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Investigation of Microorganisms and Aflatoxin (B1) In the Local Wheat Flour Produced in Iraq and The Effect of Some Factors on The Production of Poison

Iman K.L. Al-Zubaydi[1], Kadhim H. Yassen[2], Lubna A.J. Rasheed[3]

[1]Department of Biology, College of Science, Al-Mustansiriyah University, Baghdad, Iraq
[2]Ibn Khaldun University College and Al-Mustansiriyah University, Baghdad, Iraq
[3]Department of Environmental Engineering, College of Engineering, Al-Mustansiriyah University

Corresponding Author: Iman K.L. Al-Zubaydi
E-mail: Emankhalil87644@gmail.com, khzwaini@uomustansiriyah.edu.iq, dr.lubna@uomstansiriyah.ed.iq

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Abstract

It can cause scattered dust and usually loaded with various germs that adversely affect the health of silos workers in silos. The main objective of the study is to measure microbial contamination. (bacterial, fungal, mycotoxins), which are widely prevalent in Iraqi mills and negatively It affects the health of silos workers in particular and the general health of the community. The study included wheat flour, which were randomly collected for the winter seasons from 4 mills in Baghdad during December and January 2022. The results indicated that the most isolated types of wheat flour are pathological spores in addition to the presence of a percentage of microbial toxins, and the chemical analysis with HPLC during the winter season showed the presence of toxic mycotoxin contamination for a number of mills, and the khan was about(33.6 ppb) while Al Taji mills (36.2 ppb), and most of them samples contained Mold than the permissible limit, while some of the samples contained an allowable percentage of mycotoxin, the result was a heterogeneity in the dorsal shape of the isolates and also a difference in smell, texture and color difference clearly may be due to the occurrence of a type of genetic mutation on the growth of fungi in different conditions and their ability to produce toxins.

Keywords: Temperature and pH, Microorganisms Contamination, B1 afla toxins

Introduction

Wheat (bread wheat) *(Triticumaestivum L.)* It is considered one of the main commodities and plays a key role in global trade. (7), 715 million tons per year is the total world production per year, thus ranking second after corn in consumption and about (billion tons / year), and The most famous microbes are Widespread and highly common damage caused by loss of grain quality during storageperiod is the growth of the mold. (1).

A large number of research and studies have shown secondary metabolites known as mycotoxins are one of the main causes of damage to grains stored in silos, which may lead to

food and feed poisoning.. The importance of fungi is due to the fact that their enzymes are extracellular (10). Most fungi have the ability to produce high concentrations of toxins if they have the right conditions and are characterized by their ability to contaminate large quantities of stored grains in proportions exceeding the permissible limits. (8)

Mycotoxins are toxic by-substances produced from some fungi such as Aspergillus, Fusarium, Penicillium, when eating food products containing these toxins, causing a serious risk to consumer health (15). Many types of these toxins Which have been discovered, and the most important and widespread are those that negatively affect health in general. of organisms such as Aflatoxin, Ocratoxin, Patulin, Fumioncin, Zeraline, Nivanol and Diboxinvalenol, although they may appear in the food chain as a result of crop contamination with mushrooms in the field and sometimes after harvest. (8).

Aflatoxin (B1) is classified among the most dangerous types of deadly toxinsnd is a powerful carcinogen (12), fungi (e.g*., Apergillus spp, Fusarium spp, Penicillium spp*) and Bacteria (e.g., *Salmonella spp Bacillus cereus*) contaminate flour and its products and also the presence of these bacterial species more than the permissible limit causes many diseases., (17)

The main objective of this study is to measure Mold contamination associated with storage conditions measure aflatoxin B1 in Iraqi flour.

Materials and Methods

Collection Of Samples

Forty samples were collected randomly. from Iraqi mills (Dora, Taji, Rusafa, Khan), and samples were collected for the winter season 2022.

