00d
M4	2.600b	1.800d	3.000a	1.800c
M5	4.600a	3.800b	3.000a	3.00a
LSD value	0.5975	0.342	0.3580	0.532

Column means followed by a common letter are not statistically different at 5 %

The mean values of two isolates showed highly significant difference from one another, revealed that isolate P2-9 showed more aggressiveness compared to NP-4 (Table 4.9).

Table 4.9: Mean value of two isolates of *Bipolaris sorokiniana* applied for pathogenicity test

Isolates	Means values (0-5) scale	(0-3) scale
	Leaf	Root
P2-9	2.440a	2.800a
NP-4	1.760b	1.720b
LSD value	0.3915	0.2315

Column means followed by a common letter are not statistically different at 5 %

4.4.1 Standardization of test tube moist cotton swab method

Among the two inoculation procedures tested for the standardization of test tube moist cotton swab method, the procedure1 was found the best one for pathogenicity test and subsequently for aggressiveness analysis. The results were analyzed for Analysis of variance (ANOVA), (Table 4.10). It revealed that there was no significant effect of replications, while highly significant effect of the inoculation procedures and varieties and inoculation procedures x varieties interactions was observed (Table 4.10).

Table 4.10. Analysis of variance of inoculation procedure applied for standardization

Sources of variation	Degree of freedom	Mean Squares	
		Leaf (0-5) scale	Root (0-3) scale
Replication	5	0.650 NS	0.183 NS
Inoculation Procedure	1	17.361 **	2.250**
Varieties	2	12.167 **	0.750**
Inoculation Procedure x Varieties	2	4.389 **	0.083 NS
Error	25	0.303	0.157

** Mean squares of varieties, inoculation procedure and varieties x inoculation Procedure interaction is highly significant at 1%.

Data was recorded on two aspects. The efficiency of two inoculation procedures and reaction of isolate on three wheat varieties tested. The mean values for inoculation procedures presented in table (Table 4.11) showed that Procedure1 is highly significantly different then Procedure 2 and found more efficient.

Table 4.11: Mean values for two inoculation procedure applied for Standardization

Procedures	Means	
	Leaf (0-5) scale	Root (0-3) scale
1	3.278a	2.833a
2	1.889b	2.333b
LSD Value	0.440	0.351

Column means followed by a common letter are not statistically different at 5 %

The mean values of three varieties used for standardization of the inoculation procedures showed significant difference among varieties reaction (Table 4.12). The isolate P2-9 used in the study showed its aggressiveness on Wafaq-2001 followed by Inqilab-91 and least on Bhakkar-2001 (Table 4.12).

Table 4.12: Mean values for three varieties of wheat used in standardization for inoculation procedure

Varieties	inoculation procedure	
	Leaf (0-5) scale	Root (0-3) scale
Wafaq 2001	3.33 a	2.833 a
Inqilab 91	2.500 b	2.583 ab
Bhakkar 2001	1.917 c	2.333 b
LSD Value	0.463	0.333

Column means followed by a common letter are not statistically different at 5 %

The interaction means of three wheat varieties and the two inoculation procedures tested showed significant difference from one another. The results showed that the inoculation procedure1 used in the study produced significantly more aggressiveness on Wafaq 2001 variety but there was no significant difference on rest of the two varieties in comparison to Wafaq 2001. Incase of procedure2 there was no

significant difference of aggressiveness on both Wafaq 2001 and Inqilab 91 but both were significant different to Bhakkar 2001 in terms of aggressiveness. This reflected that the aggressiveness on Wafaq 2001 was significantly more compared to rest of two varieties when procedure1 was applied (Table 4.13).

Table 4.13: Mean Value for Interaction of Varieties and inoculation procedure

Varieties	Inoculation procedure 1		Inoculation procedure 11	
	Leaf (0-5) scale	Root (0-3) scale	Leaf (0-5) scale	Root (0-3) scale
Wafaq 2001	4.500a	3.00a	2.167b	2.667ab
Inqilab 91	2.833b	2.833ab	2.167b	2.333bc
Bhakkar 2001	2.500b	2.667ab	1.333c	2.000c
LSD Value	0.6545	0.022	0.0629	0.0176

Column means followed by a common letter are not statistically different at 5 %.

4.5 PATHOGENICITY

The pathogenicity of all the isolates of different fungi collected during 2004 and 2005 was conducted by using test tube moist cotton swab method, (Plate 4.14). Cultivar Wafaq-2001 was used to confirm the Koch's postulate.

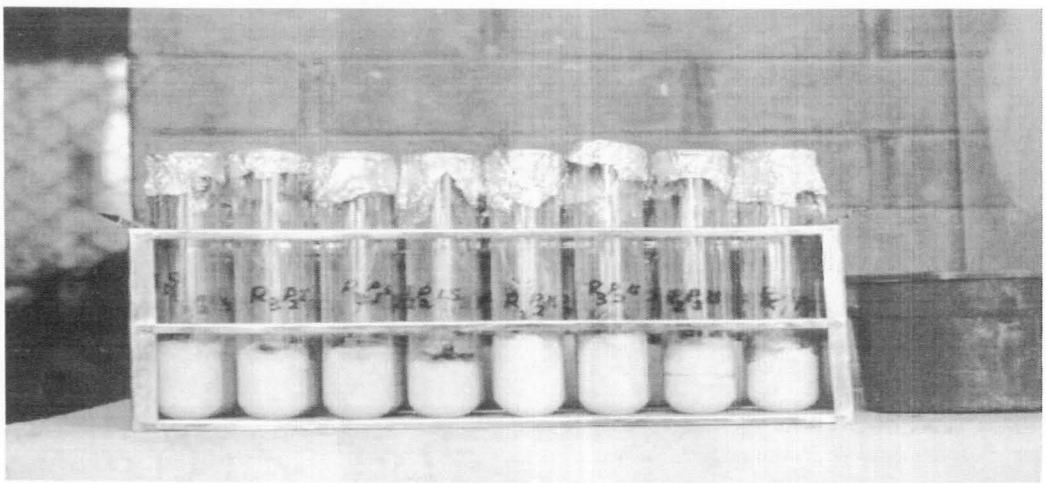


Plate 4.14. Pathogenicity test by test tube moist cotton swab method

4.5.1 Pathogenicity of foliar blight fungi isolated during 2004 and 2005

The results of the pathogenicity tests conducted on different isolates of fungi collected at both seedling and booting stage during 2004 revealed that thirty seven isolates of *Bipolaris sorokiniana* were pathogenic and produced the foliar blight symptoms (Plate 4.15). All these isolates were individually reisolated and Koch's postulates were confirmed by comparing with the mother culture and symptoms expression. None of other isolates of fungi including *Alternaria alternata, Curvularia lunata, Epicoccum purpurascens,* and *Drechslera spicifer* was proven to be pathogenic upon confirmation of Koch's postulates (Table 4.14).

Table 4.14: Pathogenicity of fungi associated with foliage of wheat collected from different wheat growing agro-ecological zones of Punjab and NWFP during 2004 and 2005.

S. No.	Fungi isolated	Year 2004	Year 2005	Pathogenicity confirmation
1	*Alternaria. alternata*	+	+	-
2	*Bipolaris sorokiniana*	+	+	+
3	*Curvularia lunata*	+	+	-
4	*Epicoccum purpurascens*	+	+	-
5	*Drechslera spicifer*	+	+	-
6	*Drechslera. rostata*	-	+	-
7	*Stemphylium Spp.*	-	+	-
8	*Cladosporium cladosporioides*	-	+	-
9	*Colletotrichum graminicola*	-	+	+
10	*Dilophospora alopecuri*	-	+	+

The results of the pathogenicity tests conducted on different isolates collected at booting stage (only) during 2005 surveys revealed that all fifty isolates of *Bipolaris sorokiniana* were pathogenic and Koch's postulates was confirmed. In addition two isolates of *Colletotrichum graminicola* were isolated one from zone 7 (Punjab) and one from zone 10(NWFP) and its pathogenicity was confirmed (Plate 4.16 and Plate 4.17). *Dilophospora alopecuri* (Plate 4.18) the cause of leaf blight on wheat was

isolated from zone 10 (NWFP) was isolated and proven to be pathogenic. Rest of the fungi isolated from wheat leaves were found non-pathogenic to wheat in our studies (Table 4.14).

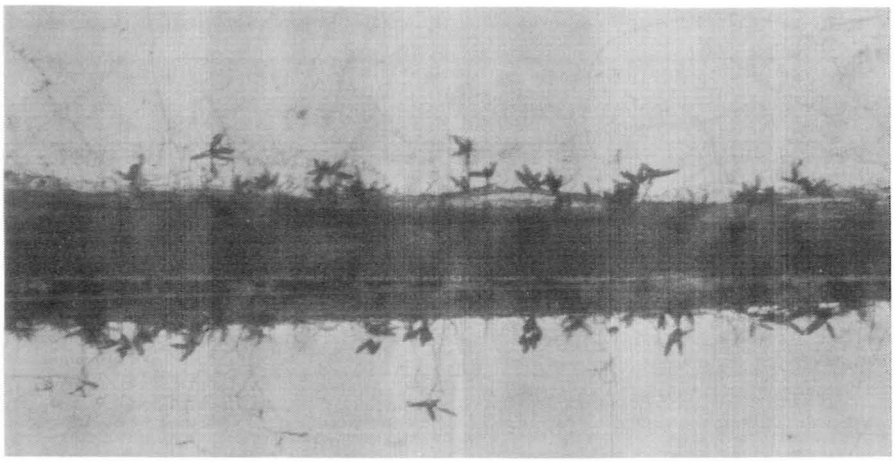


Plate 4.15. Spores of *Biploaris sorokiniana* on wheat leaf during pathogenicity

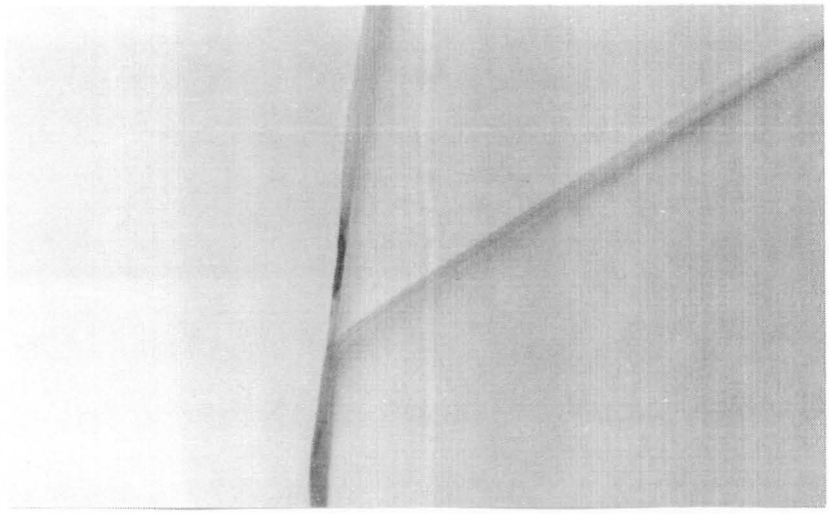


Plate 4.16. Symptom on wheat leaf during pathogenicity test caused by *Colletotrichum graminicola*

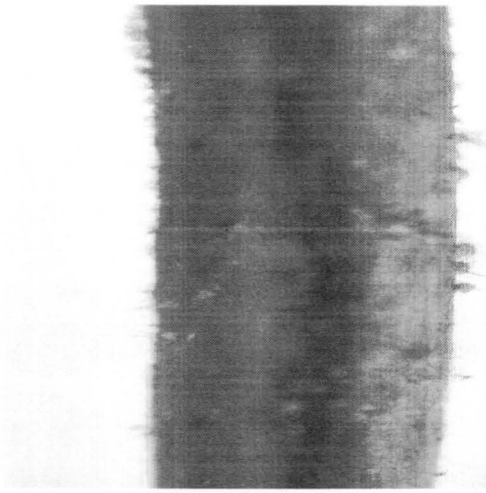


Plate 4.17. Close up of wheat leaf showing the ascervuli of *Colletotrichum graminicola*, setae at 100 x magnification

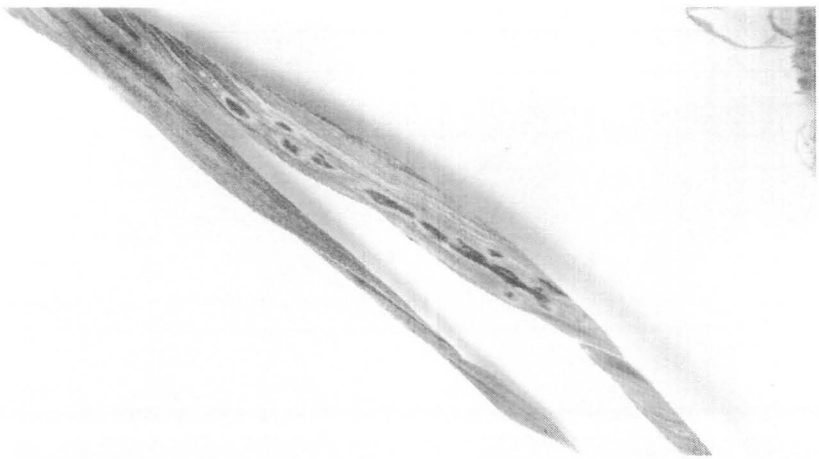


Plate 4.18 Symptoms of *Dilophospora alopecuri* on wheat leaf during pathogenicity

4.6 AGGRESSIVENESS ANALYSIS

4.6.1 Selection of wheat varieties for aggressiveness analysis

Ten commercial wheat varieties were tested against six isolates of *Bipolaris sorokiniana.* These isolates were collected during survey at the initial stage of the studies during 2004 and on the basis of these result the three varieties were selected for further analysis. The results are depicted in table 4.15. It is evident from the overall means of six isolates behavoiur on three varieties, all isolates exhibited more aggressiveness on Wafaq 2001, Inqilab 91 and Bhakkar 2001 among the ten varieties tested and hence was selected for further evaluation against the rest of the isolates of *Bipolaris sorokiniana.*

Table 4.15: Selection of wheat varieties against *Bipolaris sorokiniana* isolates

Varieties	Mean values (0-5) scale Isolates						
	P1-9	P1-6	P1-4	P1-10	NP1-2	NP1-3	Average
Bakhtawar 93	1.6	2.0	2.3	2.0	2.0	2.0	1.9
Chakwal 86	2.3	2.3	2.3	2.0	2.0	2.0	2.1
Inqilab 91	**3.0**	**2.6**	**3.0**	**2.6**	**2.6**	**1.6**	**2.5**
Kirin 95	1.0	1.6	2.0	2.0	1.6	2.0	1.7
SH 2002	2.0	2.0	2.0	2.0	2.0	1.6	1.9
Bhakkar 2001	**2.6**	**3.0**	**2.6**	**2.3**	**2.3**	**2.6**	**2.5**
Iqbal 2000	1.6	1.6	2.0	1.6	2.0	1.6	1.7
Ahaqab 2000	1.0	1.6	1.6	2.0	2.0	1.6	1.6
Wafaq 2001	**4.3**	**2.6**	**3.0**	**3.0**	**3.6**	**3.0**	**3.2**
Margalla 99	2.0	2.0	2.0	2.0	1.6	2.0	1.9

4.6.1.1 Aggressiveness analysis of *Bipolaris sorokiniana* isolates

The aggressiveness analysis of eighty seven isolates of *Bipolaris sorokiniana* collected from different agro-ecological wheat growing zones of Punjab and NWFP during 2004 (thirty seven isolates) and 2005 (fifty isolates) was done, using three commercial wheat varieties as mentioned above (section 4.6.1)

4.6.1.2 Aggressiveness analysis of isolates of *Bipolaris sorokiniana* on three commercial wheat varieties.

Total eighty seven isolates of *Bipolaris sorokiniana* isolated from foliar samples of wheat from different wheat growing agro-ecological zones during 2004 & 2005 were tested for aggressiveness. In aggressiveness test all isolates were found to be pathogenic to three varieties of wheat, however in terms of aggressiveness the isolates varied on these varieties (Appendix.11).

The aggressive reaction of eighty seven isolates of *Bipolaris sorokiniana* on these wheat varieties was subjected to Analysis of Variance (ANOVA) (Table 4.16. The results revealed that there was no significant effect of replications, however there was highly significant effect of isolates among themselves and their reaction on different varieties on which they were tested.

Table 4.16: Analysis of variance for aggressiveness of *Bipolaris sorokiniana* isolates inoculated on three commercial wheat varieties.

Sources of variation	Degree of freedom	Sum of squares	Mean square	F value	Prob
Replication	2	0.110	0.055	0.4009NS	0.0533
Isolates	86	178.715	2.078	15.172**	0.000
Varieties	2	57.083	28.542	208.3804**	0.000
Isolates x varieties	172	260.695	1.516	11.0658**	0.000
Error	520	71.223	0.137		

**F value highly significant at 1 % level. Coefficient of varaince12.94%

The effect of aggressiveness on wheat variety Wafaq-2001 was found highly significant compared to other two wheat variety (Inqilab-91 and Bhakkar-2001), whereas the aggressiveness on both these two varieties was found non significant. The mean value of these three varieties showed that isolates were more aggressive on Wafaq-2001than Inqilab-91 and Bhakkar-2001 (Table 4.17).

Table 4.17: Mean values for aggressiveness of *Bipolaris sorokiniana* isolates inoculated on three commercial wheat varieties.

Varieties	Mean Value (0-5) scale
Wafaq-2001	3.241 a
Inqilab-91	2.697 b
Bhakkar-2001	2.644 b
LSD Value	0.0637

Column means followed by a common letter are not statistically different at 5 %

To clarify the aggressiveness behaviour of eighty seven isolates of *Bipolaris sorokiniana* based on their reaction on three wheat varieties Wafaq-2001, Inqilab-91 and Bhakkar-2001, the results on these varieties were subjected to cluster analysis by the unweighted pan group method of averages using the software package statistika version,Mini tap (Fig.4.16). The dendrogram (Fig.4.16) is based on the combined (means) behaviour of *Bipolaris sorokiniana* on three wheat varieties.

