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Wheat seed contamination with seed-borne diseases in cold climatic zone of Iran

S. Mobasser^{*}, M.R. Jazayeri, F. Khazaei, L. Sadeghi

Seed and Plant Certification and Registration Institute (SPCRI), Karaj, Iran. *Corresponding author. E-mail: sa_mobasser@yahoo.com

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Abstract

In order to evaluate the informal wheat seed contamination with seed-borne diseases, two most important provinces (East Azarbaijan and Khorasan Razavi) for wheat production were selected in cold region of Iran in 2008-2009 crop seasons and ten different locations (city or village) were considered in each province and ten informal seed farmers were selected randomly. A seed sample (1 kg) was taken from planting source of each farmer. Different analysis including purity, germination and seed health (head blight, common bunt and loose smut disease) tests carried out on seed samples according to ISTA rules. Fusarium graminearum was identified as the main disease in provinces. Blotter test showed significant differences (P<0.05) among the towns but there was no meaningful difference between provinces. Washing test for T. caries showed significant difference between the two provinces (P < 0.1) but there was no meaningful difference among towns in each province. Furthermore there was significant difference between and among provinces and towns with respect to T. leavis infection. Also the data showed clear dispersal of these two species (T. caries and T. leavis). Negative and high significant correlation was observed between U. tritici and germination. So the precision study of seed borne disease in different areas of the country for producing healthy seed is recommended.

Keywords: Wheat seed; F. graminearum; T. leavis; U. tritici; T. caries.

Introduction

A little more than 9 million hectares (60% of the arable land) of total land area in Iran are planted with cereals, in which wheat occupies 6.6 million ha.

The share of the formal seed system in providing country's seed requirement varies according to the crop. For some hybrid seeds, 100% of the seed needed is produced and distributed by state companies, whereas for self pollinated crops, e.g., cereals, the formal system's share is less than 20%, in general (Mobasser, 2011). Distribution and use of cereal certified seed (e.g. wheat seed) are highly related to agro-ecological conditions. In warm areas where farmers are faced more difficulties in saving their own seeds until the next cropping season, formal seed distribution is significantly higher than in other regions (Anonymous, 2011). Also, in rain-fed cropping systems, due to shortage of certified seed of improved varieties, the use of local varieties within informal system is higher than the irrigated areas (Anonymous, 2005). Results of a survey conducted during 2001-2002 in the four most important wheat growing provinces, showed that more than 50% of irrigated and 80% of rain fed wheat growers never purchased certified seed directly (Mobasser, 2002). These farmers usually are smallholders living in very remote rural areas. On the other hand, farmers with large production areas and close to certified seed distribution centers, tended to change their seed annually. This is in contrast with the findings of Bishaw et al. (1994), Cromwell et al. (1993) and Van Gastel and Bishaw (1994) who reported that most farmers retain seed of modern varieties for a longer period, in spite of the recommendations to regularly replace their stocks with certified seed. This example shows quite a different situation of wheat seed production, distribution and marketing in Iran. Seed pathology is related to food production in various ways. First, seeds are the primary input for crop production. When seeds are sown, seed-borne pathogens may cause disease or death of the crop plants, resulting in loss of crops and food (Bishaw, 2004). Second seed may contain plant pathogens or agents that cause diseases in plants. These diseases may affect storage, vigor, germination, marketability, harvested yield, seed appearance, or contain toxins. When infected seeds are planted, the pathogens are distributed throughout the field and contaminate the land. It is important to know what levels of pathogens are in or on the seed. When levels of disease are high, it may be important to seek a different source of seed for planting. It also may be very helpful to know the present pathogens, to choose a good treatment. One of the most important things is not to introduce plant diseases which are not already in the field. Many of pathogens can persist in soil or on crop trash and become a problem for many consecutive years. The important point is that all pathogens which affect wheat are not seed-borne nor all of

wheat seed-borne diseases in a distinct region have an economic impact on yield, or produce a hazardous toxin (Prescott et al., 2006).

Fusarium graminearum Schwabe [teleomorph *Gibberella zeae* (Schwein.)], also known as *Fusarium* head blight (FHB) or scab is a destructive disease of wheat and barley in warm and humid wheat growing regions of the world (Rudd et al., 2001). The International Maize and Wheat Improvement Center (CIMMYT) have identified *Fusarium* head blight as a major factor limiting wheat production in many parts of the world (Parry et al., 1995).

