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Research Article

Role of seed borne inoculum on Karnal bunt infection risks

Ramesh Chand Sharma[1*#] and Indu Sharma[2*]

[1]*Seed Technology Centre, Punjab Agricultural University (PAU), Ludhiana-141004, India*
[2]*Dept of Plant Breeding, Genetics and Biotechnology, PAU, Ludhiana -141004, India*
[#]*Central University of Punjab, Bathinda*

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****Corresponding author***

Email: *ramesh_pau@yahoo.com*

Abstract

Field studies on the role of seed borne inoculum of Karnal bunt (KB) and infection risks were carried out at Punjab Agricultural University, Ludhiana during 2003-04 to 2011-12 using the most susceptible wheat variety, WH 542. The potential of KB infected seeds in causing the disease in subsequent years was determined by planting seed of different KB intensities (0.0 -10%) at different sites. The KB continued to appear up to five years in three of the sites, though in traces in some of the seasons. Weather conditions played a significant role in the disease development. Nevertheless, the pattern of disease occurrence was often independent of different KB intensities due to lateral movement of infective propagules across the plots. Occurrence of infection may have depended more on wind direction, random movement and chance landing of sporidia. Another study conducted from 2005-06 to 2007- 08 showed that disease occurred up to 100 m from the primary inoculum centre. In third experiment the disease incidence was observed to be comparatively higher when the bunted grains were powdered and applied at the soil surface than when used as whole grains beneath the soil. Even a single powdered infected grain, was capable of causing considerable infection around 10 m radius. The fourth and fifth experiments (2006-07 to 2007-08), respectively, carried out to ascertain the viability of sporidia in relation to nutritional component and field exposure showed that sporidia maintained their viability, respectively, for 21, 18 and 9 days in January, February and March under field conditions and also on soil extract and leaf washing agar up to 4th sub-culturing indicating their potential to survive and travel long distances.

Keywords: Karnal bunt infection risks, seed borne inoculum, sporidial dispersal, viability of Karnal bunt

1. Introduction

Karnal bunt (*Tilletia indica* Mitra) of wheat is a disease of quarantine significance. The teliospores of the pathogen perpetuate in the soil and germinate to produce a tuft of primary sporidia followed by allantoid shaped secondary sporidia which further multiply, become air borne to cause floret infection. Every year, the inoculum is added in the soil by sowing infected / infested seed. There are number of reports demonstrating the survival of teliospores for 2-7 years based on germination tests conducted under lab conditions (Aggarwal *et al*., 1977; Dhiman, 1982; Sharma and Nanda, 2002; Inman *et al*., 2008). It is, however, not documented as to how long the soil borne teliospores will be able to cause infection under natural field conditions and little is known about the inoculum density-disease relationship. Moreover, the seeds are sown upto a depth of 4-6 cm and it was assumed that the full risk of infection may not be apparent in the first year since teliospores may remain buried in the soil and only be exposed in the following years (Smilanick, *et al.,* 1985; Gill *et al.,* 1992) by routine cultural operations. Present studies, were, therefore, carried out with an objective to evaluate the potential of infected seeds in causing the disease in the subsequent years by determining the survival of seed borne inoculum in the soil; disease gradients around selected primary infection centre (PIC); infection potential

of loose or intact spores (with seed) - placed either underneath or on the surface of the soil and the viability of the infective propagules (sporidia) in the field.

2. Materials and methods

The studies were initiated in 2003-04 in the experimental area of Seed Technology Centre, Punjab Agricultural University Ludhiana, India with known history of non occurrence of the disease in the last 15 years. During 2003-04, seed of different KB intensities (0.25, 0.5, 1.0, 5.0 %) was planted in four replications with a plot size of 2 x 2.5 m in a randomized block design- designated as Site 1. Seed lot without bunted grains served as check treatment. The most susceptible variety, WH 542 was used for these studies. The seed was prepared by blending KB infected seed of grade 2-3 (Aujla *et al.,* 1989) to get the desired concentrations. The experiment was laid out in the second week of November, 2003 and harvested / threshed in May, 2004. The produce of each plot was thoroughly mixed manually with the help of a spade followed by boerner type divider. From each seed lot (replication), 3 primary samples were drawn with the help of sleeve type of trier and pooled to make the working sample for recording KB incidence. From this sample, three random samples were drawn with the help of a 100 cc beaker for recording the infection visually on the seed examination board against the artificial light and if no infection was detected, the whole lot of the working sample was transferred to a large white enameled tray to examine the trace infections against the white background under the natural sun light.

