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Incidence of Karnal bunt in eastern Uttar Pradesh and its effect on seed quality

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Abstract
Karnal bunt caused by Tilletia indica Mitra (*Neovossia indica* Mitra (Mundkur) was first recorded in April, 1930 from Botanical Research Station, Karnal (Haryana) on wheat cultivar, foundation and Punjab A. The disease was prevalent in the sub continent since long, infecting native wheats grown over North Western India but it never caused serious yield reduction. However, the disease appeared in serious proportions in early 1970s with the introduction of dwarf Mexican Wheat Varieties into India and Pakistan. Till 1974-75 the disease remained restricted to Jammu and Kashmir, Punjab and Tarai region of Uttar Pradesh. In some places, it appeared in the epidemic form in different years. In the absence of strict domestic quarantine regulation it spreaded to new areas in North Western Wheat belt. However, Madhya Pradesh, southern Rajasthan, Maharashtra and Peninsular India are free from Karnal bunt due to high temperatures. It is also reported from Nepal, Iran, Mexico, United States, South Africa, Brazil, Afganistan, Syria, Turkey, Lebnan, Sweden, Poland, Itlay.The pathogen is floral infecting organism that partially infects seeds of bread wheat, duram wheat and also the triticale. Not all the spikes on a plant are infected and within a spike only a few spikelets are bunted.

Keywords: Karnal bunt, 1000 grain Weight (g), Germination (%), Seedling vigour

1. Introduction
Wheat (*Triticum aestivum* L.) continues to be the most dynamic sector in world grain production and India is the second largest producer, preceded only by China and major contributor to the agricultural economy of the country. It is the staple food and major source of energy and nutrition of Indian diet. It is known for its remarkable adoption to a wide range of environment. Its importance derived from the properties of their glutin, cohesive network of tuft endosperm protein, starch with the expansion of fermentations dough. It is utilized for bread, cakes, cokies, noodles, pestri-products, chapatti & macaroni etc. Wheat grain contains 60-68% starch, 8.0 to 15% protein, 1.5 to 2.0% fat, 2.0-2.5% cellulose and 1.5 to 2.0% minerals [19]. Karnal bunt [14, 15], new bunt [11, 12, 13] or partial bunt [6] of wheat was first discovered by Mitra in April 1930 in the experimental seed material grown at the Botanical Station, Karnal and was reported by him in 1931. In India, Karnal bunt is particularly prevalent in the *tarai* and high fertility irrigated areas of north west plains. However, parts of Madhya Pradesh, Southern Rajasthan, Maharastra & penninsular India are free from Karnal bunt as the temperature are higher in the areas. Incidence of Karnal bunt is erratic in eastern India and seldom exceeds traces to very low level of infections. Karnal bunt is a disease of wheat, durum, rye and triticale. Though the disease native to South Asia but subsequently it has been reported from Iran, Syria, Afganistan, Iraq, Mexico, Nepal and United States. Besides yield losses, Karnal bunt can reduce wheat flour quality due to fishy, unpalatable odour and taste, if a grain lot contains 1-4 per cent infected seed. If in a grain lot 5 percent of the grain infested, the quality of the flour recovery and chemical changes in composition of flour and gluten contents cause poor dough strength. Karnal bunt is also a disease of quarantine interest and it affects the international trade of commercial wheat grain and movement of wheat germplasm throughout the world. Thus, presence of diseased grain in wheat lots can cause economic loss to wheat exporting countries. Therefore, it is important to find out the incidence and its efficacy on seed quality.

2. Material and method
Narendra Deva University of Agriculture and Technology, Kumarganj, Faizabad has a well equipped notified Seed Testing Laboratory under the Department of Seed Science & Technology.

Samples of different release and notified varieties meant for quality seed production purposes i.e. nucleus, breeder, foundation and certified seed at different seed production farms/Krishi Vigyan Kendra of the University established at different location in eastern U.P. are collected and analysed for testing against seed quality traits like germination, physical purity, genetic purity, seed vigour, seed health etc. Total 26 seeds samples comprising 11 released and notified varieties under university seed production chain were available in the seed testing lab from the produce of Rabi 2011-12. A part from the said seed samples were obtained and analysed for Karnal bunt of wheat using both the techniques recommended i.e. visual examination of dry seeds and NaOH seed soak method [2, 5]. The seeds having black point symptoms were also interrupted and the percentage was also worked out. The black pointed seeds were categorized based on the symptoms on the grain.

