ASSESSMENT OF WHEAT FOLIAR MYCOFLORA AND ITS MANAGEMENT STRATEGIES IN DISTRICT BHIMBER, AZAD KASHMIR, PAKISTAN

TANVEER, H.[1*] – ISHTIAQ, M.[1] – MEHRBAN, A.[2] – WAHEEDA, M.[1] – SHAHZAD, A.[1] – MEHWISH, M.[1]

[1]*Department of Botany, Mirpur University of Science and Technology Bhimber Campus, Bhimber Azad Kashmir, Pakistan*

[2]*Department of Chemistry, University of Gujrat, Pakistan*
(phone: +92-3469642212)

*Corresponding author
e-mail: tanveerajk@gmail.com; drishtiaqajk@gmail.com

Abstract. In this research, an analysis of wheat foliar mycoflora was explored with detection of 21 foliar fungal species from eight cultivated varieties in the wheat fields of District Bhimber of Azad Kashmir, Pakistan. Out of 21 species, 19 were isolated from three wheat varieties viz: V1 (Fareed-2006), V3 (Lasani-2008) and V6 (Aas 2011). Wheat variety V8 (Galaxy-2013) showed less number (28.5%) of fungal species invasion. *Mycosphaerella graminicola* was present ubiquitously on all eight wheat varieties with 100% prevalence while *Cephalosporium gramineum* was only found on three wheat varieties with 37.5%. The minimum occurrence was shown by fungus *Nigrospora sphaerica* (15%). In sub-division Samahni, wheat variety Fareed-2006 (V1) depicted the highest disease susceptibility with incidence of 63.3%. The variety Galaxy-2013 was found the best crop in Samahni having least incidence value of 22.3%. In sub-division Bhimber, wheat variety Seher-2006 was the most affected by mycoflora having highest disease incidence (60.0%) while least infection was measured in Galaxy-2013 (20.9%), being appropriate varietal crop for the area. In Barnala sub-division, Fareed-2006 indicated highest disease incidence (59.7%) while minimum disease incidence was measured in Galaxy-2013 (29.7%). As general conclusion Galaxy-2013 was proved as the best crop variety in the study area being nonth or least infected by fungal taxa. In second aspect of experiment comprising of optimization of management and control strategies, the parameter of grain yield was measured. As a general without any pre-treatment of seed crop, the variety V8 was the best of with yield of 1543 kg/ha, that might be due to its genetic resistance or better eco-climatic adaptability. Out of applied management strategies; use of fungicides (Quadris and Headline) spray on wheat leaves proved to be better having rise of yield i.e., 1550 kg/ha and 1560 kg/ha, respectively. The other strategy: use of biological products i.e. different plant extracts (*Acacia nilotica, Azadirachta indica, Curcuma longa, Eucaylptus citriodora, Ficus bengalensis*) spray proved that landmark rise was obtained in yield from variety V8 with 1739 kg/ha as compared to others. This rise was huge (1739 kg/ha) in comparison to the without treatment crop having 1543 kg/ha yield and it was proved pre-treatment produces better crop than control (blank) sample culminating the result that use of bio-products (plant extracts) are the best for control and management of mycoflora of wheat.
Keywords: *mycoflora, wheat varieties, foliar diseases, fungicides, biocontrol*

Introduction

Wheat (*Triticum aestivum* L.) is the largest crop present worldwide, which occupies about 217 m/hectors (FAO, 2012) and among cereal crops it is ranked third with annual production about 651 million tons in year 2010. It is likely that eco-climatic and frequently environmental changes have bad impacts on wheat yields and quality (Ortiz et al., 2008; Bender and Weigel, 2011). Climate change influenced wheat production. Therefore, better

methods are applied for disease management in near future (Ewert, 2012). Wheat has high relation to environmental and climatic conditions (Rosada et al., 2010). Excessive moisture in the field and storage, humidity, drought and temperature extremes are principal environmental factors that determine the intensity of mycotoxins contaminations (Coulombe, 1993).

This crop is also very sensitive to pests, especially pathogenic fungi that cause leaf, head, and stem diseases (Jaczewska, 2010). These diseases contribute annually to a significant loss in wheat production (Korbas, 2004). A comprehensive survey of wheat pathogens including viruses, fungi and bacteria was carried out by Bockus et al., (2010). It is estimated that 10 % of net wheat yield losses were due to fungal and bacterial pathogens and only 2% yield was reduced by viruses (Oerke, 2006). The question arises that whether these overall estimated yield reduction will be higher or lower under globally fluctuating weather which will depend on the direct effects on different pathogens and the indirect effects through the host-plant interaction.

Current research shows fungal diseases because these are major components of yield losses in wheat crop at local level as well as worldwide. On the other hand, viral and bacterial pathogens are usually less important according to disease severity (Oerke, 2006). Another reason for this study is the extremely limited literature on viral and particularly bacterial wheat diseases with respect to climate change (Juroszek and von Tiedemann, 2011).

Changes of different wheat pathogens in India and Pakistan is a future risk (Kaur et al., 2008). For example, the importance of stripe rust and Kernel bunt in Punjab, India is assumed to be reduced in the future due to temperature and humidity changes. On the other hand, the importance of leaf rust, foliar blights, Fusarium head blight, and stem rust may increase in Punjab in the future, the latter disease particularly in the absence of resistance in wheat cultivars (Kaur et al., 2008).

