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DETECTION OF SEED-BORNE MYCOFLORA ASSOCIATED WITH WHEAT VARITIES AND MANAGEMENT STRATEGIES IN DISTRICT BHIMBER AZAD KASHMIR, PAKISTAN

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Abstract

Pakistan is very fertile for wheat production but there are problems of yield loss, low grain quality and substandard fodder. Wheat (*Triticum aestivum* L.) is invaded by several fungal pathogens; many of them are seed-borne. In current study, 29 different seed-borne pathogens were isolated from eight wheat varieties of district Bhimber Azad Kashmir during the years 2014 and 2015. Mycoflora was explored by using Agar plate method (APM), standard blotter method (SBM), washing method (WM) and deep freeze blotter method (DFBM). Deep freezing method showed better results among all protocols. *Tilletia tritici* (87.5%) and *Cladosporium herbarum* (87.5%) were more prevalent fungal pathogens on the most wheat varieties. Susceptibility analysis depicted that varieties Fareed-2006, Seher-2006, Lasani-2008 and Millat-2011are most vulnerable for fungal attack and not suitable for growth in this study area. Wheat varieties V4 (Faisalabad- 2008), V7 (Punjab-2011) and V8 (Galaxy-2013) showed least prevalence and these three wheat varieties are cultivated dominantly in the study area. For control measures two chemical and biological methods were used. In chemical protocol fungicides Benlate (56.84%) and Thiabendazole (58.44%) showed good fungal control. In biological treatment, applying plant extracts showed better germination rates (79.5^{A}, 81.6^{A} and 78.2^{A}) in study area in comparison with other treatments. Fungicide treatments also gave better germination rate (77.3A), but are not recommended due to their detrimental and toxic impacts on environment and human health. This study recommends that biocontrol method for elimination of seed mycoflora which is ecofriendly, less costly and easily to handle. It depicts that seed-borne diseases severely affect the biomass and gains quality and quantity of wheat crop. As conclusion wheat variety Galaxy-2013 (0.7^{EF}) showed maximum resistance to seed mycoflora hence it is recommended as the best variety for cultivation in the study area.

Keywords: Wheat varieties; *Tilletia tritici;* Seed-borne pathogens; Fungicides; Biocontrol; Bhimber; Azad Kashmir

Introduction

Wheat (*Triticum aestivum* L.) is a globally important cereal crop which belongs to a family Poaceae. About 228 wheat varieties are cultivated worldwide while 30 different varieties of wheat are grown in Pakistan; out of these 22 are regarded as high-yielding and eight as low-yielding based on agronomic data. Among these wheat (*T. aestivum*), Spelt (*T. spelta*), Durum (*T. durum*), Emmer (*T. dicoccon*) and Einkorn (*T. monococcum*) are prominently cultivated (Nestbitt & Mark., 1999). The wheat is source of many vitamins, minerals, proteins and carbohydrates. About 100 g of hard red winter wheat contain about 12.6 g of protein, 1.5 g of total fat, 71 g of carbohydrate and 3.2 mg of iron (Lev-Yadun *et al.,* 2000; Anon., 2012).

In 2010, world production of wheat was 651 million tons, making it the third cultivated cereal crop after maize (844 million tons) and rice (672 million tons) (Belderok *et al*., 2012). Wheat is cultivated on an area of 9046 thousands hectares with an average grain yield of 2657 kg ha^{-1} and having total yield equal to 24032 thousands tones in Pakistan (Anon., 2010). There was declining trend observed in wheat yield from 2010-11(2,933 kg per acre) to 2013-14 (2,797 kg per acre) (Shahid, 2015). In Azad Kashmir wheat is grown at an area of about 92 thousand hectares with an annual production of about 113 thousand tons. This yield is very low as compared to per hectare yield of advanced countries of the world (Qureshi & Bhatti, 2001; Siddiqui and Bajwa, 2001).

Wheat crop at all stages of growth are suffered in various injuries and stresses especially fungal diseases which influenced on their normal growth and development. About 20% of wheat yield is lost due to attack of pathogens (Fakir, 1999). Wheat seeds are designated as highly effective source for transferring fungal pathogens. Seed-borne diseases have been observed to reduce growth and production of crop. There are various ways of spreading of seed fungi which reduced wheat productivity as a result of supply of the seeds that were contaminated with fungal pathogens (Agarwal & Sinclair, 1996).

District Bhimber of Azad Jammu and Kashmir is an agricultural area and wheat is main crop of the area. The local population mainly relies on wheat crop for source of food, feed and fodder. The yield production is limited due to many edaphic and epidemiological constraints. The cause of low yield may be multifarious in the area but pathogenic diseases especially fungi are root causing factors. There are different fungal species affecting on it in the area which cause various diseases, such as rust, smut, foliar blight and others (Asad *et al*., 2007; Iftikhar e*t al*., 2010). Some of these diseases are also commonly present in Bhimber, Azad Kashmir. Their control is necessary for better yield of wheat in the selected area.

