Iraqi Journal of Biotechnology, December 2018, Vol. 17, No. 3, 85-91

Comparing the fungi contamination of rice samples collected from local and non-local markets

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Received: September 13, 2018 / **Accepted**: November 28, 2018

Abstract: Rice (*Oryza sativa*) as a cereal grain, it is the most widely consumed staple food for a large part of the world's human population, especially in Asia. Fungi can grow in the rice grains with specific condition and some types of these microorganisms can produce mycotoxins, it is considerd a high risk population because this toxin associated with health effects in order to provide evidence on risk assessment and may responsible for liver cancer**.** The aim of this study to investigate the contamination level of 100 local and non-local rice samples collected from Iraqi and other countries markets and compared between the results by using pour plate method employed for the isolation of fungi. The results showed that samples from Iraqi markets (different origins) especially imported samples, were contained high content of fungi when compared with non-local samples. There are many types of fungi appeared in the collected rice samples like *Aspergillus* spp, *pencillium* spp, *fusarium* spp, *Rhizupus* spp, *Alternaria* spp and *Mucor* spp, and some samples were contained number of fungi higher than the acceptable limit of central organization for standardization and quality control in Iraq (COSQC), which is $1x10^4$ CFU/g, and that consider very dangerous for the human health.

Keywords: Rice, Fungi**,** contamination

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Introduction:

Grains are one of the important foods for growing population of human most commonly cultivated cereal grains in the world include maize, rice, wheat, barley, sorghum, millet, oats, and rye (1).

Food security, which is the condition of having enough food to provide adequate nutrition for a healthy life, is a critical issue in the developing world. About 3 billion people, nearly half the world's population, depend on rice for survival. In Asia as a whole, much of the population consumes rice in every meal and in many countries, rice accounts for more than 70% of human caloric intake (2).

Rice is the seed of the grass species *Oryza sativa*, it is a monocotyledonous angiosperm. The genus Oryza contains more than twenty species, only two of which are referred to as cultivated rice: *O. sativ*a, cultivated in South-east Asian countries and Japan, and *O. glaberrima* cultivated in West Africa (3). The rice is the agricultural commodity with the third-highest worldwide production in 2016 (741.5 million tones), after sugarcane (1.9 billion tones) and maize (1.0 billion tones). In order to meet the requirements of a growing world population, worldwide production and yield of cereals has been increased for the last 50 years (2). This important grain may become contaminated during growth, harvesting and other agricultural operations such as

processing, handling and shipping in general, members of the *Pseudomonadaendospore*-forming bacteria, yeast and molds are the most common rice microflora (4).

Sixteen genera of fungi comprising twenty seven species were found to be associated with the rice samples. Among them, the most predominant was *Bipolaris oryzae* which was associated with 82.08 per cent seed samples, followed by *Alternaria padwickii* (63.36%), *Curvularia lunata* (46.08%), *Pyricularia oryzae* (44.64%), *Alternaria alternata* (34.56%), *Fusarium moniliforme* (27.36%) and *Curvularia pallescens* (21.6%). *Aspergillus flavus* and *Curvularia oryzae* had an incidence of 15.84% (5). Sixteen fungal species comprising11 genera showed to be associated with the 5 rice varieties. The major fungi associated with rice seeds were: *Bipolaris oryzae, Curvularia lunata, Aspergillus flavus, Fusarium moniliforme, F. oxysporum and Rhizopus* spp (6).

Aim of research:

The aim of this study to investigate the contamination level of the some rice samples collected from local and non-local markets and compared between the results.

Materials and Methods:

Collection of samples:

One hundred rice samples of several kinds and trade marks were collected randomly (approximately 1kg for each sample) in the period from February to May 2017, from the local markets (Iraqi rice samples and imported rice samples) and non-local markets (other countries markets) as shown in Table (1).

Isolation fungal contamination:

Sample collected were culture on Sabouraud dextrose agar (SDA). In this study serial dilutions up to 10^4 were prepared for the determination of Fungal Count (FC). One ml of serially diluted sample was poured in each petridish (triplicate trials) for the isolation in fungi and incubated for 5 days at 25 C°. The microbial load of the rice samples were calculated per gram of rice sample (7).

Identification and characterization of fungi:

1- Morphological examination including colony morphology, colony appearance was studied in terms of shape, color, texture and their margin on SDA (8).
2- Microscopic examination was carried out to identify the hypha, septale, conidiophores, spores, basidia and cystidia as described by Odhiambo *et al.,* (2013)(9).

