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RESEARCH ARTICLE

INCIDENCE OF SEED BORNE MYCOFLORA ON MAIZE AND ITS EFFECT ON SEED GERMINATION

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ABSTRACT

Seed-borne fungi are a serious problem during storage in India. Seed infection reduces seed viability, productivity and food value. Maize is one of the most important cereal crops in the world. It has a nutritional value for both animals and humans. Isolation of seed borne mycoflora of maize varieties was studied by using blotter and agar plate methods. During isolation eight fungal species were recovered. Agar plate method yielded highest number of fungi as compared to blotter method. Frequency occurrence of *Aspergillus niger* and *A. flavus* was very high in all varieties of maize. Infection percentage was higher in non-surface sterilized seeds as compared to surface sterilized seeds. Germination percentage was same in both surface sterilized and non-surface sterilized seeds.

INTRODUCTION

India is one of the world's largest agricultural countries. Seed-borne fungi are a serious problem during storage in India. Seed infection reduces seed viability and productivity in crops, as well as adds contamination to the edible grains. Maize is one of the most important cereal crops in the world. Maize is grown all over the world, in more than 100 countries. It is one of the major crops in America, Africa and Asia. Varieties of Maize are Sweet corn, Dent corn, Flint corn, Popcorn, Flour corn and Pod corn. In India considerable work has been done for development of specialty corns such as sweet corn, pop-corn, baby-corn, quality protein maize with high lysine and tryptophan, green-eared corn, high oil corn, waxy-corn, fodder maize, etc. Hybrid maize is one the variety of maize which generally has a high yield level and that is why it is most favored by the farmers. The importance of Corn is due to its wide diverse use. It is used both as food for human and feed for animals (Krishnamurthi, 1969). Corn is directly consumed as feed and as an edible table vegetable. It is also fractionated by either dry or wet milling into food and industrial ingredients. Starch, the major constituent of the corn kernel, is used in its native form or after chemical or enzymatic modification, in foods and industrial products. Starch is also converted into glucose or fructose for use as food sweetener. Glucose can be fermented in to ethanol for beverages or into many other chemicals. Recently, it has been discovered that maize or corn can also be used in the production of fuel. During harvesting and storage, seeds may be infested by various pathogenic fungi and that often reduce both the yield and the quality of grains. Literature review indicated that fungi play an important role in the deterioration of stored grains. Seed borne fungi cause large destruction of agricultural crops. Infected seeds become intoxicated, affect germination and seedling growth in various crop and plants. Lot of seed is wasted due to contamination and it also creates pollution. This problem is very serious globally including India. To understand the nature and severity of the effect of the seed borne fungal flora, especially on germination of seeds an attempt was made to isolate the fungi associated with the grains.

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MATERIALS AND METHODS

Collection of seed samples: The seed samples were collected from store houses, market places and from farmers and preserved in cloth bags at room temperature during the studies as described by Neergaard (1973).

Determination of seed Mycoflora: Blotter technique (ISTA, 1976) and agar plate methods using five different media like Potato Dextrose Agar (PDA), Malt extract agar (MEA), Czepek's Dox agar (CZA), Rose Bengal agar (RBA) and Seed extract agar (SEA) was carried out. For isolation of seed borne mycoflora, pre-sterilized corning glass Petri-plates of 9 cm diameter were poured with 25 ml of autoclaved medium. 05 seeds per Petri-plate were spaced at equal distance aseptically. Fifty seeds were tested for each treatment. The plates were incubated at room temperature (25 ± 2^0c) under natural day night conditions. Seeds were examined after seven days of incubation and fungi were identified under microscope.

Seed sample was divided into two groups. The first group was untreated seeds and was used directly for determination of surface borne mycoflora of seeds. The second group was surface sterilized by soaking in 0.1 % solution of $HgCl_2$ for 2 minutes, subsequently thoroughly washed twice with sterile distilled water and placed on agar plates and blotter test method plates for determination of seed borne fungi. (Embaby *et al*., 2006; Amer Habib *et al*., 2007). The incidence (%) of dominant mycoflora was calculated as follows :- (Gachande and Mukadam, 2001).

$$\text{percentage Incidence} = \frac{\text{Number of seeds bearing fungal colonies}}{\text{Total number of seeds examined}} \times 100$$

