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Post Harvest Management of Karnal Bunt, A Quarantine Disease in Wheat

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ABSTRACT

Karnal bunt disease of wheat caused by *Tilletia indica* is a designated disease and a limiting factor in wheat export because most countries regulate the Karnal Bunt (KB) pathogen as a quarantine pest. KB pathogen is seed, soil and air borne. The seed must be threshed and examined for KB infection. Therefore, the post harvest management of KB in wheat seed production through mechanical processing is very important. Pre-cleaner and screen grader removed 83.1 and 61.4% of total KB infected seed present in the seed lot and reduced KB infection from 1.42 to 0.24% and 4.27 to 1.65% in 2012-13 and 2013-14, respectively but it 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 min^{-1}) and two output settings (O_1- 45 cm deck width, O_2- 43 cm deck width) of specific gravity separator were studied with an objective of getting maximum Karnal bunt free seed per unit of time. Minimum KB infection in final product (0.04 and 0.28%), maximum final output (12.58 and 12.78 kg min^{-1}) and KB free seed recovery per minute (12.58 and 12.75 kg) with 84.1 and 86.4% recovery efficiency has been obtained by the treatment $S_1F_3O_1$ (slope of deck 2^0, feeding 15 kg min^{-1}, output deck width 45 cm) in 2012-13 and 2013-14, respectively. Mechanical processing reduced KB infection by more than 93%, depending on the intensity of infection and increased seed quality i.e., seed germination improved by 7.71% and physical purity by 2.41%.

Key words: Karnal bunt, quarantine disease, wheat, mechanical processing

INTRODUCTION

Karnal bunt disease also known as kernel bunt or partial bunt caused by *Tilletia indica* Mitra, Syn *Neovossia indica* (Mitra) Mundkur is an internationally regulated disease of wheat adversely affecting the yield and trade. The disease was first reported from Karnal in India by Mitra (1931). Karnal bunt is recorded from all wheat growing states of India, maximum being in Punjab, Haryana, HP and Uttar Pardesh (Gill *et al*., 1993). 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 cropping systems and enhanced inputs, it assumed epiphytotic proportions, causing substantial losses to both quality and quantity of wheat (Singh *et al*., 1985; Gill and Aujla, 1986; Bedi, 1989).

The disease caused losses between 0.2 to 0.5% of total production (Munjal, 1975; Joshi *et al*., 1983) which increased to 20% in the year of epidemic occurrence in Uttar Pradesh in 1982 (Shukla *et al*., 1982; Singh, 1986). Freshly collected infected grains emit a foul smell, like rotten fish, due to the presence of trimethylamine and the wheat products from severely infected grains are unpalatable (Singh and Bedi, 1985). The disease apart from causing direct quantitative and qualitative losses in terms of yield and seed vigour is also responsible for rejection of grains in international trade. Flour made from bunted grains is discoloured and has an unpleasant odour and taste. Pasta products made with flour contaminated with KB spores can have an unacceptable color. Even one to four percent Karnal bunt infection renders the grains unfit for human consumption (Mehdi *et al*., 1973). Generally, wheat containing more than 3% bunted kernels is considered unfit for human consumption. An importing country insists on zero tolerance levels for fear of introducing the disease in areas where the disease is not known to occur (Martin, 1986). Strict quarantine regulations have been imposed on the import of bunted grain. In 1996, the Governments of Turkey, Morocco and Poland detained some export consignments of wheat from India. A cargo worth US $ 5 million was not allowed to be unloaded in Poland due to Karnal bunt infection. Thus, the disease greatly affects the potential export markets for India. All round increase in wheat yield, comfortable food stocks and freedom from blemishes like black tip and Karnal bunt may facilitate greater grain trade in India of both bread and durum wheat and their value added products. Karnal bunt disease is designated as high risk disease and minimum tolerance levels have been fixed for it in foundation and certified seed. Economic losses caused by the disease are mainly attributed to lower grain quality as well as quarantine restrictions. Because most countries regulate Karnal bunt pathogen as a quarantine pest, effective techniques as efficacy of mechanical processing for elimination of bunted grains were 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 2012-13 (32170 kg) and 2013-14 (38160 kg) were taken for the study. The average moisture content of the wheat seed, determined as per ISTA (1993), was 9.2 and 8.9%, respectively and hence seed drying was not considered necessary for processing. Visual evaluation of the harvested seed lot did not show any broken seed and therefore the length separator was by-passed.

