Isolation and Identification of Most Prevailing Fungal Diseases of Wheat and Maize in Tehsil Havelian, District Abbottabad

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Abstract

The present study was designed to investigate fungal diseases of wheat and maize crops of Tehsil Havelian, District Abbottabad, Pakistan. In this study seven fungal pathogens (*Puccinia triticina*, *Ustilago tritici*, *Alternaria triticina*, *Drechslera tritici repentis*, *Fusarium graminearum*, *Cladosporium herbarum*, *Curvularia lunata*) causing leaf rust, Smut, Alternaria leaf blight, Tan spot/yellow spot, Fusarium head blight, Black point in wheat and four fungi (*Biopolaris maydis*, *Alternaria alternata*, *Curvularia lunata* and *Colletotrichum graminicola*) causing Sourthen leaf blight, Alternaria leaf blight, Curvularia leaf spot and Anthracnose leaf blight in maize were isolated and identified on the basis of cultural characteristics and micrometry. In wheat samples *Puccinia recondita* and *Alternaria triticina* were most prevalent pathogens isolated in all ten sampling locations and showed maximum percent incidence of 73.33% and 90%, respectively. While *Curvularia lunata* was isolated from only one location Pangran with maximum incidence of 6.67%. Similarly in maize samples *Alternaria alternata* and *Bipolaris maydis* were dominated pathogens isolated in three locations Chamba, Sultanpur and Takia sheikhan with highest percent incidence 93.33% and minimum percent incidence was shown by *Curvularia lunata* i-e 3.33% isolated from samples of six locations. All foliar samples of wheat and maize were found infected either singly or mixed infection. Single infection percentage was found higher as compared to mixed infection.

Keywords: Wheat; Maize; Fungal Diseases; Disease Incidence

Introduction

Wheat (*Triticum aestivum*) and maize (*Zea mays*) are two important cereal crops that belong to family poaceae. Wheat is the leading grain crop of Pakistan used as major food. Similarly, Maize is also the top most crops of the world according to its total production and in Pakistan after rice and wheat it is largest grown crop.

In 2018-19 Pakistan cultivates wheat on an area of 8,470 (000, hec) with a total production 25,195 million tonnes [1], while in

Khyber Pakhtunkhwa province total area of wheat was 739570 thousand hectares and total production was 1327580 million tonnes [2].

Production of wheat worldwide is effected by many factors, diseases are considered major yield stress. However, the production is highly constrained by fungal incited diseases which cause losses 15-20% in yield [3]. Among fungal diseases, foliar pathogens are considered most important to reduce average yield of cereal crops. The reduction in yield due to leaf blight recorded in India dur-

ing 1998 and 1999 decreased the 1000 grain weight significantly from 1.2 to 26.1% [4]. Other important foliar diseases are rusts and smuts [5] which are present in almost every area of Pakistan where wheat is cultivated. During a survey of wheat fields in the surrounding areas of Islamabad in spring 2000 tan spot was found as great as spot blotch [6]. The other fungi isolated from wheat foliage in various areas of Punjab were *Alternaria alternata*, *Bipolaris sorokiniana*, *Cladosporium* spp, *Helminthosporium* spp. and *Stemphylium* spp [7].

Maize is also a major grain crop in world after wheat and rice. Maize is multipurpose cereal providing fuel and food to human beings, feed to animals and poultry, and fodder to livestock [8]. Maize covers 75% of the world food supplies and cultivated in all parts of the world. It is another cash and food crop of Pakistan where it was grown on an area of 1,318 (000, hec) in 2018-19 with a production of 6,309 million tones [1].

Maize is infected by different microbes causes a great food loss (30% total production) in the world [9]. Global losses in maize due to diseases were recorded to be 9% in 2001-03. Stalk and ear rots, Gray leaf spot, Southern leaf blight, northern leaf blight, gray leaf spots and different kinds of rust are amongst main diseases of maize [10,11].

Wheat and maize are major crops of Tehsil Havelian, District Abbottabad, Khyber Pakhtunkhwa Province. Tehsil Havelian lies in 34° 05' N latitude and 73° 16' E longitudes and is located on the Karakoram Highway about 15.5 kilometers south west of Abbottabad at 868 meter height above sea level. The area fall in subtropical humid zone with mild to warm temperature during April-May and September-October respectively; humid temperature in June-July, and cool to mild temperature during November-February. The temperature rises up to 35°C during the mid-summer months and drops below 0°C during winter months.

