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Original article

Detection of seed-borne fungal pathogens associated with wheat (*Triticum aestivum* L.) seeds collected from farmer fields and grain market

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ABSTRACT

Background: The plant pathogens significantly affect quality and marketability of several cereal crops. Of these, seed-borne phytopathogens are responsible for quality and quantity losses in crops on commercial scale. The aim of this study was to isolate and detect the seed-borne mycoflora from seed lots of various bread wheat (*Triticum aestivum* L.) genotypes widely grown in Bahawalpur division of south Punjab, Pakistan.

Methods: Eleven commercial wheat cultivars/genotypes were investigated through standard blotter paper method. The samples were randomly collected from farmer fields and grain markets. The collected samples were brought to the laboratory and analyzed with different methods to detect the mycoflora.

Results: Twenty-one fungal species were recovered from seeds lots of diversified gene pool. The most frequently isolated fungi were *Alternaria alternata*, *Fusarium monoliformae*, *Aspergillus flavus*, *Helminthosporium* spp., *Curvularia* spp., *Bipolaris sorokiniana*, *Phoma* spp. and *Penicillium spp*. The one devasting fungal specie *Alternaria alternata* (88.8%) was the dominating species in all wheat genotypes included in the study.

Conclusion: These results divulge the presence of fungal pathogens in all wheat cultivars and could exert adverse effect on their allometric traits. So, there is a potential need to control these seed borne pathogens to minimize the crop yield losses.

1. Introduction

Wheat (*Triticum aestivum* L.) is a cereal crop and used as a staple food in several parts of the world. It is an essential cereal crop for the people of Pakistan and grown during winter from under irrigated conditions with water necessities range from 20 to 21 acre feet and is categorized first as vital food crop followed by rice and maize (Hussain et al., 2012). It yields the highest grain production as compared to any other crop in the world (Gulbitti-Onarici et al., 2009; Lamoureux et al., 2005). Foliar infections caused by

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fungal pathogens often have a negative impact on the yields of wheat. Necrotic regions appear on the leaf because of an infestation, and it may be challenging to identify the primary causal pathogen based only on the apparent symptoms, particularly in the case of two strains.

Wheat crop is affected by number of seed-borne pathogens, which can reduce its global production to significant extent. In Pakistan, 50 seed-borne phytopathogens are known to infest wheat. These phytopathogens, i.e., *Alternaria alternata, Fusarium graminearum, Curvularia lunata, Bipolaris sorokinaina, Drechsleria, Helminthosporium sativum, Aspergillus* and *Penicillium* are commonly account for mycoflora of wheat seeds. The wheat seeds affected by black point diseases involve *Alternaria alternata, Curvularia lunata, Fusarium graminearum* and *Cladosporium. Bipolaris sorokinaina* causes spot blotch in cereal grains and grasses, as it is the seed and soil-borne pathogen of wheat (Abdul Rehman, 2011; Hussain et al., 2013; Mehboob et al., 2015; Raza et al., 2014).

The awareness of the associated mycoflora with the specific frequency on commercially grown varieties runs with the source to access the threat related with unwanted organisms (Dinesh et al., 2015). For the controlling of plant diseases, the main step is to use disease-free and certified seeds. The germination test (Ozaslan et al., 2017) of seeds is substantial step in detecting the seed borne pathogen linked with wheat seeds and offers valuable information about mycoflora and their effective control (Anna, 2016; Majumder et al., 2013; Pathak and Zaidi, 2013).

The aim of study was to detect the mycoflora linked with wheat grains of eleven different wheat varieties available in the market by following International Seed Testing Association (ISTA) techniques. The results would help to provide the empirical information on the most infested genotypes and possible measures to control the pathogens associated with each genotype.

2. Materials and methods

The current study was conducted in the laboratory of Plant Pathology, Regional Agricultural Research Institute, Bahawalpur, Pakistan. Isolation, multiplication, and identification of fungi was carried out by using standard laboratory protocols as followed by ISTA.