Seed processing machines: Seeds were processed in Nippon Sharyo Japan make processing plant of 1 ton h^{-1} capacity. The processing line comprised of 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 screen of air screen machines was 5.50 mm (round) and 2.30 mm (oblong), respectively, as against 2.10 mm (oblong) bottom screen recommended by 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 (Fig. 1), comprising of 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 min^{-1}) and two output settings (O_1- 45 cm, O_2- 43 cm) were kept with an objective of getting maximum Karnal bunt free seed.

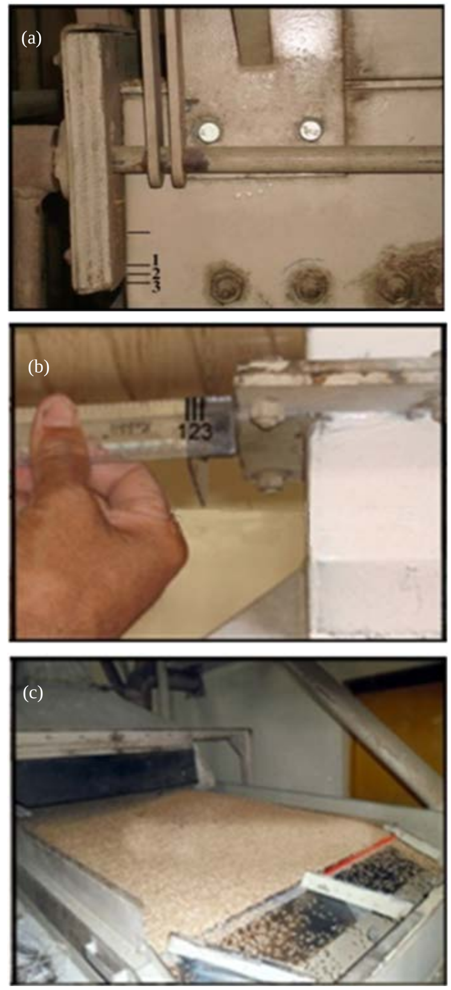

Fig. 1(a-c): Various adjustments of specific gravity separator, (a) Slop adjustment, (b) Feeding adjustment and (c) Output adjustment

Sampling and analysis: 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 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 (1999). The laboratory-based study on physical purity was measured using two replications of 50 g 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 was also counted till the completion of germination. Thereafter, a Germination Index (G.I.) was computed using the equation (Gupta, 1993) as:

$$G.I. = n/d$$

where, n = number of seedlings emerging on day 'd' and d = day after planting.

Root and shoot length of ten normal seedlings was measured 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 get 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 1 min. Two samples of 100 g 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 of Karnal bunt free seed was computed using the following equation:

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

Statistical analysis: 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

In the present study, out of the total of 32170 kg and 38160 kg seed, 28068 and 33203 kg of quality seed was received at the product outlet after processing with 87.25 and 87.01% seed recovery during 2012-13 and 2013-14, respectively. The quality parameters of specific gravity separated wheat seed lot i.e. physical purity, standard germination, test weight, germination index, dry seedling weight, vigour index I and II except seedling length were significantly better than unprocessed seed (Table 1) whereas seed material rejected by specific gravity separator was significantly poor in all the quality parameters in comparison to unprocessed, pre-cleaned, screen graded and specific gravity separated seed. The germination of unprocessed, pre-cleaned, screen graded and specific gravity separated seed was 84.20, 85.80, 88.30 and 91.30%, respectively. The physical purity of unprocessed, pre-cleaned, screen graded and specific gravity separated seed was 97.12, 97.36, 98.08 and 99.53%, respectively. Test weight of unprocessed, pre-cleaned, screen graded and specific gravity separated seed was 36.14, 37.03, 38.03 and 40.12, respectively. Similar increasing trend was observed in other seed quality parameters i.e., germination index, seedling length, seedling dry weight, vigour index I and II in different stages of processing (Table 1).

Karnal bunt infection in unprocessed seed was 1.42 and 4.27% which reduced to 0.90 and 2.08% by pre-cleaner in 2012-13 and 2013-14, respectively (Table 2). The infection decreased further to 0.24 and 1.65% after screen grading during 2012-13 and 2013-14, respectively. The average minimum and maximum temperature was 7.0 and 25.1°C during 2012-13 and 3.9 and 23.7°C during 2013-14 at 75-105

Table 1: Mean values of different quality parameters at different stages of wheat processing during 2012-13 and 2013-14

