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Screening Of Fungal Isolates For Mycotoxin Production

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Abstract:Molds are known to be responsible for the creation of hazardous metabolites known as mycotoxins when they come into contact with a wide variety of food commodities. There are around 160 different species of mold that are known to produce approximately 182 different mycotoxins and other hazardous metabolites (Moreau, 1979). Major mycotoxins are elaborated by 3 genera of fungi i.e., Aspergillus (Aflatoxins, Ochratoxins, Sterigmatocystin), Penicillium (Citrinin, Patulin, Citreoviridin, and Penicillic acid) and Fusarium (Zearalenone, Fumonisin, Trichothecenes). The capacity of moulds to produce mycotoxins differs with the nature of substrates environmental conditions and genetic set up of mould species (Krogh, 1987).

Key Words: Alatoxins, Ochratoxins, Penicillium, Fusarium, metabolites, mycotoxins, Aspergillus .

Mold produced toxins in food have been known about since biblical times; however, their role in inciting the disease syndrome was not recognized until the outbreak of "Ergotism" in cattle, which was caused by consumption of barley and rye grains infected with Claviceps purpurea. The consumption of ergot contaminated fodder caused lameness, gangrene of extremities and inflammation of the digestive tract in cattle, finally leading to death. The disease called "Alimentary Toxic Aleukia" (ATA) happens to be another most important mycotoxicosis due to Fusarium species. During 1942-47, this disease widely occurred in USSR and proved most fatal to animals (Riazanov, 1947). Since then, several mycotoxicosis seriously affecting human and animal health have been reported from various parts of the world.

Historically, the year 1960, had been the most important because of the scientific concern about mycotoxins was generated during this year. It was the year, when about a lakh beautiful game birds "Turkey" died in England following the consumption of peanut meal made toxic by Aspergillus flavus Link ex Fries. This epidemic was named as "Turkey 'X' disease" and the toxin produced by this fungus was named as aflatoxin (Blount, 1961; Sargeant et al, 1961). This sickness caused the victim to lose their appetite, become lethargic, and have weakening in their wings before ultimately leading to their demise within a week. An examination performed after death revealed that the patient had a hemorrhagic and necrotic liver in addition to enlarged kidneys. At the same time, there was a high death rate among ducklings and juvenile pheasants in the United Kingdom. In both Uganda and Kenya, there have been reports of significant duckling mortality due to an illness with identical symptoms. These reports led to the launch of multidisciplinary research projects all around the world to examine food-borne mycotoxicosis, which ultimately led to the identification of more than a dozen different mycotoxins. Toxins such as aflatoxins, ochratoxins, zearalenone, trichothecenes, patulin, and fumonisins are among those that are suspected of playing a causative role in the development of disease in both humans and animals (Krogh, 1983). Mycotoxins can take on a broad variety of chemical forms; some examples include polypeptides, alkaloids, benzoquinones, anthroquinones, xanthones, coumarins, terpenes, and derivatives of these chemicals (Moreau, 1979).

OBJECTIVE- To screen guar seed samples for association of moulds particularly species of Aspergillus, Penicillium and Fusarium.

METHODS- Survey of cluster bean (Cyamopsis tetragonaloba) fields in Agra region was

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undertaken during 2006 and 2007 for collecting post harvest and stored seed samples. The seed samples were collected from different places of five districts viz., Agra, Mathura, Firozabad, Mainpuri and Etah of Agra region (commissnary). The post harvest seeds weighing 250-500g were collected from villagers in the months of November and December. However stored seed samples of clusterbean were collected from villagers and traders in winter, summer and rainy seasons. All these samples were collected in sterilized polythene bags, which were sealed over flame and stored at 4ºC in refrigerator till analysed. In most of the cases, seed samples for association of moulds and determination of moisture content were screened within a fortnight but not later than a month.

RESULT & DISCUSSION- The results included in previous chapter about association of moulds with freshly harvested and stored clusterbean seeds, clearly indicate that species of 3 genera i.e., Aspergillus, Penicillium and Fusarium were most commonly associated with clusterbean seeds. Interestingly some speciesof thesemoulds areknown toelaborate someimportantmycotoxins. Therefore, it was thought desirable to study the mycotoxin producing potentiality of these moulds in liquid media. The present chapter deals with the findings related to production of aflatoxins, ochratoxins, zearalenone and patulin by isolates of Aspergillus flavus, A. ochraceus, Fusarium moniliforme, Penicillium patulum and P. expansum respectively. The results so obtained are preented in Table 1 to 5.

