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DETECTION OF FUNGI AND FUMONISIN B_1 IN LOCAL RICE AND EVALUATING SOME DETOXIFICATION METHODS

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الكشف عن الفطريات وسم الفيومونيسين B_1 في الرز المحلي وتقييم بعض طرائق إزالة سميته

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المستخلص:

هدف الدراسة الكشف عن التلوث الفطري وعن سم الفيومونيسين B_1 في الرز المحلي وتم اختبار خمس عزلات للفطر *Fusarium moniliform* على إنتاج سم الفيومونيسين B_1 على وسط الرز وكذلك درس تأثير خمس طرائق لإزالة سميته ، والفطريات التي تم عزلها من حبوب الرز هي *Alternaria spp.* و *Aspergillus spp.* و *Fusarium spp.* و *Penicillium spp.* و *Rhizoctonia spp.* و *Rhizopus spp.* وقد أظهرت النتائج أن الرز كان خاليا من التلوث بسم الفيومونيسين B_1 ، وتباينت العزلات الخمسة في إنتاج سم الفيومونيسين B_1 وتراوحت التراكيز المنتجة بين ٥٢٤.٣ - ٨٢٥.٣ مكغم / كغم ، و كانت نسبة إزالة السم باستخدام هيدروكسيد الكالسيوم بتركيز ١ % و ٢ % و الفحم المنشط ١ % و ٢ % و سكر الفركتوز ١ % و ٢ % وطبخ الرز بطريقتي البزل و القلي واستخدام فرن الموجات الدقيقة بمستويين من الطاقة ٨٠ واط و ١٠٠ واط هي ٨٠.٤ % و ٨٤.٣ % و ٤٥.٨ % و ٥٠.٠٤ % و ٣٠.٤ % و ٣٢.٣ % و ٧٣.٨ % و ٢٦.٢ % و ٦٩.٧ % و ٧٣.٠٨ % على التوالي.

Abstract:

The study aimed to detect fungal contamination and FB_1 concentration in local rice. Five isolations of *Fusarium moniliform* were tested for FB_1 production in rice medium. The effects of five strategies to detoxify FB_1 were also studied. The following fungal genera were isolated from the rice kernels: *Alternaria spp., Aspergillus spp., Fusarium spp., Penicillium spp., Rhizoctonia spp.* and *Rhizopus spp.* The samples of rice cleared from FB_1. The five isolations varied in FB_1 production on rice medium, and the concentration ranged from 524.3 to 825.3 µg / kg. The FB_1 eliminated percentage by using calcium hydroxide 1%, 2%, active charcoal 1 %, 2 %, fructose 1 %, 2 %, parboiling, frying and microwaving 80w, 100w were 80.4%, 84.3%, 45.8%, 50.04%, 30.4%, 32.3%, 73.8%, 26.2% and 69.7%, 73.08% respectively.

Introduction:

The rice (Oryza *sativa L.*) is one of the main cereals in the world. The rice reputes as a principal food for more than half of the world population especially in Asia and also in Iraq. The rice kernel nutritional magnitude came from containing highly percentage of carbohydrates, providing the human with sufficient energy (13), its protein also have an equiponderating content of essential amino acids, especially the Lysine, which gives the rice more nutritional value than other cereals (8). Toxin producing molds are widespread in nature, and when they are occurring in grains they often reduce both the yield and the quality of grains. These molds infect grains in the field before harvest or, if storage conditions are optimal, they may grow during storage (4). These molds can produce commonly known as mycotoxins. These mycotoxins are among the most potent mutagenic and carcinogenic substances known. They cause chronic health risks: prolonged exposure through diet has been linked to cancer and kidney, liver and immune – system diseases (20). Mycotoxins were produced by several fungi, particularly by species of *Aspergillus, Fusarium, Penicillium, Claviceps* and *Alternaria*. They comprise a group of several hundreds of chemically different toxic compounds (17) (24). The most common mycotoxins are Aflatoxins, Ochratoxins, Tricothecenes, Zearalenone and Fumonisins (12). Fumonisin B_1 (FB_1) is a recently discovered mycotoxin; it is produced by several *Fusarium* species. Since its identification FB_1 has been shown to be associated with the previously known disease equine leuconocephalomalacia a fatal neurological disorder of horses and porcine pulmonary edema (11). This toxin has also been linked to esophageal cancer in human (25). Fumonisin B_1 has also been shown to cause feed refusal, poor growth, altered serum chemistry and organ lesion in poultry (15). Although the prevention of mycotoxin contamination in the field is the main goal of agricultural and food industries, once the crop becomes infected under field conditions, fungal growth will continue during post-harvest phases and storage. Therefore several strategies for detoxification or decontamination of commodities containing mycotoxins have been reported and may be classified as chemical, physical and microbiological. Food processing that may involve physical and / or chemical decontamination could be considered as a strategy to destroy mycotoxins. Therefore this study aimed to investigate the fungal growth on the rice and to detect Fumonisin B_1 concentration by using ELISA technique, testing the rice as a medium for Fumonisin B_1 production, detoxify Fumonisin B_1 by cooking the rice in several methods and testing the ability of calcium hydroxide, active charcoal and fructose to detoxify the Fumonisin B_1.

