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Seed Borne Mycoflora of Some Commercial Wheat (*Triticum Aestivum*) Cultivars in Sindh And Balochistan

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Abstract: *A total of eighteen wheat varieties each nine variety from both Sindh including Hamal, Imdad, Abadgar, Benazir Moomal, Mehran, T.D-1, Anmol and T.J-83 from ARI Tandojam and Balochistan viz., Sarang-63, Wadahnuk-51, Sariab, Zarghoon-79, NIA-Amber, NIA-Sunari, Zardana-50, Pasban and Sarsabz from ARI Sariab Quetta respectively. The results of our experiment showed that the maximum wheat seed impurities percentage of 35.00, 25.50, 17.50, 11.60 and 10.40% were recorded for healthy, broken, malformed, Seeds debris and sclerotia in Sindh wheat variety Hamal. Minimum wheat seed impurities percentage of 90.5, 5.50, 3.00, 1.00 and 2.20% were recorded for healthy, broken, malformed, Seeds debris and sclerotia in wheat variety T.D-1. Maximum wheat seed impurities by seed test percentage of 25.00, 25.00, 12.50, 12.50 and 25.00% were recorded for healthy, broken, malformed, Seeds debris and sclerotia in Balochistan wheat variety Sarang-63. Minimum wheat seed impurities percentage of 85.5, 5.40, 4.00, 2.20 and 3.00% were recorded for healthy, broken, malformed, Seeds debris and sclerotia in wheat variety Zardana-50. The most dominant seed borne pathogen (Fussarium moniliform) infected the Sindh wheat varieties viz., Anmol (25.10%), Hamal (12.10%), Moomal (12.50%), Benazir (10.20%), Abadgar (4.50%), Mehran (3.50%), T.D-1 (1.00%), Imdad (2.40%) and T.J-83 (2.20%), respectively. Similar trend were recorded for Balochistan wheat varieties viz., Pasban (28.50%), NIA-Sunari (15.40%), NIA-Amber (15.20%), Wadahnuk-51 (12.50%), Sarsabz (8.40%), Sariab (5.20%), Zardana-50 (2.50%), Sarang-63(4.50%) and Zarghoon-79 (3.50%), respectively. The lowest seed infection from the tested wheat cultivars was obtained from the Sindh as compared to Baluchistan. The highly affected variety from the Sindh was Anmol (52%) and Hamal (49%), followed by the Moomal (45%) whereas the medium infection was found in the varieties like, Benazir (35%) followed by Abadgar (34.1%), the lowest seed borne infection was obtained from the highly cultivated varieties of Sindh like, Mehran (25%), Imdad (22.0%), T.J-83 (20%) and T.D-1 (18.3%). Amongst the varieties from Balochistan the most severely affected varieties were Pasban (55%), NIA-Sunari (53.2%), NIA-Amber (49.2%), Wadahnuk-51 (45.8%), Sarsabz (35.2%), Sariab (33.3%), Sarang-63 (22.91%), Zarghoon-70 (30%) and Zardana-50 (22.5%). Among all the isolated fungi from the seed mycoflora of wheat, the most frequently found pathogen was Fusarium monoliform (36%), followed by the Aspergillus niger (19%). Other associated fungi were in low frequency as compared to Aspergillus flavus, Alternaria alternate and Pencillium sp with frequency of 17.8%, 15.2% and 12%, respectively.*

Keywords: Seed borne mycoflora of some commercial wheat in Sind and Balochistan.

1. INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the main cereals, which rank in the first place, both in grounds and in production among the grain yields of the world. It is utilized to feed around 33% of the world populace. Wheat is the principle staple food of the general population of Pakistan and the nation's most imperative vital farming ware. It is developed by 80% of the agriculturists (more than a million), on near 40% of the aggregate developed area and 65% of the food crops area in the nation. It generally contributes 12.5% to the worth added agribusiness and 3.1% to GDP. The per capita wheat utilization of the nation (130 kg a year) is among the most elevated on the planet. Wheat is likewise the most vital single item as a wellspring of salary of the larger part of agriculturists in the rustic areas of Pakistan. Wheat production in money related terms is worth Rs.250 billion, in this way has an incredible importance and effect on the economy of the nation. One percent misfortune or addition in wheat production represents Rs.2.5 billion. Wheat being the staple food of the general masses assumes an essential part in financial improvement of Pakistan. Wheat is an oat yield of massive significance to us and the demand is satisfied at the

expense of soil poisoning and tainting through various pesticides, herbicides and over abundance of composts. Zero culturing has gotten to be mainstream among the dynamic ranchers and serves as a prudent technique to monitor soil dampness, supplements and work power. Mulching then again has advantages of monitoring dampness in the field and others. (Shakil Shaukat 2014).