No systematic work regarding isolation and identification of major fungal diseases of Wheat and Maize of Tehsil Havelian has been done so far, therefore present study was designed to diagnose the foliar fungal diseases of wheat and maize of Tehsil Havelian on the basis of characteristic symptoms in the field, microscopic slides and cultural characteristics, incidence percentage of isolated fungi and their presence as single or mixed infections.

Materials and Methods

Sample collection

Ten locations (Sultanpur, Chamba, Jangra, Pangran, Bandi Subkhan, Khokkar Maira, Langra, Takia Sheikhan, Diwal minhal, Sajjikot) in Tehsil Havelian (Figure 1) where wheat and maize are grown as major crops were selected and extensive survey was conducted during 2019. Total 300 samples of each crop i.e. wheat and maize were collected randomly from farmers field on the basis of disease symptoms following the 'Z' pattern as a sampling procedure [12]. Photography of disease symptoms and field diagnosis of diseases was made on the basis of physical observation of characteristic symptoms in the field at the time of sampling. A single sample consisted of leaves plucked from bottom, middle and top of plant showing symptoms and stored in labeled polyethylene zipper bags to indicate location, sample number and date of collection. The samples were brought to the laboratory at Hazara Agriculture research station Abbottabad (HARS) for isolation and identification of fungi associated with disease symptoms.

Figure 1: Map of Tehsil Havelian with sampling locations.

Isolation of fungus

Agar plate method was used for fungus isolation. Potato Dextrose Agar (PDA) media was prepared and autoclaved at 121°C at 15 psi pressure for 15 minutes. After autoclaving the PDA was poured @ 15 - 20 ml per petridish of 9 cm diameter under sterilized laminar flow chamber. The samples showing disease symptoms were cut into small pieces. The pieces were first surface dis-

infected with 3% sodium hypochlorite (NaOCl) solution for three minutes, washed three times with sterilized distilled water and placed on sterilized blotting paper to dry excess water. The samples were aseptically inoculated on PDA medium and incubated for seven days in growth chamber at 25°C.

Identification of fungi

After incubation the morphology of fungal colonies were observed and microscopic slides were made from the cultures, identification was made by using morphological keys of Barnett and Hunter [13] and Eillis [14] and micrometry was also done for measuring conidial size. The isolated fungi were subcultured on potato dextrose agar in order to get pure culture.

The % incidence of individual fungus was calculated by using formula:

$$\% \text{ incidence} = \frac{\text{No. of samples infected with respective fungal species} \times 100}{\text{Total number of samples}}$$

Statistical analysis

The recorded data on percent fungal incidence was subjected to analysis of variance (ANOVA) in Completely randomized design (CRD) with three replications per sample and the means were compared using least significance difference (LSD) test at 5% probability level. The statistical analysis was done using computer software Statistix 8.1.

Results

In this study a number of fungal pathogens and the respective diseases of wheat (*Triticum aestivum*) and maize (*Zea mays*) on the basis of symptoms and disease identification were made from the foliar samples collected from ten locations of Tehsil Havelian, District Abbottabad during March-May and August-October 2019, respectively.

Fungi isolated from Wheat foliage

The diseases diagnosed on the basis of visual symptoms and fungi isolated from wheat foliage were Leaf rust (*Puccinia recondita*), Smut (*Ustilago tritici*), Alternaria leaf blight (*Alternaria tritica*), Tan spot (*Drechslera tritici repentis*), Fusarium head blight (*Fusarium graminearum*) and black point (*Cladosporium herbarum* and *Curvularia lunata*).

Leaf rust

Leaf rust or brown rust (*Puccinia recondita*) is a fungal disease of wheat that was observed in all ten locations during sampling (Table 1 and 2). A significant difference ($p \leq 0.05$) was observed among all the locations regarding % incidence of *Puccinia recondita* (Table 1). Maximum incidence percentage i.e. 73.33% was observed in location Sultanpur followed by 53.33% in location Pangran whereas in location Langra its incidence was lowest (10%) followed by location Diwal (13.33%) (Table 1). The characteristic symptoms of leaf rust observed at the time of sampling in the field were Small, circular to oval orange coloured 1.5 mm long pustules on the upper side of leaves and on leaf sheath (Figure 2A). On some plants a mixture of orange and black lesions were observed. Each pustule contains thousands of urediospores, on some plants the uredinal stage turned into telial stage (Figure 2B) containing brown to black spores known as teliospores.

From the microscopic slides directly made from leaf samples collected from the field showing rust symptoms, urediospores were typically large orange-red, oval with thick rough outer wall, range from 10-20µm (Figure 4B). Teliospores were brown to dark colour, 25-35 x 15-20 µm in size (Figure 4A). The characteristics of *Puccinia recondita* matched with those reported by [13,14].