The global losses due to seed-borne diseases are estimated at 12% of potential production (Agarwal and Sinclair, 1987). FHB not only causes quantitative yield loss but may also generate problems with grain quality because of *F. graminearum* and other FHB-causing *Fusarium* spp. contaminate grain with mycotoxins such as deoxynivalenol (DON) (Rosewich et al., 2002).

Common bunt is a disease of *Triticum* species that is caused by two very closely related fungi, *Tilletia tritici* (syn. *T. caries*) and *T. laevis* (syn. *T. foetida*), which are distributed throughout the world on spring-planted and autumn-planted wheat (Wilcoxson and Saari, 1996). In Iran, bunt and smut are the most important diseases of wheat after the rusts (Zad, 1972; Khazra and Bamdadian, 1974; Sharif and Bamdadian, 1974; Akbari and Zolghadri, 1988; Mardoukhi, 1989). Common bunt causes losses of 25-30% in parts of Iran (Bamdadian, 1993a; Bamdadian, 1993b). This disease is found throughout the country, but it is concentrated in the northern and northwestern regions (Akbari and Zolghadri, 1988; Bamdadian, 1993a).

Loose smut [*Ustilago tritici* (Persoon) Rostrup] of wheat occurs wherever cultivated wheat, is grown (Anon, 1982). Since the pathogen is seed-borne, it will be spread from place to place by man; rarely is it disseminated over long distances through the air. The environment influences development of loose smut soon after infection while the pathogen spreads through the embryo and also during growth of the infected plant in the next generation (Wilcoxson and Saari, 1996). The main objective of this work was comparative study of informal wheat seed contamination with seed-borne diseases within two provinces in cold climatic zone of Iran.

Materials and Methods

In order to evaluate the informal wheat seed health quality, two most important provinces (East Azarbaijan and Khorasan Razavi) of wheat production in cold region were selected. Ten different locations that is most cultivated areas of wheat (town or village) were considered randomly in each province (Table 5). Then, in each town or village ten informal seed farmers were selected randomly. A seed sample (1 kg) was taken from each farmer from a seed lot intended for planting based on ISTA rules (ISTA, 2004). Different seed quality analysis was conducted on seed samples including purity, germination and seed health (head blight, common bunt and loose smut disease) according to ISTA rules (ISTA, 2009). Experimental data was analyzed using SPSS (SPSS Inc., 2008) software package.

Isolation of pathogens

Fusarium graminearum (Head blight)

For isolation of Fusarium, the deep-freezing blotter method and culture in agar medium were used. The test was carried out on sample of 400 seeds according to the ISTA rules (ISTA, 2009) on blotter paper in plastic containers place, 25 seeds evenly placed in each container and incubated for 24 hours at 20 °C and for 24 hours at -20 °C respectively. Then seeds incubated for 7-10 days at 20-30 °C in growth chamber under near ultraviolet (NUV) light at 12 hours interval of alternation with darkness until the colonies are about 2 cm in diameter. Then seeds were evaluated by microscope (Mather and Kongsdal, 2003; ISTA, 2009). In agar method (Potato Dextrose Agar) seeds were pre-treated with sodium hypochlorite (NaOCl) solution (1% available chlorine) for 1-5 min and then seeds removed. Aseptically 10 seeds placed to the PDA surface. Plate contain seeds incubated for 5-7 days at 20-25 °C in growth chamber under fluorescent light in 12 hours interval of alternation with darkness until the colonies are about 2 cm in diameter. Then infected seedlings were counted and the pathogens were identified based on macroscopic characteristics (appearance, color and growth type of colony) and microscopic traits (appearance of phialides, presence or absence of microconidia, macroconidia and chlamydospores) (ISTA, 2009).

Tilletia laevis and T. caries (Common bunt)

The washing test was used for recording the presence of teliospores of smuts for washing test, 50 g seeds were shaken in water and the obtained suspension was examined under microscope after concentrating the released spores by centrifuging at 2000 rpm for 10 min, filtering the fluid and re-suspending the spores in water. Drops of the suspension were transferred to

a counting chamber. Teliospores identified and classified to genus and species and also number of spores per gram seed was determined (ISTA, 2009).