All inclusive, there is most likely the quantity of individuals who depend on wheat for a generous piece of their eating routine adds up to a few billions. In this way, the nutritious significance of wheat proteins ought not be thought little of, especially in less created nations where bread, noodles and different items (e.g. bulgar, couscous) may give a considerable extent of the eating regimen. Wheat gives almost 55% of sugar and 20% of the food calories. It contains sugar 78.10%, protein 14.70%, fat 2.10%, minerals 2.10% and significant extents of vitamins (thiamine and vitamin-B) and minerals (zinc, iron). Wheat isalso a decent wellspring of follows minerals like selenium and magnesium, supplements crucial to great wellbeing riboflavin and a few minerals, sugars and so on. The horse shelter, which comprises of *pericarptesta* and *aleurone*, is likewise a dietary hotspot for fiber, potassium, phosphorus, magnesium, calcium, and niacin in little amounts (Kumar *et al.* 2011). Straw use has turned out to be progressively critical in the monetary estimation of wheat production. There are numerous employments of wheat straw, for example, deposit for preservation culturing, landscaping, buildup to lessen soil disintegration amid street or building development, mushroom production, horse bedding, roughage feeding and others.(Nathan B. Smith, 2013-14).

Wheat production increased to 25,286 thousand tons in 2014-15, as compared to 24,211 thousand tons in 2013-14, showing an increase of 4.4 percent. According to the latest Pakistan Economic Survey 2014-15 (GOP, 2014), the area under wheat cultivation increased to 9039 thousand hectares in 2014-15 from 8660 thousand hectares showing an increase of 4.4 percent over preceding year area under wheat. The production of wheat stood at 25.286 million tons during 2014-15 against 24.211 million tons last year showing 4.4 percent increase; while the yield per hectare in 2014-15 remained 2797 kg, showing no change almost over the last year yield of 2796 kg per hectare. Pakistan is among top ten wheat producing countries of the world (Khan *et al.* 2002), but the average wheat yield is much lower than other major wheat growing countries including China, USA, India etc. (Arain *et al.* 2005).

This grain is developed on more land area than some other business food. World exchange wheat is more noteworthy than for all different products joined. All around, wheat is the main wellspring of vegetable protein in human food, having a higher protein content than other real cereals, maize (corn) or rice Curtis *et al.* (2002). Wheat grain is a staple food used to make flour for raised, level and steamed breads, scones, treats, cakes, breakfast oat, pasta, noodles, couscous and for maturation to make lager, other mixed drinks, and biofuel. (Neill, 2002).

Wheat plants at all periods of growth are at risk to different injuries and stresses, which intrude with their average working and change. Consistently around 20% of the wheat that for the most part would be open for food and feed is lost in view of diseases (Fakir, 1999). Seed prosperity expect a basic part for productive improvement and yield misuse of a harvest creature bunches. Among various parts that impact seed prosperity, the most indispensable are the seed borne growths that lower seed germination, and in addition decrease seed vigor achieving low yield. Sound seed expect a key part for powerful advancement and also to expand yield of item. Seed-borne pathogens of wheat are careful to achieve assortment in plant morphology and moreover diminishing yield up to 15-90 % if untreated seeds are created in the field (Wiese, 1984). A couple seed-borne pathogens are known not associated with wheat seed which are accountable for deteriorating seed quality in the midst of limit (Kamal and Mughal (1968) and Khan *et al.* (1974); Gill & Tyagi (1970); Khan and Bhutta (1994) and Bhutta and Hussain, (1999); Singh (1983); Martin *et al.* (1984); Ghosh and Nandi (1986); Kunwar (1989); Dharmvir *et al.* (1968) noticed the nearness of a few growths, i.e., *Alternaria, Helminthosporium, Fusarium, Curvularia, Stemphylium, Rhizopus, Cladosporium, Aspergillus, Penecillium, Drechslera sorokiniana, Fusarium moniliforme, Aspergillus spp., Drechslera, Penicillium, Penicilium jenseni,* species in wheat seeds.

A study on the pretreatment with mercuric chloride showed that a number of seed-borne fungi remained the same but their incidence decreased. It indicated that these fungi were internally seed-borne and not surface contaminants Bhutta, (1998). Moreover, Gary *et al.* (1999) expressed that seed which is remotely debased or conveys parasitic or bacterial living beings on the seed coat is said to be swarmed. Seed that is inside sullied is tainted. Seed gets to be pervaded amid the developing season as seed creates and develops on the plant, amid collecting, or while away. Life forms that invade the seed coat are either systemic or non systemic. Systemic creatures lie torpid on the surface of the seed until the seed sprouts, and taint the seedling amid germination. As the seedling builds up, the parasite additionally creates. Shama, (1991) reported that contagious hyphae attack the seed chiefly through in place seed coats. The seriousness of misfortunes incited by plant pathogens is regularly identified with the product growth stage at the season of illness onset and strengthening. Once in a while, when illness starts at a propelled phase of product advancement, crop yield is not impaired (Zadoks, 1985).

