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Screening of local wheat varieties and associated seed borne infection in in-vitro study at Aligarh district

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ABSTRACT

A screening study was made in order 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 view, detailed investigation were carried out to study the implication of seed-borne fungi of wheat on seed quality parameter and to find out suitable detection methods for seed-borne infection of the three detection methods adopted for inoculation of seed mycoflora in wheat varieties. 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 detection methods, a total no of 11 genera and 20 species of fungi were isolated and identified

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Key words: Seed-borne incidence, Wheat varieties, Methods of detection, ISTA.

INTRODUCTION

Plants are always exposed to a large number of pathogenic microorganisms both in the aerial as well as in the soil environment. Thus, plants, disease causing organisms and the surrounding environment form a triangle which involves various types of associations or interactions. Plant diseases have been found to affect the growth and productivity of crop plants. Seeds play a vital role for the healthy production of a crop. They are known to carry pathogen which causes heavy yield losses. Seed pathology involves the study of living entities, environmental factor affecting adversely to the seed production and utilization, as well as disease management practices applied to seed. Seed health and it is concerned with the seed-borne microorganisms which may be associated externally, internally or as concomitant contamination as selerotia, gall, fungal bodies etc. The associated microorganisms may be pathogenic, weak parasite or saprophyte. Seed-borne microorganisms not only create problems in agricultural production but prove hazardous to animals and human beings.

The present situation regarding problems and achievements in seed pathology and domestic and international quarantine in India needs to resolve present inadequacies in this country's quarantine legislation and for

international alignment in seed health testing methods and a general information exchange on plant pathogen [32]. Seeds are regarded as highly effective means for the transfer of plant pathogens over the years. Seed-borne diseases have been found to affect the growth and productivity of crop plants [18].

Seed-borne fungi are important from economic point of view as they render losses in a number of ways. Some of the fungi infect the seed and cause discolouration of the seed [6, 5]. Several seed-borne pathogens are known to be associated with wheat seed which are responsible for deteriorating seed quality and weight during storage. Seed-borne pathogens of wheat are responsible to cause variation in plant morphology and also reducing yield up to 15-90 % if untreated seeds are grown in the field [35].

Kamal & Mughal [13] and Khan *et al*., [15] noted the presence of several fungi, i.e., *Alternaria, Helminthosporium, Fusarium, Curvularia, Stemphylium, Rhizopus, Cladosporium, Aspergillus,* and *Penicillium* species in wheat seeds. Many authors reported the incidence of *Alternaria, Curvularia, Fusarium, Aspergillus,* and *Penicilium* spp., as major storage fungi from wheat grains and reported that several species of *Aspergillus, Penicilium* are responsible for deteriorating wheat grains during storage [25, 9].

Triticum aestivum, commonly known as 'Genhu' is the important cereal crop of Indian agriculture and food security system. Wheat is one of the main staple food or feed crop of several countries of Asia, Africa and is grown in almost all the temperate and subtropical regions of the world. It achieved highest yield of 76.37 m tones each year. It is an important food crop next to cereals in India and covers an area of more than 12 million hectors. Wheat suffers from many diseases but the most destructive of them are the diseases caused by fungi, viz., rusts, smuts and bunts, leaf-spots and foot rots and mildews.

Wheat plants at all stages of growth are subjected to numerous injuries and stresses, which interfere with their normal functioning and development. Each year about 20% of the wheat that otherwise would be available for food and feed is lost due to diseases [8].

Seed borne infection of fungal pathogens are important not only for its association with the seeds which cause germination failure or causing disease to the newly emerged seedlings or growing plants but also contaminate the soil by establishing its inocula permanently [12].

Niaz *et al* [30] at Pakistan performed an experiment to detect the Seed borne mycoflora of maize was tested by using blotter, agar plate and deep freezing methods as recommended by ISTA on 100 samples collected from different places. Yago *et al* [34] at Korea reported the seed-borne mycoflora of sorghum and foxtail millet collected from different growing areas were isolated and taxonomically identified using dry inspection, standard blotter and the agar plate method.

The main aim of the present study was to enumerate the fungal species and their effect on germination associated with wheat seeds. In this study screening of five varieties of wheat and seed health testing techniques have been evaluated for their comparative efficacy in the detection of seed mycoflora which can be helpful for determining the quality of seeds in the laboratory.