Wheat varieties are affected by different diseases which reduce quantitative as well as qualitative yield losses in Pakistan. The foliar diseases like leaf and glumes blotch, powdery mildew, three types of wheat crop rusts, tan spot, head blight and smuts are the quickest spreading diseases in different wheat varieties. Yield losses reach upto 30% and depend on yield frequency according to environmental as well as wheat cultivar conditions.

Stripe rust is a more serious fungal disease of wheat crop. It infects susceptible host cultivars with symptoms occurring about one week after initial infection occurs. The disease often causes severe grain yield and quality loss as stripe rust can appear and spread rapidly when climatic conditions favor the disease. Stripe rust is reported in over 60 countries worldwide.

Fusarium culmorum, Fusarium graminearum and *Fusarium sporotrichioides* are the main producers of trichothecenes. These toxins are common fungal contaminants of cereals and occur naturally worldwide in the cultivation of wheat and other cereals (Brown et al., 2001; Champeil et al., 2004; Wagacha and Muthomi, 2007).

Therefore, management of fungal diseases is necessary for a sustainable agriculture. Crop management practices affect the development of the disease epidemics. In the humid subtropics of South Asia, there is evidence of stress conditions, which favor foliar blight (Dubin and Bimb, 1994). Factors such as minimum tillage or surface seeding, irrigation, late planting, or low soil fertility may be responsible for higher foliar blight severity in the wheat-based cropping systems of the Indo-Gangetic plains (Sharma and Duveiller, 2003). Lower disease severity with higher nitrogen application was reported by Chaurasia and Duveiller (2006) and Sharma and Duveiller (2004). However, Singh et al., (1998) reported more disease infection on higher nitrogen application. Selection and breeding for resistant cultivars is the

main disease management strategy. Planting resistant cultivars is one of the least expensive and most effective management strategies to prevent diseases.

Fungicides are widely used to manage foliar wheat diseases in several countries of world (Carmona et al., 1999). The response to fungicide application depends on the severity of specific foliar diseases, cultivar disease resistance or tolerance, management practices and environmental conditions (Roth and Marshall, 1987; Varga et al., 2005; Carignano et al., 2008). Fungicides applied at flag leaf and spike emergence of winter wheat increased mean grain weight and grain yield when they extended canopy life (Gooding, 2006). Triazoles and Strobilurins are the most common systemic fungicides used to control foliar diseases on wheat. These types of fungicides generally move upward in the transpiration stream and may accumulate at the leaf margins (Arregui and Puricelli, 2008).

Introduction of the study area

The study area viz: District Bhimber (*Figure 1*) consists of three sub-divisions (tehsils). The environmental conditions of this district are ubiquitous in three tehsils. Geographcally,the district is located between latitude 32-48˚ to 33-34˚ and longitude 73-55˚ to 74-45˚ and has an area of 1516 km^2. The climate of this area is constant. Hot summer temperature is often over 38-46˚C from May to September and cold winters (4-10˚C). The total average rain fall is about 1233 mm per month. Hail occurs occasionally during the month of February and March. Humidity remains high in rainy season and winters. Topographically, the area is semi mountainous and plain. The southern part is plain while northern part consists of rough and precipitates steep sand stone hills and rugge. The plain areas of district Bhimber are very fertile for crops cultivation.

The objective of this research was to survey foliar diseases associated with wheat varieties; to measure the incidence, prevalence and severity of foliar diseases of selected eight wheat varieties; to improve wheat yields by reducing or eradicate foliar fungal diseases through fungicides treatments and biological treatment by use of plant extracts.
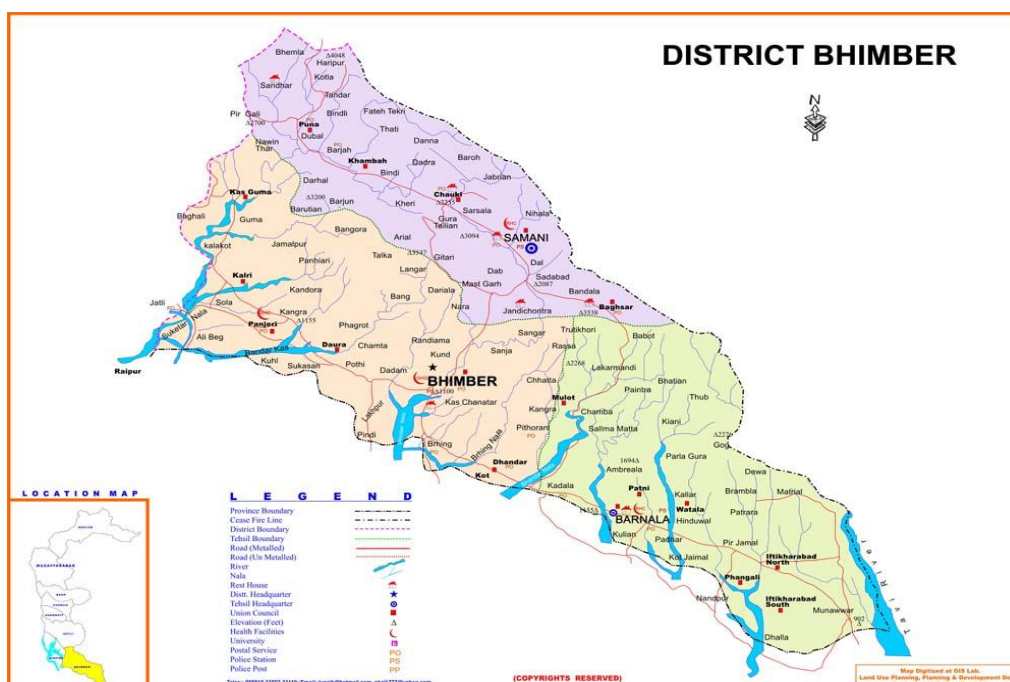
Figure 1. Map of the study area (District Bhimber)