Isolation Of Bacteria

Weigh five grams for each sample and then hang it in 45 ml of distilled water, and then leave it to homogeneity within 15 minutes, after which only 1 ml of stuck Then divide into suitably sterilized Petri dishes (nutritious agar and McConky agar) until pure isolation is obtained, and by the Vitek 2 system to diagnose it definitively. (4).

Total number of viable bacteria

The incubation period, colonies were expressed as colony-forming units per gram number of bacterial cells was calculated with a ± device at 28 degrees according to the following equation:

number of colonized in the dish(cfu/g) = Counter inverted sample dilution. x Number of bacteria (1 ml of original .(4)

Isolation Of Fungal

Wheat samples are planted in petri dishes (diameter 90 mm, 10 grains) containing (grapes agar) (PDA) and the dishes were incubated for (5-7) days at 25 ° C.There is a second The fungus was isolated by taking one gram for each sample added to 9 ml of sterile D.W with a light shake of the mixture, and then leave the mixture for 15 minutes., and then transfer to sterile Petri dishes and cool (dextrose agar Sabord) (SDA) to 45 ° C and is poured and incubated at 25°C for 5 days, Morphology characterization of fungal species and microscopic observation was done , The average number of colonies developing in each sample, the percentage of the presence of each sex and species, the frequency of isolation for each sex and the type of fungal load of the samples were calculated. (20).

$$\%\text{frequency of species} = \frac{\text{Number of species appearance in the sample}}{\text{total number of the species appearance}} \times 100\%$$

Detection of mycotoxins using HPLC technology

The examination was carried out in the laboratories of the Ministry of Science and Technology - Department of Environment and Water and according to the method provided using a high-performance liquid chromatography device (SYKAM model) (German origin), where the carrier phase was used: acetic tontrile: distilled water: (30: 70) The separation column was: (C- 18) ODS (25cm * 4.6mm) to separate the mycotoxins. A fluorescence detector (ex=365nm, em= 445nm) was used to detect the mycotoxins, where the flow rate of the carrier phase was: 0.7 ml. / min.(11)

Results and Discussion

Identification of Bacteria & Fungi for Winter Season.

The results obtained for the winter season 2022 in the table, which shows a variety of contaminated microorganisms in samples of wheat flour, which included pathogenic bacteria and fungi in 4 silos in Baghdad from (Al- Dora , AL –Taji , Al- Rusafa , Al- Khan). Microbial growth was diagnosed by studying the microscopic properties of bacterial isolates by gram dye and fungal isolates by lactophenol, so the built-in Vitek2 system was used to confirm the identification of isolated bacteria as well as the diagnosis of fungi based on morphology under the microscope.

Identification Of Bacteria (Wheat flour) winter \2022 and total bacterial count (CFU\g (Table 1)

Location	Type	Isolated bacterial specie	total bacterial count (CFU\g(
Al- Dora	Flour	*Pantoea Spp. Sphingomonas paucimobilis*	9.7×10^6
AL –Taji	Flour	*Ralstonia insidiosa Enterobacter cloacae Sphingomonas paucimobilis*	9.9×10^6
Al- Rusafa	Flour	*Sphingomonas paucimobilis Ralstonia Pickettii*	7.1×10^6
Al- Khan	Flour	*Pantoea Spp. Sphingomonas paucimobilis Enterobacter cloacae complex*	5×10^6

shown in(Table 1) For samples throughout the winter season 2022 is the *Pantoea Spp.* Isolated group of wheat flour produced from them*Pantoea Spp.* (3). as well as bacteria species belonging to the Enterobacteriaceae family. Gram negative isolated from a sample of wheat found in freshwater and soil and isolated from humans which represent as pathogenic bacteria also transmitted by snails and some beetles(13) Furthermore, Al-Taji mills selective gram-negative bacteria isolated from a sample of wheat found in freshwater and soil and isolated from humans which represent as pathogenic bacteria also transmitted by snails and some beetles, (6)). It is likely that most of the sources of these pathogenic bacteria isolated in this study are the result of irrigating agricultural land with water loaded with sewage waste and animal waste.,(5) (15). From the results obtained which noted in the Table 1 the maximum value of bacteria growth for the winter season was detected in wheat flour of AL –Taji was 9.9×10^6CFU/g While the minimum value of bacterial growth in local wheat in AL-Khan 5×10^6 CFU/g.