Stage	Physical purity (%)	Germination (%)	Test weight (g)	Germination index	Seedling length (cm)	Seedling dry weight (g)	Vigour index I	Vigour index II
Unprocessed	97.12	84.20	36.14	18.23	15.34	1.04	1291.63	87.57
Pre-cleaned	97.36	85.80	37.03	19.94	16.67	1.13	1430.29	96.95
Screen graded	98.08	88.30	38.30	20.87	18.03	1.19	1592.05	105.08
Specific gravity separated	99.53	91.30	40.12	21.59	19.11	1.23	1744.74	112.30
Rejected by SGS	87.27	72.60	24.94	14.65	11.25	0.83	816.75	60.26
CD at p=0.05	0.81	6.12	0.035	3.17	4.09	0.11	349.45	24.43

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

Years	Unprocessed	Pre cleaned	Screen graded	Average temperature (°C) 75-105 DAS Max.	Average temperature (°C) 75-105 DAS Min.	Rainfall 75-105 DAS (mm)	Average relative humidity (%)
2012-13	1.42	0.90	0.24	25.1	7.0	154.6 (7*)	96.4
2013-14	4.27	2.08	1.65	23.7	3.9	58.4 (3*)	96.2

*Effective rainy days

Days After Sowing (DAS). The rainfall during the same period was 154.6 and 58.4 mm with 7 and 3 effective rainy days, respectively.

The portion of wheat grain affected with *T. indica* is transformed into teliospores of the fungus and looks black powder on examination. The infection varies from small points of infection to completely bunted kernels. Severely damaged grains (Fig. 2) are identified very easily since the tissues along the suture and adjacent endosperm are replaced with spores. Based on the intensity of KB disease, the infection was classified into three categories, tip (<25% infection), partial (26-75% infection) and full (>75% infection). In 2012-13 tip, partial and full infections in unprocessed seed was 14, 45 and 41% as against 55, 32 and 13% of the total KB infected seed during 2013-14, respectively (Table 3). However, after specific gravity separation, none of the seeds had full infection and partial infection decreased to 13 and 6% but tip infected seeds increased to 87 and 94% during 2012-13 and 2013-14, respectively. Similarly, there was decrease in partial and full infected seeds and increase in tip infected seeds during pre cleaning and screen grading processes. In 2013-14 tip infection was four times than in 2012-13 and partial and full infection of seed was less.

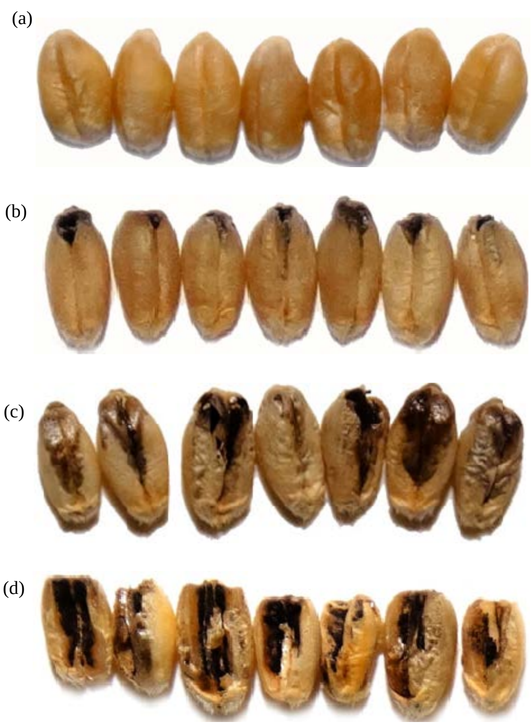

Fig. 2(a-c): Karnal bunt intensity on wheat seed, (a) Healthy seed, (b) Tip infection (<25%), (c) Partial infection (25-75%) and (d) Complete infection (>75%)

Different grades of KB infections i.e. tip, partial and full affected seed germination, vigour and test weight of wheat seeds adversely. Seed germination in tip, partial and full infection was 89.75, 66.25 and 36.50%, respectively as against 97.25% in healthy seed (Table 4). Seed vigour also showed similar trend i.e., 2159, 1401 and 599 in tip, partial and full infected seeds as against 2316 in healthy seeds. There was 38.13% loss in seed weight in full infection as against 12.64 and 0.40% loss in partial and tip infections. Seedling length was also reduced by 40.1% in full infected seed as against 26.7 and 17.7% in partial and tip infections, respectively.

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 and 2013-14. Specific gravity separator decreased KB infection in the seeds from 0.24 to 0.09% in 2012-13 (Table 5) and from 1.65 to 0.57% in 2013-14 (Table 6).