MATERIALS AND METHODS

The experiment was conducted in the laboratory of department of Plant pathology and Nematology, Aligarh Muslim University, Aligarh. To compare the efficiency of different methods for the detection of seed-borne organism, seed samples of five varieties such as PBW343,WH896, HD264, and HD273, PBW502 of this important cereal crop that is wheat (*Triticum aestivum*) were tested and analyzed.

Experiments were conducted to work out relationship between environment variables and effect of fungal diseases using grain samples of wheat (*Triticum aestivum*). Many fungal genera were found to be associated with the deteriorated samples of wheat during storage by direct observation, standard blotter method, and agar plate method, collected from different places. The germination tests were conducted on 300 seeds in accordance with ISTA rules [1]. During the present investigation following methods were used for seed testing and identification of fungal diseases.

The International Seed Testing Association (ISTA) as an internationally recognized organization providing standardized methods for routine seed quality testing, provides a number of methods of seed health testing (ISTA-SHT). Seed health testing methods applied, depended on the nature of the pathogen and on the type of plant in question.

Blotter method

The blotter method [23], [20] is one of the incubation methods where the seeds are plated on water soaked filter papers, and incubated usually for 8 days under 12th alternating cycles of light and darkness. After incubation, fungi developed on seeds are examined under different magnification of a stereomicroscope and identified. The identification of the fungi is based on the way they grow on the seeds and on the morphological characters of fruiting bodies, spores / conidia observed under a compound microscope.

Potato dextrose agar method

The agar plate method, is another popular method in which seeds are plated on an agar medium and the plated seeds are usually incubated for 5-7 days at 22-25 ^{o}C under 12th alternating cycles of light and darkness. At the end of the incubation period, fungi growing out from seeds on the medium are examined and identified. Identification is based on colony characters and morphology of sporulating structures under a compound microscope.

Deep freeze method: This method was developed by [22] to detect slow growing pathogens. Three hundred seeds of moderately infected were placed at the rate of 10 seeds per plate on moistened blotters in the way as described under Standard blotter method. The Petri plates were incubated at 20± 2ºC for 24 h under alternate cycles of 12 h NUV light and darkness, for next 24 hours the plates are incubated at –20ºC in dark and then kept back under original conditions for next five days. After eight days of incubation, the seeds were examined under stereo-binocular microscope [17].

For the surface sterilization the seed in all methods were sterilized by 0.1% mercuric chloride solution to 1 to 2 min then washed by sterilized water [11].

The frequency of the fungus was calculated by the following formula:

$$\frac{\text{No. of seeds containing a particular fungus}}{\text{Total seeds used}} \times 100$$

Statistical analysis:

Data recorded were analyzed statistically using SPSS 12.00 software (SPSS Inc. Chicago, IL, USA) wherever considered necessary. The data obtained were analyzed statistically and significance was calculated at $p< 0.05$ and $p< 0.01$ levels of probability.

RESULTS AND DISCUSSION

Intensive agricultural development is taking place in India with a view to accelerate food production for feeding the ever increasing population. All available resources are being mobilized to set up our food production and the farmers are being advised to take up to scientific farming. Increased crop productivity can be achieved by using cultivars of high yielding varieties and avoiding crop failures. This involves the demand of better quality seed in terms of germination, purity and health by the farmers.

Numerous examples exist in agricultural literature for the international spread of plant diseases as a result of the use of infected or contaminated seeds [2].

The cultivation of wheat is hampered due to seed-borne diseases. Keeping the importance of wheat for developing countries like India and also in view of the fact that quite little work has been carried out on the seed pathology and seed health-testing of this crop in India, it was considered desirable to study certain aspects of seed mycoflora so that the losses due to them could be minimized.

Seed-borne mycoflora of wheat reported by different workers include *Alternaria alternata*, *Drechslera sorokiniana*,

Fusarium moniliforme, F. avenaceum, F. graminearum, F. nivale, F. culmorum, F. equiseti, F. sporotirchioides, Cladosporium herbarum, Stemphylium botrysum [31, 10, 26].

