Available Online at ESci Journals
ESci Journal of Plant Pathology
ISSN: 2305-106X (Online), 2306-1650 (Print)
http://www.escijournals.net/EJPP

# SEED BORNE MYCOFLORA OF SOME COMMERCIAL WHEAT (*TRITICUM AESTIVUM* L.) CULTIVARS IN PUNJAB, PAKISTAN

**Muzammil Hussain, Muhammad U. Ghazanfar, Muhammad I. Hamid, Mubashar Raza**
*Department of Plant Pathology, University College of Agriculture, University of Sargodha, Sargodha, Pakistan.*

**A B S T R A C T**

Seed borne mycoflora associated with ten commercial varieties of wheat viz. Blue silver, Faisalabad 85, Manthar-3, Pak 81, Parwaz 94, Pirsabaq 2005, Punjnad-1, Sariab-92, Sh-2002 and Wafaq-2001 was investigated through standard blotter paper and agar plate method by using Mann-Whitney U test. At least eleven fungal genera were recovered from seeds. The most frequently isolated fungi were *Bipolaris sorokiniana* (11.125%), *Aspergillus flavus* (9.825%), *Alternaria alternata* (7.15%) and *Aspergillus niger* (6.225%). It is apparent from the present investigation that all commercial wheat varieties tested were contaminated by fungi. The rolled paper method was used to find out the effect of seed borne fungi on seed germination. Seeds of Pak 81, Wafaq-2001 and Blue silver were germinated in high proportion with variable number of normal and abnormal seedlings than the seeds of other varieties tested. The fungi associated with seeds of wheat cause dire diseases in wheat reducing the germination capacity.

**Keywords**: Mycoflora, Wheat, Black point, Spot blotch and Germination.

## INTRODUCTION

Wheat (*Triticum aestivum* L.) a cereal crop belonging to family *Gramineae* and known to have originated from the Fertile Crescent region of the near east, used as a staple food around the world. It ranked first as essential food crop in Pakistan followed by rice and maize but to fulfill requirement, country has to import wheat significantly from other countries (Hussain *et al*., 2012). In the year 2007, cultivated area of wheat in Pakistan was 8.49 million ha with production of about 23.52 million tons (Govt. of Pakistan, 2008).

Seed borne mycoflora is one of the major components reducing the wheat yield. Mycoflora associated with seeds both internally and externally are responsible for seed abortion, mortality of grains, reduction in germination capacity, seed necrosis and at the end cause destructive to serious diseases during different stages of plant growth (Niaz and Dawar, 2009). Yield losses due to seed borne fungi have been reported between 15 to 90% of untreated seeds grown in field (Wiese, 1998).

Seed borne pathogens of wheat include *Alternaria alternata, Cladosporium oxysporum*, *Curvularia lunata*, *Drechslera sorokiniana, D. tetramera, Fusarium graminearum, Helminthosporium sativum,* and post-harvest fungi include species of *Aspergillus* and *Penicillium* (Ilyas *et al.*, 1998). Genera of *Tilletia*, *Ustilago*, *Bipolaris*, *Fusarium*, *Alternaria*, *Drechslera*, *Stemphylium*, *Curvularia*, *Cladosporium*, *Rhizopus*, *Aspergillus* and *Penicillium* has been the most common isolated fungi from wheat seeds (Rehman *et al*., 2011). Seeds affected with black point disease of wheat comprise of *Alternaria alternata, Cladosporium cladosporioides, Curvularia lunata* and *Fusarium* spp. (Dey *et al.,* 1992). *Bipolaris sorokiniana* is a seed and soil borne pathogen, causes spot blotch in cereal grains and grasses (Wiese, 1998). Knowledge of the associated mycoflora with the particular frequency on commercially grown varieties provides with the basis to access the risk associated with undesirable organisms.