(A) Screening of isolates of Aspergilus flavus for production of aflatoxins- It has been recorded in the present investigation that Aspirgillus flavus was most frequent and abundant mould associated with different seed samples of cluster bean. In all 90 isolates of Aspergillus flavus were isolated from clusterbean seeds of 5 districts of Agra region. It is not necessary that all the strains of A. flavus are potentially able to produce aflatoxins. The genetic make-up of a strain is the most important of the many elements that influence aflatoxin production. Other factors, like as environmental conditions, can also play a role. In the current experiment, isolates of Aspergillus flavus were tested in semi-synthetic S.M.K.Y. medium for their ability to produce aflatoxin in accordance with Diener and Davis's (1970).

The perusal of Table-2 indicates that out of 40 isolates of A. flavus obtained from freshly harvested seeds of cluster bean only 23 elaborated aflatoxin, thereby suggesting aflatoxigenic nature of 57.5 percent isolates. These isolates produced aflatoxin B1 in the range of 150 to 1260ppb. However only 7 isolates, could also produce aflatoxin G1 in the range of 120-680 ppb. Interestingly, none of the isolates screened in present study could elaborate aflatoxin B2 and G2. Thus, it is clear that aflatoxin B1 is the main aflatoxin elaborated by strains of Aspergillus flavus.

Further, out of 50 isolates of Aspergillus flavus obtained form stored seed samples of cluster bean of different places, only 32 elaborated aflatoxin B1 B2 and G1 but none of the isolates produced aflatoxin G2. This study indicates that 64.0 percent isolates of Aspergillus flavus were aflatoxigenic. All the 32 aflatoxigenic isolates produced aflatoxin B1 in the range of 160 to 2590 ppb., but aflatoxin B2 (150-890 ppb.) and aflatoxin G1 (Traces to 1250 ppb) were produced by 5 and 12 isolates respectively. It is interesting to note that these isolates also produced aflatoxin B1 in more quantily (800-2000ppb). Thus, it can be said that 17 isolates of Aspergillus flavus produced aflatoxin B1, B2 and G1 and remaining 15 toxigenic isolates only produced aflatoxin B1.

The aflatoxin producing potential of isolates of Aspergillus flavus has been studied by several workers and concluded that aflatoxin production. Depends on the nature of substrate, genetic set up of strains and prevailing environmental conditions. According to research conducted by workers from the United Kingdom at the Tropical Products Institute in London, 75 percent of 43 potentially dangerous strains of Aspergillus flavus developed aflatoxins in varied concentrations. According to Austwick and Ayerst (1963), a toxigenic

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strain of A. flavus was found in 52 percent of an unrepresentative sample of isolates from multiple African nations. According to research that was conducted in Israel by Borut and Joffee (1965), 71.2 percent of the Aspergillus falvus isolates that were taken from groundnut soils and Kernels produced aflatoxin in various quantities. A summary of the investigations conducted in six countries viz., U.K., U.S.A., Holland, South Africa, Israel and India revealed that 60% isolates of A. flavus were aflatoxigenic. This report supports the findings of present investigation regarding aflatoxin producing potential of A. flavus isolates.

(B) Screening of isolates of Aspergillus ochraceus for production of ochratoxins- Ochratoxin-A was first isolated form Aspergillus ochraceus (K-804), a strain isolated by Scott (1965) from sorghum grains. Krogh (1983) suggested that members a Aspergillus ochraceus group are probably the most significant producers of ochratoxins in the tropical and semi-tropical regions. The ochratoxin producing potential vary with strain variability. Although the growth rate, morphology, and chemical composition of toxic and non-toxic strains may be identical (Stubblefield et.al., 1970), the isolate's ochratoxin-producing capacity is primarily dependent on its genetic set up, other factors influencing ochratoxin production include substrate type, temperature, and water activity.

In the current experiment, forty isolates acquired from cluster bean seeds were tested for their capacity to produce ochratoxins in YES liquid media. Fifteen of the isolates came from post-harvest conditions, while the remaining 25 were stored at room temperature. The results so obtained are incorporated in Table-3. A careful examination of this Table indicates that seven isolates from post harvest seeds (out of 15) elaborated ochratoxin A and B, thereby suggesting ochratoxigenic nature of 46.6% isolates. The ochratoxigenic isolates produced ochratoxin-A in the range of 110-1260 ppb. Interestingly only two isolates showed production of ochratoxin-B in very small amount. On the other hand, out of 25 isolates of Aspergillus ochraceus. Collected from stored seeds, only 15 showed production of ochratoxins, thus indicating occurrence of 60.0 percent ochratoxigenic isolates. These isolates produced ochratoxin-A in the range of 130-1600 ppp, while ochratoxin-B was produced in the range of traces to 210 ppb by 5 isolates, which also produced ochratoxin-A in the liquid medium.