Minimum KB infection was observed in $S_1F_3O_1$ (0.04 and 0.28% in 2012-13 and 2013-14, respectively) and $S_1F_3O_2$ (0.02 and 0.30% in 2012-13 and 2013-14, respectively) treatments. However, feed in both the treatments was 15 kg min^{-1} but final output per minute (12.58 and 12.50 kg in $S_1F_3O_1$ and $S_1F_3O_2$, respectively in 2012-13 and 12.78 and 12.70 kg in $S_1F_3O_1$ and $S_1F_3O_2$, respectively in 2013-14) of which KB free seed was 12.58 and 12.50 kg in $S_1F_3O_1$ and $S_1F_3O_2$, respectively in 2012-13 and 12.75 and 12.66 kg in $S_1F_3O_1$ and $S_1F_3O_2$ treatments, respectively in 2013-14 with recovery efficiency of 84.1 and 86.4% in 2012-13 and 2013-14, respectively in $S_1F_3O_1$ treatment as against the overall final output of 7.32 and 7.42 kg min^{-1}, KB free seed recovery of 7.31 and 7.38 kg min^{-1} and recovery efficiency of 74.63 and 76.48% during 2012-13 and 2013-14, respectively (Table 5 and 6). Though the maximum recovery efficiency (96.2 and 96.9%) was obtained by $S_1F_1O_1$ treatment but the final output per minute (4.78 and 4.80 kg) and KB free seed recovery per min (4.76 and 4.80 kg) was very low in 2012-13 and 2013-14, respectively.

Karnal bunt infection in final output was higher during 2013-14 than in 2012-13 (Table 7). A 2^0 slope of the deck gave the best results in terms of minimum Karnal bunt infection in final output (0.05 and 0.32%), maximum final output per minute (8.29 and 8.67 kg), KB free seed recovery per min (8.29 and 8.64 kg) and maximum recovery efficiency

Table 3: Intensity of Karnal bunt infection (%) at different stages of processing

	KB infection in unprocessed seed			KB infection in pre cleaned seed			KB infection in screen graded seed			KB infection in specific gravity separated seed		
Years	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

Tip: <26% infection, Partial: 26-75% infection, Full: 76-100% infection

Table 4: Effect of Karnal bunt infection on seed quality parameters

Karnal bunt infection	Test weight (g)	Loss in seed weight over healthy seeds (%)	Loss in seedling length over healthy (%)	Germination (%)	Vigour index I
Healthy	40.20	-	-	97.25	2316
Tip	40.04	0.40	17.7	89.75	2159
Partial	34.26	12.64	26.7	66.25	1401
Full	24.87	38.13	40.1	36.50	599

Tip: <26% infection, Partial: 26-75% infection, Full: >75% infection

Table 5: Recovery efficiency of Karnal bunt free seed by specific gravity separation during 2012-13

Treatments	Karnal bunt infection (%) Final output	Karnal bunt infection (%) SGS Screen	Feed/min (kg) SGS	KB free seed (%) SG	KB free seed/min (kg) SG	Final output/min (kg) SGS	KB free seed (%) SGS	KB free seed/min (kg) SGS	Recovery efficiency (%) KB free seed
$S_1F_1O_1$	0.04	0.43	5.00	99.76	4.99	4.80	99.96	4.80	96.20
$S_1F_1O_2$	0.06	0.36	5.00	99.76	4.99	4.68	99.94	4.68	93.80
$S_1F_2O_1$	0.06	0.41	10.00	99.76	9.98	8.68	99.94	8.67	87.00
$S_1F_2O_2$	0.06	0.37	10.00	99.76	9.98	8.52	99.94	8.51	85.40
$S_1F_3O_1$	0.04	0.33	15.00	99.76	14.96	12.58	99.96	12.58	84.10
$S_1F_3O_2$	0.02	0.41	15.00	99.76	14.96	12.50	99.98	12.50	83.50
$S_2F_1O_1$	0.07	0.32	5.00	99.76	4.99	4.07	99.93	4.06	81.50
$S_2F_1O_2$	0.11	0.33	5.00	99.76	4.99	4.02	99.89	4.01	80.40
$S_2F_2O_1$	0.08	0.26	10.00	99.76	9.98	7.38	99.92	7.38	74.00
$S_2F_2O_2$	0.08	0.27	10.00	99.76	9.98	7.30	99.92	7.29	73.10
$S_2F_3O_1$	0.10	0.17	15.00	99.76	14.96	10.03	99.90	10.02	67.00
$S_2F_3O_2$	0.11	0.26	15.00	99.76	14.96	10.17	99.89	10.16	67.90
$S_3F_1O_1$	0.13	0.23	5.00	99.76	4.99	3.08	99.87	3.08	61.70
$S_3F_1O_2$	0.13	0.33	5.00	99.76	4.99	2.92	99.87	2.91	58.40
$S_3F_2O_1$	0.19	0.24	10.00	99.76	9.98	6.57	99.81	6.55	65.70
$S_3F_2O_2$	0.10	0.21	10.00	99.76	9.98	6.22	99.90	6.21	62.30
$S_3F_3O_1$	0.13	0.27	15.00	99.76	14.96	8.90	99.87	8.89	59.40
$S_3F_3O_2$	0.15	0.21	15.00	99.76	14.96	9.30	99.85	9.29	62.10
Mean	0.09	0.30	10.00	99.76	9.98	7.32	99.91	7.31	74.63

