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The Effects of Fumonisin-B1 Determined Storage Wheat on Sensory and some Physiological Parameters in Rats

ABSTRACT

This study was conducted in the laboratories of the Department of Food Science / College of Agriculture/ University of Tikrit with the aim of isolating and diagnosing fungi contaminated by stored wheat grains and estimating the concentration of FB1 in it and determining its physiological effects in rats. The results of the study showed the presence of *Aspergillus* sp. molds by 38 in the tested samples, followed by *Fusarium* sp. by 28 and *Alternaria alternata* by 19 then the genus *Penicillium* sp. by 10 and *Mucor* sp. by 5. The presence of FB1 toxin in local and imported wheat samples was also investigated by estimating using ELISA (Enzyme-linked Immune Sorbent Assays) as its highest concentration was 2.240 mg/kg in local wheat from the A-Dour district, while the lowest concentration was 0.103 mg/kg in Australian wheat samples. The ability of *F. moniliforme* mold isolates to produce FB1 was also studied, it was found that they were capable of producing toxin with concentrations of 4.264 mg / kg in local wheat samples taken from Al-Dour district and 3.597 mg / kg in local wheat samples taken from Baiji district. Using HPLC (High Performance Liquid Chromatography). With regard to the sensory evaluation of wheat flour used in the study, the best results for wheat flour of Australian wheat gave the highest results which reached 24 degrees while the worst results for wheat flour of Baiji which amounted to 14 degrees for the qualities studied: color, smell. With regard to the sensory evaluation of beard made from wheat samples in this study, the highest was recorded 33 degrees for Australian wheat and lowest evaluation for Dour wheat with 24 degrees with regard to the studied qualities of color, smell, taste.

The effect of FB1 on the rate of weight gain in male rats has also been studied, the presence of this toxin in the rats' diet led to a significant decrease in weight after 21 days of the trial age, where the average weight in the T3 treatment was 141 g and the results showed a significant increase in the relative weight of the liver and Kidney in treatment T3 was 4.6g and 1.7g respectively.

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INTRODUCTION:

Mycotoxins are natural contaminants of food and feed and these substances pose a potential threat to human and animal health, its also causing deterioration in the quality of marketable products, causing huge economic losses (Rashedi, et al, 2012). Cereals, including wheat, are usually colonized by *Fusarium* species and can be contaminated with mycotoxins, which are known as secondary metabolites produced by Fungi that have a significant impact on health and productivity (Cendoya, et al, 2019).

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Among all types of *Fusarium* sp. that can be found in wheat grains and their products, it could isolate many species of this Fungus which could produces Fumonisin-B1 and that the most important *F. verticillioides* and *F. proliferatum* (Guo, et al., 2016; Amato, et al., 2015). Just as Fumonisin is found in corn and its products, it is found in wheat and its products (Cendoya, et al., 2018). The genus *Fusarium* is a plant-causing pathogen common world –wide, producing more than a hundred types of secondary metabolites that have an impact on human and animal health (Al-jibouri, 2015). The most important toxins of *Fusarium* produced in large quantities of grain are Fumonisin, Zearalenone and Trichothecenes (Sopterean and Puia, 2012). Fumonisin-B1 in terms of occurrence and toxicity is the most important followed by Fumonisin-B2 which differs structurally from B1 in the number of hydroxyl groups and their arrangement on hydrocarbon molecules (Voss, et al., 2007). The toxin causes leukemia in horses, pulmonary edema syndrome in pigs and nephrotoxicity and renal and literal toxicity activities in rats (Wan Norhasima, et al., 2009). These mycotoxins have been associated with esophageal cancer (Marasas, et al., 2001), and neural tube defects (Missmer, et al., 2005). Moreover, recent studies suggest that exposure to Fumonisin can be associated with stunting in children (Shirima, et al., 2014). Also Fumonisin-B1 cause pathological histopathological changes and hepatic, renal, neurological toxicity and effects on immune and developmental abnormalities and tumors of the liver and kidneys (Voss, et al., 2001; Gelderblom, et al., 2001; Marasas, et al., 2004). This study aimed to: 1. determine the extent of contamination of wheat grains with *Fusarium* 2. Test the ability of fungi isolates to produce FB1 toxin 3. Estimate the concentration of FB1 toxin in wheat grains using ELISA and HPLC 4. Study the effects of FB1 toxin on the sensory characteristics of flour and bread 5. Test the extent of the effect of FB1 toxin in male white rats physically.