All control measure strategies are helpful to minimizing infections from the crop seeds. The fungal pathogens that are entirely seed-borne can be controlled by fungicide treatment. Healthy seed plays an important role not only for successful cultivation but also for increasing yield of crop. Various experiments have been

conducted in the last three decades to find out the effective means of isolating and controlling seed fungi from different crops. Chemical and biological methods are found to be more effective in controlling fungal diseases of wheat seeds than other conventional methods.

The purpose of this research project was multifarious such as; (1) to investigate and identify fungi on seeds of wheat varieties in the study area, (2) to investigate incidence, severity and prevalence of mycoflora related with seeds of eight wheat varieties commonly grown in district Bhimber, Azad Jammu and Kashmir, (3) to apply different management strategies on seeds before sowing and recommended the best one method for obtaining better yield in three tehsils of district Bhimber, Azad Jammu and Kashmir.

Material and Methods

Collection of seed samples: 72 Seed samples of eight wheat varieties were collected from three tehsils of district Bhimber i.e., Samahni, Barnala and Bhimber. The seed samples were labelled, put in plastic bags, tightly sealed and stored at room temperature (25°C) in laboratory for good preservation prior to seed analysis. These seeds were grown in model plots of MUST University for mycofloral analysis and management (Nazar *et al*., 2013).

Detection of Fungi: The Seeds were tested by following techniques using blotter paper and PDA medium (Anon., 2001). This includes Agar plate method (APM), Standard Blotting Method (SBM), washing method (WM) and deep freeze blotter method (DFBM).

Standard Blotting Method: In SBM method, seeds of each variety were taken and treated with 10% NaClO for five minutes. Then seeds were dipped in distilled water for few minutes. After treatment, seeds were placed on blotter sheets. The blotter sheets having seeds were moistened with distilled water and incubated for seven days at temperature (±25˚C). At end of incubation period, fungi growing out from seeds were examined for fungal identification. The fungi were further identified upto species level, following keys of Chidambaram *et al*., (1973) and Mathur and Kongsdal (2003). Four hundred seeds were sown in 4 trays (100 seeds per tray) for germination test. Seed germination was recorded along with seed-borne infection of fungal pathogens after seven days of incubation period on wet filter paper in blotter test. Seed germination rate was calculated by following protocols of Islam *et al*., 2009 and Nazar *et al*., 2013.

The germination rate of identified fungi was calculated by the following formulae:

$$\text{Germination rate} \frac{\text{No. of seeds containing a particular fungus}}{\text{Total seeds used}} \times 100$$

Agar Plate Method: In APM method, treated and untreated seeds were placed on the PDA medium. The PDA medium prepared by mixing of 20g agar, 20g dextrose and 20g potato starch in 1000 ml of distilled water. The medium sterilized at 15 psi for 20 minutes in autoclave. 15ml media transferred in each petridish under aseptic circumstances. Seeds from each sample were surface sterilized in 1% NaOCl for three to five minutes and then given three washing treatments with distilled water. The seeds were transferred on PDA medium. Incubation conditions were similar as for blotter method. After 3-5 days, fungi were detected and identified by morphological characteristics of fungi (Domsch *et al*., 1980, Hussain *et al.,* 2011).

Seed Washing Method: Similarly in WM, seeds of each variety were washed with sterilized water. This test was used to study fungi located on surface of wheat seeds. 50g of seed samples from each variety were taken in a beaker (200 ml) that having 50 ml sterilized distilled water. The beaker was shaken on a mechanical shaker for ten minutes. The suspended spores were concentrated by centrifugation at 3000 rpm for 15 minutes. The supernatant was discarded and deposit suspended in 500 µL distilled water (Mathur & Kongsdal, 2003). The fungal spores were identified under a stereomicroscope. Then seeds were transferred on PDA medium for further analysis of fungi (Nazar *et al*., 2013). To control growth of bacteria antibiotic (streptomycin) was added in PD prior inoculation.

Deep Freeze Blotter Method: In DFBM method, a set of three blotter papers were dipped in distilled water and placed in petri dishes. 10 seeds per plate were placed on a three layered blotter paper as described in SBM. The Petri dishes were incubated for 24 h at 25°C and then transferred to -20°C in a freezer for 6 to 8 h followed by incubation at 25°C for 5 to7 days (Mathur and Kongsdales, 2003). After the incubation period, each tetri dish was examined under a stereomicroscope in order to record the incidence of different seed borne fungi. Primary identification of fungi grown on the wheat seeds was performed on basis of their typical colony characteristics and conidial morphology. The percentage of seed infection in each sample and percentage of infection in each region were determined by following formulae;

$$\text{Seed infection (Mean)} = \frac{\text{Number of seeds on which a fungal species identified}}{\text{Number of seeds tested}} \times 100$$

$$\text{Regional infection (Mean)} = \frac{\text{Frequency of sample on which a fungus identified}}{\text{Number of samples collected}} \times 100$$

For further analysis, mycelium of fungi were isolated on potato dextrose agar (PDA medium) and maintained for 5 to 7 days. Then identification was done by following protocols of Nelson *et al*., (1983), Leslie & Summerell, 2006) and online comparison.