Gary *et al*. (1999) gave more explanation of this thought the pathogens might be seed-borne or seed transmitted. A seed-borne pathogen is conveyed in or on the seed. A seed transmitted pathogen is one that is seed-borne and then creates in the seedling after germination. Seed transmitted pathogens might bring about malady in seedlings; they may grow systemically to bring about illness in the grown-up plant. Seed conveys growths, microorganisms, infections and nematodes on the seed coat or inside the seed, some of which bring about plant ailments. Soil, unless disinfected or sanitized, additionally contains growths and different creatures that assault seed and seedlings.

2. REVIEW OF LITERATURE:

Mehboob *et al*. (2015) collected wheat seed samples from different locations of three districts viz Sialkot, Narrowal and Gujranwala, were analyzed in the Seed Pathology laboratory to study the associated mycoflora by using the Standard blotter method (SBM). A total of 14 genera and 22 species of fungi were isolated, among which *Drechslera sorokiniana* was recovered with maximum mean frequency (18.1%), other pathogenic fungi include *D. tetramera* (15.66), *D. teres* (12.5), *Alternaria alternate* (9.75), *A. tritici* (4.33), *A. triticola* (6.41), *Fusarium semitectum* (10.58), *Cercospora spp*. (2.75) *F. solani* (1.08), *F. oxysporum* (1.66), Stemphylium solani (5.66), *S. botryosum* (2.55), *Cladosporium herbarium* (3.41), *Phomaspp* (6.5) and *Sclerotinia sclerotiorum* (3.25). Among five tested fungicides as seed treatment and poisoned food technique, Score® (*Difenoconazole*) and *Topsin-M*® (*Thiophanate* methyl) exhibited the best performance to inhibit the mycelial growth of *D. sorokiniana* followed by *AmistarTop* ® (*Azoxystrobin + Difenoconazole*) while *Halonil* ® (*Chlorothalonil*) and *Curzate-M* ® (*Cymoxanil + Mancozeb*) were least effective.

Rahman *et al*. (2015) selected wheat (*Triticum aestivum* L.) seeds (100 additions) on the reason of various area zones of Pakistan. Division and prominent affirmation of seed considered animals were driven by spreading surface test. An aggregate of five basic seed borne parasites including *Alternaria alternate, Aspergillus niger, Fusarium sp., Drechslera sp.* additionally, sp. were withdrawn from the wheat seeds. The rehash of event of these five seed envisioned parasites *Alternaria alternata, Aspergillus niger, Fusarium sp. Drechslera sp*. in addition, sp. was 49%, 46%, 42%, 35% and 16%, freely. Contamination rate differentiated from 0-90% in every one of the 100 wheat progressions. Among the extensions, most imperative polluting (100%) of seed envisioned improvements was recorded in 011185 and 011757 while scarcest illness (10%) was recorded in 011415 headways. Moreover, in increases amassed from Gilgit Baltistan and AJK, *Alternaria niger* and *Alternaria fusarium* were common, while in Kpk region, *Aspergillous niger* was fundamental trailed by *Alternaria alternata.*

Neha *et al.* (2014) studied to evaluate the infection and identification of different fungal genera associated with storage wheat varieties. Total loss due to seed-borne diseases is up to an extent of 30-75%. Disease free quality seed production in wheat is utmost important to sustain the production and to maintain the quality crop. Keeping this in perspective, point by point examination were done to concentrate on the ramifications of seed-borne organisms of wheat on seed quality parameter and to discover reasonable discovery strategies for seed-borne disease of the three location techniques embraced for vaccination of seed mycoflora in wheat assortments. Wheat varieties grown during the months of October to March 2011, collected from Quarsi agricultural farm, Aligarh were screened by using Blotter method, Agar plate method and Deep freeze method as recommended by ISTA. All experiments were in a completely randomized design (CRD) in three replicates. Out of the five varieties tested, PBW343 was found to be most susceptible and was associated with more fungal flora than the other varieties. Seed mycoflora of abnormal seeds was also studied. Maximum incidence of fungi was observed in case of discolored seeds followed by shrunken seeds and cracked seeds. In all the three discovery strategies, an aggregate no of 11 genera and 20 types of organisms were disengaged and distinguished.