Identification of Fungi for Winter Season

The results obtained for the winter season 2022 in the table, which shows a variety of contaminated microorganisms in samples of wheat flour, which included pathogenic fungi in 4 mills in Baghdad from (Dora, Taji, Rusafa, Khan), Microbial growth was diagnosed by studying the microscopic properties of fungal isolates by lactophenol, as well as the diagnosis of fungi based on morphology under the microscope.

(Table 2) Identification of (wheat flour) in winter \2022 and Frequency of fungi Species

Location	Type	Fungal Contamination	Frequency of Species%
Al-Dora Silo	Flour	*Asp. Niger* *Penicillium spp.* *Fumigatus Asp.*	33.30 35.45 31.25
Taji silo	Flour	*Asp.s flavus ,* *Rhizopus spp.* *Penicillium spp.*	77.78 11.11 11.11
Al-Rusafa Silo	Flour	*Rhizopus spp.* *Penicillium spp.* *Asp. flavus*	13.64 11.11 75.25
Al-Khan silo	Flour	*Asp.flavus,* *Asp. fumigtaus* *Rhizopus spp.*	77.78 11.11 11.11

The fungi referred to in Table 2 and isolated from Iraqi mills, and the results showed that the most present genera are; *Asp spp., Rhizopus spp*. and *Penicillium spp.,* which are classified among the dominant fungi in the poor storage of grain in general, knowing that wheat was newly harvested during the winter 2022, The most prevalent was *Asp.spp*, while the dominant genus was *Asp. fumigtaus* and *Asp.Flavus*, a number of fungi distinguished by rapid growth, resistance to low heat and low water, nevertheless *Rhizopus spp*., Penicillium appeared largely for grain and flour grain samples, a study conducted in the Bulgarian state (17), A number of fungal species have emerged that have been accurately isolated such as; *ASP. spp.* and *Pen. spp*. and *Rhizopus spp*. isolated from grain-loaded fungi, correspond to the results of Kumar's study (2)(12).

Presence of Aflatoxin (B1) in Iraqi Wheat flour samples

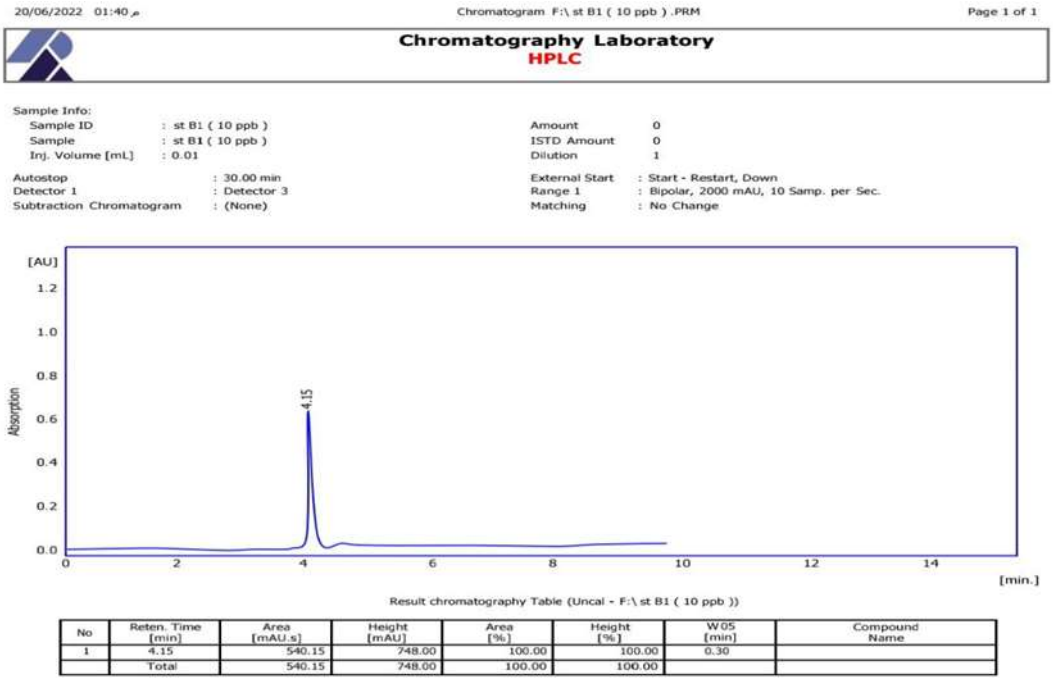
The chemical analysis process was performed using HPLC device for all the samples included in the study, where all the samples were taken and before the laboratory transplant to ensure the presence of Mycotoxin, and several types of Mycotoxins were investigated and according to standerd available in the local markets ,and Table (3) shows the chemical analysis with HPLC used to analyze samples of samples taken from silos included in the study.