SG: Screen grader, SGS: Specific gravity separator

Table 6: Recovery efficiency of Karnal bunt free seed by specific gravity separation during 2013-14

Treatments	Karnal bunt infection (%) Final output	Karnal bunt infection (%) SGS screen	Feed/min (kg) SGS	KB free seed (%) SG	KB free seed/min (kg) SG	Final output/min (kg) SGS	KB free seed (%) SGS	KB free seed/min (kg) SGS	Recovery efficiency (%) KB free seed
$S_1F_1O_1$	0.34	2.43	5.00	98.35	4.92	4.78	99.66	4.76	96.90
$S_1F_1O_2$	0.33	2.73	5.00	98.35	4.92	4.53	99.67	4.52	91.90
$S_1F_2O_1$	0.30	2.67	10.00	98.35	9.84	8.75	99.70	8.72	88.70
$S_1F_2O_2$	0.35	2.19	10.00	98.35	9.84	8.47	99.65	8.44	85.80
$S_1F_3O_1$	0.28	2.66	15.00	98.35	14.75	12.78	99.72	12.75	86.40
$S_1F_3O_2$	0.30	2.70	15.00	98.35	14.75	12.70	99.70	12.66	85.80
$S_2F_1O_1$	0.31	1.68	5.00	98.35	4.92	4.30	99.69	4.29	87.20
$S_2F_1O_2$	0.45	1.53	5.00	98.35	4.92	4.23	99.55	4.21	85.70
$S_2F_2O_1$	0.48	1.73	10.00	98.35	9.84	7.32	99.52	7.28	74.00
$S_2F_2O_2$	0.26	1.34	10.00	98.35	9.84	7.12	99.74	7.10	72.20
$S_2F_3O_1$	0.35	1.37	15.00	98.35	14.75	10.45	99.65	10.41	70.60
$S_2F_3O_2$	0.35	1.41	15.00	98.35	14.75	10.43	99.65	10.40	70.50
$S_3F_1O_1$	0.83	1.15	5.00	98.35	4.92	3.27	99.17	3.24	65.90
$S_3F_1O_2$	0.91	1.11	5.00	98.35	4.92	3.10	99.09	3.07	62.50
$S_3F_2O_1$	1.11	0.93	10.00	98.35	9.84	6.55	98.89	6.38	64.90
$S_3F_2O_2$	1.15	0.99	10.00	98.35	9.84	6.32	98.85	6.24	63.50
$S_3F_3O_1$	1.02	0.97	15.00	98.35	14.75	9.22	98.98	9.26	62.80
$S_3F_3O_2$	1.14	0.88	15.00	98.35	14.75	9.18	98.86	9.08	61.50
Mean	0.57	1.69	10.00	98.35	9.84	7.42	99.43	7.38	76.48

SG: Screen grader, SGS: Specific gravity separator

efficiency (83.10 and 87.87%) in 2012-13 and 2013-14, respectively. With increase in slope of the deck, Karnal bunt infection in final output increased (0.09 and 0.14% in slope 2 and 3, respectively during 2012-13, 0.37 and 1.03% in slope 2 and 3, respectively during 2013-14) but final output per minute (7.16 and 6.16 kg in slope 2 and 3, respectively during 2012-13; 7.31 and 6.27 kg in slope 2 and 3, respectively during 2013-14), KB free seed recovery per minute (7.15 and 6.16 kg in slope 2 and 3, respectively during 2012-13; 7.28 and 6.21 kg in slope 2 and 3, respectively during 2013-14) and recovery efficiency (71.72 and 61.70% in slope 2 and 3, respectively during 2012-13, 74.04 and 63.12% in slope 2 and 3, respectively during 2013-14) decreased significantly in both the years. On the other hand, gradual increase in feedings

Table 7: Effect of slope, feeding and output on Karnal bunt free seed recovery by specific gravity separation during 2012-13 and 2013-14