The mean values of eighty seven isolates of *Bipolaris sorokiniana* tested on three commercial wheat varieties, these isolates were classified into three major groups (on the basis of similarity group of the means according to aggressiveness behaviour), (A, B and C) (Table 4.18). Some groups were further classified into sub-groups,(the isolates showing further minor similarities within the group) group B (sub-group B1 and B2), sub groupB2 further classified into mini groups B2a and B2b, group C (sub groupC1 and C2) both divided into mini groups C1 (C1a and C1b) and C2 (C2a and C2b). Group A consisted of two isolates; both belonged to least

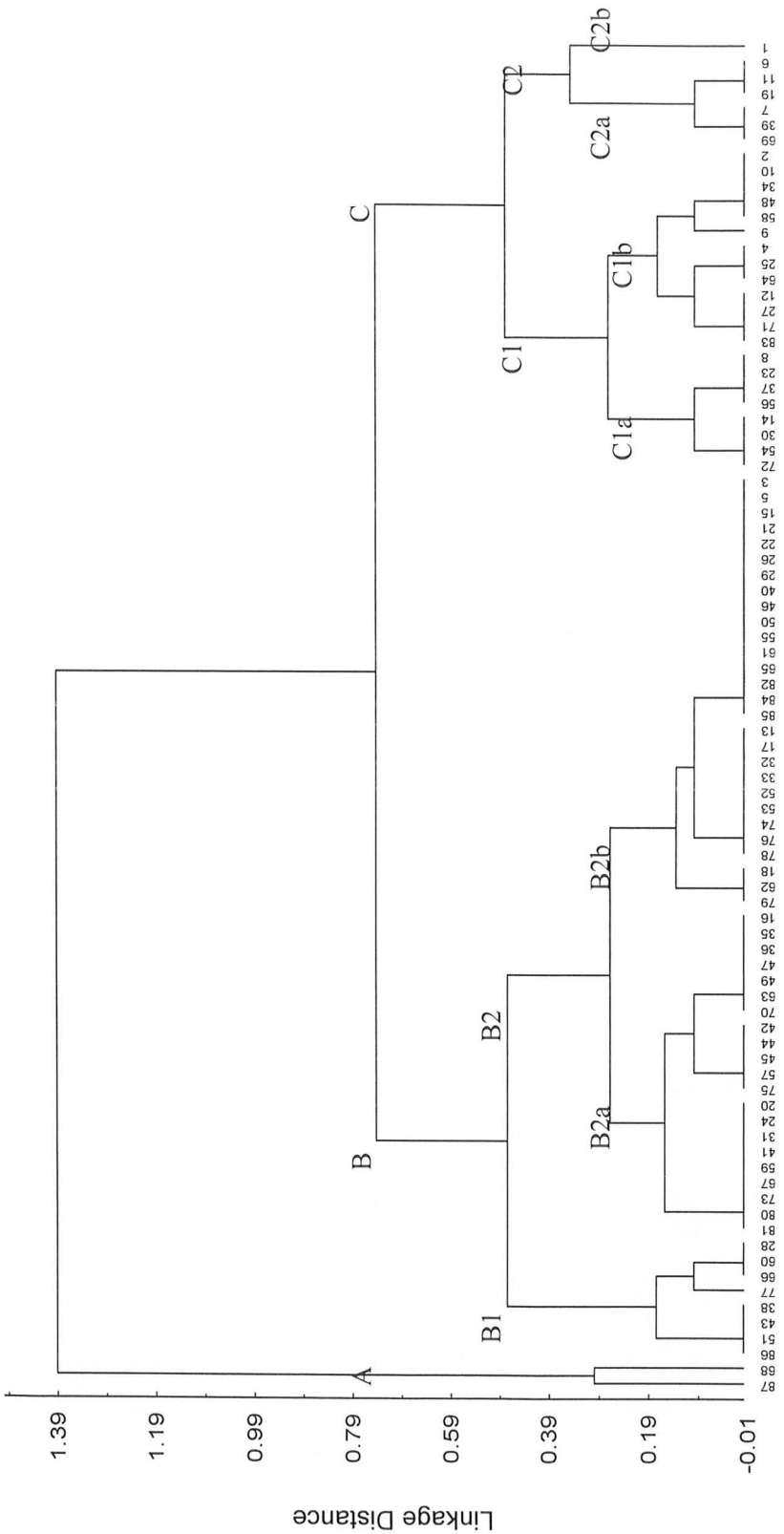


Fig.4.16. Dendogram showing different groups (on the basis of similarities) of eighty seven isolates of *Bipolaris sorokinian*

Table 4.18: Classification of eighty seven isolates of *Bipolaris sorokiniana*, based on cluster analysis of aggressiveness tests on three commercial wheat varieties

Group	Sub-Group	Category	No. of Isolates	Isolates
A	-	Least Aggressive	2	NP3-29, G5-3,
B	**B1**	Slightly Aggressive	8	P4-40, P2-28, P4-1, NP-9, NP3-12, NP3-27, SWT1-26, S-K-1.
	B2			
	B2a	Slightly Aggressive	21	P2-18, P4-12, P4-31, P1-10, P2-24, P2-25, P3-15, P3-16, P4-5, P4-9, NP1-3, NP6, NP3-9, NP3-10, NP3-16, NP3-28, NP7, NP3-6, G1-6, G1-8, SWT1-7
	B2b	Slightly Aggressive	28	P4-2, P4-17, P1-6, P4-14, P2-22, P2-23, P4-13, P4-14, P4-33, P1-4, P2-3, P2-4, P3-14, NP1-2, NP-8, NP-10, NP-11, NP3-3, NP3-13, NP3-15, NP3-21, SWT1-2, SWT1-22,G1-18 G1-2, G1-9, S-Shing-7,S-G-10
C	**C1**			
	C1a	Moderately Aggressive	8	P4-22, P2-7, P4-30 ,P1-9 ,P2-26, NP-20 ,NP3-7,NP3-5
	C1b	Moderately Aggressive	13	P2-15, P4-16 ,P4-27, P4-24, P4-29,P4-32, P4-34, P2-5, NP4, NP3-10, NP3-18, NP3-4, S-Shing-5
	C2			
	C2a	Moderately Aggressive	6	P4-18, P4-20, P4-28, P4-11, P3-5, NP3-31
	C2b	Aggressive	1	P2-9

aggressive isolates. Group B comprised of 57 isolates, out of which 8 isolates belonged to sub-group B1 and 49 belonged to sub group B2, the sub group further sub divided into mini group B2a and B2b of which 21 isolates belonged to B2a and 28 belonged to B2b.All the isolates in these groups consisted of slightly aggressive but due to slight difference in aggressiveness were further sub divided. Group C comprised of 28 isolates out of which 8 isolates belonged to sub group C1 which was further sub divided into C1a and C1b, C1a comprised of 8 isolates while C1b comprised of 13 isolates all belonged to the moderately aggressive isolates. The sub group C2 comprised of 7 isolates and was further sub divided into two mini groups

C2a and C2b out of which 6 isolates belonged to C2a and one isolate belonged to C2b.The C2a isolates are categorized to moderately aggressive while C2b one isolate belonged to aggressive group.

4.6.1.3 Frequency of different aggressiveness group of *Bipolaris sorokiniana* isolates, on three commercial varieties of wheat.

The isolates collected during 2004 and 2005 from different wheat growing zones were analyzed and categorized in four classes based on their aggressiveness: aggressive, moderately aggressive, slightly aggressive and least aggressive.

P2-9 an isolate of zone 5 was the only isolate found aggressive among the eighty seven isolates on combine mean basis on three commercial wheat varieties. The behavior of other isolates from zone 5 revealed that, nine isolates were moderately and 2 isolates were slightly aggressive. In zone 6, out of 16 isolates, 5 isolates showed moderately aggressiveness while 11 isolates showed slightly aggressiveness (Fig. 4.17). In zone 7 of Punjab 4 and 13 isolates belonged to moderately aggressive and slightly aggressive classes respectively .The detail of frequencies from different zones in Punjab NWFP is given in (Fig. 4.17)

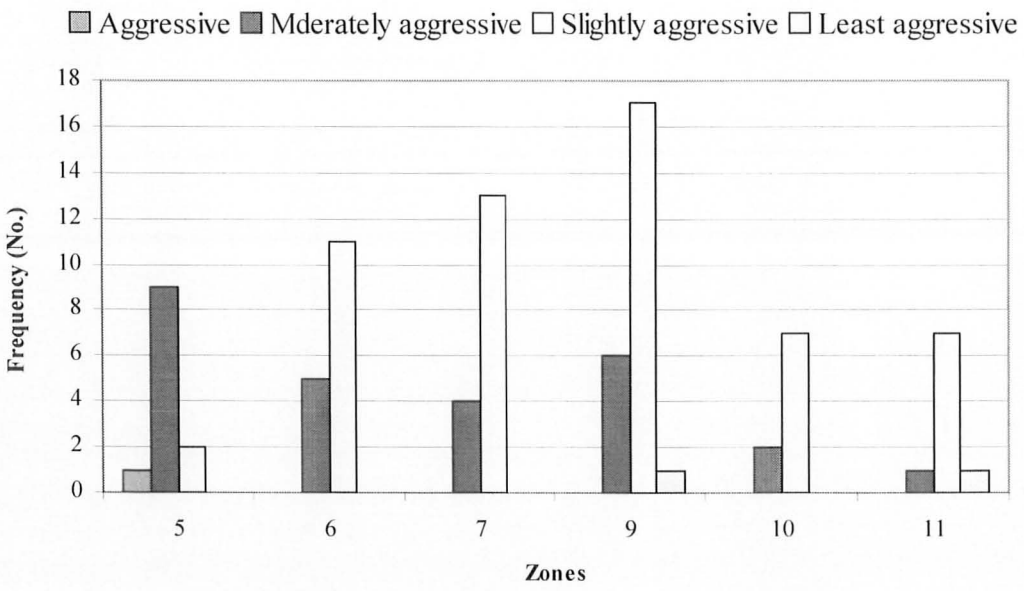


Fig.4.17. Zone wise frequency of different aggressiveness group of *Bipolaris sorokiniana* isolates, on three commercial varieties of wheat.

In zone 9 (NWFP) six isolates exhibited moderately aggressive reaction while 17 and one isolate showed slightly and least aggressiveness respectively. In zone 10 two isolates exhibited moderately and 7 slightly aggressive reaction. As regard zone 11 one isolate each exhibited moderately and least aggressive behavior while 7 isolates exhibited slightly aggressive reaction. The detail of frequencies of isolates from NWFP zones is given in (Fig. 4.17).

4.7 EPIDEMIOLOGICAL STUDIES

4.7.1 Effect of Different Temperatures on the Radial Mycelial Growth of Isolates of Bipolaris *sorokiniana*

Total 46 isolates of *Bipolaris sorokiniana* have been selected in this study to find out the most conducive temperature (20°C, 25°C and 30°C) for the radial mycelial growth. The effect of time on the growth of isolates in relation to temperature was also recorded. The data was subjected to analysis of variance (ANOVA) (Table 4.19).

Table 4.19. Analysis of variance on the effect of three temperatures regimes on the mycelial growth of isolates of *Bipolaris sorokiniana*

Sources of variation	Degree of Freedom	Sum of square	Mean square	F value	*Prob*
Isolates	45	21238.16	476.40	7581.92 **	0.0000
Days interval	2	419798.49	209899.24	3340529.57**	0.0000
Isolates x Days	90	3884.20	43.15	686.85**	0.0000
Temperatures	2	111210.95	55605.47	884956.65**	0.0000
Isolates x Temperatures	90	4424.28	49.15	782.35**	0.0000
Days x Temperatures	4	14814.18	3703.54	782.35**	0.0000
Isolates x Days x Temperature	180	2936.64	16.31	58941.64**	0.0000
Error	828	52.02	0.06	259.64**	0.0000

**F value Highly Significant at 1% level. Coefficient of Variation: 0.56%

There was highly significant effect of isolates, Days interval, temperature, Isolates x day's interval interaction, isolates x temperature interaction, Days interval x temperature and isolate x days interval x temperature interaction.

The mean values of day's interval showed the maximum growth after 12 days and the least after 4 days interval (Table 4.20).

Table 4.20: Mean values for Number of days (time interval) on the radial mycelial growth of *Bipolaris sorokiniana* at three temperatures regimes.

Days	Mean Growth (mm)
4	21.682c
8	46.537b
12	66.632a
LSD Value	0.0342

Column means followed by a common letter are not statistically different at 5 %

The mean values of three temperatures also showed significant affect on radial mycelial growth however the most suitable temperature was 25°C followed by 30°C but slow trend in growth was observed at 20°C after same day's interval (Table 4.21).

Table 4.21: Mean values of the radial mycelial growth of *Bipolaris sorokiniana* at three temperatures regimes.

Temperatures (°C)	Mean Growth (mm)
20	32.183c
25	54.806a
30	47.863b
LSD Value	0.0342

Column means followed by a common letter are not statistically different at 5 %

The mean value for interaction between the days interval and three temperatures also suggested significant difference among days and temperatures, however in all the cases i.e. after 4 days interval 8 and 12 days interval the maximum growth was observed at 25^{O}C whereas slower growth at 20^{O}C (Table 4.22).

Table 4.22: Mean values of interaction between time interval and temperatures of the radial mycelial growth of *Bipolaris sorokiniana*

Days	Temperature(^{O}C)	Mean Growth (mm)
4	20	12.941c
4	25	27.544f
4	30	24.562h
8	20	27.370g
8	25	59.989c
8	30	52.252e
12	20	56.236d
12	25	76.883a
12	30	66.776b
LSD Value		0.0593

Column means followed by a common letter are not statistically different at 5 %

The behaviour of the individual isolate at three days interval and at three temperature regimes is given in graphics (Fig. 4.18, Fig.4.19 and Fig. 4.20) The results after 4 days interval at three temperatures revealed that Isolate P4-16 has the maximum growth (17.5mm) at 20°C, the minimum growth (10.3mm) of isolate G1-6 (Appendix 18). At 25°C the maximum growth (35.4mm) of the same isolates P4-16

was observed while the minimum growth (21.9mm) of isolate G1-2 was observed (Appendix 18.) .However at 30°C the maximum growth (28.5mm) was observed of isolate P3-15 while minimum growth (19mm) of isolate G5-3 was found.(Fig.4.18., Appendix 12.).

After 8 days interval at 20°C the same isolate P4-16 has attained the maximum growth (35.4mm) whereas G1-6 having minimum growth (21.9mm) at this temperature (Appendix 13). At 25°C, in addition to P4-16 an other isolate P4-28 attained the maximum growth (66.3mm) while the minimum growth (56mm) was observed of isolate NP3-31 (Appendix 13). At 30°C the maximum growth (65mm) was observed of isolate P3-15 while minimum growth (40.1mm) was observed of isolate G1-2 (Fig. 4.19) (Appendix 13).

After 12 days interval at final reading of the experiment, at 20°C the maximum growth (67.2mm) was recorded of isolate P4-28 while minimum growth (45.3mm) was observed in isolate G1-9 (Appendix 14). At 25°C, the maximum growth (89.1mm) was recorded of isolate P4-16 while the minimum growth (67mm) was observed of isolate G5-3 (Appendix 14). At 30°C the maximum growth (82mm) was observed of isolate P4-31 while minimum growth (50.3mm) was observed of G5-3 (Fig.4.20), (Appendix 14).

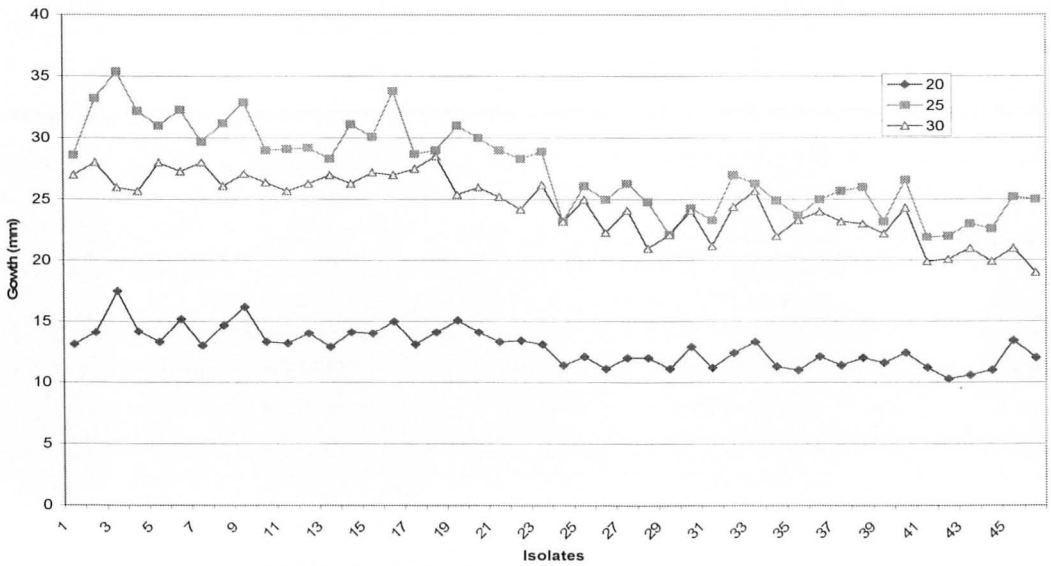


Fig.4.18. Radial mycelial growth of *Bipolaris sorokiniana* at three temperature regimes after four days interval

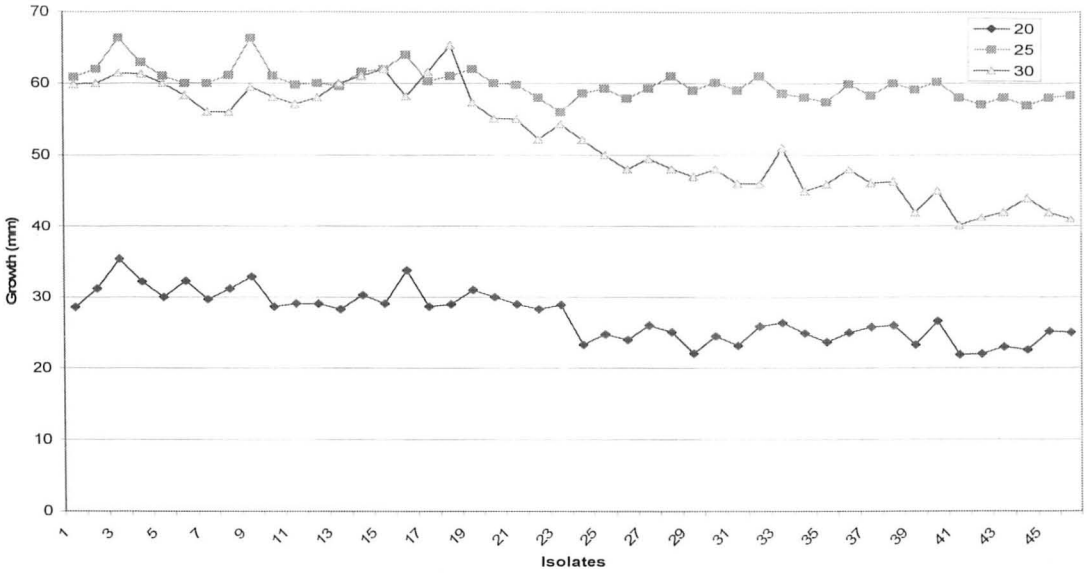


Fig.4.19. Radial mycelial growth of *Bipolaris sorokiniana* at three temperature regimes after eight days interval