(**Table 3) Determination of fungal aflatoxin toxin (B1) in wheat flour samples 2022/winter**

location	In flour B1 concentration (ppb)
Al- Dora Silo	24.7
Al- Taji Silo	36.2
Al- Rusafa Silo	18.75

Al- Khan Silo	33.6

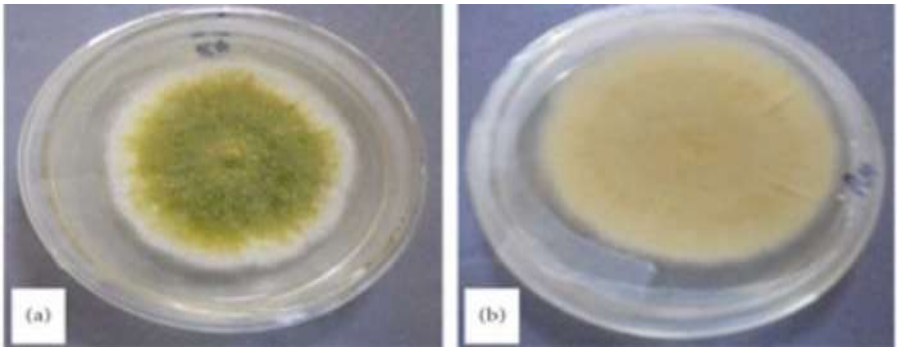
Figure1: The chemical analysis process HPLC device for B1 poison



Results of the winter season of Alkhan mills The presence of B1 poison contamination in local wheat (33.6 ppb) while the rest of the silos did not show any percentage of pollution with the mentioned poison, while the percentage of contamination of flour produced for silos (Taji,) was about 36.2 ppb as shown in the table above, and the reason for the emergence of this large percentage of pollution may be due to the quality of the water used in the process of processing wheat before the grinding process (16), the poor process of storing grain in the mill and the type of silo Storage and mill(13)(14).

The effect of certain physical factors (temperature and pH) on fungal growth and phenotypic