Figure 2: Characteristics symptoms of wheat Leaf rust (A,B); Loose Smut (C); Alternaria Leaf Blight (D,E); wheat Tan Spot (F,G) in wheat Field.

Figure 3: Pure cultures of fungi isolated from wheat foliar samples on PDA after seven days of incubation at 25°C; *Alternaria triticina* (A); *Drechslera tritici repentis* (B); *Fusarium graminearum* (C); *Cladosporium herbarum* (D); *Curvularia lunata* (E).

Figure 4: Teliospores (A) and Urediospores (B) of *Puccinia recondita*; conidia of *Ustilago tritici* (C); conidia of *Alternaria triticina* (D); Conidia of *Drechslera tritici repentis* (E); Conidia of *Fusarium graminearum* (F); Conidia of *Cladosporium herbarum* (G); Conidia of *Curvularia lunata* (H) at 400X Magnification.

Loose smut

The causing agent of loose smut is *Ustilago tritici* and during sampling the loose smut symptoms were recorded in four locations Chamba, Jangra, Pangran and Sajikot with maximum fungal incidence of 23.33% in location Jangra while minimum (10%) incidence in location Sajikot (Table 1). The disease can be identified easily at the time of heading. The infected head showed black sooty fungal spore masses instead of normal grains. All the grains in a smutted head were completely transformed to black powder (Figure 2C).

Conidia of *Ustilago tritici* were spherical to subglobose, darkly pigmented, brown in colour (Figure 4C), size ranges from 7.5 - 12.5 µm. These characteristics of *Ustilago tritici* matched with those reported by [13,14].

Leaf blight

The wheat leaf blight caused by *Alternaria triticina* was recorded from all the ten sampling locations (Table 1 and 2). The greatest % incidence of *Alternaria triticina* i.e. 90% was recorded in location Langra followed by Dewal (83.33%) (Table 1). Its characteristic symptoms on wheat observed during sampling were small, elongated colourless lesions appeared on the leaves which with the passage of time became dark brown to grey surrounded by chlorotic halo. These lesions differ in size reaching a diameter of 1cm or more (Figure 2D and 2E). The disease first appeared on lower most leaves and gradually spread to the upper leaves.

The colony of *Alternaria triticina* on PDA was black to brown colour with smooth and entire margins (Figure 3A). Conidia were acoenogenous, irregularly elliptical, both ends circular or ellipsoid and slowly tapering into a beak. Spores were pale brown to olive, which become darkish with time having 3-4 transverse and 1 longitudinal septa; length including beak was 27.5-52.5 µm, width 10-17.5 µm (Figure 4D). These characteristics of *Alternaria triticina* resembles with those reported by [13,14].

Tan spot

The prevalence of Tan spot of wheat also known as yellow leaf spot caused by fungus *Drechslera tritici repentis* (anamorph) or *Pyrenophora tritici-repentis* (telomorph) was lowest among all the isolated fungi and showed % incidence of 3.33% in only three locations (Table 1). Lesions first appeared as lens-shaped tan blotches with dark center surrounded by yellow border giving it a distinct-

Location	% incidence of fungal pathogens						
	<i>Puccinia recondita</i>	<i>Ustilago tritici</i>	<i>Alternaria tritici</i>	<i>Fusarium graminearum</i>	<i>Drechslera tritici-repentis</i>	<i>Cladosporium herbarum</i>	<i>Curvularia lunata</i>
Sultanpur	73.33a	0c	23.33e	0c	3.33a	0c	0b
Chamba	43.33b	16.67ab	40cde	0c	0a	0c	0b
Jangra	36.67b	23.33a	40cde	0c	0a	0c	0b
Pangran	53.33ab	13.33ab	30de	0c	0a	0c	6.67a
Banda	40b	0c	46.67cd	3.33c	0a	23.33ab	0b
Khokkar Maira	33.33bc	0c	50cd	30b	3.33a	13.33bc	0b
Langra	10d	0c	90a	56.67a	3.33a	30a	0b
Takia Sheikan	36.67b	0c	60bc	0c	0a	10bc	0b
Diwal	13.33cd	0c	83.33a	30b	0a	23.33ab	0b
Sajikot	13.33cd	10bc	76.67ab	36.67b	0a	6.67c	0b

Table 1: Percent incidence of fungal pathogen(s) isolated from wheat leaf samples of ten locations of Tehsil Havelian.

