MYCOTOXIN-PRODUCING POTENTIAL OF SOME ASPERGILLUS, PENICILLIUM AND FUSARIUM ISOLATES FOUND ON CORN GRAINS AND SUNFLOWER SEEDS IN EGYPT

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SUMMARY : Sixty-three isolates of Aspergillus, Penicillium and Fusarium, isolated from corn grains and sunflower seeds, were screened qualitatively for production of mycotoxins. Eighteen isolates of Aspergillus (out of 28), 18 isolates of Penicillium (out of 26) and 6 isolates of Fusarium (out of 9) proved to be toxic and produced mycotoxins. Eleven different known mycotoxins were detected in the chloroform extracts of the different isolates tested and these are aflatoxins B_1, B_2, G_1 and G_2, sterigmatocystin, ochratoxin A, citrinin, Penicillic acid, rubratoxin B, diacetoxyscripenol and zearalenone.

Key Words : Aspergillus, penicillium, fusarium, mycotoxins.

INTRODUCTION

Substantial economic losses of foods and feeds occur yearly due to deterioration by molds. In addition spoilage of stored crops is often accompanied by the formation of mycotoxins. Mycotoxins are a potential threat to both human and animal health.

The mycotoxins and mycoflora of many agricultural commodities in Egypt were previously studied in this laboratory (10,13,14,18,31,32).

This study is aimed at determination of toxicity and mycotoxin production by different isolates of the common genera : *Aspergillus, Penicillium and Fusarium* from corn grains and sunflower seeds in Egypt.

MATERIALS AND METHODS

Organism

Sixty-five mold isolates (28 Aspergillus, 26 Penicillium and 9 Fusarium) previously recovered (16) from corn grains and sunflower seeds collected in Assiut, Egypt, were tested in this study.

Cultivation of fungal isolates for mycotoxins screening

Czapek's medium fortified by 2 gm yeast extract and 10 gm peptone was used. Fifty ml of medium in 250 ml Erlenmeyer flasks were sterilized, inoculated with spore suspension (approx. 10^6 spores) of 1-2 week old culture of the tested isolate and incubated at 28°C for 10 days as stationary cultivation.

Extraction of the fungal toxins

The culture was extracted with chloroform, and the extract was concentrated in vacuo. The dry material was transferred to 1-dram vials with small amounts of chloroform, and the solution was evaporated to dryness under a stream of nitrogen. Mycotoxins were dissolved in chloroform and separated by thin-layer chromatography on Silica Gel 60-coated plates using chloroform : methanol (97:3, v/v) as the developing solvent.

The spots of aflatoxin B_1, B_2, G_1 and G_2 were removed from the plate, eluded with methanol and estimated spectrophotometrically (21). Diacetoxyscripenol was determined using 4-anisoladehyde reagent (24) and 4-(4-nitrobenzyl pyridine reagent (27). Detection of ochratoxin A, sterigmatocystin and zearalenone was carried out according to Josefsson and Müller (17). Citrinin, penicillic acid and rubratoxin B was determined according to Damodaran *et. al.* (6), Ciegler and Kurztman, (5) and Moss (19), respectively.

Bioassay method for mycotoxins

The immature brine shrimp (Artemia salina L.) was used for mycotoxins bioassay. The test has been used for aflatoxins (1) and for other mycotoxins (15,23).

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RESULTS AND DISCUSSION

A high percentage of *Aspergillus* (about 67%) were toxic and capable of producing mycotoxins (Table 1). One isolate of each of *A. candidus, A terreus and A. ustus* proved to be citrinin-producer. This toxin was previously reported to be produced by these fungal species (20). Citrinin was previously isolated from a variety of *Aspergillus and Penicillium* species. This toxin is regarded as an important mycotoxin which may be ingested by man and animals. It caused renal toxicity and kidney damage in experimental animals (3).

Table 1: Toxicity and toxins produced by different isolates of Aspergillus species.