Materials and Methods

Sample collection

In this research study, all wheat growing areas of the district were surveyed. Fungal affected wheat leaves were collected in plastic bags and stored at room temperature for assessment and analyses of fungal diseases. The leaves were collected at seedling, young and mature stages. Morphological symptoms were recorded for presence or absence of foliar diseases in wheat growing areas of district Bhimber Azad Jammu and Kashmir (AJ&K). Leaves of four common plants were collected for biological treatments against different fungi. The disease free and fresh plants were selected for this investigation. About two gram (02 g) of fresh and healthy leaves were taken for each solvent. Then, surface sterilized with 1% Sodium hypochlorite and alcohol for 2-3 minutes and the plant materials were washed thoroughly with distilled water.

The following methods were applied for assessment of foliar diseases.

Assessment of disease prevalence by diagonal transect method

Affected leaves of wheat varieties were collected from different field trails from three tehsils. The samples were collected from five points along with a diagonal transect as X- pattern (Anonymous, 2003). At each sampling site, the collected foliar samples were put in sterile flasks and transferred to laboratory for further fungal analysis, identification and purification (Iftikhar et al., 2012). The prevalence of disease was measured according to presence or absence of foliar spots in each field trail. The prevalence and severity (% age) was calculated by applying following formulae:

$$i. \, Disease \, Prevalece(\%) = \frac{Locations \, with \, filiar \, disease}{Total \, locations} \times 100 \qquad (Eq.1)$$

$$ii. \, Severity = \frac{\sum(a \times b)}{n \times z} \times 100 \qquad (Eq.2)$$

where $\sum$ (a×b) = Sum of the symptomatic plants and their corresponding scoring scales, n = Total number of sampled plants and z= Highest Score Scale.

The assessment of the disease was made in double digit system which comprises of two digits representing the vertical disease progress and percentage of the blight area covered (severity). The first digit gives the relative height of the disease in 0-9 scale as proposed by Saari and Prescot (1985) and second digit represents the percentage area covered by the blight pathogen on the flag leaf and one below it which is as under:

10%=1, 20%=2, 30%=3, 40%=4, 50%=5, 60%=6, 70%=7, 80%=8, 90%=9

As for example the disease score of 55 denotes that the first digit of 5 represents the height of the disease in' 0-9 scale whereas the second digit exhibits average disease severity in percent on the flag leaf and one below it.

Assessment of disease incidence (diagonal transect method)

A fixed plot survey was applied for wheat leaves collection. The plants within the fixed plot were assessed for foliar diseases and leaf samples were collected from

selected localities by X pattern (Sharma and Duveiller, 2003) and brought to laboratory for further fungal study (Iftikhar et al., 2012). Five plants were selected by diagonal transect method for documentation of incidence of different types of foliar diseases (Teng and James, 2001) by using following formula:

$$\text{iii. Incidence (\%)} = \frac{\text{Number of leaves showing foliar spots}}{\text{Total No. of leaves of sample}} \times 100 \quad \text{(Eq.3)}$$

Identification of fungi by agar plate method

In agar plate method (APM), collected samples were placed on sterile PDA media. The PDA media prepared by mixing of prepared PDA media in 1000 ml distilled water. The media autoclaved at fifteen (15) psi pressure for twenty (20) minutes in autoclave. 15 ml media transferred in each petridish under aseptic circumstances. Foliar samples were surface sterilized in 1% $NaOCl_2$ for three to five minutes and then given three washing treatments with distilled water. The effected parts were transferred on PDA media after distillation. After 3-5 days, fungi were detected and identified by morphological characteristics (Domsch et al., 1980, Hussain et al., 2011).

Isolated fungi were purified by sub-culturing on PDA media (Usmani and Ghaffar, 1982). The plates were incubated at room temperature for 3-5 days. After the complete growth of fungi, the fungal cultures were sealed and preserved in refrigerator for future use.

Disease management strategies

There are different management strategies were applied to control foliar diseases of wheat varieties in district Bhimber Azad Kashmir.

Fungicide treatments (chemical control)

In field trails, each wheat variety was sprayed with five fungicides i.e., Tilt (0.29 l ha^{-1}), Proline (0.76 l ha^{-1}), Strobilurins (0.89 l ha^{-1}), Quadris (0.58 l ha^{-1}) and Headline at different rates. The fungicides were applied on leaves of each variety in selected fields separately. The timing of different fungicides was different for better result. After all treatments, wait for fruit ripe and harvested the crop and calculated the yield of each wheat variety (Fakir, 1999).