Zakaria *et al.* (2014) tested grain samples of 14 Egyptian wheat cultivars for seed-borne fungi. The deep freezing method was used. Five seed-borne fungi viz., *A. flavus, A. niger, Curvularialunata, F. moniliforme and Penicillium chrysog*enum were isolated from the wheat cultivars viz., BaniSuef 4, BaniSuef 5, Gemmiza 7, Gemmiza 9, Gemmiza 10, Giza 168, Misr 1, Misr 2, Sakha 93, Sakha 94, Shandaweel 1, Sids 1, Sids 2 and Sids 3. *A. flavus, A. niger* and *F. moniliforme* were the most prevalent fungal species. Their incidence ranged from 21.0-53.5%, 16.0-37.5%, and 12.0-31.0%, respectively.

Bashir *et al.* (2012) investigated three varieties each of adjacent and improved wheat (*Triticum aestivum*) cultivars for seed-borne pathogenic mycoflora utilizing the plate structure and laid on totally randomized design. An aggregate 99 parasitic limit amassed into five irresistible species to be specific; *Rhizopus nigricans, Mucor spp, Penillium jenseni, Aspergillus niger,* and *Fusarium moloniformes* were pulled back with *Rhizopus nigricans*, and *Fusarium moloniformes* (30.30 % and 35.40%, autonomously) being the most as much of the time as could be permitted happening parasitic species and *penicillium jenseni* (7.0 %) being the base bounteous. Aftereffects of the study have additionally displayed that near to wheat cultivars were more debased than the enhanced plans. It is in this way

clear both enhanced and neighboring wheat groupings are spoiled by parasitic mycoflora.

Muzammil *et al.* (2011) investigated seed borne mycoflora associated with ten business groupings of wheat viz. Blue silver, Faisalabad 85, Manthar-3, Pak81, Parwaz 94, Pirsabaq 2005, Punjnad-1, Sariab-92, Sh-2002 and Wafaq-2001 through standard smudging surface paper and agar plate method by using Mann-Whitney U test. No under eleven parasitic genera were recovered from seeds. The most routinely separated growths were *Bipolaris sorokiniana* (11.125%), *Aspergillus flavus* (9.825%), *Alternaria substitute* (7.15%) and *Aspergillus niger* (6.225%). It is clear from the present examination that all business wheat groupings attempted were sullied by life forms. The moved paper procedure was used to find the effect of seed borne parasites on seed germination. Seeds of Pak 81, Wafaq-2001 and Blue silver were created in high degree with variable number of ordinary and bizarre seedlings than the seeds of various varieties attempted. The parasites associated with seeds of wheat cause basic ailments in wheat lessening as far as possible.

Singh *et al.* (2011) studied to identify the parasitic species and their effect on germination associated with wheat seeds. Seeds of two cultivars viz., Kundan and HUW-234 of wheat (*Triticum aestivum* L.) were accumulated in the wake of gathering from agriculture farm, Banaras Hindu University, Varanasi. These seeds were treated with potassium nitrate and dissect for seed mycoflora by agar plate procedure and smearing surface strategy. Complete sixteen infectious species were isolated from test cultivars by the standard strategies. Life forms separated and perceived were *Alternaria alternata, Alternaria solani, Aspergillus candidus, Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Aspergillus terreus, Curvularia lunata, Fusarium roseum, Fusarium semitectum, Penicillium citrinum, Penicillium rubrum, Rhizopus stolonifer*, *Trichoderma harzianum*, Dark sterile mycelium and White sterile mycelium. In the midst of detachment, the smearing surface method yielded the higher number of life forms when diverged from agar plate technique. Germination of swarmed wheat seeds was controlled by three methodologies viz., smudging surface system, multi-pot plate procedure and plastic pot strategy. Germination was decreased in the midst of limit period in light of the fact that new seeds demonstrated better germination percent i.e., from 95 to 100% than set away seeds. Nitrate treated seeds show favored germination percent over untreated seeds of both cultivars.

Rajput *et al.* (2005) tried one hundred twenty wheat seed tests assembled from Sindh wheat creating regions for infectious seed-borne pathogens by using the standard blotching surface system. Five seed borne parasites viz., *Alternaria tenuis, Aspergillus niger, Fusarium moniliforme, Curvuluri alunata and Stemphylium herhurum* were limited from 12 wheat groupings viz., Mehran, T.J-83, Soghat, Sarsabz, Anmol, Johar, C-591, Sindh-81, Pak-70, Mexipak-65, H-68 and Faisalabad-85 separately. *Alternaria tenuis* was force with a sullying range from 22.5-47.5%. Most noteworthy seed germination was seen in Anmol and slightest in Pak-70. Most compelling root and shoot length of seedlings was recorded in Anmol and Sarsabz took after by H-68 and minimum in Pak-70, Mehran-89, Soghat and Johar.