Means represented with different alphabets showed significant difference at $P \leq 0.05$ in a column or row.

tive 'eye spot' appearance (Figure 2F and 2G). Tan spot lesions appeared on both lower and upper leaf surface.

Colonies were fast growing, blackish brown in colour with black reverse and rarely whitish on PDA media (Figure 3B). Conidia are straight or lightly curved, cylindrical, with rounded apex the basal segment distinctly is cone shaped. Spore color varies from light to dark brown with 3-8 transvers or pseudosepta, spore vary in size $27.5-62.5 \times 10 - 15 \mu\text{m}$ (Figure 4E). The characteristics of *Drechslera tritici repentis* matched with those reported by [13,14].

Fusarium head blight/scab

Fusarium head blight of wheat (FHB) isolated and identified in five sampling locations (Banda, Khokkar Maira, Langra, Diwal, Sajikot) of Tehsil Havelian (Table 1 and 2) among which the significantly highest fungal incidence i.e. 56.67% was recorded from location Langra and least from location Banda (3.33%). Characteristic symptoms include premature bleaching of the diseased spikelets shortly after infection. On leaves small tan to brown lesions with yellow halos appeared. Seeds developed from the infected spikelets may be shriveled and chalky or white in appearance referred to as tombstone.

Colony on PDA was white pinkish with cottony surface (Figure 3C). Conidia were hyaline, large straight sickle shaped with 3-7

horizontal septa measuring $20 - 27.5 \times 5 - 7.5 \mu\text{m}$. One end cell is often slightly bend (Figure 4F). The characteristics were similar to those reported by [13,14].

Black point

Black point also called kernel smudge caused by *Cladosporium herbarum* and *Curvularia lunata*. Both fungi were isolated from sampling locations among which Banda, Khokkar Maira, Langra, Takia Sheikan, Diwal, Sajikot samples were found infected with *Cladosporium herbarum* with maximum % incidence of 23.33% in locations Banda and Diwal while *Curvularia lunata* was isolated only from one location Pangran with % incidence of 6.67% (Table 1 and 2). During sampling yellow lesions were observed on leaves and dark brown to black discoloration of pericarps of maturing wheat kernels.

Cladosporium herbarum formed low, thick powdery or velvety olivaceous-green to olivaceous-brown, reverse greenish black colonies on PDA (Figure 3D). Conidia were lemon shaped to cylindrical ellipsoidal with one septa, light brown to smoky brown in colour. One-celled, reaching $7.5-10 \times 2.5-5 \mu\text{m}$ (Figure 4G) These characteristics matched with those described by [13,14].

Curvularia lunata colony appeared downy, velvety black, fluffy (Figure 3E). Conidia were smooth walled olivaceous brown, obvoid

Location	Disease(s) identified	Location	Disease(s) identified
Sultan-pur	Alternaria leaf blight	Khokkar Maira	Leaf rust
	Leaf rust		Fusarium head blight/ scab
	Tan spot/yellow spot		Alternaria leaf blight
Chamba	Alternaria leaf blight		Black point
	Leaf rust		Tan spot/yellow spot
	Loose smut	Leaf rust	
Jangran	Leaf rust	Langra	Fusarium head blight
	Loose smut		Alternaria leaf blight
	Alternaria leaf blight		Black point
	Black point		Tan spot/yellow spot
Pangran	Leaf rust	Takkia sheikhan	Alternaria leaf blight
	Loose smut		Leaf rust
	Alternaria leaf blight		Black point
	Black point	Leaf rust	
Banda Subkhan	Alternaria leaf blight	Diwal	Fusarium head blight
	Leaf rust		Alternaria leaf blight
	Black point		Black point
		Sajjikot	Leaf rust
			Fusarium head blight

Table 2: Fungal diseases of wheat identified on the basis of field symptoms and pathogen characteristics among ten sampling locations of Tehsil Havelian, District Abbottabad in 2019.

to broadly club shaped and bend at subterminal cell with length 20-25 µm and width 7.5-10 µm having three septa. The subterminal cell larger than remaining cell (Figure 4H). These characteristics matched with those reported by [13,14].

Identification and Percent Incidence of fungi isolated from Maize foliar samples

Pathogen(s) from the infected foliar maize samples were isolated on PDA media and identified on the basis of macro and microscopic characteristics with reference to mycological keys of Barnett and Hunter 1998, Ellis 1982. The fungi isolated were *Bipolaris maydis*, *Curvularia lunata*, *Alternaria alternata* and *Colletotrichum graminicola*. On the basis of pathogen identification four different diseases of maize crop (Southern leaf blight, Curvularia leaf spot, Alternaria leaf blight, Anthracnose leaf blight) prevailed in the Tehsil Havelian.