Treatments	Karnal bunt infection in final output (%)		Final output/min (kg)		Karnal bunt free seed recovery/min (kg)		Recovery efficiency (%)	
	2012-13	2013-14	2012-13	2013-14	2012-13	2013-14	2012-13	2013-14
Slope 1 (2°)	0.05	0.32	8.29	8.67	8.29	8.64	83.10	87.87
Slope 2 (2.5°)	0.09	0.37	7.16	7.31	7.15	7.28	71.72	74.04
Slope 3 (3°)	0.14	1.03	6.16	6.27	6.16	6.21	61.70	63.12
Feeding 1 (5 kg min^{-1}	0.09	0.53	3.93	4.04	3.92	4.02	78.68	81.66
Feeding 2 (10 kg min^{-1})	0.10	0.61	7.44	7.42	7.44	7.36	74.55	74.84
Feeding 3 (15 kg min^{-1})	0.09	0.57	10.58	10.79	10.57	10.76	70.65	72.93
Output 1 (45 cm deck width)	0.09	0.56	7.34	7.49	7.34	7.46	75.16	77.48
Output 2 (43 cm deck width)	0.09	0.58	7.29	7.34	7.28	7.30	74.09	75.48
CD 0.05 for slope	0.02	0.09	0.155	0.162	0.152	0.160	4.12	4.27
CD 0.05 for feeding	NS	NS	0.155	0.161	0.153	0.158	2.23	2.29
CD 0.05 for output	NS	NS	NS	NS	NS	NS	0.82	0.84
CD 0.05 for interactions	NS	NS	NS	NS	NS	NS	NS	NS

(5, 10 and 15 kg min^{-1}) to specific gravity separator led to significant increase in final output per minute (3.93, 7.44, 10.58 and 4.04, 7.42, 10.79 kg during 2012-13 and 2013-14, respectively) and KB free seed recovery per minute (3.92, 7.44, 10.57 and 4.02, 7.36, 10.76 kg during 2012-13 and 2013-14, respectively) whereas there was decrease in recovery efficiency (78.68, 74.55, 70.65 and 81.66, 74.84, 72.93% during 2012-13 and 2013-14, respectively) but Karnal bunt infection in the final output was at par in all the feedings. Output treatments were statistically at par for Karnal bunt infection, final output per minute and KB free seed recovery but recovery efficiency reduced significantly with reduction in output collection deck widths as O_1 treatment (75.16 and 77.48%) and O_2 treatment (74.09 and 75.48%) during 2012-13 and 2013-14, respectively.

DISCUSSION

Seed quality parameters as germination and physical purity of unprocessed seed lot were lower to the Indian Minimum Seed Certification Standards of 85 and 98%, respectively (Tunwar and Singh, 1988) which turned to acceptable lot with 91.30% germination and 99.53% physical purity after passing through all the stages of processing. Out of total impurities of 2.88% in the unprocessed seed lot, 0.96% was removed collectively by air screen machines (pre-cleaner and grader), showing removal of 33% of total impurities present in the seed lot (Table 1). Specific gravity separator alone improved germination by 3%, followed by screen grader (2.50%) and pre-cleaner (1.60%). 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 germination of the seed lot by 7.71%. Specific gravity separation also had a positive effect on the seed test weight. Test weight was significantly higher in the seed lot after specific gravity separation than that of pre-cleaned and screen graded seed lot. Maximum increase in test weight was in seed after passing through specific gravity separator (1.82 g), followed by screen grader (1.27 g) and pre-cleaner (0.89 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, 2002; Doshi *et al*., 2013). All the quality parameters of seed improved significantly after specific gravity separation as against unprocessed seed thereby indicating that the size and density of seed are important factors for determining seed quality. This also showed importance of gravity upgradation in improving vigour of the seed lot. Seed quality parameters of the rejected component of the specific gravity separator were far below the seed standards and were also inferior to the unprocessed seed lot at each stage. These results thus showed positive impact of separation process in all the three machines and emphasized the necessity of processing.

KB disease also affects the size and density of seed. Intensity of disease in the seed was variable and very much dependent on environmental conditions at the time of flowering. The portion of wheat grain affected with *T. indica* is transformed into teliospores of the fungus and looks 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. 2) 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 revealed that with increase in disease intensity on the seed there was a gradual decrease in seed weight, seedling length, seed germination and vigour (Table 4). Singh (1980) also reported that with increase in disease intensity in the seed, there was gradual decrease in seed weight, seedling length and germination percentage. Beniwal *et al*. (2000) and Bansal *et al*. (1984) also reported reduction in 1000 grain weight ranging from 4.5-52.3% depending on severity of infection.