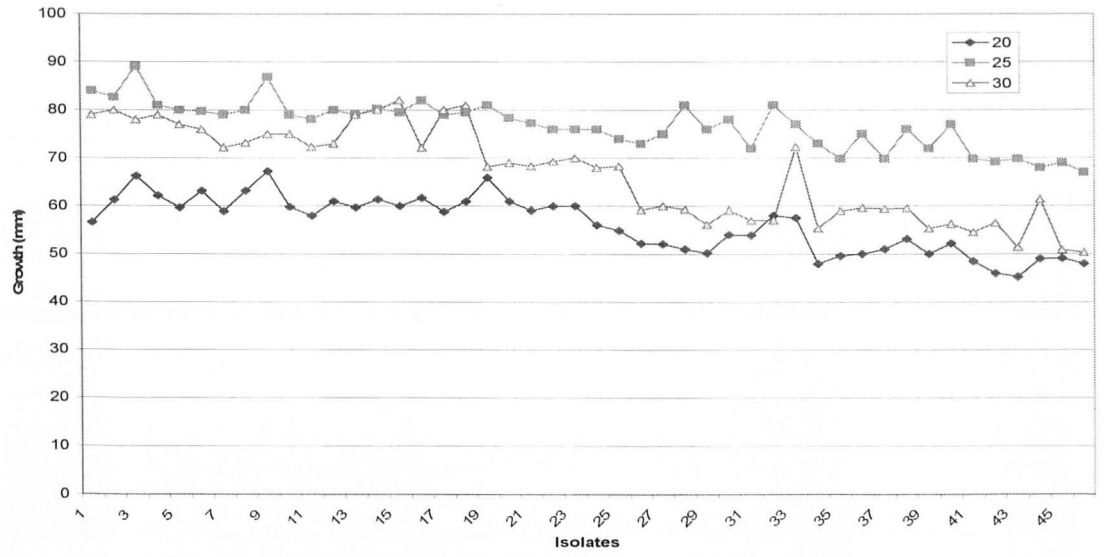


Fig. 4.20. Radial mycelial growth of *Bipolaris sorokiniana* at three temperature regimes after twelve days interval

4.7.1.1 Hosts other than wheat:

To observe the hosts of *Bipolaris sorokiniana* other than wheat; plants showing (Table 4.23) foliar blight symptoms on oat and barley were also monitored either in the wheat field or around the field. One isolate each of barley and oat were collected, purified and were inoculated first on host plant to confirm the pathogenicity then again reisolated, multiplied and inoculated on wheat plants by using test tube moist cotton swab method. The typical spot blotch symptoms were produced. After again re-isolation from wheat these isolates were individually inoculated on barley and oat plant and this inoculation again resulted in similar symptom. Apart from these hosts other hosts were also determined for the fungus *Bipolaris sorokiniana*, which revealed that the plant of the crop *Oryza sativa, Halianthus annus, Arachis hypogea* and *Cicer arientenum* were found non host of the fungus *Bipolaris sorokiniana.*

Table 4.23: Status of Hosts of *Bipolaris sorokiniana* other than wheat.

S.No.	Crop	Status
1.	*Avena sativa*	+
2.	*Hordeum vulgare*	+
3.	*Brassica compestris*	+
4.	*Glycine max*	+
5.	*Lens culinaris*	+
6.	*Vigna radiata*	+
7.	*Oryza sativa*	-
8.	*Halianthus annus*	-
9.	*Sesamum indicum*	+
10.	*Arachis hypogea*	-
11.	*Vigna mungo*	+
12.	*Sorghum bicolor*	+
13.	*Zea mays*	+
14.	*Cicer arientenum*	-
15.	*Panicum maximum*	+

4.7.1.2 Assessment of foliar blight in different production technologies

During the survey of 2004 and 2005 the samples collected from wheat crop sown after rice from the rice-wheat cropping system were analyzed to assess the prevalence of foliar blight fungi. (Appendix 15). Foliage pathogenic fungus (*Bipolaris sorokiniana*) was isolated from conventional and zero tillage practicing fields where as none of the Bed planted fields yielded any pathogenic fungi. The fungus *Bipolaris sorokiniana* was isolated from 10 fields, seven from conventional and three from zero tillage (Table 4.24).

Table 4.24: Fungi isolated from different cultivation technologies in rice wheat Cropping system during 2004 and 2005

Fungi isolated	Year2004			Year 2005		
	BP	ZT*	C	BP	Z T	C
A. alternata	3	0	3	1	1	5
B. sorokiniana	0	0	2	0	3	5
C. cladosporioides	0	0	0	0	0	1
C. lunata	1	0	0	0	0	2
D. rostata	0	0	0	0	1	1
Total	**4**	**0**	**5**	**1**	**5**	**14**

* No field was observed during 2004.
BP= Bed Planting, ZT= Zero tillage, C= Conventional

Chapter 5

DISCUSSION

Wheat being a staple food for more than 15 million people in the country plays a vital role in the economy of Pakistan. There are many reasons for the stagnation in the yields compared to many developing countries. Apart from abiotic factors there are biotic factors including involvement of microorganisms which can regularly take their toll on the crop yield wherever the crop is cultivated in Pakistan. Rusts, smuts and bunts were the major diseases of wheat occurring in Pakistan during 2000-2001 (Khan *et al.*, 2003). The foliar blight diseases have been recorded in most growing areas of India (Sharma *et al.*, 1996), Bangladesh (Alam *et al.*, 1994) and Nepal (Karki, 1996). Previously in Pakistan in the Southern province of Sindh, where winter temperatures are warmer, Helminthosporium Leaf Blight (HBL) has been noted (Bhatti and Ilyas, 1986; Hafiz, 1986). However most recently Iram and Ahmad (2005) reported 100% prevalence of foliar blight in the four districts of Punjab, during surveys of 2001 and 2002 in rice-wheat cropping system. The occurrence of foliar blight in major wheat areas of Pakistan calls for detailed survey, analysis and improved understanding of foliar blight for better crop productivity.

The present study was taken up for detailed analysis of the foliar blight pathogens prevalent in the wheat production zones of NWFP and Punjab. The understanding of the pathobiology of the isolated pathogens will bring about the information which could be used by the researchers in breeding for the improvement of wheat production.

As a result of the pathogenicity in the current study *Bipolaris sorokiniana* came out to be the only and major foliar blight pathogen, therefore the detailed studies were focused only on the isolates of this pathogen. During current study other two fungi, *Colletotrichum graminicola* and *Dilophospora alopecuri* were found pathogenic hence some basic studies on these pathogens were also conducted. The morphological studies were conducted on the eighty seven isolates of *Bipolaris sorokiniana* collected during the surveys 2004-2005. The characterization of the fungal isolates was carried out on the basis of morphology including spore

measurement, size, colour and type of the colony of each culture. Five different methods were tried to standardize the one most efficient and effective method to be used for pathogenicity and aggressiveness analysis. The aggressiveness analysis of eighty seven isolates of *Bipolaris sorokiniana* was conducted on three commercial wheat varieties. The epidemiological study was carried out to work out the most suitable temperature for the isolates of *Bipolaris sorokiniana.* The other hosts like oat and barley were found with wheat crop during surveys (showing foliar blight spots) were also collected and its pathogenicity was conducted on respective hosts and later on wheat for confirmation. Thirteen crop plants (*Brassica compestris, Glycine max, Lens culinaris*, *Vigna radiate, Oryza sativa*, *Halianthus annus*, *Sesamum indicum*, *Arachis hypogea*, *Vigna mungo*, *Sorghum bicolour*, *Zea mays, Cicer arientenum* and *Panicum maximum*) were studied to see the hosts other than wheat. The assessment of foliar fungi under different cultivation methodologies (zero tillage, on ridges, and conventional) in rice-wheat cropping system in wheat crop sown after rice was also taken into consideration.

5.1 Prevalence, Incidence, Severity and Disease Index of foliar blight of wheat during 2004 and 2005

There is a little work and scanty literature available in Pakistan regarding foliar blight diseases and its prevalence. The survey of wheat crop during 2004 was conducted at two growth stages; the seedling stage and the booting stage. The prevalence of foliar blight during seedling stage was not more than 55% which was observed in zone 10 of NWFP. (Fig.4.2 and Fig4.3). Similarly the disease incidence in all of zones surveyed ranged from to 1.9% 5.3%. (Fig.4.4 and Fig.4.5) The severity ranged from 0.1to 0.5(Fig.4.4 and Fig.4.5) while disease index ranged from 0.4 to. 1.2%. The frequency of different fungi at seedling stage was also very low as during survey the farmers informed that most of the sowing is done with fungicide treated seeds which may be the cause of low fungal isolation at this stage during our studies. Seed and soil treatment trials in Nepal and Bangladesh have shown a significant improvement in plant stand and yields (Alam *et al.*, 1994; Badaruddin *et al.*, 1994; Meisner *et al.*, 1994; Dubin and Bimb, 1991). The seed and soil borne inoculum are important in the establishment of leaf blight, seedling blight and root rot and that can be indicated by the symptoms and damage at the seedling stage and on primary leaves.

This supported the findings that seed treatment reduces the disease incidence on young plants at early developmental stages (Weisig and Fehrmann, 1993).Another reason of low frequency of foliar fungi at seedling stage might be due to unfavourable climatic conditionsThe mean temperature in the wheat production zones ranged from 14.9 to 17.3^{0}C with the average rain from 5.5 to 52.2 mm (Meteorological Deptt. Islamabad, 2005). During the survey at seedling stage which was not conducive for the establishment of foliar disease. (Fig.3.2 and Fig.3.3)

At booting stage, the prevalence, incidence, severity and disease index of foliar pathogens was higher in all the zones. (Fig.4.7, 4.8, 4.9, 4.10, 4.11, 4.12,413 and 4.14).As during this stage of the crop the environmental conditions were very conducive for the disease development. (The monthly mean temperature during March in zone 5 was 25.50C, in zone 6, 24.9^{0}C and in zone 7, 24.9^{0}C whereas in zone 9 and 10 it was 23.6^{0}C), (Fig.3.2 and Fig.3.3) which was one of the reasons for high foliar fungi as observed by (Singh and Tewari, 2001) who studied the role of environmental conditions in the development of major foliar diseases of wheat was observed during three crop seasons (1995-96, 1996-97 and 1997-98) at Pantnagar, Uttar Pradesh, India. Where minimum and maximum average temperature of 11.7^{0}C (range 10.8 – 12.5^{0}C) and 27.7^{0}C (range 26.8 - 28.5^{0}C), 39.7 mm rain and 42.8 – 88.2 relative humidity were the most favorable conditions for the faster development of the disease. (Fig.3.2 and Fig.3.3)

The frequently isolated fungi from wheat leaves collected from wheat production zones of Punjab and NWFP during 2004 were *Alternaria alternata* and *Bipolaris sorokiniana*, both during seedling and booting stage of the crop. The three leaf blight diseases spot blotch, tan spot and *Alternaria* blight were also recorded by Joshi *et al*., (1970); Nema, (1986); Sharma *et al*., (1996) in most wheat growing areas of India. Similarly a large number of blight affected wheat samples were collected from every crop season since 1988 from different coordinating centers in India and neighboring countries. After repeated isolations *Bipolaris sorokiniana, Helminthosporium spiciferum, Pyrenophora tritici-repentis, Alternaria alternata, Alternaria triticina* and *Curvularia lunata* were found to be associated with foliar blight each year (Singh *et al*., 1998). In our results during 2004 at seedling stage the

foliage samples of wheat yielded *Alternaria alternate* followed by *Epicoccum purpurascens*, *Bipolaris sorokiniana, Curvularia lunata and Drechslera spicifer* compared to booting stage when the fungi *Alternaria alternata* was followed by *Bipolaris sorokiniana*, *Epicoccum purpurascens*, *Drechslera spicifer* and *Curvularia lunata*. The low incidence of foliar fungi at seedling stage was found during 2004 which is supported by the observations of initiation of foliar blight at early stage (in December) with the increase of severity at heading and flowering stages. (Singh *et al.*, 1998). Keeping in consideration the low incidence of foliar blights at seedling stage during 2004, the survey was abandoned for the next wheat cropping season and only the survey was conducted at the booting stage of the crop. This decision is also supported by a study in which a 10 year survey conducted by Alam and Saha (1991) in Bangladesh during 1985/86-1995/96 wheat seasons reported that spot blotch damage occurred late in the season when the crop is approaching maturity. The surveys, out of six zones at booting stage in different wheat growing zones of Punjab and NWFP, Pakistan during 2005 showed 100% prevalence of foliar blights in all the zones except one. The incidence, severity, the disease index was also higher during 2005 may be due to the conducive temperature and frequent rains in this year. (Meteorological Deptt.Islamabad, 2005) as previously observed by Satvinder *et al.,* (2001) that severe leaf blight epidemic occurred on wheat in Punjab (India) and adjoining areas in 1995-96 where the incidence of foliar blight pathogens was remained low till 1995. In 1996 the leaf blight due to *Drechslera sorokiniana* and *Fusarium* species occurred widely resulting in severe set back to wheat crop mainly because of favourable environmental conditions. (Satvinder *et al.*, 2002).

The high temperatures and high relative humidity which resulted after rains favored the outbreak of the foliar blights in South Asia's (Jagdish *et al.*, 2002).The higher incidence in Kerala, India attributed mainly due to the favorable environmental conditions.(Sing *et al.,* 2001).

5.2 Isolation/frequency of fungi from foliage of wheat crop from different wheat growing Agro-ecological zones of Punjab and NWFP during 2004 & 2005

The frequency of overall fungi isolated from the wheat leaves different production zones during 2005 revealed maximum isolates of *Alternaria alternata* followed by *Bipolaris sorokiniana, Colletotrichum graminicola, Dilophospora alopecuri, Stemphylium* sp., *Cladosporium cladosporioides Drechslera spicifer* and *Epicoccum purpurascens*. As Singh *et al.* (2004) isolated more or less same fungi when they collected the leaf samples of different wheat cultivars showing blight symptoms from Jammu and Kashmir. They isolated *Bipolaris sorokiniana* and *Alternaria triticina* from 29 and 12 samples of total 88. *Alternaria alternata Chaetomium* spp., *Fusarium moniliforme, Epicoccum purpurascens, Curvularia lunata* and *Penicillium* spp. were also isolated.

During the year 2004 the frequency of fungi was lower compared to the year 2005, which might be due to the non conducive climatic conditions. This is supported by the work done by Csosz (2005) who analyzed 1879-2720 leaf samples from 8-13 stations of Hungary in March to June 2000 – 2001and found *Pyrenophora tritici-repentis, Septoria tritici* and *Bipolaris sorokiniana*. The occurrence of necrotrophic pathogens was highest (10.79%) in 2001 and lowest (2.63%) in 2002. He also reported that the occurrence and rate of the necrotrophic pathogens changed significantly among years and locations mainly due to the unprotected environment and resistance of cultivars. During the year 2004 as the rainfall was not as frequent as during the year 2005 in the wheat production Zones (Meteorological Deptt.Islamabad, 2005). Secondly may be due to the varieties difference in the different zones in this year. The leaf blight surveys conducted during the years 2000, 2001 and 2002 wheat growing seasons in India, Nepal and Bangladesh revealed that out of 198 leaf samples collected in farmer's fields *Bipolaris sorokiniana* was predominant in India and Bangladesh while *Drechslera tritici-repentis* was commonly isolated from samples collected in Nepal. The variation in the frequency of the foliar pathogens was observed between years and even during a single growing season according to climatic conditions (Mercado, 2003). In our studies out of 2500 sample, thirty seven isolates of *Bipolaris sorokiniana* were isolated during 2004 and out of 1520 samples, fifty

isolates were isolated during 2005.The higher number of isolates of *Bipolaris sorokiniana* during 2005 seems to be due to the suitable climatic conditions in that year as pathogen required 20-30^{0}C (Metha, 1998) with relative humidity 86% and 37.4mm rain (Akram and Amerika singh, 2003). The higher number of isolates (*Bipolaris sorokiniana*) in second year may also be due to the presence of other alternate hosts (barley and oats) that can serve as secondary source of inoculum (Reis, 1989; Schafer *et al.*, 2005).

On the basis of isolation studies, *Bipolaris sorokiniana* was found as a major pathogenic foliar blight pathogen isolated from eighty seven sample/ fields in both the years 2004 and 2005, which showed an increased incidence of the disease in two major wheat provinces of Pakistan, i.e., Punjab and NWFP, as compared to earlier reports of its prevalence from the southern province of Sindh, where winter temperatures are warmer (Bhatti and Ilyas, 1986; Hafiz, 1986). Iram and Ahmad, (2005) reported 19 isolates of *Bipolaris sorokiniana* from four districts of Punjab alone in rice-wheat cropping system. Recently the increasing trend of spot blotch (*Bipolaris sorokiniana*) has also been observed in different ecological zones (Iftikhar *et al.,* 2006) having mean temperatures in the range of 22-25 ^{0}C. During the current study the frequency of *Bipolaris sorokiniana* was also higher in zones 5, 6 and 7 of Punjab due to these climatic conditions (Meteorological Deptt.Islamabad, 2005). This is supported by the study that one of the major limiting factors to wheat production is very hot temperature (<22.5^{0}C) and humid climate mainly due to the disease caused by *Bipolaris sorokiniana* (Lapis, 1985; Mann, 1992; Sarri, 1986; Saunder, 1988). Now it has been reported that spot blotch has expanded to wheat crop area under optimum and irrigated conditions. (Van Ginkel and Rajaram, 1997) as was observed in our studies in zones 9, 10 and 11 of NWFP varied in climatic condition where the similar conditions prevailed as reported by Van Ginkel and Rajaram, 1988. Similarly Singh and Kumar (2005) reported leaf blight of wheat caused by *Bipolaris sorokiniana* a destructive disease of wheat. The disease occurred in all 6 agro-climatic zones in India despite variable climates prevailed in these zones.

5.2.1 Two New Additions to Foliar Blights Pathogens of wheat in Pakistan (First Reports):

During the year 2005 two foliar blight pathogens were isolated which after reviewing the literature are the first reports in Pakistan.

a) *Colletotrichum graminicola* (Ces.) Wils.

