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Detection of Presence Fungi and Aflatoxines (AFB1 and AFG1) in Rice Samples Collected from Maysan Province

Intesar Ali Mezeal
Department of Biology, College of Science, University of AL- Mustansarya, Baghdad, Iraq
Author for Correspondence:intesarali664@yahoo.com

Abstract
This study aimed to examination the frequency and capacity of fungi and aflatoxins in rice samples stored collected from some houses in Maysan province, Iraq. The levels of aflatoxins produced by isolated Aspergillus flavus were also appraised. Fifty rice samples were investigated for fungal infection. The fungi were isolated and identified were Cladosporium spp., Colletotrichum nymphaeae , Aspergillus flavus, A. niger, A. candidus, Fusarium spp., F. oxysporum ,F. graminearum , Penicillium spp. , Mucor spp. , Trichoderma sp.,Rhizopus sp., Alternaria spp. , A. alternaria and Chaetomium sp.. A.flavus recorded highest percentage of frequency was 82% flowed by A. niger and Penicillium spp., A. flavus recorded highest percentage of presence was 90.3% flowed by Cladosporium spp., 27 rice samples showed ability to produce AFB1 ,while the AFG1 was produced from 22 rice samples . Results showed that it is wide difference of aflatoxins creation was evident amongst the experienced isolates of A. flavus expending, TLC technique, it was originate that 25 isolates, among 41 were able to produce AFB1 and 12 isolates were able to produce AFG1.
Keywords: aflatoxines ,AFB1,AFG1,A.flavus,rice, Maysan province,Iraq

1- Introduction

Rice (Oryza sativa L.) is one of the most cultured nutrition harvests worldwide About 593 million tones (Mt)rice is bent yearly (FAO, 2002), It is one of the focal grains crops in Iraqi food people, altogether troubled with the quality and protection of food .Mycotoxins occurring in food possessions are secondary metabolites of filamentous fungi Mycotoxins pollute many types of food crops throughout the food hawser (Reddy *et al*., 2010). Food and Agricultural Organization of the United Nations (FAO) projected that at least 25% of the world's muesli grains are polluted by mycotoxins, comprising aflatoxins (FAO,2004). Mycotoxin-producing fungi are significant contaminant and demolishers of agricultural crops and seeds through storage, through processing and in the markets, and decrease their nourishing significance (Jimoh and Kolapo, 2008). Aflatoxins container contaminate agricultural supplies including corn, wheat, rice, peanut, and numerous other yields (Sinha and Sinha, 1991:Aly, 2002, Reddy *et al*., 2009; Yassin *et al*., 2011). Mycotoxin infection is fewer generally described for rice than for many other muesli harvests, but rice characterizes a great substrate for fungal growing and toxinogenesis later it is used as an perfect culture medium to examination the toxigenic possible of fungal isolates (Bars, 1992). Mycotoxins are toxic natural secondary metabolites created by several species of fungi (as Fusarium, Aspergillus and Penicillium genera). The attendance of mycotoxins in food and feedstuff might distress human and animal health, by way of they can cause many different antagonistic effects such as estrogenic, gastrointestinal, and kidney disorders induction of cancer, and mutagenicity (EFSA , 2012: Marin etal ,2013). From the greatest pertinent collections of mycotoxins establish in food are AFB1and AFG1 (Capriotti, *et al* ,2012). Numerous mycotoxicoses in humans and animals have been described owing to the consumption of mycotoxin-contaminated food and feedstuff (Peraica and Domijan, 2001). Among the toxigenic fungi *A. flavus* is the greatest frequently isolated and is recognized as aflatoxins manufacturer, which are carcinogenic composites. There are a amount of reports that demonstration the toxigenic possible essay of *A. flavus* isolated in different crops or foods. Adulteration of food grains as, rice is significant subject for grain quality and from customer's health opinion of view. Consequently, current study was carried out for the isolation and identification mycotoxins and their producing fungi related with rice grains during storage.

2 - Materials and Methods

2-1 Samples collection

A total of 50 stored rice samples in Iraqi houses were collected from Maysan province ,the samples were labeled, packaged in polyethylene bags, transferred to the laboratory and kept in4°C.

2-2 Isolation and identification of fungi

Ten grams of each sample (fine powder) were transferred into screw-capped medicinal bottle containing 90 mL of sterile distilled water and were mechanically homogenized at constant speed for 15 min. The sample-water suspension was allowed to stand for 10 min with intermittent shaking before being plated , serial dilutions (1:5) were prepared and 1 mL portions of suitable dilutions of the resulting samples suspension were used to inoculate Petri dishes each containing 15 mL Czapek's Dox agar medium. Plates were then incubated for 5 days at 28°C.

