Isolation of Seed borne Pathogens associated with Some Cereal grains in Khartoum State (Sudan)

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Abstract- Seed borne fungi present on or inside the seed or as contaminant with the seed debris adversely affect seed viability, germination, emergence, plant growth vigour and eventually production and productivity. In the present study, seed borne fungi of 4 imported cereal seeds (Maize and Wheat) were examined and recovered from the seeds. The seeds were highly contaminated with Four fungal species (*Aspergillus*, *Penicillum*, *Alternaia* and *Rhizopus*) were detected in (Maize and Wheat) In maize *Aspergillus* recorded the highest fungi percent in Filter paper (28.67%) and Agar method (28.33%) Followed by *Penicillium*, *Alternaria* and *Rhizopus* (26.67,17.33 and5.33) respectively. The genus *Aspergillus* and *Penicillum* were the most prevalent genera followed by *Alternaria* and *Rhizopus.*

Therefore, there is urgent need for development of proper Standard laboratory seed testing methods, fungal eradication measures, and adopting strong legislations and quarantine regulations. The use of certified and high grade seeds is a priority.

Index Terms- wheat, maize, seed pathogens, mycoflora

I. INTRODUCTION

Cereals can be defined as a grain or edible seed of the grass family Graminaceae (Bender & Bender, 1999). Cereals are grown for their highly nutritious edible seeds, which are often referred to as grains. Some cereals have been staple foods both directly for human consumption and indirectly via livestock feed since the beginning of civilization (BNF, 1994). Cereals are the most important sources of food (FAO, 2002), and cereal based foods are a major source of energy, protein, vitamins and minerals for the world population. Generally, cereals are cheap to produce, are easily stored and transported, and do not deteriorate readily if kept dry.

Wheat is a major cereal crop in many parts of the world. It belongs to the *Triticum* family, of which there are many thousands of species (Kent & Evers. 1994).It is grown as both a winter and aspring cereal and, owing to the number of species and varieties and their adaptability, it is grown in many countries around the world. The great wheat-producing countries of the world include the USA, China and Russia; extensive wheat growing occurs in India, Pakistan, the European Union (EU), Canada, Argentina and Australia. It is estimated that 556.4 million tons of wheat will have been produced in 2003, accounting for 30% of the world's cereal production (FAO. 2003). Wheat plants are largely susceptible to various pests and diseases (Prescott *et al*. 2006).

The maize kernel (the reproductive seed of the plant) has four main parts – the germ, the endosperm, the pericarp and the tip cap. Production in the USA exceeds that in any other country (Fast & Caldwell 2000). Therefore, the present study aim to detect and identify seed borne fungi associated with seed of cereals grain (via Wheat and Maize).

II. MATERIALS AND METHODS

1. Study location

This study was conducted in the laboratory of plant pathology department of plant protection, college of agricultural studies, Sudan University of science and technology during February –April, 2016.The aim of this study was to detect and identify seed borne fungi associated with seed of cereals grain (Wheat and Miaze) collected from three different locations in local market of Khartoum (Al hagyousif, Khartoum bahri and Omdurman) stored since season2015.

Collections of samples

The cereals seed stocks on shells were obtained from grains market seed stocks in each location(Al haguosif, Khartoum bahri and Omdurman).The maizeand wheat, , samples were obtained from on random and homogeneous sample of five kilo grams was secured from each of the three locations in the market .The seed samples were drawn according to international standers for seed testing association (ISTA, 1966). Collected samples were labeled and kept separately in sealed paper bag and transformed to the laboratory where they were stored at 5^{o}c refrigerator for further investigations.

All materials except seeds, which used in the experiments, were sterilized using 70% ethyl alcohol. Cotton blue and lacto phenol were used for staining of the fungal cytoplasm and for providing a light blue background, against which the walls of hyphae can readily be seen according to Aneja (2004).

Detection and isolation of seed borne fungi

Dry seed inspection

A sample of 400 seeds of each seed sample were randomly selected and examined under microscope and by magnified lens and naked eye according to the international seed testing association (ISTA) (Rules,1966). The samples were examined for impurities, plant debris, weed seed, discoloration and malformation.

