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Mycobiota and Mycotoxins Contaminating Rice Grains in El Minia, Governorate, Egypt

A.M. Moharram[1], M.M. Yasser[2], M.A. Sayed[2], O.A. Omar[3] and M.M.M. Idres[2]

[1]Department of Botany and Microbiology, Faculty of Science, Assiut University, Egypt.
[2]Department of Botany and Microbiology, Faculty of Science, Beni-Suef University, Egypt.
[3]Department of Agricultural Microbiology, Faculty of Agriculture, Minia University, Egypt.

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The mycological analysis of 51 samples of rice grains collected from different localities in El-Minia Governorate revealed the isolation of 54 species of fungi belonging to 21 genera. Most common mycobiota (geneus) were *Aspergillus* and *Penicillium* being isolated from 96.07% and 54.9% of samples contributing 63.08 % and 21.89% of total fungal count. The prevalent species were represented by *Aspergillus flavus*, *A. candidus*, *A. niger*, *Penicillium chrysogenum*, *P. islandicum* especially on dichloran rose bengal chloramphenicol agar medium (DRBC). These species in addition to some osmophilic fungi including *A. chevalieri*, *A. montevidensis*, *A. rubrum* were also common when Dichloran Glycerol agar (DG18) was used for culturing of rice samples. About 12.5% of samples analysed for natural occurrence of mycotoxins were contaminated either with Aflatoxin – B1 (100-200 μg/ kg), ochratoxin –A (50-100 μg/ kg) or sterigmatocystin (10-20 μg/ kg). These samples were of grade 3 (with more than 10% broken grains) and showed infestation with rice weevil. The majority of fungal strains tested for their mycotoxin production in liquid cultures were able to produce variable levels of aflatoxin B1, Aflatoxin G1 (*A. flavus and A. parasiticus*), Ochratoxin –A (*A. ochraceus*), terrein (*A. terreus*), gliotoxin and fumagillin (*A. fumigatus*).

Keywords: Aflatoxin B1, B2 and G1; Mycotoxins; Rice.

Rice is one of the most famous and important cereals worldwide. According to [1] the cultivated area was estimated as 156 million hectars producing about 721 million metric tons (MMT) of rice. The most important world rice producers are China (202.3 MMT), and India (154.5 MMT). Rice is one of the most important commercial crops planted in Egypt. It is a privileged source of carbohydrates and proteins. It is used for different food and nonfood products. The foods include cooked rice, rice flour, breakfast cereals, and desserts. The inedible rice hull is used as fertilizer, fuel, and others, while the bran is a source of cooking oil. Straw from the stems and leaves is used as feeding or bedding for animals and for making roofs, bricks, hats, sandals and baskets. Rice bran and straws are also used as suitable substrates in mushroom cultivation[2].

Fungal contamination of cereals is an important issue for grain quality and from consumer's health point of view. Rice is one of the famous cereals which favor mycotoxin contamination [3]. In recent years, there have been many studies from various countries on

*Corresponding author E-mail:

the occurrence of high levels of aflatoxins by fungal rice contamination[4,5,6]. In 2001, Reddy and Sathyanarayana[7] listed 143 fungal species from rice. Reddy *et al*.[8] published a review contain the important groups of mycotoxins including aflatoxins (AFS), fumonisins , ochratoxin A(OTA), deoxynivlenol, and zearalenone (ZEN) , which contaminated rice in different countries. Sempere & Santamarina[9] confirmed that the majority of mycotoxins were produced by *Aspergillus, penicillium*, and *Fusarium*, Ferre[10] further found that AFS, citrinin, deoxynivalenol, fumonisins, sterigmatocystin, ZEN, cyclopiazonicacid, gliotoxin ,patulin and some trichothecenes are the main mycotoxins that have been identified in rice with a high variable of contaminated varieties and at different infected levels.

In Egypt, several studies on fungi and mycotoxins have been focused on food grains including rice since rice is the main food of most people in the world, the presence of mycotoxin contamination can be a serious health risk, corn, soybean and peanuts[11, 12]. Therefore, the present study was carried out for identification of fungi contaminating rice grains as well as for evaluation of mycotoxin levels in the tested samples and in cultures of toxinogenic fungi.

MATERIALS AND METHODS

Collection of rice samples

A total of 51 rice samples were collected from the market at different localities of El-Minia governorate covering El-Edwa, Maghagha, Bni Mazar, Mattay, Samalott, Minia, Abu-Qurqas, Mallawi, Deir Mawas between March 2015 and March 2016(Fig.1). Collected samples were transported immediately to laboratory and kept in plastic bags at 5-7°C till mycological and mycotoxin analysis.