For the management of crop disease the major step is to use disease free and certified seed. Germination test of seeds is significant in identifying seed borne pathogen associated with wheat seeds and provides valuable information regarding mycoflora and their efficient control. The theme of study was to record the mycoflora

* Corresponding Author:
Email: muzammil0991@gmail.com

associated with commercial wheat varieties of wheat seed by using ISTA techniques and testing of seed germination.

**MATERIALS AND METHODS**

Research was conducted during the year 2011-2012 in the mycology lab. Department of Plant Pathology, University College of Agriculture (UCA), Sargodha to check the association of seed borne mycoflora with commercial varieties of wheat.

**Collection of Seed Samples:** Seeds of ten commercial varieties of wheat (400 seeds / variety) viz. Blue silver, Faisalabad 85, Manthar-3, Pak 81, Parwaz 94, Pirsabaq 2005, Punjnad-1, Sariab-92, Sh-2002 and Wafaq-2001 that were collected from National Agriculture Research Centre, Islamabad. Following formula was used to record the disease incidence.

$$\text{Incidence (\%)} = \frac{\text{No. of infected seeds}}{\text{Total no. of seeds}} \times 100$$

**Detection of Fungi:** Blotter paper method (ISTA, 1985) and agar plate method (Muskett and Malone, 1941) as suggested by International Seed Testing Association (ISTA) were used for the detection of fungi. Seeds were disinfected with chlorox 1% for 1-2 minutes and then washed three times with distilled water (Mittal *et al*., 1999).

**Blotter Paper Method:** Three whatman filter papers/blotter paper moistened in distilled water were placed on 9 cm sterilized petri dishes. Disinfected seeds were placed on blotter paper at the rate of 25 seeds per plate. The Petri dishes were then incubated at 22 ± 2 $^0$C for 7 days under alternating cycle of 12 hours fluorescent light and constant darkness. After 7 days incubated seeds were examined under steriobinocular microscope for identification of fungi.

**Agar Plate Method:** Potato dextrose agar (PDA) was used in this method for the isolation of mycoflora. 25 seeds per plate were inoculated and incubated at 22 ± 2 $^0$C. After 7 days incubated seeds were examined under steriobinocular microscope for fungi and then the isolated mycoflora were subcultured by single spore technique for macro and microscopic studies. The fungi were identified with the help of keys, monographs and text provided by several authors (Barnett and Hunter, 1972; Pedro *et al*., 2009).

**Seed Germinating Test:** Standard rolled paper method was used for testing seed germination. In this method 100 seeds of each commercial variety of wheat were placed between 2 layers of blotter paper and incubated at 22 ± 2 $^0$C for 15 days. After incubation period, germination percentage of seeds with normal and abnormal seedling was calculated and confirmed (ISTA, 1993). Abnormal seedlings having mycoflora were examined and recorded.

**Statistical Analysis:** The difference between the mean values of seed borne mycoflora isolated from the blotter paper and agar plate method was analyzed by using Mann-Whitney U test (Hart, 2001).

**RESULTS AND DISCUSSION**

From the tested wheat cultivars, a total of twelve fungal species were isolated including *Alternaria alternata*, *Aspergillus flavus*, *Aspergillus niger*, *Bipolaris sarokiniana*, *Curvularia lunata*, *Chaetomium globosum*, *Diplodia* spp., *Fusarium oxysporum*, *Macrophomina phaseolina*, *Penicillium* spp., *Rhizopus* spp. and *Trichoderma hamatum*. The mean infection of *Bipolaris sarokiniana* (27.3±2.94), *Aspergillus flavus* (20.5±2.68), *Chaetomium globosum* (15.8±1.26) and *Alternaria alternata* (14.8±2.49) on wheat seeds were high while *Aspergillus flavus* (18.8±1.90) was more frequently isolated fungus followed by *Bipolaris sarokiniana* (17.2±1.85) and *Alternaria alternata* (13.8±1.90) in blotter paper and agar plate method respectively (Table 1). Hajihasani *et al*. (2012) reported 15 fungal species comprised of *Tilletia laevis*, *T. tritici*, *Ustilago tritici*, *Fusarium graminearum*, *F. culmorum*, *Microdochium nivale*, *Bipolaris sorokiniana*, *Alternaria alternata*, *Curvularia* spp., *Aspergillus niger*, *A. candidus*, *A. flavus*, *Penicillium* spp., *Mucor* spp. and *Rhizopus* spp. from seeds of three wheat cultivars. *Fusarium* spp. and *Alternaria alternata* reduce germination percentage and induce seedling blight (Karim, 2005).