Materials and methods:

The samples of the local rice were taken from Al- Tajy silo during the spring season. Two different samples were taken, in fact 15 kg for both samples. The first sample was taken from middle of Iraqi areas while the second was taken from the south areas. The samples were randomly taken from different directions and they

were packed in a polyethylene pouch befor they were transported to the laboratory to: determine the chemical composition of kernels, to isolate the fungi genera and detect the mycotoxin Fumonisin B_1. The chemical composition of rice samples was determined by AOAC methods (1), including the moisture, ash, protein and fat. The percentage of protein was determined by using microkjeldahle technique and achieved by calculating the total nitrogen in 0.2 gm of sample. The results were multiplied with conversion factor 5.7 to obtain the total protein percentage. The fat percentage was estimated by placing 5 gm of sample in the extraction thimble of Soxhlet extraction unit and extracted by using the organic solvent hexane. The carbohydrate percentage was calculated from the equation: 100 minus the percentage of moisture, ash, protein and fat. The Fungal genera were isolated from 100 kernels by cultivating the kernels in Petri dishes each Petri dish had 10 kernels. The Potato dextrose agar medium was used to isolate the fungi after it was mixed with 300 µgm tetracycline to prevent bacterial growth. The Petri dishes were incubated at 25 C˚ for 5 – 7 days after that the fungal genera were identified with assistance of Barnett and Barry (5) classification key. The inner fungi were isolated through sterilizing the outer surface of the kernels by emerging them in 1% sodium hypochlorite solution with shaking stilly for two minutes, after that the kernels were washed three times with sterilized water, dried with sterilized filter paper and cultivated as well as the cultivation of the surface fungi (2). The total count of fungi, determined by using dilution plat count method was conducted by taking 10 gm from the sample added to a dilution bottle contained 90 ml of peptone water and it was shaken stilly well, afterward a number of decimal dilutions were conducted, then 1ml of each dilution was taken, and cultivated in a Petri dish containing the medium potato dextrose agar. One dish was kept without cultivation as a control other dishes were incubated at 25 C˚ for 5 – 7 days. After that the fungi were identified and its total count was determined (2). Fumonisin B_1 level in the rice kernels was detected by the use of ELISA technique and conducted by taking a 1 kg of the rice sample and milled it, then the toxin was extracted by using 50% acetonitrile + 50% distilled water, next the mixture of sample and extraction solution were placed in a flask shaker for 1 hr. (21). The quantum estimation of Fumonisin B_1 was conducted according to the manufacturer's recommended procedure, attached with the standard package of Fumonisin B_1 test which was acquired from Neogen Company. The results were identified by using Microwell reader device under wave length 650 nm. Five isolations of *Fusarium moniliforme* were tested for Fumonisin B_1 production on rice medium (7) and these isolations were gained from biotechnology laboratory, department of food science and biotechnology, college of agriculture, university of Baghdad. The chemical strategy for Fumonisin B_1 detoxification was achieved by applying three chemical materials: active charcoal, calcium hydroxide and fructose. They were used in two concentrations 1% and 2 %. The physical detoxification strategies were represented in three methods of cooking applied on contaminated rice kernels with Fumonisin B_1, the parboiling, frying and using the

microwave with two energy levels 80 and 100 w for 6 minutes. The data were statically analyzed by the use of completely randomized design (CRD) with significant level 0.05 (22).