Biological treatments (biocontrol)

As fungicides were sprayed, similarly different plant extracts were sprayed on all selected eight wheat varieties for reduction of fungal contaminations and better yields. This treatment method is more reliable as compared to fungicides because this method has few/no side effects Yield (kg/ha) and 1000-grain weight were measured after harvesting all plots (Pathak and Razia, 2013).

Statistical analysis

The data were evaluated by help of computer software MSTAT-C. Some data was analyzed statistically by LSD and ANOVA. Significance was calculated at $p < 0.05$ and $p < 0.01$ levels of probability. Each value is mean of three replicates (Steel *et al*., 1996; Pathak and Razia, 2013).

Results

Wheat (*Triticum aestivum*) is being attacked by several fungal diseases many of them are foliar pathogens. In this research, 21 different foliar fungal species from 40 foliar samples of eight wheat varieties were collected from three tehsils (sub-divisions) of district Bhimber Azad Jammu and Kashmir during the year 2014 and 2015 for mycofloral analysis (Table 1). The foliar samples of eight wheat varieties were collected and preserved in Laboratory of Botany, Mirpur University of Science and Technology (MUST), Bhimber Campus Azad Jammu and Kashmir for further experimental works.

Highest number of fungal species were identified from wheat variety V1 (Fareed-2006), V3 (Lasani-2008) and V6 (Aas 2011). Out of 21 fungal species 19 (90.5%) appeared on these three wheat varieties. Less number of fungal species i.e., 6 (28.5%) were isolated from wheat variety V8 (Galaxy-2013). In the current analysis, occurrence of fungal species was found variable; *Mycosphaerella graminicola* was present on all wheat varieties (100%) while *Cephalosporium gramineum* was only found on three wheat varieties with 37.5% as indicated in Table 1. The identification of fungal pathogens was presented by field photography. *Figure 2* shows the attack of *Puccinia tritici-repentis, Figure 3* the attack of pathogen *Stagonospora nodorum.* Similarly, *Figure 4* presents attack of *Puccinia striiformis* on wheat crop, *Figure 5* the attack of pathogen *Drechslera tetramera*, while *Figure 6* shows attack of various fungal species on wheat crop and *Figure 7* the presence of *Alternaria* species on wheat.

Figure 2. *Attack of Puccinia tritici-repentis*

Figure 3. *Attack of Stagonospora nodorum*

Figure 4. *Attack of Puccinia striiformis*

Figure 5. *Attack of Drechslera tetramera*

Figure 6. *Multiple fungal species attack on wheat*

Figure 7. *Attack of Alternaria species on leaf*

Table 1. *Survey/Isolation of Foliar mycoflora associated with eight wheat varieties from District Bhimber Azad Kashmir, Pakistan*

S/No	Fungi detected	Name of Wheat Varieties								Total	%
		V1	V2	V3	V4	V5	V6	V 7	V8	08	100
1	*Alternaria alternata*	+	-	+	+	+	+	-	-	5	62.5
2	*Aspergillus niger*	+	+	+	+	+	+	-	-	6	75.0
3	*Bipolaris sorokiniana*	+	+	+	+	+	+	-	+	7	87.5
4	*Blumeria graminis*	+	+	+	+	+	+	+	-	7	87.5
5	*Cephalosporium gramineum*	-	+	-	+	-	+	-	-	3	37.5
6	*Cladosporium herbarum*	+	-	+	-	+	+	-	+	5	62.5
7	*Drechslera tetramera*	+	+	+	-	+	+	-	+	6	75.0
8	*D. tritici-repentis*	+	-	+	-	+	+	-	-	4	50.0
9	*Helminthosporium sp.*	+	+	+	+	+	+	+	-	7	87.5
10	*Microdochium nivale*	+	+	+	+	+	+	-	-	6	75.0
11	*Mycosphaerella graminicola*	+	+	+	+	+	+	+	+	8	100
12	*Nigrospora sphaerica*	+	+	+	-	+	+	-	-	5	62.5
13	*Penicillium lilacinum*	+	+	+	+	+	+	-	-	6	75.0
14	*P. chrysogenum*	+	+	-	+	-	+	+	-	5	62.5
15	*Puccinia striiformis*	+	+	+	-	+	+	-	-	5	62.5
16	*P. triticina*	+	+	+	+	-	+	+	+	7	87.5
17	*P. recondite*	+	+	+	+	+	-	+	-	6	75.0
18	*Pyrenophora teres*	-	+	+	-	+	+	-	-	4	50.0
19	*P. tritici-repentis*	+	+	+	-	+	+	-	-	5	62.5
20	*Rhizoctonia cerealis*	+	-	+	-	+	-	+	+	5	62.5
21	*Stagonospora nodorum*	+	+	+	-	+	+	+	-	6	75.0
	Total Species Identified	19	17	19	12	18	19	8	6	118	
	%age of each variety	90.5	81	90.5	57	85.7	90.5	38	28.5	70.2	

Key: + = Present, - = Absent, V1= Fareed-2006, V2= Seher-2006, V3= Lasani-2008, V4= Faisalabad-2008, V5= Millat-2011, V6= Aas 2011, V7= Punjab-2011, V8= Galaxy-2013

The present mycofloral picture of District Bhimber consists of phyto-geographical snapshot of three Sub-divisions i.e., Bhimber (Bm), Samahni (Sm) and Barnala (Br). The prevalence of identified fungal species was calculated by Equation 1 and presented in tabular form (*Table 2*). The highest (100%) prevalence of fungi was observed in three locations of Sm, two locations of Bm and four locations of Br. According to wheat varieties V1 (96%) showed more prevalent as compared to others. While minimum prevalence was shown by V8 (70.7%) as mentioned in *Table 2*.