Karnal bunt infection in unprocessed seed was higher during 2013-14 than in 2012-13. During 2013-14 the experimental seed material was produced in the same field of 2012-13 as per the land requirement condition for quality seed production where KB inoculum was already available and the weather conditions were also congenial for development of the disease. The incidence of the disease was more in 2013-14 due to cooler temperature and well distributed rainfall during ear emergence initiation and 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.

In 2012-13, disease spread was low but the disease intensity high (partial and full infection) due to more number of effective rainfall days and higher average maximum and minimum temperatures. In 2013-14, tip infection was four times than in 2012-13 (Table 3) and partial and full infection of seed was less. Nagarajan (1991) and Aujla *et al.* (1977, 1986) also stated that high humidity (70% and above), low temperature (19-23°C), continuous rainy/foggy and cloudy weather from ear emergence to anthesis had favoured disease epiphytotics. Our results revealed that out of total KB infection of 1.42 and 4.27% in the unprocessed seed lot, 0.52 and 2.19% infected seeds were removed by pre-cleaner and 0.66 and 0.43% by screen grader, showing removal of 83.1 and 61.4% of total KB infected seed present in the seed lot by air screen machines in 2012-13 and 2013-14, respectively. The percentage of full and partial KB infected seeds decreased after passing through pre-cleaner, screen grader and specific gravity table but percentage of tip infected seeds increased in the final product after passing through machines in the same order. Specific gravity was able to completely remove full KB infected seeds from the final product during both the years of study due to low seed density/seed dimensions.

Karnal bunt infection in final output was higher during 2013-14 than in 2012-13 (Table 7) because of higher percentage of tip infection in seed. Tip infected seed was difficult to separate from the healthy seed as there was no or very little difference in density of the seed (Table 2). With increase in slope of the deck Karnal bunt infection in final output increased but final output per minute, Karnal bunt free seed recovery per minute and recovery efficiency decreased significantly in both the years. Therefore, minimum slope of the deck (S_1) i.e., at 2° gave highest final output/min (8.29 and 8.67 kg during 2012-13 and 2013-14, respectively) and KB free seed recovery per minute (8.29 and 8.64 kg during 2012-13 and 2013-14, respectively) as against S_2 and S_3 slopes. On the other hand increased feeding to specific gravity separator led to significant increase in final output per minute and decrease in recovery efficiency but Karnal bunt infection in final output was at par in all the feedings. Therefore, feeding of 15 kg min^{-1} (F_3) was best as it gave maximum final output per minute (10.58 and 10.79 kg during 2012-13 and 2013-14, respectively) and KB free seed recovery per minute (10.57 and 10.76 kg during 2012-13 and 2013-14, respectively). Output treatments were at par for Karnal bunt infection in the final output and final output per minute but recovery efficiency reduced significantly with reduction in output collection deck width, hence O_1 treatment (45 cm deck width) was better than O_2 treatment (43 cm deck width). Thus, specific gravity separation decreased KB infection by removing 62.5 and 63.6% of KB infected seed in 2012-13 and 2013-14, respectively than that of pre-cleaned and graded seed lots.

Treatment $S_1F_3O_1$ (slope of deck 2^0, feeding 15 kg min^{-1}, output deck width 45 cm) was found to be the best as it gave minimum KB infection in final product (0.04 and 0.28%), maximum final output per minute (12.58 and 12.78 kg) and KB free seed recovery per minute (12.58 and 12.75 kg) with 84.1 and 86.4% recovery efficiency in 2012-13 and 2013-14, respectively.

Hence, this study showed that all the three processing operations are necessary in processing wheat seed for efficient removal of KB infected seed. Mechanical processing reduced KB infection by more than 93% depending on the intensity of infection and improved seed germination by 7.71% and physical purity by 2.41%.

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REFERENCES

Abdul-Baki, A.A. and J.D. Anderson, 1973. Vigor determination in soybean seed by multiple criteria. Crop Sci., 13: 630-633.

Agarwal, V.K. and H.S. Verma, 1983. A simple technique for the detection of Karnal bunt infection in wheat samples. Seed Res., 11: 100-102.

Agrawal, R.L., 1993. Physical Purity Analysis. In: Handbook of Seed Testing, Agrawal, P.K. (Ed.). National Seed Corporation, New Delhi, India.

Aujla, S.S., Y.R. Sharma, K. Chand and S.S. Sawney, 1977. Influence of weather factors on the incidence and epidemiology of Karnal bunt disease of wheat in the Punjab. Indian J. Ecol., 4: 71-74.

Aujla, S.S., I. Sharma, K.S. Gill and A.S. Grewal, 1986. Prevalence of Karnal Bunt in Punjab as influenced by varietal susceptibility and meteorological factors. Plant Dis. Res., 1: 51-54.