MATERIALS AND METHODS:

Collection and preparation of wheat samples: six varieties of local and imported wheat grains were collected from the silos of Salahaddin Province samples, then keep in clean nylon bags until tests are carried out.

Preparation of culture media: The media was prepared according to the manufacture's instructions which are : PDA, MEA and sterilized with autoclave at 121C° and a pressure 15 lb/inch² for 15 min.

Isolation of Fungi from wheat samples: The Lacey et al. (1999) method was used to isolate *Fusarium* sp. from grains by taking 100 grain from each sample by three triplicates and sterilized with 2% Sodium hypochlorite, the grains washed by distill water three times and then dried by using filter paper after that transported to dish and incubated at 27C° for 7 days.(Evan, et al., 2000).

Purification and conservation of Fungal isolates growing on wheat grains: After the diagnosis of the Fungi a loop was taken from the Fungus spores and transferred to Petri dishes containing PDA medium and incubated at 27C° for 7 days and the process was repeated to obtain 100% pure isolates and the purified isolates preserved in test tubes with slants of PDA and kept in the refrigerator at 4C°.(Evans, et al., 1993).

Diagnosis of Fungi isolated from wheat grains: Fungal isolates were diagnosed by using optical microscopy by taking loop of the fungus on glass slices using Lacto phenol Cotton Blue Stain, and taxonomic keys were used for Fungi diagnosis (Samson, et al., 1981; Prajna, et al., 2008).

Fumonisin-B1 Determination: was extracted from wheat samples by ELISA method and according to the working steps attached with ELISA kit.

Determination of Fumonisin-B1 in the wheat samples, where the method of work with the ELISA kit was followed.

Extraction of Fumonisin-B1 from *Fusarium moniliform* cultures and determination of the most productive isolation of toxin for the purpose of other tests, where the method used by Trucksess, et al.(1984) was followed with some modifications on the method.

Quantitative estimation of Fumonisin-B1 in the High Performance Liquid Chromatography (HPLC) device, the test was performed using column C18 and mobile phase methanol: water (23:77) and PH: 3.5, color indicator O-Pthaldialdehyde with a volume of 25:25 and a follow rate of 1ml/min along a wave length of 280 nm. The samples were compared with standard toxin

supplied by Shenzhen company. The estimation was carried out in the laboratories of the ministry of Science and Technology using a device of YL 9100 German origin.

Biological experiment:

a-Preparation of feed: the feed was prepared according to National Academy of Science-National Research Council(NAS/NRC) (2002) and the feed consisted of 158.5g/kg casein, 100g/kg glucose, 50g/kg cellulose, 100g/kg corn oil, 5g/kg vitamins, 50g/kg mineral salts, 187 g/kg wheat bran, 350 g/kg corn starch. The distilled water was added to make a paste that was then placed in stainless steel utensils, dried in the oven at 50 C° and kept in polyethylene bags in the refrigerator throughout the experiment.

b-Laboratory animals: In the experiment, 24 male albino rats were used, ranging in weight from 145 to 150g, the animals were placed in special cages measuring 36×10×15 cm and sawdust added to the cages's floor, the feed placed in the designated place in iron feeders and drinking water randomly divided into three groups with eight replicates, the first group(T1), the control group, the second group(T2) in which Fumonisin-B1 with concentration of 3.597mg/kg added to feed and the third group(T3) where the same toxin added to feed with a concentration of 4.264mg/kg, the experiment was ended at 21 days.

Animal parameters assay: The parameters assay in treated animal with toxin were included the growth rate and relative organ weights.

Wheat grinding: After cleaning the wheat from impurities and gravel, the grains were sifted with a manual sieve and crushed with a mill for grains and sifted flour with a manual sieve and with 100% complete extraction, and bread was made from it with the use of flour produced by the Kuwait Flour Mills Company and bakeries for comparison with the name (Patent flour) and then a sensory evaluation was performed.

Sensory evaluation: sensory evaluation of flour and bread samples was carried out by teaching staff of Food Science Dept./College of Agriculture/ Tikrit University according to the sensory evaluation form which includes four attributes: color, smell, taste, and general acceptance using a 10 grading system (Palazoglu, et al., 2015).

Statistical analysis: the results were analyzed using a complete random design (CRD) and the results were analyzed using SAS-Statistical analysis System (2012), the mean was compared using the Lowest Significant difference(LSD) to determine the significant differences at probability level of 0.05(Al-Rawi and Abdul-Aziz, 2000).