Three replicates plates per medium were used for each sample, the obtained fungi were subjected to identification by the aid of the following references (Booth, 1971; Ellis, 1976 ; Pitt, 1979 ;Domsch et al., 1980; Kozakiewicz, 1989 ;Moubasher, 1993; Samson *et al*., 1995 Klich, 2002; Samson *et al*., 2002).

2-3 Aflatoxin exploration in rice samples

Aflatoxins were extracted from rice samples according to the method described by (Schuller *et al*., 1983). 25 g of each rice sample were added to a 250-ml conical flask containing 25 ml distilled water and 50 ml chloroform. The flasks were shaken for 30 min on a rotator shaker and the suspensions were filtered. The resulting chloroform extracts were purified according to (Takeda *et al*., 1979) , Elutes were evaporated to dryness on steam bath. Each residue was re-dissolved in 1 ml chloroform, Aflatoxins were analyzed on 20x 20 cm TLC-plastic sheets, percolated with silica gel plate type 60 F254 each sample extract containing aflatoxins was loaded on the silica gel plates Standard aflatoxins B1, and G1 supplied from Sigma Chemical Company USA were used for comparison with the unknown samples. The plates were developed in a glass jar containing chloroform-acetone (9:1, v/v) as developing solvent. Aflatoxins were quantified as described by (Shannon *et al*.,1983; FAO , 1990; FAO. 2004) using luorodensito meter (TLD-100 vitiation). Measurements were performed through fluorescence at 366 nm.

2-4 Aflatoxins in cultures of *A.flavus* isolates

The method adopted in the present investigation was carried out according to (A.O.A.C., 1984).One ml aliquot of spore suspension (10-5 spores/ml)was used to inoculate 250 ml conical flask containing 50 ml Czapek's-Dox Medium ,The cultures were incubated for 10 days in the dark at 28°C. At the end of the incubation period, each culture was filtered and the filtrate was extracted with chloroform. The extract was treated as described before , as for analysis of food samples. Mycelia growth was expressed as the dry weight of the mycelia mass collected after extraction of aflatoxins and drying at 70° for approximately24 h (Kane and Mullins, 1973).

3-Results and Discussion

3-1 Fungal infection of tested samples

Results data shown in table (1) diagnosis fifty sample of rice stored in households that have been collected randomly from some respects of the province of Maysan ,Iraq. Sampling difference in footings of the kinds of fungi isolated and the percentage of their presence within the sample's and percentage of frequency in samples were examined. The data presented isolation and identification of, *Cladosporium spp., Colletotrichum nymphaeae , A. flavus, A. niger, A. candidus , Fusarium spp., F. oxysporum F. graminearum., Penicillium spp. , Mucor spp. , Trichoderma sp.,Rhizopus sp., Alternaria spp. , A. alternaria and Chaetomium sp.*, another studies that isolation similar fungi from rice samples like *Aspergillus, Penicillium ,Fusarium, Phoma, Curvularia, Helminthosporium, Cladosporium, Arthrinium* , *Alternaria*. *Trichoderma* and *Chaetomium* (Garcia, 1986; Rama Devi *et al*., 1988; Uraguchi and Yamazaki, 1978) .Fungal species isolated from rice samples (uncooked and cooked) were found in the instruction of *Rhizopus* spp. (76%) *Aspergillus* spp(67%) *A. flavus* (42%) *Mucor* spp. (64%) and Penicillium spp. (31%) (Tahir et al 2012; Sundaram *et al* 1988). The expansion of fungi, particularly *Aspergillus*, *Fusarium* and *Penicillium* species, is an unanswered difficult in storage They are accountable for quantitative and qualitative fatalities and below convinced conditions these species can progress toxic metabolites (Makun et al 2007).

Table1: Isolated Fungi From Race Samples

fungi	% frequency	%presence
Cladosporium spp.	**50**	**88.54**
Colletotrichum nymphaeae	**44**	**12.83**
Aspergillus flavus	**82**	**90.3**
A. niger	**78**	**87.42**
A. candidus	**70**	**81.64**
Fusarium sp.	**62**	**32.09**
F. graminearum	**64**	**78.89**
F. oxysporum	**8**	**12.76**
Penicillium spp.	**76**	**85.52**
Mucor spp.	**12**	**0.54**
Trichoderma sp.	**28**	**34.48**
Rhizopus sp.	**68**	**64.32**
Alternaria spp.	**58**	**34.78**
A. alternaria	**18**	**14.53**
Chaetomium sp.	**22**	**24.74**

Results in Table 1 showed that *A. flavus* recorded highest percentage of frequency was 82% flowed by *A. niger* and *Penicillium* spp. were recorded percentage of frequency was 78% and 76% respectively. while lowest percentage of frequency recorded was 8% and 12% and 18% in the funguses *F. oxysporum* , *Mucor* spp. and *A. alternaria* respectively.

