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Post harvest management of Karnal bunt (*Tilletia indica)* in wheat by mechanical seed processing

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ABSTRACT

Karnal Bunt (*Tilletia indica* Mitra) is a designated disease and a limiting factor in wheat export because most countries regulate Karnal bunt (KB) pathogen as a quarantine pest. The seed must be threshed and examined for KB infection as detection of disease in the field is difficult. Therefore, the post harvest management of KB in wheat seed production through mechanical processing is very important. Pre-cleaner and screen grader of the processing line removed 83.1, 61.4 and 77.8% of total KB infected seed present in the seed lot and reduced KB infection from 1.42 to 0.24, 4.27 to 1.65 and 3.57 to 0.79% in 2012-13, 2013-14 and 2014-15, respectively which is much higher than the permissible limit of 0.05%. Hence, a total of 18 combinations, comprising of three deck slopes (S_1- 2^0, S_2- 2.5^0, S_3- 3^0), three feedings (F_1- 5 kg, F_2- 10 kg, F_3- 15 kg per minute) and two output settings of the deck width (O_1- 45cm, O_2- 43cm) of specific gravity separator were studied with an objective of minimizing Karnal bunt infected seed in the seed lot within permissible limits. Minimum KB infection in final product (0.04, 0.28 and 0.11%), maximum seed recovery (12.58, 12.78 and 12.72 kg/minute) with 84.1, 86.4 and 85.4% recovery efficiency was obtained by the treatment $S_1F_3O_1$ (slope of deck 2^0, feeding 15 kg/minute, output deck width 45cm) in 2012-13, 2013-14 and 2014-15, respectively. Mechanical processing reduced KB infection by more than 90%, depending on the intensity of infection, as well as improved seed quality, i.e. seed germination improved by 7.90% and physical purity by 2.22%.

Key words: Karnal bunt, Mechanical processing, Quarantine disease, Wheat

Karnal bunt is an internationally regulated disease of wheat adversely affecting the yield and trade. The disease has become a major SPS issue in the wake of recent Sanitary and Phytosanitary Agreement stipulated by WTO. Under the agreement, each member country has to undertake pest risk analysis (PRA) for all the agricultural commodities for export and import. Karnal bunt also known as kernel smut or partial bunt caused by *Tilletia indica* Mitra, syn. *Neovossia indica* (Mitra) Mundkur was first reported from Karnal in India by Mitra in 1931. Since then it is recorded from all wheat growing states of India, maximum being in Punjab, Haryana, Himachal Pradesh and Uttar Pradesh. The disease is also reported from Afghanistan, Iraq, Mexico, Sweden, Syria, Turkey, Russia and Nepal. Prior to 1970s, Karnal bunt disease occurred sporadically and remained confined to a few pockets only. But as a consequence of changed climatic conditions, cropping systems and enhanced inputs, it assumed epiphytotic proportions, causing substantial losses to both quality and quantity of wheat.

Karnal bunt pathogen not only reduces the weight and impairs viability of seeds, but also causes deterioration of flour quality due to production of trimethylamine (Singh and Bedi 1985) and is also responsible for rejection of grains in international trade. Generally, wheat containing more than 3% bunted kernels is considered unfit for human consumption. It is difficult to detect the disease in the field and KB infected seed shows no symptoms until near maturity. Developing wheat kernels are randomly infected in an ear and usually with partial infections. Karnal bunt disease is designated as high risk disease and minimum tolerance levels have been fixed by number for it in foundation (0.05%) and certified seed (0.25%). Because most countries regulate the Karnal bunt pathogen as a quarantine pest, efficacy of mechanical processing for elimination of bunted seeds was studied to retain good quality product and to prevent disruption or losses in international trade.

MATERIALS AND METHODS

Seed of wheat cultivar HD 2851 grown at ICAR-Indian Agricultural Research Institute, Regional Station, Karnal during *rabi* seasons

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2012-13, 2013-14 and 2014-15 were taken for the study. The average moisture content of the wheat seed determined as per ISTA rules (1993) were 9.2, 8.9 and 9.3%, respectively.

Seeds were processed in Nippon Sharyo (Japan) make processing plant of one tonne per hour capacity. The processing line comprised seed pre-cleaner-cum-grader, screen grader, indented cylinder grader in bypassed mode and specific gravity separator. Air screen machines (pre-cleaner and screen grader) were equipped with feed control, scalping screen, grading screen and aspiration system. Top and bottom screens of air screen machines were 5.50 mm (round) and 2.30 mm (oblong), respectively, as per the recommendations of Indian Minimum Seed Certification Standards (Tunwar and Singh 1988). The essential parts of specific gravity separator were an adjustable porous deck, fan system that forces air through the porous deck, assemblies that oscillate and incline the deck. The inclination of deck, feeding and output were adjusted for better separations of low density and diseased seeds from the lot. Total 18 combinations, comprising three slopes (S_1- 2^0, S_2- 2.5^0, S_3- 3^0), three feedings (F_1- 5 kg, F_2- 10 kg, F_3- 15 kg per minute) and two output settings (O_1- 45cm, O_2- 43cm) were kept with an objective of getting maximum Karnal bunt free seed.