Species	Code No.	Source	Mycotoxin	Tox. test*
A. candidus	160	Corn	citrinin	C
A. flavipes	123	Corn	(-)	D
A. flavus	11	Corn	aflatoxins B_1 and B_2	A
A. flavus	73	Corn	(-)	D
A. flavus	127	Sunflower	aflatoxins B_1, B_2, G_1 and G_2	A
A. flavus var. columnaris	80	Corn	aflatoxins B_1, B_2, G_1 and G_2	B
A. oryzae	78.90	Corn	aflatoxins B_1, B_2, G_1 and G_2	B
A. tamarii	19.30	Corn	(-)	C
A. zonatus	120	Corn	aflatoxins B_1, B_2, G_1 and G_2	B
A. aureolatus	31	Sunflower	sterigmatocystin	B
A. aereolatus	31, 110	Corn	sterigmatocystin	B
A. nidulans var. dentatus	30, 56, 62	Corn	(-)	D
A. quadrilineatus	32, 45	Sunflower	sterigmatocystin	B
A. quadrilineatus	86	Corn	sterigmatocystin	B
A. japonicus	18	Corn	(-)	D
A. niger	3	Corn	(-)	D
A melleus	70	Sunflower	ochratoxin A	B
A. ochraceus	97	Sunflower	ochratoxin A	B
A. terreus	124	Corn	citrinin	B
A. ustus	96	Sunflower	citrinin	B
A. sydowi	64	Corn	sterigmatocystin	C
A. wentii	118	Corn	(-)	D

*Toxicity test:
(-) Negative
A=high toxicity; more than 75% mortality of brine shrimp larvae.
B=moderate toxicity;between 50-75% mortality of brine shrimp larvae.
C=low toxicity; between 25-49% mortality of brine shrimp larvae.
D= non-toxic; less than 25% mortality of brine shrimp larvae.

Two out of the three isolates of *A. flavus* Link were toxic and produced aflatoxin. It was recognized that different strains of *A. flavus* vary tremendously in their ability to produce aflatoxin. Schroeder and Boller (26) found that of the isolates of *A. flavus* 96%, 79%, 49% and 35% isolated from peanuts, cotton seeds, sorghum and rice grains, respectively, produced aflatoxin. In this laboratory El-Kady *et. al.* (12), Youssef (29) and Zohri (30) found that about 15%, 27% and 39% of *A. flavus* strains isolated from cotton, soybean and broad bean seeds, respectively, produced aflatoxin. One of the positive isolates of *A. flavus* recorded in the present study produced aflatoxins B_1 and B_2 only.

Aflatoxin was also produced by the single isolate of *A. flavus* var. columnaris and the two isolates of A. oryzae which produced aflatoxins B_1, B_2, G_1 and G_2. *A. flavus* var. columnaris and *A. oryzae* were previously reported as aflatoxin producers (11).

Both of two isolates of *A. tamarii* neither caused mortality to the test organism nor produced aflatoxin. These findings agree with the earlier results of Tuite (28) who reported that *A. tamarii* encountered on food and feed stuffs and considered non-mycotoxigenic. However, Schroeder and Boller (26) reported that *A. tamarii* appeared to produce trace amounts of aflatoxins B_1 and B_2 in some tests, but the production was irregular and so low that it could not be definitely established.

The single isolate of *A. zonatus,* proved to be toxic and produced aflatoxins B_1, B_2, G_1 and G_2. Production of aflatoxin by an isolate of this species is reported for the first time. More recently in this laboratory, Youssef (29) reported production of aflatoxins for the first time by *A. flavo-furcatis.* The close morphological and possible genetic relationship of both *A. zonatus* and *A. flavo-furcatis* to the aflatoxin-producers, *A. flavus* and *A. parasiticus* may explain this ability in part.

All the isolates of *A. aureolatus* and *A. quadrilineatus* (three isolates of each) proved to be toxic and produced sterigmatocystin. Several species of *A. nidulans* group were recorded previously as sterigmatocystin producers (4). Production of sterigmatocystin by *A. quadrilineatus* was reported for the first time in this laboratory by El-Kady and Abdel-Hafez (8). All the isolates tested of *A. quadrilineatus* (fifteen isolates) proved to be sterigmatocystin producers. However, *A. aureolatus* has no previous history of toxicogenicity. Production of sterigmatocystin by members of this species which belong to *A. nidulans* group is reported for the first time. Sterigmatocystin was also produced by the single isolate of *A. sydowii.* This result agrees

Table 2: Toxicity and toxins produced by different isolates of *Penicillium* species.