2.1 Detection procedure of Karnal bunt

Karnal bunt infected seeds were detected by two methods i.e. visual observation of dry seed and NaOH Seed soak method. In case of first one infected seeds were spread on purity work board and seeds were visually examined for Karnal bunt infected seeds, the infected seeds showed jet black shiny colour, were collected to find out the infection percentage of seed samples. In case of any doubt, with clear cut detection of Karnal bunt infected seeds the second method was also used in which seeds were soaked in 0.2% NaOH solution at room temperature up to 24 hours. After this seeds were spreaded on blotter paper infected seeds slowed Jet black shiny colour in case of any doubt seeds were punctured with needle for exposed out the teliospores.

2.2 Methodology of Karnal bunt detection

Bunted seeds can be detected in the seed samples by visual observations aided with light. However, for exact and reliable quantitative assessment, "sodium hydroxide seed soak method" [3, 4] may be followed. Sodium hydroxide solution removes all other coatings, stain, dust, chemical etc. from the seed and causes a bit swelling of the disease even in minute cases in infection**.**

2.3 Working Sample

Foundation seed-4,000 seeds in 2 replications of 2000 seeds each Certified seed-800 seeds in 2 replications of 400 seeds each.

2.4 Procedure

- Wheat seeds are soaked in a flask/beaker containing 250ml of 0.2% NaOH solution (2g NaOH/1000 ml water) for 24 h at 25^0C.
- After 24h the solution is decanted.
- Seeds are thoroughly washed in tap water.
- Seeds are examined visually, aided with light.
- The seeds exhibiting jet black shiny appearance with hallow or without hollowness are separated.
- In case of any doubt, seeds are ruptured separately in a drop of water and observed for the release of stream of fungal spores.
- Such seeds are counted as infected seeds and results are reported in percentage.
- The NaOH seed soak method can also be used to differentiate between Karnal bunt and black point (*Alternaria alternata*), as it is often difficult to differentiate between the two diseases, particularly when Karnal bunt infection is incipient stage [9]. In black point the internal tissue of the seed is not converted into black powdery mass, thus such seeds on rupturing do not release stream of fungal spores.

The disease become evident when grain have developed. In a stool, all the ears are not infected and in a ear all the grains are not bunted. The fungus affects the grains partially and for this region the bunt is also known as partial bunt. The Karnal bunt is not easily detected prior to harvest, since it is usual for only a few kernels per spike to be effected by the disease that why for detection visual inspection for a mass of black teliospore replacing a portion of endosperm sometimes the whole endosperm with intact pericarp are even ruptured one were seen during the detection.

Disease kernels give off fishy odour when crushed. Rarely the entire grain is converted into a sorous. In badly infected spikelets the glums spread apart and quite often fall off exposing the bunted grains under field condition but it is very tedious to confirm infected ear until the person is well trained and acquainted. Normally the embryo tissue except in very severely cases is not destroyed and in most of the cases the infected grains were germinated during the testing.

3. Effect of Karnal bunt on seed quality

Since the quality of infected seed was very limited and it was not possible to record the 1000 grain weight of individual grade of different variety. Ultimately the infected seeds were mixed thoroughly and thousand grain weights of bulk seeds of infected grade wise as well as healthy seed was recorded and presented in Table-2.

In Karnal bunt embryo is attacked partially and showed that the embryo is not attacked and infected grain germinate normally producing weak seedlings indicating the poor vigour of the affected seeds [12, 17]. Therefore, to establish the fact regarding the affect of the disease on different seed quality traits was worked out during the present investigation. For this purpose infected grains from different varieties were collected separately and graded fro infection intensity as per procedure suggested [18].

For grading of infected seeds the criteria followed is given as follows –

Grade I upto ¼ of the seed converted into sorous.

Grade II more than ¼ to ½ grain converted into sorous.

Grade III more than half of the grain converted into sorous.

Germination percentage following (ISTA, 1985) was worked out on the basis of normal seedlings. 10 normal seedlings per replication were used for measuring the loss in root shoot length. Losses of seed vigour and viability in heavily infected seeds were compared and viability of healthy seeds of the same lot. The vigour index was calculated as per formula suggested by [1].