RESULTS AND DISCUSSION:

Fungi associated with wheat samples:

Data in table 1 shows that, The fungal isolated were appear in wheat samples collected from difference wheat storage (Local wheat) sources were contains *Aspergillus* sp., *Fusarium* sp., *Penicillium* sp., *Alternaria alternata*, *Mucor* sp. at percentage at 38, 28, 10, 19, 5 respectively, American wheat 35, 19, 24, 15, 7 respectively, Australian wheat 42, 7, 25, 20, 6 respectively.

Table-1: Fungi genera associated with wheat samples.

Sample	Percentage of Fungi genera%				
	<i>Aspergillus</i> sp.	<i>Fusarium</i> sp.	<i>Penicillium</i> sp.	<i>Alternaria alternata</i>	<i>Mucor</i> sp.
Local wheat	38	28	10	19	5
American wheat	35	19	24	15	7
Australian wheat	42	7	25	20	6

As for the results of isolation of Fungi from imported wheat grains, the American wheat showed an affinity with the results of previous studies, where the ratio of genus *Fusarium* 17 and genus *Mucor* 3 and did not show convergence for the genera *Aspergillus*, *Penicillium*, and *Alternaria* with percentages 23, 7, 22 respectively (Al-Humairi, 2007).

As for the Fungi associated with Australian wheat grains, the results were agreed with Al-Humairi(2007) in terms of infection with *Aspergillus sp.* where the contamination rate was 32 and the rate of *Penicillium sp.* 15. The sampling period also confirmed that the *Aspergillus* species increased their activity at relatively high temperatures, and the results showed a convergence with *Mucor sp.* with a rate of 3, *Fusarium sp.* and *Alternaria alternata* did not show convergence with the results, the ratio are 17 and 4.2 respectively. This is due to number of factors affecting crops, which increase the ability of infection such as geographical location, climate, culture media, the relationship between microorganisms and environmental conditions (Vujanovic, et al., 2012).

Determination of Fumonisin-B1 concentrations in wheat grains by ELISA technique:

Table-2 Shows the concentration of Fumonisin-B1 in local and imported wheat grains using ELISA technique. As shown, the lowest concentration was for Australian wheat samples with a concentration of 0.103 mg/kg while the highest concentration of Fumonisin-B1 in wheat grain of Dour District at the level of 2.240 mg/kg.

Table-2: Concentration of Fumonisin-B1 in local and imported wheat samples using ELISA technique.

Sample	Concentration (mg/kg) of FB1
Australian wheat	0.103
American wheat	0.798
Samarra wheat	0.312
Tikrit wheat	0.414
Baiji wheat	1.150
Dour wheat	2.240

The results of Fumonisin-B1 concentrations in local and imported wheat samples using ELISA ranged from 0.103-2.240 mg/kg, the lowest level in Australian wheat samples followed by local wheat from Tikrit, Samarra, and American wheat at a concentration less than 1 mg/kg. The concentration of this toxin in wheat samples did not exceed the limits set by FAO and WHO.(WHO, 2001), which is 2 mg/kg, also did not exceed the limits set by FDA of 2-4 mg/kg as well as limits set by the EU is 0.8-4 mg/kg.

The study also agreed with a study conducted in Serbia where the concentration of Fumonisin-B1 toxins in wheat ranged from 0.75-5.400 mg/kg (Stankovic, et al., 2012).

Determination of Fumonisin-B1 in vitro by *F. moniliforme* isolates using HPLC technique:

F. moniliforme isolates were obtained from local wheat samples taken from the Baiji and Al-Dour regions. The concentration in Baiji wheat was 3.597 mg/kg and in Al-Dour 4.264 mg/kg using HPLC technique. The results of this technique in the samples which *F. moniliforme* was isolated and after culturing on wheat media for the purpose of laboratory toxin production, the results showed that the concentration given by the isolation originating from Baiji wheat was higher than the limits allowed by FAO and WHO which is 2 mg/kg.(WHO, 2001), and it was within the limits set by FDA of 2-4 mg/kg. while the concentration of Fumonisin-B1 in Al-Dour wheat higher than the limits set by FAO, WHO and FDA.

Sensory evaluation:

a-Sensory evaluation flour of local and imported wheat:

Table (3) represent the sensory evaluation rate carried out by assessors on local and imported wheat flour samples and compared it with a flour model from local markets. The Australian flour sample gave the highest rating in terms of color, smell of 8 degrees and the same as the comparison

sample in while the lowest grades were for Baiji flour, it scored the lowest grades in terms of color, smell.

Table-3: Sensory evaluation flour of local and imported wheat.

