Mycoflora Of Maize (Zea Maize) At Different Locations In Hail Area-Saudi Arabia

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Abstract: Zea maize is one of the main cereals produced in Hail area (Saudi Arabia). The risk of mycotoxin contamination is related to mycoflora associated with corn kernel. This paper reports on isolation and identification of external and internal mycoflora of maize harvested in Hail area in 2006 – 2008. A mycological survey was carried out on 200 samples from two agricultural companies . Comparison between frequency and relative density of the prevalent genera and species was carried out. Genus Fusarium was the most prevalent component of the internal seed - borne mycoflora in the two companies, Aspergillus spp. was the most prevalent genus as external seed –borne mycoflora. The predominant species of the different genera were Fusarium moniliformme, Aspergillus flavus ,A. niger and Alternaria alternate.

Keywords: Zea maize, Seed - borne fungi, internal mycoflora, external mycoflora.

Introduction:

Maize (Zea mays L.) is a cereal crop widely cultivated throughout the world and greater weight of maize is produced each and every year than any other grain. The United States produces almost half of the world harvest whereas, other countries which grow maize are as wide spread as China, Brazil, France, Indonesia, Japan, Korea, Taiwan, Mexico, Malaysia, India, Colombia South Africa, and Egypt . These countries account for around 80% of total world production. In Saudi Arabia the production of maize was 40607 thousand tons on the production area was 6464 h., whereas in Hail area the production was 36735 ton on the production area 4313[1].The maize is also commonly known as corn. Major consuming Nations of corn are China and USA. There has been continuous increase in the consumption demand of corn mainly owning to increase in the demand from meat and starch sector. There is growing requirement of maize from poultry sector where it is being used as feed. In the presence of seed borne pathogens several types of abnormalities occur in the seeds. Such seeds are rejected by seed industries and for agricultural purposes. Considering the fact attempt has been made to study the maize seed mycoflora and their eco-friendly management. Seed borne mycoflora is one of the major components reducing the maize yield. Mycoflora associated with seeds both internally and externally are responsible for seed major step is to use disease free and certified seed [2]. Some of fungi species which are related to corn mostly belong to Fusarium spp. and Apergillus spp. There are many reports that indicate these fungi species produce dangerous mycotoxin which can be harmful for human health and animals [3]. However, considerable information about mycoflora seeds corn is available in some corn producer countries such as Argentina, South America, Canada and many other counties [4]. Fungi affect the quality of grain through increase in fatty acid, reduction in germination, mustiness and finally spoilage of grain.

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The importance of fungi is also due to production of toxins that causes health hazard in human and animals. Fungal development in grains is influenced by temperature humidity and period of storage. Survey of literature shows that a number of fungi viz., Alternaria alternata, Aspergillus spp., Bipolaris maydis, Fusarium moniliforme, Fusarium spp., Cephalosporium spp., Helminthosporium spp., Mucor sp., and Penicillium spp., have been reported from maize seed (5and 6). The aim of this study is to identify the isolated fungi associated with maize grains in Hail area , to determine the relationship between internal and external mycoflora and to establish the species of the genera which will record high distribution percentage.

Materials and Methods:-

Collection of Seed Samples: A total of 200 samples of maize grains were collected from two agricultural companies in Hail area (Nadic and Hatco companies) during 2006-2008. Samples were collected in sterile plastic bags and kept at 4°C. All the samples were subjected to mycological analysis.

Isolation of external fungi (Seed washing method): This test was used to study fungal inoculums located on the surface of maize seed. 50 g of seed samples were taken in a 200 ml beaker containing 50 ml sterilized distilled water and 1 to 2 drops of Tween 80, shaken for 10 min over a mechanical shaker. The suspended spores were concentrated by centrifugation at 3000 rpm for 15 min. [7]. From 1/10 and 1/100 dilution 0.1ml of the suspension was cultured on YGCA (Yeast Extract Glucose Chloroamphenicol Agar). The plates were incubated under altering periods of 12h darkness of day light at 28± 2°C for 4 -7 days. The fungal colonies that developed were counted and those of different species were subcultured on PDA (Potato Dextrose Agar medium) and then identified on the basis of morphology under microscope [8]