4. Result

4.1 Incidence and severity of Karnal bunt and black point in wheat varieties.

Total 26 seed samples of newly released wheat varieties grown at different locations like Faizabad, Sultanpur, Bahraich, Basti, Barabanki, Sidharth Nagar, Mahrajganj, Ghazipur and Azamgarh under quality seed production programme and submitted to the Notified Seed Testing laboratory of the Department of Seed Science and Technology, N.D. University of Agriculture & Technology, Narendra Nagar (Kumarganj)

Faizabad. A part of these samples were obtained from the set laboratory and analysed for Karnal bunt as well as black point of wheat. The black point infected seeds as well as Karnal bunt infected seeds were categorized on the basis of conversion of the grain part. Besides, both type of infected seeds were subjected to pathogenic association also.

Four samples of wheat varieties PBW 502 produced in Bahraich, Faizabad, Sultanpur and Mahrajganj districts was analyse. Out of four samples, one samples from Mahrajganj were found infected (0.05%) and other from Bahraich, Faizabad, Sultanpur were found free from Karnal bunt infection. Thus the range of karnal bunt infection was 0.00 to 0.05 per cent with average infection of 0.125 per cent. Also, all the four seed lots were found infected with black point ranging between 0.20 to 0.30 per cent. The fungi which were found associated with back point infected grain were *Alternaria alternata* and *Bipolaris sorokiniana* and *Curvularia lunata* (Table-1).

Four samples of wheat varieties PBW 550 produced in Bahraich, Faizabad, Basti, Siddarth Nagar districts were analyse. Out of four samples, one sample from Siddarth Nagar was found infected (0.10%) and the others from Bahraich, Faizabad, Basti were found free from Karnal bunt infection. Thus the range of Karnal bunt infection was 0.00 to 0.10 per cent with average infection of 0.0125 per cent. Also, all the four seed lots were found infected with black point ranging between 0.15 to 0.30 per cent. The fungi which were isolated prominently from the infected grains were *Alternaria alternata* and *Bipolaris sorokiniana*.

Table 1: Incidence and severity of Karnal bunt & Black point in wheat varieties of eastern Uttar Pradesh.

S.N.	Variety	Production site	Per cent seed infection (KB average)	Per cent seed infection (BP average)	Pathogenic associations (Fungi isolated) Descending order
1.a)	PBW502	Bahraich	Free	0.25	*Alternaria alternata Bipolaris sorokiniana*
b)	PBW502	Faizabad	Free	0.30	*Curvularia lunata*
c)	PBW502	Sultanpur	Free	0.20	*Alternaria alternata*
d)	PBW502	Maharajganj	0.05	0.30	*Alternaria alternata B. sorokiniana*
	Total/average	4 districts	0.0125	1.05/0.26	*A.alternate B.sorokiniana*
2.a)	PBW550	Bahraich	Free	0.30	*B.sorokiniana*
b)	PBW550	Faizabad	Free	0.15	*A.alternata*
c)	PBW550	Basti	Free	0.25	*B.sorokiniana*
d)	PBW550	Siddarth Nagar	0.10	0.30	*B.sorokiniana, A.alternata*
	Total/average	4 districts	0.025	1.00/0.25	*B.sorokiniana, A.alternata*
3.a)	Raj 3077	Barabanki	Free	0.60	*A.alternata, B.sorokiniana*
b)	Raj 3077	Faizabad	Free	0.20	*B.sorokiniana, A.alternata*
c)	Raj 3077	Sultanpur	Free	0.20	*B.sorokiniana, C. lunata*
	Total/average	3 districts	0.00	1.40/0.36	
4.a)	K-7903	Faizabad	0.15	0.25	*B.sorokiniana, A.alternata*
b)	K-7903	Sultanpur	Free	0.15	*B.sorokiniana*
c)	K-7903	Barabanki	0.05	0.25	*B.sorokiniana*
	Total/average	3 districts	0.067	0.65/0.21	
5.a)	HD2733	Ghazipur	Free	0.05	*B.sorokiniana*
b)	HD2733	Azamgarh	Free	0.05	*B.sorokiniana*
c)	HD2733	Siddarth Nagar	Free	0.25	*A.alternata*
	Total/average	3 districts	0.00	0.35/0.11	
6.a)	PBW 343	Faizabad	0.05	0.25	*A.alternata*
b)	PBW 343	Sultanpur	Free	0.20	*B.sorokiniana*
c)	PBW 343	Faizabad	0.15	0.35	*B.sorokiniana*
	Total/average	3districts	0.067	0.45/0.225	
7.a)	NW 1012	Faizabad	Free	0.25	*B.sorokiniana*
b)	NW 1012	Barabanki	Free	0.25	*B.sorokiniana*
	Total/average	2 districts	0.00	0.45/0.225	
8.a)	K 307	Faizabad	Free	0.10	*B.sorokiniana, A.alternata*
9.a)	K-9423	Faizabad	Free	3.30	*A.alternata, B.sorokiniana*
10.a)	DBW17	Faizabad	Free	1.15	*B.sorokiniana, A.alternata C. lunata*
11.a)	UP262	Barabanki	0.10	1.30	*A.alternata*