Fungicide treatments (Chemical control): Seeds of each wheat variety were taken and treated with six fungicides i.e., Benlate, Difenoconazole, Strobilurins, Thiabendazole, Thiram, Triticonazole and Triazoles at the rate of 2 gm per kg. The fungicides were applied on seeds of each variety in conical flasks separately. After all treatments, Seeds were placed on blotter sheets in petri plates @ 10 seeds / per plates and then moistened with distilled water. Then the treated seeds were grown as described above in SBM (Fakir, 1999).

Biological treatments (Biocontrol): The collected seeds of different varieties were treated with plant latex and plant

extract for reduction of fungal contaminations. This treatment method is more reliable as compared to fungicides because this method has few/no side effects (Pathak & Razia, 2013). The plant extract were prepared by soaking/maceration method and seeds were dipped in it for 10 minutes and grown as per above protocols.

Statistical Analysis: Data regarding germination and frequency of occurrence of various seed-borne fungi were analyzed statistically by applying SPSS software and DMR test. 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 and Discussion

Fungal Prevalence and Incidence: Wheat (*Triticum aestivum* L.) is being attacked by several fungal pathogens many of them are seed-borne. In this research, 29 different seed-borne fungal pathogens from 72 seed samples of eight wheat varieties were collected from three tehsils of Bhimber Azad Jammu and Kashmir during the year 2014 and 2015 for mycofloral analysis (Table 1). Mycoflora associated with selected wheat varieties with their infection rates and its consequent impacts on growth of seedlings was observed as indicated in Table 3. The seeds germplasm 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.

In the current analysis, occurrence of fungal species was found variable, *Cladosporium herbarum* and *Tilletia tritici* (87.5%), *Curvularia lunata* and *Rhizopus sp.* (75.5%), *Aspergillus niger, Aspergillus flavus, Drechslera hawaiiensis, Fusarium graminearum, Microdochium nivale, Nigrospora oryzae, Penicillium oxalicum, Septoria nodorum, Trichoderma hamatum* and *Ustilago tritici* (62.5%) as indicated in Table 1. This may be due to use of different varieties or similar ecoclimatic conditions. The result shows that maximum (86%) fungal pathogens were isolated from V2 (Seher-2006) and minimum (7%) fungal pathogens were isolated from V8 (Galaxi-2013) as shown in Table 1. The variety V8 produced better yield as compared to other varieties in the area because it is less affected by fungal pathogens. The wheat variety (V8) may be fit in the ecoclimatic condition of Bhimber and adopting the local climatic condition for better production. Secondly, this variety may be more disease resistant power against fungal pathogens. Therefore, attack of fungi on this variety is less as compared to other varieties. These findings were correlated with the results of Aslam *et al*., (2005). They analyzed twelve wheat varieties in Sindh province. They also explained the correlation between low and high occurrence of the wheat varieties. The similar findings on seed borne fungi of wheat crop were explored in Pakistan by Hasany *et al*., (1968), Khan *et al*., (1974), Fakhrunnisa *et al*., (2006) and Majumder *et al*., (2013).

The present mycofloral picture of District Bhimber consists of phytogeographical snapshot of three Tehsils i.e., Bhimber (Bm), Samahni (Sm) and Barnala (Br). The prevalence of identified fungal species was calculated and presented in tabular form (Table 2). The highest (63.69^{A}) prevalence of fungi was observed in Sm and lowest (50.92^{C}) in Bm. Moderate (50.92^{B}) prevalence occurred in Barnala. Statistical analysis of these three localities showed significance difference. The difference is due to their morphological and genetic diversity that make it to grow or spread in diverse type of climate/environment (Table 2). Climate of Samahni is more humid and more variable cold temperature at day and night that boosts up the growth of fungi more easily in this locality. Therefore, more fungal attack was observed in Samahni area as compared to others. Current result correlates with the similar findings of other scientists (Hussain *et al*., 2011; Karim, 2005). They previously described that climate of an area influenced on more prevalent distribution and diverse growth of fungal species.

According to isolated fungal species *Fusarium graminearum* (84.77^{A}) is more prevalent as compared to other identified species of seed mycoflora of wheat varieties. Second dominant species was *Tilletia tritici* (84.13^{A}). The prevalence of other isolated species was as *Curvularia lunata* (80.33^{AB}) followed by *Ustilago tritici* (72.2^{BC}), *Rhizoctonia solani,* (76.6^{BCD}), *Rhizopus sp.* (75.87^{BCD}), *Aspergillus niger* (74.13^{CD}), *Nigrospora oryzae* (72.5^{CD}) and so on. The lowest prevalence of *Claviceps purpurea* (22.07^{O}) was observed (Table 2). Out of 29 fungal species, 15 species in which the means are not significantly different from one another, while 14 are significantly different from each other as shown in Table 2 by different capital letters (A, B, etc.). Thus, distributions of 15 species are similar, very frequent and ubiquitous in the study area. It means that these species grow in the synonymous environmental conditions. These findings were correlated with the previous results of Aslam *et al*., (2005), Nirenberg *et al., (*1994) and Glazek, (1997). They were explains prevalence of seed mycoflora of wheat crop with statistical tools (significance difference).