2.1. Collection of wheat samples from grain market

The seed samples of eleven wheat genotype (i.e., 'Gold-2016', 'Shafaq-2006', 'Millat-2011', 'Lasaani-2008', 'Punjab-2011', 'Sahar-2006', 'TD1', 'Bhakar-2002', 'Fareed-2006', 'Galaxy-2013' and 'Aas-2011') were collected from six different wheat cultivated areas of Bahawalpur division of south Punjab in Pakistan. Similarly, three random samples were collected from local grain market according to standard sampling technique. The standard samples of 400 gm seed size were stored in craft paper bags and labeled.

2.2. Techniques used for isolation and detection of seed-borne mycoflora

For the isolation and detection of seed-borne mycoflora, the blotter paper (Habib et al., 2011; Mancini et al., 2016; Toma and Abdulla, 2013) and Agar plate method (Demeke et al., 2005; Ko et al., 2005) was followed. The collected seeds were surface sterilized with Clorox 1 % solution for 1–3 min, subsequently washed thrice with double distilled water (Butt et al., 2011; Chen et al., 2012; Habib et al., 2011).

2.3. Blotter paper method

The blotter paper method is the most popular and frequently used for the detection of several fungi, which can produce mycelial growth and fruiting structures during incubation. This method is most frequently used for the detection of seed-borne diseases. All collected seeds were plated in plastic Petri dishes, each having three layers of water soaked filter papers. The nine seeds per plate were incubated for seven days at 20–22 °C, for the period of 12 h under near the ultraviolet (NUV) light or in darkness. All slides were examined under the stereo-binocular microscope to detect the presence of mycoflora inside the seed (Abdullah and Atroshi, 2016; Butt et al., 2011).

2.4. Agar plate method

The ager plate method is used to identify and detect the microorganisms associated with seed bases on nutrient ager. The potato dextrose agar (PDA) was used in this method for the isolation of mycoflora. To obtain a better and reliable growth for various wheat mycoflora, PDA platting technique (Ahmad and Pathak, 2016; Tanveer et al., 2013) was followed. The modified PDA having (20 g potato extract, 20 g agar and 20 g dextrose) was prepared. All the medium was autoclaved and poured in 20 ml glass Petri dishes of 9-cm diameter. The nine seeds in each dish were plated with the help of sterile forceps. All Petri dishes were kept for 6 days at 20 °C under 12 h alternative cycles of near ultraviolet light and darkness. All the slides were examined under the stereoscopic microscope to observe the mycoflora inside the seed. Radial growth percentage of mycoflora prevalent inside the seed lots was also examined. The percentage and relative frequency of fungus was calculated by formula as described by Association of official seed analysts (Tanveer et al., 2013)

$$\text{Frequency of the fungus} = \frac{\text{Number of seeds containing a particular fungus}}{\text{Total seeds used}} \times 100$$

$$\text{Relative abundance of the fungi} = \frac{\text{Total no. of colonies of a fungus on seed}}{\text{Total number of colonies of all fungus}} \times 100$$

2.5. Identification of Soil-borne fungal microbes

All the corresponding samples were shifted into sterile Petri dishes for further analysis. All the cultured fungi were placed on slides for identification. All the slides covered with cover slip were stained with lactophenol-cotton blue to identify and measure the fungal structures. The identification and measurement of fungal structure with its colony morphology, spore formation and spore characteristics were examined under microscope and compared with already published literatures, identification keys and reference books (Guler et al., 2016; McClenny, 2007; Pornsuriya et al., 2008; Rønhede et al., 2005; Sanjotha et al., 2011; Tambekar and Wate, 2007).

3. Results

All fungi species associated with wheat samples were isolated by blotter paper method. The radial growth of all the isolated fungus were accomplished by PDA and its observance was by Ager plate method. The frequently isolated species included *Alternaria alternata, Fusarium* spp., *Curvularia lunata, Penicillium, Aspergillus, Helminthosporium, Drechslera, Stemphylium and Phoma* (Fig. 1).