Isolation of fungi

The method of seed-plate (direct plating) was utilized to determine the seed borne fungi on the rice grains. The grains were then plated on a suitable isolation media at a plating rate of 5 rice grains per plate and four replicates for each rice sample[13].

A general purpose enumeration agar medium, dichloran rose bengal chloramphenicol agar: DRBC, which contained (g/l of distilled water): glucose 10, peptone 5, potassium dihydrogen phosphate 1, magnesium sulphate 0.5, dichloran 0.002 (0.2% in ethanol 1ml) , rose bengal 0.025, chloramphenicol 0.1, agar 15, pH 5.6.

A selective isolation medium, dichloran 18 % glycerol agar, (DG18) were used to detect and isolate xerophilic fungi, the medium DG18 containing (g/l of distilled water): peptone 5, glycerol 220, chloramphenicol 0.1, glucose 10, potassium dihydrogen phosphate 1, magnesium sulphate 0.5, dichloran 0.002 (0.2% in ethanol 1 ml), agar 15, final pH 5.6. Both DRBC and DG18 media were prepared as described by[13].

All plates were inoculated with rice grains (5 grains/plate). Cultures in quadriplicates were incubated for 7-8 days at 30°C but plates containing DG18 were incubated for 14 days to allow growth of slow growing fungi.

Identification of isolated fungi

Fungi isolated from rice grain samples were transferred to fresh Czapek's Dox medium in Petri dishes and slant media bottles for identification. Morphological and cultural characteristics of the growing fungi were evaluated for preliminary identification. Then fungal colonies were subjected to microscopic identification according to[14-20].

Analysis of mycotoxins

The isolated fungi were screened for mycotoxin production by growing on potato dextrose broth (PDB). Erlenmeyer flasks (250 ml) containing 50 ml aliquots of potato dextrose medium were autoclaved, inoculated with fungi, and incubated for 7 days at 30 °C . After incubation period, the extraction of mycotoxins was carried out according to[21-23]. Then flask contents were blended using surface sterilized hand free blender and 50 ml of chloroform was added to flasks which were shaken for 24 hours. Cultures were filtered using whatman NO.1 filter paper. Fifty ml of this filtrate was shaken with an equal volume of chloroform for 30 min. The chloroform layer was separated using a separating funnell and filtered again over a bed of anhydrous sodium sulfate. Porcelain chips were added to flasks containing filtrates and were steam evaporated.

Estimation of mycotoxins

Thin layer chromatography (TLC) was used for detection of mycotoxins [24]. About 50 µl of chloroform extract of the mycotoxin was

applied on silica gel plates together with specific standards developed with mobile phase methanol: chloroform (4:96) and observed under long wave length UV light (365 nm) in a UV chamber CN-15. LC Vilber Lourmat, France. Qualitative detection of mycotoxins was done on the basis of their fluorescence and retention factor (RF) values [25].

Mycotoxin detection in rice samples

Twenty four samples of rice grains were chosen for this part of study. Samples were selected on the basis of their content of potentially toxinogenic fungi**.**

One hundred grams of rice grains and 150 ml chloroform were mixed in 250 ml Erlenmeyer flasks. Flasks were shaken for 24 hours then filtered through Whatman NO.1 filter paper. Detection of mycotoxins in the rice extract was done as mentioned above using TLC plates.

RESULTS

Using two isolation media (DRBC and DG18) it was possible to isolate and identify 54 fungal species attributed to 21 genera from the tested rice samples**.**

On DRBC medium

As shown in table (2) the total gross fungal population reached 653 colonies per 20 grains in all samples. The mycological analysis of 51 samples revealed the isolation of 52 species related to 20 genera of fungi. *Aspergillus* was the most dominant genus, being isolated from 49 samples matching 96.07% of rice samples and 63.08% of total fungal population. Nienghteen species of *Aspergillus* of which *A. flavus* was the most dominant (62.74% of samples). Two species of *Aspergillus* occurred in moderate incidence and these were *A. candidus* and *A. niger* (35.29% and 45.09% of samples matching 8.57% and13.93% of total fungal population respectively). *A. terreus* was isolated in low frequency being recovered from 17.64% of rice samples accounting for 2.14% of total fungal count.

The genus *Penicillium* appeared in high incidence (54.90% of samples) giving rise to 21.89% of total fungi. Eleven species of *Penicillium* were recovered, among which *P. chrysogenum* and *P. islandicum* were of low incidence (17.64% and 19.60% of rice samples respectively).