Frequency (fungal occurrence) was calculated as follows :- (Embaby *et al*., 2006).

$$\text{Fungal frequency} = \frac{\text{Total count of fungal species (TC)}}{\text{Sum of Total count of all occurred fungal species}} \times 100$$

RESULTS AND DISCUSSION

Determination of seed Mycoflora

Eight species of fungi were found to be associated with seeds of three different maize varieties like Amber, Local, and African Tall (Table 1). *Aspergillus flavus, Aspergillus niger, Aspergillus fumigatus* and *Fusarium moniliforme* were isolated from blotter technique from Local variety. *Aspergillus flavus, Aspergillus oryzae, Aspergillus niger, Aspergillus terreus, Fusarium moniliforme* and *Penicillium sp.* were isolated on PDA, *Aspergillus flavus, Aspergillus niger, Aspergillus terreus, Fusarium moniliforme* and *Fusarium solani* were found on MEA. CZA medium recovered all above mentioned fungi except *Aspergillus oryzae* and *Penicillium* sp. For African tall variety *Aspergillus flavus, Aspergillus niger, Aspergillus terreus, Fusarium solani* and *F. moniliforme* were isolated from on MEA, *Aspergillus flavus, Aspergillus oryzae* and *Aspergillus niger* occurred on RBA and *Aspergillus flavus, Aspergillus niger* and *Aspergillus terreus* on SEA and PDA. *Penicillium* sp. was isolated only on CZA. *F. moniliforme, Aspergillus flavus, Aspergillus oryzae, Aspergillus niger* were isolated by blotter technique. Amber variety yielded *Aspergillus flavus, Aspergillus niger* and *Aspergillus terreus* by blotter method, *Aspergillus flavus, Aspergillus niger, Aspergillus terreus, Aspergillus fumigatus, Fusarium moniliforme, Fusarium solani* and *Penicillium sp.* on MEA, *Aspergillus flavus, Aspergillus niger, Aspergillus terreus, Aspergillus fumigatus* and *Fusarium moniliforme* on RBA and only *Penicillium sp.* was recovered from CZA medium. No fungal species were recovered on SEA medium. PDA, MEA, CZA, RBA were found to be more favourable for the growth of seed borne fungi. SEA and blotter technique were less favourable for the isolation of seed borne fungi (Table 1). Similar results have been reported by other workers (Noble and Richardson, 1968; Esuruoso *et al*., 1975; Neergaard, 1977; Abou-Heilah, 1984; Al Kassim, 1996; Al Kassam and Monawar, 2000). The present results show that, potentially, all seeds of the tested maize varieties were liable to attacks by the seed-borne fungi. Many of the isolated fungi were reported to be toxigenic, carcinogenic and mutagenic in animals and humans (Abramson. 1997; Eriksen G.S. and Aleksander, 1998; Pieckova and Jesenka, 1999; Baliukoniene *et al*., 2003). Bakan *et al*. (2002); Verga *et al*. (2005) and Ishrat and Dawar, (2009) reported *Fusarium* and *Aspergillus* species as the common toxic fungal contaminants of maize where as Montes *et al*. (2009) reported *Fusarium*, *Penicillium*, and *Aspergillus* species as major fungi encountered in maize.

Fungal frequency on maize seeds: (Table – 2)

Total 260 isolates were recovered from 3 varieties of maize. Amber variety yielded 51 isolates, Local variety yielded 127 isolates, and African Tall variety yielded 82 isolates. Frequency of occurrence was highest in Local variety followed by African Tall variety and then by Amber variety. *Aspergillus flavus* and *Aspergillus niger* were the most frequently occurring species and isolated from all varieties of maize.