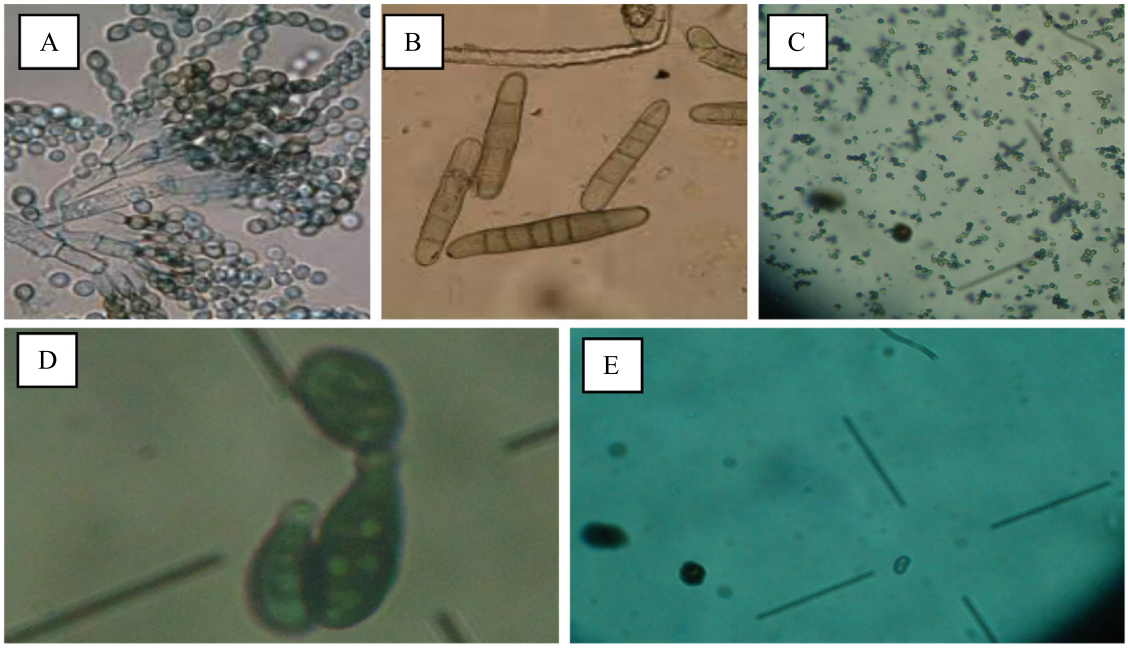

Fig. 1. Mycoflora isolated from different wheat varieties, *Penicillium* (A), *Drechslera* (B), *Aspergillus* (C), *Alternaria alternata* (D) and *Phoma* (E).

From wheat cultivar 'Gold-2016', twenty-one fungal species were isolated including *Alternaria alternata, A. tenuis, A. sesmicola, Fusarium monoliformae, Stemphylium, Phyllosticta, Curvularia, Hetrosporium, Penicillium and Cercospodium*. The most frequently isolated species were *A. alternata, A. sesmicola, Phyllosticta and Fusarium monoliformae* (Table 1). The *A. alternata, A. tenuis, Curvularia, Aspergillus, Penicillium, Fusarium monoliformae, Fusarium semitectum, Fusarium graminearum, Stemphylium* and *Bipolaris sorokiniana* were isolated from genotype 'TD1'. Most frequently isolated pathogens were *A. alternata, A. tenuis, Bipolaris sorokiniana, Fusarium semitectum, F. monoliformae and Aspergillus* (Table 1).

The isolated fungal species from the cultivar 'Millat-2011' were *A. alternata, A. tenuis, Phoma, Bipolaris sorokiniana, Stemphylium, Phyllosticta, Gonatoboryum, F. monoliformae, Curvularia* and *Penicillium*. The most frequently isolated fungi included *A. alternata, A. tenuis, Phyllosticta* and *F. monoliformae* (Table 2). In 'Shafaq-2011' cultivar, the isolated pathogens include *A. alternata, A. tenuis, Bipolaris, F. monoliformae, F. graminearum, F. oxysporum, Helminthosporium, Curvularia* and *Stemphylium*. The most frequently isolated pathogens were *A. alternata, A. tenuis* and *F. oxysporum* (Table 2).

The pathogens identified from cultivar 'Fareed-2006' were *A. alternata, A. tenuis, F. monoliformae, F. graminearum, B. sorokiniana, Drechslera, Curvularia, Helminthosporium* and *Penicillium*. The most

Table 1
Mycoflora isolated from 'Gold-2016' and 'TD1' genotypes included in the study.

