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Original Research Paper

Extracting mycotoxins from dairy products and detecting the responsible genes using polymerase chain reaction as forensic evidence in economic crimes

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Abstract: This study includes isolate and indentify of accompanying fungi to raw milk that available in the local markets of Babylon province, as well as study their ability to produce aflatoxin B1 by the Aspergillus flavus.
The results of isolation and identify appearance of numerous fungi in raw milk to them A. flavus , A.niger , Penicillium spp., Fusarium spp. and Candida spp. that the A.flavus and Candida spp. were the most visible fungi and highest frequency. The results showed by using ammonia test that 80 % of the total isolates of A.flavus were producing Aflatoxin B1, but when using the chromatography thin layer technique (TLC) there are % of its islolates were producing Aflatoxin B1. The polymerase chain reaction technique (PCR) used to detection for ability of A.flavus isolates to produce Aflatoxin B1, the result confirmed that all isolated of four tested were carriers of the gene aflD which was regulator for Aflatoxins producing with 400 base pair.

Keywords: milk , Aflatoxin B1 , gene aflD

1.Introduction

Microfungi create secondary metabolites called mycotoxin, which can kill or seriously harm both people and other animals. Certain mycotoxins or mycotoxin derivatives have been used as antibiotics due to their pharmacological action [1,2].

The hepatocarcinogenic bidihydrofurano compounds known as aflatoxins are generated by certain Aspergillus genes, particularly by strains of Aspergillus flavus and Aspergillus parasiticus [3,4]. The hydroxilated metabolites of aflatoxin B1 (AFB1), or AFM1, are present in milk products that are derived from animals that have consumed tainted feed. AFM1 in milk is produced from 0.3-6.2% of AFB1 in animal feed [5]. The International Agency for Research on Cancer (1993) first categorized AFM1's toxicity as a Group B2 agent; however, the IARC (International Cancer Research Agency, 2002). Even at low concentrations, AFM1 poses a serious risk to human health, particularly for children who are the main users of dairy products. In contrast, AFM1 remains reasonably stable during the pasteurization, sterilization, and storage of milk and milk-based products [6,7]. As a result, regulation standards have been created and global monitoring of AFM1 in dairy products has been recommended. The US Food and Drug Administration states that milk should contain no more than 0.5 mg/kg of AFM1 (US Food and Drug Administration, 1996). The European Union (EU) more strictly regulated the amount of AFM1 in milk intended for adult consumption to 0.05 mg/kg [8]. This amount cannot be greater than 0.025 mg/kg for infant food items [9].

2.Methodology

Samples collection

A total of 30 samples of different raw milk were randomly collected from the local markets in Babel province and weighed 500 ml per sample with three replicates .for the purpose of obtaining isolates from the fungus producing Aflatoxin M1.

3. Results and discussion

Isolation and Diagnosis of Fungi with Row milk

The results of isolation of fungi showed 235 isolates belonging to four different genus (Candida spp, Pencilinium spp, A. flavius and A. niger). The percentage frequency of the A.flavus fungus was in the

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Abi Gharq area for heat exposed samples (61%) while the percentage frequency of the same samples was not exposed to heat (63%)and in aofee area percentage frequency for A. flavus for heat exposed samples was (57%), while the percentage frequency of fungus for the same samples was not exposed to heat (63%). In the Wardia area, the frequency of fungus was A. flavus (52%) for heat-resistant samples, while the percentage of fungus appearance for the same samples was not exposed to heat (57%) as the tables (1, 2 and 3) and figures (1,2,3and 4) .

The reason for the emergence of Aspergillus in most samples of milk is the possession of the species the ability to produce a large number of enzymes analyzed, which is used in nutrition and growth as well as increase the capacity of spread, especially that some of them can withstand the adverse environmental conditions as they can grow in low moisture content To the relative density of the spinach they produce[10,11,12].

Detection of aflatoxin by thin layer chromatography (TLC) the isolates of A.flavus , a product of aflatoxin, which was randomly taken from different milk samples in abi Garak the isolate A2 produced 72.94% of aflatoxin B1,while in second isolate B7 71 .8% , B13 produced 72.3% and the C6 was produced 73.52% of aflatoxin M1, by comparing the color of glare, extract of each isolation and RF with the standard Aflatoxin B1. The values of the RF for the standard toxin were 73.52% [13,14,15].

In this study use all these method to diagnosis of Aflatoxin B1in raw milk which more used by people in life style without found any attention of out healthy risk for bad used any food without sterilization method such as milk product process which effect on human health may be put a law for delinquent persons.

Table 1: number of fungi isolates from raw milk from Abi Garak.

Numbers of isolates						
Total of isolate	*Candida* spp	*Pencilinum* spp	*A.flavus*	*A.niger*	sample	heat
2	1	0	0	1	1A	Use of heat
5	0	1	3	1	2A	
2	1	1	0	0	3A	
3	0	0	2	1	4A	
4	1	1	2	0	5A	
2	0	1	0	1	6A	
5	1	0	0	2	7A	
5	0	2	2	1	8A	
5	0	1	4	0	9A	
2	1	1	0	1	10A	
3	1	0	0	2	1A	Non use heat
7	0	1	5	1	2A	
4	2	2	0	0	3A	
6	1	0	3	2	4A	
9	2	1	6	0	5A	
3	2	0	0	1	6A	
3	2	1	0	2	7A	
6	2	1	2	1	8A	
8	1	2	5	0	9A	
5	2	2	0	1	10A	
89	20	18	34	18		No. of genous

