

## Mycobiota and Mycotoxins Contaminating Rice Grains in El Minia, Governorate, 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 (genus) 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 <sup>1</sup> the cultivated area was estimated as 156 million hectares 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<sup>2</sup>.

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 <sup>3</sup>. In recent years, there have been many studies from various countries on

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the occurrence of high levels of aflatoxins by fungal rice contamination<sup>4,5,6</sup>. In 2001, Reddy and Sathyanarayana<sup>7</sup> listed 143 fungal species from rice. Reddy *et al.*<sup>8</sup> published a review contain the important groups of mycotoxins including aflatoxins (AFS), fumonisins, ochratoxin A(OTA), deoxynivalenol, and zearalenone (ZEN) , which contaminated rice in different countries. Sempere & Santamarina<sup>9</sup> confirmed that the majority of mycotoxins were produced by *Aspergillus*, *penicillium*, and *Fusarium*, Ferre<sup>10</sup> further found that AFS, citrinin, deoxynivalenol, fumonisins, sterigmatocystin, ZEN, cyclopiazonic acid, 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<sup>11, 12</sup>. 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<sup>13</sup>.

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<sup>13</sup>.

All plates were inoculated with rice grains (5 grains/plate). Cultures in quadruplicates 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<sup>14-20</sup>.

### 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<sup>21-23</sup>. 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 funnel 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<sup>24</sup>. 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 <sup>25</sup>.

#### **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. Nineteen 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% and 13.93% 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* prevailed 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. montevicensis*, *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. montevicensis* 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 µg/L. *A. parasiticus* produced B1 and G1 (10-20 µg/L). *A. terreus* (2 isolates) were able to elaborate terrein (10 -300 µg/L). Ochratoxin-A (20 -200 µg/L) was produced by 3 isolates of *A. ochraceus*. Finally, *A. fumigatus* produced Gliotoxin, fumagillin (10-20 µg/L for each).

