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Assessment of Aflatoxins, Aflatoxigenic Fungi and Mycoflora Associated with Cereals

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ABSTRACT

Aflatoxins are one of the most potent toxic substances that occur naturally. These are a group of closely related mycotoxins produced by fungal species such as Aspergillus flavus and Aspergillusparasiticus. Which commonly grow on foods and feeds. Sample of cereal seeds viz., Bajra, Jowar, Maize and Wheat were collected. Dilution plate and agar-plate methods were used for isolation of mycoflora associated with collected samples. All isolates of Aspergillus flavus obtained from collected samples were screened for their aflatoxigenic potential in SMKY liquid medium. In all fourteen different fungi were isolated from cereal seeds. Aspergillus flavus, A. niger, A. ochraceus, Aspergillus sp., Fusarium sp. and Penicillium sp. were stand out common seed infesting fungi, A. flavus was dominant in all fungi and its 70.1 % strains were aflatoxigenic. Highest percentage of aflatoxigenic fungi was recorded in Maize seeds (86.20%). Aflatoxin producing potentials of Aspergillus flavus obtained from Maize (14.38 ppm) followed by Bajra (11.94ppm), Wheat (11.63 ppm), and Jowar (11.11 ppm). Analysis of cereal grain seeds for its natural aflatoxin contamination revealed that 26.25 % samples were naturally contaminated with aflatoxin. Maximum concentration of aflatoxin $B_1$was detected in Maize seeds (27.87 ppb) followed by Jowar seeds (8.38 ppb), Wheat seeds (5.61 ppb) and Bajra seeds (5.25 ppb). Cereals contaminated with aflatoxin has poses a potential threat for the life of human and animal beings.

Keywords – Cereals, Mycoflora, Aspergillus flavus, aflatoxins.

INTRODUCTION

Aflatoxins are one of the most potent toxic substances that occur naturally. These are a group of closely related mycotoxins produced by fungal species such as *Aspergillus flavus* and *Aspergillus parasiticus*. Which commonly grow on foods and feeds (Chaudhary and Kolhe, 2022; Kolhe, 2016). The cereals are common and important staple food crops for the people of the Jalgaon District of Maharashtra State. Most of the cereal crops are mainly grown as rainfed in the queer weather conditions during Kharif (rainy) season.

It is evident from the literature that, the seeds of cereal crops like Bajra, Jowar, Maize and Wheat carry number of fungi as seed mycoflora both in field as well as during the storage. The seed mycoflora associated naturally with the grains is found to be responsible for seed deterioration and also poisoning the grains. Sorghum serves as a host for over 100 pathogens, including fungi, bacteria, viruses and nematodes (Thakur *et al.*,1997). These pathogens, individually or in combination, leads to considerable losses in yield and grain quality. Seeds/ grain infected with mould are also more likely to be contaminated with mycotoxins, and these metabolites can present health hazards to consumers (Alves *et al.,*2010; Kolhe,2016; Kolhe and Ingale., 2011). Considering these facts, studies on the seed-borne mycoflora of cereals and toxins production are carried out in the present research.

MATERIALS AND METHOD

Sample of cereals, Viz., Bajra - pearl-millet (Pennisetum typhoides Burm.), Jowar (Sorghum vulgare pers.), Maize-corn (Zea mays L.,) and Wheat (Triticum aestivum L.) were randomly collected from store houses, market places of Jalgaon District, Maharashtra during February - May 2019. To minimize the loss of water content, the samples were collected in a sterile polyethylene bagsand sealed, transferred without delay to the laboratory, kept at 4ºC until analysis. Isolation of mycoflora was done by agar plate methods using peptone, glucose, rose bengal agar medium containing streptomycin. (Booth, 1971). Fungal colonies formed were identified and percent incidence of each fungus was calculated.

The isolates of *Aspergillus flavus* were screened for their aflatoxin producing potentials in SMKY liquid medium (Diener and Davis, 1966). Ten days old culture filtrates were extracted with chloroform (v/v) and qualitatively analyzed for different types of aflatoxins on TLC plates (Reddy *et al.*, 1970).

For analysis of aflatoxin contamination in seeds. The oil seeds samples were examined under U.V. light for BGYF test (Fennell *et al.*,1973). Powdered cereals seeds were extracted with methanol: water (6:4 v/v) and sodium chloride (Anon, 1975). The aqueous methanolic extraction for aflatoxin with chloroform which was processed for qualitative analysis of aflatoxin on TLC plates (Reddy *et al.*, 1970).

The TLC plates were air-dried and observed under long-wave UV light (360nm) for aflatoxins (B_1, B_2 ,G_1 &G_2). The aflatoxins were also chemically confirmed by spraying trifluoroacetic acid and 25%sulfuric acid. Each spot was scraped separately, dissolved in chilled methanol and subjected to spectrophotometric measurement at 360 nm using a temperature controlled using shimadzu UV160A Spectrophotometer (Nabney and Nesbitt, 1965).