However, considering the lateral movement of the sporidia which caused disease even in the check plots on Site 1, the bigger plots (9.0 x 4.5 m) and higher inoculum concentrations (0.25, 2.0, 5.0 and 10.0%) were used for subsequent experiments on 4 sites (designated as Sites 2,3,4,5 - each created every year since 2004-05). Bigger plots were assumed to confine the movement of sporidia within respective plots and were also considered to absorb the effect of inoculum displacement (about 0.25-0.5 m from original position) with the tractor driven harrow and cultivator (a normal tilling practice). Each plot was surrounded by a barren buffer area of 1 m with a 6 cm raised bund to restrict the movement of teliospores with irrigation water between the plots. The infection potential of the initial inoculum was determined in the current crop season as well as subsequent seasons by planting healthy seed (Table 1- potential time lines). The Site 1 was abandoned for further studies. Simultaneously effect of rainfall, temperature and humidity was also taken into account on the development of the disease.

Table 1. Time lines for assessing the viability and infection potential of seed borne inoculum

Site	Crop Seasons								
	2003-04	2004-05	2005-06	2006-07	2007-08	2008-09	2009-10	2010-11	2011-12
1	Planted infected seeds	Abadoned for further studies	-	-	-	-	-	-	-
2		Planted infected seeds	Planted healthy seeds	Planted healthy seeds	Planted healthy seeds	Planted healthy seeds			
3			Planted infected seeds	Planted healthy seeds	Planted healthy seeds	Planted healthy seeds	Planted healthy seeds		
4				Planted infected seeds	Planted healthy seeds	Planted healthy seeds	Planted healthy seeds	Planted healthy seeds	
5					Planted infected seeds	Planted healthy seeds	Planted healthy seeds	Planted healthy seeds	Planted healthy seeds

Further, the data of site 2 during 2004-05 revealed that the infective propagules still moved across the bigger plots to cause the disease, a new experiment was initiated in 2005-06 to find out as how far the infection can occur from the primary infection centre (PIC). Though the experiment could not be accommodated on a large plot to record the observations all around, however, a field measuring 110x40 m, isolated about 200 m on all sides with non wheat growing area (i.e. Agro forestry farm to western side, Central Institute of post harvest and

Engineering Technology to northern side, cotton field to eastern side and experimental area of pulses to southern side), was selected for these studies. A PIC (3 x 3 m) was created at the one end (western side) of the field so that the maximum distance could be utilized in a single direction (eastern side) for the disease spread. KB infected seeds (400 g) were ground, mixed with 500 g sand and spread uniformly on the PIC. Water was sprinkled on the PIC so that the teliospores settled down and did not drift with air. Inoculum was not added to the PIC in the subsequent years of observations.During first year of experimentation, the field was available only up to 65 m, however in the subsequent two seasons (2006-07 and 2007-08) the crop was sown up to 100 m. The samples were drawn from the centre of PIC (0 m) as well as at a gap of every 5.0 m to the Eastern side. At each intersection, four samples/ replicates (each of one square m plot) were harvested, threshed and examined.