A seed-borne pathogen present externally or internally or associated with the seed as contaminant, may cause seed abortion, seed rot, seed necrosis, reduction or elimination of germination capacity as well as seedling damage resulting in development of disease at later stages of plant growth by systematic or local infection [16, 4].

Standard Blotter method [23, 3] and PDA method [24] and [27] and Deep freezing method as suggested by ISTA were used for detection of seed-borne mycoflora.

The frequency of association of wheat seed mycoflora was influenced by varieties tested.

The highest frequency of seed mycoflora was observed on wheat variety PBW343 followed by WH896, HD264, and HD273, with lowest fungal frequency recorded from the seeds of wheat variety PBW502.

The fungi which have been detected from all the five screened varieties by Blotter method during the study were found to be associated with wheat seeds. In all five varieties tested, PBW343 was found to be most susceptible and infected with 7 fungal genera as compared to other variety. The occurrence of individual fungi varied widely. From the total population of fungi emerged in this study, variety PBW343 was found to be associated with maximum no of fungal genera ie seven. Five genera were detected from variety WH896 and HD264. In case of varieties PBW502 and HD273 only four genera of seed fungi were isolated. In variety PBW343 the most predominant fungi detected in order of prevalence were *Fusarium solani*, *Alternaria alternata*, *Aspergillus flavus*, *Rhizopus oryzae*, *Fusarium moniliforme*, , *Aspergillus niger*, *B.sorokiniana*, *Penicillium spp*, *Drechslera spp*. Maximum fungal incidence was recorded with *Fusarium solani* and minimum with *Drechslera spp*. in variety PBW343 screening test (Table 1).
As the observations reveal, more number of fungi were isolated by Blotter method as compared to Agar plate method. This may be due to the reason that some of the slow growing fungi and weak competitors could not grow in the culture plates in competitions to the fast growing fungi and selective nature of the culture medium which might not have favored the growth of such species.

Variety PBW343 was again tested to check the efficacy of different fungal flora by three isolation methods as recommended by ISTA.

It is evident from Fig (A) that 20 most frequently occurring fungi viz. *Aspergillus niger, Alternaria alternata, Aspergillus flavus, Alternaria triticina, Fusarium moniliforme, Bipolaris sorokiniana, Fusarium oxysporum, Rhizopus oryzae, Drechslera spp, Penicillium spp., Curvularia lunata, Helminthosporium spp.,* and *Cladosporium spp*. were isolated from the non sterilized seeds of variety **PBW343** with Blotter method.

For the, internal seed mycoflora of Blotter method, seeds were surfaced sterilized by dipping in 0.1% mercuric chloride. All the same fungi were isolated from sterilized seeds but in less frequency (Fig A).

In case of non sterilized seeds highest frequency and deviation (78.33) was recorded for *A.niger* followed *by A.alternata* (77.33) where as in case of sterilized seeds in blotter method mycoflora frequency again the highest frequency (72.33) was recorded for *A.niger* followed by *A.flavus* (66.66). Lowest frequency (15.66) was recorded for *Drechslera australiansis*, in non sterilized seeds however this fungus was absent in surface sterilized seeds (Fig A). Percentage of germination of seeds in Blotter method was 37% in external seed mycoflora and in case of internal seed mycoflora percentage was 46 recorded.

A perusal of (Fig B) indicates that the number of fungi detected in non sterilized seeds in PDA method was 18 and isolated mycoflora viz., *Aspergillus niger, Alternaria alternata, Aspergillus flavus, Alternaria triticina, Fusarium moniliforme, Bipolaris sorokinia, Fusarium oxysporum, Rhizopus oryzae, Drechslera spp, Penicillium spp., Curvularia lunata,*. In all of the above fungi *Helminthosporium spp.,* and *Cladosporium spp* were absent in non-sterilized seeds from PDA methods.

In case of non sterilized seeds in PDA methods highest frequency and deviation (72.66) was again recorded for *A.alternata* followed *by A.flavus* (71.33) where as in case of surface sterilized seeds fungal frequency was highest recorded for *F.moniliforme* (65.33) followed by *A.niger* (62.66). Lowest frequency (14.33) was recorded for

D.australiensis, however this fungus was absent in surface sterilized seeds (Fig B). Percentage of germination of seeds was 42% in external seed mycoflora and 48% in internal seed mycoflora were recorded in PDA method.