Ustilago nuda (Loose smut)

Embryo test was used for loose smut. For the embryo test, three samples, each containing 1050 seeds (approximate 100 g) was soaked separately in 1 liter of 5% NaOH containing 0.15 g of trypan blue at room temperature for 22-24 hours. After soaking, the entire sample was washed in water to facilitate the separation of embryos from the endosperm and passed over a set of sieves. Then the seeds for separation and clearing the embryos transferred to lactic acid-glycerol mixture. Some of mixture containing embryos placed in the grooves of the examination plates and consequently stained mycelium observed through stereomicroscope. Finally the total number of embryos and the number infected by *Ustilago nuda* counted in each groove (ISTA, 2009).

Results and Discussion

As it can be seen from table 1, according to results of the questionnaires, the seed source of majority of the interviewed farmers, is the previous harvest, however its amount in Khorasan Razavi (79.38%) is slightly bigger than East Azerbaijan (63%). This pattern is similar in most years, with local traders or markets accounting for less than 15%. However, a non-random barley diffusion study in Syria reported that about half of barley farmers used their own seed saved from the previous season, and 37% of them purchased seed from neighbors for planting (Aw Hassan et al., 2008). The role of nearby farmers (friends, related and pioneer farmers) in providing the needed seed for planting is considerable.

Less usage of mechanization in cultural practices is an indicator of subsistence farmers in all of world. The foundation of a good crop is laid by proper planting. Moreover, most mistakes that made at planting cannot be corrected, so it is very important to get it right. The great parts of farmers in both provinces (80.21% and 57% in Khorasan Razavi and East Azerbaijan, respectively) as shown in table 2, sowing their informal seed by hand which is followed by a disc plow to bury the seeds. In this method, the seeds are settled in different depth, most of them cannot emerge from the soil, great part remain in surface of soil and are consumed by birds, causing higher seeding rate.

Sites	No of interviewed farmers	Previous harvest	Friends & Related	Pioneer farmers	Cooperatives	Previous harvest & related	Previous harvest & Pioneer farmers	Previous harvest & Cooperatives	Other sources
East Azerbaijan Khorasan Razavi	100 97	63 79.38	10 6.19	12 0.00	2 3.09	3 10.31	2 0.00	5 0.00	3 1.03
Table 2. Planting Methods of Informal Wheat Seed by the Farmers $(\%)$.	ethods of Inform	nal Wheat See	ed by the Fa	armers (%).					
Sites	No of interviewed farmers	ewed farmers		Handy Sowing	Broadcast		Line Planting B	Broadcast & Line Planting	anting
East Azerbaijan	-	00	4.)	57.00	4.00		35.00	4.00	
Khorasan Razavi	5	5	~	80.21	15.63		4.17	0.00	

Table 1. Declared Source of Informal Wheat Seed by the Farmers (%).

Processing is the first post-harvest activity in farmers' seed management. It includes activities such as handling (transporting/receiving), seed drying, cleaning and treating. Seed cleaning has a dual purpose: it removes noncrop seed materials from the harvested material, such as straw, stones and weed seeds, thus reducing the bulk to be stored; and it also selects the seeds on the basis of physical characteristics such as size, shape, density and color, thus removing small and shriveled seeds and improving the seed quality. For most crops, seed cleaning is no different to the cleaning of food grain for consumption, so that local methods for cleaning food grain are well suited for seed cleaning. Such methods include winnowing, sieving and hand-picking. Winnowing removes the light particles like straw and dust, and it can be used to remove seeds with a low density (low weight per volume: empty or 'soft' seeds). Sieving selects the seed on the basis of shape and size.

After cleaning, the seed may or may not be treated, depending on the local need to control plant diseases. Chemical seed treatment has become routine practice for many crop seeds in formal seed systems, and increasingly also in farmers' seed production, and is seen as offering the cheapest, safest and most efficient form of the plant protection (Louwaars and Amekinders, 2008). Table 3 shows only 24% of East Azerbaijan and about 2% Khorasan Razavi farmers are planting their informal seed after machine cleaning but traditionally a significant portion of them trying to clean their own seed before planting by sieving.

Sites	No of interviewed farmers	Hand sieving	Hand sieving + seed treatment	Machine cleaning	Machine cleaning + seed treatment	seed treatment without cleaning	Any action before planting
East Azerbaijan	100	30.00	26.00	9.00	15.00	12.00	8.00
Khorasan Razavi	97	36.08	20.62	1.03	1.03	23.71	17.52

Table 3. Different seed preparation methods used by informal wheat seed farmers.