3. MATERIALS AND METHODS

Sample Collections

During 2014 and 2015, a total of 18 samples (200 seeds each varieties) of wheat from active silos in Sindh and Balochistan province, including Mehran,T.J83, Anmol, Mamool, Abadgar, Benazir, T.D-1, Hamal and Imdad, from ARI Tandojam and Sarang-63, Wadahnuk-51, Sariab, Zarghoon-79, Sarabaz-60, NIA-amber, NIA-sunari, Zardana-50 and Pasban, collected from ARI Sariab Quetta. Samples were submitted to the mycology laboratory at the A.R.I Tandojam, by the aim of assessing any possible fungal contaminations, aflatoxin determination, and fungal identification. The samples were stored at 4°C until the beginning of laboratory analysis.

Isolation and identification of the fungus

Isolation was done from 200 seeds of each variety under aseptic conditions by standard blotter method. After 6-7 days incubation, the fungi associated with seeds were identified on the basis of their typical colony characteristics and conidial morphology (Sejiny *et al.,* 1984).Germination studies were conducted by taking 200 seeds per variety, surface sterilized with 0.01% mercuric chloride and plating them in sterilized Petri dishes with three layers of blotter papers moistened with sterilized water. The Petri dishes will be kept for 7 days at 25±1ºC.

The frequency of the most pre dominant fungi was calculated according to the formula.

$$\text{Colonization (\%)} = \frac{\text{Number of pieces colonized by pathogen}}{\text{Total number of colonies}} \times 100$$

Selection and multiplication

After identification of fungi associated with wheat seed most predominant and ruinous fungus i.e (*Alternaria spp, Aspergillus niger, Fusarium spp, Aspergillus flavus and Penicillium spp*) were multiplied and maintained for future use. Moreover, colony growth response of fungus was also observed against different nutrient media like, PDA.

Disease Severity

Percentage of plant that are disease is known disease severity. Percent estimates of disease severity were converted by angular transformation before statistical analysis (Little & Hills 1978). To calculate disease severity and number of healthy leaves per plant:

DS = å %TLB / Lvs HLvs = (100% - DS / 100) x Lvs

where, DS = disease severity (percentage)

%TLB = estimated percentage taro leaf blight per leaf

Lvs = number of leaves per plant

HLvs = effective number of healthy leaves per plant

4. RESULTS

Survey and sampling of different commercial wheat cultivars

Survey of different commercial cultivars of wheat was done from different localities of Sindh and Balochistan during the year 2015, in order to find out the seed borne mycoflora of wheat. During the survey the different commercial wheat varieties were observed from the both of the provinces visited. The varieties from Sindh were, Mehran,T.J-83, Anmol, Moomal, Abadgar, Benazir, T.D-1, Hamal and Imdad, from ARI Tandojam whereas the cultivars observed from Balochistan provinces were, Sarang-63, Wadahnuk-51, Sariab, Zarghoon-79, Sarsabaz-60, NIA-amber, NIA-sunari, Zardana-50 and Pasban, collected from ARI Sariab Quetta. All the samples taken were brought to the Plant Pathology Laboratory Department of Plant Pathology, Faculty of Crop Protection, Sindh Agriculture University Tandojam for isolation and identification of the seed borne mycoflora associated with the seed of wheat. The results of our survey showed that almost every wheat variety was affected by severe seed borne infection.

Percentage of impurities revealed from wheat seed varieties of Sindh (seed dry test) n=100

Seed impurities of Sindh wheat varieties viz., Hamal, Imdad, Abadgar, Benazir, Moomal, Mehran, T.D-1, Anmol and T.J-83 were observed and the data (Table-1) indicated that healthy wheat seed percentage of 35.00, 40.00, 45.00, 55.8, 60.10, 60.50, 90.5, 74.00, and 85.00%;, broken percentage of 25.50, 20.00, 15.00, 10.40, 20.60, 17.40, 5.50, 15.10, and 7.80%; malformed percentage of 17.50, 18.60, 15.00, 13.10, 8.40, 6.80, 3.00,6.30 and 3.90%; seed debris percentage of 11.60, 12.00, 15.20, 15.60, 4.50, 6.80, 1.00, 2.40 and 2.20%; sclerotia percentage of 10.40, 9.40, 9.80, 5.10, 6.40, 8.50, 0.00, 2.20 and 1.10% for varieties Hamal, Imdad, Abadgar, Benazir, Moomal, Mehran, T.D-1, Anmol and T.J-83, respectively. Maximum wheat seed impurities percentage were observed in wheat variety Hamal and minimum wheat seed impurities percentage were recorded for wheat variety T.D-1. One-way (ANOVA) indicates non-significant difference in seed impurities among all wheat varieties.