The disease caused by *Colletotrichum graminicola* is known as Anthracnose. The disease occurs worldwide but damage reported in isolated areas where it reduces yield as much as 25% by affecting the plant vigor and grains (Wiese, 1998). The disease was found during survey (March, 2005) of foliar diseases of wheat in Punjab and NWFP.

b) *Dilophospora alopecuri* (Fr.) Fr. (Syn. *D. graminis* Desm, *Dilophia graminis* Fuckel)

This is a seed borne pathogen and causes leaf spot (twist) disease of wheat and other cereals. Previously disease was reported in United States, Cananda and caused minor losses in parts of Europe and later from India in 1961 (Wiese, 1998). Later the pathogen has been found in Ladakh district of Jammu and Kashmir (Dar *et al.*, 1995). The symptoms produced spots and leaf distortion (Wiese, 1998). These symptoms were noted during a survey (July, 2005) of foliar diseases of wheat in Gulmit Gojal, Hunza valley of Northern Areas. The incidence of the disease was very low (in traces). *Dilophospora alopecuri* was identified after visual and microscopic examination and in-vitro pathogenicity.

Another frequently isolated fungus in our study was *Alternaria alternata.* The results of primary studies on the isolation and identification of wheat black embryo in Xinjiang, China yielded many *Alternaria* spp. and *Bipolaris sorokiniana.* (Luan-Feng Gang *et al.*, 2004) However Singh and Srivastava, (1997) reported that the relative frequency of *Alternaria triticina* seems to be declining, possibly due to the availability of more resistant varieties. Most recently Singh *et al.* (2004) analyzed 88 samples of wheat and isolated *Alternaria triticina* from 12 samples which reflected 13% incidence of the disease due to two pathogens. *Bipolaris sorokiniana* is found to be major pathogenic foliar blight fungus in our studies. Therefore further investigations

were focused on eighty seven isolates. This pathogen is also reported as a principal fungus involved in the seedling blight and root rot in wheat in Pakistan. (Kishwar *et al.*, 1992). Other workers reported it in winter wheat because of high precipitation and high temperatures during the growing period of the season (Anonymous, 1986; Hafiz, 1986).

5.3 Standardization of Methodology for Conducting Pathogenicity

Among the five in-vitro methods, four were conducted in pots and one in test tubes. Out of five methods test tube moist cotton swab method was found the most effective and efficient method to conduct pathogenicity, aggressiveness analysis and in vitro screening. In our studies this method was specially compared with the method used by Lamari and Bernier (1989) in which the plants at seedling stage planted in pots needed to be sprayed with spore suspension with a particular concentration. For the maintenance of humidity the plants needed to be covered with plastic bags for 30 hours. Compared to the test tube method where there is no need to maintain humidity. In test tube method there is no need to water, because once the cotton in the tube has been moistened the maximum disease infection has been obtained after incubated at 25 ± 2^0C. The additional advantage of the test tube method is that we can observe the root infection in the same experiment. All the seed borne and soil borne fungi can be tested by this method.

5.4 Pathogenicity

During pathogenicity, all eighty seven isolates of *Bipolaris sorokiniana* of years 2004 and 2005 confirmed its pathogenic nature in addition to *Colletotrichum graminicola* (2, isolates) (Wiese, 1998) and *Dilophospora alopecuri* (I, isolate) (Dar *et al.*, 1995; Wiese, 1998). Other isolated fungi like *Alternaria alternata* (181 isolates), *Curvularia lunata* (5 isolates), *Epicoccum purpurascens* (13 isolates) , *Drechslera spicifer* (4 isolates) and one isolate each of *Drechslera rostrata, Stemphylium* sp. and *Cladosporium cladosporioides* were proved non-pathogenic to wheat in our studies. Among a number of fungi including *Bipolaris sorokiniana, Alternaria triticina, Alternaria alternata, Epicoccum purpurascens, Curvularia lunata, Penicillium* sp. and *Acremonium strictum* upon pathogenicity test only

Bipolaris sorokiniana and *Alternaria. triticina* were found pathogenic while the other mycoflora produced no symptoms (Singh *et al.*, 2004). In another study wheat samples collected from various agroclimatic regions in India; *Bipolaris sorokiniana* and *Alternaria triticina* were the major isolations in addition to *Alternaria alternata, Fusarium moniliforme, Epicoccum purpurascens* and *Penicillium* spp. Upon pathogencity test only *Bipolaris sorokiniana* and *Alternaria triticina* were found pathogenic under artificial inoculation test on wheat. (Mahto *et al.*, 2002). Singh *et al.*, (1998) found that *Bipolaris sorokiniana* and *Alternaria triticina* to be pathogenic among *Bipolaris sorokiniana, Helminthosporium spiciferum, Pyrenophora tritici-repentis, Alternaria alternata, Curvularia lunata* and *Alternaria triticina.* Whereas he observed that few isolates of *Helminthosporium spiciferum* and *Alternaria alternata* were pathogenic. As later in 2001-2002, 9 isolates of *Alternaria alternata* were found pathogenic to wheat (Iram and Ahmad, 2005). These pathogens are reported to be opportunistic or facultative in nature. They are encountered sporadically and are seldom important on national scale, but can cause severe losses in individual fields (Joshi *et al.*, 1986; Joshi *et al.*, 1978; Nema, 1986). Mahto *et al.*, (2002) conducted pathogenicity on these organisms and found them as saprophytes. One isolates of *Bipolaris sorokiniana* isolated each from oat and barley were also tested for pathogenecity on wheat and host plants as a part of epidemiological studies and in either case they were found pathogenic which confirmed the findings of Balogh *et al.*, (1991) who reported oat and barley as hosts for *Bipolaris sorokiniana*.

During in-vitro pathogenicity test *Colletotrichum graminicola* produced the identical symptoms on wheat plant as observed on original disease plant samples. Observations were consistent with others (Wiese, 1998). To our knowledge there is no previous report of *Colletotrichum graminicola* on wheat in Pakistan. The similar result was obtained during pathogenicity of *Dilophospora alopecuri* and observations were consistent with Wiese, 1998. Therefore both the fungi are the first reports (Iftikhar *et al.*, 2006) as members of leaf spotting complex (*Bipolaris sorokiniana, Pyrenophora tritici-repentis*) on wheat plant in wheat growing areas (Bhatti and Ilyas, 1986); Hafiz, 1986; Ali *et al.*, 2001).

5.5 Phenotypic Characteristics of *Bipolaris sorokiniana*

Bipolaris sorokiniana was characterized by measuring the size and by noting the shape and septation of the conidia of different isolates belonged to different ecologies. The conidia of isolates collected during 2004 were slightly curved with brown to olivaceous brown colour having septation ranged from 3-12. While the conidia collected in 2005 were dark brown coloured, slender and gently curved few are straight while few were light brown to brown coloured having septation ranged from 2-9. The dimensions of the conidia collection during 2004, ranged from 20-95µ, (mean length as 35-65µ), the width 10-30µ, (mean 13.3-25µ). While the conidia collected in 2005 the length ranged from 25-90µ, (mean 31-73µ), width 10-25µ, (mean 11.6-25µ).The other workers reported more or less the same measurements, colour and septation of the conidia like, Sivanesan and Holliday, (1981) reported the straight to curved conidia having 3-12 septation, olive brown measuring 40-120 x 17-28µ in length and width. While Barba *et al.* (2004) studied the morphology of *Bipolaris sorokiniana* on different substrates and found the conidia measuring 38 -9 2 x 15-21.9µ with 5-7 pseudosepta. In another study Luttrell, (1955) collected different isolates of *Bipolaris sorokiniana* from different hosts found the measurement varied in length 36-129µ, (mean 56-104µ), width 14-30µ, (means 17.7-23.5µ) with 2-12 septa.

The colour of the colony of both the year collection (2004 and 2005), exhibited four distinct colours. Among these the black colonies sporulate profusely and having suppressed type of growth. The black colour was due to the dense sporulation, the others showing grayish to brownish colour and few were of albino type exhibiting whitish growth indicating less sporulation. Our findings confirming the observations of Chand *et al.,* (2003), who studied the variability, in natural populations of the pathogen (*Bipolaris sorokiniana*) of wheat based on colony morphology, the isolates were classified into five groups. The majority (44.63%) of the isolates in the natural population was of black suppressed type. Whereas Maraite et al., (1997) observed the colour of the colonies on minimal medium varied from white to light pink and in few instances dark green while studying the 27 isolates of *Bipolaris sorokiniana.* The colony colour has a strong correlation with aggressiveness as Chand *et al.* (2003) found the black group was most aggressive and was identified as the epidemic

population. In our study most of the black coloured isolates were more aggressive on wheat variety Wafaq-2001 compared to other two varieties. Few isolates that were of black in colour showed aggressive behaviour also to other two varieties (Inqalib-91 and Bhakker-2001) in addition to Wafaq-2001. All white or albino type of isolates were indiscriminately slightly or least aggressive to all three varieties may be due to the reason of less conidial formation and slow sporulation rate, as the white isolates producing very few conidia.(Chand *et al.*, 2003).

5.6 Aggressiveness Analysis

The aggressiveness study was conducted only on the eighty seven isolates of *Bipolaris sorokiniana* based on understanding that this fungus is known pathogen of wheat and is prevalent in all the wheat growing areas surveyed. Although two isolates of *Colletotrichum graminicola* and one isolate of *Dilophospora alopecuri* were also found pathogenic but these were not included in aggressiveness analyses due to low incidence of these two fungi. Similarly the other fungi associated with foliage of wheat were found non-pathogenic. Hence were not studied further.

The aggressiveness data of eighty seven isolates of *Bipolaris sorokiniana*, was analyzed using analysis of variance test. For elaboration cluster analysis was done by constructing similarity dendrogram on combine mean values of all the isolates on three wheat varieties. The use of analysis of variance method determined the specificity of host and pathogen. However significant varieties x isolates interaction may occur in non-specific pathosystems, resulting from varieties x environment, isolate x environment and isolate x variety x environment interactions (Kulkarni and Chopra, 1982; Norwood *et al.*, 1984; Winer, 1984). Some factors are very important in aggressiveness tests like difference in microclimate, uniformity of inoculum coverage and condition of the inoculum (Peever *et al.*, 2002). These conditions were critically kept under consideration in the present investigation. Every time fresh inoculum by single spore culture was prepared and discs of the inoculum were taken from actively grown part of the culture and placement of inoculum near by seed vicinity. In the test tube method it was assumed that the microclimatic conditions prevailed in the test tube were alike which led to successful results.

It was observed that out of eighty seven isolates, belonging to different ecological zone (5,6,7,9,10 and 11) fifty seven isolates exhibited slightly aggressive, twenty seven moderately aggressive, two least aggressive and one aggressive behaviour. This picture of aggressiveness depicts that although the isolates collected from different ecologies, majority of the isolates exhibited slightly aggressive behaviour on three wheat varieties. As observed by Mikhailova *et al.* (2002) while studying the aggressiveness behaviour of 11 isolates of *B. sorokiniana* collected at different geographical location in Russia on 10 varieties of wheat showed significant difference in fungal strains behaviour. Whereas, Duveiller and Altamirano (2000) isolated 27 isolates of *Bipolaris sorokiniana* from roots, leaves and grains of spring wheat collected at a site in Mexico showed no clear difference between groups of isolates. They reported this behaviour of the fungus appeared as a continuum of isolates differing in aggressiveness while this work was conducted with isolates from a single site. Similar observations were recorded by Rasmussen *et al.,* (2002) that the stability of resistant genetic strains remained essential considering that *Bipolaris sorokiniana*, the principal pathogen forms a continuum of strains differing in aggressiveness. The different aggressive behaviour of the pathogen has no specific link between its aggressiveness and genotype (Maraite *et al.*, 1997) which has also been observed in our study. The pathogen collected from one genotype gave indifferent results on different genotypes including the original one. This is supported by the findings of (Maraite *et al.*, 1997) who collected the isolates of *Bipolaris sorokiniana* from background of one genotype and when inoculated these isolates on a wide range of genotypes under controlled conditions found also different results.

In another perspective if we compare the severity of isolates exhibiting aggressive reaction in-vitro to that observed in field it also varied. Taking the example of P2-9 isolate its severity in the farmer's field was 2 compared to in-vitro study in which it exhibited aggressive reaction rated to 4. This may be due to the effect of variable environmental conditions and inoculum pressure, which reflected the different disease severity in controlled and field conditions as observed by Jain and Prabhu (1976) who narrated that, for the success of an individual isolate there is a need of its interaction with the environment and the inoculum pressure.

On the other hand the two least aggressive isolates G5-3 and NP3-29 were Albino brownish type in colour respectively. As already reported by Chand *et al.*, (2003) that the isolates with low frequency having white colour produces less conidia, showed low aggressive behaviour. This is also in consistence with our studies.

5.7 Epidemiological Studies

The epidemiological study was focused on, to see the effect of three temperature regimes on the radial myclial growth of *Bipolaris sorokiniana* with the objective to find out the most suitable temperature for its growth. In addition, assessment of foliar blight in rice-wheat area, in the wheat crop sown after rice and host other then wheat of the fungus *Bipolaris sorokiniana* was studied.

5.7.1 The effect of Different temperature Regimes on the radial mycelial growth of *Bipolaris sorokiniana*

The isolates collected from different ecologies were selected on the basis of their aggressiveness reaction (aggressive to moderately aggressive and least aggressive reaction) on three wheat varieties representing all the zones of Punjab and NWFP.

The results revealed that 20°C is not at all suitable temperature for any of the isolates. The maximum growth (86.9mm) of isolate P4-28 was recorded after 12 days interval at 25°C in contrast to 75mm and 67.2 mm at 30°C and 20°C respectively. Our findings are inconsistent with Xiao *et al.*, 1997 who reported that the temperature between 23 to 25.8°C is the most suitable for *Bipolaris sorokiniana* and it becomes problem for wheat production in China. This was one of the reason that in our experiment we did not go beyond 30°C and below 20°C.Akram and Amerika singh, (2003) observed that there was high disease severity in Taria region of India when the temperature 27.6°C with r.h 86.4% and 37.4mm rains occur compared to the period when temperature ranged from 12°C to 31.8°C. Similar observations were recorded by Maraite *et al.* (1997) who noted the slow growth of different isolates of *Bipolaris sorokiniana* from world collection at 20°C. If we correlate this effect of temperature with the disease development in the field and that of incubation we followed in our experiments all were incubated at 25 °C.Singh and Tewari, (2001) found the most favorable temperature for the rapid disease development due to *Bipolaris sorokiniana*

ranged between 26.8 to 28.5°C. Similar correlation is evident as observed during phase 1 seedling stage survey might be due to unfavourable climatic conditions. The mean temperature in the wheat production zones ranged from 14.9 to 17.3^{0}C with the average rain from 5.5 to 52.2 mm (Meteorological Deptt. Islamabad, 2005) during the survey at seedling stage which was not conducive for the establishment of foliar disease.

5.8 Host of *Bipolaris sorokinaina* other than Wheat.

During our studies among fifteen hosts plant studied to see the host of *Bipolaris sorokinaina* other than wheat, it was found that *Oryza sativa, Halianthus annus, Arachis hypogea* and *Cicer arientenum* were found to be the non host of *Bipolaris sorokinaina*.These results were found in contradictory to the findings of Bakonyi., 1997 who found, *Halianthus annus* and *Cicer arientenum* as the secondary hosts for this fungus. Whereas the *Oryza sativa* as already reported by Duveiller and Gilchrist, 1994 that the spot blotch may be present in small amount on rice even though it is basically a non-host of the disease. The rice may then serve as a "green bridge" to the subsequent wheat crop. However it was observed that although the fungus was found to grow on the host crop plants it also acted as a weak parasite, reflected in the contrasting symptoms it produced in the wheat plant. Zillinsky, 1983 reported *Bipolaris sorokinaina* affects small grain cereals and a wide variety of other grasses act as a major potential hosts, while it can affect many other hosts but acts as a weak parasite.

5.9 Assessment of foliar blight in different production technologies

The rice wheat cropping system in different parts of the world is reported to be responsible for the increase of foliar blight, particularly with reference to *Bipolaris sorokiniana*. Diman *et al.* (1994) reported the expansion of spot blotch in moderate and temperate regions of irrigated rice-wheat producing areas. In these rice wheat growing areas, the spot blotch may be present in small amount on rice even though it is basically a non-host of the disease. The rice may then serve as a "green bridge" to the subsequent wheat crop (Duveiller and Gilchrist, 1994). In particular, rice-wheat rotations in which zero or minimum tillage is adopted this disease has increased

(Saunders, 1989). In our studies total 10 isolates of *Bipolaris sorokiniana* (2 isolates, during 2004 and 8 isolates, during 2005 were isolated in the rice wheat area. The rice wheat area was surveyed especially with emphasis to assess prevalence of different foliar blight on wheat crop sown after rice, where different production technologies are practiced. The results of both the years in rice-wheat area revealed that maximum number of isolates of *Bipolaris sorokiniana* were isolated from conventional followed by zero tillage practice as compared to bed planting. Where no isolation of fungus *Bipolaris sorokiniana* was made. Less or more similar trend has been observed by Sutton and Vyn, (1990) who contradict with the results and findings of other researchers and found higher disease levels in conservation and zero tillage fields. Whereas Kuroswski *et al.*, 2005 studied the effects of tillage systems and found no significant influence on the occurrence of both types of net blotch. There was found insignificant difference in the intensity of leaf blotch On the contrary Bailey *et al.*, (2001)while studying the effect of tillage and plant diseases, its severity and prevalence on spring wheat and other crops with zero tillage and conventional tillage in Indian, Head SK from 1992 to 1995 found unchanged severity of leaf spot. They found *Bipolaris sorokiniana* was lower under both type tillage system and *Fusarium* spp were higher under zero tillage than conventional. In our finding if we consider the prevalence of *Bipolaris sorokiniana* (being the pathogenic fungus) then 'bed planting' would seem to be the most suitable practice to adopt in rice-wheat cropping systems.

CONCLUSIONS

The following conclusions have been drawn from the studies conducted on pathobiology of foliar blight of wheat in the Punjab and NWFP areas of Pakistan.