Table 2. *Prevalence (%) of foliar diseases of eight wheat varieties from different locations of district Bhimber Azad Jammu and Kashmir, Pakistan*

Area	Locations	Wheat varieties with disease prevalence (%)								Means
		V1	V2	V3	V4	V5	V6	V7	V8	
Samahni	5	100	100	100	100	100	100	100	100	100
Bandala	5	100	100	100	100	100	100	100	100	100
Chowki	5	100	100	100	100	100	100	100	100	100
Jandala	5	100	80	60	40	80	100	60	20	67.5
Poona	5	80	60	60	20	100	80	40	20	57.5
Bhimber	5	100	60	60	80	80	60	40	40	65
Bring	5	80	60	80	60	80	80	20	20	60
Sokasan	5	100	100	100	100	100	100	100	100	100
Punjairi	5	100	100	100	100	100	100	100	100	100
Kasgumma	5	80	100	80	20	60	80	40	40	62.5
Bernala	5	100	100	100	100	100	100	100	100	100
Chanb	5	100	100	100	100	100	100	100	100	100
Kadala	5	100	100	100	100	100	100	100	100	100
Tander	5	100	80	80	60	100	80	20	20	67.5
Thub	5	100	100	100	100	100	100	100	100	100
%age of each variety		96	89	88.3	78.7	93.3	92	74.7	70.7	

Key: + = Present, - = Absent, V1= Fareed-2006, V2= Seher-2006, V3= Lasani-2008, V4= Faisalabad-2008, V5= Millat-2011, V6= Aas 2011, V7= Punjab-2011, V8= Galaxy-2013

Disease incidence was measured by help of Equation 3. In tehsil Samahni, wheat variety (WV) Fareed-2006 (V1) prevailed highest disease incidence (63.3%) followed by Seher-2006 (60.3%), Aas-2011 (59.6%) and so on. The minimum disease incidence was measured in Galaxy-2013 (22.3%). These results indicated that wheat variety Galaxy-2013 was more suitable for high growth and better yields production of wheat crop in the study area. This WV showed low fungal infection rate (*Table 3*). In tehsil Bhimber, WV Seher-2006 showed highest disease incidence (60.0%) while minimum disease incidence was reflected in Galaxy-2013 (20.9%). In Bernala tehsil, variety (WV) Fareed-2006 (V1) indicated highest disease incidence (59.7%) while minimum disease incidence was measured in Galaxy-2013 (29.7%) (*Table 3*).

The comparative analysis according to disease severity among three tehsils of District Bhimber was also under investigation. The Severity was measured by use of Equation 2. According to 0-9 rating scales, foliar symptoms of diseases assessed through visual basis i.e., 0= no symptoms, 1= 10-20% spots on leaves, 2=20-30% spots, 3= 30-40% spots, 4= 40-50% and 5= 50-60% spots, 6= 60-70% spots, 7=70-80% spots, 8= 80-90% spots and 9= 90-100% (*Table 4*).

Table 3. *Incidence of foliar diseases of eight wheat varieties in district Bhimber Azad Kashmir*

Tehseels	Locations	*Incidence (%)								Mean
		V1	V2	V3	V4	V5	V6	V7	V8	
Samahni	Samahni city	81.7	75.0	78.3	56.7	78.3	73.3	46.7	38.3	66.0
	Bandala	71.7	70.0	60.0	45.0	56.7	61.7	45.0	30.0	55.01
	Chowki	65.0	58.3	60.0	40.0	56.7	56.7	35.0	21.7	49.2
	Poona	48.3	46.7	45.0	20.0	48.3	48.3	18.3	8.3	35.4
	Jandala	50.0	51.7	48.3	26.7	48.3	58.3	23.3	13.3	39.9
	Mean	**63.3**	**60.3**	**58.3**	**37.7**	**57.6**	**59.6**	**33.7**	**22.3**	**49.2**
Bhimber	Bhimber city	70	65.0	66.7	56.7	66.7	60.0	51.7	33.3	58.7
	Bring	58.3	55.0	51.7	50.0	55	55.0	45.0	13.3	47.9
	Sokasan	35.0	50.0	55.0	35.0	53.3	45.0	30.0	16.7	40.0
	Punjairi	60.0	61.7	30.0	30.0	38.3	50.0	26.7	18.3	39.4
	Kasgumma	55.0	68.3	53.3	33.3	46.7	48.3	30.0	23.3	44.8
	Mean	**55.7**	**60.0**	**51.3**	**41.0**	**52.0**	**51.7**	**36.7**	**20.9**	**46.2**
Barnala	**Barnala city**	76.7	71.7	73.3	60.0	71.7	71.7	53.3	36.7	64.4
	Chanb	61.7	63.3	60.0	48.3	56.7	58.3	46.7	21.7	52.1
	Kadala	63.3	55.0	53.3	36.7	60.0	53.3	35	13.3	46.2
	Tander	48.3	45.0	40.0	23.3	26.7	33.3	18.3	67	37.7
	Thub	48.3	50.0	55.0	35.0	43.3	43.3	26.7	10.0	38.95
	Mean	**59.7**	**57.0**	**56.3**	**40.7**	**51.7**	**51.9**	**36.0**	**29.7**	**47.8**