Table-1 Percentage of impurities revealed from wheat seed varieties of Sindh seed dry test) n=100

Varieties	Seed impurity				
	Healthy	Broken	Malformed	Seed Debris	Sclerotia
Hamal	35.00	25.50	17.50	11.60	10.40
Imdad	40.00	20.00	18.60	12.00	9.40
Abadgar	45.00	15.00	15.00	15.20	9.80
Benazir	55.8	10.40	13.10	15.60	5.10
Moomal	60.10	20.60	8.40	4.50	6.40
Mehran	60.50	17.40	6.80	6.80	8.50
T.D-1	90.5	5.50	3.00	1.00	0.00
Anmol	74	15.10	6.30	2.40	2.20
T.J-83	85	7.80	3.90	2.20	1.10

SE± = 1.3922

LSD @ 0.05 = 58

P-value = 0.2532

Percentage of impurities revealed from wheat seed varieties of Balochistan (seed dry test) n=100

Seed impurities of Balochistan wheat varieties viz., Sarang-63, Wadahnuk-51, Sariab, Zarghoon-79, NIA-Amber, NIA-Sunari, Zardana-50, Pasban and Sarsabz were observed and the data (Table-2) indicated that healthy wheat seed percentage of 25.00, 33.10, 44.50, 55.8, 60.10, 65.50, 85.5, 75.00, and 80.00%; broken percentage of 25.00, 20.60, 18.70, 15.50, 10.40, 5.20, 5.40, 10.40 and 8.00%; malformed percentage of 12.50, 17.50, 12.50, 13.10, 10.10, 10.60, 4.00, 5.10 and 6.50%; seed debris percentage of 12.50, 15.40, 9.30, 11.50, 10.00, 8.60, 2.20, 4.50 and 3.10%; sclerotia percentage of 25.00, 13.40, 15.00, 4.10, 9.40, 11.10, 3.00, 5.00 and 2.40% for varieties Sarang-63, Wadahnuk-51, Sariab, Zarghoon-79, NIA-Amber, NIA-Sunari, Zardana-50, Pasban and Sarsabz, respectively. Maximum wheat seed impurities percentage were observed in wheat variety Sarang-63 and minimum wheat seed impurities percentage were recorded for wheat variety Zardana-50. One-way (ANOVA) indicates non-significant difference in seed impurities among all wheat varieties.

Table-2 Percentage of impurities revealed from wheat seed varieties of Balochistan seed dry test (n=100)

Varieties	Seed impurity				
	Healthy	Broken	Malformed	Seed Debris	Sclerotia
Sarang-63	25.00	25.00	12.50	12.50	25.00
Wadahnuk-51	33.10	20.60	17.50	15.40	13.40
Sariab	44.50	18.70	12.50	9.30	15.00
Zarghoon-79	55.8	15.50	13.10	11.50	4.10
NIA-Amber	60.10	10.40	10.10	10.00	9.40
NIA-Sunari	65.50	5.20	10.60	8.60	11.10
Zardana-50	85.5	5.40	4.00	2.20	3.00
Pasban	75.00	10.40	5.10	4.50	5.00
Sarsabz	80.00	8.00	6.50	3.10	2.40

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SE± = 1.5876
LSD @ 0.05 = 3.2339
P-value = 0.0655

Seed borne pathogens in seeds of nine wheat varieties of Sindh using standard blotter test

The data (Table-3) showed that the varieties Anmol, Hamal, Moomal, Benazir, Abadgar, Mehran, T.D-1, Imdad and T.J-83 were infected against seed borne pathogens viz., *Fusarium moniliform*, with infection percentage of 25.10, 12.10, 12.50, 10.20, 4.50, 3.50, 1.00, 2.40 and 2.20%; *Asperillus niger* with infection percentage of 12.60, 08.50, 7.40, 05.10, 3.40, 2.40, 1.20, 2.80 and 2.00%; *Aspergillus flavus* with infection percentage of 13.50, 10.10, 5.20, 06.40, 5.40, 5.10, 2.50, 4.10 and 3.30%: *Alternaria alternate* with infection percentage of 09.40, 05.40, 03.20, 3.50, 2.10, 1.10, 00, 1.80 and 1.00%; *Pencillium sp* with infection percentage of 05.20, 3.20, 1.50, 1.20, 1.20, 1.50, 00, 1.60, and 1.00%, respectively. One-way (ANOVA) indicates significant difference in seed borne pathogen among all wheat varieties.

