IN-VITRO STUDY ON PREVALENCE OF MYCOFLORA IN WHEAT SEEDS

P. Adhikari[1], G. B. Khatri-Chhetri[1], S. M. Shrestha[1] and S. Marahatta[2]
Institute of Agriculture and Animal Sciences, Tribhuvan University, Nepal
[2]Agriculture and Forestry University, Chitwan, Nepal

ABSTRACT

A study on incidence of mycoflora was carried out by blotter method in wheat seeds. Forty seed sample of wheat (*Triticum aestivum*) was collected, ten varieties/genotypes each from Chitwan; Kaski; Banke and Lalitpur. The study was done at Seed Quality Control Center, Hariharbhawan, Lalitpur, Nepal, during August to December, 2013, with the objective of determining fungi associated with wheat seeds *in-vitro*. A total of eighteen species of fungi within thirteen genera were detected. *Alternaria alternata* and *Bipolaris sorokiniana* were predominant in all the varieties/genotypes and locations. Percentage frequency and type of fungi detected varied with variety and location. Among all fungi, mean frequency (76.00%) and relative abundance (55.15%) were highest of *Alternaria alternata*. Among the seeds of different location, *B. sorokiniana* was found highest from the seed of Banke and *A. alternata* from seeds of Lalitpur. Lowest percentage frequency of fungi was found in genotype BL 4009 (3.06%) from Chitwan and in variety Nepal 297 (6.25%) from Kaski, and highest in genotype BL 1177 (16.40%) from Banke. Nepalese wheat seeds appeared prevailed with many pathogenic fungi. Therefore, selection of wheat seeds from resistant varieties and areas with lower temperature and relative humidity, and their treatment is suggested to reduce disease and increase yield.

Key words: Varieties, Genotypes, Blotter test, Pathogen, Seed infection.

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 *Alternaria alternata, Drechslera sorokiniana, Fusarium moniliforme, F. avenaceum, F. graminearum, F. nivale, F. culmorum, F. equiseti, F. sporotirchioides, Cladosporium herbarum* (Khan, 1992). Shrestha *et al*. (1998) and Shakya (1997) reported presence of *Bipolaris sorokiniana* in seed samples of wheat collected from different parts of Nepal. 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. 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). Therefore, 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. Two hundred gram wheat seeds of forty seed samples were collected from 4 research institutes (10 varieties/genotypes each from Khumaltar, Lalitpur; Rampur, Chitwan; Lumle, Kaski and Khajura, 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 and rinsing in distilled water. Three layers of blotting paper were placed in the labeled, plastic petri-dishes and moistened with distilled water.

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

S.No.	Khumaltar	Rampur	Lumle	Khajura
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

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 20 ºC 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 (number of seeds out of one hundred in which at least a fungal species appeared) and relative abundance (number of fungi of a particular genus and/or species as a percentage of total number of fungi observed in one hundred seeds) were calculated (in percent) by using the following formula (Naqvi *et al.*, 2013):

$$\text{Percentage frequency} = \frac{\text{No. of seeds on which fungus appears}}{\text{Total number of seeds}} \times 100$$

$$\text{Relative abundance} = \frac{\text{No. of a particular fungi}}{\text{Total no. of all fungi}} \times 100$$

After computation of percentage frequency and relative abundance of each fungus in each variety/genotype, mean percentage frequency and relative abundance of all 10 varieties/genotypes of each location were calculated.

RESULTS AND DISCUSSION

The study detected a total of thirteen genera and eighteen species of fungi in seeds of forty wheat varieties/genotypes. Percentage frequency of *Alternaria alternata* was highest, followed by *Bipolaris sorokiniana* and *Cladosporium herbarum* on seeds of four locations. Small value of incidence of *Fusarium graminearum* was observed in all locations except Lalitpur, where it was not detected. 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*, *Drechslera*, *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).

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

Varieties / genotypes	*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

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

Varieties/ genotypes	*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

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

Varieties/ genotypes	*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

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

Varieties/ genotypes	*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

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*

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.

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

Among the weak pathogens, incidence of *A. alternata* was maximum in all locations with a mean frequency and relative abundance of 70.59% and 55.15%, respectively (Table 6), while incidence of other 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. 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*).

Greater relative abundance of *B. sorokiniana* from seeds of Banke (34.69%) and Chitwan (32.57%) indicated that *B. sorokiniana* was more prevalent than other fungi at those locations. 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. Though Bhrikuti and Gautam are recommended as resistant variety to leaf blight but they were found susceptible in Lalitpur, Chitwan and Banke. Result showed that none of the variety/genotype was free from seed mycoflora.

CONCLUSION

Nepalese wheat seeds found to be associated with many fungi, but their presence varied with varieties/genotypes and locations. Result indicated that none of the variety/genotype was resistant to *B. sorokiniana* infection. All wheat variety/genotypes tested have various percentage frequency of the pathogen. Variety/Genotypes with low frequency of seed infection may be grown in disease prone area and also wheat seeds should be produced in areas with relatively lower temperature and relative humidity for low seed infection with fungi.