Methods for the detection of seed borne fungal pathogens

The seed samples were tested by the standard filter paper and agar methods for fungi identified and their percentage of occurrence was calculated by applying following formula:

Pf= (No. of seeds on which fungus appear/Total number of seeds)x100.

All the detection methods of seed borne fungi as described by ISTA. The normal and discolored seeds were tested separately for seed borne fungi.

Blotter method

For the detection of seed borne fungi, standard blotter method as described by the international seed testing association (ISTA, 1996), was used for the detection of the seed-borne fungi associated with each seed samples. All the samples were platted on moisture filter papers (dia-9.0.cm) in 9.0cm sterilized plastic petri-dishes. five seeds were plated from each sample, 3arranged at the periphery of plate and 2 at the center, each sample was replicated four times and then kept in dark place for seed germination after seven days of incubation, seeds were the examined for fungal growth under steromicroscope. Fungi identification by habit character was supplemented by microscopic examination of spores and fungi bodies using acompoundmicroscope. Other identification used method according to were Agarwal *et al*., (1989); Buregers *et al*.,(1994)., Mathursk, SD Mathur, PNeergard (1975)., and Mathur and Kongsdal (2003). In cadence levels were recorded as the percentage of infected seeds in a sample.

Agar method

All seed samples was pre-treated with sodium hypochlride (NaOCl)1% for 5minutes then washed three times with sterilized distilled water(SDW) and dried between tow filter papers. The seed samples, (five of each crop) seeded in PDA medium (Potato Dextrose Agar), in sterilized glass petri-dishes.

The plates were incubated for seven days in incubator at 25C°. Then the seeds were examined under light microscopes using sli

Slide preparation and identification

The samples of fungus were taken randomly from each crop samples. These samples were identified on the basis of colony characteristics and microscopic examinations standard books and research paper were consulted during the examination of those fungi (Aneja, 2004.,Barntt and Hunter, 1999., and Rifai,.1969). The binocular compound microscope was also used to determine the type of fungus in each plate.

Data analysis

All the collected data were determined by Analyses of Variance (ANOVA) using a completeiy randomized desing. The significance ($p<0.05$) of differences between treatments were determined, using the Duncan's Multiple Range (DMR) test of Statistical Analysis.

III. RESULT AND DISCUSSION

This study was conducted under laboratory conditions, during February –April, 2016 to investigate the occurrence seed borne mycoflora associated with seeds samples of four food crops collected from three local markets in Khartoum State in Sudan. The methods used in detection that Dry inspection, Blotter method and agar plate method,

4.1 Dry Seed Inspection

Dry inspection of the Cereal grains collected from different areas of Khartoum state discolored seeds. Besides that, impurities were mixed with all seed samples. These were identified as dust particles, stones, pieces of straw and plant debris. However, with the exception of the few discolored seeds no disease like symptoms was observed in the dry inspected seeds.(Table 1)

Table 1. Dry inspection for Maize and Wheat crops

Crop	Healthy	Unhealthy (%)	Malformation seeds (%)	Plant debris (%)	Total (%)
Maize	73	22	5	0	100
Wheat	70	15	9	6	100

Blotter method and agar plate method in maize

Two samples of cereal grain collected from seed market, fungi isolated include Aspergillus, Penicillium, Alternaia, Rhizopus.Table (2),plate (1and 2).Four fungal species (Aspergillus, Penicillum, Alternaia and Rhizopus) were detected in Miaze. The fungi Aspergillus recorded the highest fungi percent in Filter paper (28.67%) and Agar method(28.33%) Followed by Penicillium, Alternaria and Rhizopus(26.67,17.33and5.33) respectively