It was observed that most frequently isolated fungi from wheat varieties is *Fusarium moniliform.* Where as the less frequently isolated fungi was *Pencillium spp* in all nine wheat varieties of Sindh. Furthermore, the wheat variety Anmol is most prominent with higher infection percentage against *Fusarium moniliform*. Minimum infection rate was determined for wheat variety T.D-1 for all seed borne pathogens.

Table-3: Percentage of seed borne pathogens in seeds of nine wheat varieties of Sindh using standard blotter test (n=100)

Seed borne pathogens	Varieties								
	Anmol	*Hamal*	*Moomal*	*Benazir*	*Abadgar*	*Mehran*	*T.D-1*	*Imdad*	*T.J-83*
Fusarium moniliform	25.10	12.10	12.50	10.20	4.50	3.50	1.00	2.40	2.20
Aspergillus niger	12.60	08.50	7.40	05.10	3.40	2.40	1.20	2.80	2.00
Aspergillus flavus	13.50	10.10	5.20	06.40	5.40	5.10	2.50	4.10	3.30
Alternaria alternate	09.40	05.40	03.20	3.50	2.10	1.10	00	1.80	1.00
Pencillium sp	05.20	3.20	1.50	1.20	1.20	1.50	00	1.60	1.00

SE± = 2.1705
LSD @ 0.05 = 4.4020
P-value = 0.0001

Seed borne pathogens in seeds of nine wheat varieties of Balochistan using standard blotter test

Table-4: Percentage of seed borne pathogens in seeds of nine wheat varieties of Balochistan using standard blotter test (n=100)

Seed borne pathogens	Varieties								
	Pasban	NIA-Sunari	NIA-Amber	Wadahnuk-51	Sarsabz	Sariab	Zardana-50	Sarang-63	Zarghoon-79
Aspergillus niger	28.50	15.40	15.20	12.50	8.40	5.20	2.50	4.50	3.50
Fusarium moniliform	15.20	12.30	10.20	08.40	5.50	4.30	2.40	4.80	3.20
Pencillium sp.	15.40	14.50	8.40	10.80	7.60	8.40	3.10	5.40	4.60
Aspergillusflavus	14.20	10.20	05.50	7.20	4.50	4.10	1.50	2.80	2.00
Alterneria alternate	10.00	8.40	4.10	3.50	2.40	2.10	00	2.00	1.80

The data (Table-4) showed that the varieties Pasban, NIA-Sunari, NIA-Amber, Wadahnuk-51, Sarsabz, Sariab, Zardana-50, Sarang-63, Zarghoon-79 were infected against seed borne pathogens viz., *Asperillus niger* with infection percentage of 28.50, 15.40, 15.20, 12.50, 8.40, 5.20, 2.50, 4.50 and 3.50%; *Fusarium moniliform* with infection percentage of 15.20, 12.30, 10.20, 08.40, 5.50, 4.30, 2.40, 4.80 and 3.20%; *Pencillium sp.* with infection

percentage of 15.40, 14.50, 8.40, 10.80, 7.60, 8.40, 3.10, 5.40 and 4.60%: *Aspergillus flavus* with infection percentage of 14.20, 10.20, 05.50, 7.20, 4.50, 4.10, 1.50, 2.80 and 2.00%; *Alternaria alternate*. with infection percentage of 10.00, 8.40, 4.10, 3.50, 2.40, 2.10, 00, 2.00, and 1.80%, respectively. One-way (ANOVA) indicates significant difference in seed borne pathogen among all wheat varieties.

It was observed that most frequently isolated fungi fromwheat varieties is *Aspergillus niger.* Where as the less frequently isolated fungi was *Alterneria alternate* in all eight wheat varieties of Balochistan. Furthermore, the wheat variety Pasbanis most prominent with higher infection percentage against *Aspergillus niger*. Minimum infection rate was determined for wheat variety Zardana-50 for all seed borne pathogens. **Table-4**

Percentage of seed borne pathogens in seeds of nine wheat varieties of Balochistan using standard blotter test (n=100)

SE±	= 2.1500
LSD @ 0.05	= 4.3604
P-value	= 0.0000

Severity of seed mycoflora isolated from different wheat seed varieties of Sindh and Balochistan

Among the visited wheat varieties from Sindh, the highly affected varieties were Anmol (52%) and Hamal (49%), followed by the Moomal (45%) whereas the medium infection was found in the varieties like, Benazir (35%) followed by Abadgar (34.1%), the lowest seed borne infection was obtained from the highly cultivated varieties of Sindh like, Mehran (25%), T.D-1 (18.3%), Imdad (22%) and T.J-83 (20%). Amongest the varieties from Balochistan the most severely affected varieties were Pasban (55%), NIA-Sunari (53.2%), NIA-Amber (49.2%), Wadahanuk-51 (45.8%), Sarsabz (35.2%), Sariab (33.3%), Saraang-63 (22.91%), Zarghoon-79 (30%) and Zardana-50 (22.5%) Figur-1 & 2.