Each of *Alternaria* and *Cladosporium* were found to contaminate low number of rice samples (15.68% and 21.56%) participating in fungal population with 2.45% and 3.98% respectively.

On DG18

A total of 47 species of fungi belonging to 16 genera were isolated. *Aspergillus* was the most dominant genus (49 samples matching 96.07% of rice samples) representing 65.51% of total fungal counts. It was represented by 19 species, of which *A. candidus* and *A. niger* prevaled in 52.94% and 50.98% of samples matching 8.28% and 6.93% of total fungal counts respectively as shown in Table (2).

Six species of *Aspergillus* appeared in moderate incidence and these were *A. chevalieri, A. flavus, A. flavus var. columnaris, A. montevidensis, A. rubrum and A. versicolor* (31.37%, 37.25%, 25.49%, 45.09%, 33.33% and 31.37% respectively). The following 5 species of *Aspergillus* (*A. clavatus, A. fumigatus, A. ochraceus, A. oryzae and A. terreus*) were found to contaminate low number of rice samples (15.68%, 15.68%, 15.68%, 15.68% and 17.64%) participating in fungal population with 1.55%, 1.65%, 1.55%, 0.93% and 1.65% respectively.

The genus *Penicillium* appeared in high incidence (62.74% of samples) giving rise to 18.42% of total fungi. Eleven species of *Penicillium* were recovered, among which, *P. aurantiogriseum, P. chrysogenum, P. citrinum, P. duclauxii, P. glabrum, P. islandicum* and *P. oxalicum* were of low incidence (13.72%, 17.64%, 21.56%, 15.68%, 13.72%, 15.68% and 15.68% respectively).

Each of *Alternaria* and *Cladosporium* occurred in moderate incidence (25.49 % and 27.45%) participating in fungal population with 3.51% and 4.03% respectively. *Lichtheimia corymbifera* was found to contaminate low number of rice samples (15.68%) accounting for 1.86% of total fungal count.

The majority of fungi isolated on DRBC were also recovered on DG18 but the following differences were observed:

a- Each of *A. chevalieri, A. montevidensis* and *A. rubrum* were isolated in moderate frequency on DG18 but they were of rare incidence on DRBC. This is often due to the osmophilic character of these species.

b- *Aspergillus niger* which occurred in high frequency (50.95%) on DG18 , was found to be of moderate incidence on DRBC (45.09%). On the other hand *A. flavus* occurred in high incidence on DRBC (62.74%) but was moderately found on DG18 (37.25%).

c-Each of *A. clavatus*, A. *fumigatus* and *A. ochraceus* were of low frequency on DG18 but they were rare on DRBC. *Aspergillus oryzae* occurred in low incidence on DG18 (15.68%), while it was absent on DRBC.

d- *Cladosporium* genus occurred in low incidence on DRBC (21.56%) but was moderately found on DG18 (27.45%).

e- Each of *Penicillium aurantiogriseum, P. citrinum, P. duclauxii, P. glabrum* and *P. oxalicum*, were isolated in low frequency on DG18 but they were of rare incidence on DRBC.

f- *Fusarium* genus and *F. semitectum* which occurred in low incidence on DG18 (15.68%) was found to be of rare frequency on DRBC (9.80% and 5.88%).

g- *Lichtheimia corymbifera* appeared in low frequency on DG18 (15.68%) but was rarely found on DRBC (7.84%), *Alternaria alternata* was isolated in moderate frequency on DG18 (25.49%) but it was of low incidence on DRBC (15.68%).

Natural occurrence of mycotoxins

With reference to Table (3), twenty four samples of rice were randomly selected and analyzed for natural occurrence of mycotoxins, only 3 samples were found contaminated with 3 different types of mycotoxins namely streigmatocystin (10-20 µg/ kg), ochratoxin-A (50-100 µg/ kg) and aflatoxin B1(100-200 µg/ kg) ranging from 10 to 200 µg/ kg. These samples were collected from El-Minia City (NO.33), Mattay (NO.19) and Samalott (NO.22), respectively as shown in Table 3. Other tested rice samples were free from any mycotoxin contamination. All of the toxin contaminated samples were of grade 3 where more than 10% of grains were broken. Moreover, samples 19 and 22 were infested with rice weevil. The presence of *A. flavus* is of main concern because the fungus is a potent aflatoxin producer.

