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Study on Prevalence of Mycoflora in Wheat Seeds

Pratishtha Adhikari[1], Gopal Bahadur Khatri-Chhetri[1], Sundar Man Shrestha[1], Santosh Marahatta[2]

[1]*Institute of Agriculture and Animal Sciences, Tribhuvan University, Nepal*
[2]*Agriculture and Forestry University, Nepal*

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* Corresponding Author:
E-mail: adhikari_pk@yahoo.com

ABSTRACT

Forty seed sample of wheat (*Triticum aestivum*) were collected from four locations viz. Chitwan, Kaski, Banke and Lalitpur and tested by blotter method at laboratory during 2013 for determining fungal pathogens associated with wheat seeds in Nepal. Eighteen species representing thirteen genera of fungi were recovered from the seed. *Alternaria alternata* and *Bipolaris sorokiniana* were predominant in all the varieties/genotypes from all the locations, where *B. sorokiniana* was strongly pathogenic in wheat crop. Percentage frequency and type of fungi detected varied with variety and locations. *Bipolaris sorokiniana* was highest (64.40%) in Banke than remaining three locations. Seeds of Chitwan had lowest percentage (5.50%) of seed infection as compared to other locations. Relative abundance of *Alternaria alternata* (55.10%) was highest as it was the most prevalent component of seed borne mycoflora, followed by *Bipolaris sorokiniana* (34.69%) and *Cladosporium herbarum* (7.19%). Differences in quantity of precipitation and relative humidity might be the possible reason for variation in frequency and type of fungi detected in wheat seeds of four locations.

Introduction

Wheat is third most important cereal crop in Nepal in terms of area (760,000.0 ha) and production (1,882,000.0 mt) with productivity of 2.47 ton per hectare (MOAD, 2013). It is the major winter cereal grown from terai (plain area) to hills, and share of terai to the total area and production of nation is 59.3% and 69.3%, respectively (Khanal et al., 2012). Annual average increase in wheat production is 10%, however, in 2012/13; a marginal increment of 2% was recorded. Seed is one of the important inputs for cultivation, as it determines potential production and productivity of the crops (Friis-Hansen, 1995).

There are several factors limiting wheat yield. Among them diseases incidence and their poor management is one of the important factors in Nepal (Rosyara, 2002). Seed may be passive carrier of pathogens. Seeds infected by pathogens in the field may survive and become sources of primary inoculum in the next generation. They may cause seed abortion, seed rot, seed necrosis, reduction or elimination of germination as well as seedling damage resulting in development of disease at later stages of plant growth by systemic or local infections (Khanzada et al., 2002; Bateman and Kwasna, 1999). Some common seed borne fungi isolated from wheat seeds were *Absidia* sp., *Alternaria alternata*, *Aspergillus* sp., *A. candidus*, *A. flavus*, *A. niger*, *A.sulphureus*, *Cephalosprium* sp., *Chaetomium globosum*, *Cladosporium herbarum*, *Curvularia lunata*, *Drechslera halodes*, *D. hawaiiensis*, *D. tetramera*, *Fusarium moniliforme*, *F. oxysporum*, *F. pallidoroseum*, *F. subglutinans*, *Penicillium* spp., *Rhizoctonia solani* and *Rhizopus* sp. (Fakhrunnisa and Ghaffar, 2006). These pathogens also affect grain quality and human health. Barabara et al. (2004) reported quality and nutritional composition of wheat reduced by fungal infection. Glutein content in fusarious wheat was lower in comparison to healthy wheat seeds (Dexter et al., 1996).

Pathogen free seeds are vital to have desired germination, emergence, healthy seedlings and plant population. Early identification of seed borne pathogens is important for timely management of diseases and to avoid epidemics. The objective of seed health testing is to identify the healthy and pure seeds that can be sown in the field, which ultimately results in production of healthy food, healthy seed crops, and improved yields in terms of quality and quantity. Early identification of seed borne pathogens allows timely management of diseases and helps to avoid epidemics (Nafula, 1997). It is also essential to carry out seed health testing to check the spread of many seed borne diseases to new areas. The unrestricted movement and exchange of germplasm are vital for the process in crop improvement programs, but the movement of germplasm may result also in spread of diseases (Warham et al., 1996). The present study was carried out to identify fungi prevailing in wheat seeds used commonly in Nepal.