Similarly in Deep freeze method from non sterilized seeds highest frequency and deviation (37.33) was recorded for *A.alternata* followed *by A.niger* (31.33) where as in case of surface sterilized seeds fungal frequency again the highest 30.66 was recorded for *A.alternata* followed by *A.niger* (25.33). Lowest frequency (10.66) was recorded for *Drechslera spp* from non sterilized seeds and from sterilized seeds lowest frequency (0.00) was recorded from *Drechslera spp,* including *Helminthosporium spp.,* and *Cladosporium spp* (Fig C). Percentage of germination of seeds was 28% in non sterilized seed and 20% in sterilized seed were recorded in Deep freeze method.

In blotter method the fungi isolated and identified were 12 from the untreated seeds of variety **WH896**.Similarly from the PDA method 9 fungi were isolated and identified [28].

Deep freeze method provided very low germination due to frozen or dead embryos. Results revealed that the deep freezing method was best for the isolation of deep seated and pathogenic fungi viz., *Fusarium* spp. while blotter method was found suitable for germination test and for isolation of *Aspergillus* spp. [25] isolated *Alternaria, Curvularia, Fusarium, Aspergillus,* and *Penicilium* spp., as major storage fungi from wheat grains.

Mycoflora were found to differ both in quantity and quality in different procedures. From Blotter method, 10 fungi from external seed surface and eight fungi from surface sterilized seed were detected in variety HD264. In case of external surface, the following fungi *Fusarium moniliforme, Rhizopus spp., Mucor spp., Alternaria alternata, Aspergillus niger, A. flavus, Curvularia lunata, Drechslera spp., Alternaria spp. and Penicillium spp.* were isolated [29].

It was also evident from these results that inoculum pressure can be directly associated with the intensity of disease development since in our experiment highest frequency of fungal flora was recovered from the variety PBW343 that also showed minimum germination. [7] also reported reduction in the germination of wheat seed due to fungi colonizing during storage. [19] also isolated *Aspergillus* spp., *Penicillium,* spp., followed by *A. alternata* from 50% samples of the stored wheat seeds.

These results clearly show that the seed surface of wheat is adhered by a large number of fungal species which can be reduced by surface sterilization of seeds before planting. The reduction of frequency rate of fungi from sterilized sunflower seeds was also found by [33]. Seed surface sterilization with 0.1 or 0.2% (w/v) $HgCl_2$ for 3 min significantly decreased *Alternaria alternata, Fusarium spp*. and *Epicoccum purpurescens*; however, [30] observed that the surface disinfection of seed with 1% NaOCl reduced the incidence of *Aspergillus spp., Chaetomium spp., Cladosporium spp., Rhizopus spp. and Cephalosporium spp*.

Surface sterilization also has the advantage of minimizing competition among fungi on the seed [14]. [21] at Serbia made a study on seed-borne fungi of some cereals. He isolated a total of 41 species of fungi from seed samples of barley, maize, soybean, and sunflower. The majority of detected species occurred on barley (35 of 41 species or 87.8%) comparing to soybean (17 of 41 species or 41.5%), sunflower (16 of 41 species or 39.0%) and maize (15 of 41 species or 36.9 %).

From these finding it is concluded that the seed health testing is a primary need to avoid crop failure. Various seed health testing methods and techniques provide information about the health condition of the seeds, it determines the quality of the seeds and about the associated pathogens which can cause a crop failure and also provide an inoculum for further spread of the pathogen and discuses to the new areas. Thus, such studies, methods and techniques are prerequisite for the production of enough food and feed and for the development of a prosperous country.