Key: *Incidence=Percentage shows wheat plants infected with foliar diseases

Table 4. *Disease Severity of foliar diseases of eight wheat varieties in district Bhimber Azad Kashmir*

Tehseels	locations	*Severity (0-9)								Mean
		V1	V2	V3	V4	V5	V6	V7	V8	
Samahni	Samahni city	8.0	6.3	7.0	4.3	6.0	6.0	4.0	3.0	5.6
	Bandala	7.3	5.7	5.0	3.3	5.3	5.7	2.7	1.7	4.6
	Chowki	5.0	4.0	6.0	4.3	5.7	5.0	2.7	3.0	4.5
	Poona	5.0	3.3	4.0	4.0	3.7	3.7	3.3	1.3	3.5
	Jandala	5.3	4.0	5.3	3.0	5.3	4.3	2.3	1.7	3.9
	Mean	**6.1**	**4.6**	**5.5**	**3.9**	**5.2**	**4.9**	**3.0**	**2.1**	**4.4**
Bhimber	Bhimber city	8.0	6.0	6.0	3.7	5.0	6.0	4.0	2.7	5.2
	Bring	7.3	5.3	5.0	3.0	4.7	5.3	2.3	1.0	4.2
	Sokasan	6.7	6.3	6.7	4.7	5.3	4.0	2.3	2.0	4.7
	Punjairi	4.7	5.3	7.7	2.3	3.0	3.7	3.0	1.0	3.8
	Kasgumma	6.0	7.0	6.0	2.0	4.0	5.7	2.0	1.3	4.3
	Mean	**6.5**	**5.9**	**6.3**	**3.1**	**4.4**	**4.9**	**2.7**	**1.6**	**4.4**
Barnala	**Bernala city**	7.7	7.0	7.7	4.7	6.3	6.3	4.0	2.7	5.8
	Chanb	6.7	6.7	6.0	4.0	4.7	5.3	2.3	2.0	4.7
	Kadala	5.0	7.7	6.0	4.0	6.7	5.3	3.0	3.0	5.1
	Tander	5.3	4.0	3.3	1.7	3.3	3.3	1.7	1.7	3.0
	Thub	7.3	7.0	5.0	2.7	5.7	4.7	2.7	1.3	4.5
	Mean	**6.4**	**6.5**	**5.6**	**3.4**	**5.3**	**4.9**	**2.7**	**2.1**	**4.6**

*Severity= 0-9 rating scales show foliar symptoms of diseases assessed through visual basis i.e., 0= no symptoms, 1= 10-20% spots on leaves, 2=20-30% spots, 3= 30-40% spots, 4= 40-50% and 5= 50-60% spots, 6= 60-70% spots, 7=70-80% spots, 8= 80-90% spots, 9= 90-100%

In tehsil Samahni, highest severity was shown by WV Fareed-2006 (V1) as 6.1 on severity scale. The minimum severity was shown by Galaxy-2013 (2.1). Less severity on wheat variety produced better yields. In tehsil Bhimber, variety (WV) Seher-2006

(V1) indicated highest disease severity (6.5) while minimum disease severity was measured in Galaxy-2013 (1.6). In Barnala tehsil, highest severity was shown by V1 and V2 (6.4, 6.5) while minimum disease severity was seen in Galaxy-2013 (2.1) as indicated in *Table 4*.

Management strategies

Foliar diseases of WV were managed by applying fungicides treatments through chemical spray and biological treatments by different plant extracts spray. In this research, five different fungicides were used for chemical treatment to wheat leaves at early growth stages/before appearance of fungal disease symptoms. These fungicides reduced fungal infections and improved wheat yields.

The total yields of eight WV were measured before and after treatment for comparative analysis. Without treatments, highest yield was obtained from V8 (1543 kg ha^{-1}). On the other hand, low yield was received from V1 (1210 kg ha^{-1}) and V2 (1245 kg ha^{-1}) as indicated in *Table 5*. After fungicides spray highest yield was obtained from V8 (1706 kg ha^{-1}) which is better than untreated samples. Similarly, 1602 kg ha^{-1} yield was produced by V4 while lowest yield was received from V1 (1362 kg ha^{-1}) that is also better from untreated samples as mentioned above (*Table 6*).