In spite of difficulty of seed treating by individual wheat farmers, considerable percentage of the farmers (53% in East Azerbaijan and 45.36% in Khorasan Razavi) use to treat their own seed before planting. The amount of informal wheat seed which is treated prior to planting in Pakistan (Tetlay et al., 1992) and Syria (Bishaw et al., 2011) are lower.

The results showed that the diameter of *F. graminearum* colonies varied from 3/9 to 5/1 cm. The mycelium was light yellow to grayish red (Figure 1). The spordokhiums were established in the center of small mass of spores. According to the specifications listed in the key resources (Nelson et al., 1983; Bergess et al., 1994) the fungus was identified *F. graminearum* Schwabe. So *F. graminearum* was recognized as main cause disease in two provinces of East Azarbaijan and Khorasan Razavi at this study. Results of variance analysis of blotter test showed significant differences (P<0.05) among the towns but meaningful difference was not observed between two provinces (Table 4).

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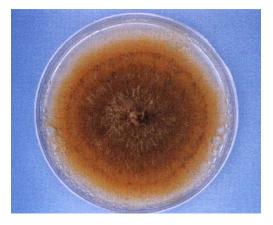


Figure 1. Fusarium graminearum isolate of colony morphology on PDA after 15 days at 25 $^{\circ}$ C.

Table 4. Variance analysis of wheat infection between provinces and among towns.

Source	DE		Me	an Square	
Source	DF	U. tritici	T. leavis	T. caries	F. graminearum
Province	1	0.077	16680410.44**	14535455.12**	105.03
Town (Province)	19	0.087	2495315.61*	914559.52	804.73^{*}
Error	175	0.077	1428049.6	1220995.8	438.08

Many species of *F. graminearum* causes ear blight and its symptoms are almost similar and are considered as the main cause of most disease in many countries (Clear and Abramson, 1986). Francis and Burgess (1977) divided the species *F. graminearum* into two groups 1 and 2. Group 2

isolates produce perithecia in nature, while group 1 isolates, which cause crown rots in semiarid areas, do not produce any perithecia. Giberella. zeae produces deoxynivalenol and zearalenone in the kernels of corn and wheat (Miller, 1994), and accumulation of these mycotoxins in the grain increases the importance of this pathogen to agriculture. In eastern Canada, head blight and ear rot epidemics occur sporadically at intervals of several years (Satton, 1982). Presence of *F. graminearum* has been reported at Mazandaran area by Ershad (1995) and displayed that the first group attacked the root & crown while second group attacked to the wheat ear.

The results showed that the highest and lowest infection percent of wheat fields were related to Bardskan (61.5%) and Sabzevar areas in Khorasan province (22.5%) respectively (Table 1). According to same planted cultivar (Roushan) in both areas, the probability difference in the aspect of infection level is related to rotation of wheat with sugar beet instead of maize in Sabzevar. In Ethiopia, several seed-borne fungi were recorded from wheat seed samples where 84%, 31%, 74%, 13%, 52% and 31% of samples were infected by *Cochliobolus sativum*, *F. avenaceum*, *F. graminearum*, *F. nivale*, *F. poae* and *Septoria nodorum*, respectively. Among all Fusarium species, *F. graminearum* was predominant (Bishaw, 2004). There were significant difference (P<0.001) in the mean infection levels across different regions and districts in Ethiopia and Syria for wheat and barley (Bishaw, 2004).

It was also found that the highest and lowest infection level of wheat fields in East Azarbaijan provinces were in Miyaneh (54.1%) and Charimagh (29.9%) respectively (Table 5). The cooler climate of Charimagh compared to Miyaneh region probably is the main cause of high level of seed infection. The other reason might be related to rotation of wheat with maize in Miyaneh. One of the other reasons can be related to the kind of planted seeds.

Infection, mainly by ascospores and less commonly by macroconidia, is favored by warmth and persistent surface wetness. Symptoms, which may appear within 2 days of infection or up to several weeks later, are blighted florets or spikelets (rarely an entire ear) and pink mold. Later in the season perithecia may appear as raised black spots (Teich and Hamilton, 1985).