(C) Screening of isolates of Fusarium moniliforme for production of zearalenone- It is quite evident from the observations of preceding chapter that among species of Fusarium, the Fusarium moniliforme was more frequent and abundant in both post harvest and stored seed samples of clusterbean. Fusarium species are known to produce a wide variety of mycotoxins, which mainly include three most important toxins viz., zearalenone (ZEN), deoxynivalenol (DON) and trichothecene (T-2) (Hussain et.al., 1989). Out of these, zearalenone is a toxic metabolite with strong estrogenic activity that affects the reproductive system of swine, cattle, human beings and other laboratory animals (Blaney et.al., 1984). Further it is not necessary that all the strains of Fusarium species are potentially able to produce zearlaenone but the zearalenone producing potential varies with the strain variability. In the present investigation, 53 isolates of Fusarium moniliforme were obtained from clusterbean seeds of different places. Since, Fusarium moniliforme is an important zearalenone producer, it was thought desirable to screen its various isolates for zearlenone producing capacity using most rice medium (Scott et.al., 1973). The results so obtained are presented in Table 4.

The observations presented in Table-4 indicates that out of 23 isolates of Fusarium moniliforme obtained from freshly harvested seeds, only 12 showed production of zearalenone in the range of 160 to 1060 ppb., thereby suggesting toxigenic nature of 52.1 percent isolates. Like wise, 20 isolates (out of 30) obtained from stored clusterbean seeds elaborated zearalenone in the range a 240 to 1450 ppb., thereby indicating toxin

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producing ability of 66.6 percent isolates. In all, 32 isolates (out of 53) isolates were found to elaborate zearalenone in varying quantity.

Reports regarding the production of zearalenone by various species of Fusarium are on record from almost all over the world (Joffe, 1960). Further, zearalenone producing Fusarium species have been isolated from numerous natural substrates but they abundantly occur in the cereal crops. Interestingly, the present finding about zearalenone production by isolates of Fusarium moniliforme obtained from clusterbean seeds is probably the first report from India.

(D) Screening of isolates of Penicillium expansum and P. patulum for production of patulin- Patulin was frist isolated from Penicillium patulum by Birkinshow et.al., (1943). In 1952, an out break of feed poisoning in dairy cattle occurred in Japan (Hori and Yamamoto, 1953). The mass death of 118 cows was attributed to the consumption of mouldy malt-feed infected with toxigenic strain of Penicillium patulum. Since then, several workers have reported production of Patulin by species of Penicillium. Fungicapable of producing patulin include Penicillium patulum, P. claviforme, P. cyclopium, P. lanosum P. lapidosum, P. melinil and P. urticae, Aspergillus clavatus, A. giganteus A. terreus and Byssochlamys nives (Wilson, 1976). However, in nature, the major producer of patulin is probably Penicillium expansum and the most suitable natural source is apple juice (Pohland and Allen, 1970). In view of above facts, it was considered important to screen isolates of Penicillium expansum and P. patulum for patulin production. The results so obtained are presented in table - 5.

The perusal of Table-5 indicates that a total of 36 and 28 isolates of Penicillium expasum and P. patulum respectively were screened for patulin production in YES liquid medium following Schwenk et.al., (1958). Out of 16 isolates of Penicillium expansum obtained from clusterbean seeds just after harvest, only 9 produced patulin in the range of 230-540 ppb., thereby suggesting toxigenic potential of 56.2% isolates. On the other hand 15 isolates. (out of 20) obtained from stored clusterbean seeds, produced patulin in the range of 270-860 ppb., thus indicating toxigenic nature of 75.0 per-cent isolates. In all 24 isolates (out of 36) were found capable of producing patulin in variable quantity.

Further, a total 28 isolates (10 from post harvest stage and 18 from stored seeds) of Penicillium patulum were screened for patulin production. This study revealed that 16 isolates showed variable amount of patulin in culture medium, which suggest that 57.1% isolates of Penicillium patulum were toxigenic in nature. In this study five isolates (out of 10) obtained from freshly harvested seeds produced patulin in the range of 170-480 ppb, thereby indicating toxigenic nature of 50 per-cent isolates of Penicillium palulum. Like wise, 11 isolates (out of 18) obtained from stored seed samples of clusterbean showed patulin production in the range of 200-640 ppb, thereby suggesting toxigenic nature of 61.1 percent isolates.

In India, aflatoxigenic nature of isolates of Aspergillus flavus from different food stuffs has been determined by several workers. From cereals and millets, about 10.8 to 100% incidence of aflatoxigenic strains of A. flavus has been recorded by Bilgrami et.al., (1980), Mishra et.al., (1979), Agarwal et.al., (1983), Singh et.al., (1985), Singh and Bedi (1986), Prasad et.al., (1986), and Mishra (2008). Like wise ochatoxin formation by isolates of Aspergillus ochraceus, isolated from different sources has been studied by Gupta (1992a). This study clearly indicated that isolates obtained form stored seeds produced more quantity of mycotoxins as compared to isolates obtained from seeds just after harvest.