Third experiment was initiated in 2006-07 to find out the effect of mode of application as well as form of inoculum on disease development. Four treatments of inoculum levels viz., 1,5,10 and 25 infected grains were taken for this study. The inoculum was applied in two forms i.e. either whole infected grains as such or equivalent of crushed (powdered) infected grains and in two modes i.e. either on the soil surface or underneath the soil. These experiments were conducted on different plots, each of 21x21m size with a single PIC (10x10cm) in the middle of the plot. These plots were further surrounded by 4 rows of oats (presumably to serve as barrier) to eliminate chances of contamination, if any, from the adjoining plots. For the addition of powdered inoculum on the soil surface, the infected seeds were ground in the mixer, thoroughly mixed with 50g sand and the required quantity of the inoculum was spread uniformly over the PIC. A mist of water was sprayed over the centre so that the teliospores settled down and did not drift with air. A boundary bund was also raised around the PIC to prevent the movement of spores by irrigation. For the underground application, the 5 cm upper soil layer of the PIC was removed, the inoculum was spread and again covered with the soil. Likewise, the whole infected grains were added in the same way but the soil surface was covered with nylon netting temporarily and guarded till they got degraded. One plot served as check in each treatment where inoculum was not added. To record the KB infection, samples were drawn from the centre (0 m) as well as at four transects (replicates) to the East, West, North and South from the middle of the PIC at 2.5, 5.0, 7.5 and 10.0 m all around by harvesting a one square m plot at each intersection. However, for recording the data from the PIC, the one square meter plot (including PIC) in the middle of the field was divided in to four equal squares and treated as four replications for sampling. The experiment was repeated in 2007-08.

In view of horizontal spread of the disease, two more experiments were initiated in 2006-07 to ascertain the viability and infectivity of infective propagules (sporidia) in relation to nutritional component and field exposure. The effect of nutritional component on the subsequent multiplication and pathogenic potential of sporidia was studied by serial sub - culturing on different media. The cultures were raised on PDA, soil extract agar, wheat leaf washing agar and water agar and were serially sub-cultured after every 15 days. The pathogenic potential of the subsequent generations was evaluated by harvesting sporidia from each generation/medium for artificial inoculations. For preparing soil extract, one litre of distilled water was added to ½ Kg of field soil, kept overnight and supernatant filtered through ordinary coarse filter paper. In order to prepare leaf washing agar, 100 g of fresh leaves of highly susceptible wheat variety (WH 542) were washed using 200 ml of distilled water (DW). The sporidial suspension was prepared by pouring 10 ml of DW in each test tube, scratching the cultures with camel hair brush followed by sieving through double layer muslin cloth. Fifty ml of inoculum was used for inoculating twenty ears. The experiment was repeated in 2007-08.

To record the viability under field exposures, sporidia were showered from the KB cultures (grown on PDA) onto the petri plates that were placed under the crop canopy in the months of January, February and March. Overhead shelter was provided to protect the plates from rain. Two plates were removed daily and 20 ml sporidial suspension was prepared by pouring 10 ml tap water in each plate. The plates were required to be scrapped at the bottom by a camel hair brush to remove the firmly sticking sporidia. Ten ear heads were inoculated every time at boot leaf stage using hypodermal syringe. The experiment was repeated in 2007-08.

3. Results and discussion

In the first experiment initiated during 2003-04 at Site 1, the disease appeared in traces (1-2 infected grains/kg) in the plots sown with seed lots having 0.25 and 5.0% infected grains and also in check. The experiment was considered of preliminary nature but the observations provided an indication that the disease occurrence was independent of disease intensities and would have depended more on the wind direction and random movement and chance of landing of the sporidia from the PIC. Contrary to earlier reports and belief, it was hypothesized that there was a limited role of vertical movement of sporidia in the disease development and the air borne sporidia were presumed to have a lateral movement and could land away from PIC to cause infection. In view of above, the plot size at site 1 was considered inadequate and abandoned for further studies.

Table 2. Development of Karnal bunt disease in different years by sowing seeds of different infection levels