Samples	Color (10)	Smell (10)	Total
Flour model	8	8	16
Australian flour	8	8	16
American flour	5	7	12
Samarra flour	7	7	14
Al-Dour flour	5	6	11
Tikrit flour	6	7	13

b-Sensory evaluation of bread from local and imported wheat flour:

Table-4 Shows the sensory evaluation of local and imported wheat bread compared with wheat flour from local markets, and showed that Australian wheat bread gave the highest grades from other samples in terms of color, smell, taste. Al-Dour flour showed the lowest grade compared to other bread samples of (6) for all traits, as for the wheat flour of Baiji was not suitable for making bread.

Table-4: Sensory evaluation bread of local and imported wheat.

Samples	Color (10)	Smell (10)	Taste (10)	Total
Bread to compare	8	8	8	24
Australian bread	9	8	8	24
American bread	7	6	6	19
Samarra bread	8	7	7	22
Dour bread	6	6	6	18
Tikrit bread	7	6	6	19

The reason for the varying degrees of sensory evaluation of flour and bread may be damage to the grain when stored in bad conditions, including changes in the chemical and physical properties of starch and the breakdown of proteins by proteinase enzyme by breaking the peptide bonds causing low tensile strength of wheat gluten as well as damage caused by microorganisms such as fungi (Al-Fen, 2013).

Effect of Fumonisin-B1 on the rate of weight gain in male rats:

The results in table-5 Show the effect of Fumonisin-B1 feeding to the rats on the growth rate rats which feeding for 21 days. The results were show that's FB1 was caused in decreased significant ($P < 0.05$) in body weight of rats feed of FB1 at concentration (T2) and with (T3) compared with the control group of rats.

Table-5: Effect of Fumonisin-B1 on the weight increase rate in male rats.

Rat weight(gm/rat/week)			
Treatment	1-7	8-14	15-21
T1	148.5a	151.2a	153.3a
T2	147.0a	146.2b	145.0b
T3	145.0b	142.0c	141.0c

LSD for average weight of increase rate in male rats 2.807

*T1: control group, T2: Fumonisin-B1 with a concentration, of 3597mg/kg, T3: Fumonisin-B1 with a concentration of 4264mg/kg.

*Different characters in one column indicate significant differences at a probability level ($P < 0.05$).

* Each value in the table represents a rate of eight replicates.

The results agree with Qiang, et al. (2011) that the rate of weight gain after 14 days of feeding rats on contaminated fodder with fumonisin-B1 at a concentration of 7 mg/kg decreased significantly compared to the control group. The reason of decreasing in weight gain that this toxin inhibit the formation of sphingolipids in the cell membrane of the cell leads to the failure of physiological activity of cell and thus deteriorates growth rates (Riley, et al., 1993).

Effect of Fumonisin-B1 on the relative weights of the rat internal organs:

The results in table-6 showed the effect of Fumonisin-B1 on the relative weight of the liver and kidney of male rats. The FB1 caused significant increase in the relative weight of liver and kidney at concentration(T2) and (T3) compared with control group of rats.

Table-6 Effect of Fumonisin-B1 on the relative weights of the rat internal organs

The relative weight of the internal organs of rats		
Treatment	Liver(gm)	Kidney(gm)
T1	3.3 a	0.5 a
T2	3.7 b	0.8 b
T3	4.6 b	1.7 b
LSD for average weight of organs and treatments 0.426		

*T1: control group, T2: Fumonisin-B1 with a concentration, of 3.597mg/kg, T3: Fumonisin-B1 with a concentration of 4.264mg/kg.

*Different characters in one column indicate significant differences at a probability level($P < 0.05$).

* Each value in the table represents a rate of eight replicates.

These results are consistent with previous studies that have shown that fumonisin-B1 causes a decrease in growth rates and enlargement of the internal organs as well as liver damage with abnormal mitosis of bile and liver tissue and reduced immunity (Mobio, et al., 2000). The results also agreed with previous studies that the effect of fumonisin-B1 on the internal organs of broiler chicks when fed for 21 days led to significant increase in liver and kidney weight when compared with organ values in control group chicks (Al-Jubouri, 2002). The reason for the increase in weight of the liver is due to the accumulation of fat in it to reduce the formation of low-density lipoproteins (LDL), which is responsible for the transfer of fat from the liver to other tissues and thus block the fat in the liver and cause fatty liver with imbalance of water balance and osmotic pressure in the cells, What explains the weight increase of kidney is damage to the renal tubules due to toxins and get an imbalance in the work of the kidney and thus enlarged (Kubena, et al., 1997).