TABLE-1

ISOLATES OF DOMINANT FUNGI SCREENED FOR MYCOTOXIN PRODUCTION

S.No.	Nme of Fungi	Post harvest samples	Stored samples
1.	*Aspergillus flavus*	40	50
2	*Aspergillus ochraceus*	15	25
3	*Penicillium patulum*	10	18
4	*Penicillium expansum*	16	20
5	*Fusarium moniliforme*	23	30

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TABLE-2

AFLATOXIN PRODUCING POTENTIAL OF ISOLATES OF ASPERGILLUS FLAVUS OBTAINED FROM CLUSTER BEAN SEEDS.

S. No.	Seed sample	Total No. of isolates screened	No. of aflatoxigenic isolates	% occurrence of toxigenic isolates	Range of aflatoxins (in ppb)			
					B_1	B_2	G_1	G_2
1.	Post harvest (just after harvest)	40	23	57.5	150-1260 (23)	-	120-680 (7)	-
2.	Stored	50	32	64.0	160-2590 (32)	150-800 (5)	Traces-1250 (12)	-
Figures in parenthesis indicate the no. of isolates								

TABLE-3

OCHRATOXIN PRODUCING POTENTIAL OF ISOLATES OF ASPERGILLUS OCHRACEUS OBTAINED FROM CLUSTER BEAN SEEDS

S. No.	Seed sample	Total No. of isolates screened	No. of toxigenic isolates	%of toxigenic isolates	Range of ochratoxins (in ppb)	
					OTA	OTB
1.	Post harvest	15	07	46.6	110-1260 (07)	Traces (2)
2.	Stored	25	15	60.0	130-1600 (15)	Traces-200 (5)
OTA=ochratoxin A; OTB=ochratoxin B						

TABLE-4

ZEARALENONE PRODUCING POTENTIAL OF FUSARIUM SEEDSMONILIFORME ISOLATES OBTAINED FROM CLUSTERBEAN

S. No.	Seed sample	Total No. of isolates screened	No. of toxigenic isolates	%of toxigenic isolates	Range of zearalenone (in ppb)
1.	Post harvest	23	12	52.1	160-1090
2.	Stored	30	20	66.6	240-1450

TABLE-5

PATULIN PRODUCING POTENTIAL OF ISOLATES OF PENICILLIUM EXPANSUM AND PENICILLIUM PATULUM OBTAINED FROM CLUSTERBEAN SEEDS

S. No.	Seed sample	No. of isolates screened	No. of toxigenic isolates	%of toxigenic isolates	Range of patulin (in ppb).
(A) *Penicillium expansum*					
1.	Post harvest	16	09	56.2	230-540
2.	Stored	20	15	75.0	270-980
(B) *Penicillium patulum*					
1.	Post harvest	10	05	50.0	170-480
2.	Stored	18	11	61.1	200-640

CONCLUSION- In the present investigation, 40 isolates of Aspergillus ochraceus (15 from post harvest and 25 from stored seeds) were screened for their ability to produce ochratoxin A and B in YES liquid medium. Perusal of table 10 indicates that 7 isolates (out of 15) obtained from freshly harvested seeds elaborated ochratoxin A in the range of 110-1260 ppb, thereby suggesting ochratoxigenic nature of 46.6% isolates. On the other hand 15 isolates of Aspergillus ochraceus (out of 25) obtained from stored seed samples showed production of ochratoxin A and B in the range of 130-1600ppb and traces to 210ppb respectively thus indicating ochratoxigenic nature of 60% isolates. Further, ochratoxin B was produced by those isolates of Aspergillus ochraceius which produced ocluratixin -A in more quantity. The observations presented in table 3 indicates that out of 23 isolates of Fusarium moniliforme obtained from freshly harvested seeds, only 12 showed production of zearalenone in the range of 160-1060 ppb, thus suggesting toxigenic nature of 52.1% isolates. Likewise 20 isolates (out of 30) of Fusarism moniliforme isolated from stored seeds. Elaborated zearalenone in the range of 240-1450ppb., hence indicating toxin producing ability of 66.6 percent isolates. Thus, in all 32 isolates (out of 53) of F. moniliforme were found to elaborate zearalenone in varying quantity. The perusal of table 5 indicates that out of 16 isolates of Penicillium expansum obtained from freshly harvested cluster bean seeds, only a produced patulin in the range of 230-540ppb. While 15 isolated from stored samples of cluster bean seeds produced patulin in the range of 270-860 ppb. Further, 16 isolates (out of 28) of Penicillium patulum produced patulin in the range of 170-640 ppb. Thereby suggesting toxin producing ability of 57.1% isolates. The present study clearly indicated that isolates of Aspergillus flavus, A. ochraceus, Fusarium moniliforme, Penicillium expansum and P. patilum obtained from stored seed samples produced more quantity of mycotoxins as compared to the isolates obtained from seed samples collected after just harvest.

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