Total no. of seed samples = 26
Total no. of districts = 9
No. of infection range =0.05-0.15%

Three samples of wheat variety Raj 3077 produced in Barabanki, Faizabad and Sultanpur districts were analyse. Out of three samples, all were found free from Karnal bunt infection. Interestingly, all the three samples were found infected with black point ranging between 0.20 to 0.60 per cent. The fungi which were isolated from the infected grains were *Alternaria alternata*, *Bipolaris sorokiniana* and *Curvularia lunata*.

Three samples of wheat varieties K.7903 (Halna) produced in Faizabad, Sultanpur and Barabanki districts were analyse. Out of three samples two samples were found infected one from Faizabad (0.15%) and the other from Barabanki (0.05%). The produce of Sultanpur was found free from Karnal bunt infection. Thus, the range of Karnal bunt seed infection was 0.00 to 0.15% per cent with average infection of 0.66 per cent. Also all the three seed lot were found infected with black point

ranging between 0.15 to 0.25 per cent. The fungi which were isolated prominently from the infected grain were *Alternaria alternata* and *Bipolaris sorokiniana*.

Three samples of the wheat variety HD 2733 produced in Ghazipur, Azamgarh and Siddarth Nagar districts were analyse. All the samples were found free from Karnal bunt infection. But, all the three samples were found infected with black point ranging between 0.05 to 0.25 per cent. The fungi isolated from infected grains were *Biopolaris sorokiniana* and *Alternaria alternata*.

Three samples of wheat variety PBW 343 produced in Faizabad and Sultanpur districts were analyse. Out of three samples, two sample from Faizabad were found infected (0.05%) and other 0.15% produce from Sultanpur was found free from Karnal bunt infection. Per cent with average infection of 0.066 per cent. Also all the three seed lots were found infected with black point ranging between 0.20 to 0.25 per cent. The fungi isolated from the infected grains were *Alternaria alternata* and *Bipolaris sorokiniana*.

One sample of wheat varieties K-307 produced in Faizabad district was analysed and found free with Karnal bunt infection. But, the same was found infected with black point (0.10%). The fungi isolated from the infected grains were *Bipolaris sorokiniana* and *Alternaria alternata*.

One sample of wheat variety K-9423 produced in Faizabad district was analysed and found free with Karnal bunt infection. But, it was found infected with black point which was 3.30 per cent. The fungi isolated from the infected grains were *Alternaria alternata* and *Bipolaris sorokiniana*.

One sample of wheat variety DBW-17 produced in Faizabad was analysed and found free with Karnal bunt infection. But, infected with black point, which was 1.15 per cent. The fungi isolated from infected grains were *Bipolaris sorokiniana, Alternaria alternata* and *Curvularia lunata*.

One sample of wheat variety UP-262 produced in Barabanki was analyse and found infected with Karnal bunt which was 0.10 per cent, and black point which was 1.30 per cent respectively. The fungi isolated prominently from the infected grain was *Alternaria alternata*.

4.2 Effect of Karnal bunt (*N.indica*) on seed quality parameters.

The infected seeds separated from different seed lots/varieties were graded on the basis of portion converted into sorous (black powdery mass), the basis of grading is given below: –

Grade I Upto ¼ of the seeds converted into sorous.

Grade II More than ¼ to ½ grain converted into sorous.