The variety Seher-2006 (V2) prevailed highest infection rate (20.7^{A}) followed by Fareed-2006 (17.3^{B}), Lasani-2008(16.8^{B}), Millat-2011(14.3^{C}) and Aas-2011(12.9^{D}). The minimum infection rate was measured in Punjab-2011 (2.3^{E}), Faisalabad- 2008 (1.7^{E}) and Galaxy-2013 (0.7^{EF}). These results indicated that these three wheat varieties are more suitable for high growth and better yields production of wheat crop in the study area because these WV showed low fungal infection rate (Table 3). This is may be due to wheat-friendly environment or due to resistant against fungal pathogens. Similar results were obtained by Weber *et al*., (2001), Khanzada *et al*., (2002) and Bateman & Kwasna, (1999). They described that seed-borne diseases have been found to affect the growth and productivity of crop plants. Hence fungal pathogens do hamper number of grains, weight and quality of wheat crop.

The highest infection rate was shown by *Fusarium graminearum* (18.22^{A}) and *Tilletia tritici* (17.59^{AB}). The minimum infection rate (3.09^{F}) was shown by *Drechslera tetramera* (Table 3). Less infected wheat variety showed better yields. It means that seed-borne diseases have been found to affect the biomass and net gains of wheat crop. These results were correlated with Khanzada *et al*., (2002). Thus minimum infection rate or disease free wheat varieties showed better economic potential which is basic requirement for increasing population in this world. Therefore, we should try to sow the disease resistant WV for better crop production.

Table 1. Detection of Seed Borne Mycoflora Associated with Wheat Varieties of District Bhimber Azad Kashmir.

S/No	Fungi detected	Name of Wheat Varieties								Total	%age
		V1	V2	V3	V4	V5	V6	V 7	V8	08	100
1	*Aspergillus niger* Tiegh.	+	+	+	-	-	+	+	-	5	62.5
2	*A. flavus* (McAlpine) Al-Musallam	+	+	+	-	-	+	+	-	5	62.5
3	*Alternaria alternata* (Fr.) Keissl.	+	-	-	+	+	+	-	-	4	50.0
4	*A.clamydophora*	-	+	+	-	+	-	-	-	3	37.5
5	*A. triticina*	-	+	+	-	+	-	-	-	3	37.5
6	*Bipolaris sorokiniana*	+	+	+	-	-	+	-	-	4	50.0
7	*Botrytis cinerea*	+	+	-	-	+	+	-	-	4	50.0
8	*Cladosporium herbarum*	+	+	+	+	+	+	+	-	7	87.5
9	*Curvularia lunata*	+	+	+	-	+	+	+	-	6	75.5
10	*Claviceps purpurea*	+	-	+	-	-	+	-	-	3	37.5
11	*Drechslera hawaiiensis*	+	+	+	-	+	+	-	-	5	62.5
12	*D. tetramera*	-	-	-	-	+	+	-	-	2	25.0
13	*D. tritici-repentis*	+	+	+	-	-	-	-	-	3	37.5
14	*D. australiensis*	-	-	-	+	+	+	-	-	3	37.5
15	*Fusarium graminearum*	+	+	+	-	+	+	-	-	5	62.5
16	*F. avanaceum*	-	+	-	-	+	+	+	-	4	50.0
17	*F. moniliforme*	-	+	+	-	-	-	-	+	3	37.5
18	*Microdochium nivale*	+	+	+	-	+	+	-	-	5	62.5
19	*Mucor* sp.	+	+	+	+	-	-	-	-	4	50.0
20	*Nigrospora oryzae*	+	+	+	-	+	+	-	-	5	62.5
21	*Penicillium* sp.	+	+	+	-	-	-	+	-	4	50.0
22	*P. oxalicum*	+	+	+	-	+	+	-	-	5	62.5
23	*Rhizoctonia solani*	+	+	-	-	+	+	-	-	4	50.0
24	*Rhizopus* sp.	+	+	+	+	+	+	-	-	6	75.5
25	*Septoria nodorum*	+	+	+	-	+	+	-	-	5	62.5
26	*Trichothecium roseum*	-	+	+	-	+	+	-	-	4	50.0
27	*Trichoderma hamatum*	+	+	+	+	-	-	+	-	5	62.5
28	*Tilletia tritici*	+	+	+	-	+	+	+	+	7	87.5
29	*Ustilago tritici*	-	+	+	+	+	+	-	-	5	62.5
	Total Species Identified	21	25	23	07	20	22	08	02	128	55.20
	%age of each variety	72	86	79	24	69	76	27	7	55	

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

Table 2. Prevalence of identified fungi of wheat varieties from three localities of district Bhimber AJK.