Bansal, R., D.V. Singh and L.M. Joshi, 1984. Effect of Karnal-bunt pathogen *Neovossia indica* (Mitra) mundkur on weight and viability of wheat seed. Indian J. Agric. Sci., 54: 663-666.

Bedi, P.S., 1989. Impact of new agricultural technology on karnal bunt of wheat in punjab. Pl. Dis. Res., 4: 1-8.

Beniwal, M.S., P. Chawla, S. Madan and R. Singh, 2000. Effect of Karnal bunt on grain weight and quality of wheat. Ann. Biol., 16: 101-104.

Doshi, J.S., V.B. Patel, J.B. Patel and J.A. Patel, 2013. Quantification of quality improvement in wheat seed processing. J. Agric. Eng., 50: 83-86.

Gill, K.S. and S.S. Aujla, 1986. Breeding for Karnal bunt resistance in wheat. Crop Improvement, 19: 109-118.

Gill, K.S., I. Sharma and S.S. Aujla, 1993. Karnal bunt and wheat production. Punjab Agricultural University, Ludhiana, India, pp: 1-153.

Gomez, K.A. and A.A. Gomez, 1984. Statistical Procedures for Agricultural Research. 2nd Edn., John Wiley and Sons, New York, USA., ISBN-13: 9780471870920, Pages: 680.

Gupta, P.C., 1993. Seed Vigour Testing. In: Handbook of Seed Testing, Agrawal, P.K. (Ed.). National Seed Corporation, New Delhi, India.

ISTA., 1993. International rules for seed testing. Seed Sci. Technol., 21: 1-288.

ISTA., 1999. International rules for seed testing, rules 1999. Seed Sci. Technol., 24: 1-335.

Joshi, L.M., D.V. Singh, K.D. Srivastava and R.D. Wilcoxson, 1983. Karnal bunt: A minor disease that is now a threat to wheat. Bot. Rev., 49: 309-330.

Martin, P.M., 1986. Quarantine of Karnal bunt in Canada. Proceedings of the 5th Biennial Smut Workshop, April, 28-30, 1986, Ciudad Obregon, Sonora Mexico.

Mehdi, V., L.M. Worhi and Y.P. Abrol, 1973. Studies on chapatti making quality. VI. Effect of wheat grains with bunts on the quality of chapaties. Bull. Grain Tech., 11: 195-197.

Mitra, M., 1931. A new bunt on wheat in India. Ann. Applied Biol., 18: 178-179.

Munjal, R.L., 1975. Status of Karnal bunt (*Neovossia indica*) of wheat in northern India during 1968-1969 and 1969-1970. Indian J. Mycol. Plant Pathol., 5: 185-187.

Nagarajan, S., 1991. Epidemiology of Karnal bunt of wheat incited by *Neovossia indica* and an attempt to develop a disease prediction system. Wheat Special Report No. 4, International Maize and Wheat Improvement Center (CIMMYT), Mexico, March 1991, pp: 1-70.

Shukla, D.N., N. Singh and S.N. Bhargava, 1982. Wheat varieties affected by Karnal bunt. Vijnana Parishad Anusandhan Patrika, 25: 39-40.

Singh, D., 1980. Note on the effect of Karnal bunt infection on the Vigour of wheat seed. Seed Res., 8: 81-82.

Singh, D.V., K.D. Srivastava and L.M. Joshi, 1985. Present status of Karnal bunt of wheat in relation to its distribution and varietal susceptibility. Indian Phytopathol., 38: 507-515.

Singh, P.P. and P.S. Bedi, 1985. Effect of Karnal bunt infection on gluten characteristics and protein fractionation of wheat grains. Ann. Biol., 1: 223-225.

Singh, D.V., 1986. Bunts of Wheat in India. In: Problems and Progress of Wheat Pathology in South Asia, Joshi, L.M., D.V. Singh and K.D. Srivastava (Eds.). Malhotra Publishing House, New Delhi, India, ISBN-13: 9788185048031, pp: 125-165.

Sinha, J.P., B.S. Modi, R.P. Nagar, S.N. Sinha and M. Vishwakarma, 2001. Wheat seed processing and quality improvement. Seed Res., 29: 171-178.

Sinha, J.P., M.K. Vishwakarma, S.S. Atwal and S.N. Sinha, 2002. Quality improvement in rice (*Oryza sativa*) seed through mechanical seed processing. Indian J. Agric. Sci., 72: 643-647.

Tunwar, N.S. and S.V. Singh, 1988. Indian minimum seed certification standards. Central Seed Certification Board, Department of Agriculture and Cooperation, Ministry of Agriculture, Government of India, pp: 1-388.