Sr. No.	Pathogens isolated	S1	S2	S3	S4	S5	S6	S7	S8	S9	%
Gold 2016											
1	*Alterneria tenuis*	+	+	–	+	+	+	+	–	+	77.7
2	*Alterneria sesmicola*	+	+	+	+	–	+	+	+	+	88.8
3	*Alterneria alternate*	–	+	+	+	+	+	+	+	+	88.8
4	*Hetrosporium*	+	+	–	–	+	–	–	–	+	44.4
5	*Cercospodium*	+	+	+	+	–	+	–		–	55.5
6	*Curvularia*	–	+	–	+	–	+	+	+	+	66.6
7	*Phyllosticta*	+	–	+	+	+	–	+	+	+	77.7
8	*Stemphylium*	–	+	–	+	+	–	–	+	+	55.5
9	*Fusarium monoliformae*	+	+	+	–	+	+	+	–	–	66.6
10	*Pencillium*	–	+	+	–	–	+	+	–	–	44.4
TD1											
1	*Fusarium semitectum*	+	–	+	–	+	–	+	+	+	66.6
2	*Fusarium monoliformae*	+	+	–	+	+	+	–	+	+	77.7
3	*Fusarium graminearum*	+	–	+	+	–	–	+	–	–	44.4
4	*Alternaria alternate*	+	+	+	+	+	+	–	+	+	88.8
5	*Drechslera*	–	+	+	–	–	–	+	+	–	44.4
6	*Alternaria tenuis*	+	+	–	+	+	+	+	+	+	88.8
7	*Bipolaris sorokiniana*	+	+	+	–	–	+	+	+	+	77.7
8	*Curvularia*	+	–	–	+	+	–	+	+	+	66.6
9	*Stemphylium*	+	+	–	–	–	+	–	–	–	33.3
10	*Pencillium*	–	–	+	–	–	+	–	+	–	33.3
11	*Aspergillus*	+	–	+	+	+	+	+	–	+	77.7

S = Sample, + = Presence of particular fungus, – = Absence of particular fungus, The S1-S6 denote samples collected from field, whereas S7-S9 are the samples collected from grain market.

Table 2
Mycoflora isolated from 'Millat-2011' and 'Shaafaq-2011' wheat genotypes included in the study.

Sr. No.	Pathogens isolated	S1	S2	S3	S4	S5	S6	S7	S8	S9	%
Millat-2011											
1	*Alterneria alternate*	+	+	+	+	+	+	+	–	+	88.8
2	*Alterneria tenuis*	+	+	+	–	+	+	–	+	+	77.7
3	*Bipolaris*	+	+	–	+	–	+	+	+	–	66.6
4	*Stemphylium*	–	+	+	–	+	–	+	+	+	66.6
5	*Phoma*	+	+	–	+	–	+	–	–	+	55.5
6	*Phyllosticta*	+	–	+	+	+	–	+	+	+	77.7
7	*Gonatoboryum*	+	+	–	+	–	+	+	–	–	55.5
8	*Fusarium monoliformae*	+	–	+	–	+	–	+	+	+	66.6
9	*Curvularia*	+	–	+	+	–	–	–	+	–	44.4
10	*Pencillium*	+	+	–	–	–	+	+	–	–	44.4
Shaafaq-2011											
1	*Alterneria alternate*	+	+	+	+	+	+	+	+	–	88.8
2	*Alterneria tenuis*	+	+	–	+	+	+	+	+	+	77.7
3	*Bipolaris sorokiniana*	–	–	+	–	+	–	+	+	+	55.5
4	*Fusarium monoliformae*	+	+	–	+	–	–	+	–	+	55.5
5	*Fusarium gramiearum*	–	–	+	+	+	+	–	+	+	66.6
6	*Fusarium oxysporum*	+	–	–	–	+	+	–	–	–	33.3
7	*Helminthosporium*	–	+	+	+	+	+	+	+	+	88.8
8	*Curvularia*	+	–	–	+	+	+	+	+	–	66.6
9	*Stemphylium*	+	+	–	+	+	+	–	–	+	66.6

S = Sample, + = Presence of particular fungus, – = Absence of particular fungus, The S1-S6 denote samples collected from field, whereas S7-S9 are the samples collected from grain market.

frequently isolated pathogens were *A. alternata, A. tenuis, Fusarium spp* and *Drechslera* (Table 3). Similarly, the isolated pathogens from 'Sehaar-2006', include *A. alternata, Helminthosporium, Brasilaria, Paecilomyces, Sclers, Curvularia, Stemphylium* and *B. sorokiniana.* The most frequently isolated pathogens include *A. alternata, Curvularia* and *B. sorokiniana* (Table 3).