- The most suitable stage of survey to assess the distribution of diseases and collection of foliar blight diseased samples of wheat was the booting stage of the crop in different agro ecological zones of NWFP and Punjab.
- A high prevalence of foliar blight was observed in zones (5& 6) in Punjab and zone (9) in NWFP.
- The highest incidence of foliar blight was observed in the year 2005 probably due to the weather conditions that year.
- *Bipolaris sorokiniana* (leaf blotch) was found the most dominant pathogen causing foliar blight in all agro ecological zones. (5, 6, 7,9,10 and 11).
- Two fungi namely *Colletotrichum graminicola* and *Dilaphospora alopecuri* were identified first time as a foliar blight causing pathogens on wheat in Pakistan.
- The in-vitro tests tube moist cotton swab method was standardised for pathogenicity test and aggressiveness analysis of the pathogen.
- The phenotypic characteristic showed that the aggressive isolates were of black coloured with suppressed type of colony growth and the least aggressive one were white albino with fluffy colony growth.
- P2-9 was identified to be an aggressive isolate on three commercial wheat varieties (Wafaq-2001, Inqlab 91 and Bhakhar 2001).
- The most conducive temperature for pathogen (*Biploris sorokiniana*) growth was 25°C.
- Out of the three technologies studied (the bed planting, zero tillage and conventional) followed in rice-wheat cropping system for wheat crop sown after

rice. The bed planting sowing technology was found to be most suitable one on minimize the pathogen, causing foliar blight.

- *Oryza sativa, Halianthus annus, Arachis hypogea* and *Cicer arientenum* were found the non hosts of *Bipolaris sorokiniana* while in addition to wheat the crop plants (*Brassica compestris, Glycine max, Lens culinaris, Vigna radiata, Sesamum indicum, Vigna mungo, Sorghum bicolor, Zea mays, Avena sativa,Hordeum vulgare* and *Panicum maximum*) were found to be the hosts of *Bipolaris sorokiniana*. However it was observed that although the fungus was found to grow on the host crop plants it also acted as a weak parasite, reflected in the contrasting symptoms it produced in the wheat plant.

Researchable Issues:

- The effect of a range cultivation techniques on soil borne pathogen (*Bipolaris sorokiniana*) followed in the rice wheat requires further studies.
- Relationship of *Pyrenophora tritici-repentis* and *Bipolaris sorokiniana* needed further investigation.
- Interaction of foliar blight disease with wheat rust and powdery mildew also need to be studied.

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APPENDICES

Appendix 1: Incidence, Severity and Disease index of different fungi at seedling stage in different wheat growing agro-ecological zones of Punjab during 2004.

Zone	Location	Districts	Fungus	Incidence%	Severity (0-5)	Disease Index%
7	Rawat	Rawalpindi	*Alternaria alternata*	0	0	0
7	Shani Sheralam	Rawalpindi	*Alternaria alternata*	0	0	0
7	Mandera	Rawalpindi	0	0	0	0
7	Nattiakalam	Rawalpindi	0	0	0	0
7	Rama	Rawalpindi	0	0	0	0
7	Gujar Khan	Rawalpindi	0	0	0	0
7	Naran Mughlan	Rawalpindi	*Alternaria alternata*	0	0	0
7	Kalas	Rawalpindi	0	0	0	0
7	Taxila	Rawalpindi	0	0	0	0
7	Wah Cantt	Rawalpindi	*Bipolaris sorokiniana*	10	1	2
7	Hassan Abdal	Rawalpindi	*Bipolaris sorokiniana*	10	1	2
7	Fateh Jang	Attock	0	0	0	0
7	Jand	Attock	0	0	0	0
7	Attock	Attock	0	0	0	0
7	Chakwal	Chakwal	0	0	0	0
7	Dhudial	Chakwal	0	0	0	0
7	Chah Umran	Chakwal	0	0	0	0
7	Chak Baqar Shah	Chakwal	0	0	0	0
7	Doman	Chakwal	0	0	0	0
7	Talagang	Chakwal	*Alternaria alternata*	10	1	2
7	Sarai Alamgir	Jehlum	*Alternaria alternata*	10	1	2
7	Jehlum	Jehlum	*Alternaria alternata*	10	1	2
7	Dina	Jehlum	*Alternaria alternata*	10	1	2
7	Kachian	Khushab	*Alternaria alternata*	10	1	2
7	Kattha	Khushab	0	0	0	0
7	Sahu Wala	Khushab	0	0	0	0
7	Khushab	Khushab	0	0	0	0
7	Sargodha	Sargodha	0	0	0	0
7	Sargodha by pass	Sargodha	0	0	0	0
7	Bonjha Minhas	Sargodha	0	0	0	0
7	Danda Wala	Sargodha	*Bipolaris sorokiniana*	10	2	4
7	Chiniot	Jhang	0	0	0	0
7	Chanab Nagar	Jhang	*Alternaria alternata*	10	1	2

7	Dhok Chashma	Mianwali	0	0	0	0
7	Magian Kanhar	Mianwali	0	0	0	0
7	Dhok Pathan	Mianwali	0	0	0	0
7	Majju Chak	Gujranwala	0	0	0	0
7	Tahrain Wala	Gujranwala	0	0	0	0
7	Gujranwala	Gujranwala	0	0	0	0
7	Wazirabad	Gujranwala	0	0	0	0
7	Sialkot	Sialkot	0	0	0	0
7	Daska	Sialkot	0	0	0	0
7	Pasroor	Sialkot	0	0	0	0
7	Sarian Wali	Sialkot	0	0	0	0
7	Gujrat	Gujrat	0	0	0	0
7	Lala Musa	Gujrat	0	0	0	0
7	Kharian	Gujrat	0	0	0	0
6	Muridke	Sheikhupura	0	0	0	0
6	Sheikhupura	Sheikhupura	0	0	0	0
6	Gojra More	T.T.Singh	*Curvularia lunata*	10	1	2
6	Kot Sahi Nagar	Faisalabad	0	0	0	0
6	Faisalabad	Faisalabad	*Bipolaris sorokiniana*	10	2	4
6	Satiana	Faisalabad	0	0	0	0
6	Tandlianwala	Faisalabad	0	0	0	0
6	Lahore	Lahore	*Alternaria alternata*	10	2	4
6	Thokar Niaz Baig	Lahore	*Alternaria alternata*	10	1	2
6	Pattoki	Kasur	*Epicoccum purpurascens*	10	1	2
6	Manga Mandi	Kasur	*Epicoccum purpurascens*	10	1	2
6	Kore Shah	Okara	*Epicoccum purpurascens*	10	1	2
6	Gogera Bangla	Okara	0	0	0	0
6	Okara	Okara	*Alternaria alternata*	10	1	2
6	Sahiwal	Sahiwal	0	0	0	0
6	Radharam	Sahiwal	0	0	0	0

Appendix 2: Incidence, Severity and Disease index of different fungi at seedling stage in different wheat growing agro-ecological zones of NWFP during 2004.

Zone	Location	Districts	Fungus	Incidence%	Severity (0-5)	Disease Index%
9	Akora Khattak	Nowshera	*Bipolaris sorokiniana*	10	1	2
9	Risal Pur	Nowshera	0	0	0	0
9	Aza Khail	Nowshera	0	0	0	0
9	Jahangira	Nowshera	*Curvularia lunata*	10	1	2
9	Mardan	Mardan	*Epicoccum purpurascens*	10	1	2
9	Gujar Garhi	Mardan	*Epicoccum purpurascens*	10	1	2
9	Takht Bai	Mardan	*Alternaria alternata*	10	1	2
9	UAP	Peshawar	0	0	0	0
9	Pabbi	Peshawar	0	0	0	0
9	Suffaid Dri	Peshawar	0	0	0	0
9	Charsadda	Charsadda	*Alternaria alternata*	10	1	2
9	Shahal Alampur	Charsadda	*Alternaria alternata*	10	1	2
9	Tangi	Charsadda	0	0	0	0
9	Umerzai	Charsadda	0	0	0	0
9	Mattoini	Kohat	0	0	0	0
9	Kohat	Kohat	*Bipolaris sorokiniana*	10	1	2
9	Gombut	Kohat	0	0	0	0
9	Khushal Garh	Kohat	0	0	0	0
10	Jalala	Malakand	0	0	0	0
10	Dargai	Malakand	0	0	0	0
10	Sakhakot	Malakand	0	0	0	0
10	But Khela	Malakand	*Drechslera spicifer*	10	1	2
10	Gagota	Mingora	*Drechslera spicifer*	10	1	2
10	Landakai	Mingora	0	0	0	0
10	Kota	Mingora	*Curvularia lunata*	10	1	2
10	Abuha	Mingora	*Curvularia lunata*	10	1	2
10	Gurati	Mingora	*Epicoccum purpurascens*	10	1	2
10	Mingora	Mingora	*Epicoccum purpurascens*	10	1	2
10	Sangate	Mingora	*Epicoccum purpurascens*	10	1	2

Appendix 3: Incidence, Severity and Disease index of different fungi at booting stage in different wheat growing agro-ecological zones of Punjab during 2004.

Zone	Location	Districts	Fungus	Incidence%	Severity (0-5)	Disease Index%
5	Kabirwala	Khanewal	*Alternaria alternata*	50	1	4
5	Jahanian	Khanewal	*Bipolaris sorokiniana*	20	1	6
5	Khanewal	Khanewal	*Bipolaris sorokiniana*	60	2	10
5	Mian Channun	Khanewal	*Alternaria alternata*	10	1	2
5	Dera Tooro	Khanewal	*Alternaria alternata*	10	1	2
5	Duniapur	Lodhran	*Alternaria alternata*	10	1	4
5	Lodhran	Lodhran	*Alternaria alternata*	10	1	2
5	But Kot	Bahawalpur	0	0	0	0
5	Bahawalpur	Bahawalpur	*Alternaria alternata*	20	1	2
6	Chiniot	Jhang	*Bipolaris sorokiniana*	40	1	2
6	Chenab Nagar	Jhang	*Drechslera spicifer*	10	1	2
6	Manchuwala	Jhang	*Epicoccum Purpurascens*	10	1	2
6	Degree Sherge	Jhang	*Alternaria alternata*	50	2	4
6	Chak Noor	Jhang	*Alternaria alternata*	10	1	2
6	Shor Kot	Jhang	*Bipolaris sorokiniana*	30	3	18
6	Faisalabad UAF	Faisalabad	*Epicoccum purpurascens*	10	1	2
6	Faisalabad	Faisalabad	*Bipolaris sorokiniana*	20	3	50
6	Satiana	Faisalabad	*0*	0	0	0
6	Tandlian Wala	Faisalabad	*Alternaria alternata*	10	1	2
6	Dallowal	Faisalabad	*Bipolaris sorokiniana*	10	1	6
6	Gojra More	T.T. Singh	*Alternaria alternata*	10	1	2
6	Kot Sahi Nagar	T.T. Singh	0	0	0	0
6	Chak 345 JB	T.T. Singh	*Alternaria alternata*	10	1	4
6	Lahore	Lahore	*Alternaria alternata*	10	1	2
6	Thokar Niaz Baig	Lahore	*Alternaria alternata*	10	1	2
6	Sheikhupura	Sheikhupura	*Bipolaris sorokiniana*	10	1	2
6	Muridke	Sheikhupura	*Alternaria alternata*	10	1	2
6	Killar Pind	Sheikhupura	*0*	*0*	*0*	*0*
6	Manga Mandi	Kasur	*Alternaria alternata*	10	1	2
6	Pattoki	Kasur	*Alternaria alternata*	10	1	2
6	Okara	Okara	0	0	0	0
6	Kore Shah	Okara	0	0	0	0
6	Gogera Bangla	Okara	*Alternaria alternata*	10	1	2
6	Sahiwal	Sahiwal	*Bipolaris sorokiniana*	30	1	6
6	Chicha Watni	Sahiwal	*Alternaria alternata*	10	1	2
6	Harrapa	Sahiwal	*Alternaria alternata*	30	1	4
6	Radharam	Sahiwal	*0*	0	0	0
7	Sohawa	Rawalpindi	*0*	0	0	0
7	Taraki	Rawalpindi	*0*	0	0	0
7	Gujar Khan	Rawalpindi	*0*	0	0	0
7	Mandra	Rawalpindi	*0*	0	0	0
7	Rawat	Rawalpindi	*0*	0	0	0
7	Dina	Jehlum	*0*	0	0	0
7	Jehlum	Jehlum	*0*	0	0	0

7	Sarai Alamgir	Jehlum	*0*	0	0	0
7	Lala Musa	Gujrat	*0*	0	0	0
7	Gujrat	Gujrat	*0*	0	0	0
7	Kharian	Gujrat	*0*	0	0	0
7	Qiladidar Singh	Gujranwala	*Alternaria alternata*	10	1	2
7	Gujranwala	Gujranwala	*Bipolaris sorokiniana*	20	1	2
7	Wazirabad	Gujranwala	*0*	*0*	*0*	*0*
7	Tilla wala	Gujranwala	*Alternaria alternata*	10	1	2
7	Keyella	Hafizabad	*Alternaria alternata*	10	1	2
7	Hafizabad	Hafizabad	*Bipolaris sorokiniana*	10	1	2
7	Ottianwala	Hafizabad	*Alternaria alternata*	20	1	2
7	Jarianda	Hafizabad	*Alternaria alternata*	10	2	4
7	Daska	Sialkot	0	0	0	0
7	Sialkot	Sialkot	0	0	0	0
7	Pasroor	Sialkot	*Bipolaris sorokiniana*	10	1	2
7	Kotti Bawaj	Sialkot	*Alternaria alternata*	10	1	2
7	Badiana	Sialkot	*Bipolaris sorokiniana*	20	2	4
7	Dhudial	Norowal	*Alternaria alternata*	10	1	2
7	Jattli	Chakwal	*0*	*0*	*0*	*0*
7	Dhudial	Chakwal	*0*	*0*	*0*	*0*
7	Chakwal	Chakwal	*0*	*0*	*0*	*0*
7	Talagang	Chakwal	*0*	*0*	*0*	*0*
7	Khushab	Khushab	*0*	*0*	*0*	*0*
7	Kachina	Khushab	*Alternaria alternata*	10	1	2
7	Kattha	Khushab	*0*	*0*	*0*	*0*
7	Sahu Wala	Khushab	*0*	*0*	*0*	*0*
7	Sargodha	Sargodha	*Bipolaris sorokiniana*	*20*	*1*	*2*
7	Sargodha Bypass	Sargodha	*Alternaria alternata*	10	1	2
7	Bonjha Minhas	Sargodha	*Alternaria alternata*	10	1	2
7	Danda Wala	Sargodha	*0*	*0*	*0*	*0*
7	Bhalwal	Sargodha	*Bipolaris sorokiniana*	*10*	*2*	*4*

Appendix 4: Incidence, Severity and Disease index of different fungi at booting stage in different wheat growing agro-ecological zones of NWFP during 2004.

Zone	Location	Districts	Fungus	Incidence%	Severity (0-5)	Disease Index%
9	Akora Khattak	Nowshera	*Bipolaris sorokiniana*	80	2	12
9	Risal Pur	Nowshera	*0*	0	0	0
9	Aza Khail	Nowshera	*Alternaria alternata*	40	1	6
9	Jahangira	Nowshera	*Alternaria alternata*	20	1	4
9	Sheran Kot	Nowshera	*Alternaria alternata*	20	1	2
9	Mardan	Mardan	*Bipolaris sorokiniana*	90	3	26
9	Sirdary	Mardan	*Alternaria alternata*	10	1	26
9	Gujar Garhi	Mardan	*Alternaria alternata*	20	1	4
9	Takht Bai	Mardan	*Alternaria alternata*	20	1	4
9	Charsadda	Charsadda	*Bipolaris sorokiniana*	80	2	16
9	Shahalampur	Charsadda	*Alternaria alternata*	20	1	4
9	Tangi	Charsadda	*Alternaria alternata*	10	1	2
9	Umerzai	Charsadda	0	0	0	0
9	Gulbilala	Charsadda	*Alternaria alternata*	10	1	4
9	Peshawar	Peshawar	*Alternaria alternata*	10	1	2
9	Pabbi	Peshawar	*Bipolaris sorokiniana*	60	1	2
9	Suffaid Dri	Peshawar	0	0	0	0
9	Aman Garh	Peshawar	*Alternaria alternata*	10	1	2
9	Sirdiab	Peshawar	*Bipolaris sorokiniana*	90	3	20
9	Mattoni	Kohat	0	0	0	0
9	Kohat	Kohat	*Bipolaris sorokiniana*	50	2	6
9	Gombut	Kohat	*Alternaria alternata*	10	2	2
9	Khushalgarh	Kohat	*Bipolaris sorokiniana*	10	1	4
9	Gundiliopayan	Kohat	0	0	0	0
9	Gondiala	Kohat	*Alternaria alternata*	10	1	4
10	Jalala	Malakand	*Alternaria alternata*	20	1	4
10	Dargai	Malakand	*Bipolaris sorokiniana*	60	1	4
10	Sakhakot	Malakand	*Alternaria alternata*	10	1	4
10	Batkhela	Malakand	*Alternaria*	10	1	4

			alternata			
10	Batkhela 1	Mingora	*Alternaria alternata*	10	1	2
10	Batkhela 2	Mingora	0	0	0	0
10	Shorgarh	Mingora	*Alternaria alternata*	10	1	2
10	Dippo	Mingora	0	0	0	0
10	Abboha	Mingora	0	0	0	0
10	Ghanam	Mingora	0	0	0	0
10	Tariq Abad	Mingora	*Curvularia lunata*	10	1	2
10	Khundke	Mingora	0	0	0	0
10	Cashghe	Mingora	0	0	0	0
10	Khota	Mingora	*Drechslera spicifer*	10	1	2
10	Ghamghari	Mingora	0	0	0	0
10	Kalam	Mingora	*Epicoccum purpurascens*	10	1	2
10	Bahrain	Mingora	*Epicoccum purpurascens*	10	1	2
10	Satamadyan	Mingora	*Alternaria alternata*	10	1	2
10	Madyan 1	Mingora	*Alternaria alternata*	10	1	2
10	Madyan 2	Mingora	*Alternaria alternata*	10	1	2
10	Gharhi	Mingora	*Bipolaris sorokiniana*	20	1	4
10	Parhatali	Mingora	*Alternaria alternata*	10	1	2
10	Narinalike	Mingora	*Alternaria alternata*	10	1	2
10	Sheen	Mingora	*Alternaria alternata*	10	1	2
10	Dilburabad	Mingora	*Alternaria alternata*	10	1	2
10	Suhdara	Mingora	*Bipolaris sorokiniana*	100	1	12
10	Badargam	Mingora	*Alternaria alternata*	10	1	4
10	Janabad	Mingora	*Alternaria alternata*	10	1	2
10	Bechophoka	Mingora	*Alternaria alternata*	10	1	2
10	Bumain	Mingora	*Alternaria alternata*	10	1	2
10	Kotkala	Mingora	*Bipolaris sorokiniana*	10	1	2
10	Bahikhela	Mingora	*Bipolaris sorokiniana*	20	1	2
10	Pirchala	Mingora	0	0	0	0
10	Biakola	Mingora	*Bipolaris sorokiniana*	10	1	2
11	Sasi	Skardu	*Alternaria alternata*	10	1	2
11	Shatate	Skardu	*Bipolaris sorokiniana*	20	1	4
11	Shamgarh	Skardu	*Alternaria alternata*	10	1	2

11	Randoo	Skardu	*Alternaria alternata*	10	1	2
11	Khumbobaba	Skardu	*Alternaria alternata*	10	1	2
11	Baghida	Skardu	*Bipolaris sorokiniana*	20	1	4
11	Tangus	Skardu	*Alternaria alternate*	10	1	2
11	Surray	Skardu	*Alternaria alternata*	10	1	2
11	Surdas	Skardu	*Alternaria alternata*	10	1	2
11	Kachina	Skardu	*Alternaria alternata*	10	1	2
11	Shiger	Skardu	*Alternaria alternata*	10	1	2
11	Churkashing	Skardu	*Alternaria alternata*	10	1	2
11	Banazir Chowk	Skardu	*Alternaria alternata*	10	1	2
11	Huttu	Skardu	*Alternaria alternata*	10	1	2
11	Skardu	Skardu	*Alternaria alternata*	10	1	2
11	Chargha	Skardu	*Alternaria alternata*	10	1	2
11	Hassanabad	Skardu	*Bipolaris sorokiniana*	20	1	4
11	Thurga	Skardu	*Alternaria alternata*	10	1	2
11	Golla	Skardu	*Alternaria alternata*	10	1	2
11	Ghonai	Skardu	*Alternaria alternata*	10	1	2
11	Yogho	Skardu	*Alternaria alternata*	10	1	2
11	Braka	Skardu	*Alternaria alternata*	10	1	2
11	Kirin	Skardu	*Alternaria alternata*	10	1	2
11	Kachora	Skardu	*Bipolaris sorokiniana*	10	1	4

Appendix 5: Incidence, Severity and Disease index of different fungi at booting stage in different wheat growing agro-ecological zones of Punjab during 2005.