Seeds of blue silver, Faisalabad 85 and Wafaq-2001 were infected by seed borne mycoflora more than 70% while the Pirsabaq 2005 (56.25%) and Sh-2002 (59.75%) were least infected by seed borne fungi (Table 2). The incidence of *Bipolaris sarokiniana* (11.125%) a pathogen of spot blotch of wheat was most prevalent fungus on the seeds of all varieties (Fig. 1 and 2). Iftikhar *et al*. (2006) found that wheat variety Wafaq 2001 was susceptible to *Bipolaris sarokiniana* during artificial inoculation test. Incidence of *Aspergillus flavus* (9.825%), *Chaetomium globosum* (7.3%), *Alternaria alternata* (7.15%), *Aspergillus niger* (6.225%), *Penicillium* spp. (5.7%), *Rhizopus* spp. (4.8%) and *Fusarium oxysporum* (3.3%) were high on wheat seeds (Fig. 2) due to which low germination as well as emergence of abnormal

Table 1: Fungi on wheat seeds detected by Blotter Paper and Agar Plate Method.

| Mycoflora | Blotter paper method | | Agar plate Method | | Difference |
|---|---|---|---|---|---|
| | Mean± S.E | Max-Min | Mean± S.E | Max-Min | |
| *Alternaria alternata* | 14.8±2.49 | 31-05 | 13.8±1.90 | 25-07 | NS |
| *Aspergillus flavus* | 20.5±2.68 | 35-09 | 18.8±1.90 | 30-11 | NS |
| *Aspergillus niger* | 13.2±1.45 | 20-07 | 11.7±1.33 | 18-07 | NS |
| *Bipolaris sorokiniana* | 27.3±2.94 | 42-14 | 17.2±1.85 | 25-08 | S |
| *Curvularia lunata* | 7.00±1.04 | 12-02 | 7.60±1.29 | 15-02 | NS |
| *Chaetomium globosum* | 15.8±1.26 | 22-09 | 13.4±0.93 | 18-09 | NS |
| *Diplodia* spp. | 0.00±0.00 | 00-00 | 5.80±1.59 | 15-00 | S |
| *Fusarium oxysporum* | 5.50±1.58 | 14-03 | 7.00±1.79 | 16-00 | NS |
| *Macrophomina phaseolina* | 6.10±1.80 | 16-04 | 8.30±1.57 | 18-03 | NS |
| *Penicillium* spp. | 11.6±1.42 | 18-04 | 11.2±1.66 | 22-05 | NS |
| *Rhizopus* spp. | 9.10±1.28 | 14-06 | 10.1±1.94 | 19-00 | NS |
| *Trichoderma hamatum* | 0.00±0.00 | 00-00 | 3.10±1.53 | 14-04 | S |

(Mean value with their standard error, n=10), Significant and non-significant difference between the mean values of mycoflora isolated from blotter paper and agar plate method represents 95% confidence level.
Max= Maximum value of infected seeds, Min= Minimum value of infected seeds.