## DISCUSSIONS

These results showed that the total fungal counts which were produced 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 <sup>26, 27</sup> 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.* <sup>28</sup> 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.* <sup>29</sup> 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 <sup>11</sup> 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
<i>Lichtheimia corymbifera</i>	15	2.29	4	7.84	18	1.86	8	15.68
<i>Alternaria alternata</i>	16	2.45	8	15.68	34	3.51	13	25.49
<i>Aspergillus</i>	412	63.08	49	96.07	633	65.51	49	96.07
<i>A. aegyptiacus</i>	2	0.3	1	1.96	-	-	-	-
<i>A. amstelodami</i>	5	0.76	5	9.8	2	0.2	2	3.92
<i>A. candidus</i>	56	8.57	18	35.29	80	8.28	27	52.94
<i>A. chevalieri</i>	17	2.6	3	5.88	44	4.55	16	31.37
<i>A. clavatus</i>	11	1.68	4	7.84	15	1.55	8	15.68
<i>A. flavipes</i>	7	1.07	3	5.88	10	1.03	5	9.8
<i>A. flavus</i>	137	20.98	32	62.74	48	4.96	19	37.25
<i>A. flavus var. columnaris</i>	8	1.22	3	5.88	61	6.31	13	25.49
<i>A. fumigatus</i>	9	1.37	4	7.84	16	1.65	8	15.68
<i>A. montevidensis</i>	5	0.76	4	7.84	57	5.9	23	45.09
<i>A. niger</i>	91	13.93	23	45.09	67	6.93	26	50.98
<i>A. ochraceus</i>	5	0.76	5	9.8	15	1.55	8	15.68
<i>A. oryzae</i>	-	-	-	-	9	0.93	8	15.68
<i>A. parasiticus</i>	2	0.3	2	3.92	6	0.62	5	9.8
<i>A. rubrum</i>	10	1.53	3	5.88	84	8.69	17	33.33
<i>A. sydowii</i>	1	0.15	1	1.96	11	1.13	5	9.8
<i>A. tamaraii</i>	1	0.15	1	1.96	6	0.62	4	7.84
<i>A. terreus</i>	14	2.14	9	17.64	16	1.65	9	17.64
<i>A. versicolor</i>	29	4.44	5	9.8	85	8.79	16	31.37
<i>A. wentii</i>	2	0.3	2	3.92	1	0.1	1	1.96
<i>Cladosporium</i>	26	3.98	11	21.56	39	4.03	14	27.45
<i>C. cladosporioides</i>	8	1.22	4	7.84	19	1.96	6	11.76
<i>C. herbarum</i>	10	1.53	5	9.8	12	1.24	6	11.76
<i>C. sphaerospermum</i>	8	1.22	4	7.84	8	0.82	4	7.84
<i>Cochliobolus</i>	2	0.3	2	3.92	-	-	-	-
<i>C. lunatus</i>	1	0.15	1	1.96	-	-	-	-
<i>C. spicifer</i>	1	0.15	1	1.96	-	-	-	-
<i>Fusarium</i>	10	1.53	5	9.8	24	2.48	8	15.68
<i>F. semitectum</i>	4	0.61	3	5.88	22	2.27	8	15.68
<i>F. verticillioides</i>	6	0.91	2	3.92	2	0.2	2	3.92
<i>Geosmithia</i> sp.	1	0.15	1	1.96	-	-	-	-
<i>Geotrichum candidum</i>	5	0.76	3	5.88	1	0.1	1	1.96
<i>Gliocladium roseum</i>	1	0.15	1	1.96	4	0.41	3	5.88
<i>Hyalodendron</i> sp.	-	-	-	-	1	0.1	1	1.96
<i>Mucor circinelloides</i>	8	1.22	3	5.88	8	0.82	3	5.88
<i>Nigrospora oryzae</i>	2	0.3	2	3.92	11	1.13	6	11.76
<i>Paecilomyces</i> sp.	1	0.15	1	1.96	4	0.41	3	5.88
<i>Penicillium</i>	143	21.89	28	54.9	178	18.42	32	62.74
<i>P. aurantiogriseum</i>	6	0.91	4	7.84	22	2.27	7	13.72
<i>P. chrysogenum</i>	12	1.83	9	17.64	20	2.07	9	17.64
<i>P. citrinum</i>	21	3.21	5	9.8	30	3.1	11	21.56
<i>P. corylophilum</i>	2	0.3	1	1.96	3	0.31	3	5.88
<i>P. crustosum</i>	17	2.6	6	11.76	15	1.55	6	11.76
<i>P. duclauxii</i>	3	0.45	2	3.92	13	1.34	8	15.68
<i>P. glabrum</i>	10	1.53	5	9.8	14	1.44	7	13.72
<i>P. islandicum</i>	38	5.81	10	19.6	23	2.38	8	15.68
<i>P. oxalicum</i>	7	1.07	3	5.88	12	1.24	8	15.68
<i>P. pinophilum</i>	1	0.15	1	1.96	1	0.1	1	1.96
<i>P. thomii</i>	26	3.98	4	7.84	25	2.58	3	5.88
<i>Rhizopus oryzae</i>	4	0.61	2	3.92	6	0.62	2	3.92
<i>Scopulariopsis koningii</i>	1	0.15	1	1.96	1	0.1	1	1.96
<i>Quambalaria cyanescens</i>	1	0.15	1	1.96	-	-	-	-
<i>Trichoderma harzianum</i>	2	0.3	2	3.92	3	0.31	1	1.96
<i>Trichurus spiralis</i>	1	0.15	1	1.96	-	-	-	-
<i>Ulocladium chartarum</i>	1	0.15	1	1.96	1	0.1	1	1.96
<i>Wallemia sebi</i>	1	0.15	1	1.96	-	-	-	-
Total fungal counts	653	-	-	-	966	-	-	-

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

rice from El-Sharkia, El-Dakahlia, El-Gharbia, and Kafr El-Shekh governorates and recorded an average of  $6.79 \times 10^4$  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.*<sup>12</sup> 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<sup>30,31,32,33, 2, 28, 34, 35, 36, 37</sup>. While Sales and Yoshizawa<sup>38,39</sup> studied occurrence of *Aspergillus* section *Flavi* in rice from Philippines. Toman *et al.*<sup>40</sup> 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.