Identification of fungi:

Isolates of fungi were identified according to the following authorities: Fusarium spp., according to Nelson et al. [9]; Penicillium spp., Aspergillus spp., and other fungi according to Pitt and Hocking [10]. The isolation frequency (Fr) and relative density (RD) of species were calculated according to González et al. [11] as follows:

$$Fr\ (\%) = \frac{\text{No. of samples of occurrence of a species}}{\text{Total No. of samples}} \times 100$$

RD (%) = No. of isolated genus or species x 100
Total No. of isolated fungi

Statistical analysis:
Asymptotic tests for equality of proportions were used to compare internal and external frequencies and relative densities. [12], and the Fischer exact test was used to analyze possible differences in the isolation frequencies of fungal species. The analysis was performed by using software SPSS [13].

Results:
Comparison between relative density and frequency of external and internal mycoflora associated with maize grains, which were collected from two agricultural companies in Hail area revealed four genera such as Aspergillus, , Alternaria, Penicillium and Fusarium and are shown in table1. Based on the percentage frequency and relative density the members of genus Fusarium spp. were predominantly isolated from maize grains as internal mycoflora at all locations (Fr. range 8.0 - 10% and R.D. 2.5 -3.5 as external mycoflora and internal mycoflora Fr .22.1 – 45% and R.D. 10.8 – 25%) . The second most prevalent genus as internal mycoflora was Alternaria spp. (Fr.20 -27.5% and RD. 10.25 - 17.5%) as external mycoflora for internal mycoflora (Fr. 35-45% and R.D. 20%) . The most predominant external mycoflora of the mold was Aspergillus spp (Fr.27.5- 37.5 and R.D.15.13 – 23.8%) and for internal mycoflora relative density and frequency were slightly low (Fr. 16 – 18.4% and R.D. 12 – 15.3%). Penicillium sp. recorded the lowest value of external and internal mycoflors . Aspergillus spp. showed significant different at P=0.05 between frequencies of the different species, only, no significant difference between retative densities was shown (table 2.). Other genera isolated as significant components of the internal and external mycoflora included Fusarium spp., but no significant difference between the species of Alternaria spp. (table 3 and 4).

Table (1): *Average Frequency and Relative Density between External and Internal Mycoflora of Maize Grains at Different Locations in Hail area*

Genera of external mycoflora	Hatco		Nadic	
	R.D.%	Fr.	R.D.%	Fr.
Aspergillus spp.	23.8**	37.5 *	15.13 **	27.5 *
Penicillium sp.	10**	17 *	5.5**	15 *
Alternaria spp.	17.5**	27.5 *	10.25**	20*
Fusarium sp.	2.5**	8 *	3.5**	10 *
Total No. of isolates	102		82	
Total No. of grains	100		100	
Genera of internal mycoflora	Hatco		Nadic	
	R.D.%	Fr.	R.D.%	Fr.%
Aspergillus spp.	15.3**	18.4*	12**	16 *
Penicillium sp.	8.3**	16.7*	0**	0*
Alternaria spp.	20**	35*	20**	45 *
Fusarium sp.	10.8**	22.1*	25**	45*
Total No. of isolates	82		106	
Total No. of grains	100		100	

Fr: Frequency RD: Relative density
* Significant difference between Fr. of external and internal mycoflora at P= 0.05
** Significant difference between RD. of external and internal mycoflora at P= 0.05

Table 2: *Comparison Between Relative Density and Frequency of Different Species of Genus Aspergillus spp.*

Different Species of Aspergillus	External Fungi			Internal Fungi		
	R.D%	Fr.%	Total No. of Isolates	R.D%	Fr.%	Total No. of Isolates
A.flavus	54.1	75.5*	100	55.4	57.2*	57.7
A.niger	24.3	45.5*	45	31.4	32.7*	32.7
A.nidules	13.5	27.5*	25	6.3	6.7*	6.7
A.terrus	8.1	20.4*	15	6.3	6.7*	6.7