Table 5. *Total yields of untreated field trials of different wheat varieties in district Bhimber Azad Kashmir, Pakistan*

S/No	Field Trails	Wheat Varieties with their yield (kg ha^{-1}) before fungicide treatment								Means
		V1	V2	V3	V4	V5	V6	V7	V8	
1	T1	1150	1260	1300	1460	1430	1340	1480	1460	1360 [a]
2	T2	1200	1280	1250	1390	1340	1270	1420	1489	1330 [ab]
3	T3	1220	1190	1360	1450	1210	1340	1390	1570	1341 [a]
4	T4	1180	1298	1230	1270	1195	1290	1550	1620	1329 [ab]
5	T5	1300	1200	1175	1520	1290	1380	1500	1580	1368 [a]
	Average	1210 [e]	1245 [e]	1263 [d]	1418 [b]	1293 [d]	1324 [c]	1468 [b]	1543 [a]	

Key: T1= Trail 1, T2= Trail 2, T3= Trail 3, T4= Trail 4, T5= Trail 5

Table 6. *Fungicides treatment on leaves of wheat varieties and measured total yield increase (kg ha^{-1}) from Bhimber district Azad Jammu and Kashmir, Pakistan*

S/No	Fungicide	Wheat Varieties with yield increase (kg ha^{-1}) after fungicides treatment								Means
		V1	V2	V3	V4	V5	V6	V7	V8	
1	F1	1300	1380	1490	1600	1580	1440	1610	1690	1511 [b]
2	F2	1350	1560	1385	1580	1500	1390	1580	1700	1506 [b]
3	F3	1410	1480	1520	1450	1450	1460	1550	1680	1500 [ab]
4	F4	1290	1500	1500	1685	1470	1400	1745	1810	1550 [a]
5	F5	1460	1520	1460	1695	1495	1530	1670	1650	1560 [a]
	Average	1362 [e]	1488 [c]	1471 [c]	1602 [ab]	1499 [c]	1444 [cd]	1631 [b]	1706 [a]	

Key: F1= Tilt (0.29 l ha-1), F2= Proline (0.76 l ha-1), F3= Strobilurins (0.89 l ha-1), F4= Quadris (0.58 l ha-1), F5= Headline (0.58 l ha-1)

The impact of fungicides on yield rate of wheat varieties were also calculated and documented in *Table 6*. After spray of fungicide Quadris (0.58 l ha-1) and Headline

(0.58 1 ha-1) on wheat trails, maximum yield was produced as compared to other fungicides i.e., 1550 kg ha^{-1}, 1560 kg ha^{-1}. This means that the fungicide F4 and F5 are more effective to combat and reduce fungal diseases from surfaces of wheat leaves (*Table 6*).

On the other hand, after different plant extracts treatment/spray on wheat variety trails was showed maximum yields was obtained from V8 (1739 kg ha^{-1}) as compared to others WV. Lowest yield was obtained by WV Seher-2006 (1348 kg ha^{-1}) as mentioned in *Table 7*.

Table 7. *Treatment of leaves of wheat varieties with different crude plant extracts in Bhimber district Azad Jammu and Kashmir, Pakistan*

S/N	Plant Name	Wheat varieties spared with different plants crude extracts								Mean
		V1	V2	V3	V4	V5	V6	V7	V8	
1	*Acacia nilotica*	1350	1400	1490	1590	1570	1540	1650	1680	1534 [ab]
2	*Azadirachta indica*	1330	1500	1370	1570	1560	1400	1590	1750	1509 [b]
3	*Eucaylptus citriodora*	1420	1450	1550	1490	1400	1450	1565	1685	1501 [b]
4	*Ficus bengalensis*	1250	1470	1580	1630	1480	1460	1770	1870	1564 [a]
5	*Curcuma longa*	1390	1570	1420	1590	1500	1580	1685	1710	1556 [a]
	Average	1348 [f]	1478 [e]	1482 [e]	1574 [c]	1502 [d]	1486 [e]	1652 [b]	1739 [a]	

Discussion

Wheat (*Triticum aestivum*) is suffered from many diseases some of them are foliar pathogens. In this current research, 21 different foliar fungal species were identified from eight wheat varieties. The foliar samples of eight wheat varieties were collected and brought to Laboratory of Botany, Mirpur University of Science and Technology (MUST), Bhimber Campus Azad Jammu and Kashmir for experimental works.

Out of 21 fungal species 19 (90.5%) were isolated from V1 (Fareed-2006), V3 (Lasani-2008) and V6 (Aas-2011). these Highest number of fungal species were depicted that the ecoclimatic conditions of district Bhimber were more suitable for fungal growth and reproduction during wheat seasons. Secondly, these three wheat varieties have less resistance against fungal pathogens. Therefore fungal spores were easily attack on leaves of the varieties (Arregui and Puricelli, 2008). Low fungal frequencies (6 species) were identified from wheat variety V8 (Galaxy-2013). This means that V8 variety is more susceptible to fungi and more disease resistance as compared to other WV. Hence, it is more suitable for sowing in the study area. Similar observations were analyzed by Ortiz et al. (2008).

In current analysis, fungal pathogen *Mycosphaerella graminicola* was isolated from all wheat varieties. This may be due to high moisture contents and optimum temperature in District Bhimber Azad Kashmir. As previously same climatic study was conducted by Wagacha and Muthomi (2007). The fungi *Cephalosporium gramineum* was only present on three wheat varieties with 37.5% as indicated in *Table 1*. This fungal species did not spread more in the study area due to unfavorable environmental conditions.

The present mycofloral picture of District Bhimber consists of a comparative study of phyto-geographical diversity and comparative mycological analysis of three sub-divisions (tehsils) of district Bhimber Azad Kashmir, i.e. Bhimber (Bm), Samahni (Sm) and Barnala (Br). Similar prevalence of fungi in the three areas depicts their ubiquity in all ecosystems of this climate. According to wheat varieties V1 is more vulnerable to fungal diseases while minimum dominancy was shown by V8 as mentioned in *Table 2*. These findings were similar to above described results as discussed previously by Singh et al., (1998).