S.No	Fungi Identified	Localities			Total Prevalence (Mean)
		Samahni (Sm)	Bhimber (Bm)	Barnala (Br)	
1	*Aspergillus niger*	86.2	65.2	71.0	74.13^{CD}
2	*A. flavus*	65.3	42.0	56.2	54.5^{F}
3	*Alternaria alternata*	68.1	49.2	50.1	55.8^{F}
4	*A. clamydophora*	44.0	27.0	38.3	36.43^{KL}
5	*A. triticina*	47.1	20.2	26.1	31.13^{MN}
6	*Bipolaris sorokiniana*	52.0	30.1	35.0	39.0^{IJK}
7	*Botrytis cinerea*	39.4	18.0	25.1	27.5^{N}
8	*Cladosporium herbarum*	88.2	59.2	67.2	71.53^{D}
9	*Curvularia lunata*	91.0	72.0	78.0	80.33^{AB}
10	*Claviceps purpurea*	32.2	15.0	19.0	22.07^{O}
11	*Drechslera hawaiiensis*	50.1	41.3	43.0	44.8^{GH}
12	*D. tetramera*	38.0	17.4	25.0	26.8^{NO}
13	*D. tritici-repentis*	49.0	36.3	40.2	41.83^{HIJ}
14	*D. australiensis*	41.0	22.0	36.1	33.03^{LM}
15	*Fusarium graminearum*	94.2	78.1	82.0	84.77^{A}
16	*F. avanaceum*	77.4	50.3	61.0	62.9^{E}
17	*F. moniliforme*	56.0	31.1	47.3	44.8^{GH}
18	*Microdochium nivale*	79.2	54.4	60.2	64.6^{E}
19	*Mucor* sp.	60.4	39.3	48.1	49.27^{G}
20	*Nigrospora oryzae*	81.0	67.1	69.4	72.5^{CD}
21	*Penicillium* sp.	35.1	18.4	27.0	26.83^{NO}
22	*P. oxalicum*	34.0	21.0	30.2	28.4^{MN}
23	*Rhizoctonia solani*	89.4	66.4	74.0	76.6^{BCD}
24	*Rhizopus* sp.	87.2	68.4	72.0	75.87^{BCD}
25	*Septoria nodorum*	52.0	35.2	43.0	43.4^{HI}
26	*Trichothecium roseum*	76.1	54.0	62.0	64.03^{E}
27	*Trichoderma hamatum*	44.3	30.2	37.0	37.17^{JKL}
28	*Tilletia tritici*	93.2	78.2	81.0	84.13^{A}
29	*Ustilago tritici*	90.2	69.2	72.2	77.2^{BC}
Total prevalence (Average)		63.49^{A}	44.01^{C}	50.92^{B}	52.81

Key: Capital letters (A, B….etc) on total calculated values show the significance difference among fungal species and selected localities

Table 3. Total Infection of Mycoflora Associated with Eight Wheat Varieties of District Bhimber, AJK.