Results and discussion:

In current study the culture of rice samples showed many types of fungi were dominant like *A. flavus* (28%), *A. niger* (27%), *pencillium* spp (24%) and yeast (19%), while other fungi in less account, 2% for *Fusarium* spp and 1% for each *mucor* spp, *A. clavatus* and *Alternaria* spp (Figure, 1). *A. flavus* was the common fungi isolate recorded 28 (28%) while the *Mucor, A.clavatus* and *Alternaria* were the lowest fungi isolate recorded 1 (1%). This study was agreed with other studies like Ashfaq *et al*.,

(2015)(10) in Pakistan confirm that many types of fungi appeared in their study but the *Aspergillus* spp were the dominant in all rice samples. Also Abu-Taleb *et al,* (2012)(11) observed that many species of fungi in cereal samples (Barly, rice, wheat and maiz) has been collected from Saudi Arabia, and reported that the highest percentage was for *Aspergillus* spp. Sibounnavong and Soytong, (2013) (12) found 18% of samples with *A. flavus* and 17.6% of samples infected with *A.niger* in rice variety of Karnataka Ponni in India.

Table (1): The variety and source of Rice Samples

N	Variety	Source	N	Variety	Source	N	Variety	Source
1	Roya staon	Baghdad	37	Hama	Sulaymaniyah	74	Billion	Baghdad
2	Mahmood	Baghdad	38	Fader	Sulaymaniyah	75	Rose brand	Baghdad
3	Thai white	Baghdad	39	pirlanta	Turkey	76	Mahmood	Baghdad
4	Dar aldeafa	Baghdad	40	Osmancik	Turkey	77	Joker	Baghdad
5	Rawaan	Baghdad	41	Goze	Turkey	78	Thawooz	Baghdad
6	Hassony	Baghdad	42	Reis	Turkey	79	Al maha	Baghdad
7	Inci	Baghdad	43	Ovadan	Turkey	80	Al basha	Baghdad
8	Karam albasha	Baghdad	44	Besefalak	Turkey	81	Shalezar	Iran
9	Joker	Baghdad	45	Pirinc osmancik	Turkey	82	Naser kalamy	Iran
10	Jovial	Baghdad	46	Gonen baldo	Turkey	83	Kalat	Iran
11	Eagle star	Baghdad	47	Trakya baldo	Turkey	84	Aklemia	Iran
12	Baghdad	Baghdad	48	Jasmine	Turkey	85	Stara	Iran
13	Thia rice	Baghdad	49	Maxlmally	Turkey	86	Mehssan	Iran
14	Amber(najaf)	Baghdad	50	Richmnd	Turkey	87	Krdea	Iran
15	Arganten rice	Baghdad	51	PNC	Turkey	88	Nema	Iran
16	Uruguay rice	Baghdad	52	4PNCA	Turkey	89	Thia jasmine	u.k
17	Khazar	Baghdad	53	Fresco	Turkey	90	Risotto	u.k
18	Al kaaf	Baghdad	54	Captin aref	Baghdad	91	Paeha	u.k
19	Jasmine	Baghdad	55	8 star	Baghdad	92	Abuaraba	Baghdad
20	Shahean	Baghdad	56	panda	Baghdad	93	Dar adeafa	Baghdad
21	Moafq	Baghdad	57	korja	Baghdad	94	Global	Baghdad
22	Karam albasha1	Baghdad	58	Saqer	Baghdad	95	Gul wing	Baghdad
23	Amber	Meshkab	59	Joker	Baghdad	96	Sfl spey	Baghdad
24	Al raqi	Baghdad	60	White thi	Baghdad	97	Cabten areaf	Baghdad
25	Al sonono	Baghdad	61	Hasson	Baghdad	98	Ricea	Ukraine
26	Argentina rice	Baghdad	62	Mawasam	Baghdad	99	Roma2	Romania
27	Argentina rice	Baghdad	63	Hana	Baghdad	100	Roma1	Romania
28	Aldoha	Egypt	64	Zand	Baghdad			
29	Elmatbakh	Egypt	65	313	Baghdad			
30	forat	Holy najaf	66	Eagle	Baghdad			
31	Amber	Holy najaf	67	Xxxl basha	Baghdad			
32	Jasmine	Holy najaf	68	Al awal	Baghdad			
33	Uruguay rice	Baghdad	69	Tiryaki	Baghdad			
34	Uruguay rice	Baghdad	70	Karam al basha	Baghdad			
35	Richmond	Kazakhstan	71	K.H.M	Baghdad			
36	Star	Sulaymaniyah	72	Karam	Baghdad			
			73	Narjes	Baghdad			