Mycotoxins produced by fungal strains

Twenty eight fungal isolates belonging to *A. flavus* (21 isolates), *A. ochraceus* (3), *A. parasiticus* (1), *A. fumigatus* (1) and *A. terreus* (2) were analyzed for mycotoxin production using TLC technique. as general these strains were able to produce aflatoxins, Ochratoxin-A, fumagillin, Gliotoxin and terrein, respectively (Table 4).

All isolates of *A. flavus* produced aflatoxins in variable degrees as shown in Table 4. Levels of aflatoxins (B1, B2, G1, and G2) at levels ranging from 10 to 300 ug/L. *A. parasiticus* produced B1 and G1 (10-20 ug/L). *A. terreus* (2 isolates) were able to elaborate terrein (10 -300 ug/L). Ochratoxin-A (20 -200 ug/L) was produced by 3 isolates of *A. ochraceus*. Finally, *A. fumigatus* produced Gliotoxin, fumagillin (10-20 ug/L for each).

DISCUSSIONS

These results showed that the total fungal counts which were prduced on two medium types were revealed the most predominant genus were *Aspergillus, Penicillium, Alternaria, cladosporium and Fusarium* respectively.

This finding corresponding with previous studies recorded by [26, 27] who investigated the mycobiota of rice which were grown on two isolation media including DRBC (dichloran rose-bengal chloramphenicol agar) and DG18 (dichloran 18% glycerol agar) media and he reported that a total of sixty two species related to 34 genera. The broadest species spectrum were from the genera *Aspergillus , Penicillium, Eurotium* followed by *Fusarium , Cladosporium and Cochliobolus*. Rice grains were mostly contaminated *by Aspergillus candidus, A. flavus, A. niger, A. amstelodami, A. rubrum, Penicillium citrinum, P. oxalicum* and *Talaromyces spp*.

In Swed, Fredlund et al. [28] collected 99 rice samples from the Swedish retail market. *Aspergillus* was the most common fungal genus identified but also *Penicillium, Eurotium, Cladosporium, Wallemia, Alternaria, Epicoccum* and *Trichotecium* were isolated . *A. flavus* presented in 21% of the samples.

From Egyptian paddy rice, Abdel-Hafez et al.[29] isolated *Aspergillus flavus*, *A. sydowii, A. terreus, A. fumigatus* and *A. ochraceus*) and *Penicillium* species (*P. chrysogenum and P. corylophilum*) along with *Fusarium oxysporum, Alternaria alternata, Cladosporium cladosporioides, Trichoderma viride* and *Mucor racemosus*. Abd-Allah & Ezzat [11] Sampled Paddy

Table 1. Total count (colonies/20 grains) and number of species (NS) of fungal species isolated from rice grains

Sample No	Grade	Locality	Level of break	DRBC		DG18	
				TC	NS	TC	NS
1	3	El-Edwa	High	13	4	20	9
2	2	El-Edwa	Moderate	23	9	9	8
3	3	El-Edwa	High	4	11	27	11
4	3	El-Edwa	High	18	7	15	7
5	3	El-Edwa	High	30	7	49	7
6	3	El-Edwa	High	9	6	32	9
7	1	Maghagha	Low	25	6	15	4
8	3	Maghagha	High	13	6	34	8
9	2	Maghagha	Moderate	11	6	10	10
10	3	Maghagha	High	22	6	23	7
11	2	Maghagha	Moderate	10	5	1	5
12	2	Bni-Mazar	Moderate	29	7	32	7
13	2	Bni-Mazar	Moderate	18	4	18	5
14	1	Bni-Mazar	Low	7	11	12	5
15	1	Bni-Mazar	Low	1	7	1	7
16	3	Bni-Mazar	High	14	2	13	2
17	1	Bni-Mazar	Low	14	4	6	5
18	3	Mattay	High	22	6	23	3
19	3	Mattay	High+ Rice weevil	15	2	7	4
20	1	Mattay	Low	10	4	36	8
21	3	Samalott	High	27	2	28	5
22	3	Samalott	High + Rice weevil	2	2	9	6
23	3	Samalott	High	2	2	25	3
24	1	Samalott	Low	1	1	-	-
25	3	Samalott	High	9	1	35	1
26	2	Samalott	Moderate	16	7	11	5
27	3	Samalott	High	21	7	7	11
28	3	El- Minia	High	38	8	17	8
29	3	El- Minia	High	18	4	30	10
30	3	El- Minia	High	3	2	9	9
31	2	El- Minia	Moderate	9	4	11	10
32	3	El- Minia	High	2	5	22	11
33	3	El- Minia	High	12	1	33	7
34	1	El- Minia	Low	9	2	14	6
35	3	El- Minia	High	2	1	32	6
36	3	El- Minia	High	7	4	42	3
37	1	El- Minia	Low	11	4	10	7
38	3	Abu-Qurqas	High	10	3	27	12
39	1	Abu-Qurqas	Low	16	3	9	5
40	2	Abu-Qurqas	Moderate	22	8	12	4
41	3	Abu-Qurqas	High	17	8	15	1
42	3	Abu-Qurqas	High	3	4	32	11
43	3	Mallawi	High	15	3	5	7
44	2	Mallawi	Moderate	6	3	12	6
45	2	Mallawi	Moderate	16	2	24	13
46	3	Mallawi	High	9	1	13	12
47	1	Mallawi	Low	3	3	9	11
48	3	Mallawi	High	3	4	39	9
49	3	Deir Mawas	High	8	3	22	9
50	1	Deir Mawas	Low	20	6	6	5
51	2	Deir Mawas	Moderate	8	1	23	14