Doot American		Ustilago tritici	ritici	Tiletia leavis	avis	T. caries	es	F. graminearum	earum
cast Azaroaijan	-	Number of samples	Mean Infection						
Bonab	-	=	0.24	=	127.4	=	144.32	=	46.55
Shabestar	2	10	0.00	10	697.5	10	1106.25	10	33.30
Kaleibar	с	10	0.08	10	223.7	10	507.50	10	41.50
Ahar	4	10	0.00	10	181.2	10	1.25	10	43.70
Sarab	5	12	0.03	12	132.3	12	1078.13	12	45.75
Marand	9	6	0.06	6	466.7	6	1206.94	6	34.44
Miyaneh	7	10	0.05	10	197.5	10	533.75	10	54.10
Marage	8	8	0.25	8	148.4	8	675.00	8	49.13
Chareimagh	6	10	0.05	10	297.5	10	107.50	10	29.90
Hashtrod	10	10	0.02	10	168.7	10	750.00	10	40.20
	Total	100	0.08	100	260.4	100	608.50	100	41.91
Khorasan Razavi									
Ghochan	-	15	0.100	15	1641.63	15	0.00	15	50.80
Forbate jam	7	10	0.050	10	2132.50	10	0.00	10	49.30
Forbate Heidariye	з	10	0.190	10	1314.75	10	1.25	10	46.00
Mashhad	4	7	0.014	7	483.93	7	32.14	7	46.14
Sabzevar	5	10	0.020	10	1205.00	10	0.00	10	22.50
Kashmar	9	8	0.063	8	156.25	8	0.00	8	25.75
Bardskan	7	2	0.150	2	18.75	2	0.00	2	61.50
Chenaran	8	ŝ	0.000	3	1758.33	3	16.67	3	40.33
Rashtkhar	6	10	0.320	10	776.25	10	2.50	10	36.80
Neishabor	10	11	0.245	11	328.41	=	0.00	=	46.09
Sarakhs	=	10	0.180	10	141.25	10	86.25	10	52.90
	E	20							

In Lambton County, Ontario (Teich and Nelson, 1984), blight incidence was lower where wheat was planted after soybeans or small grains rather than after corn, where nitrogen and phosphorus fertilization were adequate, and where weed density was low. There was not any correlation between head blight incidence attributable to normal versus nitrogen fertilizer application which our finding is in line with Teich and Hamilton (1985) report (Table 3). Planting wheat after corn increases the incidence of head blight and mycotoxin deoxynivalenol ([DON] vomitoxin; 3a, 7a, 15trihydroxy-12, 13-epoxytrichothec-9-en-8-one) concentration more than any other factor studied. Plowing under corn Stover before planting wheat and treating wheat seed with Vitaflo 280 (carbathiin plus thiram; Uniroyal Chemical, Elmira, Ontario) was associated with less blight. None of the other cultural practices studied or soil test parameters were shown to affect head blight or DON concentration (Teich and Hamilton, 1985). Giberela zeae over winters on corn stalks and wheat straw and produces perithecia and sporodochia that give rise to ascospores and macroconidia, respectively. The fungus also may survive on weeds and natural vegetation (Jenkinson and Parry, 1994). Although control measures for head blight have been proposed for many years, data supporting their effectiveness are rare. Boewe (1960) proposed plowing in residues of host crops, rotation to avoid planting wheat after corn (Zea mays L.), another host, and treating seed to kill spores on seed surfaces. Wiese (1977) suggested that some cultivars are infected less frequently than others because of physical barriers to floret and spikelet infection and that chemical seed treatment are a partial deterrent.

Results of variance analysis of washing test for infection rate of *T. caries* between two provinces were significant different (P<0.1%) but there was no meaningful difference among various towns in each province. However there was significant difference between and among provinces and towns with the respect of *T. leavis* infection (Table 4).

Five species of *Tilletia* are described in Iran. The data also made clear that these two species (*T. caries* and *T. leavis*) were different in their distribution. Our finding showed that *T. caries* and *T. leavis* were dominant species in East Azarbijan and Khorasan Razavi provinces, respectively (Table 5). In the other study it has been reported that *T. laevis* and *T. controversa* were most common and second most common infection respectively (94 and 4.5% of the samples) in Iran meanwhile the lowest

infection was related to T. caries (0.1% of the samples) (Sharifnabi and Hedjaroude, 1992). The reported dominance of T. laevis may also reflect the predominance of bread wheat's in the area and at higher elevations of northern crop areas. We observed that there was negative and high significant correlation between head blight and common bunt infection (Table 6). Bishaw (2004) reported 68 and 14% of the samples from wheat were infected with common bunt and loose smut, respectively in Syria. Neither T. laevis nor T. tritici predominated in Syria, but their presence was correlated with the host species. Only 3% of samples tested contained a mixture of both pathogens. The highest severity was a 60% incidence in the bread wheat cultivar Mexipak and a 33% incidence in the durum wheat landrace cultivar Shyhani (Mamluke et al., 1990). In Ethiopia there was a significant difference (P<0.001) infection levels for most of the pathogens from different seed source compared to Syria where there was no significant difference for seed samples from different source (Bishaw, 2004). Investigation of variance analysis of embryo testing for level of infection of U. tritici had recognized no significant difference between and among provinces and towns.