Location	% incidence of isolated Fungi			
	<i>Bipolaris maydis</i>	<i>Curvularia lunata</i>	<i>Alternaria alternata</i>	<i>Colletotrichum graminicola</i>
Sultanpur	93.33a	0d	6.67e	43.33abc
Chamba	50cd	0d	93.33a	0d
Jangra	86.67ab	10cd	3.33e	33.33b
Pangran	0e	86.67a	43.33cd	0d
Banda Subkhan	70bc	0d	10e	60a
Khokkar Maira	53.33cd	3.33cd	33.33d	46.67ab
Langra	76.67ab	0d	40cd	36.67b
Takia Sheikan	93.33a	26.67b	3.33e	26.67c
Diwal	46.67d	16.66bcd	70b	0d
Sajikot	53.33cd	20bc	56.67bc	6.67d

Table 3: Percent incidence of fungal pathogen(s) isolated from foliar samples of Maize in Tehsil Havelian.

Means represented with different alphabets showed significant difference at $P \leq 0.05$ in a column or row.

Sourthen corn leaf blight

Southern corn leaf blight is one of major fungal disease of maize caused by fungus *Bipolaris maydis* also known as *Cochliobolus heterostrophus* in its teleomorph state (previously known as *Drechslera maydis* or *Helminthosporium maydis*) and it was observed in nine locations during sampling except in location Pangran (Table 3). The maximum % incidence of 93.33% was recorded in samples of location Sultanpur and Takia Sheikan followed by

location Jangra (86.67%), however location Diwal showed least incidence (46.67%) of *Bipolaris maydis* (Table 3). The characteristic disease symptoms observed on corn leaves at the time of sampling were brown coloured oblong, oval or diamond shaped lesions which with the passage of time became elongated and were confined by leaf veins. The length of lesions was up to 2-3cm and their width was 2-6mm. Lesions were usually present on lower leaves and move towards the upper leaves (Figure 5A).

Bipolaris maydis produced light grey to black colour colony on PDA (Figure 6A). Conidia have 4 - 10 septations, spindle shaped brown color with tapering to round ends. A small, slightly papillate hilum was observed at the base of conidia. The size is 17.5-105 x 7.5-17.5µm (Figure 7A) The characteristics of *Bipolaris maydis* were found very close with those described by [13,14].

Curvularia leaf spot

Curvularia lunata the causal organism of Curvularia leaf spot was recorded in six locations during sampling with maximum % incidence of 86.67% from location Pangran (Table 3) while in location Khokhar Maira only 3.33% incidence of *Curvularia lunata* was recorded. The samples of locations Sultanpur, Chamba, Banda Subkhan and Langra did not found infected with *Curvularia lunata* (Table 3). At the time of sampling brown to tan lesions 0.5cm in diameter were observed (Figure 5B). Lesions often have reddish brown margins and encircled by a yellow halo in the mid to upper canopy of maize crop.

Cultural characteristics of *C. lunata* include gray to grayish black colony on potato dextrose agar medium (Figure 6B). Microscopically conidia were clavate, three septate, basal and apical cell pale brown, central cell was darker and larger (Figure 7B). The conidia were variable in size 22.5-32.5x 7.5-15µm. The characteristics of *Curvularia lunata* matched with those described by [13,14].

Alternaria leaf blight

The *Alternaria alternata* causes Alternaria leaf blight or leaf spot of maize was isolated and identified in all ten locations of Tehsil Havelian with highest % incidence of 93.33% from location Chamba followed by location Diwal in which % incidence of *Alternaria alternata* was 70% (Table 3 and 4). The least incidence of 3.33% was recorded from locations Jangra and Takia Sheikan (Table 3). Lesions on maize leaf incited by *Alternaria alternata* observed at the time of sampling were small, oblong brown Lesions encircle by a yellow halo. As disease progresses, lesions merge into brown necrotic areas, which expand for some distance but are restricted by the leaf veins (Figure 5C).

Colony morphology of *Alternaria alternata* on potato dextrose agar at initial stage was light grey which then turned to dark green or blackish with white margins (Figure 6C). The conidia were variable in size, dark brown in colour, ovate to ellipsoidal, muriform with a short, cylindrical beak but were often beakless. Conidia showed 3 -6 transverse septa with no or one longitudinal septa (Figure 7C). They range in size from 20-52.5µm x 7.5-15µm. The characteristics of *Alternaria alternata* matched with those described by [13,14].