Zone	Location	Districts	Fungus	Incidence%	Severity (0-5)	Disease Index%
5	Kabirwala	Khanewal	*Alternaria alternata*	20	1	2
5	Khanewal	Khanewal	*Bipolaris sorokiniana*	80	2	10
5	Kacha Khuh	Khanewal	*Bipolaris sorokiniana*	50	1	4
5	Mian Channun	Khanewal	*Bipolaris sorokiniana*	80	2	20
5	Jahanian	Khanewal	*Alternaria alternata*	80	1	10
5	Iqbal Nagar	Khanewal	*Alternaria alternata*	80	2	20
5	Lar	Bahawalpur	*Alternaria alternate*	50	1	6
5	Butkot	Bahawalpur	*Alternaria alternata*	50	1	8
5	Bahawalpur	Bahawalpur	*Bipolaris sorokiniana*	50	1	10
5	Lodhran	Lodhran	*Bipolaris sorokiniana*	80	2	28
5	Duniapur	Lodhran	*Bipolaris sorokiniana*	50	1	2
5	Chak P114	Multan	*Alternaria alternata*	40	1	2
5	Multan	Multan	*Bipolaris sorokiniana*	50	2	6
5	Ahmedpur	Muzaffar Garh	*Bipolaris sorokiniana*	60	2	12
5	Muhammad Wala	Muzaffar Garh	*Bipolaris sorokiniana*	70	2	20
5	Muzaffar Garh	Muzaffar Garh	*Bipolaris sorokiniana*	60	2	20
6	Chiniot	Jhang	*Bipolaris sorokiniana*	60	2	10
6	Chenab Nagar	Jhang	*Alternaria alternata*	30	1	2
6	Mancha Wala	Jhang	*Bipolaris sorokiniana*	90	2	24
6	Mallhu More	Jhang	*Bipolaris sorokiniana*	100	3	58
6	Dergie Shergee	Jhang	*Bipolaris sorokiniana*	70	2	20
6	Chak Noor	Jhang	*Alternaria alternata*	60	2	4
6	Shorkot	Jhang	*Alternaria alternata*	50	1	2
6	UAF	Faisalabd	*Alternaria alternata*	10	1	2
6	Faisalabad	Faisalabd	*Bipolaris sorokiniana*	90	3	56
6	Dallowal	Faisalabd	*Alternaria alternata*	10	1	4

6	Satiana	Faisalabd	*Alternaria alternata*	10	1	2
6	Tandlianwala	Faisalabd	*Alternaria alternata*	20	1	2
6	Gojra More	TTSingh	*Alternaria alternata*	10	1	2
6	Kot Sahi Nagar	TTSingh	*Alternaria alternata*	10	1	2
6	Chak No 345	TTSingh	*Alternaria alternata*	10	1	2
6	Lahore	Lahore	*Alternaria alternata*	10	1	2
6	Thokar Niaz Baig	Lahore	*Alternaria alternata*	10	1	
6	Kalashahkaku	Lahore	*Alternaria alternata*	10	1	2
6	Kalashahkaku 2	Lahore	*Alternaria alternata*	40	2	8
6	Kalashahkaku 3	Lahore	*Stemphylium Sp.*	10	1	2
6	Kalshahkaku 4	Lahore	*Drechslera rostata*	20	1	10
6	Mangamandi	Kausar	*Bipolaris sorokiniana*	80	2	14
6	Pattoki	Kausar	*Bipolaris sorokiniana*	80	3	42
6	Phool Nagar	Kausar	*Bipolaris sorokiniana*	80	3	24
6	Okara	Okara	*Bipolaris sorokiniana*	90	2	20
6	Kore Shah	Okara	*Alternaria alternata*	10	1	2
6	Gogera Bangla	Okara	*Alternaria alternata*	20	2	4
6	Sahiwal	Sahiwal	*Bipolaris sorokiniana*	80	2	18
6	Chicha Watni	Sahiwal	*Alternaria alternata*	10	1	4
6	Harrapa	Sahiwal	*Alternaria alternata*	60	1	4
6	Radharam	Sahiwal	*Alternaria alternata*	20	1	2
6	Pakpattan	Pakpatan	*Alternaria alternata*	*20*	*1*	*4*
7	Nattiakalam	Rawalpindi	*0*	*0*	*0*	*0*
7	Rama	Rawalpindi	*0*	*0*	*0*	*0*
7	Gujar Khan	Rawalpindi	*0*	*0*	*0*	*0*
7	Naran Mughlan	Rawalpindi	*0*	*0*	*0*	*0*
7	Kalas	Rawalpindi	*0*	*0*	*0*	*0*
7	Taxila	Rawalpindi	*Alternaria alternata*	*10*	*1*	*2*
7	Wah Cantt	Rawalpindi	*Alternaria alternata*	*10*	*1*	*2*
7	Hassan Abdal	Rawalpindi	*Alternaria alternata*	*10*	*1*	*2*
7	Fateh Jang	Attock	*Alternaria alternata*	*10*	*1*	*2*

7	Jand	Attock	*Alternaria alternata*	*10*	*1*	*2*
7	Attock	Attock	*Alternaria alternata*	*10*	*1*	*2*
7	Jattli	Chakwal	*Bipolaris sorokiniana*	*80*	*3*	*58*
7	Dhudial	Chakwal	*Bipolaris sorokiniana*	*90*	*2*	*18*
7	Dhudial	Chakwal	***Colletotrichum graminicola***	*10*	*1*	*2*
7	Chakwal	Chakwal	*Alternaria alternata*	*100*	*2*	*20*
7	Chahumra	Chakwal	*Alternaria alternata*	*70*	*2*	*20*
7	Chak Baqer Shah	Chakwal	*Alternaria alternata*	*60*	*1*	*6*
7	Doman	Chakwal	*Alternaria alternata*	*50*	*1*	*6*
7	Walana	Chakwal	*Alternaria alternate*	*70*	*1*	*2*
7	Walana	Chakwal	*Epicoccum purpurascens*	*10*	*1*	*2*
7	Talagang	Chakwal	*Alternaria alternata*	*60*	*1*	*2*
7	Chinji	Khushab	*Alternaria alternata*	*50*	*1*	*2*
7	Kattha Sakral	Khushab	*Alternaria alternata*	*40*	*1*	*2*
7	Khushab	Khushab	*Alternaria alternata*	*40*	*1*	*2*
7	Khushab	Khushab	*Cladosporium cladosporioides*	*10*	*1*	*2*
7	Taji Sharif	Khushab	*Alternaria alternata*	*40*	*1*	*2*
7	Kachina	Khushab	*Alternaria alternata*	*40*	*1*	*2*
7	Sohowala	Khushab	*Alternaria alternata*	*40*	*1*	*2*
7	Sargodha	Sargodha	*Alternaria alternata*	*90*	*2*	*20*
7	Sargodha 2	Sargodha	*Alternaria alternata*	*40*	*1*	*2*
7	Bonjha Minhas	Sargodha	*Alternaria alternata*	*30*	*1*	*2*
7	Dandawala	Sargodha	*Alternaria alternata*	*20*	*1*	*2*
7	Bhalwal	Sargodha	*Alternaria alternata*	*20*	*1*	*2*
7	Ottian	Hafizabad	*Alternaria alternata*	*20*	*1*	*6*
7	Tarianda	Hafizabad	*Alternaria alternata*	*10*	*1*	*2*
7	Jabraan	Hafizabad	*Bipolaris sorokiniana*	*20*	*1*	*4*
7	Attawa	Hafizabad	*Bipolaris sorokiniana*	*20*	*1*	*4*
7	Kelleye	Hafizabad	*Alternaria alternata*	*10*	*1*	*2*
7	Kot Lodha	Hafizabad	*Alternaria*	*10*	*1*	*2*

			alternata			
7	Dera Sandian	Sialkot	*Alternaria alternata*	*10*	*2*	*16*
7	Badiana	Sialkot	*Bipolaris sorokiniana*	*20*	*1*	*6*
7	Dusapur	Sialkot	*Alternaria alternata*	*10*	*1*	*4*
7	Nazim Abad	Sialkot	*Alternaria alternata*	*10*	*1*	*4*
7	Killar Pind	Sialkot	*Alternaria alternata*	*10*	*1*	*2*
7	Pasroor	Sialkot	*Alternaria alternata*	*20*	*1*	*4*
7	Kottibawaj	Sialkot	*Alternaria alternata*	*10*	*1*	*2*
7	Dhudial	Narowal	*Bipolaris sorokiniana*	*20*	*2*	*4*
7	Galoti Morr	Gujranwala	*Alternaria alternate*	*10*	*1*	*2*
7	Qazi Kot	Gujranwala	*Alternaria alternata*	*10*	*1*	*2*
7	Aiman Abad	Gujranwala	*Alternaria alternata*	*10*	*1*	*2*
7	Killa Didar Singh	Gujranwala	*Alternaria alternata*	*10*	*1*	*2*
7	Gujranwala	Gujranwala	*Alternaria alternata*	*10*	*1*	*2*
7	Tella Wala	Gujranwala	*Alternaria alternata*	*10*	*1*	*2*
7	Sheikhupura	Sheikhupura	*Bipolaris sorokiniana*	*20*	*1*	*4*
7	Khanpur Mallian	Sheikhupura	*Alternaria alternata*	*10*	*1*	*2*
7	Muridke	Sheikhupura	*Alternaria alternata*	*10*	*1*	*2*
7	Jehlum	Jehlum	*Alternaria alternata*	*10*	*1*	*2*
7	Dinna	Jehlum	*Alternaria alternata*	*10*	*1*	*2*

Appendix 6: Incidence, Severity and Disease index of different fungi at booting stage in different wheat growing agro-ecological zones of NWFP during 2005.

Zone	Location	Districts	Fungus	Incidence%	Severity (0-5)	Disease Index%
9	Akora Khattak	Nowshera	*Bipolaris sorokiniana*	30	1	4
9	Risal Pur	Nowshera	*Alternaria alternata*	20	1	4
9	Aza Khail	Nowshera	*Alternaria alternata*	10	1	2
9	Jahangira	Nowshera	*Alternaria alternata*	10	1	2
9	Sheran Kot	Nowshera	*Alternaria alternata*	10	1	2
9	Mardan	Mardan	*Bipolaris sorokiniana*	20	1	6
9	Sirdary	Mardan	*Bipolaris sorokiniana*	10	1	2
9	Gujar Garhi	Mardan	*Alternaria alternata*	20	1	4
9	Takht Bai	Mardan	*Bipolaris sorokiniana*	10	2	8
9	Rajjar	Charsadda	*Bipolaris sorokiniana*	20	2	8
9	Charsadda	Charsadda	*Bipolaris sorokiniana*	20	1	4
9	Shahalampur	Charsadda	*Alternaria alternata*	10	1	2
9	Shabqadar	Charsadda	*Bipolaris sorokiniana*	30	2	8
9	Tangi	Charsadda	*Alternaria alternata*	10	1	2
9	Gulbilala	Charsadda	*Bipolaris sorokiniana*	10	1	2
9	Pabbi	Peshawar	*Bipolaris sorokiniana*	20	1	4
9	Suffaid Dri	Peshawar	*Alternaria alternata*	10	1	2
9	Amaan Garh	Peshawar	*Alternaria alternata*	10	1	2
9	Badaber	Peshawar	*Bipolaris sorokiniana*	10	1	2
9	Sirdiab	Peshawar	*Bipolaris sorokiniana*	30	1	12
9	Kohat	Kohat	*Bipolaris sorokiniana*	10	1	4
9	Gombut	Kohat	*Bipolaris sorokiniana*	10	1	2
9	Khushalgarh	Kohat	*Bipolaris sorokiniana /Colletotrichum graminicola*	10	1	2
9	Gundiliopayan	Kohat	*Alternaria alternata*	10	1	2
9	Gondiala	Kohat	*Alternaria alternata*	10	1	2

9	Jande	Kohat	*Bipolaris sorokiniana*	10	1	2
10	Jalala	Malakand	*Bipolaris sorokiniana*	10	1	4
10	Dargai	Malakand	*Alternaria alternata*	10	1	2
10	Saffakot	Malakand	*Bipolaris sorokiniana*	20	1	2
10	Gulmit	Gilgit	*Dilophospora alopecuri*	10	1	2
10	Nagar Gulmit	Gilgit	*Alternaria alternata*	10	1	2
10	Naseer Abad	Gilgit	*Alternaria alternata*	10	1	2
10	Murtaza Abad	Gilgit	*Alternaria alternata*	10	1	2
10	Ali Abad	Gilgit	*Alternaria alternata*	10	1	2
10	Sheeshkot	Gilgit	*Alternaria alternata*	10	1	2
10	Pasu	Gilgit	*Alternaria alternata*	10	1	2
10	Khyber	Gilgit	*Alternaria alternata*	10	1	2
10	Sust	Gilgit	*Alternaria alternata*	10	1	2
11	Shing-5	Skardu	*Bipolaris sorokiniana*	10	1	2
11	Skardu Khaploo	Skardu	*Bipolaris sorokiniana*	10	1	2
11	Hassan Abad	Skardu	*Alternaria alternata*	10	1	2
11	Kotabela	Skardu	*Bipolaris sorokiniana*	10	1	2
11	Braksan	Skardu	*Bipolaris sorokiniana*	10	1	2
11	Radio Pakistan	Skardu	*Alternaria alternata*	20	1	4
11	Benazir Chowk	Skardu	*Alternaria alternata*	10	1	2
11	Kachora	Skardu	*Bipolaris sorokiniana*	10	1	4

Appendix 7: Dimension of conidia of *B. sorokiniana* cultured on PDA isolated from different wheat growing agro ecological zones of Punjab and NWFP during 2004

Zones	Isolates	Length Micron	Width micron	Mean length micron	Mean width micron	No. of Septa (Range)Nos
5	P2-9	80-70-45	20-20-15	65.0	18.3	2-6
5	P2-15	55-30-20	20-15-10	35.0	15.0	2-5
6	P1-6	65-50-30	30-25-20	48.3	25.0	2-5
6	P2-7	75-65-40	25-20-15	60.0	20.0	2-6
6	P2-14	70-65-35	25-20-10	55.0	18.3	2-6
6	P2-18	80-70-35	20-20-20	61.6	20.0	2-7
6	P2-22	80-70-40	25-20-10	63.3	18.3	3-7
6	P2-23	60-35-25	20-15-10	40.0	15.0	2-6
7	P1-4	65-45-30	20-15-10	46.6	15.0	3-5
7	P1-9	80-50-40	25-30-20	56.6	25.0	3-5
7	P1-10	70-60-40	30-20-20	56.6	23.3	3-6
7	P2-3	75-60-40	25-20-15	58.3	20.0	3-6
7	P2-4	80-75-40	25-20-20	65.0	21.6	3-7
7	P2-5	75-60-45	25-20-20	60.0	21.6	2-7
7	P2-24	60-50-30	25-20-10	46.6	18.3	2-6
7	P2-25	65-50-30	25-20-15	48.3	20.0	3-5
7	P2-26	65-60-40	20-20-15	55.0	18.3	2-6
7	P2-28	55-40-30	20-15-15	41.6	16.6	2-7
9	NP1-2	70-55-30	20-25-15	51.6	20.0	2-6
9	NP1-3	60-65-30	15-25-10	51.6	16.6	2-7
9	NP-4	70-60-35	20-15-15	55.0	16.6	3-7
9	NP-6	85-60-30	20-20-15	58.3	18.3	3-9
9	NP-8	90-55-30	20-20-15	58.3	18.3	3-6
9	NP-9	65-55-35	20-15-15	51.6	16.6	3-7
9	NP-10	55-40-25	15-15-15	40.0	15.0	3-13
9	NP-11	95-60-40	20-15-15	65.0	16.6	2-8
9	NP-20	80-65-30	20-20-15	58.3	18.3	3-7
10	NP-7	70-50-30	20-20-15	50.0	18.3	2-5
10	SWT1-2	65-55-40	20-15-10	53.3	15.0	2-6
10	SWT1-7	65-55-30	20-20-10	48.3	16.6	2-8
10	SWT1-22	60-55-30	20-15-15	48.3	16.6	3-7
10	SWT1-26	60-60-35	25-20-10	51.6	18.3	2-5
10	G1-18	70-60-40	20-20-15	56.6	18.3	2-6
11	G1-2	80-65-45	20-20-15	63.3	18.3	2-8
11	G1-6	80-70-40	20-20-15	63.3	18.3	2-5
11	G1-8	70-60-40	20-20-15	56.6	18.3	2-6
11	G1-9	55-50-35	15-15-10	40.0	13.3	5-7

Appendix 8. Cultural Characteristics of *B. sorokiniana* Isolates collected during 2004.and aggressiveness relationship with three commercial wheat varieties