Results showed that *A. flavus* recorded highest percentage of presence was 90.3% flowed by *Cladosporium* spp., *Penicillium* spp. and *A. niger* were recorded percentage of presence was 88.54 % , 85.52 % and 87.42 % respectively. while lowest percentage of presence recorded was 0.54 % , 12.83 % and 14.53 in the funguses *Mucor* spp., *F. oxysporum* and *A. alternaria* respectively. Sales and Yoshizawa (2005a, 2005b) described the incidence of *A. flavus* and *A. parasiticus* in rice bran (14%) and rough rice (78%). Notwithstanding such studies, there has been no attempt to investigate fungal contamination in entirety in seeds, hull, rice kernel, and kernel powder of rice cultivars grown-up crossways the country in dissimilar ecosystems . Gautam *et al*.,(2012) originate that all the samples were establish contaminated with one or more fungal genera *like Aspergillus* ,*Rhizopus*, *Penicillium, Fusarium, Curvularia, Cladosporium* and *Alternaria*. *A. flavus*, *A. niger* and *Rhizopus stolonifer* were isolated with highest frequency and density.

3-2 Aflatoxins in Rice samples

Results in Table 2 showed that the ability to produce AFB1 in Rice samples recorded in of 27 rice samples ,while the AFG1 was recorded only in 22 rice samples , the samples (ME4, ME5, ME9, ME10, ME13, ME14, ME15, ME18 ME21, ME27, ME29, ME32, ME33, ME41, ME45, ME47) were not produced any afatoxins (neither AFB1 nor AFG1).

The highest concentration of AFB1 was recorded in M11 , M25 and M34 samples wre 800 , 790 and 780 (µg/kg dry wt.) respectively, and less percentage was as shown in M3 , M23 and M46 samples were 20 and 30 (µg/kg dry wt.) respectively (table 2) , about AFG1 the highest concentration was recorded in M37 was 780 (µg/kg dry wt.) and less percentage was as shown in M19, M24 and M7 samples were 20 and 40 (µg/kg dry wt.) respectively. If the average temperature throughout summer is very hot ,the adjustable climatic conditions of the area may kindness the probabilities of fungal and mycotoxin contamination of storage nutrition grains (Essono *et al*., 2007), Gautam *et al*.,(2012) establish afterward mycotoxins examination only Afaltoxins B1 and B2 were sensed in around 72% samples, many studies suggests that discovery of fungi and aflatoxins postures a danger for consumer's health and it is essential to checkered the rice grains before permitting distribution. Liu *et al* (2006) and Konishi *et al*., (2006) intentional aflatoxins and additional mycotoxins in rice from China and Japan correspondingly while, Mangala *et al*., (2006) , Taligoola *et al*.,(2010,2011) and Surekha *et al*., (2011) also described toxigenic fungi and mycotoxins in rice.

Table.2 Aflatoxins in Rice samples

Samples	Concentration of AFB1(µg/kg dry wt.)	Concentration AFG1(µg/kg dry wt.)
ME1	**0.0**	220
ME2	**650**	600
ME3	**20**	**0.0**
ME4	**0.0**	**0.0**
ME5	**0.0**	**0.0**
ME6	**400**	**740**
ME7	**480**	**40**
ME8	**80**	**700**
ME9	**0.0**	**0.0**
ME10	**0.0**	**0.0**
ME11	**800**	**620**
ME12	**540**	**0.0**
ME13	**0.0**	**0.0**
ME14	**0.0**	**0.0**
ME15	**0.0**	**0.0**
ME16	**120**	**580**
ME17	**620**	**0.0**
ME18	**0.0**	**0.0**
ME19	**720**	**20**
ME20	**430**	**180**
ME21	**0.0**	**0.0**
ME22	**600**	**0.0**
ME23	**30**	**660**
ME24	**340**	**20**
ME25	**780**	**420**
ME26	**0.0**	**60**
ME27	**0.0**	**0.0**
ME28	**0.0**	**720**
ME29	**0.0**	**0.0**
ME30	**0.0**	**600**
ME31	**100**	**0.0**
ME32	**0.0**	**0.0**
ME33	**0.0**	**0.0**
ME34	**790**	**400**
ME35	**580**	**0.0**
ME36	**670**	**0.0**
ME37	**0.0**	**780**
ME38	**400**	**0.0**
ME39	**350**	**0.0**
ME40	**600**	**100**
ME41	**0.0**	**0.0**
ME42	**0.0**	**420**
ME43	**780**	**0.0**
ME44	**240**	**80**
ME45	**0.0**	**0.0**
ME46	**30**	**720**
ME47	**0.0**	**0.0**
ME48	**740**	**0.0**
ME49	**140**	**0.0**
ME50	**0.0**	**540**

