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EFFICIENT APPROACHES FOR THE MANAGEMENT OF KARNAL BUNT OF WHEAT CAUSED BY *NEVOSSIA INDICA*

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A B S T R A C T

Evaluation of various wheat varieties against Karnal bunt disease showed that seven cultivars were susceptible, and the same number were moderately susceptible. Fourteen varieties among all were highly resistant whereas nineteen depicted resistant response against the *Nevossia indica*. Sowing time showed a varying response on disease incidence while the fungicidal chemistries showed that shelter and dolomite were the best at the dose of 45 ug/ml and 100 ug/ml and while 100 µg/ml of Antracol and Alert+ also demonstrate similar results. Protection application of dolomite and shelter showed 62 and 64% decrease in the incidence of Karnal Bunt disease. As eradicative spray dolomite and shelter were used and their application lead to 39 and 41% reduction of disease respectively. Antracol was found least effective as curative spray with 24.07% against the Karnal Bunt disease comparatively. Hence, breeding resistant varieties is the need of the hour for the management of the wheat crop as it is cheaper, safe, and environment friendly approach not only for the farmer, climate and the country. Current research open avenues to exploit more ways for the Karnal bunt management by adopting biological control strategies.

Keywords: Fungicides, Screening, Chemotherapy, Protective, Curative.

INTRODUCTION

Wheat, (*Triticum aestivum* L.) being a leading food grain of human being which has been under cultivation for at least 6000 years. Its straw is also utilized by animals as feed. It plays a central role in the agricultural planning and policies development. Any harm to wheat crop either by biotic and abiotic factors leads to huge damage for country's economy as this is the staple food for the people of Pakistan. Wheat crop is attacked by a few but important pathogens i.e. fungi and bacteria. Wheat is attacked by *Nevossia indica* (Mitra) causing an economically important disease Karnal bunt. First time the disease was reported Karnal (India) in 1930 that's why called Karnal Bunt (Mitra, 1931). *Nevossia indica* Mitra [syn. *Neovossia indica* (Mitra) Mundkur] is a fungus of phylum basidiomycota, etiolating the disease in many countries like India, Pakistan, Nepal, Afghanistan, Iraq and Mexico (Singh *et al*., 1989). Environmental variation plays a crucial role in prevalence and surveillance of *Nevossia indica.* Southern Punjab, dry and arid zone provide the same environmental condition for prevalence of *Nevossia indica* inoculum.

Karnal bunt spread in wheat seed lots was analyzed and tested in Pakistan, to determine the presence of fungus *Nevossia indica* by dry examine technique from 1993 to 1997. From 730 wheat seed samples, from middle Punjab and Northwest parts of Pakistan, 3% infection was found in various seed lots, however, Southern Punjab areas of the Pakistan showed infection free seed. Incidence of KB disease displayed a lagging trend (up to 0.5%) at the level of country (Bhutta *et al*., 1999). Infestations of the disease are reported from multiple areas of the Punjab in 1986 and 1987. When

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climatic condition favors fungus, it infects both durum and bread wheat. Teliospores germinate on wheat flowers and develop the pro-mycelium having abundance of sporidia (sickle shaped) specially when temperature prevails between 20 to 25ºC. These primary sporidia protuberate for development of secondary sporidia at later stage. Rain splashes or wind assist sporidia to run on to new wheat barbs in the field where development of germ tube from secondary sporidial formation occurs which grows and enter palea, lemma, and glumes (natural openings) of ear heads. Hyphae enter in to ovary with commencement of intercellular growth (Goates, 1988).

Low temperature, light showers and high humidity at flowering stage increase severity of the disease. High temperature ranges, sun shines, and dry spans are un-facilitated factors in disease development. Soil borne, and seed borne infection usually act as an ingenuity in the disease epidemics. Along with seed and soil borne infection, infective secondary sporidia can germinate and spread onto the surface of leaves and glumes in resistant varieties. This miracle yields a good source of inoculum for airy infection (Dhaliwal *et al*., 1989). Disease losses are not in the form of yield strictly due to being negligible, but they do have solemn effect on the international marketing as causing poor quality seed. Pathogen attack reduces the flour quality as result of which grain weight affected (Singh *et al*., 1990). Teliospores are the main thing to be identified for Karnal bunt disease to prevail in the field. In Pakistan under maximum pressure of inoculum wheat varieties are being tested in screening and evaluation continuous its programs in several institutes, no variety/ line could be announced as immune or resistant or free from infection. Various genotypes with their great potential to cope with disease which having paul 14178, 14106, 14130, 14195, 14095 and 14245 are reported but some other like pau 14091, 14249, 14228, and 14160 proved to moderately resistant to karnal bunt stress (Dhaliwal *et al*., 1989). Based on cited reviews, and facts, current research was conceptualized to study chemotherapeutic and varietal solution of the disease.