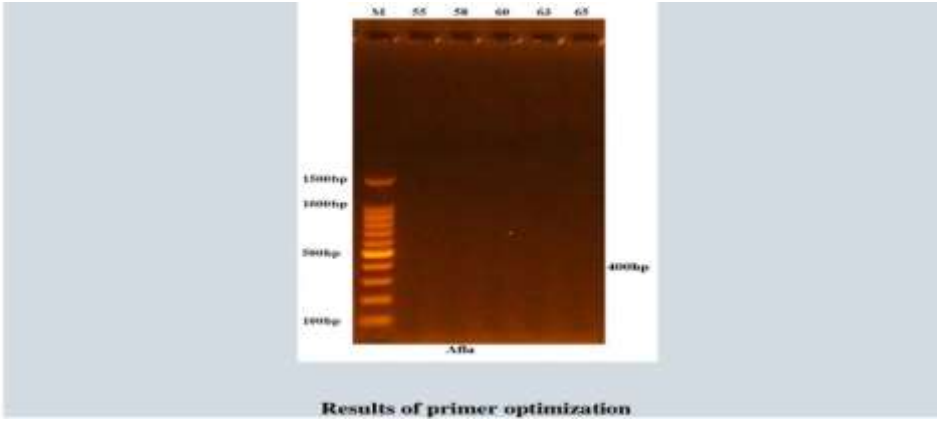
Some isolates of *Aspergillus flavus* fungus taken from models containing a high amount of aflatoxin toxin previously measured by HPLC examination have been conducted multiple operations of sub culture and have been developed at a temperature ranged between (28-30) C^0 and pH (7-8) for 10 days continuously, the result was a heterogeneity in the dorsal shape of the isolates and also a difference in smell, texture and color difference clearly as in Figure (1), The reasons may be due to the occurrence of a type of genetic mutation.



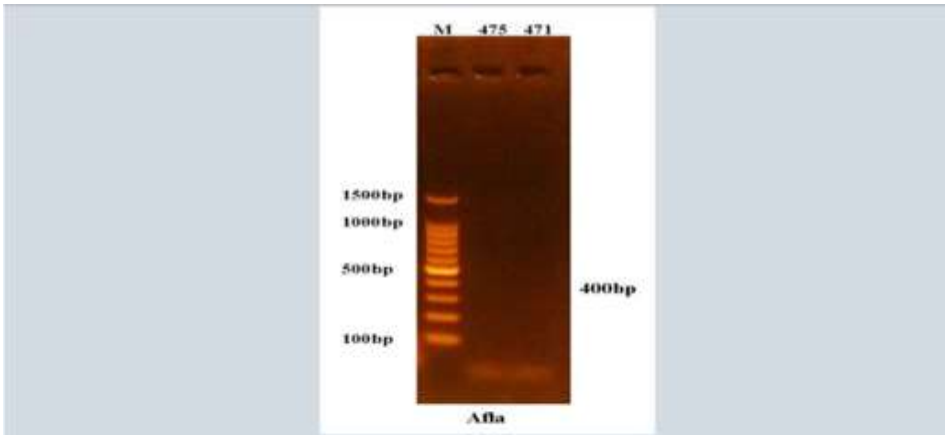
(Figure 1) ***a: Aspergillus flavus first isolates, b: Aspergillus flavus after sub culture and different (pH, Temperature)***

Molecular diagnostics

The results of the amplification of the *Agla* primer for fungal species showed on 1.5% agarose electrode gel for *A. flavus* isolates which were grown at a temperature of 30 °C and pH 8 and on the medium, PAD The results of the PCR examination showed the absence of the gene responsible for the production of the toxin and the reason may be due to the difference in the temperature of the insulation and also a difference in the pH used and the incubation period that lasted for 10 days on the isolates before the electrophoresis procedure, knowing that these isolates have shown heterogeneity In the phenotypic characteristics of the difference in color, appearance, texture and smell, which calls for the need for more genetic tests and deeper studies in this range to find ways to reduce the production of mycotoxins, especially in food and grains, and this is consistent with a study conducted before(19) (18), on the growth of fungi in different conditions and their ability to produce toxins and the heterogeneity in the production of mycotoxins in different development conditions(9).



Results of primer optimization



Results of the amplification of *Agla* primer of fungal species were fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes 475-471 resemble PCR products.

(Figure 2) the amplification of Agla primer of fungal species For two isolated strain of *Aspergillus flavus* resemble PCR products

Conclusion

It is important for silo workers to follow safety guidelines, use personal protective equipment (PPE) and receive appropriate training. Regular medical examinations and regular periodic visits to hospitals and health centers can help reduce the risks of direct handling of microbial contaminants and flying dust from transporting, storing and grinding grain and preserving the health of the community.

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