Results and discussion:

The chemical analysis of rice kernels composition demonstrates that carbohydrate, moisture, protein, fat and ash were varied for each sample (fig. 1). The rice species have different composition depends on its classes, nature of the soil, environmental conditions and the fertilizer kind (14).

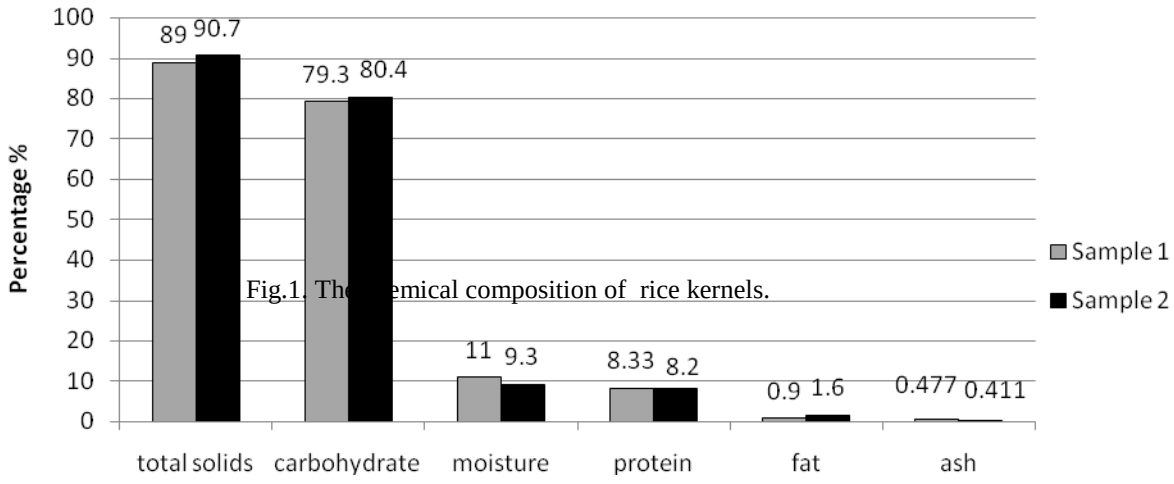

Fig.1. The chemical composition of rice kernels.

Numbers of fungal genera were isolated from rice kernels and these fungi were the following: *Alternaria spp.*, *Aspergillus spp.*, *Fusarium spp.*, *Penicillium spp.*, *Rhizoctonia spp.* and *Rhizopus spp.* The prevalence was to the genera *Penicillium* and *Aspergillus* and some of these fungi genera were found in one sample but not found in the other, like *Fusarium spp.* were isolated only from sample 1 while *Alternaria spp.* and *Rhizoctonia spp* .were isolated from sample 2 only. The sterilization of the outer surface of kernels encouraged the growth of some genera which were not capable to grow, when the kernels were cultivated directly without sterilization, and this was ascribed to the competition occurred among the fungal genera on the nutrients (table 1).

Table 1. The fungal genera, isolated from the surface and the inner of rice kernels.

Fungal genera	Sample 1		Sample 2	
	Surface	Inner	Surface	Inner
Alternaria spp.	-	-	-	+
Aspergillus spp.	*+++	++	+	+
Fusarium spp.	+	-	-	-
Penicillium spp.	+	+	+++	+++
Rhizoctonia spp.	-	-	-	++
Rhizopus spp.	-	+	+	-

***Numerous growth +++, middle growth ++, few growth +, no growth –**

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The determination of total count of fungal genera showed that *Penicillium spp.* had the uppermost number of isolated fungi then the *Aspergillus spp.* and *Rhizopus spp.* (table 2). Previous studies reported that fungal genera, invaded the stored cereals were the same in most parts of the world, and the major of these fungi, invade a rice kernels were *Aspergillus, Penicillium, Alternaria, Fusarium, Cladosporium* and *Rhizopus*, and these which have been transported from field to stowage included: *Fusarium moniliforme* and *Helmenthosporium* (10).