Table 2: Infected and healthy seeds percentage of wheat varieties.

| Wheat varieties | Infected seed (%) | Healthy seed (%) |
|---|---|---|
| Blue silver | 77.25 | 22.75 |
| Faisalabad 85 | 71.50 | 28.50 |
| Manthar-3 | 61.50 | 38.50 |
| Pak 81 | 60.00 | 40.00 |
| Parwaz 94 | 63.00 | 37.00 |
| Pirsabaq 2005 | 56.25 | 43.75 |
| Punjnad-1 | 64.50 | 35.50 |
| Sariab-92 | 61.00 | 39.00 |
| Sh-2002 | 59.75 | 40.25 |
| Wafaq-2001 | 71.50 | 28.50 |

seedlings were most common. *Alternaria alternata* was a frequently isolated fungus and it was involved in black point disease of wheat (Fig. 1). Özer (2005) reported *Alternaria alternata* was the most dominant fungus isolated from black pointed seeds. Zare *et al*., (2006) also reported an average infection level of *Fusarium culmorum* upto 15.5%. *Aspergillus niger* and *Penicillium* spp. have been found to reduce the seed germination and seed loss during storage (Ijaz *et al.,* 2001). *Alternaria alternata* and *Curvularia lunata* caused delay in seed germination due to rot of seeds. *Aspergillus flavus* produces toxic chemicals that result in decrease of shoot and root elongation (Shakir and Sultan, 2000). Table 3 indicates the germination of naturally infected seeds of all commercial varieties was more than 85% but the numbers of emergence of abnormal seedlings were more than the normal seedlings.

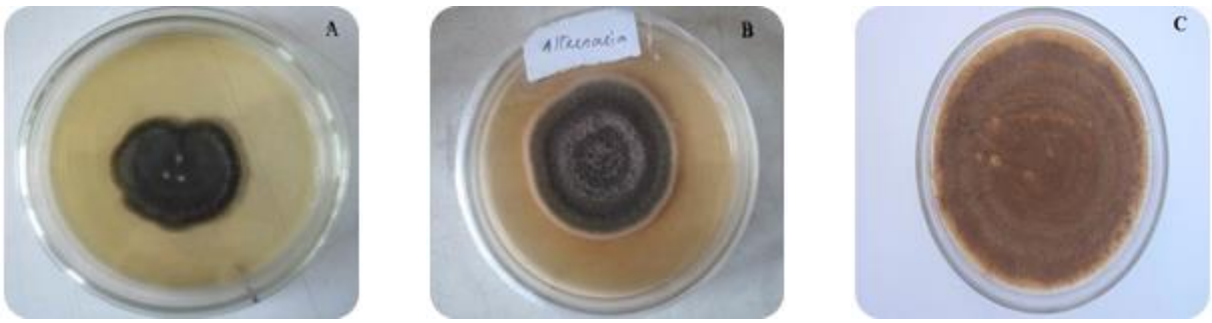


Figure 1: Colony morphology of mycoflora isolated from wheat seeds. A. *Bipolaris sorokiniana* a pathogen of spot blotch of wheat (5 dpi) B. *Alternaria alternata* dominantly involved in black point disease of wheat (5 dpi) C. *Aspergillus flavus* (5 dpi) associated with wheat seeds particularly involved in reduction of seed germination.

Table 3: Percentage seed germination, number of normal and abnormal seedlings and fungal pathogens isolated from abnormal seedlings emerged from naturally infected seeds of ten commercial varieties of wheat.

| Wheat varieties | Germinated seeds (%) | Seedlings condition(%) | | Fungal pathogens isolated from abnormal seedlings |
|---|---|---|---|---|
| | | Abnormal | Normal | |
| Blue silver | 92 | 74 | 18 | *Aspergillus flavus*, *Curvularia lunata., Chaetomium globosum*, |
| Faisalabad 85 | 86 | 61 | 25 | *Alternaria alternata., Aspergillus niger, Fusarium oxysporum*, *Macrophomina phaseolina*, |
| Manthar-3 | 88 | 71 | 17 | *Alternaria alternata.*, *Aspergillus flavus*, *Bipolaris sorokiniana*, *Chaetomium globosum*, |
| Pak 81 | 94 | 75 | 19 | *Aspergillus niger*, *Fusarium oxysporum*, *Bipolaris sorokiniana*, |
| Parwaz 94 | 85 | 67 | 18 | *Alternaria alternata.*, *Aspergillus flavus*, |
| Pirsabaq 2005 | 89 | 78 | 11 | *Alternaria alternata.*, *Aspergillus niger*, *Curvularia lunata*, |
| Punjnad-1 | 87 | 71 | 16 | *Alternaria alternata.*, *Bipolaris sorokiniana*, *Chaetomium globosum*, |
| Sariab-92 | 91 | 70 | 21 | *Aspergillus flavus*, *Aspergillus niger*, *Fusarium oxysporum*, |
| Sh-2002 | 90 | 81 | 09 | *Alternaria alternata.*, *Aspergillus flavus*, |
| Wafaq-2001 | 93 | 76 | 17 | *Aspergillus niger*, *Bipolaris sorokiniana* |