S/N	Detected Fungi	Wheat Varieties With Their Infection Rates (%age)								
		V1	V2	V3	V4	V5	V6	V7	V8	Total Infection
1	*Aspergillus niger*	23.25	22.0	22.5	0.0	0.0	20.5	12.0	0.0	12.53^{ABCD}
2	*A. flavus*	20.5	19.0	18.0	0.0	0.0	17.25	9.5	0.0	10.53^{ABCD}
3	*Alternaria alternata*	18.5	0.0	0.0	1.5	17.5	13.75	0.0	0.0	6.41^{DE}
4	*A. clamydophora*	0.0	22.5	21.5	0.0	19.0	0.0	0.0	0.0	7.87^{CDE}
5	*A. triticina*	0.0	32.0	28.25	0.0	23.75	0.0	0.0	0.0	10.5^{ABCD}
6	*Bipolaris sorokiniana*	28.75	26.5	21.75	0.0	0.0	17.25	0.0	0.0	11.78^{ABCD}
7	*Botrytis cinerea*	32.0	23.0	0.0	0.0	21.75	19.5	0.0	0.0	12.03^{ABCD}
8	*Cladosporium herbarum*	22.75	19.5	19.0	5.0	17.5	15.25	4.25	0.0	12.91^{ABCD}
9	*Curvularia lunata*	27.5	23.75	22.5	0.0	22.0	19.0	13.25	0.0	16.0^{ABC}
10	*Claviceps purpurea*	14.25	0.0	14.0	0.0	0.0	12.0	0.0	0.0	5.03^{EF}
11	*Drechslera hawaiiensis*	11.0	10.0	10.75	0.0	7.75	9.25	0.0	0.0	6.09^{DE}
12	*D. tetramera*	0.0	0.0	0.0	0.0	13.75	11.0	0.0	0.0	3.09^{F}
13	*D. tritici-repentis*	38.5	32.0	24.0	0.0	0.0	0.0	0.0	0.0	11.81^{ABCD}
14	*D. australiensis*	0.0	0.0	0.0	2.25	25.5	18.0	0.0	0.0	5.72^{EF}
15	*Fusarium graminearum*	30.5	31.0	28.75	0.0	30.75	24.75	0.0	0.0	18.22^{A}
16	*F. avanaceum*	0.0	33.75	0.0	0.0	25.5	21.5	14.5	0.0	11.91^{ABCD}
17	*F. moniliforme*	0.0	38.75	33.0	0.0	0.0	0.0	0.0	14.0	10.72^{ABCD}
18	*Microdochium nivale*	19.0	16.5	21.25	0.0	15.75	15.5	0.0	0.0	11.0^{ABCD}
19	*Mucor* sp.	22.0	20.0	20.5	12.5	0.0	0.0	0.0	0.0	9.37^{BCD}
20	*Nigrospora oryzae*	27.5	23.25	22.25	0.0	22.0	18.0	0.0	0.0	14.12^{ABC}
21	*Penicillium* sp.	10.25	10.0	8.0	0.0	0.0	0.0	0.75	0.0	3.62^{F}
22	*P. oxalicum*	18.75	16.5	15.75	0.0	13.25	13.0	0.0	0.0	9.66^{BCD}
23	*Rhizoctonia solani*	35.5	30.75	0.0	0.0	33.0	28.0	0.0	0.0	15.91^{ABC}
24	*Rhizopus* sp.	31.75	25.0	23.25	14.0	20.25	12.5	0.0	0.0	15.84^{ABC}
25	*Septoria nodorum*	21.5	16.0	15.75	0.0	14.0	11.25	0.0	0.0	9.81^{BCD}
26	*Trichothecium roseum*	0.0	27.0	26.75	0.0	22.75	15.0	0.0	0.0	11.44^{ABCD}
27	*Trichoderma hamatum*	19.5	18.0	16.0	5.75	0.0	0.0	3.0	0.0	7.78^{CDE}
28	*Tilletia tritici*	30.5	30.0	22.5	0.0	23.0	18.75	9.75	6.25	17.59^{AB}
29	*Ustilago tritici*	0.0	35.0	32.75	9.25	27.25	23.5	0.0	0.0	15.97^{ABC}
Total Infection (Mean)		17.3^{B}	20.7^{A}	16.8^{B}	1.7^{E}	14.3^{C}	12.9^{D}	2.3^{E}	0.7^{EF}	10.87

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

Management Strategies: Seed diseases of WV were managed by applying fungicides treatment and biological treatment. In this research, seven different fungicides were used for seed treatment before sowing. These fungicides reduced fungal infections and improved yields. The impact of fungicides on germination rate of wheat varieties were calculated and documented in Table 4. Fungicide Thiabendazole (F4) showed maximum germination rate (58.44^{A}) and gradually reduced the germination rate as Benlate (56.84^{AB}), Triticonazole (55.78^{BC}), Difenoconazole (54.19^{CD}), Strobilurins (53.25^{D}), Thiram (48.00^{E}) and Triazoles (46.75^{F}). This means that the fungicide F4 is more effective to combat and reduce fungal diseases from surfaces of wheat seeds. On the other hand, after fungicides

treatment wheat variety V4 (Faisalabad-2008) and V8 (Galaxy-2013) had showed maximum germination rate (69.67^{A}, 70.92^{A}) as compared to others. Therefore these two varieties were more suitable for producing better yields in District Bhimber Azad Kashmir (Table 4). The fungicides treatment against wheat diseases were also applied by Sharf *et al*., (2011). They also obtained better yield of wheat after fungicide treatment. The fungicides treatment showed some harmful impacts on environment as well as on human health. Therefore biocontrol methods are better for fungal control as compared to fungicides. Similar control measures were applied by Mathur *et al*., (1993).

To explore the impacts of isolated mycoflora on wheat seed germination in different treatments, an analysis of variance (ANOVA) was formulated. Its statistical data indicated that the effect of different fungal species was quite dynamic and variable not only for taxa differences but also with climatic variations (Table 2). For further comprehensive analysis least standard deviation (LSD) and DMR test was determined for all the sampling sites in comparison with all available fungal species using different treatments during seed germination (Table 5). In this context, six different management strategies were used for the control of fungal infection on seed before sowing. Plant extracts were more suitable because plant extracts showed maximum germination rates (79.5^{A}, 81.6^{A} and 78.2^{A}) in three tehsils of district Bhimber Azad Kashmir as compared to other treatments. Similarly fungicides treatment germination rate was 77.3^{A} followed by garlic extracts (75.8^{B}), plant latex (71.1^{C}), distilled water (62.3^{D}) and hot water treatment (63.3^{D}) as indicated in Table 5. Plant extracts and fungicides treatment indicated best germination. These two are not significantly different from each other. But Plants extract did not show side effects on wheat crop. Secondly, it is cheap and easy treatment for farmer. There this biological technique is more suitable for better wheat disease control/fungal spore before sowing. Islam & Razia, (2009) were also measured infection rate of wheat seed fungi. Pathak *et al*., (2013) and Majumder *et al*., (2013) analyzed the wheat management strategies and compiled results which were similar to the current research work. They described that biological treatment is better than fungicides treatment, because it has no hazardous impacts on human health and on environment.