Table 2. Fungal genera obtained from Maize grains collected from study area

Method	Filter Papper			Agar Method		
Fungi	MIAZEB	MIAZEH	MIAZEO	MIAZEB	MIAZEH	MIAZEO
Aspergillus	42.00^{a}	28.67^{a}	22.00^{a}	28.33^{a}	28.33^{a}	11.00^{a}
Penicillum	11.67^{b}	23.00^{ab}	26.67^{a}	15.33^{a}	13.00^{ab}	1.33^{a}
Alternaria	17.33^{b}	14.33^{ab}	15.33^{ab}	15.33^{a}	5.67^{b}	1.33^{a}
Rhizopus	0.00^{b}	0.00^{b}	0.00^{b}	5.33^{a}	3.33^{b}	0.67^{a}
CV%	9.20	9.88	6.05	19.08	9.95	23.07
SE±	8.58	12.11	7.32	24.96	9.65	6.82

Key:
O= Omdurman
H= Al hagyousif
B= Khartoum bahri

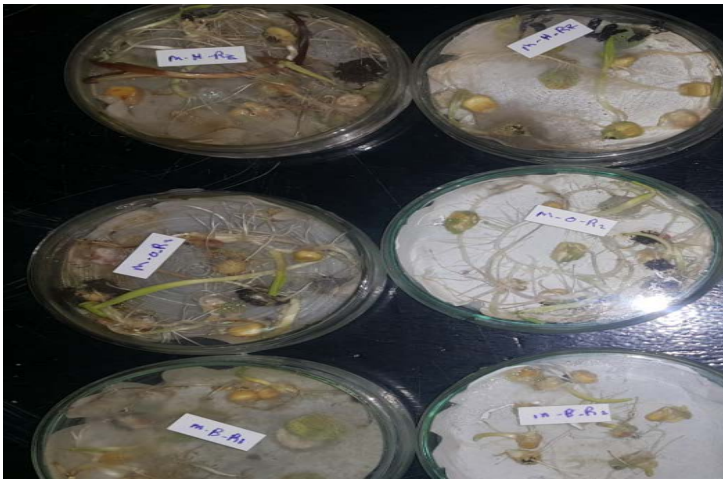

Plate 1. Seeds tested by the filterpapper for maize seeds

Plate 2. Seeds tested by the Blotter method for maize seeds

Detection of Fungi from wheat Seeds

Fungi species detected from wheat seeds are shown in Table (3).The most common fungi species detected from the wheat seeds is *Aspergillusniger* whether tested by the Blotter or Agar methods. Other fungi detected include Penicillium, Rhizopus and Alternaria (Plate 3 and 4)

Table 3. fungal genera obtained from Wheat grains collected from study area

Method	**Filter Papper**			**Agar Method**		
Fungi	**Wheat B**	**Wheat H**	**Wheath O**	**Wheat B**	**Wheat H**	**Wheath O**
Aspergillus	11.67[a]	8.00[a]	18.00[a]	27.33[a]	21.33[a]	19.67[a]
Penicillum	10.67[a]	1.33[b]	15.33[a]	12.33[ab]	9.00[ab]	18.67[ab]
Alternaria	3.67[a]	0.33[b]	11.00[a]	0.33[b]	4.33[ab]	7.67[ab]
Rhizopus	0.00[a]	0.00[b]	0.00[a]	0.00[b]	0.33[b]	3.33[b]
CV%	10.14	30.31	12.09	12.08	11.10	7.64
SE±	5.69	2.57	10.14	10.38	7.94	6.81