The isolated pathogens from 'Lasaani-2008' cultivar were *A. alternata, A. tenuis, A. sesmicola, F. semitectum, F. monoliformae, and B. sorokiniana, Trincrium, Drechslera* and *Helminthosporium.* The most frequently isolated pathogens in this cultivar included *A. spp, F. monoliformae and Helminthosporium* (Table 4). From 'Punjab-11', the isolated pathogens included *A. alternata, A. tenuis, Sclerochia, Drechslera, F. monoliformae, Stemphylium, Helminthosporium, Curvularia* and *B. sorokiniana.* The most frequently isolated fungi included *Alternaria spp, F. monoliformae* and *Helminthosporium* (Table 4).

From 'Bhakkar-2002' wheat cultivar, the isolated fungi included *A. alternata, A. tenuis, A. sesmicola, F. monoliformae, F. graminearum, B. sorokiniana, Curvularia. Helminthosporium, Penicillium and Aspergillus.* The most frequently isolated fungi were *Alternaria* spp. and *Bipolaris sorokiniana* (Table 5). In 'Galaxy-2013', the isolated fungi included *Fusarium monoliformae, F. nivale, A. alternata, A. tenuis, Phoma, B. sorokiniana, Helminthosporium and Drechslera.* The most frequently isolated pathogens included *A. alternata, F. monoliformae* and *B. sorokiniana* (Table 5).

Furthermore, the isolated seed borne pathogens from 'Aas-2011' included *A. alternata, A. tenuis, Phoma, B. sorokiniana, Stemphylium, Phyllosticta, Gonatoboryum, F. monoliformae, Curvularia* and *Penicillium.* The most frequently isolated pathogens were *A. alternata, Penicillium, F. monoliformae* and *B. sorokiniana* (Table 6).

4. Discussion

During this study of seed-borne pathogens, different species of mycoflora, i.e., *Alterneria alternata, Alterneria tenuis, Fusarium monoliformae, Fusarium graminearum, Bipolaris sorokiniana, Curvu-*

Table 3
Mycoflora isolated from 'Fareed-2006' and 'Sehar-2006' genotypes included in the study.

Sr. No.	Pathogens isolated	S1	S2	S3	S4	S5	S6	S7	S8	S9	%
Fareed-2006											
1	*Alterneria alternata*	+	+	+	+	+	+	–	+	+	88.8
2	*Alterneria tenuis*	+	+	+	+	+	+	+	+	–	88.8
3	*Fusarium monoliformae*	+	–	+	+	–	+	+	–	+	66.6
4	*Fusarium gramiearum*	+	–	+	+	–	+	–	+	+	66.6
5	*Bipolaris sorokiniana*	+	+	–	–	+	–	+	+	–	55.5
6	*Helminthosporium*	+	+	–	–	+	–	–	+	–	44.4
7	*Curvularia*	+	–	+	–	–	–	–	–	–	22.2
8	*Drechsleria*	+	–	+	+	+	–	–	+	+	66.6
9	*Pencillium*	+	–	+	–	+	–	–	+	+	55.5
Sehar-2006											
1	*Alterneria alternata*	+	+	+	+	+	+	–	+	+	88.8
2	*Helminthosporium*	+	+	+	–	–	+	+	–	+	66.6
3	*Brasilaria*	–	+	–	–	–	–	+	–	+	33.3
4	*Paecilomyces*	+	–	–	+	+	+	–	+	+	66.6
5	*Sclers*	+	+	–	–	–	+	–	+	–	44.4
6	*Curvularia*	+	–	+	+	+	+	+	+	+	88.8
7	*Stemphylium*	+	–	+	+	+	+	+	+	+	88.8
8	*Bipolaris sorokiniana*	+	+	+	+	+	+	+	–	–	77.7

S = Sample, + = Presence of particular fungus, – = Absence of particular fungus, The S1-S6 denote samples collected from field, whereas S7-S9 are the samples collected from grain market.

