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Seed borne disease in formal and informal wheat (*Triticum aestivum* L.) seed production systems in three provinces of Iran

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Abstract

For investigation of Seed-borne fungi diseases in formal and informal wheat seed production systems, a research was carried out in laboratory of Seed and Plant Certification & Registration Institute (SPCRI), Karaj, Iran, 2011-12. The studied areas was consisted of three provinces (Tehran, Kermanshah and West Azarbaijan) included Four districts and two seed production systems (formal and informal). 24 seed samples were collected from harvested seed loads. *Tilletia laevis*, *Ustilago tritici*, *Fusarium graminearum*, *Fusarium culmorum*, *Fusarium avenaceaum, Fusarium poa, Bipolaris sorokiniana* and *Alternaria alternate* were the most important fungal species detected in the samples. The lowest and highest infection rate of seed to *T.laevis* was observed in West Azarbayejan and Tehran respectively. *Alternaria alternata, Bipolaris sorokiniana and Fusarium* spp by 23.5, 6.5 and 46.54 % infection rate were the predominant fungi respectively. The lowest and highest *fusarium* spp. infection was related to Tehran (67 %) and West Azarbayejan (21.88%) respectively. The informal seed quality was at least as good as formal seed. It seems that no meaningful difference between two seed systems can be related to proper management and seed dressing in informal seed system. Such attempt on seed mycoflour is necessary for national seed health standards and disease management programs.

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Introduction

Seed health is one of the most important attributes of seed quality. Seeds can serve as a vehicle for dissemination of plant pathogens, causing serious disease outbreaks. Infected seeds may fail to germinate, produce abnormal seedlings and have low germination or low seedling vigor affecting grain yield and quality (Bishaw *et al.,* 2013).

Since a large portion of wheat seed needed for planting in Iran is provided from farmers own saved seeds and lower portion of seed is prepared from formal seed system and no special measure is taken for evaluation of seed infection rate by pathogens. Therefore, conducting a survey for comparison the informal and formal seed production is necessary to get better understanding about the seed production condition in the country. So, one of the basic strategies to produce non-infected seeds is the identification of seed-borne pathogenic agents in wheat-growing fields in both seed production systems.

If infested grain is used as seed, not only would the seed-borne diseases reduce crop yield but also it would be a source of disease inoculums (Clear and Patrick, 1993). It is important to know what levels of pathogens are in or on the seed. In high contamination level, it may 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. Seed-borne fungi are one of the most important biotic constrains in seed production worldwide. They are responsible for both pre and post-emergence death of grains, affecting seedling vigor, germination rate as well as plant morphology (Niaz and Dawar, 2009).

Mobasser *et al.* (2012) by study wheat seed contamination with seed-borne diseases in six wheat growing areas of the country showed that loose smut, common bunt and head blight are the limiting factors for wheat production both in the formal and informal seed systems. Since seed health testing is not done regularly for wheat seed, so little is known about the status of seed borne disease in formal and informal seed production systems in wheat-growing regions of the country. Seed health testing to detect seed-borne pathogens is an important step in the management of crop diseases.

Seed borne diseases of wheat like loose smut (*Ustilago tritici*), head blight or scab (*Fusarium* spp.), Karnal bunt (*Tilletia indica*), common bunt (*Tilletia laevis*), ear cockle (*Clavibacter tritici* and *Anguina tritici*) are considered as the constraints in wheat cultivation that affect crop yield and grain quality (Kumar *et al.,* 2008).

Fungi are the principal organisms associated with crop seeds. A complex of seed-borne fungi including genera of *Tilletia*, *Ustilago*, *Bipolaris*, *Fusarium*, *Alternaria*, *Drechslera*, *Stemphylium*, *Curvularia*, *Cladosporium*, *Rhizopus*, *Aspergillus* and *Penicillium* has been convincingly reported as the most frequent seed-borne fungi of wheat throughout the world (Hashmi and Ghaffar, 2006; Rehman *et al.,* 2011).