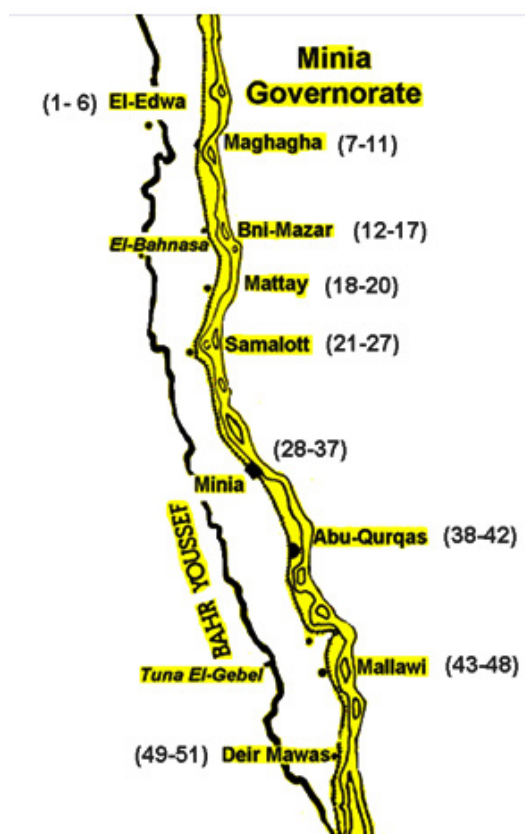
The natural occurrence of mycotoxins in rice has been studied in different countries of the world: In Nigeria,<sup>2</sup> studied fungi and some mycotoxins contaminating rice., high levels of AFB1 contamination in rice have also been reported at  $200.19 \pm 320.98 \mu\text{g}/\text{kg}$ <sup>2</sup> and  $37.2 \pm 14.0 \mu\text{g}/\text{kg}$ <sup>41</sup>. Also,<sup>42</sup> determined the mycobiota associated with rice (*Oryza sativa*), maize (*Zea mays*), and millet (*Pennisetum typhoides*) in storage.

While<sup>43</sup> 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;<sup>44,45,6,46,47</sup>. In India;<sup>48,49,50,51</sup>. In Korea;<sup>52,53</sup>. In Spanish;<sup>54,55,10</sup>. In Turkey;<sup>56,57</sup>. In Canada,<sup>3</sup>. In South America,<sup>58</sup>. In Pakistan,<sup>59</sup>.

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

AUMC, Assiut university mycological centre.



In South Vietnam, Trung *et al.*<sup>33</sup> 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<sup>60</sup>.

Detection of aflatoxins as a primary mycotoxin in stored rice was also reported by demonstrated time to time by<sup>61, 62, 48</sup>. 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.*<sup>44</sup>,<sup>63</sup> studied aflatoxins and other mycotoxins in rice from China and Japan, respectively. Subsequently, Tanaka *et al.*<sup>64</sup> 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.*<sup>65,27</sup> moreover reported toxigenic fungi and mycotoxins in rice.

Earlier, Madsen & Rasmussen<sup>66</sup> reported the presence of AFB1 contamination in milled rice.

Prasad *et al.*<sup>67</sup> 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.*<sup>68, 40, 69, 60, 70-73</sup>, 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<sup>74</sup>, and have also been shown in this work to be major contaminants of rice in El-Minia Government.