Fr: Frequency RD: Relative density
* Significant difference between Fr. of external and internal species of Aspergillus at P= 0.05
** Significant difference between RD. of external and internal species of Aspergillus at P= 0.05

Table 3: *Comparison Between Relative Density and Frequency of Different Species of Genus Alternaria spp.*

Different Species of Alternaria	External Fungi			Internal Fungi		
	R.D%	Fr.%	Total No. of Isolates	R.D%	Fr.%	Total No. of Isolates
A. alternata	47.4	55.5	45	76.8	89	70
A. raphani	52.6	65.5	50	23.9	36.6	22

Fr : Frequency RD: Relative density
* Significant difference between Fr. of external and internal species of Alternaria at P= 0.05
** Significant difference between RD. of external and internal species of Alternaria at P= 0.05

Table 4: *Comparison Between Relative Density and Frequency of Different Species of Genus Fusarium spp.*

Different Species of Fusarium spp.	External Fungi			Internal Fungi		
	R.D%	Fr.%	Total No. of Isolates	R.D%	Fr.%	Total No. of Isolates
F.moniliforme	62.1**	52*	18	83.1**	92.3*	98.4
F. oxysporum	0.00**	0.00*	0.00	4.6**	6.2*	10
F.poae	0.00**	0.00*	0.00	4.6**	6.2*	10

Fr: Frequency RD: Relative density
* Significant difference between Fr. of external and internal species of Fusarium at P= 0.05
** Significant difference between RD. of external and internal species of Fusarium at P= 0.05

Discussion:

It has long been noted that seed-borne fungal pathogens are responsible for reducing seed quality, protein and carbohydrate contents, reduction or elimination of germination capacity as well as seedling damage, which result in the reduction of crop yield [8 and 9] Over the last decades, many studies have been made to test and detect seed-borne diseases of maize throughout the world. In the present study, seed-borne mycoflora associated with local maize grains were isolated and identified. The predominant external mycoflora were Aspergillus niger and Aspergillus flavus , this results agreed with that reported by Sreenivasa [10] and [11] who stated that high degree of mould contamination in stored grains and animal feeds is a measure of their quality assurance. High level of contamination of Saudi maize with mold may be due to unsuitable conditions of storage for grains or may be due to or contaminated farm equipment or in the soil [12]. Surveys conducted worldwide also revealed that, A. flavus and A. niger were known to frequently contaminated maize and were able to produce mycotoxins such as aflatoxins [13]. These species are considered the most important fungus species because their spores are easily transmitted via seeds due to cracks [14]. Further, mycological analysis of maize samples for other seed borne fungi revealed that species of Fusarium (F. moniliforme, F.oxysporium and F. poae) were predominant as internal mycoflora. The most

predominant one was F. moniliforme, this results is agreed with that reported by Sreenivasa [15] who reported the occurrence of Fusarium moniliforme in preharvest maize ear and it was predominant in infected ear kernel. F. moniliforme is consider to be common Fusarium occurring in tropical and subtropical with corn in USA [15]. F. moniliforme is one of the dangerous fungi to human and animals because it produce toxic metabolites such as fumonisin which is considerd the main reason of various cancers in digestive system [16]. Species of the genera Atlernaria were predominant as internal mycoflora . Alternaria were saprophytic or weak parasitic fungi ,so they infect maize grains in the field or during storage so they are consider as pre-harvest and post-harvest infectors [17]. Similar to the findings of our study, [18] whom showed in their research that Alternaria spp. was the most prevalent fungi in harvested wheat, sorghum and maize dusts from Egypt [19].

Conclusion:

The study has provided for the first time, information on the external and internal mycoflora of freshly harvested corn kernels in Hail area. Fungi associated with corn that should be of concern due to their toxigenic potential include F.moniliform, F.oxysporium and F. poae, mold was detected as Aspergillus fiavus and A.niger other genux was Alternaria spp.This data is of immense value for assessing the possible health hazard in humans and animals upon consumption of such contaminated maize grains by toxigenic mould. The results of this study are highly useful for further studies on toxin producing fungi and their epidemiological significance in corn crops grown in Hail area and elsewhere of Saudi Arabia.

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