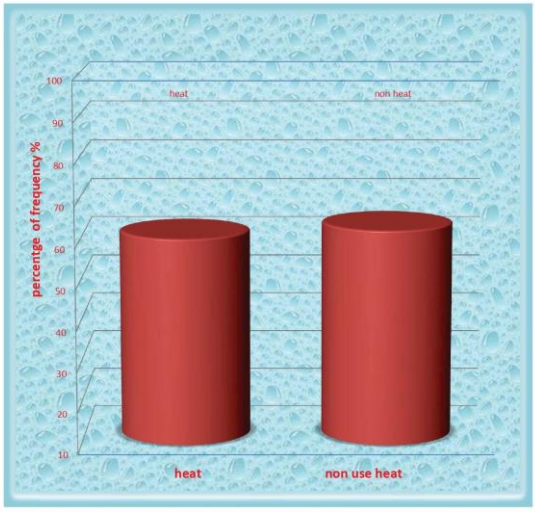

Fig 1. Percentage frequency of *A flavus* isolates in raw milk from Abi Garak for heat exposed samples (61%) and non heat exposed (63%).

Table 2: number of fungi isolates in raw milk from Abi Aofee.

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Numbers of isolates						
Total of isolate	*Candida* spp	Penicillium spp	*A.flavus*	*A.niger*	sample	Heat
2	1	0	0	1	1B	Use of heat
4	1	0	2	1	2B	
2	**0**	2	0	0	3B	
2	2	0	0	0	4B	
5	0	1	3	1	5B	
2	1	1	0	0	6B	
4	1	0	2	1	7B	
5	0	0	4	1	8B	
3	1	2	0	0	9B	
1	0	1	0	0	10B	
4	1	1	2	0	11B	
2	0	1	0	1	12B	
6	1	2	3	0	13B	
5	2	1	0	2	1B	Non use heat
8	1	2	4	1	2B	
3	1	2	0	0	3B	
4	2	1	0	1	4B	
8	1	1	4	2	5B	
4	2	2	0	0	6B	
9	0	2	6	1	7B	
7	1	0	5	1	8B	
2	2	0	0	0	9B	
2	1	0	0	1	10B	
6	1	1	4	0	11B	
2	1	0	0	1	12B	
7	1	1	5	0	13B	
109	25	24	44	16		No. of genous

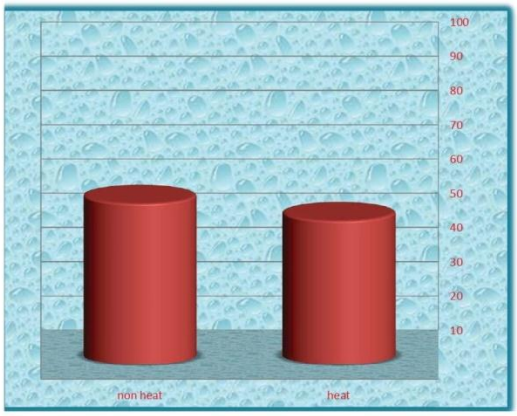


Fig 2. Percentage frequency of *A flavus* isolates in raw milk from Aofee for heat exposed samples (63%) and non heat exposed (57%).

Table 3: number of fungi isolates in raw milk from Abi wardia.

Numbers of isolates						
Total of isolate	*Candida* spp	*Penicilinum* spp	*A.flavus*	*A.niger*	sample	Heat
3	1	0	1	1	1C	Use of heat
3	2	1	0	0	2C	
4	1	0	3	0	3C	
3	1	1	0	1	4C	
3	3	0	0	0	5C	
7	1	0	4	2	6C	
3	1	2	0	0	7C	
6	1	0	3	2	1C	Non use heat
5	2	1	0	0	2C	
6	1	1	4	0	3C	
3	1	1	0	1	4C	
4	4	0	0	0	5C	
9	1	0	6	2	6C	
3	1	2	0	0	7C	
62	21	9	21	9		No. of genous

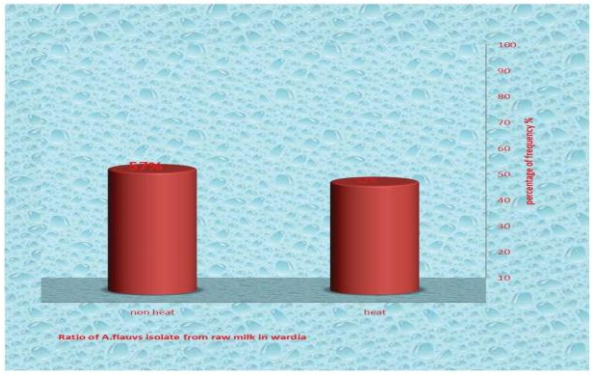
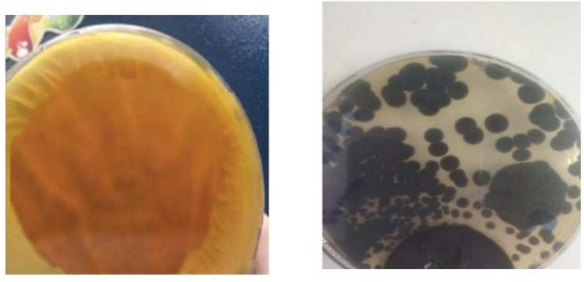


Fig 3. Percentage frequency of *A flavus* isolates in raw milk from wardia for heat exposed samples (57%) and non heat exposed (52%).



A B

Fig 4. *A flavus* (A) isolates of aflatoxin B1 high quantities. Isolates without aflatoxin B1 (B).

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Conclusion

By examining several different samples of milk, we found many types of Pathogenic fungi. The percentage of fungi found in heat-exposed milk samples was the proportion of raw lipids not exposed to heating.The fungi produced for aflatoxin toxin were detected and we had samples produced by using ammonia detection technique and TLC technology.

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