Figure 5: Characteristics symptoms of Sourthen leaf blight of maize (A); Curvularia leaf spot (B); Alternaria Leaf Blight (C); Anthracnose leaf blight (D) in maize field.

Figure 6: Pure cultures of fungi isolated from maize foliar samples on PDA after seven days of incubation at 25°C; Colony of *Bipolaris maydis* (A); *Curvularia lunata* (B); *Alternaria alternata* (C); *Colletotrichum graminicola* (D).

Figure 7: Conidia of *Bipolaris maydis* (A); conidia of *Curvularia lunata* (B); Conidia of *Alternaria alternata* (C); Conidia of *Colletotrichum graminicola* (D) at 400X magnification.

Anthracnose leaf blight

Anthracnose leaf blight of maize is important foliar disease caused by fungus *Colletotrichum graminicola* (anamorph) or *Glomerella graminicola* (teleomorph) was isolated and identified in seven sampling locations i.e Sultanpur, Jangran, Pangran, Banda subkhan, Khokkar maira, Langra, Takkia sheikhan and Sajjikot with highest percent incidence (60%) in location Banda subkhan while in location Sajjikot its incidence was 6.67% (Table 3 and 4). The anthracnose leaf blight is characterized by oval to elongated brown lesions (0.5-2cm) on leaves. These lesions gradually enlarge and become tan at the center surrounded by yellow or reddish brown zone these lesions fuse together covering all the leaf and entire leaf become blighted (Figure 5D). Anthracnose leaf blight initially showed lesions on lower leaves, as the disease boost spots move towards the upper leaves.

The colony colour of *Colletotrichum graminicola* on PDA was white (Figure 6D). Conidia were hyaline, non septate, falcate to fusiform, slightly curved and tapered toward the tips with an average length 12.5 - 45µm and average width 2.5 to 7.5µm (Figure 7D). The characteristics of *Colletotrichum graminicola* matched with those described by [13,14].

Single or mixed infection of wheat and maize crops

Foliar samples of wheat and maize from of all ten sampling locations were found infected either with single or mixed infection.

Location	Disease(s) identified	Location	Disease(s) identified
Sultanpur	Southern leaf blight	Khokkar Maira	Southern leaf blight
	Alternaria leaf blight		Anthracnose leaf blight
	Anthracnose leaf blight		Alternaria leaf blight
Chamba	Alternaria leaf blight	Langra	Curvularia leaf spot
	Southern leaf blight		Southern leaf blight
Jangran	Alternaria leaf blight	Takkia sheikhan	Anthracnose leaf blight
	Southern leaf blight		Alternaria leaf blight
	Anthracnose leaf blight		Southern leaf blight
	Curvularia leaf spot		Curvularia leaf spot
Pangran	Alternaria leaf blight	Diwal	Anthracnose leaf blight
	Curvularia leaf spot		Southern leaf blight
Banda Subkhan	Southern leaf blight	Sajjikot	Curvularia leaf spot
	Anthracnose leaf blight		Alternaria leaf blight
	Alternaria leaf blight	Sajjikot	Southern leaf blight
			Anthracnose leaf blight
			Alternaria leaf blight
			Curvularia leaf spot

Table 4: Fungal diseases of maize identified on the basis of field symptoms and pathogenic characteristics among ten sampling locations of Tehsil Havelian, District Abbottabad in 2019.

Single infection percentage was found high as compared to mixed infections. For wheat among the ten locations 100% single infection was observed in Sultanpur, Chamba, Jangra while the high percentage of mixed infection(s) were found in Langra (66.6%) followed by Diwal and Sajjikot (43.33%) (Table 5). Regarding maize all the ten locations showed single as well as mixed infections. Max-

imum single infection percentage was observed in Diwal (66.66%) followed by 63.33% in Sajjikot, Pangran, Jangran and, Khokkar maira, while minimum infection (50%) was recorded in Sultanpur, Chamba and Langran. The highest percentage of mixed infection was observed in Langra i-e 50% and low 33.33% was in locations Diwal, Khokkar maira, Pangran and Jangra (Table 6).

Location	Single infection	Mixed/Multiple infection
Sultanpur	100%	0%
Chamba	100%	0%
Jangra	100%	0%
Pangran	96.66	3.33%
Banda Subkhan	63.33%	23.33%
Khokkar Maira	70%	26.66%
Langra	33.33%	66.66%
Takkia Sheikhan	93.33%	6.66%
Diwal	56.66%	43.33%
Sajjikot	56.66%	43.33%

Table 5: Single and Mixed infection of wheat fungal pathogen(s) in Tehsil Havelian.