MATERIALS AND METHODS

Study sites: Screening of wheat cultivars was steered at Ayub Agriculture Research Institute (AARI), Faisalabad while *in vitro* aspects were performed at Department of Plant Pathology, Bahauddin Zakariya University, Multan.

Extraction and detection techniques for *Nevossia indica*: Infection of karnal bunt disease in growing field cannot be easily detected and identified. Infected spikes cannot be differentiated from healthy ones only based on symptoms, but only in severe condition of disease. The other grain diseases may baffle with karnal disease mistakenly like bunt of rye weed, common bunt, dwarf bunt, and black point (Duran, 1987). Grains should be away from ear heads for confirmation test as described in diagnosis protocol by "European and Mediterranean plant protection organization" (EPPO). EPPO described practice was followed for testing *N. indica* (OEPP//EPPO, 1991b) for quarantine operations. Crop inspection was done before, after and at the stage of heading. Seed shower test was done for accuracy; 100 seeds in submerged form in water were taken in test tube having five replications. Tubes were rotated on rotary shaker for 10 minutes and attained spore suspension was centrifuged for ten minutes at 3000 rpm. In all test tubes Sedimented *N. indica* was obtained as pellet, were examined for the presence of teliospores in sample under compound microscope. Soaking method which is a simple laboratory method was also used for detection and estimation of partially bunted spores. Cracks on the surface and within endosperm at embryo, end of kernel or along the kernel cleavage revealed black powdery mass inside them. Seeds having black sori of fungus cracked in small quantity of distilled water for discharge of spores as torrent flow of teliospores. All bunted seeds collected during field inspection and collection were passed through this procedure. Collection of teliospores under high magnification of spores appeared as dark brown, tuberculate, spherical to ovate in projection with size 30-40 µm verified as *N. indica* (Inman *et al*., 2003).

Germplasm collection: 50 genotypes were obtained from Regional Agriculture Research Institute Bahawalpur, AZRI and wheat research institute Faisalabad. These genotypes (Table 1) were trialed out for their ability to resist against the karnal bunt pathogen in artificial condition of inoculation under huge inoculum pressure. Carefully chosen varieties were grown in two dates of sowing. Varieties were sown at field area of AARI, Faisalabad. All varieties were sown with 10 cm plant spacing and 30 cm row to row spacing in a randomized complete block design with three replicates.

Table 1. Germplasm collected for the management of Karnal bunt

Sr. No.	Variety/ Line	Source	Sr. No.	Variety/ Line	Source
1	10025	*WRI	26	0989172	***RARI
2	10031	WRI	27	09B9110	RARI
3	10104	WRI	28	10B9346	RARI
4	10110	WRI	29	10B2003	RARI
5	10193	WRI	30	PAK-81	WRI
6	10217	WRI	31`	E.NO.310	WRI
7	10355	WRI	32	V-10287	WRI
8	10370	WRI	33	V-09136	WRI
9	11149	WRI	34	V-11168	WRI
10	AS 2002	WRI	35	V-11172	WRI
11	11153	WRI	36	V-12001	WRI
12	11154	WRI	37	V-08203	WRI
13	11156	WRI	38	V-09802	WRI
14	11160	WRI	39	V-09087	WRI
15	11161	WRI	40	AS-2002	WRI
16	11164	WRI	41	Auqab-2000	WRI
17	11166	WRI	42	V-10300	WRI
18	09BT043	RARI	43	V-08314	WRI
19	10BT002	RARI	44	V-11183	WRI
20	WL-711	WRI	45	BHK-02	AZRI
21	9452	WRI	46	Blue Silver	WRI
22	NR-399	WRI	47	BWP-2000	RARI
23	NR-400	WRI	48	BWP-97	RARI
24	TW-86014	**AZRI	49	Chanab-2000	WRI
25	TW-96018	AZRI	50	WL-711	WRI

*WRI= Wheat research institute, Faisalabad. **AZRI= Arid Zone research Institute, Bakhar,
***RARI= Regional agriculture research institute, Bahawalpur