Zone	Isolates	Type of growth	Colony color	Aggressiveness (0-5)		
				Wafaq-01	Inqalab-91	Bhakhar-01
5	P2-9	Suppressed	Black	5	4	3
5	P2-15	Suppressed	Grayish black	2.6	4.6	3
6	P1-6	Suppressed	Grayish black	2.6	2.6	3
6	P2-7	Suppressed	Black	3.3	3	3
6	P2-14	Suppressed	Black	3.3	2.6	2.6
6	P2-18	Suppressed	Black	3	3.3	1
6	P2-22	Suppressed	Black	3.6	3.6	1
6	P2-23	Suppressed	Black	2.3	3.3	3.3
7	P1-4	Suppressed	Brownish	3	3	2.6
7	P1-9	Suppressed	Black	4.3	2.6	2.6
7	P1-10	Suppressed	Black	3	2.6	2.3
7	P2-3	Suppressed	Black	3	2	3.3
7	P2-4	Suppressed	Black	3.3	3	2
7	P2-5	Suppressed	Black	3.3	4	3
7	P2-24	Suppressed	Grayish black	1	3.6	2.6
7	P2-25	Suppressed	Brownish	2.6	2.3	2.3
7	P2-26	Suppressed	Grayish Black	3	3.6	2.6
7	P2-28	Fluffy	Albino	2.6	1.3	2.6
9	NPI-2	Suppressed	Grayish Black	3.6	2.6	2.3
9	NP1-3	Suppressed	Grayish Black	3	1.6	2.6
9	NP-4	Suppressed	Black	4.3	3.6	2.3
9	NP-6	Suppressed	Grayish Black	2.3	2	3
9	NP-8	Suppressed	Grayish Black	3	2	3.6
9	NP-9	Fluffy	Albino	2	2	2.3
9	NP-10	Suppressed	Grayish Black	2	3.3	3
9	NP-11	Suppressed	Grayish Black	3	3	2.3
9	NP-20	Suppressed	Black	3.3	3	3
10	NP-7	Suppressed	Grayish Black	3	2	2.3
10	SWT1-2	Suppressed	Grayish Black	3.3	2.6	2.3
10	SWT1-7	Suppressed	Grayish Black	3	2.3	2.3
10	SWT1-22	Suppressed	Grayish Black	2	3.3	3
10	SWT1-26	Fluffy	Albino	2	2.3	2.3
11	G1-18	Suppressed	Grayish Black	3.3	2	3
11	G1-2	Suppressed	Grayish Black	2	3.3	3.6
11	G1-6	Suppressed	Grayish Black	2.3	2.3	3.3
11	G1-8	Suppressed	Grayish Black	3.3	1.6	3
11	G1-9	Suppressed	Grayish Black	2	3	3.6

Appendix 9. Dimension of conidia of *B. sorokiniana* cultured on PDA isolates from different wheat growing agro ecological zones at Punjab and NWFP during 2005

Zones	Isolates	Length micron	Width Micron	Mean length micron	Mean width micron	No. of Septa
5	P4-2	80-75-60	25-20-20	71.6	21.6	2-7
5	P4-16	90-70-60	20-20-15	73.3	18.3	2-10
5	P4-17	75-60-45	20-20-15	60.0	18.3	2-6
5	P4-18	85-75-55	25-20-15	71.6	20.0	2-8
5	P4-20	70-60-35	20-20-15	55.0	20.0	2-7
5	P4-22	60-50-30	20-20-20	46.6	20.0	2-6
5	P4-24	80-70-45	25-20-20	65.0	21.6	2-6
5	P4-27	80-60-35	20-20-15	58.3	20.0	3-9
5	P4-28	80-70-65	25-20-20	71.6	21.6	3-6
5	P4-29	90-60-55	20-20-15	68.3	20.0	2-7
6	P4-11	90-65-45	25-20-15	66.6	16.6	2-9
6	P4-12	65-50-35	20-15-15	50.0	16.6	2-5
6	P4-13	70-60-40	20-15-15	56.6	18.3	3-6
6	P4-14	85-65-40	25-20-10	63.3	18.3	3-8
6	P4-30	85-75-40	25-20-10	66.6	21.6	3-7
6	P4-31	75-60-40	25-20-20	58.3	15.0	2-6
6	P4-32	65-40-20	20-15-10	41.6	16.6	2-5
6	P4-33	70-40-25	20-20-10	45.0	16.6	3-6
6	P4-34	85-60-55	20-15-15	66.6	11.6	3-6
6	P4-40	55-45-25	15-10-10	41.6	16.6	2-5
7	P3-5	70-65-45	20-20-15	60.0	16.6	3-9
7	P3-14	65-55-35	20-20-15	55.0	16.6	2-8
7	P3-15	80-55-30	20-15-15	55.0	18.3	3-9
7	P3-16	75-50-30	20-20-15	51.0	18.3	2-8
7	P4-1	60-55-35	20-20-15	50.0	18.3	3-7
7	P4-5	55-35-20	20-15-10	36.6	16.6	2-7
7	P4-9	60-30-20	15-10-10	36.6	11.6	2-6
9	NP3-3	70-50-40	20-15-10	53.3	15.0	3-7
9	NP3-7	50-45-35	15-15-15	43.3	15.0	2-6
9	NP3-9	45-40-25	15-15-10	36.6	13.3	2-7
9	NP3-10	70-60-35	20-15-15	55.0	16.6	2-7
9	NP3-11	70-40-30	20-20-10	46.6	16.6	2-7
9	NP3-12	55-50-30	15-15-10	45.0	13.3	2-7
9	NP3-13	65-50-30	20-20-10	48.3	16.6	2-6
9	NP3-15	65-50-30	15-15-10	48.3	13.3	2-7
9	NP3-16	60-50-30	20-15-10	46.6	15.0	4-8
9	NP3-18	65-55-30	15-15-15	50.0	15.0	2-6
9	NP3-21	60-50-30	15-15-10	46.6	13.3	2-5
9	NP3-27	70-60-40	20-20-15	56.6	18.3	3-7
9	NP3-28	70-60-45	25-20-20	58.3	21.6	2-8
9	NP3-29	40-35-25	15-15-10	33.3	13.3	3-5
9	NP3-31	80-60-30	25-20-10	58.3	18.3	2-9
10	NP3-4	80-60-35	20-20-15	58.3	18.3	3-8
10	NP3-5	75-65-40	25-20-20	60.0	21.6	2-6
10	NP3-6	80-65-50	25-20-20	65.0	25.0	2-7
11	S-Shing-5	80-50-50	20-20-15	60.0	18.3	2-7
11	S-Shing-7	75-60-35	20-20-15	56.6	18.3	2-7
11	S-G-7	50-40-30	25-20-10	40.0	18.3	2-5
11	S-K-1	60-35-30	20-20-10	41.6	13.3	2-5
11	G5-3	55-30-20	15-10-10	35.0	11.6	2-5

Appendix 10. Cultural Characteristics of *B. sorokiniana* Isolates collected during 2005 and aggressiveness relationship with three commercial wheat varieties.

ZONE	ISOLATES	Type of growth	COLONY COLOUR	Aggressiveness (0-5)		
				WAFEQ01	INQ-91	BHAKK01
5	P4-2	Suppressed	Black	3.3	2	2
5	P4-16	Suppressed	Black	5	2	3
5	P4-17	Suppressed	Black	4	2.6	2
5	P4-18	Suppressed	Black	4	3	4
5	P4-20	Suppressed	Black	4	3.3	4
5	P4-22	Suppressed	Grayish Black	3	3	3
5	P4-24	Suppressed	Black	4.6	3	3
5	P4-27	Suppressed	Black	5	2.3	3
5	P4-28	Suppressed	Black	4.6	4	2.3
5	P4-29	Suppressed	Black	4.6	2	3
6	P4-11	Suppressed	Black	4	3	3.3
6	P4-12	Suppressed	Black	4	3	3.3
6	P4-13	Suppressed	Grayish Black	4	2.6	1
6	P4-14	Suppressed	Black	3.6	3	2
6	P4-30	Suppressed	Black	4	3	2
6	P4-31	Suppressed	Black	4	2	3
6	P4-32	Suppressed	Black	3	4	2
6	P4-33	Suppressed	Grayish Black	2.6	3	3
6	P4-34	Suppressed	Black	4	3.3	3
6	P4-40	Suppressed	Brownish	3	2	2.3
7	P3-5	Suppressed	Black	3.3	4	4
7	P3-14	Suppressed	Grayish Black	3.6	3	2
7	P3-15	Suppressed	Grayish Black	4	3	1
7	P3-16	Suppressed	Grayish Black	2.3	4	1.3
7	P4-1	Suppressed	Brownish	3	1.3	2
7	P4-5	Suppressed	Grayish Black	2.3	2.3	3
7	P4-9	Suppressed	Grayish Black	1.6	3	3
9	NP3-3	Suppressed	Black	3.6	2	3
9	NP3-7	Suppressed	Black	3.6	3	3
9	NP3-9	Fluffy	Albino	2.6	2.3	2.6
9	NP3-10	Suppressed	Black	3.6	3.3	3.3
9	NP3-11	Suppressed	Grayish Black	4	2	2
9	NP3-12	Suppressed	Grayish Black	3	2	2
9	NP3-13	Suppressed	Grayish Black	3	2.6	3

9	NP3-15	Suppressed	Black	3.3	2.6	3
9	NP3-16	Suppressed	Grayish Black	3.3	2	2
9	NP3-18	Suppressed	Black	4	3	3
9	NP3-21	Suppressed	Grayish Black	4	1.6	3
9	NP3-27	Fluffy	Albino	2.3	2.6	2
9	NP3-28	Suppressed	Grayish Black	3	2	3
9	NP3-29	Suppressed	Brownish	2	1	2
9	NP3-31	Suppressed	Black	5	3	3.3
10	NP3-4	Suppressed	Black	3.6	3	3
10	NP3-5	Suppressed	Black	4	2.3	3
10	NP3-6	Suppressed	Grayish Black	3.6	2	2.3
11	SSHING-5	Suppressed	Black	3.6	2	4
11	SSHING-7	Suppressed	Black	3	2.6	3
11	S-G-10	Fluffy	Albino	2.6	3	3
11	S-K-1	Fluffy	Albino	2	2.3	2
11	G5-3	Fluffy	Albino	1	2	1

Appendix 11: Means of aggressiveness analysis of 87 isolates of *Bipolaris sorokiniana* on three commercial wheat varieties.

Zones	Isolate	Wafaq 2001	Inqilab 91	Bhakar 2001	Means Scale (0-5)
5	P2-9	5	4	3	4
5	P2-15	2.6	4.6	3	3.4
5	P4-2	4.6	2	2	2.8
5	P4-16	5	2	3	3.3
5	P4-17	4	2.6	2	2.8
5	P4-18	4	3	4	3.6
5	P4-20	4	3.3	4	3.7
5	P4-22	3	3	3	3
5	P4-24	4.6	3	3	3.5
5	P4-27	5	2.3	3	3.4
5	P4-28	4.6	4	2.3	3.6
5	P4-29	4.6	2	3	3.2
6	P1-6	2.6	2.6	3	2.7
6	P2-7	3.3	3	3	3.1
6	P2-14	3.3	2.6	2.6	2.8
6	P2-18	3	3.3	1	2.4
6	P2-22	3.6	3.6	1	2.7
6	P2-23	2.3	3.3	3.3	2.9
6	P4-11	4	3	3.3	3.6
6	P4-12	4	3	1	2.6
6	P4-13	4	2.6	2	2.8
6	P4-14	3.6	3	2	2.8
6	P4-30	4	3	2	3
6	P4-31	4	2	2	2.6
6	P4-32	4	3	3	3.3
6	P4-33	2.6	3	3	2.8
6	P4-34	4	3.3	2.3	3.2
6	P4-40	3	2	2	2.3
7	P1-4	3	3	2.6	2.8
7	P1-9	4.3	2.6	2.6	3.1
7	P1-10	3	2.6	2.3	2.6
7	P2-3	3	2	3.3	2.7
7	P2-4	3.3	3	2	2.7
7	P2-5	3.3	4	3	3.4
7	P2-24	1	3.6	2.6	2.4
7	P2-25	2.6	2.2	2.3	2.4
7	P2-26	3	3.6	2.6	3
7	P2-28	2.6	1.3	2.6	2.1
7	P3-5	3.3	4	4	3.7
7	P3-14	3.6	3	2	2.8
7	P3-15	4	3	1	2.6
7	P3-16	2.3	4	1	2.5
7	P4-1	3	1.3	2	2.1

7	P4-5	2.3	2.3	3	2.5
7	P4-9	1.6	3	3	2.5
9	NP1-2	3.6	2.6	2.3	2.8
9	NP1-3	3	1.6	2.6	2.4
9	NP-4	4.3	3.6	2.3	3.4
9	NP-6	2.3	2	3	2.4
9	NP-8	3	2	3.6	2.8
9	NP-9	2	2	2.3	2.1
9	NP-10	2	3.3	3	2.7
9	NP-11	3	3	2.3	2.7
9	NP-20	3.3	3	3	3.1
9	NP3-3	3.6	2	3	2.8
9	NP3-7	3	3	3	3
9	NP3-9	2.6	2.3	2.6	2.5
9	NP3-10	3.6	3.3	3.3	3.4
9	NP3-11	4	2	2	2.6
9	NP3-12	3	2	2	2.3
9	NP3-13	3	2.6	3	2.8
9	NP3-15	3.3	2.6	3	2.9
9	NP3-16	3.3	2	2	2.4
9	NP3-18	4	3	3	3.3
9	NP3-21	4	1.6	3	2.8
9	NP3-27	2.3	2.6	2	2.3
9	NP3-28	3	2	3	2.6
9	NP3-29	2	1	2	1.6
9	NP3-31	5	3	3.3	3.7
10	NP-7	3	2	2.3	2.4
10	NP3-4	3.6	3	3	3.2
10	NP3-5	4	2.3	3	3.1
10	NP3-6	3.6	2	2.3	2.6
10	SWT1-2	3.3	2.6	2.3	2.7
10	SWT1-7	3	2.3	2.3	2.5
10	SWT1-22	2	3.3	3	2.7
10	SWT1-26	2	2.3	2.3	2.2
10	G1-18	3.3	2	3	2.7
11	G1-2	2	3.3	3.6	2.9
11	G1-6	2.3	2.3	3.3	2.6
11	G1-8	3.3	1.6	3	2.6
11	G1-9	2	3	3.6	2.8
11	S-Shing-5	3.6	2	4	3.2
11	S-Shing-7	3	2.6	3	2.8
11	S-G-10	2.6	3	3	2.8
11	S-K-1	2	2.3	2	2.1
11	G5-3	1	2	1	1.3

Appendix 12. Effect of temperature on the radial mycelial growth (mm) of isolates of *B.Sorokiniana* collected from different wheat growing agro-ecological zones of Punjab and NWFP during 2004 and 2005 at 20°c

ZONE	ISOLATES	4DAYS	8 DAYS	12 DAYS
5	P2-9 R1	13.5	29.3	57
	R2	13	28.7	56.6
	R3	12.9	28	56.4
5	P4-2 R1	14	31.2	61.2
	R2	14.5	31.9	62
	R3	13.9	30.5	60.9
5	P4-16 R1	17.8	35.4	66.8
	R2	17.9	35.9	66.5
	R3	16.9	34.9	65.8
5	P4-17 R1	14.5	32.5	62.3
	R2	14.2	31.9	62.1
	R3	14.1	32.4	62.4
5	P4-18 R1	13.6	30.0	59.6
	R2	13.4	30.1	60
	R3	13.1	30.0	59.6
5	P4-20 R1	15.6	32.4	63.2
	R2	14.9	32.6	63.5
	R3	15.2	32.0	62.9
5	P4-24 R1	12.9	29.2	58.9
	R2	13.2	30.0	59.0
	R3	13	30.0	59.0
5	P4-27 R1	14.2	30.8	63.0
	R2	15.1	31.0	63.4
	R3	15	31.9	63.4
5	P4-28 R1	16.2	33.0	67.5
	R2	16.4	33.0	67.2
	R3	16.1	32.9	66.9
5	P4-29 R1	13.4	28.9	60.0
	R2	13.5	28.6	59.8
	R3	13	28.7	59.6
6	P4-11 R1	13.2	29.0	58.0
	R2	13.4	29.4	58.0
	R3	13	29.1	58.0
6	P4-12 R1	14.1	29.1	61.0
	R2	14.1	29.3	61.2
	R3	14	29.0	61.0
6	P4-13 R1	13	28.6	59.9
	R2	13	28.5	59.8
	R3	12.8	28.0	59.6
6	P4-30 R1	14.3	30.6	61.7
	R2	14.1	30.4	61.4
	R3	14	30.0	61.3
6	P4-31 R1	14.2	29.5	60
	R2	14	29.0	60
	R3	14	28.9	60
6	P4-34 R1	15	34.0	62
	R2	15.1	33.9	62.1

	R3	15	33.6	61
7	P1-9 R1	13.5	29.0	59
	R2	13	29.0	59
	R3	12.9	28.3	58.5
7	P3-15 R1	14.1	29.2	61
	R2	14.2	29.0	61
	R3	14	29.0	61
9	NP-4 R1	15.1	31.1	66
	R2	15.2	31.0	66
	R3	15	31.0	65.9
9	NP3-11 R1	14.2	30.0	61
	R2	14	30.0	61
	R3	14.3	30.2	61
9	NP3-18 R1	13.4	29.0	59.2
	R2	13.5	29.0	59.1
	R3	13.2	29.1	59
9	NP3-21 R1	13.9	28.9	60.2
	R2	12.7	28.0	60
	R3	12.6	28.0	60
9	NP3-31 R1	13.2	29.0	60
	R2	13.1	29.0	60
	R3	13	28.9	60
5	P2-15 R1	11.3	23.1	56.2
	R2	11.5	23.5	56.1
	R3	11.6	23.0	56
6	P1-6 R1	12.4	25.5	55
	R2	12.1	25.1	54.9
	R3	12	24.0	54.8
6	P2-23 R1	11.2	24.0	52.5
	R2	11.3	24.1	52.2
	R3	11	23.9	52
6	P4-33 R1	12.1	26.2	52.2
	R2	12	26.0	52.1
	R3	11.9	26.9	52.3
7	P2-24 R1	12.1	25.1	51.3
	R2	12	25.3	51
	R3	12	25.0	51
7	P2-25 R1	11.3	22.0	50.1
	R2	11	22.3	50.6
	R3	11.2	22.1	50.1
7	P2-28 R1	13.1	24.8	54
	R2	13	24.6	54
	R3	12.7	24.3	54
7	P3-16 R1	11.1	23.0	53.8
	R2	11.4	23.4	53.9
	R3	11.2	23.4	54
7	P4-5 R1	12.7	26.0	58.1
	R2	12.4	26.0	58
	R3	12.3	25.9	57.9
7	P4-9 R1	13.4	26.3	57.8
	R2	13.4	26.7	57
	R3	13.2	26.4	57