Table 1: Screening of five wheat varieties by blotter method (Observations based on 200 seeds)

SN	Wheat varieties	Total no of seeds studied	Isolated Fungi	No of infected grain with fungi	Percentage of infection
1	WH896	200	*A.niger,*	83	41.5
			A. flavus	31	15.5
			F.moniliforme	45	22.5
			A. alternata	67	33.5
			C. lunata	29	14.5
2	HD264	200	*A.niger,*	37	18.5
			A. flavus	41	20.5
			F.moniliforme	27	13.5
			A. alternata	29	14.5
			C. lunata	9	4.5
3	PBW502	200	*A.niger,*	13	6.5
			A. flavus	11	5.5
			F.moniliforme	19	9.5
			A. alternata	7	3.5
4	HD273	200	*A.niger,*	5	2.5
			A. flavus	7	3.5
			F.solani	11	10.5
			A. alternata	15	7.5
5	PBW343	200	*A. alternata,*	123	62.5
			F.moniliforme,	93	46.5
			F. solani,	137	68.5
			R. oryzae,	105	52.5
			A.niger,	73	36.5
			A. flavus,	121	60.5
			D.australiensis,	47	23.5
			Penicillium spp.,	49	24.5
			B.sorokinia	57	28.5

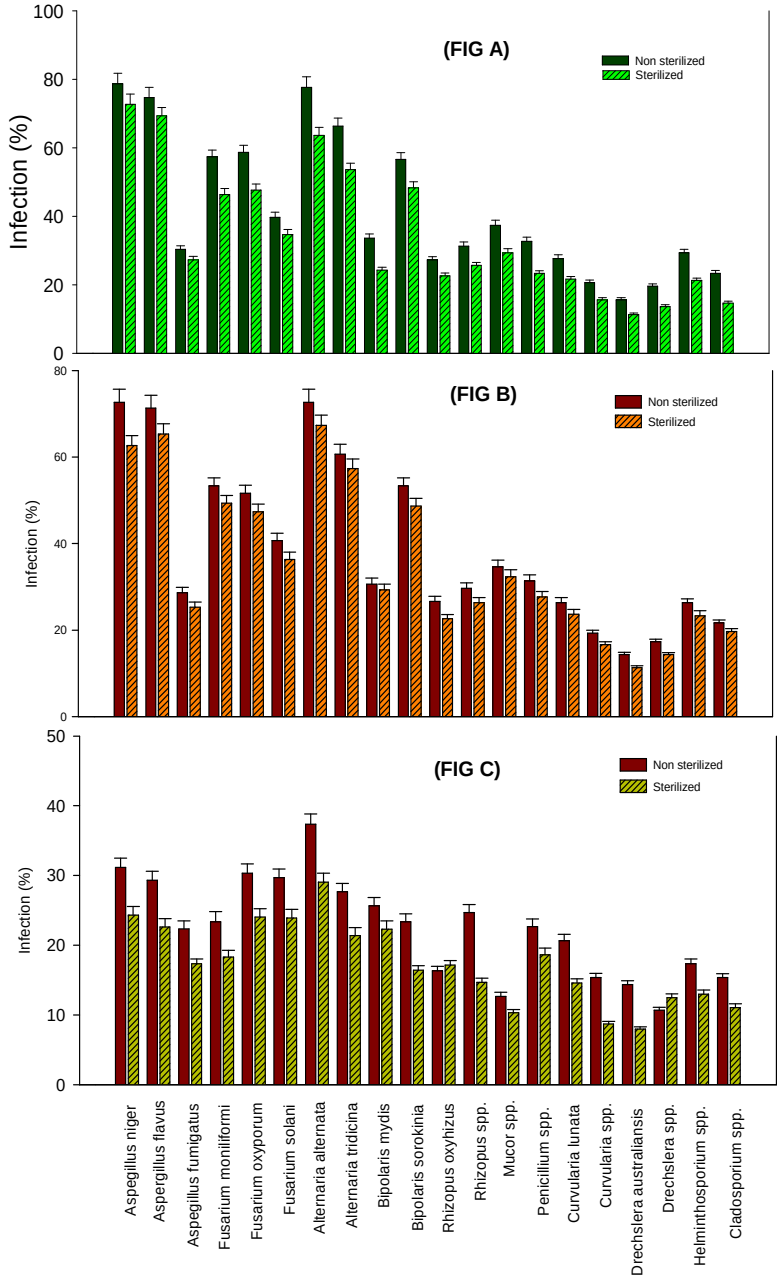

FIG A - BLOTTER METHOD, FIG B - PDA METHOD, FIG C - DEEP FREEZE METHOD SHOWING FRQUENCY OF OCCURRENCE OF SEED BORNE FUNGI.

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