Table 1. Mycoflora associated with seeds of maize varieties

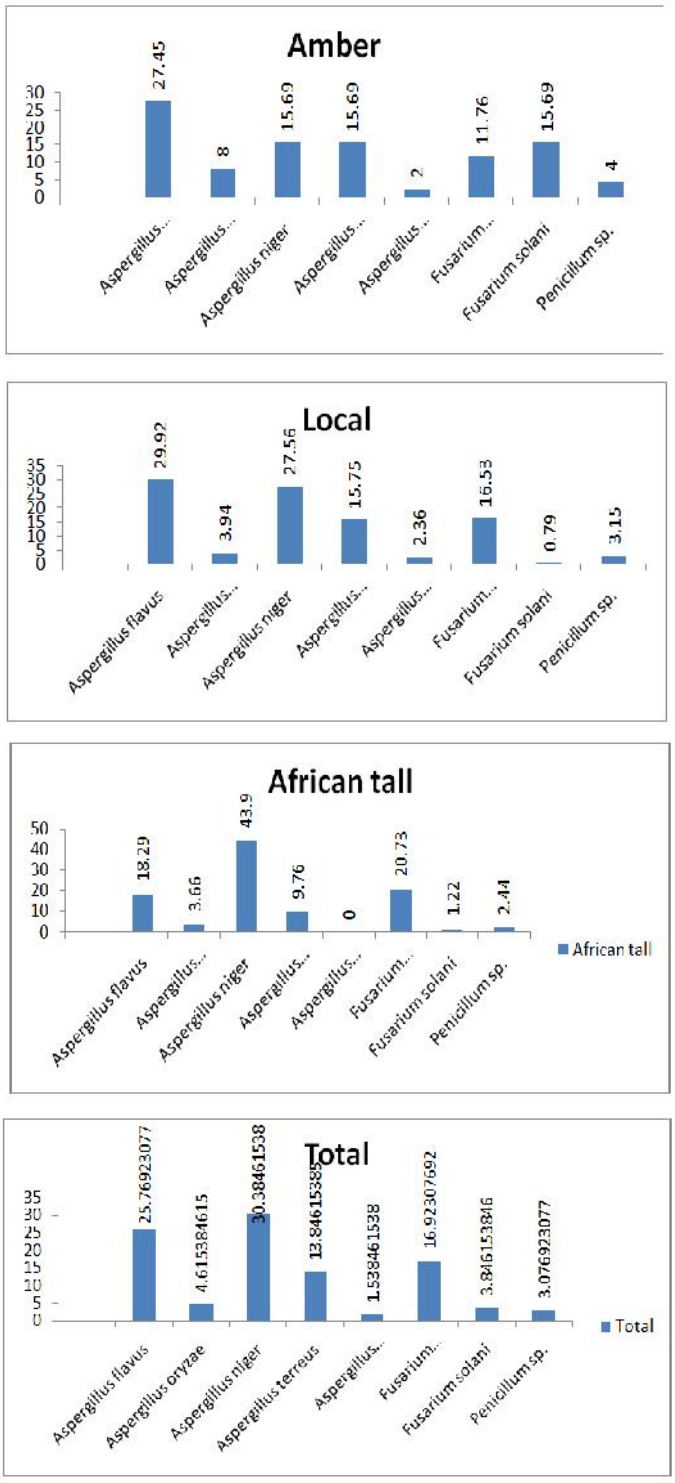
S.No.	Name of the fungus	BLOTTER			MEA			CZA			RBA			SEA			PDA		
		A	L	T	A	L	T	A	L	T	A	L	T	A	L	T	A	L	T
1	*Aspergillus flavus*	*	*	*	*	*	*	–	*	–	*	*	*	–	*	*	*	*	*
2	*Aspergillus oryzae*	–	–	*	–	–	–	–	–	–	–	–	*	–	–	–	*	*	–
3	*Aspergillus niger*	*	*	*	*	*	*	–	*	–	*	*	*	–	*	*	*	*	*
4	*Aspergillus terreus*	*	–	–	*	*	*	–	*	–	*	*	–	–	*	–	*	*	*
5	*Aspergillus fumigatus*	–	*	–	*	–	–	–	*	–	*	–	–	–	–	–	–	–	–
6	*Fusarium moniliforme*	–	*	*	*	*	*	–	*	–	*	*	–	–	–	–	*	*	–
7	*Fusarium solani*	–	–	–	*	*	*	–	*	–	–	–	–	–	–	–	*	–	–
8	*Penicillium sp.*	–	–	–	*	–	–	*	–	*	–	–	–	–	–	*	–	*	–