Table 2. The total count of fungal genera isolated from rice kernels samples.

Fungal genera	(Total count) × 10^3 / gm	
	Sample 1	Sample 2
Aspergillus spp.	0.019	0.002
Penicillium spp.	0.97	0.0033
Rhizopus spp.	0.0088	0.001

***Results represent the average of three frequents.**

The detection of FB_1 levels by using ELISA technique showed that the rice samples were cleared from toxin, because the fungus, producing FB_1 like *F. moniliform* or *F. proliferatum* was not found in the samples of rice, in addition the highly percentage of moisture in the rice samples stifled the toxin production, and this have been confirmed by Miller (16). Then the five isolations of *Fusarium verticillioides* (Syn., *moniliforme*) were tested for FB_1 production on rice kernels as a medium, and the result demonstrated that the rice kernels were a good medium for FB_1 production and there was an obvious diversity among the isolations in FB_1 production (fig. 2). These results are in general agreed with Desjardins *et. al.* (6) reporting that the fungus *F. moniliform* produced highly quantities of FB_1 reaching to 2980 µg / kg when cultivated in a rice medium. In

addition there is more than a factor that overrules the toxin production such as water activity (a_w), oxygen pressure, temperature and relative humidity (16).

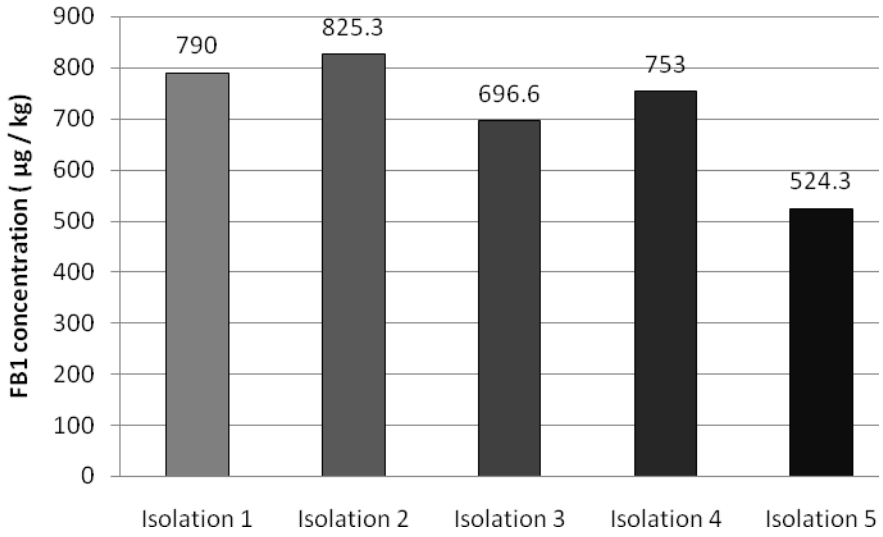

Fig. 2. FB_1 concentrations was produced by five isolations of *F. moniliforme* on rice kernels

The effects of chemical detoxification method on FB1 concentration are presented in table 3. The calcium hydroxide 1% demonstrated a highly activity in detoxification of FB_1 followed by the active charcoal 1% then the fructose 1%. They decreased the toxin concentration from 790 µg / kg to 154.6, 428, 549.6 µg/ kg respectively. In addition the double volume of these eliminatory materials from 1% to 2% interpreted to increase the elimination percentage upward (1.9 - 4.24) % (fig 3). Physical detoxification methods revealed that cooking of rice by parboiling was more sufficient than frying, because they decreased the concentration of toxin from 790 µg / kg to 206.6, 583 µg / kg respectively (table 3), and the elimination percentages were 73.8%, 26.2 % respectively (fig 3). Two microwaves energy levels, 80 w and 100 w acted in elimination of the toxin by decreasing the toxin concentration from 790 to 239.3, 212.6 µg / kg respectively (table 3) and the elimination percentages were 73.8%, 69.7 % respectively. The statically analysis confirms the significance of the treatment at (19.65) in elimination of toxin unless the fructose treatment shows no significance. It was reported that heat and calcium hydroxide solution can eliminate 95 % of FB_1 from contaminated cereals because of the hydrolysis of $FB_1$by the base effect (18). Yahya *et al.*(26) illustrated that elimination percentage of FB_1 reached to 54.7, 58,2 % when it was treated with two concentrations 1% , 2 % of active charcoal respectively and that agreed with Galvano *et. al.*(9) study, mentioning that the effect of active charcoal came from deposited of the FB_1 molecules in the distance among active charcoal granules, or by forming a chemical bonds among the active groups of active charcoal and the toxin (12). The mechanism of detoxification of FB_1 by fructose ascribed to the