ACKNOWLEDGEMENT

The authors heartily acknowledge Seed Quality Control Centre, Hariharbhawan, Lalitpur for providing research laboratory, and National Maize Research Program, Rampur, Chitwan; Regional Agricultural Research Stations, Lumle, Kaski and Khajura, Banke, and National Agricultural Research Council, Khumaltar, Lalitpur, for providing wheat seeds of various varieties/genotypes for the study.

REFERENCE CITED

Alam, K. B. and N. K. Saha. 1991. Helminthosporium leaf blight of wheat a new problem in Bangladesh. Paper presented at international Workshop on Helminthosporium blight. CIMMYT, Mexico, D. F. 12 pages.

Barabara, K., T. Mona, S. Estein and A. Birgitte. 2004. *Alternaria* and *Fusarium* in Norwegain grains reduced quality –a matched pair sample study. International Journal of Food Microbiology.93: 51-62.

Bateman, G. L. and H. Kwasna. 1999. Effects of number of winter wheat crops grown successively on fungal communities on wheat roots. Applied Soil Ecology. 13: 271-82.

Clear,R. M. and S. K. Patrick'Can. 1993. Prevalence of some seed borne fungi on soft white winter wheat seed from Ontario, Canada. Plant Disease Survey. 73: 143-149.

Dexter, J. E., R. M. Clear, and K. R. Preston. 1996. Fusarium head blight: effect on the milling and baking of some Canadian wheats. Cereal Chemistry. 73: 695-701.

Duveiller, E. and L. Gilchrist. 1994. Production constraints due to *Bipolaris sorokiniana* in wheat: Current situation and future prospects. In: D. A. Saunders and G. P. Hettel (ed.) Wheat in Heat-Stressed Environments: Irrigated, DryAreas and Rice-Wheat Systems. CIMMYT, Mexico D.F. pp. 343-352.

Friis-Hansen, E. 1995. Seeds for African peasants: Peasants' needs and agricultural research – the case of Zimbabwe. The Nordic African Institute. 227 pages.

Khan, S. A. J. 1992. Studies on fungi causing seed-borne diseases of wheat and rice and their control. Ph. D. Thesis, University of Karachi, Karachi, Pakistan. P. 17.

Khanal, N. P., K. L. Maharjan and A. Sapkota. 2012. Technical efficiency in wheat seed production: A case study from terai region of Nepal. Journal of International Development and cooperation. 19: 41-50.

Khanzada, K. A., M. A. Rajput, G. S. Shah, A. M. Lodhi and F. Mehboob. 2002. Effect of seed dressing fungicides for the control of seed borne mycoflora of wheat. Asian Journal of Plant Science. 1: 441-444.

Mathur, S. B. and O. Kongsdal. 2003. Common laboratory seed health testing methods for detecting fungi (1st ed). International Seed testing Association.

MOAD. 2013. Selected indicators of Nepalese agriculture and population.

Nafula, M. M. 1997. Assessment of seed borne pathogens for some important crops in western kenya. KARI-Katumani, PO Box, 340-90100.

Naqvi, S. D. Y., T. Shiden, W. Merhawi and S. Mehret. 2013. Identification of seed borne fungi on farmer saved sorghum (*Sorghum bicolor* L.), pearl millet (*Pennisetum glaucum* L.) and groundnut (*Arachis hypogaea* L.) seeds. Agricultural Science Research Journals. 3: 107-114.

Rosyara, U. R. 2002. Physio-morphological traits associated with resistance and tolerance to Helminthosporium leaf blight in spring wheat. M. Sc. Thesis, Tribhuvan University, Department of Plant Breeding, IAAS, Chitwan, Chitwan, Nepal. P. 2.

Saari, E. E. 1998. Leaf blight disease and associated soil borne fungal pathogens of wheat in South and Southeast Asia. In: Duveiller, E., H. J. Dubin, J. Reeves and A. McNab (ed.) Helminthosporium Blights of Wheat: Spot Blotch and Tan Spot. CIMMYT, Mexico D.F. pp. 37-51.

Salgado, J. D., M. Wallhead, L. V. Madden and P. A. Paul. 2011. Grain harvesting strategies to minimize grain quality losses due to Fusarium head blight in wheat. Plant Dis. 95: 1448-1457.

Shrestha, K. K., R. D. Timilal, B. N. Mahto and H. P. Bimb. 1998. Disease incidence and yield loss due to foliar blight of wheat in Nepal. *In:* E. Duveiller, H. J. Dubin, J. Reeves, and A. McNab (ed.) Helminthosporium Blights of Wheat: Spot Blotch and TanSpot. CIMMYT,Mexico D.F. pp. 67-72.

Shakya, P. B. 1997**.** Effect of black point disease on yield and quality of wheat seed. Nepal Agriculture Research Journal.1.

Warham, E. J., L. D. Butler and B. C. Sutton. 1996. Seed testing of maize and wheat: A laboratory guide. CIMMYT, Mexico. P. 84.

Wiese M. V. 1987. Compendium of Wheat Diseases (2nded). The American Phytopathological Society, St Paul. USA.