Key:
O=Omdurman
H=Al hagyousif
B=Khartoum bahri

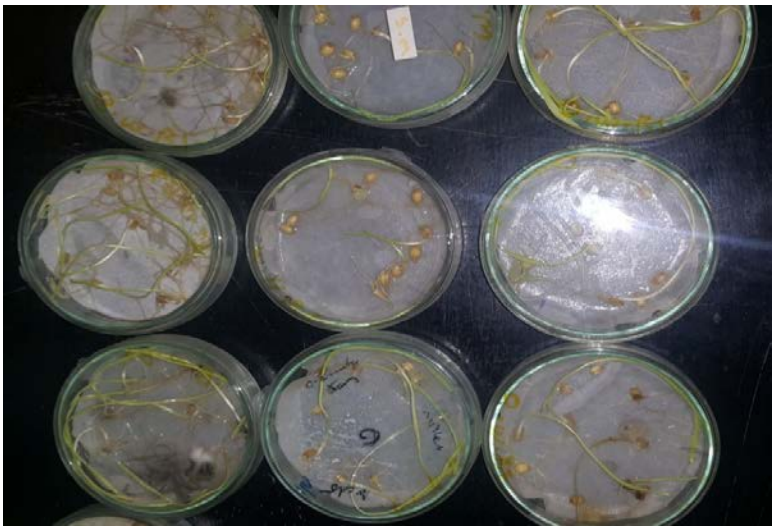

Plate 3. seeds tested by the Blotter test for wheat seeds

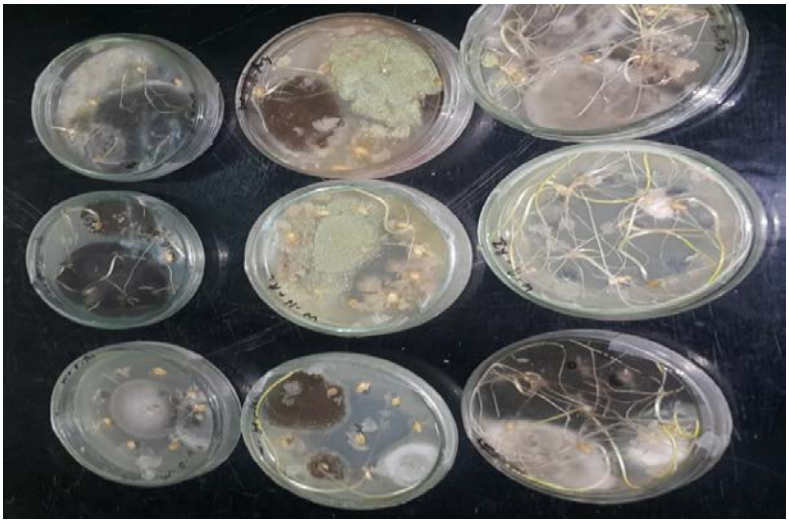

Plate 4. seeds tested by Agar plate method for wheat seeds

IV. DISCUSSION

This study was conducted in the laboratory of plant pathology department of plant protection, college of agricultural studies, Sudan University of science and technology during February –April, 2016.The aim of this study was to detect and identify seed borne fungi associated with seed of cereals

grain(Wheat and Miaze) collected from three different locations in local market of Khartoum(Al hagyousif, Khartoum bahri and Omdurman) stored since season 2015.The dry inspection tests revealed the presence of a few (less than1%) discolorated seeds. In similar tests Neegaard (1977) reported that dry seeds may show symptoms in varying degrees due to necrosis or discoloration from stain produced by various seed-borne micro-organisms. The results of the present study revealed a high incidence of seed-borne *Aspergillus niger* and A. flavus. Working with the seeds of sorghum in the Sudan, Abuagla (2001)also reported high incidence of A. niger and A. flavus. Moreover, a high incidence of A. niger and A. flavus from seeds stored in high temperature. In comparison of the different methods used in the present study the Agar plate tests gave excellent mycelial growth and conidial sporulation of the fungi detected. As explained by Mrs. Awatif (Personal Communication) such results are expected as the agar media provide the necessary nutrients for the growth of the fungi. The results of the present work also revealed that beside A. nigerother less prevalent fungi include Pencilliumsp, Alternaria and Rhizopus. As previously stated by Malone and Muskett (1964) and Reddy (1987) such results are expected from seeds stored under high temperature and high humidity conditions. Hence, the need for proper storing, seed health testing and seed sterilization should be a routine practice before sowing of above seeds.

V. Conclusion

1. In view of the abundant seed contamination reported in the present study and in other previous studies it is high time that the seed health testing should be applied before sowing both local and imported seeds.
2. The result obtained in this study showed the need for further research and investigation to provide a satisfactory explanation to these results.

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