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## REFERENCES

1. FAO (Food and Agriculture Organization). Rice marked monitor. *Trade and markets division*, 2012; **15**(1):1-33.
2. Makun HA, Gbodi TA, Akanya OH, Salako EA, Ogbadu GH. Fungi and some mycotoxins contaminating rice (*Oryza sativa*) in Niger state, Nigeria. *Afr J Biotechnol* 2007; **6**(2):099-108.
3. Bansal J, Pantazopoulos P, Tam J, Cavlovic P, Kwong K, Turcotte A-M, Lau BP-Y, Scott PM. Surveys of rice sold in Canada for aflatoxins, ochratoxin A and fumonisins. *Food Addit Contam* 2011; PartA **28**(6): 767- 774.
4. Suarez-Bonnet E, Carvajal M, Mendez-Ramirez I, Castillo Urueta P, Cortes-Eslava J, Gomez-Arroyo S, Melero-Vara JM. Aflatoxin (B1, B2, G1, and G2) contamination in rice of Mexico and Spain from local sources or imported. *J Food Sci* 2013; **78**: 1822-1829. .
5. Ok HE, Kim DM, Kim D, Chung SH, Chung M, Park KH, Chun HS. Mycobiota and natural occurrence of aflatoxin, deoxynivalenol, nivalenol and zearalenone in rice freshly