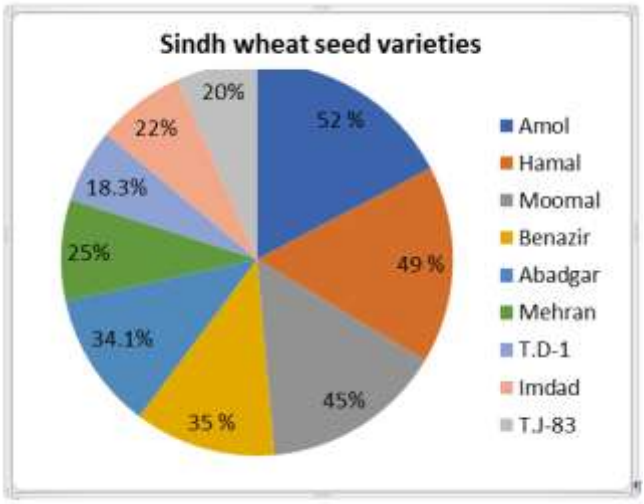

Figure-1: Severity of seed mycoflora isolated from different wheat seed varieties of Sindh (n=200 seeds)

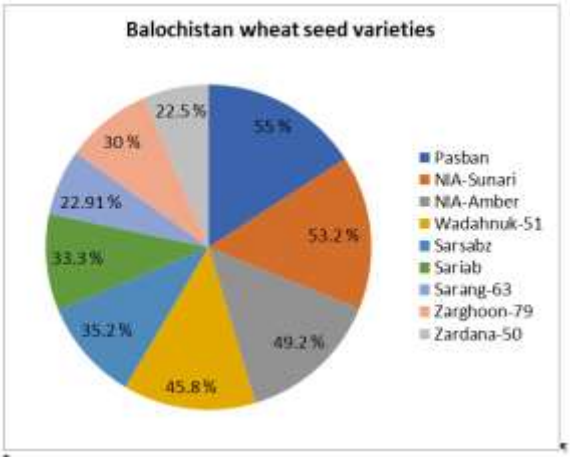

Figure-2:Severity of seed mycoflora isolated from different wheat seed varieties of Balochistan (n=200 seeds)

Isolation and identification

The isolation and identification process reveals the association of more than one fungi from the seeds of the tested wheat varieties. A number of the pathogens were found to be associated with the seeds of the tested wheat varieties like, *Alternaria alternate spp, Aspergillus niger, Fusarium moniliform, Aspergillus flavus and Penicillium spp*). All the isolated fungi were identified through the morphological and colony characteristics of their colonies with the help of the keys of the identification by (Sejiny *et al.*, 1984). Among all the most predominant fungi were*Fusarium moniliform, Aspergillus niger* and *Aletrnaria alternate.*

Frequency of the most dominant fungi

Among five isolated fungi from the seed mycoflora of wheat seed, the most dominant pathogen i.e. *Fusarium moniliform* followed by *Aspergillus niger, Aspergillus flavus, Alternaria alternate* and *Pencillium sp.* with average frequency percentage of 36, 19, 17.8, 15.2 and 12%, respectively (Figure-3).

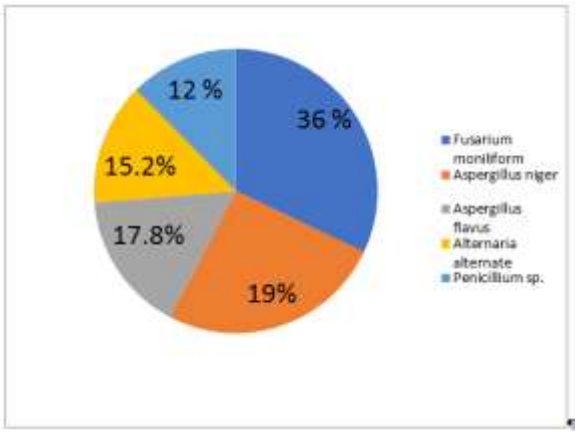

Figure-3: Frequency of different fungi associated with seed mycoflora of wheat varieties from Sindh and Balochistan

Conclusions

In conclusion, from the present investigation, it is clear that seed-borne fungi are a threat to the health of wheat grains. Due attention should be paid to the health status of wheat grains prior to sowing.

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