Ten samples, weighing 500 g each, were drawn for quality assessment at different stages, viz. unprocessed, product outlets of pre-cleaner, screen grader and specific gravity separator at intervals of 10 min. These 10 primary samples collected from each stage were mixed separately using mixer and then divided by seed divider. Three replications weighing 1.0 kg each were drawn from the mixture for each stage, and classified as representative samples. These representative samples were used for physical purity, germination, test weight, germination index and vigour index evaluation as per ISTA rules (1999). The laboratory-based study on physical purity was measured using two replications of 50g each with Complete Randomized Design. Pure seed, inert matter and other crops seed were separated using purity board and physical purity was calculated on weight basis (Agrawal 1993). Four replications of hundred seeds each were kept between wet paper towels in the germinator for 8 days at 20° C and 95% RH. The number of normal seedlings, abnormal seedlings and dead seeds were counted and recorded. Germination percent was expressed on the basis of normal seedlings. The 1000-seed weight of each sample was measured. Number of seedlings emerging daily were counted and germination index (G.I.) was computed (Gupta 1993) as: G.I. = n/d ... (1) where, n = number of seedlings emerging on day'd', and d = day after planting. Root and shoot length was measured in ten normal seedlings from every replication of each treatment taken at random without damaging their root system. Ten normal seedlings from every replication of each treatment taken at random were dried at 80° C in oven for 24 h to obtain dry weight of seedlings. Seed vigour index - I and II were derived by multiplying per cent germination with total seedling length (cm) and dry weight of seedlings (g), respectively (Gupta 1993, Abdul-Baki and Anderson 1973). For output efficiency of the specific gravity separator, three samples were taken from the final product outlet to calculate the amount of seed cleaned in one minute. Two samples of 100g each were drawn from product and reject outlets of specific gravity separator to study Karnal bunt infection which was detected by sodium hydroxide seed soak technique (Agarwal and Verma 1983). In this technique, wheat seeds are soaked overnight in 0.2% solution of NaOH. Next morning the seeds were taken out of solution and spread on a blotter sheet to remove excess moisture. On examination, the KB infected seeds gave appearance of shiny jet-black colour. The infection was confirmed as stream of jet-black spores oozed out when bunted seed were pricked under a microscope. The seeds discoloured due to other reasons turn brown to dull black in colour. Karnal bunt infection was calculated on number basis and expressed in percentage. Thereafter, recovery efficiency was computed using the following equation:

$$\text{Recovery efficiency (\%)} = \frac{\text{Final output (100 – KB infection (\%) in final output)}}{\text{Feeding (100 – KB infection (\%) in feeding)}} \times 100$$

Data were subjected to analysis of variance using completely randomized design (Gomez and Gomez 1984). Critical differences at 5% probability level were calculated and used for interpretation.

RESULTS AND DISCUSSION

The intensity of Karnal bunt disease was variable and very much dependent on environmental conditions at the time of anthesis and it also affected the size and density of seed. The portion of wheat seed affected with *T. indica* is transformed into teliospores of the fungus and looks like black powder on examination. The infection of individual kernels varies from small points of infection to completely bunted kernels. The embryo is largely undamaged except when infection is severe. Severely damaged grains (Fig 1) are identified very easily since the tissues along the suture and adjacent endosperm are replaced with spores. The seeds with small lesions produce normal seedlings, whereas those with severe infection have poor germination and produce weak, distorted seedlings.