reduction of a primary amine group in toxin molecule and converting it to a non toxic compound (18). The FB_1 molecule quite stable to heat and the temperatures / times required for thermal decomposition of FB_1 generally exceed commercial cooking parameters (23). In contrast, Pineiro *et.al.* (19) found that frying, polenta or autoclaving cornmeal, produce reductions in FB_1 of 70 – 80 % with no conversion to the hydrolyzed form. Microwaving of foods also appears to significantly reduce mycotoxins residues. It was found that the percentage of the added water goes to increase absorption of the microwaving waves and gains a highly energy cause in decomposition of mycotoxins (3).

Table 3. The effects of five detoxification methods in FB_1 concentration are present.

Treatments		FB_1 conc. Before addition µg / kg	FB_1 conc. After addition µg / kg
Calcium hydroxide	Conc. 1%	790	154.6
	Conc. 2%	790	123.3
Active charcoal	Conc. 1%	790	428
	Conc. 2%	790	394.6
Fructose 1%	Conc.1%	790	549.6
	Conc. 2%	790	534.3
Cooking	Parboiling	790	206.6
	Frying	790	583
Microwaves	80w	790	239.3
	100w	790	212.6

***Results represent the average of three frequents.**
***LSD (19.65).**

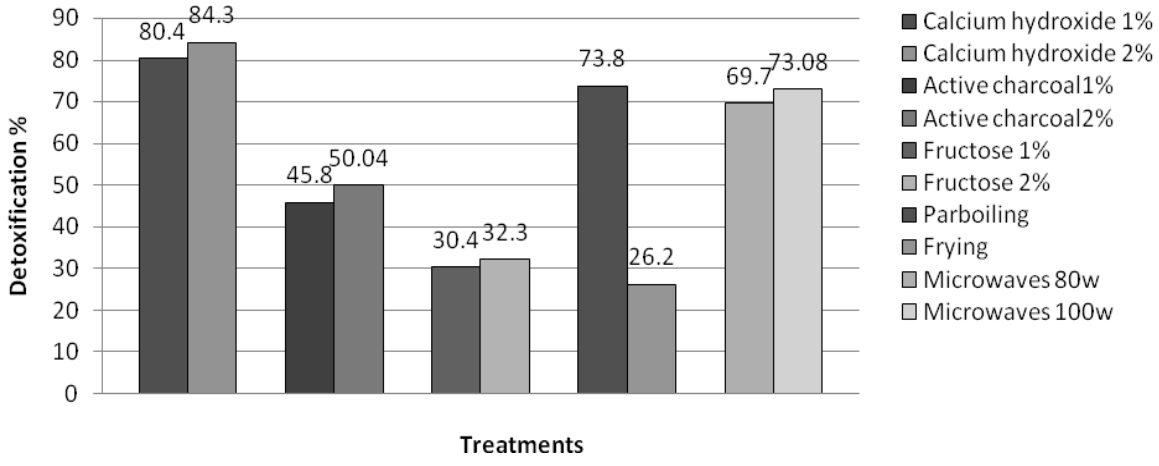

Fig. 3. Detoxification percentage by using different methods

Conclusion:

The study demonstrates that the samples of local rice are free from FB_1 but also it is a very good medium for FB_1 production if it contains a toxic producing fungus. Using calcium hydroxide, active charcoal and fructose at two concentrations 1% and 2% are result in detoxifying FB_1 by 80.4 %, 84.3%, 45.8%, 50.04%, and 30.4%, 32.3% respectively. The cooking of rice by parboiling and frying result in detoxifying the FB_1 by 73.8%, 26.2% respectively. The using of microwave oven results in decomposition of FB_1 by 69.7 % in 80 w energy level and 73.08 % in 100 w.

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