This high contain of fungi especially *A.flavus* may lead to produce many types of mycotoxins like aflatoxins that responsible for many of health effect like cancer. Fakruddin *et al*., (2015)(13) showed the *A. flavus* is

one of the major producers of aflatoxin and can contaminate wide range of agricultural commodities either in field or in stores. The current results revealed that 14% of rice samples had collected from local and non-local markets were contain $1x10^3$- $9x10^5$ CFU/gm range of fungi, whereas the maximum acceptable range of central organization for standardization and quality control in Iraq (COSQC) was FC=$1x10^4$ CFU/gm, this mean there were many samples above this limit like, Karam albasha $1x10^5$ CFU/gm, Hana $2x10^4$ CFU/gm and Thawooz $3x10^4$ CFU/gm. The high contamination may came from the farm through dust, harvesting instruments or bad handling or from bad storage conditions as high humidity, temperature and bad ventilation.

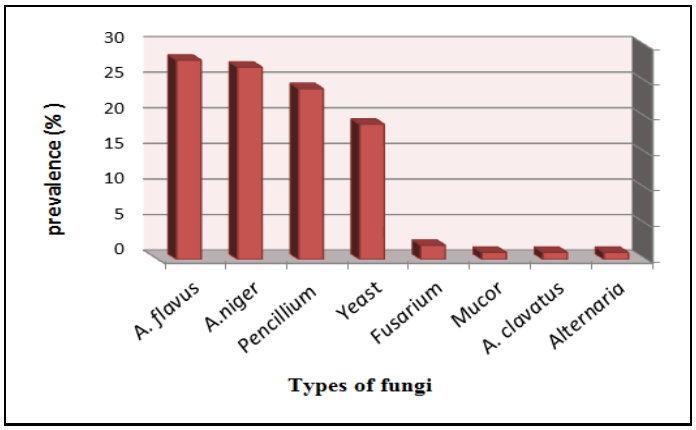

Figure (1): The Dominant Fungi Appeared in Collected Rice Samples

In this study *A.flavus* was the most common isolate the percentage recorded 28% of rice samples of local markets and non-local markets. This mold responsible for production of aflatoxins which is very dangerous for consumer and may lead to liver cancer (14). The Table (2) showed the results of total fungi for the Iraqi samples, these samples have been planted, harvested and marketing in Iraq. The results appeared that the average of fungal count was $2.4x10^4$ CFU/gm.

Table (2): Microbial Contamination of Rice Iraqi Samples Using Sabaurod Dextrose Agar

N	Sample	CFU/ gm
1	Amber(najaf)	$1x10^3$
2	Amber	$1x10^1$
3	Forat	$4x10^2$
4	Amber 1	$5x10^3$
5	Jasmine	$5x10^3$
6	Jasmine 1	$3x10^3$
	Average	$2.4x10^4$

SAB=Sabaurod Dextrose Agar

Table (3) Showed the results of samples have been planted harvested not in Iraq but sold in Iraq markets. The results investigated that fungal count was $9.6x10^4$ CFU/gm.

Table (4) showed the results of total fungi for the non-local rice samples, these samples has been planted, harvested and marketed not in Iraq but in different countries like Turkey, Iran, Ukraine, Spain, Romania, Italy, Egypt and Kazakhstan. The result revealed that the average of fungal count was $2.1x10^3$ CFU/gm.