The analysis of different grades of infection of KB on wheat seed, i.e. tip, partial and full, revealed that with increase in disease intensity on the seed there was a gradual decrease in seed weight, seedling length, seed germination

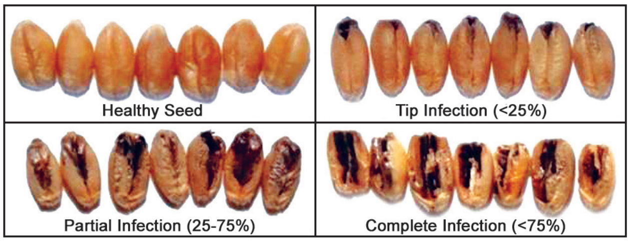

Fig 1 Karnal bunt intensity on wheat seed

and vigour as also reported by Kumar *et al.* (2015). Seed germination in tip, partial and full infection was 89.25, 65.25 and 24.75%, respectively as against 96.50% in healthy seed (Table 1). Seed vigour also showed similar trend, i.e. 2147, 1396 and 356 in tip, partial and full infected seeds as against 2307 in healthy seeds. There was 38.13% loss in seed weight in full infection as against 9.89 and 0.27% loss in partial and tip infections. Seedling length also decreased by 57.1, 29.7 and 15.3% in full, partial and tip infected seed, respectively. Beniwal *et al.* (2000) and Bansal *et al.* (1984) also reported reduction in 1000-grain weight ranging from 4.5 to 52.3 per cent depending on severity of infection. Increase in disease severity resulted in proportional decrease in seed weight, germination and vigour.

Karnal bunt infection in raw seed (unprocessed) was higher than the permissible limit in all the three years of study (Table 2) due to cooler temperature and well distributed rainfall during anthesis stage. It has already been highlighted (Gill *et al*. 1993) that it is not the amount of rainfall but the distribution of rainfall, which accounts for the KB outbreaks. More rainfall occurring for a short period may not be of much significance for the disease, but intermittent rainfall (may be very less) uniformly distributed for longer time from boot stage to spike emergence stage enhances the probability of disease occurrence.

It was observed that out of total KB infection of 1.42, 4.27, and 3.57% in the unprocessed seed lot, 0.52, 2.19 and 1.72% infected seeds were removed by pre-cleaner; and 0.66, 0.43 and 1.06% by screen grader. Pre-cleaner and screen grader were found to remove 83.1, 61.4 and 77.8% of total KB infected seed present in the seed lot and reduced KB infection from 1.42 to 0.24, 4.27 to 1.65 and 3.57 to 0.79% in 2012-13, 2013-14 and 2014-15, respectively. Though it is fit for use as grain but it is much higher than the permissible limit of 0.05 and 0.25% in foundation and certified seed, respectively.

After specific gravity separation, there was further decrease in KB infection (from 0.24 to 0.09% in 2012-13, 1.65 to 0.57% in 2013-14 and 0.79 to 0.19% in 2014-15) and removal of 62.5, 65.4 and 75.9% of KB infected seed in 2012-13, 2013-14 and 2014-15, respectively. Hence, this showed that all the three processing operations are necessary in processing of wheat seed for removal of KB infected seed and mechanical processing reduced KB infection by 93.7, 86.7 and 94.7% in 2012-13, 2013-14 and 2014-15, respectively depending on the intensity of infection.

Specific gravity separator was observed to further improve the seed quality by removing KB infected seed in 18 treatment combinations used in the study during 2012-13, 2013-14 and 2014-15 (Table 3). Minimum KB infection in final product (0.04, 0.28 and 0.11%), maximum seed recovery (12.58, 12.78 and 12.72 kg/min) with 84.1, 86.4 and 85.4% recovery efficiency has been obtained by the treatment $S_1F_3O_1$ (slope of deck 2^0, feeding 15 kg/min, output deck width 45cm) in 2012-13, 2013-14 and 2014-15, respectively. Though the maximum recovery efficiency (96.2, 96.9 and 95.2%) was obtained by $S_1F_1O_1$ treatment but seed recovery was very low (4.78, 4.80 and 4.73 kg/minute) in 2012-13, 2013-14 and 2014-15, respectively. Tip infected seed is difficult to separate from the healthy seed as there is no or very little difference in density of the seed (Table 4).

A 2° slope of the deck gave the best results in terms of minimum Karnal bunt infection in final output (0.05, 0.32 and 0.13%), maximum final output per minute (8.29, 8.67 and 8.64Kg) and maximum recovery efficiency (83.10, 87.87 and 88.36%) in 2012-13, 2013-14 and 2014-15, respectively (Table 5). With increase in slope of the deck, Karnal bunt infection in final output increased but final output per min and recovery efficiency decreased significantly in all the years. On the other hand, increased feeding to specific gravity separator led to significant increase in final output per min and decrease in recovery efficiency but Karnal bunt infection in final output was at par in all the feedings as also reported by Kumar *et al.* (2015). Therefore, feeding of 15 kg per min was best as it gave maximum final output per min (10.58, 10.79 and 10.67 kg) in 2012-13, 2013-14 and 2014-15, respectively. Output treatments were at par for Karnal bunt infection in the final output and final output per min but recovery efficiency reduced significantly with reduction in output collection deck width, hence O_1 treatment (45cm deck width) was better than O_2 treatment (43cm deck width).