CONCLUSIONS:

Through the results of isolation and diagnosis of fungi, it was found that local and imported wheat samples were contaminated with a number of fungi species, most of which are characterized by the production of mycotoxins: *Aspergillus*, *Fusarium*, *Penicillium*, *Alternaria*, *Mucor*. The results of estimating FB1 with ELISA technique showed that the highest concentration was for local wheat samples in the Al-Dour district and the lowest concentration for Australian wheat samples and it was found through a study of the ability of fungi *F. moniliforme* isolates to produce FB1 in a laboratory using HPLC technique that the highest concentration recorded by local wheat samples of Al-Dour district and less Concentration of wheat samples of Baiji district. From the results of the sensory evaluation of flour and bread for local wheat, it was found that the best quality flour is wheat Samarra wheat and the worst quality of baiji wheat flour, As for imported wheat flour, the highest result was Australian wheat, followed by American wheat, As for the sensory evaluation of bread, the results showed that the highest degree was for baking Samarra flour followed by Al-Dour bread flour, whereas imported wheat bread distinguished Australian bread, followed by the American. A study of the effect of FB1 showed a significant decrease in the rate of weight gain and relative weight gain of the liver and kidney.

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تحديد تأثيرات سم الفيومونيسين B1 المعزول من الحنطة المخزونة على الصفات الحسية وبعض الصفات الفسلجية في الجردان

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قسم علوم الأغذية- كلية الزراعة- جامعة تكريت

المستخلص

أُجريت هذه الدراسة في مختبرات قسم علوم الأغذية/كلية الزراعة/جامعة تكريت بهدف عزل وتشخيص الفطريات الملوثة لحبوب الحنطة المخزونة وتقدير تركيز سم الفيومونيسين B1 فيه وتحديد تأثيراته الفسلجية في الجردان. قد أظهرت نتائج الدراسة تواجداً للاعفان *Aspergillus sp.* ونسبة 38 في العينات المفحوصة تلاه *Fusarium sp.* بنسبة 28 والنوع *Alternaria alternata* بنسبة 19 ثم الجنس *Penicillium sp.* بنسبة 10 و *Mucor sp.* بنسبة 5. كما تم التحري عن تواجد السم الفطري الفيومونيسين B1 في عينات الحنطة المحلية والمستوردة من خلال تقديره باستعمال تقنية ELISA حيث بلغ أعلى تركيز له 2.240 ملغم/كغم في الحنطة المحلية المسحوبة من قضاء الدور في حين أن أقل تركيز كان 0.103 ملغم/كغم في عينات الحنطة الأسترالية. تم دراسة قابلية عزلات العفن من النوع *F. moniliform* على إنتاج السم الفيومونيسين B1 مختبرياً حيث تبين قابليتها على إنتاج هذا السم الفطري وبتراكيز وصلت الى 4.264 ملغم/كغم في عينات الحنطة المحلية المأخوذة من قضاء الدور و 3.597 ملغم/كغم في عينات الحنطة المحلية المأخوذة من قضاء بيبي وذلك باستعمال تقنية الكروماتوغرافيا السائل عالي الاداء (HPLC). فيما يخص التقييم الحسي لطحين الحنطة المستخدمة في الدراسة فإن أفضل النتائج كانت لطحين الحنطة الأسترالية وبلغت 24 درجة وأسوأها لطحين حنطة بيبي والتي بلغت 14 درجة فيما يخص الصفات المدروسة وهي اللون والرائحة. فيما يخص التقييم الحسي للخبز فقد تم تسجيل أعلى درجة تقييم 33 درجة للطحين الأسترالي وأدنى تقييم لخبز طحين الدور وهو 24 درجة فيما الصفات المدروسة وهي اللون والرائحة والطعم. كما تم دراسة تأثير سم الفيومونيسين B1 في معدل الزيادة الوزنية في ذكور الجردان حيث أدى وجود هذا السم في عليقة الجردان الى انخفاض معنوي في الوزن وذلك بعد مرور 21 يوم من عمر التجربة حيث بلغ معدل الوزن في المعاملة (T3) 141 غم وأظهرت النتائج ارتفاع معنوي في الوزن النسبي للكبد والكلى في المعاملة (T3) والذي بلغ 4.6 غم و 1.7 غم على التوالي.

الكلمات المفتاحية: سم الفيومونيسين، الصفات الحسية، الصفات الفسلجية، الجردان.