Location	Single infection	Mixed/Multiple infection
Sultanpur	50%	46.66%
Chamba	50%	46.66%
Jangra	63.33%	33.33%
Pangran	63.33%	33.33%
Banda Subkhan	60%	40%
Khokkar Maira	63.33%	33.33%
Langra	50%	50%
Takkia Sheikhan	53.33%	46.66%
Diwal	66.66%	33.33%
Sajjikot	63.33%	36.66%

Table 6: Single and Mixed infection of fungal pathogen(s) of maize crop of Tehsil Havelian.

Discussion

There are many factors involved in crop yield losses in which diseases play a significant role. Fungal pathogens not only damage

crops but also causes major losses in food production. In the present study a number of fungal pathogens were isolated from foliar samples of Wheat and Maize.

Puccinia recondita causes leaf rust is most common wheat rust and is most prevalent wheat disease in the sampling areas of Tehsil Havelian (Table 1). The fungus is an obligate parasite and produces orange coloured uredia on wheat leaves as most predominant characteristic which with time change color from orange to black (telia) (Figure 2A,B). Similar field symptoms were reported by [15-17] who concluded that leaf rust is characterized by uredinial stage.

Weather plays a key role in development of leaf rust. Moderate nights and warm days are ideal for rust development. Its incidence percentage was higher in location Sultanpur and it may due to the fact that this area falls in the mild to warmer part of Tehsil Havelian. [18,19] reported that wheat leaf rust is favored by three important weather factors that are moisture, temperature and wind. Temperature affects spore germination, infection, and host resistance. The relative humidity played an important role in the penetration of fungus haustorium because it makes the leaf tender due to moisture content.

Ustilago tritici the causal organism of loose smut of wheat was detected in only four sampling locations i.e Chamba, Jangra, Pangran and Sajjikot of Tehsil Havelian with maximum incidence 23.33% in Jangra (Table 1). Loose smut is seed borne fungal disease and the fungus continues to survive within the embryo of infected wheat seed as dormant mycelia and spread to next crop through infected seed [20]. In this location at the time of sampling wheat crop was at grain filling stage and showed heads covered with black powdery masses instead of healthy grains (Figure 2C). The possible reason for this high incidence in Jangra samples was that the farmers grow uncertified seed.

Alternaria leaf blight in wheat is the most prevalent fungal disease among the sampling areas (Table 1). This fungus is both soil-borne and seedborne, however, seed borne transmission can be the most likely mechanism in the sampling locations because the local farmers commonly used uncertified seed. *Alternaria triticina* produced black to brown colonies with smooth and entire margins on PDA (Figure 3A). [21] reported that *Alternaria* colonies on potato dextrose agar are discrete or effuse, dark blackish to black with smooth borders.

Drechslera tritici repentis caused tan spot of wheat was isolated from samples of three locations Sultanpur, Khokkar maira and Langra with very low % incidence i.e. 3.33% (Table 1). The spores germination of *Drechslera tritici repentis* is favoured by rain fall, significant dew and high canopy humidity with wide temperature range above 10°C. The infection spreads in the form of conidia which dispersed by wind [22,23]. In these locations heavy rain-fall with thunderstorms occurred in March to April which favors the tan spot. The fungus formed characteristic oval shaped lesions surrounded by a yellow ring on both upper and lower surfaces of leaves in wheat (Figure 2F,G) which were similar to the symptoms reported by [24-26].

The *Fusarium graminearum* causes Fusarium head blight in wheat was isolated in five locations (Table 3) with high% incidence percentage (56.67%) in location langra. The sampling was done in April and during this month temperature in langra was warm to cool, rainfall occurred in this month. [27] reported that FHB infection is favored by high relative humidity (>90%) and moderately warm temperatures (between 15 to 30°C). These conditions present before, during, and after flowering favor inoculum production, floret infection, and colonization of developing grains. (28,29) reported that *Fusarium* is a soilborne fungus its Conidia overwinter in soil or in crop residue in the field and serves as inoculum for next season crop spread.

Fusarium graminearum showed tan to brown lesions with yellow halos on leaves. [30] reported that the leaves of infected plants lose turgidity, turn light green to greenish-yellow to brown, and finally collapse and die. In culture it showed white cottony colonies on PDA media. Conidia were hyaline, large straight sickle shaped with 3-5 horizontal septa (Figure 4F). Similar cultural characteristics were described by [13,14,31].