* Present A – Amber L – Local T – African tall

Table 2. Frequency (fungal occurrence) of seed born fungi of maize varieties

Sr. No.	Name of the fungus	Amber		Local		African tall		Total	
		TC	%	TC	%	TC	%	TC	%
1	*Aspergillus flavus*	14	27.45	38	29.92	15	18.29	67	25.76923077
2	*Aspergillus oryzae*	4	8	5	3.94	3	3.66	12	4.615384615
3	*Aspergillus niger*	8	15.69	35	27.56	36	43.9	79	30.38461538
4	*Aspergillus terreus*	8	15.69	20	15.75	8	9.76	36	13.84615385
5	*Aspergillus fumigatus*	1	2	3	2.36	0	0	4	1.538461538
6	*Fusarium moniliforme*	6	11.76	21	16.53	17	20.73	44	16.92307692
7	*Fusarium solani*	8	15.69	1	0.79	1	1.22	10	3.846153846
8	*Penicillium sp.*	2	4	4	3.15	2	2.44	8	3.076923077
	Total ----------	51	100	127	100	82	100	260	100

Frequency of *Aspergillus flavus* was 27.45 % in Amber variety, 29.92% in local variety, 18.29 % in African Tall variety and total frequency percentage was 25.77. Percent frequency of *Aspergillus niger was* 15.69 % in Amber *variety*, 27.56 % in Local variety, 43.90 % in African Tall variety and total frequency was 30.38 %.



Graph 1 - Mycoflora associated with seeds of maize varieties

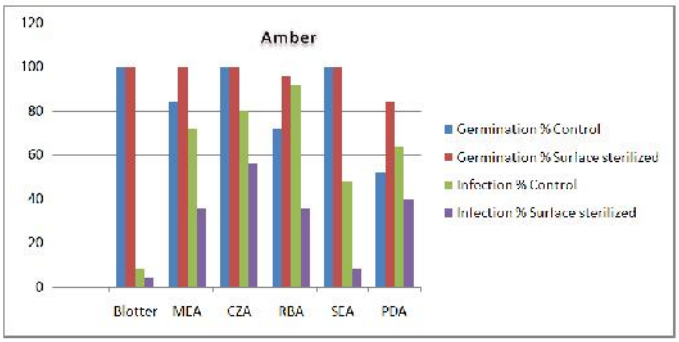
Aspergillus oryzae, *Aspergillus fumigatus*, *Penicillium* sp. And *Fusarium solani*occurred in lesser frequency in all varieties of maize. Frequencies of *A. terreus* and *F. Moniliforme* were moderate. Ishrat Niaz and Shahnaz Dawar (2009) reported that *A. niger*, *A. flavus*, *A. wentii*, *Fusarium oxysporum*, *F. solani*, *Penicillium citrinum*, *Macrophaminaphaseolina* and *Rhizopus oryzae* as the most frequent species. Similar results were obtained in different plant seeds by Ibeth *et al*.1991; Tseng *et al*.1995; Ruiz *et al*. 1996; Embaby *et al*., 2006. Montes *et al*. (2009) reported infestation of *Fusarium*, *Penicillium* and *Aspergillus* species in northern Mexico maize.

Germination and infection percentage of maize varieties

Amber variety: - (Table 3A):100 % seed germination was recorded in Amber variety under surface sterilized and non-surface sterilized grains when studied on CZA and SEA medium and blotter test. Germination percentage of non-sterilized grains was recorded 52 % to 84 % and 84% to 100% for surface sterilized grains on MEA, RBA and PDA.

Table 3A. Germination and infection percentage of amber variety on different media

Variety : Amber								
Media	Germination				Infection			
	Control		surface sterilized		control		surface sterilized	
	TC	%	TC	%	TC	%	TC	%
Blotter	25	100	25	100	2	8	1	4
MEA	21	84	25	100	18	72	9	36
CZA	25	100	25	100	20	80	14	56
RBA	18	72	24	96	23	92	9	36
SEA	25	100	25	100	12	48	2	8
PDA	13	52	21	84	16	64	10	40



Graph 3A: **Germination and infection percentage of amber variety on different media**

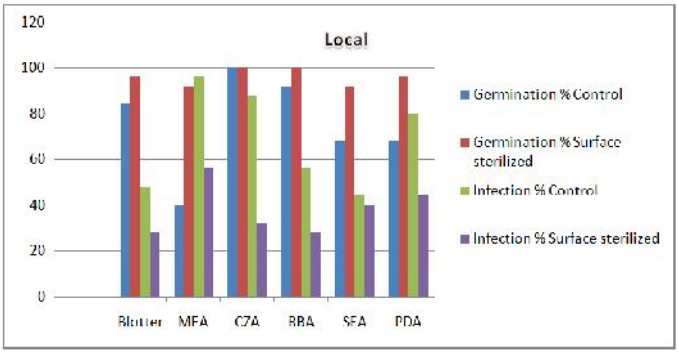
Infection percentage was recorded 08% to 92% in untreated seeds and 40% to 56% in surface sterilized seeds. Thus infection percentage appeared to decrease when seeds were surface sterilized.

