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MYCOFLORA AND MYCOTOXINS OF PEANUT (*ARACHIS HYPOGAEA L.*) SEEDS IN EGYPT. II – MYCOTOXIN PRODUCTION BY THERMOPHILIC OR THERMOTOLERANT FUNGI

By

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ABSTRACT

Twelve species and one variety which belong to 6 genera were identified from 40 peanut seed samples using a dilution–plate method on 1% glucose–Czapek's medium at 45°C. *Aspergillus* (6 species and 1 variety) followed by *Mucor* (1 species) were the most frequent genera. From the preceding genera, *A. fumigatus* was extremely dominant, followed by *Mucor pusillus, A. niger, A. terreus and A. nidulans.*

Fifty–four isolates belonging to the previous species were screened for the production of mycotoxins at 28 and 45°C. Thin layer chromatographic (TLC) analysis revealed that 7 isolates belonging to *A. flavus, A. nidulans and A. versicolor* produced aflatoxins B_1 and B_2, sterigmatocystin and versicolorin A, respectively at 28°C but none of the isolates tested produced any detectable amount of mycotoxins when grown at 45°C.

INTRODUCTION

The study of seed and grain–borne fungi is very significant due to the production of toxic substance (mycotoxins) by associated fungi and the relationships of these toxins to diseases (mycotoxicoses) of animals, fowls and humans when they use these seeds and grains as food or feed. Thermophilic fungi have a maximum temperature for growth at or above 50°C and a minimum temperature at or above 20°C, and thermotolerant fungi have maxima near 50°C but minima well below 20°C (Cooney and Emerson, 1964).

Thermophilic fungi were isolated from seeds and grains and from other sources (Clarke *et al.*, 1966; Flannigan, 1974; Kuthubutheen, 1979 and others).

In Egypt, some investigations were made for isolation of thermophilic fungi from peanut seeds (Moubasher *et al.*, 1980) and from other seeds (Abdel–Hafez and Shoreit, 1986).

Mycotoxin production by mesophilic fungi of different genera and species were extensively studied in this and other laboratories (El–Kady and Abdel–Hafez, 1981; El–Kady and El–Maraghy, 1982; Schroeder and Kelton, 1975 and others).

The present investigation is focussed on the thermophilic fungi associated with peanut seeds and their ability for production of mycotoxins.

MATERIALS AND METHODS

Forty samples of mature peanut fruits, 1/2 Kg each, were collected from markets in different places of Egypt as shown in Figs. I and II. In the laboratory, shells were unfolded and seeds were released under sterile conditions.

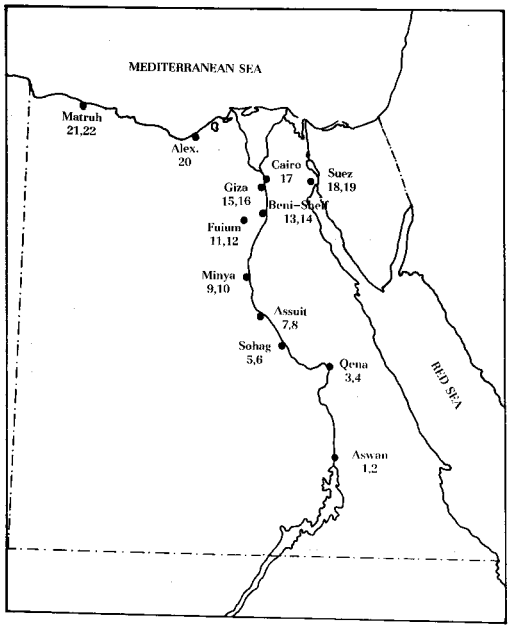

Fig. I : Outline map showing the places in Upper Egypt from which samples were collected.