Materials and Methods

The study was carried out in mycology laboratory of Seed Quality Control Centre, Hariharbhawan, Lalitpur, Nepal, during August to December, 2013. 200 grams each of 40 sample of wheat were collected from 4 research institutes (10 varieties/genotypes each from Lalitpur, Chitwan, Kaski and Banke) of Nepal (Table 1) for the study. Isolation and identification of seed borne fungi were done by blotter method described by International Seed Testing Association (Mathur and Kongsdal, 2003). Plastic petri-dishes were cleaned by washing with detergent solution and rinsing with tap water, and finally sterilized by just dipping in 4% NaOCl. Three layers of blotting paper were placed in the labeled, plastic petri-dishes and moistened with distilled water.

Twenty five seeds per petri-dish were placed in equidistance, fifteen seeds in outer ring, nine in middle ring and one at the center. One hundred seeds formed one replication and four replications were maintained per variety/genotype. The petri-dishes were incubated at 20ºC under alternate cycles of 12 hours near ultra violet light and darkness. Five, seven and nine days after incubation, the seeds were observed under stereo-binocular microscope for presence/absence of fungi. A binocular, compound microscope was used for identification of fungi. Identification was done based on morphology of spores and mycelia as described by Mathur and Kongsdal (2003).

Percentage frequency (PF) and relative abundance (RA) of fungi were calculated by using the following formula (Naqvi et al., 2013):

$$PF=\frac{\text{No. of seeds on which fungus appears}}{\text{Total number of seeds}} X\ 100$$

$$RA=\frac{\text{No.of a particular fungi}}{\text{Total no.of all fungi}} X\ 100$$

Table 1 List of wheat varieties/genotypes collected from four locations.

S.No.	Lalitpur	Chitwan	Kaski	Banke
1	Bijaya: variety	BL 4009: genotype	Tribeni: variety	NL 1171: genotype
2	RR 21: variety	NL 1191: genotype	BL 4061: genotype	NL 1177: genotype
3	Pasang Lhamu: variety	BL 4461: genotype	Nepal 297: variety	NL 1093: genotype
4	Annapurna 1: variety	Bhrikuti variety	Annapurna 2: variety	BL 4361: genotype
5	Annapurna 4: variety	Bijaya: variety	Lumbini: variety	BL 4350: genotype
6	Achyut: variety	Aditya: variety	Annapurna 1: variety	BL 4341: genotype
7	Nepal 297: variety	NL 1097: genotype	NL 1078: genotype	NL 1164: genotype
8	Bhrikuti: variety	Gautam: variety	Achyut: variety	Bhrikuti: variety
9	Gautam: variety	BL 3599: genotype	Gautam: variety	BL 3978: genotype
10	WK 1204: variety	NL 1094; genotype	Kanti: variety	BL 4347: genotype

Results and Discussion

The study detected a total of thirteen genera and eighteen species of fungi in seeds of forty wheat seed samples. Percentage frequency of *Alternaria alternata* was highest, followed by *Bipolaris sorokiniana* and *Cladosporium herbarum* on seeds of four locations. *Alternaria alternata* and *Bipolaris sorokiniana* were predominant among all the fungi detected in wheat seeds. Clear and Patrick'Can (1993) reported 35 fungal genera with 59 species from wheat grain samples with the important genera *Alternaria*, *Bipolaris sorokiniana*, *Fusarium graminearum*, *Aspergillus*, *Cladosporium*, *Epicoccum*, *Nigrospora* and *Septoria nodorum.*

Presence of fungi in seeds varied with varieties/genotypes and location. Lowest percentage frequency was found in the genotype BL 4009 (3.06%) from Chitwan and highest in BL 1177 (16.40%) from Banke (Table 2, 4). Among varieties, minimum percentage frequency appeared in Nepal 297 (6.25%) from Kaski and maximum in Gautam (13.25%) from Lalitpur (Table 3, 5).

Several fungi appeared to be associated with wheat seeds used in various locations. Frequency of *Bipolaris sorokiniana* appeared lowest in Lalitpur (16.68%), followed by Chitwan (18.93%), Kaski (24.57%) and Banke (64.40%), while relative abundance was minimum in Lalitpur (13.32%), accompanied by Kaski (17.20%), Chitwan (32.40%) and Banke (34.69%), with a mean frequency and relative abundance of 29.83% and 24.40%, respectively (Table 6). The result showed that the seeds from Banke and Chitwan were highly infected with *B. sorokiniana*, but the seeds also from other locations were not safe with regard to the pathogen. Higher incidence of *B. sorokiniana* would be due to higher mean temperature and relative humidity in Banke (20.61°C and 93.30%) and Chitwan (16.32°C and 93.97%) than in Lalitpur (14.54°C and 72.44%, respectively). Temperature in Kaski was 14.30°C, but relative humidity was not available. Saari (1998) reported that high temperature with high relative humidity at the growing period of wheat results in the spot blotch disease. Alam and Saha (1991) mentioned that infection of seed depends upon the prolonged wet weather just before the harvest or high relative humidity with frequent rains at grain filling period. Pickett and Pruitt (2010) reported that continuous cultivation of the same kind of crop leads to build up of pathogens.