Inoculum preparation and Teliospore mass culturing: For inoculum formation and mass culturing, infected kernels were utilized to confirm the diversity and mixed population of the fungus by means of the technique of Bonde *et al*. (1996). From obtained bunted grains teliospores suspensions were primed, along with isolates of fungi. Infected grain's pericarp was cross sectioned and teliospores of the pathogen *N. indica* were scrapped and detached off from the bunted grains. These ruptured grains were subjected for shaking in tween-20 solution of detergent with 2-3 drop and 100 ml water for about 20 seconds. Centrifugation of prepared solution was done in a conical centrifuge tube at 3000 rpm to attain teliospores sedimentation in the form of pellet. Screening of the teliospores obtained through 100mm sieve was made to make the suspension free of kernel debris. Teliospores passed away and the debris cling in the sieve membrane. For teliospores retention insurance in solution, teliospores were again screened through 50mm mesh size sieve. Surface sterilization was carried out by using the sodium hypochlorite (NaOCl) 0.5% solution to free the grains from the superficial microbes (Bonde *et al.*, 1999) and centrifugation was done for 2 min at 3000 rpm. Extra disinfectant was decanted and retained teliospores were rinsed twice in the decontaminated distilled water. To expel out the chances of contamination, similar procedure was done again by disinfecting with Chlorax (commercial bleach) at the time of centrifugation. Excess bleach was removed and the final concentration of teliospores was made by re-suspending it in distilled water. Plates of agar water medium and 1-2 drops of teliospores suspension were added by micropipette in the agar plates having water agar composed of 20-gram agar dissolved in 1000 ml distilled water. Incubation was done for 15 days at 21±2ºC. Fungus colonized in 9-10 days by subjecting to light source through fluorescent lamp with 12-hour cyclic round. For preparing actively sporulating inoculum, fresh colonies from medium were obtained from small pieces for designing plugs or inoculum bits having size of 4-6mm diameter. To the lower side of the lid of readily prepared PDA (in 100 ml Erlenmeyer flasks bits were placed by sticking inside it and in some plates bits placed on the lid. Little amount of dH_2O was added to both mediums.

Inoculation of germplasm: On an average, 15 plants of every genotype were selected for testing the efficiency of inoculation at extreme extent adopting two methods i.e., spray method of inoculation and boot method of inoculation.

Spray method of inoculation: Spray inoculation was used for investigation of the worth of inoculation and this technique was utilized and assessed morphologically. Fifteen heads of each cultivar at best suiting stage for spray inoculation were inoculated as of partial emerged stage of ear GS-55. Each head was sprayed properly and wholly with proper tagging and labeling by mentioning the date of inoculation. Each head having inoculation covered with sealable bags and whole plant with polyethylene sheet was maintained to favor humidity for maximum infection spread. After three days, polyethylene bags were put out from plants. Spray inoculation require more humidity than boot inoculation and therefore in field less in resource domino effect was observed. Plants were placed under intensive care to conserve the fertilizer requirement, water, and light. Treated heads were plucked from plant, rubbed and threshed with mini wheat thresher in the month of April at the time of crop maturity. Incidence percentage was judged for infection quality through spray type of inoculation. Fine smog was dispersed from flag leaf sheath and on open ear parts as well. Control was in form of wheat heads sprayed through sterilized distilled water.

Boot inoculation: Designated genotypes were subjected for sowing at two different dates with 30 days interval. Freshly prepared sporidial suspension with 10,000 sporidia/ml of water of *N. indica* were injected inside the plant to perform boot cavity. For specification of time of inoculation injected plants were labeled with stain coding ribbon. Control was prepared by injecting sterilized water (1 ml) to the plants. Plants were hedged in polythene bags to afford and sustain adequate moistness for fungus to bourgeon inside the ear of wheat genotype. Inoculation was ended in the dusk time. Plants were sustained with suggested prescriptions of fertilizers and water. Spikes were gathered, threshed through hand and evaluated for bunted grains at the stint of maturity. Incidence and reaction of disease were calculated using the disease rating scale of Bonde *et al*. (1996).

Disease Assessment: While recording for the incidence and intensity of disease, samples were gathered at the phase of maturity, labeled with the name of genotype in the craft paper bags, collection date and site. During collection of 5-8 ears which were reserved as single sample and the ears were gathered by the stem right beneath the stem. These were winnowed by hand or with mini thresher. Mature kernels' seeds were detached from the ears and collected separately with full isolation and concern. Disease data comprised of infected and uninfected grains from every spike was collected through the assistance of the succeeding formula.

$$\text{Disease incidence} = \frac{\text{No of seeds infected}}{\text{Total no ofseeds}} \times 100$$

Degree of impairment due to karnal bunt was evaluated using rating scale consist of 5 points used by Bonde *et al*. (1996) (Table 2). Wheat seeds were judged and tallied visually for individual grouping. For assessment of point infection, magnifying glass was used, or seeds were examined under binocular microscope at necessity.