RESULT AND DISCUSSION

Isolation of mycoflora: From the results it is clear that, total fourteen fungi were isolated from the seeds of all the test cereals. Among the fourteen fungi, maximum thirteen fungi were recorded from the seeds of Maize and minimum seven fungi from the seeds of Wheat. Table 1 records the fungi (in *%)* isolated from various seeds of cereals. It is obvious that aspergilli outnumbered other genera, viz. *Alternaria, Cladosporium*, *Curvularia, Fusarium, Mucor, Penicillium and Rhizopus sp. Aspergillus flavus* was found dominant on all the test cereals. Depending on percent incidence different seeds of cereals can be arranged in the following decreasing sequences: -

Maize > Jowar > Wheat > Bajra

Aspergillus flavus, A. niger and *A. ochraceus* emerge as the most common seed infesting fungi of the test cereals. *Aspergillus candidus, A. terreus and A. Parasiticus*on other hand, were encountered only in Maize seeds. After *Aspergilli,* the most common infestant was *Fusarium, Penicillium sp*. and *Rhizopus sp.*(isolated from all cereals), followed by *Mucor.* (isolated from Bajra,Jowar and Maize seeds), *Altenaria sp.* and *Curvularia sp*. (isolated from Bajra and Maize seeds), *Cladosporium sp.*(isolated from only Bajra seeds). Chaudhari and Kolhe, 2017 reported that seventeen different fungi were isolated from various oil seeds in Jalgaon District, Maharashtra. The susceptibility of sorghum to various molds has been well documented (Thakur *et al*.,2006). Species of *Aspergillus*, *Fusarium, Alternaria,* and *Curvularia* have been frequently recovered from the contaminated grains/ seeds (Sreenivasa *et al*.,2010).

Af1atoxigenic potentials of *Aspergillus flavus* isolates obtained from cereals:

Altogether 87 isolates of *Aspergillus flavus* obtained from various cereals and screened for their aflatoxin producing potentials in SMKY liquid medium (table 2),

only 61 isolates were aflatoxin producers. The incidence of toxigenic isolates varied with the commodities from which they were isolated. Depending upon the presence of toxigenic *A. flavus* isolates various cereals could be arranged in the following decreasing sequence: -isolates were able to produce AFG_1 along with AFB_1 and B_2. Only three isolates could produce all four types of aflatoxins (B_1, B_2, G_1 and G_2). Table 2 also reveals that AFB_1 producing potentials of toxigenic *A. flavus* isolates varied with the various cereals; while isolates from Maize seeds were highly aflatoxigenic, isolates recorded from Jowar seeds were least. Raper and fennell (1965) also reported that all *A. flavus* isolates are not toxigenic.

Table 1 : Mycoflora associated with cerealsand their percentage incidence.

Mycoflora	Percent incidence in oil seeds			
	Bajra	Jowar	Maize	Wheat
Aspergillus flavus	33	45	58	37
A. niger	30	34	1 1	24
A. candidus	_	_	1	_
A. terreus	_	_	2	_
A. ocharaceus	5	3	1 0	3
A. parasiticus	_	_	2	_
Aspergillus sp.	3	6	3	1 0
Alternaria sp.	2	_	2	_
Cladosporium sp.	1	_	- -	_
Curvularia sp.	1	_	2	_
Fusarium sp.	1 0	2	4	13
Mucor sp.	6	2	2	_
Penicillum sp.	4	3	2	3
Rhizopus sp.	4	5	1	1

Table 2: Aflatoxin producing potentials of *Aspergillus flavus* obtained from cereals:

Cereals	Number of isolates		Number of Isolates producing aflatoxin				Amount of aflatoxin B_1(mean) ppm
	Screened	Toxigenic (percent)	B_1	B_1+B_2	$B_1+B_2+G_1$	$B_1+B_2+G_1+G_2$	
Bajra	16	08 (50.0)	2	6	--	--	11.94
Jowar	23	18 (78.)	8	7	2	1	11.11
Maize	29	25(86.2)	13	7	3	2	14.38
Wheat	19	10(52.6)	7	2	1	--	11.63
Total	87	61(70.1)	30	22	6	3	

Table 3: Aflatoxin contamination in cereals

cereals	Number of samples							Aflatoxin B_1 Concentration (mean) ppb
	Screened for aflatoxin contamination	Positive BGYF test	Positive to aflatoxin contamination (Percent)	Samples Positive For				
				B_1	$B_1 + B_2$	$B_1+B_2+G_1$	$B_1+B_2+G_1+G_2$	
Bajra	2 0	1 2	3 (15.0)	1	2	-	-	5 .25
Jowar	2 0	9	4 (20.0)	1	2	1	-	8 .38
Maize	2 0	1 5	9 (45.0)	5	2	1	1	25 .87
Wheat	20	10	5 (25.0)	2	1	-	-	5 .61
Total	80	46	21 (26.25)	9	7	2	1	-

In 1965, Hiscocks noted that some isolates of *A. flavus* produced either B or G toxins, but majority of them produced both toxins. None of the isolates produced B_2, G_1 and G_2 in absence of B_1(Lillehoj *et al.,* 1977). It has been suggested that toxigenic nature of the isolates is possibly governed by their genetic makeup (Ciegler, 1977). Besides, genetical factors might also be responsible for the variations in aflatoxin production by different strains of *A. flavus* (Maggon *et al.,* 1969; Ciegler, 1977).