Table 3B. Germination and infection percentage of local variety on different media

Variety : Local								
Media	Germination				Infection			
	Control		surface sterilized		control		surface sterilized	
	TC	%	TC	%	TC	%	TC	%
Blotter	21	84	24	96	12	48	7	28
MEA	10	40	23	92	24	96	14	56
CZA	25	100	25	100	22	88	8	32
RBA	23	92	25	100	14	56	7	28
SEA	17	68	23	92	11	44	10	40
PDA	17	68	24	96	20	80	11	44

Local variety: -(Table 3B):100 % germination was recorded under surface sterilized and non-surface sterilized condition on CZA. Germination was recorded between 40% to 92% when seeds were non-surface sterilized and 92% to 100% for surface

sterilized grains on blotter test, MEA, RBA, SEA and PDA. Infection percentage in non-surface sterilized seeds of Local variety was recorded from 44% to 96% and 28% to 56 % in surface sterilized seeds in all experiments.

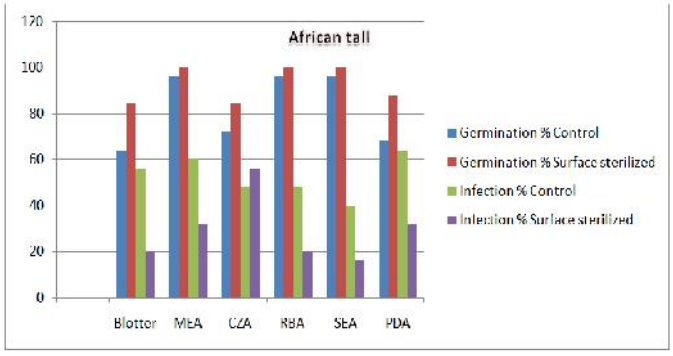


Graph 3B. Germination and infection percentage of local variety on different media

African Tall: (Table 3C): Germination was recorded 64 % to 96% when seeds were non-surface sterilized and 84% to 100% when seeds are surface sterilized.Infection percentage in non-surface sterilized seeds of African Tall variety records 40% to 64% while surface sterilized seeds it was 16% to 56% as studied by different methods.

Table 3C. Germination and infection percentage of african tall variety on different media

Variety : African tall								
Media	Germination				Infection			
	Control		surface sterilized		control		surface sterilized	
	TC	%	TC	%	TC	%	TC	%
Blotter	16	64	21	84	14	56	5	20
MEA	24	96	25	100	15	60	8	32
CZA	18	72	21	84	12	48	14	56
RBA	24	96	25	100	12	48	5	20
SEA	24	96	25	100	10	40	4	16
PDA	17	68	22	88	16	64	8	32



Graph 3C. Germination and infection percentage of african tall variety on different media

Data in Table 3A, 3B and 3C shows that surface sterilized seeds led to higher percentage of germinated seeds in all varieties of maize and infection percentage was reduced when the seeds were surface sterilized. Though the germination percentage was found to be high in both seed groups the seedling development appeared to be reduced due to associated mycoflora of seeds. The higher germination percentage may be attributed to the ability of fungi to produce plant growth regulators (Ibatsam *et al*., 2013). The adverse effect of the fungi after germination may refer to the production of toxic substances by them at mycelia stage (Jalander and Gachande, 2012; Subramanyam 1991; Gupta and Chouhan 1970; Dharamvir 1973). The observations also suggest that more colonies were obtained from agar plate method as compared to blotter technique. Similar results were obtained by El-Nagerabi and El-Shafie (2000); Kumud *et al*. (2004); Embaby *et al*. (2006) who reported that for isolation of fungi agar plate was better method than blotter test. Their reports also propose that disinfected seeds gave less fungal species and colonies and gave higher percent of germination compared with non-disinfected seeds.

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