Table 2. Disease rating scale for karnal bunt of wheat

Infection Category	% Grain Infection	Level of Resistance or Susceptibility
0	No Infection	*HR
1	1	*R
3	1.1-2	*MR
5	2.1-5	*MS
7	5.1-10	*S
9	>10	*HS

HR= Highly resistant, R= Resistant, MR= Moderately resistant, MS= Moderately susceptible, S= Susceptible, HS= Highly susceptible

Chemotherapy of Karnal bunt of wheat

Evaluation of fungicides with various dosage (In-vitro): 10 fungicides were assessed with various concentration i.e., 40, 60, 80 and 100 contraries to *N. indica* colony growth by adopting zone inhibition technique. Each concentration was prepared by adding measured quantity of 10 fungicides in disinfected water at the rate of same dosage mentioned above (Table 4). PDA (20 g agar, dextrose 20 g and starch 20 g, agar) liquefied in sterilized water to meet the volume 1000 ml of almost 20 ml was transferred into divided cliques of petri-plates and permitted to congeal. Secondary sporidial culture (grown on PDA slants in 250ml conical flasks) of *N. indica* was scratched with a

disinfected scalpel and homogenized thoroughly in sterilized water 200ml and reserved in a sterilized beaker of 1000ml. Suspension was sifted aseptically by using muslin cloth and thinned further to gain 10,000 sporidia/ ml of water. 1ml of the suspension was released individually in 90 mm diameter PDA plates and spread constantly on the surface of PDA media with a sterilized (L-shaped) glass rod. As a control, 4 PDA petri dishes were occupied with sterilized water. Each Petri plate was categorized with the dosage rates and fungicides name. Either control or fungicide solution containing petri plates with wells solution were placed at 5ºC in a refrigerator for 24 hours to let the fungicide solution diffused into the solidified medium of the Petri-plates. Incubation of plates was maintained at 20ºC. After 6 incubation days, inhibition zone diameter around each well of *N. indica* colony was measured for each concentration of trialed fungicide.

Table 3. Fungicides assessed counter to *Nevossia indica* under in vitro and in vivo by foliar spray

Sr. No.	Common name	Chemical name & % composition	Formulation (wp)	Rate g/100 l
1.	Protocol Precombi	Thiophanate Methyl + Diethofen carb	65	490 g/ 100 l
2.	Agrohit	Diamethomorph + Mancozeb	50	360g/ acre
3.	Dolomite	Metalaxyl + Mancozeb	58	245g/ acre
4.	Alert plus	Fosetyl aluminium	70	360g/ acre
5.	Crest		50	90 g/ acre
6.	Anthacal	Antracol	75	255g/ 100 l
7.	Shelter	Mancozeb	80	575g/ acre
8.	Thiomil	Thiophaneti methyle	70	210g/ acre
9.	Reconil-M	Chlorothalonil w/w + Mancozeb	70	320g/ acre
10.	Aliette	Fosetly aluminium	80	2.4 g/l

Control of Karnal bunt of wheat by eradicative and protective spray of test fungicides (In-vivo) Protective spray: AS-2002 (Highly susceptible cultivar of wheat) was sown in distance of 1.53 x 0.92-meter sub-plotting, with 60 cm plot to plot distance, 15cm plant to plant distance in a row and 30cm row to row displacement in subplot in 3 replicates. At boot stage of crop, each sub plot was sprayed with all 10 fungicides (Table 3) with suggested prescription as stated by manufacturer, but the control plot was sprayed with water only. Experimentation was planned as Randomized Complete Block Design (RCBD) arrangement with each conduct was replicated four times. In each subplot each 20 heads were then inoculated through booting procedure with *N. indica* sporidial suspension 3ml (10000 spores/ml), after period of 48 hours of spray of fungicide. Control plot was inoculated by only sterile water. Each plant and plot were marked and categorized with specific treatment applied. Optimal irrigation and further agronomic practices were subjected in each plot to maintain a healthy crop stand. When crop achieved physiological maturity, it was picked and threshed manually. Each inoculated head was observed for assessment of grain infection data.