Site*/ (Plot size)	Seed infection levels (%)	2003-04	2004-05	2005-06	2006-07	2007-08	2008-09	2009-10	2010-11	2011-12
1	0.25	traces	-	-	-	-				
Abadoned after first season/ (2.5x2m)	0.5	0.0	-	-	-	-				
	1.0	0.0	-	-	-	-				
	5.0	traces	-	-	-	-				
	0.0	traces	-	-	-	-				
2.	0.25		0.16	0.06	0.15	0.00	0.00			
(9 x 4.5 m)	2.00		0.08	0.04	0.12	0.00	0.00			
	5.00		0.14	0.04	0.22	0.00	0.00			
	10.00		0.10	0.03	0.32	0.00	Traces			
	0.00		0.24	0.02	0.25	0.00	Traces			
3.	0.25			0.01	0.70	0.00	0.20	0.00		
(9 x 4.5 m)	2.00			0.02	0.90	0.00	0.10	0.00		
	5.00			0.02	0.50	0.00	0.10	0.00		
	10.00			0.02	0.50	Traces	0.10	Traces		
	0.00			0.05	0.70	0.00	Traces	Traces		
4.	0.25				0.10	0.00	0.00	Traces	Traces	
(9 x 4.5 m)	2.00				0.05	0.00	0.00	Traces	Traces	
	5.00				0.08	0.00	0.00	0.00	0.00	
	10.00				0.10	0.00	Traces	0.00	0.00	
	0.00				0.08	0.00	0.00	0.00	Traces	
5.	0.25					Traces	0.00	0.00	0.20	0.00
(9 x 4.5 m)	2.00					Traces	Traces	Traces	0.10	0.00
	5.00					0.00	0.00	Traces	0.20	0.00
	10.00					0.00	0.00	Traces	0.20	0.00
	0.00					Traces	Traces	Traces	0.10	0.00

Traces: **Site 1: 1-2 infected grain/1Kg; Site 2-5: 1-2 infected grains/12kg*

In the subsequent years, the plot size for other four sites (2-5) was kept as 9.0 x 4.5m. The KB continued to appear up to five years in three of the sites, though in traces in some of the seasons (Table 2). At site 2, the disease appeared in each of the first three years in considerable measure, no disease in 4th year and in traces in 5th year. The overall disease infection in the first year ranged between 0.08 to 0.24 % in different treatments followed by low incidence (0.02 -0.06 %) in the 2nd year (2005-06), yet again the higher incidence (0.12 to 0.32 %) in the 3rd year, since its creation in 2004-05. The site 3, created in 2005-06, exhibited substantial incidence of disease in the 1st (0.01-0.05%), 2nd (0.5-0.9%) and 4th year (Traces-0.2%) but in traces in 3rd and 5th year. The site 4, created in 2006-07, showed the considerable disease in the first year (0.05-0.1%) but not in subsequent years. At site 5, created in 2007-08, the

disease was detected in negligible levels except in 4th year where it ranged from 0.1 to 0.2%. Though, there are number of reports demonstrating the survival of teliospores for 2-7 years, under different conditions, based only on germination tests (Aggarwal *et al*., 1977; Dhiman, 1982; Sharma and Nanda, 2002; Moosawi-Jorf and Farrokhi-Nejad, 2007; Inman *et al*., 2008), the occurrence of disease under natural field conditions (up to 5 years after sowing infected seeds) is being documented for the first time. Weather conditions played a significant role in the disease development. as evident from the fact that during 2007-08 to 2009-10 and 2011-12 either the disease was non-existent or negligible as the weather conditions were highly unfavourable (Table 3).

Table 3. Climatic conditions of three critical months of Karnal bunt disease development during 2003-04 to 2011-12

Year	Rainfall (mm)			Average temperature (°C)			Average relative humidity (%)		
	Jan	Feb	March	Jan	Feb	March	Jan	Feb	March
2003-04	67.8	0.0	0.0	12.5	15.8	22.4	87.0	73.0	62.0
2004-05	48.9	47.4	42.2	10.9	13.7	19.7	78.0	79.0	72.0
2005-06	16.8	0.0	32.5	13.3	19.4	19.5	70.9	72.3	66.0
2006-07	10.0	84.7	41.3	11.8	15.2	19.3	73.0	80.0	68.0
2007-08	16.3	3.2	0.0	11.5	13.3	22.2	71.0	72.0	65.0
2008-09	17.4	5.4	3.2	13.7	15.8	20.6	78.6	76.9	64.1
2009-10	18.4	25.0	1.0	11.4	15.1	22.5	84.2	72.4	64.9
2010-11	5.4	44.2	6.5	11.9	14.4	20.2	81.3	89.5	72.5
2011-12	52.6	0.3	0.0	11.7	13.1	19.1	78.2	70.0	62.3

There was no correlation between disease occurrence and various intensities of seed born inoculum used. This has probably occurred due to the movement of sporidia with air currents from the adjoining plots. Overall the data in table 2 signifies less disease during those years when environmental conditions were not favourable for the disease development. However, there was no relationship between the seeds sown with different infection levels and the disease occurrence. Data on average rainfall, temperature and relative humidity has been given in table 3. Earlier studies depicted the significance of high relative humidity, lower maximum temperature and higher number of rainy days prevailing at heading stage during February-March (Nagarajan *et al.,* 1997; Jhorar *et al.,* 1993, Sharma *et al*., 2008).