Grade 1= less than 5%, grade 2 = from 5% to 10% and grade 3 = more than 10%.

Table 2. Total count (TC), percentage total count (%TC), Incidence (I) out of 51 samples and percentage incidence (%I) of fungal species recovered from rice samples on DRBC and DG18 Media

Fungal species	Types of media							
	DRBC				DG18			
	TC	%TC	I	%I	TC	%TC	I	%I
Lichtheimia corymbifera	15	2.29	4	7.84	18	1.86	8	15.68
Alternaria alternata	16	2.45	8	15.68	34	3.51	13	25.49
Aspergillus	412	63.08	49	96.07	633	65.51	49	96.07
A. aegyptiacus	2	0.3	1	1.96	-	-	-	-
A. amstelodami	5	0.76	5	9.8	2	0.2	2	3.92
A. candidus	56	8.57	18	35.29	80	8.28	27	52.94
A. chevalieri	17	2.6	3	5.88	44	4.55	16	31.37
A. clavatus	11	1.68	4	7.84	15	1.55	8	15.68
A. flavipes	7	1.07	3	5.88	10	1.03	5	9.8
A. flavus	137	20..98	32	62.74	48	4.96	19	37.25
A. flavus var. columnaris	8	1.22	3	5.88	61	6.31	13	25.49
A. fumigatus	9	1.37	4	7.84	16	1.65	8	15.68
A. montevidensis	5	0.76	4	7.84	57	5.9	23	45.09
A. niger	91	13.93	23	45.09	67	6.93	26	50.98
A. ochraceus	5	0.76	5	9.8	15	1.55	8	15.68
A. oryzae	-	-	-	-	9	0.93	8	15.68
A. parasiticus	2	0.3	2	3.92	6	0.62	5	9.8
A. rubrum	10	1.53	3	5.88	84	8.69	17	33.33
A. sydowii	1	0.15	1	1.96	11	1.13	5	9.8
A. tamarii	1	0.15	1	1.96	6	0.62	4	7.84
A. terreus	14	2.14	9	17.64	16	1.65	9	17.64
A. versicolor	29	4.44	5	9.8	85	8.79	16	31.37
A. wentii	2	0.3	2	3.92	1	0.1	1	1.96
Cladosporium	26	3.98	11	21.56	39	4.03	14	27.45
C. cladosporioides	8	1.22	4	7.84	19	1.96	6	11.76
C. herbarum	10	1.53	5	9.8	12	1.24	6	11.76
C. sphaerospermum	8	1.22	4	7.84	8	0.82	4	7.84
Cochliobolus	2	0.3	2	3.92	-	-	-	-
C. lunatus	1	0.15	1	1.96	-	-	-	-
C. spicifer	1	0.15	1	1.96	-	-	-	-
Fusarium	10	1.53	5	9.8	24	2.48	8	15.68
F. semitectum	4	0.61	3	5.88	22	2.27	8	15.68
F. verticillioides	6	0.91	2	3.92	2	0.2	2	3.92
Geosmithia sp.	1	0.15	1	1.96	-	-	-	-
Geotrichum candidum	5	0.76	3	5.88	1	0.1	1	1.96
Gliocladium roseum	1	0.15	1	1.96	4	0.41	3	5.88
Hyalodendron sp.	-	-	-	-	1	0.1	1	1.96
Mucor circinelloides	8	1.22	3	5.88	8	0.82	3	5.88
Nigrospora oryzae	2	0.3	2	3.92	11	1.13	6	11.76
Paecilomyces sp.	1	0.15	1	1.96	4	0.41	3	5.88
Penicillium	143	21.89	28	54.9	178	18.42	32	62.74
P. aurantiogriseum	6	0.91	4	7.84	22	2.27	7	13.72
P. chrysogenum	12	1.83	9	17.64	20	2.07	9	17.64
P. citrinum	21	3.21	5	9.8	30	3.1	11	21.56
P. corylophilum	2	0.3	1	1.96	3	0.31	3	5.88
P. crustosum	17	2.6	6	11.76	15	1.55	6	11.76
P. duclauxii	3	0.45	2	3.92	13	1.34	8	15.68
P. glabrum	10	1.53	5	9.8	14	1.44	7	13.72
P. islandicum	38	5.81	10	19.6	23	2.38	8	15.68
P. oxalicum	7	1.07	3	5.88	12	1.24	8	15.68
P. pinophilum	1	0.15	1	1.96	1	0.1	1	1.96
P. thomii	26	3.98	4	7.84	25	2.58	3	5.88
Rhizopus oryzae	4	0.61	2	3.92	6	0.62	2	3.92
Scopulariopsis koningii	1	0.15	1	1.96	1	0.1	1	1.96
Quambalaria cyanescens	1	0.15	1	1.96	-	-	-	-
Trichoderma harzianum	2	0.3	2	3.92	3	0.31	1	1.96
Trichurus spiralis	1	0.15	1	1.96	-	-	-	-
Ulocladium chartarum	1	0.15	1	1.96	1	0.1	1	1.96
Wallemia sebi	1	0.15	1	1.96	-	-	-	-
Total fungal counts	653	-	-	-	966	-	-	-