(B) Evaluation of Curative spray: A distinct similar RCBD experiment was designed alongside with caring treatment with a variety (AS-2002) for the evaluation of curative spray. At booting stage of wheat crop, 20 heads were inoculated with the spore suspension of *N. indica* 3ml in every replication with spore count i.e., 10,000 spores/ml. Similar water sprayed subplot was kept as control. Curative fungicides were spurted after 48 hours of the process of inoculation with indorsed prescription. Later tagged and grouped field were watered, and homologous agronomic practices were provided. Injected head was reaped, manually threshed at maturity and percent infection of seed for every spray application was evaluated.

STATISTICAL ANALYSIS

Data analysis was done by least significance difference test (LSD) to envisage the variance among datasets (Steel *et al*., 1997).

RESULTS

Screening of different varieties of wheat against Karnal bunt disease and chemotherapic control was carried out in Ayub Agriculture Research Institute, Faisalabad. Genotypes were grown at two different dates of sowing. The results were recorded showing that 14 varieties as highly resistant, 19 as resistant, 7 as moderately resistant, 7 as moderately susceptible and 2 are susceptible against karnal bunt disease. And one variety displayed no growth at all. 14 highly resistant varieties contrary to karnal bunt disease were Chenab, V-1168, V-08203, V-09087, 10193, NR-399, NR-400, 09B9110, E.NO.310,11153, 11161, V-10306, 10110 and 10104 while 19 varieties showed resistant response were 11156, 11164, 11166, V-08314, V-11183, BHK-02, BWP-2000, 09BT002, 09BT043, BWP-97,V-11172, V0980210025, , 09B9172, 10B9346, V-10287,10031,

10217 and 10355. These varieties i.e., TW-96018, V-09136 11154, 11160, 11161, TW-86014 and Uqab-2000 exhibited less than 1% infection against karnal bunt disease and recommended moderately resistant. Similarly, AS 2002, TW-86014, 10370, 11149, Pak-81, WL-711 and AS-2002 showed moderately susceptible response. Remaining all varieties were designated as susceptible as they showed up to 10% infection.

Table 4. Response of varieties against karnal bunt at First sowing date

S. No.	Varieties/Lines	Bunted Seeds	Healthy Grains	Total Grains	Disease (%)	Disease Reaction
1	10025	1	942	943	0.10	R
2	10031	1	654	655	0.15	R
3	10104	0	720	720	0	HR
4	10110	0	547	547	0	HR
5	10193	0	655	655	0	HR
6	10217	2	777	779	0.25	R
7	10355	4	697	701	0.57	R
8	10370	18	733	751	2.45	MS
9	11149	15	534	549	2.80	MS
10	AS 2002	20	800	820	2.5	MS
11	11153	0	377	377	0	HR
12	11154	11	570	581	1.92	MR
13	11156	5	804	809	0.62	R
14	11160	14	875	889	1.6	MR
15	11161	0	559	559	0	MR
16	11164	3	422	425	0.71	R
17	11166	6	746	752	0.80	R
18	09BT043	5	667	672	0.74	R
19	10BT002	3	487	490	0.61	R
20	WL-711	27	362	389	6.94	S
21	9452	13	1130	1143	1.15	MR
22	NR-399	0	374	374	0	R
23	NR-400	0	629	629	0	R
24	TW-86014	11	525	536	2.01	MS
25	TW-96018	9	708	717	1.27	MR
26	0989172	6	633	639	0.94	R
27	09B9110	0	620	620	0	HR
28	10B9346	5	588	593	0.85	R
29	10B2003	1	643	644	0.15	R
30	PAK-81	25	810	835	3.0	MS
31	E.NO.310	0	578	578	0	R
32	V-10287	1	447	448	0.22	R
33	V-09136	2	188	180	1.06	MR
34	V-11168	0	661	661	0	HR
35	V-11172	3	856	859	0.35	R
36	V-12001	-	-	-	-	-
37	V-08203	0	296	296	0	HR
38	V-09802	1	453	454	0.22	R
39	V-09087	0	352	352	0	HR
40	AS-2002	15	670	685	2.2	MS
41	Auqab-2000	4	345	349	1.15	MR
42	V-10300	0	578	578	0	HR
43	V-08314	4	574	578	0.70	R
44	V-11183	3	358	361	0.84	R
45	BHK-02	2	494	496	0.40	R
46	Blue Silver	32	381	412	8.39	S
47	BWP-2000	3	551	554	0.54	R
48	BWP-97	1	534	535	0.18	R
49	Chanab-2000	0	487	487	0	HR
50	WL-711	11	287	298	3.98	MS

Similarly, results of the second date of sowing showed 14 varieties as highly resistant, 13 as resistant, six displayed moderately resistant response, 12 showed moderately susceptible response and 3 were seemed to be susceptible to karnal bunt disease. None of the varieties showed highly susceptible response against karnal bunt disease of wheat.