In the second experiment (Table 4), it was found that the surface borne teliospores either in the form of whole grains or powdered have greater role in the disease development as the average disease incidence was comparatively higher when the powdered inoculum equivalent to 25, 10, 5, and 1 grains, respectively, was added at the soil surface during 2006-07. However, during 2007- 08 the disease appeared in traces (1-2 bunted grains / 12Kg) only in 2 plots where powdered inoculum equivalent to 25 and 10 infected grains was added on the soil surface. All the samples collected from the check plots during 2006-07 were free from the disease except for two samples which showed infection in traces whereas no bunted grain was detected in the year 2007-08 in any of the samples due to non-conducive weather conditions. The results in Table 4 further corroborated and reaffirmed our observations on lateral and random movement of sporidia and their chance landing on host, as the pattern of disease occurrence was often independent of the different KB intensities imposed in each plot and infective propagules still moved across the plots. The infection was detected up to 10 m from the PIC. The present studies have established the significant role of lateral movement of the sporidia from the PIC which might have depended more on wind direction rather than earlier advocated stepwise-vertical-movement of sporidia (hypothesized as monkey jump movement) from soil surface to lower leaves and subsequently after multiplication to upper leaves and finally to ear heads (Dhaliwal and Singh, 1988; Kumar *et al.,* 2006). This finding is of utmost significance for understanding and conducting the epidemiological studies on disease spread. Therefore, the occurrence of KB infections should not be looked just in or near the PIC but invariably far beyond that as has been substantiated.

Yet in another experiment (Table 5), on disease gradient analysis, conducted for three seasons (2005-06 to 2007-08) the incidence of the disease was higher near to PIC and gradually declined progressively with moving away from it.

Table 4. Effect of form and location of inoculum application on development of Karnal bunt

Inoculum load	Form/location of inoculum	Disease spread (% incidence) from the PIC (m)					Average (%)
		0.0	2.5	5.0	7.5	10.0	
25 infected grains or equivalent	1 Whole grain (BS)	0.00	0.04	0.01	0.01	0.00	0.01
	2 Whole grain (OS)	0.00	0.23	0.10	0.10	0.10	0.11
	3 Powdered grain (BS)	0.10	0.10	0.18	0.25	0.15	0.16
	4 Powdered grain (OS)	0.60	0.28	0.38	0.30	0.10	0.33
Clean seed	5 Check	0.00	0.00	0.00	0.00	0.00	0.00
10 infected grains or equivalent	1	0.00	0.10	0.05	0.03	0.00	0.04
	2	0.30	0.20	0.18	0.10	0.08	0.17
	3	0.00	0.35	0.20	0.05	0.10	0.14
	4	0.40	0.38	0.18	0.08	0.10	0.23
Clean seed	5	0.00	0.00	0.00	Traces	Traces	Traces
5 infected grains or equivalent	1	0.00	0.00	0.00	0.00	0.00	0.00
	2	0.30	0.00	0.00	0.00	0.00	0.06
	3	0.50	0.12	0.25	0.00	0.00	0.17
	4	0.30	0.33	0.28	0.10	0.05	0.21
Clean seed	5	0.00	0.00	Traces	Traces	0.00	Traces
1 infected grain or equivalent	1	0.00	0.00	0.00	0.00	0.00	0.00
	2	0.00	0.00	0.00	0.00	0.00	0.00
	3	0.00	0.10	0.00	0.00	0.00	0.02
	4	0.00	0.10	0.08	0.03	0.00	0.04
Clean seed	5	0.00	0.00	0.00	Traces	0.00	Traces