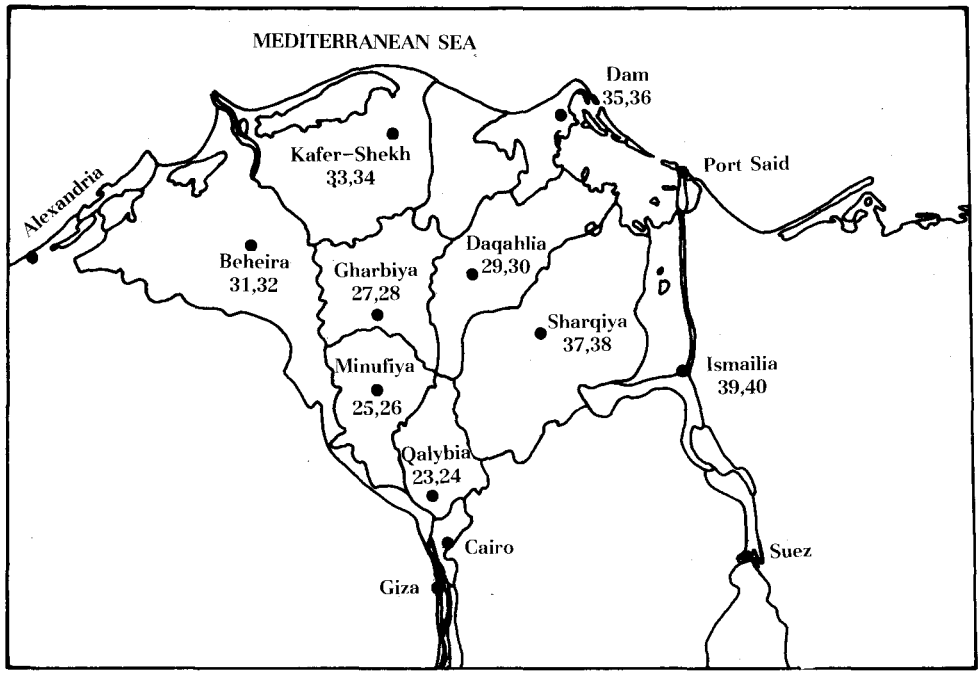

Fig. II : Outline map showing the places in the Delta area from which samples were collected.

Peanuts were plated out as described by Christensen (1963). Modified Czapek's medium was used in which glucose (10 g/1) replaced sucrose. Five plates were used for each sample. The plates were incubated at 45°C for 7 days, during which the growing fungi were counted, isolated and identified.

Cultivation for toxin production

Isolates were inoculated into autoclaved 250 ml Erlenmeyer flasks containing 50 ml of the following medium: sucrose, 30; peptone, 10; $NaNO_3$, 2; K_2HPO_4, 1; yeast extract, 1; KCl, 0.5; $MgSO_4$, 0.5; Fe SO_4, 0.01 (g/1 of distilled water). After inoculation with one ml. of a spore suspension of 2-week-old cultures of the pure organism, the flasks were incubated as stationary cultures at 28 or 45°C for 7 days.

Extraction and determination of mycotoxins from the fungal cultures

After incubation, the contents of each flask (medium + mycelium) were homogenized with 50 ml chloroform for 3 min in a high speed blendor (16000 r.p.m.). The extraction procedure was repeated three times. The chloroform extracts were collected, washed and then dried over anhydrous sodium sulphate, filtered, and then concentrated to near dryness under vacuum. The chloroform extracts were analyzed for the presence of known mycotoxins, using thin layer chromatographic plates (precoated silica gel plate type 60 F254, MERCK) according to the method previously used in this laboratory (El–Kady and El–Maraghy, 1982) and the quantities of the mycotoxins were determined in the extracts as described by Coomes *et al.,* (1965) and Schroeder and Kelton (1975).

RESULTS AND DISCUSSION

1– Thermophilic or thermotolerant fungi of peanut seed

The moisture content of the seeds tested was generally low and fluctuated between 0.8–5.3%.

Twelve species and one variety which belong to 6 genera were collected on 1% glucose–Czapek's agar medium at 45°C of which *Aspergillus* followed by *Mucor* were the most dominant genera. The previous two genera were the most common genera in Egyptian seeds and soils (Abdel–Fattah *et al.,* 1977; Moubasher *et al.,* 1982 and Mazen and Shaban, 1983).