Table 5. Reaction of wheat varieties against karnal bunt disease at sowing date

Sr. No.	Varieties/Lines	Bunted Seeds	Healthy Grains	Total Grains	Disease (%)	Disease Reaction
1	10025	7	184	191	3.8	MS
2	10031	8	258	266	3.1	MS
3	10104	4	299	303	1.33	MR
4	10110	2	248	250	0.8	R
5	10193	8	296	304	2.7	MS
6	10217	4	173	177	2.31	MS
7	10355	2	288	290	0.69	R
8	10370	0	210	210	0	HR
9	11149	7	284	291	2.46	MS
10	AS 2002	25	800	825	3.1	MS
11	11153	14	387	401	3.61	MS
12	11154	2	263	265	0.76	R
13	11156	0	610	610	0	HR
14	11160	4	187	191	2.13	MS
15	11161	1	350	351	0.28	R
16	11164	0	442	442	0	HR
17	11166	0	491	491	0	HR
18	09BT043	2	245	247	0.81	R
19	10BT002	0	346	346	0	HR
20	WL-711	30	362	392	8.2	S
21	9452	1	72	73	1.38	MR
22	NR-399	0	104	104	0	HR
23	NR-400	1	227	228	0.44	R
24	TW-86014	7	204	210	3.43	MS
25	TW-96018	0	169	169	0	HR
26	0989172	7	640	647	1.09	HR
27	09B9110	0	167	167	0	HR
28	10B9346	0	288	288	0	HR
29	10B2003	3	260	363	1.15	MR
30	PAK-81	27	815	842	3.3	MS
31	E.NO.310	0	244	244	0	HR
32	V-10287	1	262	163	0.38	R
33	V-09136	0	176	176	0	HR
34	V-11168	0	302	302	0	HR
35	V-11172	-	-	-	-	-
36	V-12001	11	225	236	4.88	MS
37	V-08203	3	296	399	1.01	MR
38	V-09802	0	-	-	-	-
39	V-09087	05	162	167	3.08	MS
40	AS-2002	17	660	677	2.5	MS
41	Auqab-2000	3	300	303	1.0	MR
42	V-10300	0	199	199	0	HR
43	V-08314	0	378	378	0	HR
44	V-11183	2	325	327	0.61	R
45	BHK-02	3	455	458	0.68	R
46	Blue Silver	18	237	255	7.59	S
47	BWP-2000	4	540	544	0.74	R
48	BWP-97	3	520	523	0.05	R
49	Chanab-2000	2	359	361	0.55	R
50	WL-711	17	331	348	5.1	S

When sowing accomplished at the date 13-11-2012, 10 varieties exposed as highly resistant to disease, 23 were found resistant, moderately resistant were seven, seven were founded as moderately susceptible and 3 expressed as susceptible to disease, during sowing on the day of 13-12-2012, 14 cultivars were highly resistant to KB disease, thirteen were resistant, moderately resistant were 6, moderately susceptible were 12 and three appeared as susceptible to disease. Sown varieties on 13-11-2012 provided better outcomes as compared to varieties sown on the day 13-12-2012.

Chemotherapeutic management of Karnal bunt disease of wheat: In vitro assessment of fungicides against *Nevossia indica:* Efficacy of fungicides for growth inhibition of *N. indica* elevated by snowballing the prescription rates as expressed in results showed dolomite and Shelter at their prescription rates 45 ug/ml and 100 ug/ml were equally active bestowing to hinder the growth of *N. indica* with 7.75 and 7.77 diameter zones inhibiting of fungi at 90µg/ml and 3.50- 3.35-mm diameter at 45 ug/ml. Antracol, Alert+ and Crest showed similar results at rate of 100µg/ml and formed small inhibition zones as compared to dolomite and shelter. It was experimentalized that efficiency of crest, Antracol and Alert+ was statistically at par. Antracol and Alert+ at the rate of 100 µg/ml exposed similar results as dolomite and shelter at the rate of 40ug/ml.