Wheat variety V4 (Faisalabad- 2008), V7 (Punjab-2011) and V8 (Galaxy-2013) were prevailed minimum seeds infection with fungi as shown in Fig 1. Therefore these three varieties are cultivated dominantly in district Bhimber for producing better yield of wheat. The impacts of fungicides on germination rate of wheat varieties were calculated and documented in Fig. 2. Similar resulted have produced in past work of Iram & Ahmad, 2005 and our findings are supportive of those results.

These three selected areas showed maximum germination rate of wheat varieties V7 and V8. Although, these three wheat varieties (Faisalabad-2008, Punjab-2011 and Galaxy-2013) showed better controls of mycoflora and produced better yields in the selected area. Therefore these three varieties should be cultivated in district Bhimber Azad Kashmir for better yields in future.

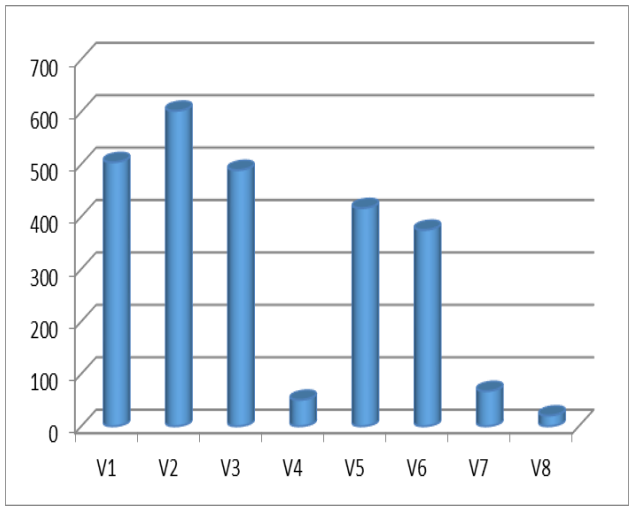

Fig. 1. Wheat varieties with their infection rates (%) collected from district Bhimber Azad Kashmir, Pakistan

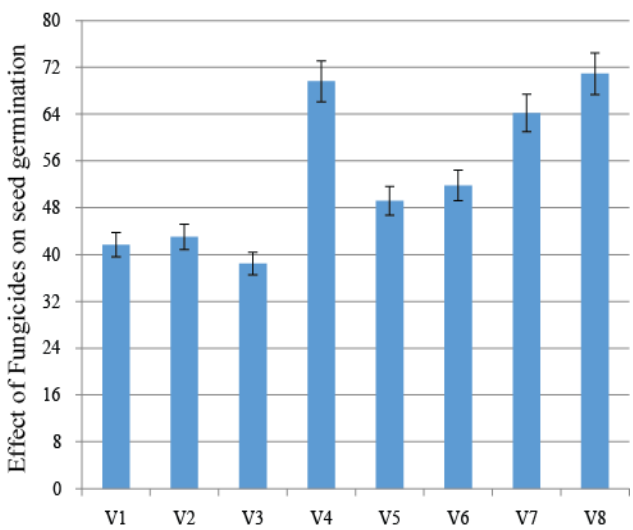

Fig. 2. Effect of Fungicides treatment on seed germination rate of eight wheat varieties cultivated in district Bhimber Azad Kashmir, Pakistan

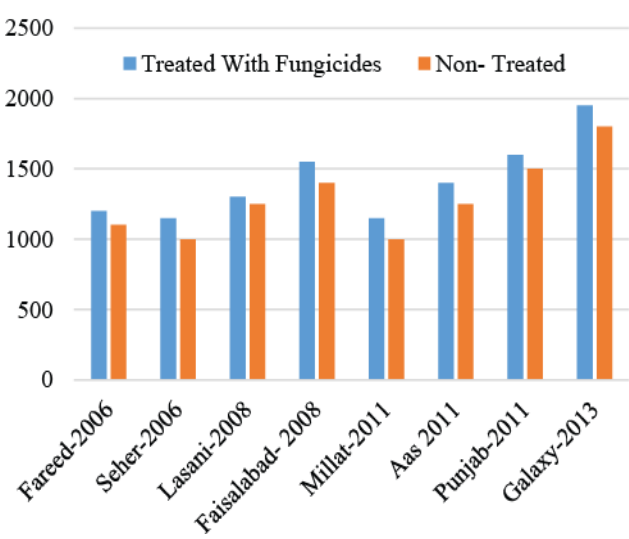

Fig. 3. Comparative analysis of treated and untreated seed samples of wheat in field trails and their effect on yields in district Bhimber Azad Kashmir, Pakistan

Table 4. Fungicides treatments on seeds of 8 wheat varieties in laboratory and measured germination rate (%age) from Bhimber district Azad Jammu and Kashmir, Pakistan.

