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Incidence of Karnal bunt (*Tilletia indica* Mitra) of wheat in southern Punjab, Pakistan

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Abstract

Wheat crop is cultivated as a staple food in Pakistan covering the largest area than any other cereal crop and contributes 60% of the daily dietary diet. Significant yield losses are reported due to different biotic and abiotic stresses in southern Punjab. Karnal bunt (*Tilletia indica*) is a quarantine importance disease becoming the challenge that deteriorates its quality and ultimately unacceptable for human consumption and export. The current study was designed to conduct the karnal bunt survey in six high yielding districts of South Punjab i.e. Bahawalpur, Muzaffar garh, Khanewal, Vehari, Lodhran and Rahimyar Khan during three consecutive wheat cropping seasons 2014-17. Maximum seed production will be obtained from these districts for next crop seasons. Two hundred and sixteen samples were collected during the survey from one hundred and twenty one wheat grains heaps from sixteen tehsils of six districts. Maximum disease incidence (3.24%), (1.38%) and (1.4%) was observed in village Januwala of district Bahawalpur (2014-15), in Dahanot of district Lodhran (2015-16) and in Derawar Fort of district Bahawalpur (2016-17) respectively. Over all the intensity of the disease is very low in southern Punjab which is a good sign. Regular monitoring of the disease to identify the Karnal bunt free areas by conducting extensive surveys should be encouraged in other areas of wheat also.

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Introduction

Within agriculture, on a global scale, wheat (*Triticum aestivum* L.) is the most important single product cultivated both as a food and source of income in many countries of the world. This observation also explains the role that wheat plays in geopolitics. Wheat is dietary staple and one of the main agricultural crops in Pakistan, with 80 percent of farmers growing it on an area of around nine million hectares (close to 40 percent of the country's total cultivated land) during the winter or "Rabi" season. This crop alone contributed about 10 percent of value added in agriculture and 2.1 percent of the country's gross domestic product (GDP) in 2015. Marketing year (MY) 2015/16 production is adjusted upwards to 25.1 million metric tons. Marketing year (MY) 2016/17 consumption is forecast at 24.5 million metric tons and production is forecast at 25.3 million metric tons, marginally higher than a year ago (FAO, 2017).

Wheat crop is exposed to many biotic and abiotic stresses during its lifecycle. Among all biotic stresses, kernel bunt (*Tilletia indica)* is a fungal pathogen causing Kernal bunt of wheat seeds. The first report of a new bunt disease in wheat came from the region of Faisalabad (Pakistan) in 1909 but documented in 1930 when observed in f Karnal (Mitra, 1931) while the Mexico was the non-Asian country where the disease was observed in 1972 within the state of Sonora EPPO, (1991).

The fungus can be grow in all seed, air and soil borne natures and can survive up to four year (Krishna and Singh 1983). Although it is considered as minor disease but due to varietal reshuffle, intensive cultivation and high input technology factors are responsible for becoming it as major threat. Disease incidence varies significantly each year depends on favorable weather conditions during heading. Initial infection occurs when temperature is fairly cool about 20 to 25°C (Bonde *et al.*, 1998) and high relative humidity which permits secondary sporidia to cause disease (Bansal *et al.*, 1984 and Asma *et al.,* 2012). Total losses in India during severe epidemics have been around 0.3 to 0.5 per cent with incidence as high as 89 per cent in some fields (Joshi *et al.,* 1983). Karnal bunt (*Tilletia indica)* is considered the disease of quarantine importance and affects the quality and economic losses (Datta *et al.*, 2000 and Asma *et al.,* 2012). Several surveys were conducted in the province of Punjab and Incidences of the disease has increased with the passage of time and it has affected both the quality and quantity in the form of losses and poses a threat to wheat production even in the dry and hot region of the Punjab (Khan *et al.*, 2010 and Asma *et al.*, 2012). The main purpose of this study was to determine the prevalence and incidence of karnal bunt in different districts to identify the disease free areas as these districts are the main areas for seed multiplication and identify the resistant level among different wheat varieties planted in southern Punjab.