- harvested in South Korea. *Food Control*, 2014; **37**: 284–291.
6. Lai XW, Liu RC, Ruan CQ, Zhang H, Liu CL. Occurrence of aûatoxins and ochratoxin A in rice samples from six provinces in China. *Food Control 2015a*; **50**: 401– 404.
  7. Reddy OR, Sathyanarayana N. Seed-borne fungi of rice and quarantine signiûcance. In: Sreenivasaprasad S, Johnson R, editors. Major fungal diseases of rice, recent advances. Dordrecht, The Netherlands: Kluwer Academic Publishers 2001; p367.
  8. Reddy KRN, Reddy CS, Abbas HK, Abel CA, Muralidharan K. Mycotoxigenic fungi, mycotoxins, and management of rice grains. *Toxin Rev* 2008; **27**: 287- 317.
  9. Sempere F, Santamarina MP. Efûcacy of *Trichoderma harzianum* in suppression of *Fusarium culmorum*. *Ann Microbiol* 2010; **60**: 335–340.
  10. Ferre FS. Worldwide occurrence of mycotoxins in rice. *Food control* 2016; **62**: 291-298.
  11. Abd-Allah EF, Ezzat SM. Natural occurrence of citrinin in rice grains and its biocontrol by *Trichoderma hamatum*. *Phytoparasitica* 2005; **33**: 73– 84.
  12. El-shanshoury AR, El-sabbagh SM, Emara HA, Saba HAE. Occurrence of moulds, toxicogenic capability of *Aspergillus flavus* and levels of aflatoxins in maize, wheat, rice and peanut from markets in Central Delta Provinces, Egypt. *Int J Curr Microbiol App Sci* 2014; **3**: 852- 865.
  13. Pitt JI, Hocking AD. Fungi and food spoilage . 3<sup>rd</sup> Edition. Springer Science and Business Media 2009; 519 pages.
  14. Domsch KH, Gams W, Anderson T. Compendium of soil fungi. 2<sup>nd</sup> Edition. IHW Verlag. Eching Germany 2007; 672 pages.
  15. Ellis MB. Dematiaceous hyphomycetes. Commonwealth Mycological Institute. Kew, Surrey, England 1971; 608 pages.
  16. Ellis MB. More dematiaceous hyphomycetes. Commonwealth Mycological Institute, England 1976; 481 pages.
  17. Leslie JF, Summerell BA. *Fusarium* laboratory manual. Blackwell publishing, John Wiley and Sons 2006; 338 pages.
  18. Moubasher AH. Soil fungi of Qatar and other Arab countries. The Scientific and Applied Research Centre, University of Qatar 1993; 566 pages.
  19. Pitt JI. The genus *Penicillium* and its teleomorphic states *Eupenicillium* and *Talaromyces*. Academic Press Inc (London) LTD 1979; 634 pages.
  20. Raper KB, Fennell DI. The genus *Aspergillus*. Baltimore, Maryland: Williams and Wilkins Company 1965; 686 pages.
  21. Jackson LK, Ciegler A. Production and analysis of citrinin in corn. *Appl Environ Microbiol* 1978; **36**: 408- 411.
  22. Trantham AL, Wilson DM. Fluorometric screening method for citrinin in corn , barley and peanuts . *J Assoc off Anal Chem* 1984; **6**: 37- 40.
  23. Zohri AA. Mycoflora and mycotoxins of some meat products. Ph.D. Thesis, Botany Department, Faculty of Science, Assiut University, Assiut, Egypt 1990.
  24. El-Kady IA, Moubasher MH. Toxicogenicity and toxin of *Stachybotrys chartarum* isolates from wheat straw samples in Egypt. *Experimental Mycology* 1982; **6**: 25- 31.
  25. Scott PM, Lawrence JW, Walbek W. Detection of mycotoxins by thin layer chromatography :application to screening of fungal extracts. *Appl Microbiol* 1970; **20**: 839- 842.
  26. Taligoola HK, Ismail MA, Chebon SK. Mycobiota associated with rice grains marketed in Uganda. *J Biol Sci* 2004; **4**(1): 271-278.
  27. Taligoola HK, Ismail MA, Chebon SK. Mycobiota and aflatoxins associated with imported rice grains stored in Uganda. *Czech Mycol* 2011; **63**: 93- 107.
  28. Fredlund E, Thim A-M, Gidlund A, Brostedt S, Nyberg M, Olsen M. Moulds and mycotoxins in rice from the Swedish retail market. *Food Addit Contam* 2009; **26**(04):527- 533.
  29. Abdel-Hafez SII, El-Kady IA, Mazen MB, El-Maghraby OM. Mycoflora and trichothecine toxins of paddy grains from Egypt. *Mycopathologia* 1987; **100**:103- 112.
  30. Tonon SA, Marucci RS, Jerke G, Garcia A. Mycoflora of paddy and milled rice produced in the region of Northeastern Argentina and Southern Parguary. *Int J Food Microbiol* 1997; **37**: 231- 235.
  31. Butt AR, Yaseen SI, Javaid A. Seed-borne mycoflora of stored rice grains and its chemical control. *J Anim Plant Sci* 2011; **21**(2): 193- 196.
  32. Shanakht H, Shahid AA, Ali SW. Characterization of fungal microbiota on rice grains from local markets of Lahore. *Journal of Hygienic Engineering and Design* 2014; **9**: 35- 40.
  33. Trung TS, Bailly JD, Querin A, Bars PL, Guerre P. Fungal contamination of rice from South Vietnam, mycotoxinogenesis of selected strains and resided in rice. *Revue Med Vet* 2001; **152**: 555- 560.
  34. Ma³gorzata P. Contamination of breakfast cereal products by fungi and mycotoxins – a potential risk for consumer’s health *Biotechnol Food Sci*, 2013; **77**(1):3-10.
  35. Stanciu O, Banc R, Cozma A, Filip L, Miere