9	NP-6 R1	11.7	25.0	48
	R2	11.4	24.9	48
	R3	11.0	24.8	48.1
9	NP-9 R1	11.0	24.5	50
	R2	11.1	23.7	49
	R3	11.0	23.0	49.8
9	NP-10 R1	12.2	25.1	50
	R2	12.1	25.1	50
	R3	12.0	25	50
9	NP3-9 R1	11.9	25.9	51
	R2	11.2	25.8	51
	R3	11.3	25.7	51
9	NP3-27 R1	12.1	26.1	53
	R2	12.1	26.1	53.2
	R3	12	26.0	53.1
10	SWT1-22 R1	11.7	23.4	50
	R2	11.4	23.1	50
	R3	11.9	23.4	50.1
10	SWT1-26 R1	12.8	27	52.6
	R2	12.4	26.7	52.1
	R3	12	26.1	52.1
11	G1-2 R1	11.5	22	48.5
	R2	11.2	21.9	48.6
	R3	11	21.8	48.5
11	G1-6 R1	10.2	22	46
	R2	10.4	22	46.1
	R3	10.4	22.1	46.1
11	G1-9 R1	10.8	23	45.6
	R2	10.7	23.1	45.2
	R3	10.5	23	45.1
11	S-G-10 R1	11	22.8	49
	R2	11.2	22.7	49
	R3	11	22.4	49
11	S-K-1 R1	13.5	25	49.2
	R2	13.4	25.6	49.3
	R3	13.4	25	49
11	G5-3 R1	12.1	25	48
	R2	12	25	48
	R3	12	25.1	48

Appendix 13. Effect of temperature on the radial mycelial growth (mm) of isolates of *B.Sorokiniana* collected from different wheat growing agro-ecological zones of Punjab and NWFP during 2004 and 2005 at 25°c

ZONE	ISOLATES	4DAYS	8 DAYS	12 DAYS
5	P2-9 R1	29.3	61.2	84
	R2	28.7	60.9	84.3
	R3	28	60.7	83.9
5	P4-2 R1	33.2	62	82.9
	R2	33.1	62	82.4
	R3	33.4	62	82.3
5	P4-16 R1	35.4	66.4	89.4
	R2	35.9	66.2	89
	R3	34.9	66	88.9
5	P4-17 R1	32.5	63	81
	R2	31.9	62.9	81
	R3	32.4	63	81
5	P4-18 R1	31	61	80
	R2	31.1	61.2	80.1
	R3	30.9	60.9	80
5	P4-20 R1	32.4	60	79
	R2	32.6	60	79.1
	R3	32	60	79
5	P4-24 R1	29.2	59.9	79.1
	R2	30	60.1	79
	R3	30	60	79
5	P4-27 R1	30.8	61	80
	R2	31	61	80
	R3	31.9	61.5	80
5	P4-28 R1	33	66.8	87
	R2	33	66.8	87
	R3	32.9	66	86.9
5	P4-29 R1	29.1	61	79
	R2	29	61	79
	R3	28.9	61	79
6	P4-11 R1	29	59.9	78
	R2	29.4	59.8	78
	R3	29.1	60	78.5
6	P4-12 R1	29.1	60	80
	R2	29.3	60	80
	R3	29	60	80
6	P4-13 R1	28.6	59.8	79
	R2	28.5	59.7	79.1
	R3	28	59	79.1
6	P4-30 R1	31.2	62	80.6
	R2	31.1	62	80.4
	R3	31	61	80
6	P4-31 R1	30	62	80
	R2	30.1	62.1	79.8
	R3	30	62	78.9
6	P4-34 R1	34	64	82
	R2	33.9	64.1	82

	R3	33.6	64.1	82.1
7	P1-9 R1	29	60.8	79
	R2	29	60.1	79.1
	R3	28.3	60.1	79
7	P3-15 R1	29.2	61.2	80
	R2	29	61	79.6
	R3	29	61	79
9	NP-4 R1	31.1	62.2	81
	R2	31	62.1	81
	R3	31	62	81
9	NP3-11 R1	30	60	78
	R2	30	60	78.6
	R3	30.2	60	78.5
9	NP3-18 R1	29	59.8	77
	R2	29	59.7	77.5
	R3	29.1	60	77.4
9	NP3-21 R1	28.9	58	76
	R2	28	58	76.1
	R3	28	58.1	76
9	NP3-31 R1	29	58	76
	R2	29	58	76.1
	R3	28.9	58	76
5	P2-15 R1	23.1	56	76.1
	R2	23.5	56.2	76
	R3	23	56	76
6	P1-6 R1	26	58.7	74
	R2	26.3	58.9	74.2
	R3	26.1	58.1	74
6	P2-23 R1	25	57.9	73
	R2	25	57.9	73
	R3	25.2	57.9	73.1
6	P4-33 R1	26.2	59.1	75
	R2	26	59	75
	R3	26.9	60	75.2
7	P2-24 R1	24.9	61.6	81
	R2	24.8	61.4	81.1
	R3	24.6	61.5	81.1
7	P2-25 R1	22	59	76
	R2	22.3	59.1	76
	R3	22.1	59	76.1
7	P2-28 R1	24.8	60.4	78
	R2	24.6	60.2	78.1
	R3	24.3	60.1	78
7	P3-16 R1	23	59	72
	R2	23.4	59.2	72
	R3	23.4	59.1	72.1
7	P4-5 R1	27	61	81
	R2	27	61	81
	R3	27.2	61.3	81.1
7	P4-9 R1	26.3	58.6	77
	R2	26.7	58.4	77
	R3	26.4	59	77.2

9	NP-6 R1	25	58.4	73
	R2	24.9	58	73
	R3	24.8	58	73
9	NP-9 R1	24.5	57.8	70
	R2	23.7	57.3	69.9
	R3	23	57.2	69.8
9	NP-10 R1	25.1	60	75
	R2	25.1	60	75
	R3	25	59.9	75.1
9	NP3-9 R1	25.9	58.7	70
	R2	25.8	58	69.7
	R3	25.7	58.4	69.8
9	NP3-27 R1	26.1	60	76
	R2	26.1	60	76.2
	R3	26	60	76
10	SWT1-22 R1	23.4	59.2	72
	R2	23.1	59.1	72
	R3	23.4	59.3	72.1
10	SWT1-26 R1	27	60.5	77
	R2	26.7	60	77.1
	R3	26.1	60	76.9
11	G1-2 R1	22	58	70
	R2	21.9	58	69.9
	R3	21.8	58.2	69.7
11	G1-6 R1	22	57.1	69.9
	R2	22	57.1	68.8
	R3	22.1	57	68.9
11	G1-9 R1	23	58	70
	R2	23.1	58.1	69.9
	R3	23	58	69.5
11	S-G-10 R1	22.8	57	68
	R2	22.7	56.9	68
	R3	22.4	56.8	68.2
11	S-K-1 R1	25	58	69
	R2	25.6	58	69.1
	R3	25	58	69.1
11	G5-3 R1	25	58.7	67
	R2	25	58	67.1
	R3	25.1	58.1	67.1

Appendix 14. Effect of temperature on the radial mycelial growth. (mm) of isolates of *B.Sorokiniana* collected from different agro-ecological zones of Punjab and NWFP during 2004 and 2005at 30°c

ZONE	ISOLATES	4DAYS	8 DAYS	12 DAYS
5	P2-9 R1	27	59	79
	R2	27.1	59	79.1
	R3	27.3	60	79
5	P4-2 R1	28	60	80
	R2	28.1	60.1	80.1
	R3	28	60	80
5	P4-16 R1	26.2	62	78
	R2	26.1	61.9	78.1
	R3	26	62	78
5	P4-17 R1	25.9	61	79
	R2	25.8	61.8	79.1
	R3	25.6	61.1	79
5	P4-18 R1	28	60	77
	R2	27.9	60	77.2
	R3	28.1	60	77.2
5	P4-20 R1	27.5	58.6	76
	R2	27.5	58.2	76.1
	R3	27	58.3	76.1
5	P4-24 R1	28.1	56	72
	R2	28	56	72.7
	R3	28.2	56.1	72
5	P4-27 R1	26.5	56	73
	R2	26.2	56.1	73.2
	R3	26	56	73.4
5	P4-28 R1	27.1	59	75
	R2	27	59.9	75
	R3	27.2	59.6	75
5	P4-29 R1	26.4	58.2	75
	R2	26.5	58.3	75.1
	R3	26.5	58	75
6	P4-11 R1	25.8	57.2	72.2
	R2	25.9	57	72
	R3	25.6	57.2	72.7
6	P4-12 R1	26.7	58	73
	R2	26	58.1	73
	R3	26.1	58	73
6	P4-13 R1	27.2	60	79
	R2	27	60.1	79
	R3	26.9	60	79.1
6	P4-30 R1	26.5	61	80
	R2	26	61	80.1
	R3	26	61	80
6	P4-31 R1	27.5	62	82
	R2	27	62.1	82.1
	R3	27	62	82
6	P4-34 R1	27.5	58	72.7

	R2	26.9	58.5	72
	R3	26.8	58	72
7	P1-9 R1	27.3	61.1	80.1
	R2	27.9	61.8	80
	R3	27.4	61.9	80
7	P3-15 R1	28	65	81
	R2	28.1	65.9	81
	R3	28.6	65	81
9	NP-4 R1	25.5	57	68
	R2	25.7	57.4	68
	R3	25	57.5	68.5
9	NP3-11 R1	26	55	69
	R2	26.2	55.5	69.1
	R3	26.5	55	69
9	NP3-18 R1	25.1	55	68.9
	R2	25.2	55.1	68
	R3	25.3	55	68
9	NP3-21 R1	24	52.1	69
	R2	24.5	52.3	69.9
	R3	24	52.2	69
9	NP3-31 R1	26	54.6	70
	R2	26.1	54.3	70.1
	R3	26.5	54	70.1
5	P2-15 R1	23.5	52.1	68
	R2	23.1	52.2	68
	R3	23	52.2	68.1
6	P1-6 R1	25.1	50	65.9
	R2	25.1	50.1	65
	R3	25	50.1	65
6	P2-23 R1	22.7	48.2	59.6
	R2	22.2	48.2	59
	R3	22	48	59
6	P4-33 R1	24	49.7	60
	R2	24.8	49.9	60.1
	R3	24.3	49	60
7	P2-24 R1	21	48.2	59.9
	R2	21.2	48.2	59
	R3	20.9	48	59
7	P2-25 R1	22	47	56
	R2	22.3	47.2	56.2
	R3	23	47	56.2
7	P2-28 R1	24	48.2	59.5
	R2	24.2	48	59
	R3	24	48	59
7	P3-16 R1	21.5	46.1	57
	R2	21	46.2	57.1
	R3	21	46	57
7	P4-5 R1	24.8	46.1	57.1
	R2	24	46	57
	R3	24.5	46	57
7	P4-9 R1	25.9	51	72.7
	R2	25.8	51.1	72

	R3	25.6	51	72
9	NP-6 R1	22	45	55.5
	R2	22.2	45.2	55.5
	R3	22	45	55
9	NP-9 R1	23.5	46	58.8
	R2	23.5	46.1	58.9
	R3	22.9	46	59
9	NP-10 R1	24.1	48.2	60
	R2	24.1	48	60
	R3	24	48	60.1
9	NP3-9 R1	23.5	46.3	59
	R2	23	46	59.1
	R3	23	46	59
9	NP3-27 R1	23	46.4	59.8
	R2	23	46.5	59.8
	R3	23	46	59.7
10	SWT1-22 R1	22.7	42	55.5
	R2	22	42.1	55.5
	R3	22	42	55
10	SWT1-26 R1	24.5	45	56
	R2	24.5	45	56.2
	R3	24	45.2	56
11	G1-2 R1	20	40	54.8
	R2	19.9	40.3	54.8
	R3	19.9	40	54
11	G1-6 R1	20	41	56
	R2	20.2	41.2	56.6
	R3	20.2	41.6	56
11	G1-9 R1	21	42	51.4
	R2	21	42.1	51.4
	R3	21	42.2	51.4
11	S-G-10 R1	19.9	44	61.8
	R2	20	44.1	61
	R3	20	44	61
11	S-K-1 R1	21	42	50.9
	R2	21.1	42.1	51
	R3	21	42	51
11	G5-3 R1	19	41	50.1
	R2	19.2	41.1	50.1
	R3	19	41	50

Appendix 15: Fungi isolated in different production technologies followed in rice-wheat cropping system in wheat crop sown after rice during 2004 and 2005

Zone	location	District	cultivation fallowed	Fungi isolated
7	Killa Dedar Sing	Gujranwala	Bed Planting	*Alternaria alternata*
6	Killar pind	Sheikupura	Bed Planting	0
6	Kala shah Kakoo	Lahore	Bed Planting	*Alternaria alternata*
6	Kala shah Kakoo	Lahore	Bed Planting	*Alternaria alternata*
6	Muridke	Sheikupura	Bed Planting	*Alternaria alternata, Curvularia sp*
7	Sattian	Sialkot	Bed Planting	*Alternaria alternata*
7	Jarinawalla	Sialkot	Bed Planting	*Alternaria alternata*
7	Dera Sandia	Sialkot	Bed Planting	*Alternaria alternata*
7	Badiana	Sialkot	Bed Planting	*Alternaria alternata*
7	Ottianwalla	Hafizabad	Beds Planting	*Alternaria alternata*
7	Tellawalla	Gujranwala	coventional	*Alternaria.alternata*
7	Keyella	Hafizabad	coventional	*Bipolaris sorokiniana*
7	Jarianda	Hafizabad	coventional	*Alternaria.alternata*
7	Daska	Sialkot	coventional	0
7	Sialkot	Sialkot	coventional	0
7	Kolti Bawaj	Sialkot	coventional	*Alternaria alternata*
7	Badiana	Sialkot	coventional	*Bipolaris sorokiniana*
6	Muridke	Sheikupura	coventional	*Alternaria.alternata*
6	Kala shah Kakoo	Lahore	coventional	*Alternaria alternate*
6	Sheikupura	Sheikupura	coventional	*Bipolaris sorokiniana*
7	Pasroor	Sialkot	coventional	*Bipolaris sorokiniana*
7	Nazamabad	Sailkot	coventional	*Alternaria alternata*
7	Galoitoamoore	Gujranwala	coventional	*Alternaria alternata*
7	Aminabad	Gujranwala	coventional	*Alternaria alternata*
7	Jabaran	Hafizabad	coventional	*Bipolaris sorokiniana*

7	Hafizabad	Hafizabad	coventional	*Bipolaris sorokiniana*
7	Kotlodha	Hafizabad	coventional	*Bipolaris sorokiniana*
7	Gujrat	Gujrat	coventional	*Curvularia sp,Cladosporium sp,D rostata Alternaria.alternaria*
7	Lalamusa	Gujrat	coventional	*Curvularia sp, Alternaria alternata*
7	Pasoor	Sialkot	Zero tillage	*Alternaria alternata*
7	Gujranwala	Gujranwala	Zero tillage	*Bipolaris sorokiniana*
7	Hafizabad	Hafizabad	Zero tillage	*Bipolaris sorokiniana*
6	Sheikupura	Sheikupura	Zero tillage	*Bipolaris sorokiniana*
6	Kala shah Kakoo	Lahore	Zero tillage	*D. rostata*

Appendix 16: Mean Monthly Temperature/ Rainfall during wheat growing season in different agro-ecological zones during 2004 and 2005.

Zones	Temperature (°C)						Rainfall (mm)					
	2004			2005			2004			2005		
	Jan	Feb	Mar	Jan	Feb	Mar	Jan	Feb	Mar	Jan	Feb	Mar
5	13.3	17.2	25.5	12.2	14.3	23.5	9.7	2.1	0.0	37.9	57.4	53.8
6	13.0	16.6	24.9	11.2	13.7	25.4	17.1	10.0	0.0	16.8	43.7	66.5
7	13.3	17.3	24.9	11.9	13.7	24.9	23.1	5.5	0.0	66.3	48.8	67.4
9	12.0	14.9	23.6	10.4	11.6	23.3	68.0	52.0	0.0	131.0	112.2	139.2
10	-	-	22.6July	-	-	22.0	-	-	0.0	-	-	
11	-	-	20.1July	-	-	23.0	-	-	0.0	-	-	

Source: Meteorological Department Islamabad 2004-2005.

List of Publications

The Following research publications has originated from thesis research work:

A. Published:

Iftikhar,S., **Asad,S.**, Munir,A., Ahmad,I. and Sultan,A. **2006**.Prevalence and distribution of foliar blight pathogens of wheat in different agro-ecological zones of Pakistan with special reference to *Bipolaris sorokiniana.* **Pakistan Journal of Botany.38 (1):205-210.**

Asad,S., Sultan,A., Iftikhar, S., Munir,A., Iftikhar,A. and Ayub,N. **2006**.First report of Dilophospora leaf spot (Twist) disease of wheat in Pakistan. **Pakistan Journal of Botany 39(4):1387-1389**

Asad,S., Sultan,A., Iftikhar, S., Munir,A., Iftikhar,A. and Ayub,N.**2007.**Pathogenic diversity in Bipolaris sorokiniana isolates collected from different wheat growing areas of Punjab and NWFP of Pakistan. **Pakistan Journal of Botany 39(6):2225-2231.**

Iftikhar, S., **Asad, S.**, Munir, A., and Ahmad, I. **2008**. Selection of in-vitro technique for pathogenicity and screening of wheat cultivars against *Bipolaris sorokiniana.* Pakistan. **Pakistan Journal of Botany 40(1):415-420.**

Iftikhar,S., **Asad,S.**, Sultan,A.Munir,A, and Iftikhar,A.**2006.** Occurrence of *Colletotrichum graminicola* on wheat in Pakistan.**Archieve Journal of Phytopathology and plant proection. (ID-number2006/31). (In press)**

B. Submitted:

Asad, S., Iftikhar, S., Munir, A., Ahmad, I and Ayub, N. **2007**. Incidence of *Bipolaris sorokiniana* (spot blotch of wheat) in different agro-ecological zones of Pakistan. (Sarhad Journal Agriculture)

Iftikhar, S., **Asad, S.**, Munir,A., Ahmad, I. **2007.** Characterization of *Bipolaris sorokiniana* isolated from different ecological zones of wheat production in Pakistan. (Submitted- Pak. Journal of Botany)