Table 2 Percentage frequency of fungi in seeds of 10 wheat varieties/genotypes from Chitwan

V/G	AA	BS	RS	AF	BiS	CL	PS	EN	AN	FM	CH	FG	Mean
BL 4009	13.75	10.50	0.00	6.75	2.25	1.50	0.00	0.00	1.50	0.50	0.00	0.00	3.06
BL 3599	19.25	13.75	7.00	5.00	0.50	1.00	0.00	0.00	0.00	0.00	0.00	0.00	3.88
BL 4461	13.75	19.00	6.25	5.25	4.50	2.50	0.00	0.00	0.00	0.25	0.00[b]	0.00	4.29
NL 1097	17.00	27.00	10.25	8.50	2.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.40
NL 1094	29.75	9.25	6.50	7.00	7.25	2.25	0.00	3.75	0.75	0.00	0.00	0.00	5.54
Bijaya	24.00	17.75	4.25	7.50	4.50	1.25	6.75	0.50	0.00	0.00	0.00	0.25	5.56
NL 1191	44.25	20.00	3.00	0.00	8.25	0.00	0.00	1.50	0.00	0.00	0.00	0.00	6.42
Bhrikuti	26.00	29.75	11.25	9.75	2.25	1.00	0.00	0.00	0.00	0.00	0.75	0.00	6.73
Gautam	33.25	21.75	9.50	8.00	4.75	2.25	0.00	0.00	1.25	0.25	0.00	0.00	6.75
Aditya	40.25	20.50	8.50	4.00	8.75	3.00	0.00	0.75	2.50	0.00	0.00	0.00	7.35

V/G: *Varieties/genotypes,* AA: *Alternaria alternata,* BS: *Bipolaris sorokiniana,* RS: *Rhizopus spp.,* AF: *Aspergillus flavus,* BiS: *Bipolaris spicifera,* CL: *Curvularia lunata,* PS: *Penicillium* spp., EN: *Epicoccum nigrum,* AN: *Aspergillus niger,* FM: *Fusarium moniliforme,* CH: *Cladosporium herbarum,* FG: *Fusarium graminearum*

Table 3 Percentage frequency of fungi in seeds of 10 wheat varieties/genotypes from Kaski

V/G	AA	TS	BS	CH	EN	FM	AF	FS	FG	AS	AL	CL	Mean
Nepal 297	48.50	8.00	15.75	0.75	1.50	0.00	0.00	0.00	0.25	0.25	0.00	0.00	6.25
Annapurna2	69.00	19.00	16.00	5.25	0.00	1.50	0.00	0.00	0.00	0.00	0.00	0.00	9.23
Lumbini	71.50	8.25	15.25	7.50	2.25	6.00	0.00	0.00	1.50	0.25	0.00	0.00	9.36
Achyut	74.25	14.50	13.00	11.25	2.25	0.00	0.00	3.50	0.00	0.00	0.00	0.00	9.90
Tribeni	79.75	20.50	16.25	16.75	3.00	0.00	6.75	0.00	0.00	1.50	0.00	0.00	12.04
Kanti	74.25	35.75	24.25	17.75	2.25	0.00	0.00	0.00	2.75	0.00	0.00	0.00	13.08
Annapurna1	73.75	43.50	28.00	13.25	2.25	1.00	0.00	0.00	0.00	0.00	0.00	0.00	13.48
BL 4061	73.75	55.50	31.50	0.00	1.50	0.00	0.00	0.00	0.75	0.25	0.25	0.00	13.63
Gautam	74.00	45.25	30.75	11.50	2.50	0.00	0.00	0.75	0.00	1.25	0.00	0.25	13.85
NL 1078	75.75	22.25	55.00	14.25	3.00	0.50	0.00	2.00	0.00	0.00	0.00	0.00	14.40

V/G: *Varieties/genotypes,* AA: *Alternaria alternata,* TS: *Trichothecium* sp., BS: *Bipolaris sorokiniana,* CH: *Cladosporium herbarum,* EN: *Epicoccum nigrum,* FM: *Fusarium moniliforme,* AF: *Aspergillus flavus,* FS: *Fusarium semitectum,* FG: *Fusarium graminearum,* AS: *Acremonium strictum,* AL: *Alternaria longissima,* CL: *Curvularia lunata*