- D, Manes J, Loghin F. Occurrence of *Fusarium* mycotoxins in wheat from Europe – A review. *Food Technol* 2015; **XIX** (1):35- 60.
36. Christensen CM, Kaufmann HH. Microflora: In storage of cereal grains and their products. CM Christensen (Ed) American Association of Cereal Chemists 1974; 159- 191.
37. Christensen CM. Field and storage fungi. In: Beuchat LR, ed. Food and everage Mycology 2nd ed New York 1987; 211- 232.
38. Sales AC, Yoshizawa T. Mold counts and *Aspergillus* section *Flavi* populations in rice and its byproducts from the Philippines. *J Food Prot* 2005a; **68**(1):120- 125.
39. Sales AC, Yoshizawa T. Updated profile of aflatoxin and *Aspergillus flavi* contamination in rice and its byproducts from the Philippines. *Food Addit Contam* 2005b; **22**(5):429– 436.
40. Toman J, Malir F, Ostry V, Grosse Y, Dvorak V, Roubal T, Neuchlova L. The occurrence of ochratoxin A in white and parboiled rice. *Food Chemistry and Safety Czech J Food Sci* 2016; **34**: 32- 38.
41. Makun HA, Dutton MF, Njobeh PB, Mwanza M, Kabiru AY. Natural multi-occurrence of mycotoxins in rice from Niger State, Nigeria. *Mycotoxin Res* 2011; **27**: 97– 104.
42. Amadi JE, Adeniyi DO. Mycotoxin production by fungi isolated from stored grains. *Afr J Biotechnol* 2009; **8**(7): 1219- 1221.
43. Egbuta MA, Mwanza M, Njobeh PB, Phoku JZ, Chilaka CA, Dutton MF. Isolation of filamentous fungi species contaminating some Nigerian food commodities. *J Food Research* 2015; **4**(1):38- 50.
44. Liu Z, Gao J, Yu J. Aflatoxins in stored maize and rice grains in Liaoning Province, China. *Journal of Stored Products Research* 2006; **42**: 468-479.
45. Sun G, Wang S, Hu X, Su J, Zhang Y, Xie Y, Zhang H, Tang L, Wang JS. Cocontamination of aûatoxin B1 and fumonisin B1 in food and human dietary exposure in three areas of China. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess* 2011; **28**: 461- 470.
46. Lai XW, Zhang H, Liu RC, Liu CL. Potential for aûatoxin B1 and B2 production by *Aspergillus ûavus* strains isolated from rice samples. *Saudi J Biol Sci*, 2015b; **22**: 176–180.
47. Sun XD, Su P, Shan H. Mycotoxin contamination of rice in China. *J Food Sci* 2017; **82**(3): 573- 584.
48. Reddy KRN, Reddy CS, Muralidharan K. Detection of *Aspergillus* spp. and aflatoxin B1 in rice in India. *Food Microbiol* 2009; **26**: 27- 31.
49. Gautam AK, Gupta H, Soni Y. Screening of fungi and mycotoxins associated with stored rice grains in Himachel Pradesh. *International J Theoretical App Sci* 2012; **4**(2):128-133.
50. Uma V, Wesely EG. Seed borne fungi of rice from South Tamil Nadu. *J Acad Indus Res* 2013; **1**(10): 612- 614.
51. Almeida MI, Almeida NG, Carvalho KL, Goncalves GAA, Silva CN, Santos EA, Garcia JC, Vargas EA. Cooccurrence of aûatoxins B1, B 2, G 1 and G2, ochratoxin A, zearalenone, deoxynivalenol, and citreoviridin in rice in Brazil. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess* 2012; **29**: 694– 703.
52. Park JW, Choi SY, Hwang H J, Kim YB. Fungal mycoflora and mycotoxins in Korean polished rice destined for humans. *Int J Food Microbiol* 2005; **103**: 305- 314.
53. Oh JY, Sang MK, Oh J-E, Lee H, Ryoo MI, Kim KD. Microbial population, aflatoxin contamination and predominant *Aspergillus* species in Korean stored rice. *Plant Pathol J* 2010; **26**(2):121- 129.
54. González L, Juan C, Soriano JM, Moltó JC, Mañes J. Occurrence and daily intake of ochratoxin A of organic and non-organic rice and rice products. *Int J Food Microbiol* 2006; **107**: 223- 227.
55. Cantalejo MJ, Carrasco JM, Hernandez E. Mycoflora of seeds and feeds from the Spanish market. *J Basic Microbiol* 1997; **37**: 395- 402.
56. Aydin A, Aksu H, Gunsen U. Mycotoxin levels and incidence of mould in Turkish rice. *Environ Monit Assess* 2011; **178**: 271–280.
57. Buyukunal SK, Kahraman T, Ciftcioglu GR. Occurrence of AF, AFB1, OTA in rice commercialized in Eastern Turkey. *Polish J Environ Stud* 2010; **19**(5): 907–912.
58. Morrison DM, Chester I, Samuela CAN, Ledoux DR. The determination of aflatoxins in paddy and milled fractions of rice in Guyana. *Int J Biol, Biomolecular, Agric, Feed Biotechnol Engineering* 2016; **10**(11):725- 729.
59. Iqbal SZ, Asi MR, Hanif U, Zuber M, Jinap S. The presence of aûatoxins and ochratoxin A in rice and rice products: a survey. *Food Chem* 2016; **210**:135- 140.
60. Sani AM, Sheikhzadeh M. Aflatoxin degradation in rice.” *Nutrition and Food Sci* 2017; **47**(4): 469- 476.
61. Reddy CS, Reddy KRN, Raja Kumar N, Laha GS, Muralidharan K. Exploration of aflatoxin contamination and its management in rice. *J Mycol Plant Pathol* 2004; **34**(3): 816- 820.
62. Reddy KRN, Reddy CS, Muralidharan K. Characterization of aflatoxin B1 produced by *Aspergillus flavus* isolated from discolored rice grains. *J Mycol Plant Pathol* 2005; **35**(3): 470- 474.