Species	Code No.	Source of Isolation	Toxins produced	Toxicity test*
p. puberulum	505	Corn	penicillic acid	B
P. puberulum	510	Corn	(-)	D
P. canescens	570	Corn	(-)	D
P. jenseni	571,608,660	Corn	citrinin	C
P. kapuscinskii	564,586	Corn	citrinin	C
P. nigricans	512,600	Corn	(-)	D
P. chrysogenum	575,593	Corn	citrinin	C
P. citrinum	635	Corn	citrinin	C
P. corylophilum	592	Corn	citrinin	B
P. corylophilum	597	Sunflower	citrinin	C
P. meleagrinum	502	Sunflower	(-)	D
P. Steckii	532	Sunflower	citrinin	C
P. Steckii	547	Corn	citrinin	C
P. duclauxi	515,585,588	Corn	(-)	D
P. funiculosum	545,584	Corn	ochratoxin A	B
P. purpurogenum	544	Corn	rubratoxin B	B
P. rubrum	604,610	Corn	rubratoxin B	C

*Footnote as in Table 1.

with the finding of El-Kady and Abdel-Hafez (8). Negative results obtained with the three isolates of *A. nidulans var.* dentatus agree regarding sterigmatocystin production with the earlier findings of El-Kady and Abdel-Hafez (8).

One isolate of each of *A. melleus* and *A. ochraceus* produced ochratoxin *A. Ochratoxin* A has been isolated from a variety of plant products in both America and Europe. Most of the field contamination has occurred in cereals especially corn, oats, wheat and barley (25).

One isolate of *Penicillium puberulum* produced penicillic acid. There are no reports of mycotoxic diseases in man or in domestic and laboratory animals due to contamination of food or feed-stuffs with penicillic acid. However, penicillic acid has been found to cause local tumors when injected in rats and mice (7).

The production of citrinin by all the isolates of P. chrysogenum, P. citrinum, P. corylophilum, P. jenseni and P. steckii agrees with the finding of Scott *et. al.* (25). In addition P. kapuscinsii which has no previous history of toxicogenicity, proved to be citrinin producer. This is the first report for production of citrinin by a member of this species.

Rubratoxin B was produced by all the isolates tested of P. purpurogenum and P. rubrum. These results agree with the findings of Natori *et. al.* (22).

Both isolates of *P. funiculosum* proved to be toxic and produced Ochratoxin A. These results agree with the findings of Carlton and Krogh (2), who classified *P. funiculosum* as ochratoxins-producer (Table 2).

Table 3 shows that only one isolate of *Fusarium equiseti* was able to produce diacetoxyscirpenol. Previous reports proved that members of *F. equiseti* are trichothecene-producers (9). Diacetoxyscirpenol was produced alone by one isolate of *F. oxysporum* and together with zearalenone by two isolates of this species.

Only two isolates *F. moniliforme* produced zearalenone (Table 3). Screening for the ability of different isolates of *F. moniliforme* isolated from Egyptian cereal grains and cotton seeds indicated that about 31% and 37% of the total isolates produced zearalenone, respectively (9).

Data obtained in this work regarding production of aflatoxin by most members of *A. flavus* group, sterigmatocystin in most members of A. nidulans group and citrinin by most members of divaricate and velutina asymmetrica of *Penicillium* appear to support the opinion about the ability to produce a particular secondary metabolite is indicative of, but does not confirm, species or group relationships. Data obtained in this investigation, proved that most of fungi tested have some degree of toxicity and produced one or more of 11 mycotoxins identified during this work. These data strengthen our initial concern that potential hazard to human and animal health may exist due to the presence of toxigenic fungi in corn grains and sunflower

Table 3: Toxicity and toxins produced by different isolates of *Fusarium* species.

Species	Code No.	Source of Isolation	Toxins produced	Toxicity test*
F. equiseti	2020	Corn	diacetoxyscirpenol	B
F. equiseti	2022, 2026	Corn	(-)	C
F. moniliforme	2005	Corn	(-)	D
F. moniliforme	2013, 2040	Corn	zearalenone	B
F. oxysporum	2030	Corn	diacetoxyscirpenol	A
F. oxysporum	2033	Corn	diacetoxyscirpenol + zearalenone	C
F. oxysporum	2038	Corn	diacetoxyscirpenol + zearalenone	B

*Footnote as in Table 1.

seeds, and hence special attention must be paid to the conditions under which such grains and seeds will support toxin formation.

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