These results indicated that wheat variety Galaxy-2013 was more suitable for high growth and better yields production of wheat crop in the study area. This WV showed low fungal infection rate and fewer incidences due to better and quicker adaptation in the area (Sharma and Duveiller, 2003).

The comparative analysis according to disease severity among the three sub-divisions of district Bhimber was also carried out. The Severity was measured according to 0-9 rating scales which showed foliar symptoms of diseases assessed through visual basis i.e., 0= no symptoms, 1= 10-20% spots on leaves, 2=20-30% spots, 3= 30-40% spots, 4= 40-50% and 5= 50-60% spots, 6= 60-70% spots, 7=70-80% spots, 8= 80-90% spots and 9= 90-100% (Bockus *et al.,* 2010). Highest severity was calculated from WV Fareed-2006 (V1) on severity scale. This is also due to low resistance. Secondly, it may be due to repetition every year in the study area. Therefore, it becomes more vulnerable to fungi (Chakraborty et al*.,* 2011).

The severity was also minimum on Galaxy-2013. Less severity on wheat variety produced good yields. Similar findings were obtained in other locations. This means that overall results can be explained by that WV Galaxy-2013 was first time introduced in the study area. Therefore, it was less effective less as compared to the other seven WVs. Therefore former should be preferred to this variety in the study area for better food and fodder yields (Fernandes et al., 2004).

Management strategies

The population on this planet increases day by day and agricultural lands have been reduced. This is an alarming horizon for future. Demand for food is increasing day by day. Therefore we should carefully think on this issue: what is a good way to increase food and fodder yields? We should control foliar diseases of WV by applying fungicides treatments through chemical spray and biological spray treatment of different plant extracts. In this research, five different fungicides were used as chemical treatment on wheat leaves at appearance of fungal disease symptoms. Spray of fungicides reduced fungal infections especially and improved wheat yields (Varga et al., 2005; Carmona et al., 1999).

The total yields of eight WVs were measured before and after treatment for comparative analysis. Without treatments low yield was received from V1 (1210 kg ha^{-1}) and V2 (1245 kg ha^{-1}) as indicated in *Table 5*. After fungicides spray the highest yield was obtained fromV8 which was better than untreated samples. Similar research was conducted by Gooding (2006).

The impact of fungicides on yield rate of wheat varieties were also calculated and documented. After spray of fungicides on wheat trails, maximum yield was produced as compared to other fungicides. This means that the fungicides F4 and F5 are more effective to combat and reduce fungal diseases from surfaces of wheat leaves (Oerke, 2006; Ortiz et al., 2008).

On the other hand, after different plants extracts (*Acacia nilotica, Azadirachta indica, Curcuma longa, Eucaylptus citriodora, Ficus bengalensis*) treatment on wheat variety trails good yields were produced. This means that after biocontrol greater yields of wheat was obtained according to requirements. Similar biocontrol was applied by Benkeblia (2004). Finally, biological treatments are recommended to WV after sowing because this technique does not have fatal impact on health and environments. Similar work was done by Aqil et al., (2010). This biological method is cheap and easily manageable compared to every former. Previously, similar biological treatments were launched by Iftikhar et al., (2012) and Pathak and Razia (2013).

Fungicides treatments also resulted more net yields but they polluted the environment, produced harmful impacts on flora and fauna. Secondly, they also were costly applications. Therefore, we should prefer this new technique of plant extracts for fungal foliar disease management.

Conclusion

From these findings it is concluded that the control measures of fungal pathogens is a basic need to avoid crop failure. It is desirable that after sowing wheat crop, spreading of pathogens in wheat growing areas are treated with fungicides and biological treatments to attain maximum yield of wheat crops. In current research attention has been given to use biological techniques for foliar treatment to protect them against fungal pathogens. Chemical fungicides can control the crop diseases, but they have bad effects on plants, animals and human health and also on our environment. Since these fungicides are likely to have hazardous impacts on human life and environment, it is therefore necessary to search for better control measures that are cheap, ecologically sound and environmentally safe to eliminate or reduce the incidence of these pathogens and for the improvement of wheat quality and quantity, so as to obtain healthy and strong wheat plants for fodder as well as better yields of different varieties of wheat crop. Therefore biological treatment of wheat fungal diseases are recommended for obtaining better yields of wheat crop in future in the examined area.

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APPENDIX

Appendix 1. *Wheat variety Fareed-2006 (V1)*

Appendix 2. *Wheat variety Seher-2006 (V2)*

Appendix 3. *Wheat variety Lasani-2008 (V3), Wheat variety Faisalabad- 2008 (V4)*

Appendix 4. *Wheat variety Millat-2011(V5)*

Appendix 5. *Wheat variety Aas 2011 (V6)*

Appendix 6. *Wheat variety Punjab-2011 (V7)*

Appendix 7. *Wheat variety Galaxy-2013 (V8)*

Appendix 8. *Spray of Fungicides in field trails*

Appendix 9. *Spray of Plants extracts on wheat trails*