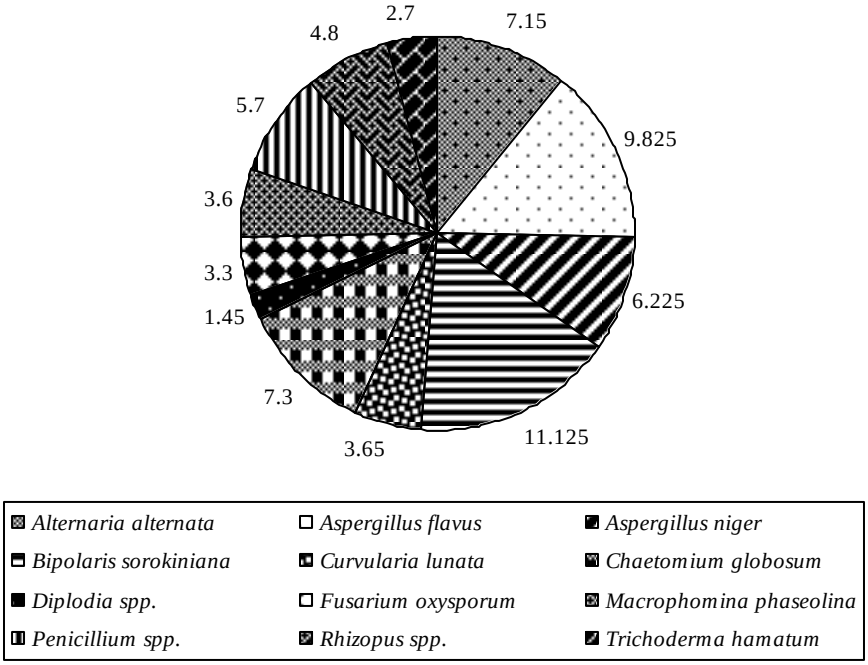


Figure 1 Mean Percentage (%) Incidence of Wheat Seed-Borne Mycoflora.

The highest germinated seeds were recorded from 3 varieties Pak 81 (94%), Wafaq-2001 (93%), Blue silver (92%) while the lowest from Parwaz 94 (85%). The germination percentage was satisfactory but the number of abnormal seedlings were found higher in Sh-2002 (81%), Pirsabaq 2005 (78%), Wafaq-2001 (76%) while the lowest number came from seeds of Faisalabad 85 (61%) and Parwaz 94 (67%) whereas, the other

commercial varieties of wheat had intermediate seed germination percentage and abnormal seedling.

The result of the study divulges that presence of fungal pathogens on all varieties of wheat seeds have adverse effect on growth of wheat plant. Hence farmers need to manage the seed borne mycoflora by applying fungicides and biological compounds to minimize crop losses and ultimately to increase quality of produce.

**REFERENCES**


Barnett, H.L. and B.B. Hunter. 1972. Illustrated genera of imperfect fungi, Burgess. Pub.Co., Minneapolis, Minnesota. 241 pp.

Dey, T.K., N. Chowdhury, A. Ayub and B.K. Goswami. 1992. Black point of wheat: Occurrence, effect of fungicidal seed treatment on germination and quality characters. *Bangladesh J. Bot.*, 22(1): 27-32.

Govt. of Pakistan. 2008. Economic Survey of Pakistan Ministry of Food, Agriculture and Livestock. Federal Bureau of Statistics.