Maize>Bajra>Wheat>Jowar

Toxigenic isolates of*A. flavus* obtained from various cereals, elaborated different components of aflatoxins in liquid medium. Aflatoxin B_1 was elaborated by all toxigenic isolates of *A. flavus* (87). None of the isolates elaborated aflatoxin B_2, G_1 or G_2 in absence of AFB_1; 30 isolates could produce AFB_1 only, 22 isolates were capable of elaborating both AFB_1 and B_2 while 6.

Aflatoxin contamination in cereals:

Out of a total 80 samples screened for their aflatoxin contamination, only 46 samples were found positive to BGYF test (table 3). But extraction studies revealed that only 19 samples were positive for aflatoxins. Result shows that about 45% Maize samples were contaminated with aflatoxin followed by Jowar (20%), Wheat (25%), and Bajra (15%). In case of Maize seeds all the four types of aflatoxins were identified; AFB_1+B_2 were present in Bajra and Wheat seeds, whereas Jowar seeds was positive for AFB_1+B_2 + G_1. Based on the concentrations of AFB_1 (ppb) recorded, samples of cerealscould be arranged in the following decreasing orders:

Maize>Jowar>Wheat>Bajra

The presence of variable amount of aflatoxin in different types of samples could be due to environmental factors, toxigenic potential of the fungal strains and composition of substratum (Nagarajan and Bhat, 1973; Bilgrami, 1984). Natural contamination of aflatoxin is known to be greatly influenced by the environmental factors (Davis and Diener, 1970; Boller and Schroeder, 1974). Oxygen availability, temperature, moisture content, type and nature of the fungal strains are known to play a dominant role in determining the extend of aflatoxin elaboration in the grains (Detroy, *et al.* 1971). The loss of food grains under storage depends on various factors which vary from place to place under changing environmental conditions. The type of microorganism dominating in grains and nearby atmosphere and also condition of food grains particularly the moisture contain and chemical composition are very important factors the losses of food grain in some country such as India and certain countries in Africa and South America may be 30% of the total annual harvest (Neergaard,1977). Ezekiel and Sombie, (2014) reported that The incidence of aflatoxin and fungal load were determined in 30 samples of five commonly consumed maize-based breakfast cereals (cornflakes and golden morn) and wheat-based pastas (macaroni, noodles and spaghetti) retailed within Ogun State, Nigeria. Aflatoxin was quantified in all food samples at concentrations ranging 0.8–3.5 ppb (mean = 1.3 ppb). Golden morn had a mean aflatoxin concentration of 2.3 ppb, a level significantly ($p < 0.05$) higher than all other food commodities. Furthermore, the maize-based breakfast cereals (mean = 1.7 ppb) had significantly ($p < 0.05$) higher aflatoxin concentrations than the wheat-based pastas (mean = 1.2 ppb). Chaudhari and Kolhe,2017 reported that seventeen different fungi were isolated from oil seeds of Jalgaon District, Maharashtra. and maximum concentration of aflatoxin $B_1$was detected in peanut seeds (215.87 ppb) followed by Alsi seeds (95.25 ppb), sesame seeds (92.89 ppb), mustard seeds (84.38 ppb) and safflower seeds (58.61 ppb). Kolhe *et al.*,1994 reported that maximum concentration of AFB_1 was 515.86 ppb in Peanut cake from Jalgaon District, Maharashtra. The evidence regarding the potent carcinogenicity of aflatoxins has forced government regulatory agencies to establish very low tolerances in food including peanuts and related products. The European Union upper limit for aflatoxins in peanut is 2 µg/ kg for aflatoxin $B_1$and 4 µg/kg for total aflatoxins (B1+B2+G1+G2) (Commission of the European communities, 2006). The Food and Drug Administration (FDA) in the United States regulates the amount of allowable aflatoxin contamination as 20 ppb in crops or 0.5 ppb in milk for humans. Concentrations can be higher for animals. In India, any food containing more than 30 ppb aflatoxins are rejected (Van Egmond and Jonker, 2005; Klich, 2007; Payne and Yu, 2010). *Aspergillus, Fusarium, Alternaria,* and *Curvularia sp*. found that, infection appearing in seed coat, endosperm and embryo.Cereals contaminated with aflatoxin has poses a potential threat for the life of human and animal beings. Therefore, need for regular monitoring of aflatoxin contamination in cereals for quality control,

and to develop method which can reduce the chances of aflatoxin production during storage and transport.

CONCLUSION

Our results show that about 23.7 % of the 80 samples tested contained detectible levels of aflatoxin B_1 in cereal grain seeds, with the highest concentrations found in Maize seeds. Detection of the aflatoxins in contaminated seeds is a serious issue as it can adversely affect the health of consumers. Therefore, its management is urgently required.

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