Germination and physical purity of unprocessed seed was lower than Indian Minimum Seed Certification Standards of 85% and 98%, respectively (Tunwar and Singh 1988). The seed lot which were below minimum standards of physical purity and germination turned to acceptable

Table 1 Mean effect of Karnal bunt infection on seed quality parameters

KB infection	Test weight (g)	Per cent loss in seed weight over healthy seeds	Per cent loss in seedling length over healthy	Per cent germination	Vigour index I
Healthy	40.12			96.50	2307
Tip	40.01	0.27	15.3	89.25	2147
Partial	36.15	9.89	29.7	65.25	1396
Full	24.63	38.61	57.1	24.75	356

Tip = < 25% infection; Partial = 26-75% infection; Full = 76-100% infection

Table 2 Percent infection of Karnal bunt at different stages of processing

Year	Un-processed	Pre-cleaned	Screen graded	Rainfall 75-105 DAS (mm)	Average temp. (°C)	
					Max.	Min.
2012-13	1.42	0.90	0.24	154.6 (7*)	25.1	7.0
2013-14	4.27	2.08	1.65	58.4 (3*)	23.7	3.9
2014-15	3.57	1.85	0.79	115.8 (5*)	22.1	10.8

* Effective rainy days

Table 3 Recovery efficiency of Karnal bunt free seed by Specific gravity separation during 2012-13, 2013-14 and 2014-15

Treatment	Karnal bunt infection (%) in final output			Karnal bunt free seed (kg) in final output/minute			Recovery efficiency (%)		
	2012-13	2013-14	2014-15	2012-13	2013-14	2014-15	2012-13	2013-14	2014-15
$S_1F_1O_1$	0.04	0.34	0.14	4.80	4.76	4.72	96.2	96.9	95.2
$S_1F_1O_2$	0.06	0.33	0.16	4.68	4.52	4.56	93.8	91.9	92.0
$S_1F_2O_1$	0.06	0.30	0.12	8.67	8.72	8.69	87.0	88.7	87.6
$S_1F_2O_2$	0.06	0.35	0.13	8.51	8.44	8.45	85.4	85.8	85.2
$S_1F_3O_1$	0.04	0.28	0.11	12.58	12.75	12.71	84.1	86.4	85.4
$S_1F_3O_2$	0.02	0.30	0.11	12.50	12.66	12.63	83.5	85.8	84.8
$S_2F_1O_1$	0.07	0.31	0.19	4.06	4.29	4.18	81.5	87.2	84.3
$S_2F_1O_2$	0.11	0.45	0.21	4.01	4.21	4.11	80.4	85.7	82.9
$S_2F_2O_1$	0.08	0.48	0.18	7.38	7.28	7.34	74.0	74.0	74.0
$S_2F_2O_2$	0.08	0.26	0.19	7.29	7.10	7.26	73.1	72.2	73.1
$S_2F_3O_1$	0.10	0.35	0.15	10.02	10.41	10.19	67.0	70.6	68.5
$S_2F_3O_2$	0.11	0.35	0.16	10.16	10.40	10.15	67.9	70.5	68.2
$S_3F_1O_1$	0.13	0.83	0.28	3.08	3.24	3.10	61.7	65.9	62.5
$S_3F_1O_2$	0.13	0.91	0.25	2.91	3.07	3.01	58.4	62.5	60.7
$S_3F_2O_1$	0.19	1.11	0.30	6.55	6.38	6.55	65.7	64.9	65.7
$S_3F_2O_2$	0.10	1.15	0.27	6.21	6.24	6.21	62.3	63.5	62.3
$S_3F_3O_1$	0.13	1.02	0.23	8.89	9.26	8.89	59.4	62.8	59.4
$S_3F_3O_2$	0.15	1.14	0.26	9.29	9.08	9.29	62.1	61.5	62.1
Mean	0.09	0.57	0.19	7.29	7.38	7.34	74.29	76.28	75.20

Table 4 Intensity of Karnal bunt infection (%) at different stages of processing

Year	Unprocessed			Pre-cleaned			Screen graded			Specific gravity separated		
KB infection	Tip	Partial	Full	Tip	Partial	Full	Tip	Partial	Full	Tip	Partial	Full
2012-13	14	45	41	43	34	23	59	29	12	87	13	0
2013-14	55	32	13	67	23	10	78	17	05	94	06	0
2014-15	34	46	20	57	29	14	79	15	06	93	07	0

Tip = < 25% infection; Partial = 26-75% infection; Full = 76-100% infection

Table 5 Mean recovery efficiency of Karnal bunt free seed by Specific gravity separation during 2012-13, 2013-14 and 2014-15