Table 4 Percentage frequency of fungi in seeds of 10 wheat varieties/genotypes from Banke

V/G	AA	BS	CH	EN	FM	BiS	CL	FS	SB	AS	US	AL	FG	Mean
BL 4341	86.25	52.50	8.75	7.75	3.00	2.50	0.50	0.00	0.00	1.25	0.75	0.00	0.00	12.56
NL 1171	91.50	60.25	8.00	3.75	1.50	1.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	12.85
NL 1164	91.50	61.75	10.25	8.75	2.25	1.50	0.00	0.00	0.00	0.00	0.00	0.00	0.50	13.58
BL 4316	93.25	54.75	9.00	7.25	5.75	4.75	5.50	0.00	0.00	0.00	0.00	0.00	0.00	13.87
BL 1093	91.00	73.00	12.50	6.25	0.00	0.00	1.25	0.00	0.00	0.00	0.00	0.50	0.00	14.19
Bhrikuti	88.50	70.25	13.75	7.75	0.75	2.75	1.00	0.25	0.50	0.00	0.50	0.25	0.00	14.33
NL 4347	90.50	45.25	25.00	20.00	0.25	5.25	1.75	0.50	0.25	0.00	0.00	0.00	0.00	14.52
BL 3978	94.00	90.75	6.25	3.25	5.00	2.75	0.00	0.00	0.00	0.00	0.00	0.00	0.00	15.54
BL 4350	82.75	73.25	28.00	12.50	7.25	0.00	0.25	0.75	0.50	0.25	0.00	0.00	0.00	15.81
BL 1177	90.50	62.25	35.25	23.25	1.75	0.00	0.00	0.00	0.25	0.00	0.00	0.00	0.00	16.40

V/G: *Varieties/genotypes,* AA: *Alternaria alternata,* BS: *Bipolaris sorokiniana,* CH: *Cladosporium herbarum,* EN: *Epicoccum nigrum,* FM: *Fusarium moniliforme,* BiS: *Bipolaris spicifera,* CL: *Curvularia lunata,* FS: *Fusarium semitectum,* SB: *Stemphylium botryosum,* AS: *Acremonium strictum,* US: *Ulocladium* sp. AL: *Alternaria longissima,* FG: *Fusarium graminearum*

Table 5 Percentage frequency of fungi in seeds of 10 wheat varieties/genotypes from Lalitpur

V/G	AA	BS	CH	EN	US	BiS	SB	CL	FM	AS	Mean
Pasang Lhamu	72.25	10.00	13.50	7.00	7.50	0.00	2.50	0.25	0.00	0.00	11.30
Nepal 297	96.25	18.00	2.25	1.00	0.00	2.00	0.50	0.25	0.00	0.00	11.95
Bijaya	97.75	11.75	6.00	1.75	0.00	2.50	0.00	0.25	0.00	0.00	12.00
Achyut	98.00	13.2	4.50	2.25	2.00	0.00	0.00	0.00	0.50	0.50	12.10
WK 1204	99.00	13.50	8.25	0.00	0.00	1.50	0.25	0.00	0.25	0.00	12.28
Annapurna 4	99.25	10.75	6.50	2.75	6.00	1.00	0.00	0.00	0.00	0.00	12.63
RR-21	95.00	21.25	6.25	0.00	1.25	2.75	0.25	0.00	0.00	0.00	12.68
Annapurna 1	97.25	23.50	5.25	0.50	0.00	1.00	1.00	0.25	0.00	0.00	12.88
Bhrikuti	93.50	23.25	11.25	1.00	0.00	2.25	0.00	0.75	0.00	0.00	13.20
Gautam	90.75	21.50	14.25	3.25	0.00	0.00	0.25	2.00	0.50	0.00	13.25

V/G: *Varieties/genotypes,* AA: *Alternaria alternata,* BS: *Bipolaris sorokiniana,* CH: *Cladosporium herbarum,* EN: *Epicoccum nigrum,* US: *Ulocladium* sp., BiS: *Bipolaris spicifera,* SB: *Stemphylium botryosum,* CL: *Curvularia lunata,* FM: *Fusarium moniliforme,* AS: *Acremonium strictum*

Table 6 Percentage frequency and relative abundance of fungi in forty seed samples from four locations.