Grade III More than half of the grain converted into sorous.

The effect of Karnal bunt infection grade wise on 1000 grain weight, germination per cent, seedling vigour, increase/decrease in test wt., germination and seedling vigour were worked out and compared all the grades individually with healthy of the bulk seeds prepared through blending technique. The max. 1000 grain wt. (38.43 gm) was recorded in case of healthy seed lots followed by grade-I infection (37.5gm) and the minimum in grade-III (32.03gm). Thus there was significant reduction in grain wt. But the difference between grade-I & grade-II infected seeds regarding the 1000 grain wt. was non-significant (Table-2).

Table 2: Effect of Karnal bunt (*N. indica*) on seed quality parameters.

Infection Grade	1000 grain wt. (g.) Average	Germination (%) Average	Seedling vigour Average
Grade I	37.5	85.33 (67.48)*	1970
Grade II	36.87	82(65.05)	1802.67
Grade III	32.03	35.66(36.63)	1474.00
Healthy	38.43	85(67.24)	1996.67
CD at 5%	1.697	3.955	315.79

*Values in parenthesis are transform values

The maximum seed germination (85.33%) was recorded in case of grade-I infected seeds followed by the healthy seed lots (85%), whereas it was minimum in case of grade-III infected seeds. The differences regarding the germination per cent of grade-I, grade-II & healthy seed lot was non-significant. The minimum seed germination (35.366%) was recorded in grade-III infected seed also which varied significantly from grade-I, Grade-II as well as healthy seed lot. Thus, the conspicuous effect of Karnal bunt infection on seed germination depending upon the disease severity of individual grain was remarkable.

The maximum seedling vigour (1996.67) was recorded in case of healthy seed lot followed by grade-I (1970). The minimum seedling vigour (1474) was recorded in case of grade-III infected seed lot. The differences among grade-I, grade-II as well as healthy seed lot was non-significant although there was significant variation from grade-III infected seed lot.

Overall, upto 23.13gm decrease in 1000 grain weight as a whole was recorded. However, the average of all the three grades was 7.71gm, means the decrease in 1000 grain wt. due to Karnal bunt infection was 7.71gm. The maximum loss in test weight (-16.67%) was recorded in IIIrd grade infected seeds & minimum (-2.41%) in case of grade-I infected seeds (Table-3).

Table 3: Percent increase/decrease in seed quality parameters due to Karnal bunt infection.

Infection grade	1000 grain wt.	Germination	Seedling vigour
Grade I	-2.41	+0.38	-1.33
Grade II	-4.05	-3.52	-9.71
Grade III	-16.67	-58.05	-26.17
Total	23.13	-61.19	-37.21
Average	7.71	-20.39	-12.40

Likewise, in case of germination 0.38 per cent increase was recorded in case of grade-I infected seeds, may be due to experimental error, whereas 3.52 and 58.05 per cent decrease in germination in case of grade-I and grade-II infected seeds was recorded respectively. The average loss in germination per cent was 20.39 per cent. Regarding, the seedling vigour upto 26.17 per cent loss was observed in case of grade-III infected seeds although, it was minimum (1.33%) in case of grade-I

infected seed, the average per cent decrease in case of seedling vigour was 12.42 per cent. Thus, we can say that Karnal bunt infection hampers various seed quality parameter i.e. test weight, germination and seedling vigour. In general with the exception regarding the increase in germination per cent in case of grade-I infected seeds seems to be a experimental error.