High incidence= 50-100%; Moderate =25-< 50%; Low=13-< 25%; Rare=1-12

rice from EI-Sharkia, E1-Dakahlia , EI-Gharbia, and Kafr E1-Shekh governorates and recorded an average of 6.79 x 10^4 fungal spores per gram rice. The fungal isolates were 47 species belonging to twenty eight genera. *Aspergillus, Cladosporium and Penicillium* were the most predominant genera. *Aspergilli* were represented by 22 species, *Aspergillus niger* and *A. flavus* had the highest occurrence. Also in previous studies on Egypt rice, were reported El-Shanshoury et al. [12] surveyed the incidence and load of fungi and aflatoxins in cereal grains and peanut, collected from some markets in central Delta provinces, Egypt.

Also some studies about the contamination of storage rice in many regions as compared to this study [30,31,32,33, 2, 28, 34, 35, 36, 37].While Sales and Yoshizawa [38,39] studied occurrence of *Aspergillus* section *Flavi* in rice from Philippines. Toman et al. [40] collected 60 samples of white and parboiled rice purchased on the Czech food market. Ochratoxin-A (OTA) analysis showed that 58 samples (96.7%) were found to be positive. OTA levels in white and parboiled rice fluctuated from 0.05 to 0.17 ng/g.

The natural occurrence of mycotoxins in rice has been studied in different countries of the world : In Nigeria, [2] studied fungi and some mycotoxins contaminating rice., high levels of AFB1 contamination in rice have also been reported at 200.19± 320.98µg/ kg [2] and 37.2±14.0µg/ kg [41]. Also, [42] determined the mycobiota associated with rice (*Oryza sativa*), maize (*Zea mays*), and millet (*Pennisetum typhoides*) in storage.

While [43] screened samples of rice, maize, cocoa and cocoa-based powder beverage collected from different markets and stores in south-western Nigeria.

In China, different studies were showed about rice and other cereal contamination with mycotoxins; [44, 45, 6, 46, 47]. In India; [48, 49, 50, 51]. In Korea; [52, 53]. In Spanish; [54, 55, 10]. In Turkey; [56, 57]. In Canada, [3]. In South America,[58]. In Pakistan,[59].