Fusarium graminearum Schwabe [teleomorph *Gibberella zeae* (Schwein.)], which is 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. 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).

Loose smut [*Ustilago tritici* (Persoon) Rostrup] of wheat occurs wherever cultivated wheat, is grown. 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.

The aim of this study was to compare and identify seed-borne mycoflora of wheat in formal and informal fields of some areas in the country. This information is necessary for growers to understand the seed-borne fungal pathogens status infecting wheat and their frequency to effectively apply control methods.

Materials and methods

In order to evaluate wheat seed health quality, 24 seed samples were collected randomly from formal and informal wheat seeds from different locations of wheat production in semi-arid area including Tehran province (Karaj, Hashtgerd-Nazarabad, Varamin and rey), West Azarbaijan province (Orumiyeh, Oshnavieh, Naghadeh and Miandoab) and Kermanshah province (Sarab Niloufar, Mahidasht, Kozaran and Ravansar). Two wheat cultivars (cv. Pishtaz for Tehran and Kermanshah and cv. Zarin for West Azarbaijan), were used for conducting the research. The laboratory tests were conducted in Seed and Plant Certification and Registration Institute (SPCRI), Karaj, Iran, 2011-12.

The formal and informal seed samples (1 kg) were used for the isolation and identification of seed-borne fungi using the seed washing, deep freezing blotter and embryo count methods described by the International Seed Testing Association (ISTA, 2012). The status of climate temperature, precipitation and elevation above sea level in studied areas during crop season is presented (Table 1). Also the chemical application against disease in studied areas is presented (Table 2).

Isolation and identification of fungi

Seed washing method

The washing test was used for recording the presence of teliospores of bunts (*Tilletia* spp.) which their spores are located on the surface of wheat seeds. 50 g seeds were shaken in water and 1-2 drops Tween 20 and the obtained suspension was examined under microscope by a counting chamber after concentrating the released spores by centrifuging at 3000 rpm for 15 min, filtering the fluid and re-suspending the spores in water. Teliospores identified and classified to genus and species on the basis of their morphology under microscope and also number of spores per gram seed was determined (ISTA, 2012).

Deep freeze blotter method

400 seeds of each sample were planted on 3-layered water- soaked blotters (filter paper) and incubated 24 hours in freezer and for 7-10 days at 20ºC under 12 hour alternating cycles of Near Ultra Violet (NUV) light and darkness and then fungi developed on each seed were examined under microscope and identified (ISTA, 2012). For complementary identification of the fungi up to species level, agar method, seeds were pre-treated with sodium hypochlorite (NaOCl) solution (1% available chlorine) for 1-5 min and aseptically placed to the Potato dextrose agar (PDA) surface. Plates 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. The pathogen was identified based on macroscopic characteristics (appearance, color and growth type of colony) and microscopic traits (spores, conidia, conidiaphores and mycelium) by referring to ISTA (2012).

Embryo count method

Embryo test was used for loose smut. Samples, each containing 1050 seeds were soaked separately in 1 liter of 5% NaOH at room temperature for 22-24 hours. After soaking, the entire sample was washed through 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 tritici* counted in each groove (ISTA, 2012).

Statistical analysis

Data on seed-borne diseases were not normally distributed, so the NPAR1WAY procedure of SAS (SAS Institute Inc, 2002).was used to perform a nonparametric comparison of means (Kruskal-Wallis test). When the Kruskal-Wallis H test was significant, differences between treatment means were identified using pairwise multiple comparisons.

Results

Identified Fungal Species

Fungal species including *Tilletia laevis, Ustilago tritici, Fusarium graminearum, F.culmorum,F. avenaceaum, F.poa, F.loagsethia, Bipolaris sorokiniana, Alternaria alternatae* were isolated and identified from the seeds of 24 wheat samples.