	Germination	Ν	Р	К	U. tritici	T. laevis	T. caries	F. graminearum
Germination	1							
Ν	-0.164*	1						
Р	-0.082	0.618^{**}	1					
Κ	-0.198**	0.223^{**}	0.207^{**}	1				
U. tritici	-0.256**	-0.001	-0.007	0.141^{*}	1			
T. laevis	0.067	-0.019	0.024	0.057	-0.004	1		
T.caries	-0.053	0.004	-0.026	0.008	-0.076	-0.102	1	
F. graminearum	0.091	-0.020	-0.009	0.031	0.057	-0.022	-0.187**	1

Table 6. Simple correlation coefficient of wheat infection with germination, nitrogen, phosphorus, potassium.

The highest and lowest infection level of *U. tritici* in Khorasan Razavi were observed from Rashtkhar (32%) and Chenaran (without infection) respectively. The most level of infection (25%) of *U. tritici* at East Azarbaijan was found in Maraghe, while the least infection (0.0%) was related to Ahar (Table 5). There was negative and high significant correlation (-0.256^{**}) between *U. tritici* and germination (Table 6).

In Iran, loose smut is widely dispersed, but is more prevalent in northern areas around the Caspian Sea (Shariff and Bamdadian, 1974; Bamdadian, 1993a; Bamdadian, 1993b). Incidence of disease ranges up to 2% in susceptible cultivars. The occurrence of this disease has been reported on rye in Turkey (Wilcoxson and Saari, 1996). Loose smut occurs naturally on Aegilops spp. and on wheat almost everywhere under cultivation in Syria (Mamluk et al., 1990). The disease occurs sporadically in Syria, with incidence at about 5% (Azmeh and Kousaji, 1982; Mamluk et al., 1990; Mamluk et al., 1992). Common bunt incidence may be reduced by seeding shallow and significantly reduced or controlled by seeding into warm soil (Purdy and Kendrick, 1963; Gaudet et al., 1990).

Hyphae infect plants shortly after the seed germinates and before seedlings emerge, which makes it possible to control by fungicide seed treatments because a high concentration of the fungicide is placed directly in the infection court.

It is more common in regions with a cool, moist climate during flowering of the host while in dry warm climates, economic losses may occurs. Since percentage infection equals loss in yield, and since most of the monetary return from any one field goes into cost of production, even 1-2% infection can reduce profit to the farmer by 5-20% (Wilcoxson and Saari, 1996). Several systemic seed treatment, such as carboxin, difenoconazole, triadimenol and others are highly effective for controlling the disease and have eliminated losses where they are commonly utilized (Hoffmann and Waldher, 1981; Gaudet et al., 1989; Williams, 1990). These chemicals adequately control disease that can result from both seed-borne and soilborne teliospore inoculum. Even where seed treatments are commonly used, a residual amount of inoculums is often still present that can cause disease outbreaks when seed treatments are not used regularly (Wilcoxson and Saari, 1996).

Conclusion

Our finding shows that informal wheat seed have meet the least acceptable quality (national standard) in the aspect of traits as germination, broken seed and infection with seed-borne diseases compared to minimum requirements of the National Seed Standards. As most of farmers in the studied area used to saw their own seed and they know the value and importance of seed cleaning and treating prior to planting, so doing special management as village based seed cleaners, providing the proper chemicals and seed treaters, makes possible the informal seed be able to yield without significant decreasing of production in comparing certified seed. Hence certified seeds propagated at special districts and its movement to other locations for planting intensifies the disease and weeds in country movement. So suitability of informal seed using mentioned methodologies will be resulted in the alleviation of vulnerability of wheat seed production system in the country. So in order to produce healthy seeds and without infection, a precise study of seed borne disease in different areas of the country is recommended.

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