Table 3. Natural occurrence of mycotoxins in rice grain samples

Rice samples (Locality)	Mycotoxins	Level (ug/ kg)
Sample2 (El-Edwa)	-ve	-ve
Sample5 (El-Edwa)	-ve	-ve
Sample6 (El-Edwa)	-ve	-ve
Sample7 (Maghagha)	-ve	-ve
Sample8 (Maghagha)	-ve	-ve
Sample10 (Maghagha)	-ve	-ve
Sample13 (Benimazar)	-ve	-ve
Sample14 (Benimazar)	-ve	-ve
Sample16 (Benimazar)	-ve	-ve
Sample19 (Mattay)	Ochratoxin-A	50– 100
Sample21 (Samalott)	-ve	-ve
Sample22 (Samalott)	Aflatoxin B1	100-200
Sample23 (Samalott)	-ve	-ve
Sample25 (Samalott)	-ve	-ve
Sample29 (EL-Minia)	-ve	-ve
Sample30 (EL-Minia)	-ve	-ve
Sample33 (EL-Minia)	Sterigmatocystin	20-Oct
Sample38 (Abu-Qurqas)	-ve	-ve
Sample41 (Abu-Qurqas)	-ve	-ve
Sample42 (Abu-Qurqas)	-ve	-ve
Sample 43(Mallawi)	-ve	-ve
Sample 45 (Mallawi)	-ve	-ve
Sample46 (Mallawi)	-ve	-ve
Sample49(Deir Mawas)	-ve	-ve

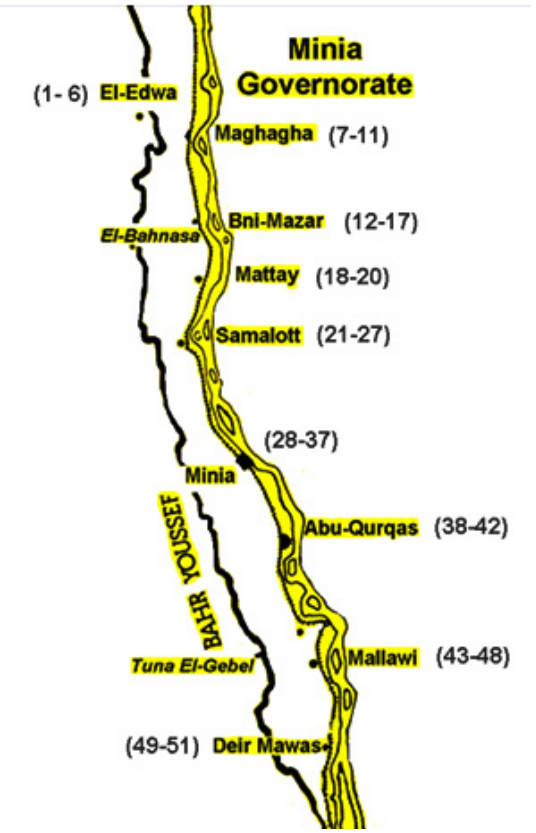

Fig. 1. Map of El-Minia Governorate showing different places from which rice samples were collected

Table 4. Mycotoxins produced by fungal strains (positive strains)

Fungal Species	Strain No. and Locality	Mycotoxins detected	Level (ug/L)
A. flavus	AUMC no. 11399	Aflatoxin B1	10-20
	El-Edwa	Aflatoxin G1	10-20
A. flavus	-7	AflatoxinB1	10-20
	Maghagha	AflatoxinG1	10-20
A. flavus	AUMC no.11394 Maghagha	AflatoxinB1	200-300
		Aflatoxin B2	200-300
		AflatoxinG1	10-20
		AflatoxinG2	10-20
A. flavus	-13	Aflatoxin B1	20-50
	Bni-Mazar	Aflatoxin G1	20-50
A. flavus	-14	AflatoxinB1	50-100
	Bni-Mazar	Aflatoxin B2	200-300
		AflatoxinG1	10-20
		AflatoxinG2	10-20
A. flavus	-18	Aflatoxin B1	10-20
	Mattay	Aflatoxin G1	10-20
A. flavus	AUMC no. 11395	Aflatoxin B1	200-300
	Mattay	Aflatoxin B2	200-300
		AflatoxinG1	10-20
		AflatoxinG2	10-20
A. flavus	AUMC no. 11400 Mattay	Aflatoxin B1	10-20
A.flavus	AUMC no. 11396 Samalott	AflatoxinB1	50-100
		Aflatoxin B2	50-100
		AflatoxinG1	10-20
A. flavus	-25 Samalott	Aflatoxin B1	50-100
A. flavus	(29) El-Minia	AflatoxinB1	200-300
		Aflatoxin B2	200-300
		AflatoxinG1	20-50
		AflatoxinG2	20-50
A. flavus	(30) El-Minia	Aflatoxin B1	10-20
		Aflatoxin G1	10-20
A. flavus	AUMC no.11398 El-Minia	Aflatoxin B1	10-20
		Aflatoxin G1	10-20
A. flavus	(33) El-Minia	AflatoxinB1	200-300
		Aflatoxin B2	200-300
		AflatoxinG1	10-20
		AflatoxinG2	10-20
A. flavus	(34) El-Minia	Aflatoxin B1	20-50
		Aflatoxin G1	10-20
A. flavus	(37) El-Minia	Aflatoxin B1	50-100
A. flavus	(38) Abu-Qurqas	Aflatoxin B1	10-20
		Aflatoxin G1	10-20
A. flavus	-42 Abu-Qurqas	Aflatoxin B1	50-100
A. flavus	(46) Mallawi	Aflatoxin B1	10-20
		Aflatoxin G1	10-20
A. flavus	(50) Deir Mawas	Aflatoxin B1	10-20
		Aflatoxin G1	10-20
A. flavus	(51) Deir Mawas	Aflatoxin B1	10-20
		Aflatoxin G1	10-20
A. fumigatus	AUMC no.11372 El-Minia	Gliotoxin	10-20
		Fumagillin	10-20
A. ochraceus	-4 El-Edwa	OchratoxinA	20-50
A. ochraceus	AUMC no. 11382 El-Minia	OchratoxinA	20-50
A. ochraceus	-34 El-Minia	OchratoxinA	100-200
A. parasiticus	(8) Maghagha	Aflatoxin B1	10-20
		Aflatoxin G1	10-20
A. terreus	-2 El-Edwa	Terrein	200-300
A. terreus	-12 Bni-Mazar	Terrein	10-20