63. Konishi S, Nakajima M, Tabata S, Ishikuro H, Tanaka T, Norizuki H. Occurrence of aflatoxins, ochratoxin A and fumonisins in retailed foods in Japan. *J Food Prot* 2006; **69**: 1365- 1370.
64. Tanaka K, Sago Y, Zheng Y, Nakagawa H, Kushiro M. Mycotoxins in rice. *Int J Food Microbiol* 2007; **119**: 59- 66.
65. Taligoola HK, Ismail MA, Chebon SK. Toxigenic fungi and aflatoxins associated with marketed rice grains in Uganda. *J Basic Appl Mycol* 2010; **1**: 45- 52.
66. Madsen B, Rasmussen G. Aflatoxin B1, B2, G1 and G2 in maize, rice, millet, buckwheat, lentils and beans etc in 1987 and 1988. *Levnedes middel styrelsen* 1990; 200(33) 9.
67. Prasad T, Sinha RK, Jeswal P. Seed mycoflora of cereals and aflatoxin contamination under storage systems. *J Indian Bot Soc* 1987; **66**: 156– 160.
68. Kushiro M. Historical review of researches on yellow rice and mycotoxigenic fungi adherent to rice in Japan. *JSM Mycotoxins* 2015; **65**(1):19-23.
69. Xiang DS, Ping S, Hong S. Mycotoxin contamination of rice in China. *J Food Sci* 2017; **82**(3): 573- 584.
70. Urooj R, Achakzai K, Zaman M, Bibi A, Afreen T, Iram S. Occurrences of mycotoxins contamination in crops from Pakistan. A Review, *American-Eurasian J Agric Environ Sci* 2015; **15**(7): 1289- 1297.
71. Mukhtar H, Farooq Z, Manzoor M. Determination of aflatoxins in super kernel rice types consumed in different regions of Punjab, Pakistan. *The J Anim Plant Sci* 2016; **26**(2):542- 548.
72. Aingkharat T, Taraporn J, Orawan A. Mycotoxins in feed and feed ingredients 2015-2016. *Thai J Vet Med Suppl* 2017; **47**: 313.
73. Majeed S, De Boevre M, De Saeger S, Rauf W, Tawab A, Fazel-e-Habib, Rahman M, Iqbal M. Multiple mycotoxins in rice: Occurrence and health risk assessment in children and adults of Punjab, Pakistan. *Toxins* 2018; **10**, 77:1- 30.
74. Bhat RV, Vasanthi S. Food safety in food security and food trade. Mycotoxin food safety risk in Developing Countries. *Food Agric Environ*, 2003; IFPRI Brief 3 of 17.