Table 1. Status of temperature, precipitation and elevation above sea level in studied areas during crop season

province	Town	Maximum temperature	Minimum temperature	Mean temperature	of Precipitation	Elevation above sea level
Tehran	Karaj	22.4	1.3	10.6	174.1	1380
	Hashtgerd&Nazarabad	21.2	1.2	10.4	181.3	1191
	Varamin	19.9	6.7	13.3	148.0	918
	Rey	18.6	6.5	12.5	159.0	1060
Kermanshah	Sarab Niloufar	18.5	1.9	10.1	293	1331
	Mahidasht	18.8	2.0	10.4	344.2	1420
	Kozaran	18.8	1.7	10.2	273.3	1368
	Ravansar	16.8	4.1	10.3	378.2	1362
West Azerbaijan	Orumiyeh	13.3	0.4	6.8	217.5	1328
	Oshnavieh	13.2	0.5	6.9	327.1	1415
	Naghadeh	13.9	2.3	8.0	214	1307
	Miandoab	14.7	1.9	8.2	192.0	1300

Tilletia laevis incidence

A significant difference ($P<0.1\%$) among provinces about infection rate of *T. laevis* was observed. The highest and lowest infection rate of *T.laevis* were140 and 43.5 spores per gram seed in samples of West Azarbayejan and Tehran provinces respectively (Table 3).

Ustilago tritici incidence

In this survey no mycelium of *Ustilago tritici* was detected except for one seed lot in Tehran which had 2 in 1000 seeds (0.02%) and also no significant difference were shown among provinces and seed systems.

Table 2. Chemical application against disease in studied areas.

province	Chemical application	No action
Tehran and alborz	95.8	4.2
Kermanshah	81.3	18.8
West Azarbaijan	87.5	12.5
Informal seed production	80.5	11.8
Formal seed production	95.8	19.5
Average	88.2	4.2

Bipolaris sorokiniana incidence

A meaningful difference ($P<0.1\%$) among the provinces but not between the seed systems was observed (Table 3).

Seed heavily was infected with *B. sorokinana* that may also result in seedling blight and root rot in plants grown from them.

Fusarium spp incidence

A significant difference ($P<0.1\%$) among the provinces but not between the seed systems was observed (Table 3). The average of infection level of Fusarium spp. was 46.54 % in three provinces with a minimum and maximum level in Tehran (67.0%) and West Azarbaijan (21.88%) provinces respectively (Table 3). Varamin in Tehran province by 80% and Naghade in West Azarbaijan by 19% had the highest and lowest infection levels to this agent. Only 16% of the entire sample tested had F. graminearum (infection mean rate 0.37 %) which was observed in Tehran and West Azarbaijan provinces samples (Table 3).

Table 3. The severity of wheat seed-borne fungi diseases in provinces, districts and seed systems tested in 2011-12.

Treatment	*Tilletia Laevis*	*Ustilago tritici*	*Alternaria alternata*	*Bipolaris sorokiniana*	*Fusarium graminearum*	*Fusarium* spp
Provinces and districts						
Tehran	43.50B	0.03	20.00B	13.88A	0.50	67.00A
Karaj	69.0	0.10	25.00	8.50	1.00	63.00
Hashtgerd&Nazarabad	27.0	0.00	20.00	8.00	1.00	67.00
Varamin	31.0	0.00	12.00	22.00	0.00	80.00
Rey	47.0	0.00	23.00	17.00	0.00	58.00
Kermanshah	55.50B	0.00	5.63C	5.13B	0.00	50.75A
Sarab Niloufar	67.0	0.00	7.00	3.50	0.00	25.00
Mahidasht	68.0	0.00	7.00	0.00	0.00	54.50
Kozaran	46.0	0.00	8.00	0.00	0.00	69.00
Ravansar	41.0	0.00	0.00	17.00	0.00	54.00
West Azerbaijan	140.00A	0.00	44.88A	0.50B	0.63	21.88B
Orumiyeh	164.0	0.00	46.50	0.00	1.50	22.50
Oshnaviyeh	92.0	0.0	48.00	0.00	1.00	23.50
Naghadeh	142.0	0.00	46.00	2.00	0.00	19.00
Miandoab	162.0	0.00	39.00	0.00	0.00	22.50
Kruskal-Wallis statistic (*H*)	14.03^{**}	2.00^{ns}	18.72^{**}	11.74^{**}	2.30^{ns}	14.09^{**}
Seed system						
informal	79.50	0.02	23.83	8.33	0.16	47.75
formal	79.83	0.00	23.17	4.67	0.58	45.33
Kruskal-Wallis statistic (*H*)	0.07^{ns}	1.00^{ns}	0.003^{ns}	0.34^{ns}	1.24^{ns}	0.03^{ns}