Aspergillus was the most common genus and was represented in 92.5% of the samples comprising 72.9% of total fungi. From this genus 6 species and one variety were identified. *A. fumigatus* was the dominant species and was isolated from most of the samples (87.5%) constituting 40.2% of the total *Aspergillus* and 29.3% of the total fungi. *A. fumigatus* has long been known as a thermophilic fungus, but Coony and Emerson (1964) and Moubasher *et al.,* (1982) considered it as thermotolerant as it has a maximum near to 50°C, but a minimum well below 20°C. This species was the most frequent fungus isolated from Egyptian covered and uncovered peanut seeds (Moubasher *et al.,* 1979) and from cultivated desert and salt marsh soil in Egypt and some Arabic countries (Moustafa *et al.,* 1982; Abdel–Hafez *et al.,* 1983; and Mazen and Shaban, 1983). *A. niger, A. terreus and A. nidulans* were isolated in moderate frequency and emerged in 30%, 30% and 27.5% of the samples comprising

38%, 13.3% and 4.3% of the total *Aspergillus* and 27.7%, 9.7% and 3.1% of the total fungi. The previous three species were dominant, but with variable frequency and density in Egyptian seeds and soils (Abdel–Fattah *et al.,* 1977; Moubasher *et al.,* 1982 and Mazen and Shaban, 1983). The remaining *Aspergilli* (3 species and 1 variety) were rare as shown in Table 1.

Table 1

Propagules (calculate per g dry seeds) in samples and number of cases of isolation NCI (out of 40) of fungal genera and species on 1% glucose–Czapek's agar at 45°C.

Genera and species	Propagules	NCI
Propagules	2967	
Aspergillus	2156	37
A. fumigatus Fresenius	868	35
A. niger Van Tieghem	823	12
A. terreus Thom.	288	12
A. nidulans (Eidam) Wint.	93	11
A. nidulans var. latus Thom & Raper	35	4
A. quadrilineatus Thom & Raper	33	4
A. flavus Link	10	3
A. versicolor (Vuill.) Lindt	6	3
Mucor pusillus Lindt	660	16
Chaetomium thermophilum La Touche	22	3
Malbranchea sulfurea (Miehe) Cooney and Emerson	10	2
Paecilomyces variotii Bainier	10	2
Sporotrichum thermophilum Apinis	50	1

Mucor pusillus was isolated in moderate occurrence and encountered in 35% of the samples giving rise to 22.2% of total fungi. Moubasher *et al.* (1979) isolated this species in moderate or low frequencies from covered and uncovered peanut seeds at 45°C. This species was also recovered from soils of some Arab countries on glucose–Czapek's agar medium at 45°C (Moustafa *et al.,* 1976; Abdel–Fattah *et al.,* 1977; Moubasher *et al.,* 1982; Abdel–Hafez *et al.,* 1983 and Mazen and Shaban, 1983).

The remaining 4 species were rare (Table 1), of which *Chaetomium thermophilum, Malbranchea sulfurea and Sporotrichum thermophilum* were truly thermophilic as recommended by several workers (Apinis, 1972; Cooney and Emerson, 1964; Evans, 1971; Moubasher *et al.,* 1982; Moustafa *et al.,* 1976; and Ward and Cowley 1972).

2- Mycotoxins of thermophilic or thermotolerant fungi of peanut seeds

Fifty-four isolates belonging to the previous species were screened for mycotoxin production at 45°C and 28°C . Results obtained (Table 2) show that all the isolates tested grew well at the two incubation temperatures. Incubation at 28°C proved to be more suitable for mycelial growth of this group of fungi which were isolated at 50°C, but grew better at 28 than 45°C. They are therefore thermotolerant according to the definition of Cooney and Emerson (1964). TLC revealed that none of the isolates tested produced detectable amounts of mycotoxins when grown at 45°C.

Four isolates of *A. nidulans* out of 8 tested at 28°C produced high levels of sterigmatocystin (50 to 275 mg/l). El-Kady and Abdel-Hafez (1981) reported that all strains (15 isolates) of *A. nidulans* (Eidan) Wint. tested proved to be sterigmatocystin producers and produced high levels of the toxin (48 to 495 mg/ 1) in YES medium (2% yeast extract + 15% sucrose) when incubated at 28°C without shaking for 10 days.