AUMC, Assiut university mycological centre.

In South Vietnam, Trung et al. [33] screened twenty five samples of Vietnamese rice coming from the Mekong delta for fungal contamination. Ergosterol content measurement and total fungal load determination indicated that moulds contamination was quite weak. Identification of fungal species revealed that *Aspergillus* was the most common genus (43.75 % of isolates) followed by *Fusarium* (21.8 %) and *Penicillium* (10.9 %). The presence of toxigenic strains such as *A. flavus, A. ochraceus* and *P. citrinum* was confirmed by cultures. Fungal strains were tested for their toxinogenic potential and the results showed that 80 % of *A. flavus* strains were able to synthesized cyclopiazonic acid (levels up to 32.3 ppm), all strains of *A. ochraceus* produced ochratoxin A (one at 178 ppm) and the studied strain of *P. citrinum* was moderately toxigenic for citrinin. Also two rice samples were found contaminated with high level of ochratoxin A (21.3 and 26.2 ppb). This contamination can probably be linked to unfavorable post harvest storage and climatic conditions.

Sani and Sheikhzadeh discussed the different methods of aflatoxin (AFT) degradation in rice. Mycotoxins are mainly present in cereal grains such as rice and are not completely destroyed during their processing and cooking [60].

Detection of aflatoxins as aprimary mycotoxin in stored rice was also reported by demonstrated time to time by [61, 62, 48]. These mycotoxins were detected on the basis of fluorescence and retention factors (R.F.) values. Presence of them was confirmed by long wave UV light.

The retention factors of all the mycotoxins produced were determined. This was done by thin layer chromatography. Liu *et al*, Konishi *et al*[44, 63] studied aflatoxins and other mycotoxins in rice from China and Japan, respectively. Subsequently, Tanaka *et al*[64] who find the variation in mycotoxin contamination in rice from different regions may be due to differences in toxigenic microflora influenced by different agricultural practices and the differences in climate and also their storage conditions, Taligoola *et al*[65,27] morover reported toxigenic fungi and mycotoxins in rice.

Earlier, Madsen & Rasmussen [66] reported the presence of AFB1 contamination in milled rice. Prasad *et al*[67] screened sixty five samples of stored rice and twelve were positive for aflatoxin. Levels of aflatoxins ranged between 84 to 2830 µg/ kg mycelium.

Recently, Kushiro ; Toman *et al*; Xiang *et al*.; Sani & Sheikhzadeh ; Urooj et al.;Mukhtar et al. ;Aingkharat *et al* ;Majeed *et al* [68, 40, 69, 60, 70-73], were studies contamination of storage rice and other cereals by different fungal toxins.

CONCLUSIONS

Our results suggest that there is a need for proper storage of rice seed to minimize the fungal contamination and their mycotoxin production. Aflatoxins and ochratoxin are among the five most significant and abundant mycotoxins contaminating foods and food stuffs in the world [74], and have also been shown in this work to be major contaminants of rice in El-Minia Government.

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