Table (3): Microbial Contamination of Imported

N	Source of rice	CFU /gm	N	Source of rice	CFU /gm
1	India	$1x10^1$	**63**	India	$2x10^4$
2	India	0	**64**	India	$1x10^2$
3	Thailand	$1x10^1$	**65**	India	$2x10^1$
4	India	$1x10^1$	**66**	Urguay	$1x10^1$
5	India	$3x10^1$	**67**	India	$1x10^1$
6	India	$4x10^1$	**68**	India	$1x10^1$
7	Thailand	$1x10^1$	**69**	India	$1x10^2$
8	India	$1x10^1$	**70**	India	$1x10^1$
9	India	$1x10^1$	**71**	India	$1x10^1$
10	India	$8x10^1$	**72**	Thiland	$1x10^1$
11	Thailand	$1x10^1$	**73**	Thiland	$8x10^1$
12	India	$1x10^1$	**74**	India	$7x10^1$
13	Thailand	$3x10^1$	**75**	India	$1x10^1$
15	Arganten	$3x10^1$	**76**	India	$3x10^1$
16	Uruguay	$3x10^1$	**77**	India	0
17	India	$1x10^1$	**78**	India	$1x10^4$
18	India	$3x10^2$	**79**	Thiland	0
20	India	$1x10^2$	**80**	India	0
21	India	$4x10^1$	**89**	Thiand	0
22	India	$2x10^1$	**92**	India	$3x10^2$
24	India	$4x10^1$	**93**	India	$3x10^2$
25	India	$5x10^5$	**94**	Vietnam	$2x10^2$
26	Argentina	$1x10^1$	**95**	Uruguay	$3x10^2$
27	Argentina	$2x10^1$	**96**	USA	$2x10^3$
33	Uruguay	$1x10^3$	**97**	India	$2x10^3$
34	Uruguay	$1x10^1$		**Average**	**$9.6x10^4$**
38	India	0			
54	India	$5x10^3$			
55	Pakitan	$1x10^1$			
61	India	$2x10^1$			
62	India	$1x10^1$			

Rice Samples Collected from Local Markets

Table (4): Microbial Contamination of non-Iraqi

N	Source of rice	CFU /gm	N	Source of rice	CFU /gm
28	Egypt	$6x10^2$	**82**	Iran	$1x10^3$
29	Egypt	$7x10^1$	**83**	Iran	$5x10^2$
35	Almond	0	**84**	Iran	$5x10^2$
39	Turkey	0	**85**	Iran	$1x10^2$
40	Turkey	0	**86**	Iran	$1x10^2$
41	Turkey	0	**87**	Iran	$4x10^2$
42	Turkey	0	**88**	Iran	$1x10^2$
43	Turkey	0	**89**	U.K	0
44	Turkey	0	**90**	U.K	$1x10^2$
45	Turkey	0	**91**	U.K	0
46	Turkey	0	**98**	Ukraine	$1x10^2$
47	Turkey	0	**99**	Romania	$1x10^2$
48	Turkey	0	**100**	Romania	$3x10^3$
49	Turkey	0		**Average**	**$2.1x10^3$**
50	Turkey	0			
51	Turkey	$2x10^1$			
52	Turkey	$1x10^1$			
53	Turkey	0			
81	Iran	$2x10^2$			

Rice Samples:

The comparison between the average of fungal contamination for the Iraqi samples, Imported samples and non-Iraqi samples was revealed that, the Imported samples contain high average of fungal contamination ($9.6x10^4$ CFU/gm), follow by Iraqi samples (**(**$2.4x10^4$ CFUgm) but the less a count was in non-Iraqi samples ($2x10^3$ CFU/gm) with significant $p \leq 0.05$ (Figure, 2 and Table, 5).

Table (5): Fungal Contamination Average of local and non Local Rice Samples

N	Type of sample	Average CFU/gm
1	Iraqi samples	**$2.4x10^4$ ***
2	Imported samples	**$9.6x10^4$ ***
3	Non-Iraqi samples	**$2.1x10^3$**

*** Significant ($p \leq 0.05$)**

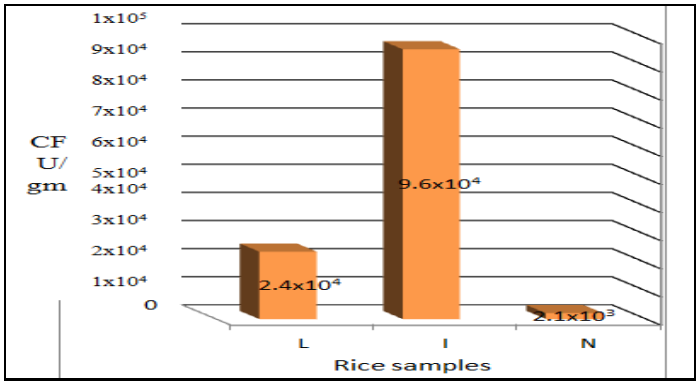

Figure (2): Compression Fungal Contamination Average of Iraqi samples (L), imported samples (I) and non-Iraqi samples (N).

From the present study a conclusions can be stated that the contamination of rice samples were high in samples collected from local markets comparison with non-local markets, and it was found the *A. flavus* was predominant in these samples.

There is default with the official authorities who responsible for follow up the health condition of the food in local markets especially with cereals which store for long time.

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