Materials and methods

The wheat growing areas of southern Punjab (Pakistan) were surveyed during three consecutive wheat cropping seasons, 2014-17. Random survey of wheat at threshing time were conducted in sixteen (16) tehsils of six districts in Southern Punjab i.e, Bahawalpur (Bahawalpur, Khairpur, Ahmadpur, Ouchsharif and Yazman Mandi), Muzafrgarh (Alipur and Muzafrgarh), Khanewal (Jahaniya and Khanewal), Vehari (Malsi and Vehari), Lodhran (Lodhran, Dunyapur and Kahrorpacca) and Rahimyar khan (Rahimyar khan, Sadqabad and Liaqatpur).Two hundred and sixteen samples of wheat grain, measuring about 200 to 300gm of seed of seventeen available wheat varieties (Punjab-11, Faisalabad-08, Fareed-06, Galaxy-13, Sehar06, Gold-16,AARI-11, Ujala-16, Millat-11, AS-02, Aas-11, Johar 16, Shafaq-06, Kohinoor, Lasani-08, TD-1 and Inqlab-91) along with advance lines and wheat seed mixtures were taken.

The collections of samples were done in labeled paper bags to indicate locations, number, date of collection and varieties name. Samples were taken from 4 to 5 places randomly of each unclean heaps. Two thousand seeds of each variety were taken for visual testing under the stereoscopic binocular microscope for categorizing diseased and healthy seeds. Healthy seeds were those with smooth coat, light brown to light yellow color without fungal propagates. Infected seeds were those with wrinkled seed coats or those with fungal propagates.

To confirm infection, infected seeds were further tested by applying soaking method as described by Dipali *et al.,* 2013. In this method wheat seed samples were soaked in 0.2% NaOH solution for 24 h at 20°C. Karnal Bunt infected seed became black in color as compared to the normal seed. For further clearance, bunted portion of each seed was crushed with needle to prepare the slide and was examined under microscope for teliospores presence.

Percent Incidence of KB infection from each sample was calculated from the total no. of abnormal grains used for analyzed of KB infection and Mean Disease Prevalence in each district was also calculated.

Percent Incidence of KB infected samples = n/N× 100

Where n = number of bunted seeds in one sample and N = the total number of seeds in one sample × 100.

Mean Disease Prevalence =

$$\frac{\text{No. of disease samples in a District}}{\text{Total No. of samples in a district}} \times 100$$

To determine the level of resistance/susceptible of each cultivar, a modified rating scale (0-5) was used for karnal bunt (*T. indica*), based on Aujla *et al.* (1989) and Bonde *et al.* (1996).

Coefficient of infection	Susceptibility category	Abbreviation
0	Highly resistant	(1R)
0.1-5.0	Resistant	(2R)
5.1-10.0	Moderately susceptible	(1S)
10.1-20.0	Susceptible	(2S)
20.1 & above	Highly susceptible	(3S)

Results and discussion

Three years data revealed that maximum disease prevalence was observed in district Lodhran (91.66%) while lowest in Rahimyar Khan (68.18%). The highest kernel bunt incidence was observed from the samples collected Ahmedpur which was in the range of 0.29-3.24% while lowest in Liaqatpur. Wheat Grain samples from all these visited tehsils were found infected with Kernel Bunt disease but disease incidence level was not in sever condition in any districts (Table 1).

Table 1. Karnal Bunt (KB) status in different Tehsils of Southern Punjab from 2015-2017.