Table 4
Mycoflora isolated from 'Lasani-2008' and 'Punjab-2011' wheat genotypes included in the study.

Sr. No.	Pathogens isolated	S1	S2	S3	S4	S5	S6	S7	S8	S9	%
Lasani-2008											
1	*Alterneria alternate*	+	+	+	+	+	+	+	–	+	88.8
2	*Alterneria tenuis*	+	+	+	+	+	+	+	+	–	88.8
3	*Alterneria sesmicola*	+	+	–	+	+	+	+	+	+	77.7
4	*Fusarium semitectum*	+	+	–	–	+	+	–	+	+	66.6
5	*Fusarium monoliformae*	–	+	+	+	+	–	+	+	+	77.7
6	*Bipolaris sorokiniana*	+	–	+	+	–	+	+	–	–	55.5
7	*Trincrium*	+	+	+	–	+	–	–	–	+	55.5
8	*Drechslera*	+	+	+	–	–	–	+	–	–	44.4
9	*Helminthosporium*	+	–	+	+	+	+	+	+	+	88.8
Punjab-2011											
1	*Alterneria alternata*	+	+	+	+	+	+	–	+	+	88.8
2	*Sclerochia*	+	+	+	–	+	–	+	+	+	77.7
3	*Drechsleria*	–	+	–	–	+	–	+	–	+	44.4
4	*Stemphylium*	+	–	+	+	+	–	–	+	–	55.5
5	*Helminthosporium*	+	+	–	+	+	+	+	+	+	88.8
6	*Alterneria tenuis*	+	–	+	+	+	+	+	+	+	88.8
7	*Fusarium monoliformae*	+	–	+	+	+	+	+	+	+	88.8
8	*Bipolaris sorokiniana*	–	+	+	+	–	+	+	+	–	66.6
9	*Curvularia*	+	–	+	+	–	+	+	+	+	77.7

S = Sample, + = Presence of particular fungus, – = Absence of particular fungus.

Table 5
Mycoflora isolated from 'Bhakar-2002' and 'Galaxy-2013' wheat genotypes included in the current study.

Sr. No.	Pathogens isolated	S1	S2	S3	S4	S5	S6	S7	S8	S9	%
Bhakar-2002											
1	*Alterneria alternate*	+	+	+	+	–	+	+	+	+	88.8
2	*Alterneria tenuis*	+	–	+	+	–	+	+	+	+	77.7
3	*Alterneria sesmicola*	+	–	+	+	–	–	+	+	–	55.5
4	*Fusarium monoliformae*	+	+	–	–	+	–	+	–	+	55.5
5	*Fusarium gramiearum*	+	+	–	–	+	–	–	+	–	44.4
6	*Bipolaris sorokiniana*	–	+	+	+	–	+	+	+	+	77.7
7	*Curvularia*	+	–	–	–	–	+	+	–	+	44.4
8	*Helminthosporium*	+	–	+	–	+	–	+	–	–	44.4
9	*Aspergillus*	+	–	+	+	+	–	–	+	+	66.6
10	*Pencillium*	+	–	+	–	+	–	–	+	+	55.5
Galaxy-2013											
1	*Fusarium monoliformae*	+	+	+	+	–	+	–	+	–	66.6
2	*Fusarium nivale*	+	+	+	–	–	–	–	–	–	33.3
3	*Alternaria alternata*	+	+	–	+	+	+	+	+	+	88.8
4	*Alternaria tenuis*	+	–	–	–	+	+	+	+	+	66.6
5	*Phoma*	–	–	+	–	+	+	–	–	–	33.3
6	*Bipolaris sorokiniana*	+	+	+	+	+	–	+	–	+	77.7
7	*Helminthosporium*	–	–	–	+	–	+	+	–	+	44.4
8	*Drechsleria*	+	+	+	–	+	+	–	–	+	66.6

S = Sample, + = Presence of particular fungus, – = Absence of particular fungus, The S1-S6 denote samples collected from field, whereas S7-S9 are the samples collected from grain market.

Table 6
Mycoflora isolated from 'Aas-2011' wheat genotype included in the study.