S/No	Fungicides	Wheat Varieties with Seed Germination Rate (Percentage Value)								Means
		V1	V2	V3	V4	V5	V6	V7	V8	
1	F1	45.25	46.50	38.25	75.00	54.00	55.75	65.00	75.00	56.84^{AB}
2	F2	42.00	44.00	40.00	70.25	50.25	52.25	62.75	72.00	54.19^{CD}
3	F3	41.75	42.75	38.75	68.50	48.75	50.00	65.00	70.50	53.25^{D}
4	F4	50.25	48.25	42.00	74.00	53.00	56.00	68.00	76.00	58.44^{A}
5	F5	39.75	40.50	36.75	65.25	44.25	48.00	61.50	66.75	48.00^{E}
6	F6	42.50	43.00	43.25	72.00	52.00	54.25	67.00	72.25	55.78^{BC}
7	F7	30.75	36.25	30.75	62.75	42.50	46.75	60.25	64.00	46.75^{F}
Average		41.7^{E}	43.0^{E}	38.5^{F}	69.6^{A}	49.2^{D}	51.8^{C}	64.2^{B}	70.9^{A}	53.32

Key: F1= Benlate, F2= Difenoconazole, F3= Strobilurins, F4= Thiabendazole, F5= Thiram, F6= Triticonazole, F7= Triazoles

Table 5. Effect of different treatments on seed germination (%age) of eight wheat varieties from different localities of Bhimber, Azad Kashmir.

S/No	Locations	Wheat Variety	Different management Strategies						Means
			Plant Extracts	Plants Latex	Garlic Extracts	Distilled Water	Hot Water	Fungicides	
1	Samahni	V1	66.8	61.3	65.3	50.1	49.4	65.6	59.7
		V2	75.0	66.4	73.5	57.3	56.8	74.6	67.3
		V3	73.4	66.8	70.1	61.7	60.2	72.6	67.5
		V4	88.6	82.7	85.5	70.2	70.1	87.8	80.8
		V5	71.2	58.9	68.5	52.2	50.2	69.7	61.8
		V6	76.7	73.5	75.2	66.8	68.7	74.5	72.6
		V7	92.2	83.5	90.2	73.5	72.3	92.7	84.1
		V8	92.7	84.7	88.5	73.7	71.9	91.0	83.8
Means			79.5^{A}	72.2^{B}	77.1^{A}	63.2^{C}	62.4^{CD}	78.6^{A}	**72.2^{B}**
2	Bhimber	V1	71.7	65.1	73.5	56.8	53.3	75.2	65.9
		V2	78.5	70.1	79.5	65.0	63.3	79.8	72.7
		V3	76.0	71.9	73.2	64.0	64.8	75.1	70.8
		V4	90.2	84.1	88.4	72.0	71.8	89.3	82.6
		V5	73.9	63.4	71.9	58.8	56.7	74.2	66.5
		V6	78.3	75.2	76.2	71.7	71.0	78.8	75.2
		V7	92.7	85.5	90.8	75.4	74.8	92.7	85.3
		V8	92.2	85.3	90.3	74.7	75.4	90.7	84.8
Means			81.6^{A}	75.1^{B}	80.4^{A}	67.3^{C}	66.4^{C}	81.9^{A}	**75.5^{A}**
3	Bernala	V1	68.1	63.4	67.1	51.8	51.2	68.8	61.7
		V2	73.5	69.3	71.7	59.6	69.3	73.1	69.4
		V3	72.3	64.7	67.7	60.6	59.7	71.7	66.1
		V4	86.8	80.2	83.8	68.7	68.3	86.7	79.1
		V5	70.2	56.3	67.0	50.2	49.4	63.3	59.4
		V6	75.2	71.3	73.5	65.0	65.3	74.8	70.8
		V7	90.1	81.7	88.0	72.0	71.3	90.0	82.2
		V8	89.3	82.0	87.7	70.8	71.8	89.7	81.9
Means			78.2^{A}	71.1^{C}	75.8^{B}	62.3^{D}	63.3^{D}	77.3^{A}	**71.3^{C}**

Capital letters (A, B….etc) show significance difference among three localities wheat varieties

Conclusions

From these findings it is concluded that the seed health testing is a basic need to avoid crop failure and it is desirable that seeds of crop plants should invariably be tested for seed health before sowing so as to assess spreading of pathogens in wheat growing areas and be treated with fungicides and biological treatments to attain maximum yield of wheat crops. In current research attention has been given to use biological products for seed treatment to protect them against seed-borne pathogens. Chemical fungicides can control the plant disease, but they produce bad effects on plants, animals and human health and also create bad effects on our surrounding 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 seed-borne pathogens and for the improvement of seed quality and emergence of plants, so as to obtain healthy and strong plants as well as better yields of different varieties of wheat crop. Therefore, it is recommended that biological treatment of wheat fungal diseases for obtaining better yields of wheat crop in future for the selected area.

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