Sr. No	Districts	Mean Disease Prevalence (%)	Tehsils	No. of Samples	% KB Incidence On Different Verities.	Coefficient of Infection (CI).
1.	Lodhran	91.66	Lodhran	7	0-1.38 (6)	0-0.25
			Kahrorpacca	9	0-1.36 (7)	0-0.33
			Dunyapur	8	0-13-0.86 (12)	0.03-0.25
2.	Rahimyar Khan	68.18	Rahimyar Khan	16	0-1.17 (8)	0-0.48
			Sadiqabad	24	0-1.15 (10)	0-0.42
			Liaqatpur	13	0-0.23 (15)	0-0.07
3.	Bahawalpur	79.41	Bahawalpur	16	0-1.16 (9)	0-0.30
			Khairpur	4	0-1.09 (11)	0-0.26
			Ahmadpur	25	0.29-3.24 (1)	0-1.25
			Ouchsharif	4	0-1.45 (5)	0-0.37
			Yazman Mandi	43	0-1.38 (6)	0-0.37
4.	Muzafrgarh	88.88	Muzafrgarh	14	0-1.60 (4)	0-0.78
			Alipur	4	0-0.69 (14)	0-0.47
5.	Khanewal	86.95	Khanewal	15	0-2.03 (2)	0-0.85
			Jahaniya	9	0.16-1.65 (3)	0-0.50
6.	Vehari	80.00	Malsi	5	0-0.70 (13)	0-0.19

Seventeen (17) cultivated wheat varieties i.e, Punjab-11, Faisalabad-08, Fareed-06, Galaxy-13, Sehar-06, Gold-16, AARI-11, Ujala-16, Millat-11, AS-02, Aas-11, Johar-16, Shafaq-06, Kohinoor, Lasani-08, TD-1 and Inqlab-91 in sixteen (16) tehsils of six districts, revealed that high disease incidence prevailed in tehsil Ahmadpur on variety Fareed-06 (3.24%). Kohinoor on the other hand showed low disease incidence of 0.14% at tehsil Yazman Mandi. In district Lodhran, the disease incidence was in the range 0.86 to 1.36% on varieties Sehar-06, mixture and Lasani-08 respectively. Similarly in district Rahimyar Khan,

maximum disease incidence of 1.17%, 1.15% and 0.23% was observed on varieties Sehar-06, TD-1 and Ujala-16. Similarly, in district Bahawalpur, maximum disease incidence of 1.16%, 1.38%, 3.24% and 1.45% was observed on varieties Galaxy-13, Mixture, Fareed-06 and Sehar-06. While In Muzafrgarh district, maximum disease incidence was in the range of 1.60% and 0.96% respectively on varieties AS-2002 and Ujala-16. The varieties Seher-06 and Galaxy-13 showed disease incidence in the range of 1.65 to 2.03%. In District Vehari maximum disease incidence was recorded on a mixture type variety in Malsi with 0.70%.

Table 2. Incidence (%) of cultivated wheat varieties to Karnal bunt in different districts of Southern Punjab**.**

Sr.No.	Districts	Tehsils	Varieties	Incidence (%)
1.	Lodhran	Lodhran	Punjab-11, Faisalabad-08, Galaxy-13, Sehar-06, Mixture.	Sehar-06 (1.29)
		Kahrorpacca	Gold-16, Sehar,-06, Ujala-16, Mixture.	Mixture (1.36)
		Dunyapur	Lasani-08, Galaxy-13, Ujala-16, Faisalabad-08,Aas-11, Johar-16, Mixture.	Lasani-08 (0.86)
2.	Rahimyar Khan	Rahimyar Khan	Faisalabad-08, Galaxy-13, Sehar-06, Mixture, Aas-11,Inqlab-91,TD-1, AS-02, AARI-11	Sehar-06 (1.17)
		Sadiqabad	Galaxy-13, TD-1, Punjab-11, Johar-16, Aas-11, Ujala-16, Mixture,	TD-1 (1.15)
		Liaqatpur	Lasani-08, Johar-16, Ujala-16,	Ujala-16 (0.23)
3.	Bahawalpur	Bahawalpur	Galaxy-13, Gold-16, Johar-16, Faisalabad-08, Lasani-08,	Galaxy-13 (1.16)
		Khairpur	Galaxy-13, Johar-16, Mixture,	Mixture (1.09)
		Ahmadpur	Sehar-06, AS-02, Aas-11, Fareed-06,AARI-11	Fareed-06 (3.24)
		Ouchsharif	Johar-16, Mixture, Ujala-16, Galaxy-13, Sehar-06, Aas-11, Faisalabad-08, Mixture,	Sehar-06 (1.45)
		Yazman	Gold-16, Galaxy-13, Johar-16, Mixture, Kohinoor	Mixture (1.38)
4.	Muzaffargarh	Muzaffargarh	Millat-11, Faisalabad-08, Sehar-06, AS-02, Aas-11,Galaxy-13,Punjab-11,AARI-11,Fareed-06, Shafaq-06	AS-02 (1.60)
		Alipur	Ujala-16, AS-02, Johar-16	Ujala-16(0.69)
5.	Khanewal	Khanewal	Sehar-06, Faisalabad-08, Millat-11, Punjab-11	Sehar-06 (2.03)
		Jahanya	Ujala-16, Faisalabad-08, Galaxy-13, Johar-16, Punjab-11,	Galaxy-13 (1.65)
6.	Vehari	Malci	Sehar-06, Mixture	Mixture (0.70)