^{ns} not significant at the 0.05 probability level.

*Significant at the 0.05 probability level.

Significant at the 0.01 probability level.

In this study grains infected by *F. graminearum* as the most pathogenic species was reduced from 54.00 to 66.75 % compared to control.

Means within a column and study factor followed by the same letter are not significantly different according to all pairwise comparisons among the means conducted using a Kruskal-Wallis test at the 0.05 probability level of significance.

Discussion

Based on the results, the frequencies of *T.laevis* teliospores was between 0.2 to 7.3 spores per seed in each wheat seed, which is consider trace amounts of spores that mostly because of chemical application in studied areas as spores on the seed are readily controlled by a range of contact and systemic fungicides. So much attention should be paid for seed treatment in the areas that common bunt infection has been reported. Spores on the seed are readily controlled by a range of contact and systemic fungicides. As teliospores typically are viable for only two years in soil under field conditions but can remain viable for many years on stored seed. So, considerable yield losses can occur due to high inoculum concentrations and disease-conductive conditions. In addition, grain quality is also reduced due to the poor palatability of products made from highly contaminated grain, which causes an off color and odor in the finished product.

No mycelium of *Ustilago tritici* was detected except for one seed lot in Tehran which had 2 in 1000 seeds (0.02%) and also no significant difference were shown among provinces. Since loose smut is a seed borne disease, control measures should be focused on the planted seed. Certified seed fields are inspected for loose smut and strict standards are enforced. Seed from fields with loose smut are rejected. Thus, using certified seed is a highly effective way to avoid loose smut. Organic producers who use farmer saved seed, should never plant seed from a crop infected with loose smut as it is a seed-borne disease and survives inside the seed.

Based on the results significant difference for the infection rate of *Alternaria* species was observed among provinces in a way that the highest (44.8%) and lowest (5.63%) mean infection rate of seed to *Alternaria* spp. was observed in West Azarbaijan and Kermanshah samples respectively. The higher rate of seed contamination with *Alternaria* in West Azarbaijan is a result of raining and lowers temperature at harvest time.

It has reported that *Alternaria alternata*, *Bipolaris sorokiniana* and *Fusarium* spp. by 55.1, 7.1 and 3.1%, respectively, were the predominant fungi isolated from wheat seed loads in Iran and *Cladosporium Rhizopus, Curvularia, Aspergillus, Stemphylium, Penicillium* and *Chaetomium* were of minor importance (Khanzada *et al.*,1980). Pathogenic fungi may reduce germination or increase the incidence of seedling blight. Black point associated with *Alternaria spp.* doesn't have detrimental effect on germination of the affected kernels, but *C. sativus* reduced germination (Fernandez *et al.,* 1994; Huguelet and Kiesling, 1973).

Seed heavily was infected with *B. sorokinana* that may also result in seedling blight and root rot in plants grown from them.

Shriveled wheat seed caused by heavy black point infections under field conditions can result in a decrease in kernel weight. However, positive associations of kernel discoloration with kernel size have been found in sampled wheat, and suggest that the larger kernels are more likely to force open the glumes, which facilitates fungal penetration and infection.