Hajihasani, M., A. Hajihassani and S. Khaghani. 2012. Incidence and distribution of seed-borne fungi associated with wheat in Markazi Province, Iran. *African Journal of Biotechnology*., 11(23): 6290-6295.

Hart, A. 2001. Mann-Whitney test is not just a test of medians: differences in spread can be important. *BMJ: British Medical Journal*, *323*(7309): 391-393.

Hussain, A., A. Saboor, M.A. Khan, A.Q. Mohsin and F. Hassan. 2012. Technical efficiency of wheat production in rain-fed areas: a case study of Punjab, Pakistan. *Pak. J. Agri. Sci*., 49(3): 411-417.

Iftikhar, S., S. Asad, A. Munir, I. Ahmad and A. Sultan. 2006. Prevalence and distribution of foliar blight pathogens of wheat in different agro ecological zones of Pakistan with special reference to *Bipolaris sorokiniana*. *Pak. J. Bot*., 38(1): 205-210.

Ijaz, A., S.A. Anwar, A. Riaz, and M.S.A. Khan. 2001. Seed borne pathogens associated with wheat seed and their role in poor germination*. Pak. J. Phytopathol.,* 13: 102-106.

Ilyas M.B, S.A.A. Bokhari, and M.A. Khan. 1998. Fungi detected from wheat seeds exhibiting black points symptoms and their control by seed treatment. *Pak. J. Phytopathol.,* 10: 86-89.

ISTA. 1985. International Seed Rules for Seed Testing. Seed Science and Technology 21: (Supp.) 289p.

ISTA. 1993. International Rules for Seed Testing. Rules Amendments. *Seed Sci. Technol*. 29: 1-127.

Karim, M. 2005. Prevalence of fungi associated with seeds of some minor cereals. *An M.S. Thesis*. Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh. p. 97.

Mittal, R.K., H.J. Hansen, K. Thomsen, M. Marzalina de, K.C. Khoo de, N. Javanthi de, K.F.Y. Tsna de, and B. Krishnapillav. 1999. Effect of seed treatments and storage temperature on storability of Syzgium cuminii seeds. TUFRO Seed Symposium 1998, Recalcitrant seeds, Proceedings of the Conference Kaula Lampur Malaysia, 12 -15 Oct 1998-99.30(1): 53-63.

Muskett, A.E. and J.P. Malone. 1941. The Ulster method for the examination of flax seed for the presence of seed borne parasites. *Ann. App. Biol*. 28: 8-13.

Niaz, I., and S. Dawar. 2009. Detection of seed borne mycoflora in maize (*Zea mays* L.). *Pak. J. Bot*. 41(1): 443-451.

Özer, N. 2005. Determination of the fungi responsible for black point in bread wheat and effects of the disease on emergence and seedling vigour. *Trakya Univ J Sci*. 6(1): 35-40.

Pedro, W.C., J.M. Verkley, J.Z. Groenewald and R.A. Samson. 2009. Fungal Biodiversity. Pub. Co. CBS-KNAW Fungal. Biodiversity centre utrecht, The Netherlands.

Rehman, A., K. Sultana, N. Minhas, M. Gulfraz, G.K. Raja, and Z. Anwar. 2011. Study of most prevalent wheat seed-borne mycoflora and its effect on seed nutritional value. *Afr. J. Microb. Res*. 5(25): 4328-4337.

Shakir, A.S. and Sultan, M. K. 2000. Studies on seed-borne fungi of certain vegetables grown in Punjab with special reference to *Alternaria radicina*. *Pak J Agri. Sci*. 37(3-4): 202.

Wiese, M.V. 1998. *Compendium of wheat diseases* (3rd Ed). St. Paul, USA: APS Press, 112pp.

Zare, L., M. Harsini, S. Hosseini-bai, and B. Motieshare. 2006. Contamination of seed harvested wheat loads to a number of seed-borne fungal pathogen. In Proceeding 17th Iranian Plant Protection Congress. p. 24.