Fungi	Frequency (%)				Relative abundance (%)			
	Lalitpur	Chitwan	Kaski	Banke	Lalitpur	Chitwan	Kaski	Banke
Bipolaris sorokiniana	16.68	18.93	24.57	64.40	13.32	32.40	17.20	34.69
Alternaria alternata	93.90	26.10	71.50	89.97	76.00	40.40	55.10	49.11
Cladosporium herbarum	7.80	0.08	9.80	15.70	6.03	0.12	6.44	7.91
Bipolaris spicifera	1.30	4.50	0.00	2.05	0.99	5.72	0.00	1.10
Fusarium moniliforme	0.13	0.10	0.90	2.80	0.39	0.22	0.66	1.36
Curvularia lunata	0.38	1.48	0.03	1.12	0.09	3.43	0.01	0.62
Fusarium semitectum	0.00	0.00	0.62	0.15	0.00	0.00	0.43	0.09
Fusarium graminearum	0.00	0.03	0.53	0.05	0.00	0.03	0.37	0.03
Stemphylium botryosum	0.47	0.00	0.00	0.15	0.29	0.00	0.00	0.09
Aspergillus flavus	0.00	6.20	0.68	0.00	0.00	7.40	0.46	0.00
Rhizopus sp.	0.00	6.70	0.00	0.00	0.00	7.90	0.00	0.00
Penicillium sp.	0.00	0.68	0.00	0.00	0.00	1.06	0.00	0.00
Aspergillus niger	0.00	0.60	0.00	0.00	0.00	0.68	0.00	0.00
Trichothecium sp.	0.00	0.00	27.20	0.00	0.00	0.00	17.82	0.00
Epicoccum nigrum	1.95	0.65	2.05	10.10	1.56	0.73	1.33	4.83
Ulocladium sp.	1.68	0.00	0.00	0.13	1.31	0.00	0.00	0.07
Acremonium strictum	0.05	0.00	0.35	0.15	0.39	0.00	0.23	0.07
Alternaria longissima	0.00	0.00	0.03	0.08	0.00	0.00	0.02	0.04
Mean	6.91	3.67	7.68	10.38	5.58	5.56	5.56	5.56

Among the weak pathogens, incidence of *A. alternata* was maximum in all locations with a mean frequency and relative abundance of 70.37% and 55.15%, respectively (Table 6), while incidence of other non pathogenic and weakly pathogenic mycoflora was negligible (less than 9.00%). Among the storage fungi, prevalence of *Aspergillus flavus* and *Rhizopus* spp. was high in Chitwan and negligible at other locations. This reflects the storage condition in which seeds are kept. Bashir and Kutama (2012) reported that prevalence of storage fungi could be due to poor method of storage. (i.e. high temperature and high RH) or contaminated farm equipments or soil. *Bipolaris spicifera, Cladosporium herbarum, Curvularia lunata, Epicoccum nigrum, Stemphyliumbotryosum, Ulocladium* sp., *Acremonium strictum, Alternaria longissima* and *Trichothecium* sp. are weak parasitic and saprophytic fungi. Most of these fungi cause sooty head molds. Hershman (2011) mentioned that wet and humid weather during the latter stages of grain development and crop maturation results in development of head mold fungi on senescing and damaged wheat heads. Cultural practices also increase the inoculum of seed borne mycoflora. Vrandečićet al. (2013) reported that the number of grains infected with *Fusarium* species were significantly lower with conventional tillage compared to reduced tillage. Suproniene et al. (2011) reported increase in grain infection by *Alternaria, Aspergillus* and *Cladosporium* species under no tillage condition. High fertilizer rates also resulted in an increase in grain infection by *Fusarium* and *Penicillium* species. Removal of the previous crop straw from the fields helps to decrease the fungal infection in cereals (Suproniene et al., 2011).

Mean frequency of all mycoflora was also highest in Banke (10.38%), while relative abundance was similar in all 4 locations (i.e.3.87% to 5.58%). According to Duveiller and Gilchrist (1994), Salgado et al. (2011) and Wiese (1987), the most important seed borne fungal diseases of wheat were Helminthosporium leaf blight (caused by *Bipolaris sorokiniana* and *Pyrenophora tritici-repentis*), Fusarium head blight (incited by *Fusarium* spp.) and Black point/smudge (caused by *Alternaria*, *Fusarium*, *Cochliobolus*, *Aspergillus*, *Cladosporium*, *Penicillium*, *Rhizopus* and *Stemphylium*).

Conclusion

Nepalese wheat seeds found to be associated with many fungi, but their presence varied with varieties/genotypes and locations. The study indicated that wheat seeds should be produced in areas with relatively lower temperature and relative humidity for low seed infection with fungi.

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