Karnal bunt has been reported previously from Punjab (Bahawalpur, Bahawal nagar, Chakwal, Dera Ghazi Khan, Faisalabad, Gujrat, Gujranwala, Jhang, Khanewal, Kasur, Layyah, Lodhran, Multan, Muzaffargarh, Rawalpindi, Sahiwal, Sialkot, and Vehari) and Khyber Pakhtunkhwa (Charsada, Hatian, Jhamra, Mardan, Nowshera, Peshawar, and Takhtbhai) with different intensities (Mirza, 2005; Shakoor, 2009). The presence of disease in all three forms makes it a serious threat to global wheat production. To export or import the wheat having kernel bunt spore is rejected by the countries. Karnal bunt is considered a reason of economic losses and the disease has been distributed from India to the other regions of the world which were free of Kernel bunt.

The incidence of kernel bunt on the varieties BARS-2009, Seher-06, Pak-81, Bakhtawar-90, Faislabad-08, Waten-94, Inqilab-91 and Aas-11 when surveyed different districts of Punjab i.e Chakwal, Jhelum, Rawalpindi, Sahiwal, Bahawal Nagar, Bahawalpur and Faisalabad during the wheat season 2012. The incidence of the disease was very low when sample analyzed (Aasma *et al.,* 2012). The variety AS-02 is considered highly susceptible to Karnal bunt when tested under field condition. The bead making quality of AS-02 is also poor due to the presence of durum wheat in his blood. But due to high yielding it is still planted on large scale but susceptible to other diseases like leaf and yellow rusts. Result of both the studied conducted in the past and presently indicating that the infection of kernel bunt is present in the resistant range with very low severity.

The economic free trades between the different countries of the world increasing the potential risks to introduce the disease entrance in the free territories. A regular survey in agricultural fields is the way to identify the disease free areas for wheat production. The continuous survey, Seher-06 and Galaxy-13 was found ineffective where planted. These already found high susceptible to rusts and spot blotch. The disease incidence in all the districts is very low which is good sign and all the lines are in the resistant range.

The presence of two to three infected seeds can infect the lot and ultimately transfer and increased when planted in the next season.

Overall scenario depicts the healthy picture as despite the fact of high disease prevalence and incidence level, still the varietal response does not touch the susceptible level. It is obvious from the data that the environmental conditions as well as the genetic resistance among the varieties cultivated in these localities do not permit the disease to spread up to the maximum extent.

The presence of the diseases in wheat growing areas of southern Punjab indicate that there is a dire need to monitor the disease on regular basis and extensive survey should be conducted to identify the karnal bunt disease free zones to avoid future threat and the production of export quality wheat.

Over all the study is providing the complete scenario in southern Punjab and indicating that overall situation of Karnal bunt incidence and coefficient in the fields is very low which is a good sign but we should be very careful and continues survey should be conducted across the wheat growing areas of Pakistan for early warning and adopting the strategies to avoid future threat.

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