5. Discussion

Since the information on Karnal bunt incidence in the districts surveyed are based on variable number of wheat grain samples received seed testing laboratory of N.D. University of Agriculture & Technology, Kumarganj, Faizabad. Karnal bunt is widely distributed in various western and eastern districts of Uttar Pradesh, while northern hill and southern dry areas are generally free from the disease. Present investigations showed that the wheat variety K-7903 was infected from Karnal bunt with average infection was 0.66 per cent followed by PBW-343 with average infection 0.15 per cent, where as trace level of Karnal bunt infection were found in PBW-502, PBW-550 and UP-262. The wheat cultivars, DBW-17, K-307, NW-1012, HD-2733 and Raj 3077 were found free from Karnal bunt infection, while the districts wise survey concluded that Sultanpur district was free from Karnal bunt infection. An extensive survey in eastern Uttar Pradesh from 2003-2007 and find out the frequency of infected samples were high in the eastern part of state during 2006 (30.90%) followed by 2005 (25.46%) and was less in 2004 (17.98%) and in 2003 (18.36%). Range of Karnal bunt prevalence in the samples were high in Basti (25.00-84.61%) followed by Bahraich (30.77-77.78%), Mahrajganj (40.00-75.00%) and rest collected samples varied from 5.45-48.00% [23]. Various other workers have listed the varieties having low disease incidence [21, 22, 24]. The effect of Karnal bunt on 1000 grain wt., germination, vigour. Increase/decrease 1000 grain wt., germination, vigour were worked out based on grading of infected seeds. The maximum 1000 grain wt. was recorded in case of healthy seed lots. Followed by grade-I infection and minimum in grade-III where as the overall, upto 23.13 gm. decrease in 1000 grain wt. as a whole was recorded. The maximum loss in grain wt. was recorded in III grade infected seeds and minimum in grade-I infected seeds.

Likewise, the maximum seed germination was recorded in case of grade-I infected seeds followed by the healthy seed lots where as it was minimum in case of grade-III infected seeds. The differences regarding the germination per cent of grade-I, grade-II & healthy seed lot was non-significant. The minimum seed germination was recorded in grade-III infected seed also which was grade-I, grade-II as well as healthy seed lot. On the other hand, germination per cent increased in case of grade-I infected seeds where as decrease in case of grade-II and grade-III infected seeds recorded respectively.

Regarding the seedling vigour was high in healthy seed lot followed by grade-I. The minimum seedling vigour was recorded in case of grade-III infected seed lot and another view maximum seedling vigour reduction per cent observed in grade-III while minimum in case of grade-I infected seeds with average per cent decrease in case of seedling vigour was 12.42.Various types of qualitative and quantitative losses have been reported by various worker [8, 16]. Regarding the deterioration in seed quality like 1000 grain weight, germination and vigour, various workers have given variable reports [7, 10, 18, 20, 25].

6. Cnclusion

Out of 11 varieties evaluated PBW-502, PBW-550, K-7903, PBW-343 and UP-262 were found having the Karnal bunt infection ranging between 0.05 to 0.15 per cent. Varieties like Raj-3077, HD-2733, NW-1012, K-307, K-9423 and PBW-17 remained free from KB infection from all the producing sites. The roll of production site was also evident. Most of these varieties which were found infected are already reported to be susceptible from KB point of view. All the varieties taken into consideration showed more or less the black point infection also. Raj-3077 exhibited maximum black point infection on average basis whereas K-9423 showed the maximum infection followed by UP-262 and PBW-17 on individual sample basis. The Karnal bunt infected grains when subjected to blotter test for fungal association, the fungi like *Alternaria alternata*, *Bipolaris sorokiniana* and *Curvularia lunata* were found prominently associated with infected grains.

Regarding the effect of Karnal bunt on seed quality parameters i.e. 1000 grain weight, germination % & seedling vigour it was evident from the data that the maximum reduction in seed wt. was in case of grade-III infected seed which was 32.03 gm as compared to 38.43 from healthy seed lot. Upto 16.67 per cent weight loss in case of grade-III infected seed was recorded as compared to 4.05 and 2.41 per cent in grade-II & grade-I infected seed lots respectively when compared to the healthy lot.

Regarding the germination per cent it was maximum in healthy as well as grade-I infected seed (both significantly at par) and the minimum in grade-III infected seed lot. Accordingly the maximum loss upto 58.05 per cent was recorded in the grade-III infected seed because maximum portion of the seeds were converted into black powdery masses with or without hollowness of the seed.

The seedling vigour was also adversely affected and the maximum vigour 1996.67 was observed in healthy category seed followed by grade-I & grade-II infected seed which were also significantly at par to each other and the minimum seedling vigour was observed in case of grade-III infected seed as was observed in other quality trait also. When the values were calculated on percentage basis the seedling vigour reduced adversely by 26.17 per cent in grade-III infected seed whereas it was minimum 1.33 per cent reduction in case of grade-I infected seed lot. Thus on an average 7.71 per cent loss in 1000 grain weight, 20.39 per cent in germination and 12.40 per cent in case of seedling vigour due to the Karnal bunt infection of various categories was evident.

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