Sr. No.	Pathogens isolated	S1	S2	S3	S4	S5	S6	S7	S8	S9	%
1	*Alterneria alternate*	+	+	+	+	+	+	–	–	+	77.7
2	*Alterneria tenuis*	+	–	–	+	–	+	+	+	+	66.6
3	*Bipolaris*	+	+	+	–	+	–	+	+	–	66.6
4	*Stemphylium*	–	+	+	–	–	+	+	+	–	55.5
5	*Phoma*	+	–	–	+	–	+	–	–	+	44.4
6	*Phyllosticta*	+	–	–	+	+	–	–	–	+	44.4
7	*Gonatoboryum*	+	–	+	+	+	–	+	–	–	55.5
8	*Fusarium monoliformae*	+	+	+	+	+	+	+	–	+	88.8
9	*Curvularia*	–	+	+	–	+	+	+	–	+	66.6
10	*Pencillium*	+	+	–	–	+	+	+	+	+	77.7

S = Sample, + = Presence of particular fungus, – = Absence of particular fungus, The S1-S6 denote samples collected from field, whereas S7-S9 are the samples collected from grain market.

laria, Helminthosporium, Aspergillus, Penicillium, Phoma, Stemphylium, Trincrium, Drechslera, Brasilaria, Paecilomyces and *Sclers* were identified and isolated. This study was in line with the findings of Hussain et al. (2013), who isolated seed-borne mycoflora including *A. alternata, B. sorokiniana, Aspergillus flavus* and *Aspergillus niger* from different commercial wheat varieties. Similarly, the genotypes 'Gold-2016', 'TD1', 'Shafaq-2011' and 'Millat-2011' were mostly infected by *A. alternata* (88 %), *A. tenuis* (77 %),

Helminthosporium (70 %), *Curvularia* (77 %), *F. monoliformae* (60 %), *Aspergillus* (44 %) *and Penicillium* (55 %). Remaining genotypes including 'Punjab-11', 'Sehar-2006', 'Lasani-2008', 'Fareed-2006', 'Aas-2011', 'Bhakar-2002' and 'Galaxy-2013' were also infected by wheat pathogens. In general, the results of our research indicate that mixed infections of two or more pathogens were widespread, despite the fact that these infections might be missed by eye examination.

This discovery unquestionably has repercussions for the control of wheat illnesses in the future and may also be applicable to the cultivation of other crops. Fungal infections have disastrous repercussions on both human and animal health as well as global food security. Fungal diseases result in losses for cereal crops connected with decreased production yield and harmed grain quality. These effects result in yearly losses for producers and farmers in the billions of dollars, as well as higher prices for consumers. Global activities concentrate on integrated breeding techniques to enhance resistance, cutting-edge monitoring and detection technologies, and molecular understanding to find disease biomarkers to eliminate fungal infections of cereal crops. However, such studies are less concentrated in Pakistan.

In the earlier studies (Hajihasani et al., 2012; Majumder et al., 2013; Mobasser et al., 2012), a total of fifteen different fungal species were isolated from seeds. These species included *Tilletia laevis, T. tritici, Ustilago tritici, F. graminearum, F. culmorum, Microdochium nivale, B. sorokiniana, A. alternata* was determined to be the predominant fungus that was isolated from the black pointed seeds in the prior investigation (Fernandez and Conner, 2011). In the same vein, the amount of infection caused by *Fusarium* spp. was 60 %.

The *A. niger* and *Penicillium* spp. have been reported to reduce the seed germination and seed loss during storage (Ijaz et al., 2001; Jamadar and Chandrashekhar, 2015). The *A. alternata and Curvularia lunata* cause delay in seed germination due to rot of seeds. *Aspergillus flavus* produce toxic chemicals that results in decrease of shoot and root elongation (Devi and Jesumaharaja, 2020; Hussain et al., 2013).

5. Conclusion

It is concluded that several seed-borne pathogens are responsible for quality and quantity losses in tested wheat genotypes. Most of the isolated pathogens are soil-borne and some have association with seeds. The maximum efforts should be taken to use disease-free seeds to minimize the quality and quantity losses. This study demonstrated that isolated fungal species have significant relationship in term of causing diseases in wheat and can lead to changes in rhizosphere. This high inoculum density and maximum fungal biomass in wheat can act as substantial indicator of maximum disease development. This increased fungal abundance association in seeds of wheat can contribute to maximum fungal saprophytes.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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