Two isolates out of 3 tested belonging to *A. flavus* produced aflatoxins B_1 and B_2 at 28°C with concentrations of 15 and 45 mg/l. Aflatoxins were only produced at 28°C and synthesis of the toxin was completely inhibited at 45°C. The observations recorded in this investigation verify those of Schindler *et al.* (1967) and Shih and Marth (1974). They reported that highest yields of toxin were obtained at 24 or 25°C, whereas maximum mould growth occurred at 29 or 35°C depending on the particular isolate. Other studies however have demonstrated maximum production up to 35°C (Detroy *et al.,* 1971). Diener and Davis (1967) reported that the highest temperature at which the toxin was formed was 41.5°C on groundnuts. However, Shih and Marth (1974) indicated that incubation at 35 or 45°C suppressed production of aflatoxins and suggested that a key enzyme responsible for aflatoxin synthesis is inactive above 35°C.

One isolate out of 3 tested which belonged to *A. versicolor* produced a trace of versicolorin A.

All the three toxins detected in this investigation (versicolorin A, sterigmatocystin and aflatoxins) are coumarins ; there are structural similarities between the three. Hsieh *et al.* (1973) have demonstrated that ^{14}C – sterigmatocystin was converted to (^{14}C) aflatoxin B_1 by resting cultures of *A. parasiticus.* Biollaz *et al.* (1968) suggested that both sterigmatocystin and aflatoxin might be formed from versicolorin A. This suggestion was confirmed recently by Lee *at al.* (1975). Our results demonstrated that similarities exist between the synthesis of the different coumarin toxins detected in this investigation. It could be concluded that not only was aflatoxin production inhibited at 45°C but that various members of the coumarin family were also retarded at high temperatures.

Table 2

Types and concentrations (mg/l) of mycotoxins produced by *Aspergillus* species at 28°C, and the mycelial dry weight (gm/50 ml medium) at 28°C and 45°C.

Aspergillus Species	Type of mycotoxin produced	Myctoxin (mg/l)	Mycelial dry weight (gm/50 ml medium)	
			at 28°C	at 45°C
Aspergillus nidulans (Eidam) Wint.	Sterigmatocystin	50	0.296	0.192
A. nidulans (Eidam) Wint.	Sterigmatocystin	100	0.320	0.260
A.nidulans	Sterigmatocystin	275	0.360	0.220
A. nidulans	Sterigmatocystin	250	0.360	0.220
A. flavus Link	Aflatoxins B_1 & B_2	15	0.670	0.560
A. flavus	Aflatoxins B_1 & B_2	45	0.840	0.790
A. versicolor (Vuill.) Lindt	Versicolorin A	trace	o.360	0.220

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الفلورا الفطرية وسموم الفطريات لبذور الفول السوداني في مصر
٢ - انتاج السموم الفطرية بواسطة الفطريات المحبة أو المتحملة للحرارة

سعد شحاتة محمد المراغي و عثمان محمد عثمان المغربي

تم عزل إثنى عشر نوعاً وسلالة واحدة تنتمي إلى ستة أجناس فطرية من أربعين عينة لبذور الفول السوداني والتي جمعت من مناطق مختلفة بجمهورية مصر العربية وذلك بإستخدام طريقة التخفيف والوسط الغذائي شبكس ١٪ - جلوكوز عند درجة حرارة ٤٥°م.

ثبت أن الأسبير جيللس والميوكر كانت أكثر الأجناس الفطرية إنتشاراً بينما كانت أسبير جيللس فيوميجاتس ، ميوكر بيسلس ، أسبير جيللس نيجر ، أسبير جيللس تيريس وأسبير جيللس نيديولنس أكثر الأنواع الفطرية شيوعاً.

درست مقدرة أربعة وخمسون معزولة تنتمي إلى الفطريات السابقة على إنتاج السموم الفطرية وذلك بتنمية المعزولات عند درجتي حرارة مختلفة وهي ٢٨°م ، ٤٥°م وقد أظهر التحليل الكروماتوجرافي مقدره سبعة معزولات تنتمي لفطره أسبير جيللس فلافس، أسبير جللس نيديولنس وأسبير جيللس فيرسيكولر على إفراز سموم الأفلا توكسينات B_1 & B_2 والأستيرجماتوسيستين والفيرسيكلورين A على التوالي بينما أظهر التحليل الكروماتوجرافي عدم مقدرةالمعزولات المختبرة على إنتاج السموم الفطرية عند تنميتها عند درجة حرارة ٤٥°م .