Based on the results the average of infection level of *Fusarium* spp. was 46.54 % in three provinces with a minimum and maximum level in Tehran (67.0%) and West Azarbayejan (21.88%) provinces respectively. Since *Fusarium* is a seed borne, air borne and soil borne disease, so presence of this pathogen in soil of studied areas in Tehran province might is the reason for its higher seed contamination.

It has shown that up to 17 species of *Fusarium* can be readily isolated from grain cereals (Parry *et al.,* 1995). Yield reductions in small grain cereals following naturally occurring epidemics of FHB have been observed in many countries with estimated losses in the order of 15- 50 % (Mc Mullen *et al.,* 1997). More precise data relating to the effects of FHB on wheat and barley yield have been obtained from inoculated trials (Mesterhazy, 1978).

In this study grains infected by F. graminearum as the most pathogenic species was reduced from 54.00 to 66.75 % compared to control.

The most important source of *Fusarium* for wheat crops is the seed but the fungus can also survive on debris in the soil. In seasons where weather conditions are wet during flowering and grain formation, spores are splashed from lower in the canopy causing ear blight and seed-borne infection. In such seasons seed-borne infection can pose a serious threat to crop establishment unless seed is treated to control *Fusarium.*

Healthy seed is an important input for crop production and so reduction of yield loss caused by seed borne fungi is one way to contribute to the food security in the world.

The infection rate of seed lots from the formal seed fields was consistently lower than informal seed fields except for *F. graminearum*. In summary, the health of formal seed was consistently better than informal seed for *Alternaria alternate*, *Bipolaris sorokiniana* and *Fusarium graminearum* that can be related to good management in formal seed production fields.
Seed quality of farmers' saved seed in many cases is not considered as a problem. Farmers' seed production is based on experimentation and experience that farmers acquired over a long period of time. Based on the results the informal seed quality was at least as good as formal seed and no significant differences was observed between two seed systems. This does however not mean that there are no problems with the quality of farmers' seed. Moreover, seed quality does not give any indication about availability and farmers' access to seed.

Seed quality problems can be due to suboptimal seed production, selection and storage Practices. Suboptimal practices and conditions potentially affect all aspects of seed quality; i.e. health, vigor, purity and genetic quality. The availability of inputs for crop production and storage facilities are important limitations for farmers in producing quality seeds. Problems with seed quality may however vary from place to place depend the crop, the environmental conditions and farmers' knowledge.

Some factor(s) related to moisture, temperature, and plant growth or senescence triggers the infection that results in symptom expression. High humidity or rainfall from anthesis to soft dough, high nitrogen fertility, excessive late season irrigation, and lodging usually are associated with high levels of the disease.
Black point can be partially controlled by reducing irrigation frequency after heading and by reducing nitrogen rates, without sacrificing either yield or quality. Because black point can occur at damaging levels in some seasons despite modifications in cultural practices, the best option for control is to combine reduced input practices with black point resistant cultivars. Current cultivars differ in the level of resistance or tolerance to the disease, although there are no completely resistant cultivars available. Black point may occur in dry land and irrigated fields in some years despite implementation of cultural management practices. The best cultural preventive practice for irrigated wheat is reducing irrigation frequency after heading. Our results indicate the incidence and geographical distribution of the seed borne fungi in three important provinces (Tehran, Kermanshah and West Azarbaijan) in Iran. This study will served as a foundation for further studies on determination of economically importance of these fungi and their management in their growing conditions.

Conclusion

Based on the results it is recommended that farmers use formal seed for the next planting season and if they use their own saved seed, it is necessary to implement proper management and good dressing to decrease the incidence of disease. As most of farmers in the studied areas used their own seed, so doing special management as village based seed cleaners, providing the proper chemicals and seed treaters, makes possible the informal seed be able to reduce the rate of seed borne disease in comparing formal seed. So suitability of informal seed using mentioned principles will be resulted in decrement of plant diseases infection and consequently higher seed yield. Also the precision study of seed borne disease in different areas of the country in formal and informal seed production systems in a large scale, for producing healthy seed is recommended.

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