Table 6. Mean inhibition zones of *Nevossia indica* colony by different fungicides at different quantity amended in PDA medium

Fungicides	Mean Inhibition zone (mm) at 4 dosage rates			
	40 ppm	60 ppm	80 ppm	100 ppm
Crest	2.52 h*	3.40 ef	3.75 e	3.77 e
Dolomite	3.50 ef	5.70 c	7.75 a	7.77 a
Agrohit	0.25 l	0.50 l	0.5 0 l	0.5 l
Alert plus	0.00	0.00 l	2.00 j	3.1 fg
Shelter	3.35 ef	4.30 d	6.45 b	7.75 a
Reconil	0.00 l	1.00 k	1.00 k	1.00 k
Aliette	0.00 l	0.00 l	0.00 l	0.00 l
Antracol	2.25 ij	2.8 0 gh	3.50 ef	3.5 ef
Protocol pre-combi	0.00 I	0.00 l	0.00 l	0.00 l
Thiomil	0.25 l	0.25 I	0.5 I	0.5 I
Control (Water)	0.00 l	0.00 l	0.00 l	0.00 l

*Values followed by same letters are statistically significant

In vivo control of Karnal bunt disease: The efficacy assessment of fungicides in field as protective and as eradicative spray expressed that protective spray provided better outcomes than eradicative sprays in handling Karnal bunt disease of wheat field (Table 7). Protective sprays of Dolomite and Shelter had statistically similar effect and frolicked most important role in monitoring the karnal bunt disease. Both caused 62 and 64% decrease in karnal bunt disease incidence respectively. When dolomite and shelter were used as eradicative spray, these caused 39 and 41% lessening of disease respectively and variance between efficiency of both were statistically at balance. Protective spray of antracol, alert plus, agrohit and crest had transitional effect on disease lessening. Curative spray of Antracol was least effective relatively causing 24.07% drop in karnal bunt disease. Curative sprays of Alert plus, crest and agrohit were unsuccessful to handle kernel bunt disease. Curative along with protective spray of protocol pre-combi, alliette, thiomil, and reconil were unsuccessful in monitoring the infection of karnal bunt disease.

Table 7. Percent drop of wheat kernals infections by *Nevossia indica* by eradicative and protective spray of various fungicides.

Fungicides	Spray of fungicides before Inoculation		Spray of fungicides after Inoculation	
	Mean percent kernel infection	Percent decrease in kernel infection over control	Mean percent kernel infection	Percent decrease in kernel infection over control
Crest	22.45 efg*	33.79	28.81 bc	7.30
Dolomite	12.73 h	62.45	19.01 fg	38.84
Agrohit	22.16 efg	34.65	28.64 bc	7.82
Alert plus	27.49 cd	18.93	28.89 bc	7.04
Shelter	12.24 h	63.90	18.50 g	40.48
Reconil	33.01 ab	2.71	26.41 cde	14.98
Aliette	30.86 abc	8.99	30.97 abc	0.35
Antracol	19.94 fg	41.19	23.59 def	24.07
Protocol pre-Combi	33.39 ab	1.53	30.10 abc	3.15
Thiomil	32.43 ab	4.36	28.87 bc	7.08
Water (control)	33.91a	0.00	0.00 abc	0.00

*Values followed by same letters are statistically significant

DISCUSSION

Breeding program for resistance against karnal bunt of wheat is very crucial activity and optimum method to handle the disease. There is well known vulnerability of bread wheat to Karnal bunt instigating infected heights more than 55% under non-natural inoculation so, it is essential to carry on guessing new varieties counter to the disease in a diversity of agro-ecological areas. Resistant genes of karnal bunt, identification and tagging is very vital for resistant breeding and for evolving resistant cultivars by introducing these resistant genes. As we have information that wheat is an exportable product, so quarantine constraint of KB pathogen made deadly consequence on the country's economy. Kaur and Nanda (2002) performed a trial on karnal bunt disease of wheat. They detected that flour obtained from kernels have infection level 3% provided off color and loathsome odor. Hence, deliberating to present necessities, a new disease rating scale which was subjected and cultivars containing 0% grain infection were declared as highly resistant, below 1% grain infection disease were measured resistant, cultivars containing 2.1-5% infection were decided as moderately susceptible, cultivars partaking 5.1-10% grain infection were decided as susceptible and varieties with more than 10 % infected grains were deliberated as highly susceptible. In current scenario when sowing was accomplished on first sowing date, 10 wheat varieties were derived as highly resistant to KB disease, 23 were resistant, 7 were moderately resistant, 7 were moderately susceptible and 3 varieties were susceptible to disease whereas on second sowing date, 14 varieties were highly resistant, 13 were resistant, six were moderately resistant, twelve were moderately susceptible and three were susceptible to disease. In a field trial for screening steered by Gogoi *et al*. (2002) they demanded that 160 lines out of 350 higher genetic pillories were hygienic from KB disease. Hoffmann (1983) stated that 26 lines of wheat displayed none of the traces of disease and 58 lines disclosed only 0-5% infection, under mock epiphytotic conditions. An experiment was performed by NARC Islamabad reporting that 38 lines were highly susceptible but 5 lines which persisted unaffected from KB infection. This inadequacy of resistance in marketable grown cultivars security to KB disease across the boundary and in the country has previously been testified.