BS – Beneath the soil, OS – On soil surface; Traces: 1-2 grains/12kg

Table 5. Infection gradient of Karnal bunt from primary infection centre (PIC)

Distance from PIC (m)	Average infection (%)		
	2005-06	2006-07	2007-08
0	6.7	0.4	0.0
5	4.1	1.2	0.0
10	3.8	0.9	0.0
15	2.2	0.6	0.0
20	2.7	0.9	0.07
25	1.8	0.7	0.13
30	0.6	0.7	0.07
35	0.6	0.2	0.0
40	0.5	0.2	0.03
45	0.9	0.2	0.03
50	0.9	0.4	0.03
55	0.6	0.2	0.0
60	0.4	0.1	0.0
65	0.3	0.3	0.0
70	-	0.4	0.0
75	-	0.4	0.0
80	-	0.6	0.0
85	-	0.6	0.0
90	-	0.5	0.0
95	-	0.2	0.0
100	-	0.1	0.0

Disease was found to occur up to 100 m from PIC indicating the high potential and viability of sporidia to travel far away from their site of production. This finding is again very important from the epidemiological point of view. While recording the effect of nutritional component on the pathogenic potential of the sporidia, it was found that sporidia maintained their viability and pathogenecity for quite long(up to 4th sub-culturing) on soil extract and leaf washing agar indicating that sporidia have every chance to survive and travel to long distances under field conditions (Table 6).

Table 6. Effect of serial sub - culturing on pathogenic potential of sporidia produced on different media

Serial transfer every 15 days	% Karnal bunt											
	Potato Dextrose Agar			Soil extract Agar			Leaf washing agar			Water Agar		
	2006-07	2007-08	Average	2006-07	2007-08	Average	2006-07	2007-08	Average	2006-07	2007-08	Average
I	36.7	29.8	33.2	24.4	19.4	21.9	22.8	14.8	18.8	15.7	11.7	26.7
II	34.8	25.9	30.8	11.7	9.8	10.8	9.4	6.3	7.8	7.6	4.6	6.1
III	38.5	29.4	34.0	6.7	5.0	5.8	4.6	2.9	3.8	3.4	1.0	2.2
IV	36.2	22.6.	29.4	1.2	0.9	6.4	0.8	0.5	4.2	0.0	0.05	0.2
V	34.7	27.8	31.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
VI	33.8	26.3	30.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

The exposure of sporidia under crop canopy further indicated that, though, there was a progressive loss in viability with the enhancement of exposure period, the sporidia could remain viable for 21 days in January, 18 days in February and only 9 days in March when the monthly average temperatures were recorded at 11.8, 15.2 and 19.3 in 2006-07 and 11.5, 13.3 and 22.2 in 2007-08, respectively (Table 7). These findings further support that sporidia can maintain their longevity for quite long during the vulnerable stage of the crop.

Though there occurs large scale variation between the replications and no correlation could be drawn within the various treatments as the disease is highly dependent upon the weather conditions, microclimate, wind direction and random movement and chance of landing of sporidia on the host.

Table 7. Effect of sporidial exposure (under crop canopy in different Months) on their pathogenic potential

Inoculations after days of exposure	Karnal bunt (%)								
	January			February			March		
	2006-07	2007-08	Average	2006-07	2007-08	Average	2006-07	2007-08	Average
0	31.6	9.4	20.5	35.2	27.6	31.4	22.8	19.0	20.9
3	30.0	7.4	18.7	24.7	26.4	25.5	8.6	11.3	10.0
6	26.4	6.2	16.3	10.8	20.2	15.5	4.2	5.2	4.7
9	21.5	5.8	13.6	7.4	14.8	11.1	0.5	2.8	1.6
12	12.6	4.6	8.6	4.8	7.3	6.1	0.0	1.0	0.5
15	8.5	3.8	6.1	3.2	1.4	2.3	0.0	0.8	0.4
18	7.8	2.8	5.3	1.8	0.08	1.3	0.0	0.0	0.0
21	0.8	1.8	1.3	0.0	0.02	.01	0.0	0.0	0.0

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