Treatment	Karnal bunt infection in final output (%)			Final output/minute (kg)			Recovery efficiency (%)		
	2012-13	2013-14	2014-15	2012-13	2013-14	2014-15	2012-13	2013-14	2014-15
Slope 1 (2^0)	0.05	0.32	0.13	8.29	8.67	8.64	83.10	87.87	88.36
Slope 2 (2.5^0)	0.09	0.37	0.18	7.16	7.31	7.22	71.72	74.04	75.17
Slope 3 (3^0)	0.14	1.03	0.27	6.16	6.27	6.22	61.70	63.12	62.60
Feeding 1 (5 kg/min)	0.09	0.53	0.21	3.93	4.04	3.96	78.68	81.66	79.61
Feeding 2 (10 kg/min)	0.10	0.61	0.20	7.44	7.42	7.45	74.55	74.84	74.93
Feeding 3 (15 kg/min)	0.09	0.57	0.17	10.58	10.79	10.67	70.65	72.93	71.59
Output 1 from 45cm deck width	0.09	0.56	0.19	7.34	7.49	7.41	75.16	77.48	76.03
Output 2 from 43cm deck width	0.09	0.58	0.19	7.29	7.34	7.31	74.09	75.48	74.72
CD (P=0.05) for Slope	0.02	0.09	0.04	0.155	0.162	1.59	4.12	4.27	4.21
CD (P=0.05) for Feeding	NS	NS	NS	0.155	0.161	1.61	2.23	2.29	2.27
CD (P=0.05) for Output	NS	NS	NS	NS	NS	NS	0.82	0.84	0.85
CD (P=0.05) for Interactions	NS	NS	NS	NS	NS	NS	NS	NS	NS

Table 6 Mean values of different quality parameters at different stages of wheat processing during 2012-13, 2013-14 and 2014-15

Stage	Physical purity (%)	Germ. (%)	Test weight (g)	Germ. Index	Seedling length (cm)	Seedling dry weight (g)	Vigour index I	Vigour index II
Unprocessed	96.85	83.90	35.94	18.21	15.49	1.01	1299.61	84.74
Pre-cleaned	97.52	85.40	37.13	19.89	16.17	1.12	1380.92	95.65
Screen graded	98.12	88.20	38.10	20.81	17.83	1.17	1572.61	103.19
Specific gravity separated	99.07	91.80	40.02	21.54	19.01	1.21	1745.12	111.08
Rejected by SGS	84.29	73.80	24.72	14.62	11.25	0.78	830.25	57.56
CD (P=0.05)	0.83	6.07	0.79	3.11	3.09	0.10	342.15	22.83

limits of 99.07% physical purity and 91.80% germination after mechanical seed processing. It was observed that out of total impurities of 3.15% in the unprocessed seed lot, 1.27% was removed collectively by air screen machines (pre-cleaner and grader), showing removal of 40.31% of total impurities present in the seed lot. Physical purity after specific gravity separation was significantly higher than that of pre-cleaned and graded seed lot. This shows necessity of all the three processing operations in processing wheat seed for higher seed quality. Specific gravity separator alone improved germination by 3.60%, followed by screen grader (2.80%) and pre-cleaner (1.50%). Germination was at par among processing machines, which reflected that the original seed lot had less proportion of seed with poor germination. Moreover, all the three machines together improved the seed lot germination by 7.90% and physical purity by 2.22%. Test weight was also found to be significantly higher in the seed lot after gravity separation (Table 6). Maximum rise in test weight was observed after passing through specific gravity separator (1.92 g), followed by screen grader (0.97 g) and pre-cleaner (1.19 g). This implied that significant difference existed in size and aerodynamic behaviour of quality seed and rejected seed/impurities. Similar results were observed in wheat by earlier researchers (Sinha *et al*. 2001, Sinha *et al*. 2002, Doshi *et al*. 2013).

Germination, germination index, seedling length, dry seedling weight, vigour index-I and vigour index-II at different stages of processing were found to be statistically at par, and remarkable improvement in desired quality parameters of the seed lot was observed through processing (Table 6). All the quality parameters of seed improved significantly after specific gravity separation as against raw (unprocessed) seed thereby indicating importance of gravity upgradation in improving vigour of the seed lot.

Hence, the study revealed that mechanical processing reduced KB infection by more than 90%, depending on the intensity of infection. It not only increased the grain quality making it fit for human consumption but also enhanced seed quality (seed germination improved by 7.90% and physical purity by 2.22%). Karnal bunt being a seed borne disease, its management through mechanical processing will increase the availability of healthy seed in the seed multiplication chain.

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