Subjection of systemic fungicide counter to Karnal Bunt of wheat were resulted ineffective because of two major restriction first one is that commercially available systemic fungicides could not continue to bout long contrary to KB blossom infection, secondly *N. indica* Karnal Bunt etiole is soil-borne in nature, not seed-borne as in circumstance of dwarf bunt (Hoffmann, 1983; Bryson, 2002). Henceforth, it is unproductive to put on systemic fungicide to wheat at end growth stages that destructively retort as high level of deposit in the grain at harvest. Opposite to it contact fungicides, because of not having systemic nature, would perhaps have slight scums, as the lingering chemical would be detached when the grain is winnowed. In the present research trial, Dolomite and Shelter having Mancozeb (have its place to Ethylene Bisdithio Carbamate EBDCs, non-

systemic, surface contact fungicide) as vigorous component is a trace of expectation for handling Karnal Bunt disease and might be utilized on wheat crop without serious venomousness.

For the previous 2-3 decades Karnal Bunt has supposed a disquieting condition in the Punjab and informed to etiolate up to 30% grain losses, reliant upon the cultivar used. This demands for rheostat of disease either adopting chemotherapy or breeding program for host resistance. Meanwhile resistance, commercially accessible cultivars, foundation counter to KB is insufficient, thus chemotherapeutic regulatory measure against the disease can be achieved by foliar application of connection fungicides at suitable plant stage of growth (Ilays *et al*., 1989a). Foliar application may guard the plants from KB infections or may be eradicate the established infection (Varshney *et al*., 2004). Contrasting reports for chemical treatment against KB are existing currently as well. Bryson *et al*. (2002) said fungicides application are not active against KB disease. Although lagging in the disease was accomplished by the applying foliar carbendazim (Krishna and Singh 1982). Chemical treatment of seed was not favored founded on some comprehensive reasons. Chemical covering of seed had not handled natural and non-natural infection of KB on wheat seed. Disobligingly, they summarized the tillering capability of plants, also tardy heading of about a week. Additionally, the infection on spike of wheat is originated by airborne sporidia which might reached from other fields of far-flung areas and the outcome of seed covering fungicide persevere no lengthier up to the heading or booting growth stage of wheat crop.

In vitro management of KB fungus *N. indica* by means of fungicides is as correspondingly active as for in vivo regulation of the KB infection. Though, defensive applications of fungicides were comparatively more active in handling wheat grain infection the as compared to eradicative applications. To the degree that time of foliar application is concerned (curative or protective spray) our discoveries are in corresponding to that of Jafari *et al*. (2000) where application of chemicals Metalaxyl and Mancozeb gave the unsurpassed control hostage to the downy mildews when applied prior to inoculation sooner than poster infection (curative application). Additionally, they also determined from their research that these fungicides own a broad range bustle in opposition to fungal pathogen: Ascomycota, Basidiomycota, and Oomycota. Shelter and Dolomite fungicides which are chiefly a intermingling of Metalaxyl and Mancozeb gave the suitable outcome when utilized as a protective spray in the field contrary to the KB of wheat. In current state, due to insufficiency of resistance cultivars chemical treatment is not rare (Singh *et al*., 1985; Ilyas *et al*., 1989a). Singh *et al*. (1985) conveyed that a solo spray of or Benomyl Bavistin or Dithane M-45 at the matching boot leaf stage was discovered active against KB. Krishna and Singh (1982) clinched from their trial that Bayleton and Bavistin (Derosal-60), when subjected as foliar spray at boot leaf stage previous (protective spray) to inoculation manage the karnal bunt disease. Singh *et al*. (1985) also specified that spray of mancozeb or carbendazim, may avoid karnal bunt disease of the wheat.

CONCLUSION

No variety is found to be resistant against this disease due to the changing climate. Fungicides are an option for the management of the disease to some extent.

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