3-3 Secretion of *A. flavus* isolates for AFB1 and AFG1

Results showed in Table 3 that it is a wide variation of aflatoxins production was obvious among the tested isolates of *A. flavus* using TLC technique, it was found that 25 isolates, among 41 were able to produce AFB1 and 12 were able to produce AFG1 ,There was a variable in the toxin concentration produced by each isolate.

Table 3: Secretion of *A. flavus* isolates for AFB1 and AFG1

A.flavus isolates	Concentration of AFB1 (µg/g dry wt.)	Concentration of AFG1 (µg/g dry wt.)
A. flavus ME1	0.0	0.0
A. flavus ME2	220	130
A. flavus ME5	0.0	0.0
A. flavus ME6	0.0	140
A. flavus ME7	90	0.0
A. flavus ME8	0.0	147
A. flavus ME9	0.0	0.0
A. flavus ME10	0.0	0.0
A. flavus ME11	320	120
A. flavus ME12	130	0.0
A. flavus ME15	0.0	0.0
A. flavus ME16	0.0	95
A. flavus ME17	230	0.0
A. flavus ME18	0.0	0.0
A. flavus ME19	310	0.0
A. flavus ME20	80	0.0
A. flavus ME21	0.0	0.0
A. flavus ME22	280	0.0
A. flavus ME23	0.0	100
A. flavus ME24	160	0.0
A. flavus ME25	315	81
A. flavus ME26	0.0	0.0
A. flavus ME28	0.0	134
A. flavus ME29	0.0	0.0
A. flavus ME32	0.0	0.0
A. flavus ME33	0.0	0.0
A. flavus ME34	310	74
A. flavus ME35	125	0.0
A. flavus ME36	275	0.0
A. flavus ME37	0.0	122
A. flavus ME38	0.0	0.0
A. flavus ME39	0.0	0.0
A. flavus ME40	195	0.0
A. flavus ME41	0.0	0.0
A. flavus ME42	0.0	98
A. flavus ME43	300	0.0
A. flavus ME44	0.0	0.0
A. flavus ME47	0.0	0.0
A. flavus ME48	295	0.0
A. flavus ME49	0.0	0.0
A. flavus ME50	0.0	102

The highest concentration of AFB1 was recorded in *A. flavus* ME11 , *A. flavus* ME25, *A. flavus* ME19, *A. flavus* ME34 and *A. flavus* ME43 was 320 , 315 , 310 and 300 (µg/kg dry wt.) respectively, and less concentration was as shown in *A. flavus* ME7 , and *A. flavus* ME20 were 80 and 90 (µg/kg dry wt.) respectively (Table3) , about AFG1 the highest concentration was recorded in *A. flavus* ME8 and *A. flavus* ME6 were 147 and 140 (µg/kg dry wt.) respectively, and less concentration was as shown in *A. flavus* ME34 and *A. flavus* ME25 was 74 and 81 (µg/kg dry wt.) respectively. This is owing to genetic difference for these strains, which is reflected in the quantity and quality of the product by the poison metabolic pathways for fungi tested variation(Lee and Hagler,1991). Aflatoxins are the greatest imperative naturally occurring mycotoxins in agricultural foodstuffs. Aflatoxins are produced by numerous species of *Aspergillus ,A. flavus, A. nomius, A. ochraceoroseus* (Samson and Hong,2006; Varga and Samson,2008) . Lima *et al*. (2000) informed that of the 19 isolates of *A. flavus* improved in rice, 52.6% were aflatoxigenic, producing aflatoxins foremost practices used for rice cultivation are disseminating, harvesting and storage. The varied climatic conditions with deference to elevation moisture, temperature and relative humidity deliver variation in rice cultivation practices (Makun *et al*,2007 : Reddy *et al*,2009). *A. flavus* is the foremost producer of the well-known carcinogenic aflatoxins, the

attendance of this fungus and aflatoxins is of enormous worry in footings of food safety since it's the most strong naturally happening toxic and hepatocarcinogenic compounds ,The fungi *A. flavus A. parasiticus,A. niger* and *A. ochraceus* have been conveyed previous by Reddy *et al*., (2006) of which *A. flavus* have remained identified as the main quality preventive, creating aflatoxin polluted seeds when in storage .

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