Black point of wheat is a seedborne disease and is caused by multiple fungi among which *Cladosporium herbarum* and *Curvularia lunata* were detected in sampling locations (Table 1). No distinguishing foliar symptoms were observed except tan or yellow dull colour instead of healthy green colour. Their presence in leaf samples may be due to the infected seed used or may be due to soil infestation in which crops were planted. The farmers of these locations usually use uncertified seed which can be the main reason for prevalence of many seedborne fungal diseases in Tehsil Havelian. Sampling was done during March and April and at this time en-

vironmental condition was wet which favors the disease at grain filling stage. On PDA media *Cladosporium herbarum* showed green to greenish black colony (Figure 3D), while *Curvularia lunata* produced velvety black colonies (Figure 3E), same colony characteristics were described by [32], respectively.

In maize crop four fungal pathogens were isolated and identified from foliar samples (Table 3). *Bipolaris maydis* is fungal pathogen which causes southern corn leaf blight in maize was found in nine locations with maximum percent incidence 93.33% in location Sultanpur. Temperature and amount of rainfall is critical in dissemination and survival of *Bipolaris maydis*. The sampling was done in August and monsoon rains occurred during this month. [33] reported that high humidity with low temperature and cloudy weather favours southern corn leaf blight along with heavy dew on host plant also increase disease severity. Once plant is infected it will develop oval, diamond shaped lesions on their leaf surfaces. *Bipolaris maydis* produced light grey to black colour colony on PDA (Figure 5A). [34] also reported of black to grey coloured colony of *Bipolaris maydis* on PDA.

Curvularia lunata is fungal pathogen in maize causing Curvularia leaf spot and during sampling it was isolated from six locations with maximum incidence 86.67% in location Pangran (Table 3). Characteristic disease symptoms are tan lesions (0.5cm) with reddish brown borders (Figure 5B) similar symptoms are also reported by [35]. Pathogen remains viable in crop debris which is most important source of infection. Spores spread by wind or splash with water and infect maize in wide temperature range. During continuous wet period large number of conidia formed in the disease lesions, blown onto other maize plants to form new infection.

Alternaria alternata caused Alternaria leaf blight in maize and showed small lesions on infected maize leaves (Figure 5C and Table 3 and 4) was isolated from nine sampling locations (Table 4). Majority of the farmers used uncertified seed which can be a possible reason of its prevalence because *Alternaria* is a seedborne fungus and overwinters as dormant mycelium in the seed.

Colletotrichum graminicola causes anthracnose leaf blight in maize is isolated in six locations (Table 3) with highest percent incidence 60% in location Banda subkhan. The sampling was done

in October and the environmental condition of Banda subkhan was moderate, nights are cool as compared to days which was favorable for infection of Anthracnose leaf blight. Further at this time the maize crop was near to maturity in this location. The anthracnose leaf blight is characterized by oval to elongated brown lesion on leaves (0.5-2cm) (Figure 5D). Similar characteristic symptoms of Anthracnose leaf blight of maize were described by [36]. Its colony and conidial characters (Figure 6D and 7D) resembles to those described by [13,14,37], who concluded that the conidia of *C. graminicola* are falcate shaped slightly curved and show no septations.

All foliar samples of wheat and maize crop collected from ten locations of Tehsil Havelian were found infected either singly or with mixed infections (Table 5 and 6). It may be due to that mostly farmers of Tehsil Havelian have land holdings of less than one hectare, they do not purchase certified disease free seed from authentic seed companies and rely on their own produce as seed sources for next season crop. Further the farmers do not use any fungicide spray as preventive control measures.

Conclusions and Recommendations

It is concluded from present study that the wheat and maize crops of Tehsil Havelian, District Abbottabad were found infected with fungal diseases either singly or mixed infection. From wheat samples six fungal pathogens *Puccinia recondita*, *Ustilago tritici*, *Alternaria trititica*, *Drechslera tritici repentis*, *Fusarium graminearum*, *Cladosporium herbarum* and *Curvularia lunata* and in maize four pathogens *Helminthosporium maydis*, *Curvularia lunata*, *Alternaria alternata* and *Colletotrichum graminicola* were isolated. These diseases are a great threat to future yield production.

There is need of further research work on molecular level to identify the specific fungal isolates present in the study area. Majority of the farmers have little knowledge about fungal diseases and their efficient control therefore it is recommended that farmers should be educated through trainings and seminars etc. for proper disease control measures and the use of healthy certified seed for their crop production.

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