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Research Article

Isolation and Diagnosis of Filamentous Fungi from Dairy Products and Detection their Toxicity

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Abstract
Dairy products provide a favourable habitat for the growth and survival of many microorganisms including fungi because of their physical and chemical characteristics. Isolates were characterised both macroscopically and microscopically,out of 20 analysed samples 6 species belonging to 5 genera were isolated. *Aspergillus niger*, *Aspergillus flavus*, *Penicillium expansum*, *Cladosporium sp.*, *Fusarium oxysporum* and *Alternaria alternata.* The study show most of dairy products contaminated with these fungi .the predominant fungus was penicillium (42.85%) followed by Aspergillus (32.14%), Cladosporium (14.30%), Fusarium (7.14%) and finally alternaria (3.57%).Also detection of toxicity was carried out and the genus Aspergillus *flavus* was highly toxic followed by *Aspergillus niger* and *Fusarium oxysporum* with moderate toxicity, *Penicillium expansum* and *Alternaria alternata* with low toxicity and finally *Cladosporium sp.* with no toxicity.

1. Introduction

Milk is an essential food in the human diet and has great value as a healthy and nutritious food; In the early years of human life, milk & dairy products are significant nutritional fact in the adult diet (1), due to their chemical &physical properties, dairy products provide a suitable environment for the growth and survival of many microorganisms such as fungi (2) Identification of fungal microbiota be beneficial for improving production processing, quality of dairy products and safety of consumers. The diversity and count of fungi in dairy products along with the microbial quality of the utilized milk, treatment heat, production and maturity conditions, all of them different greatly. Certain yeasts & molds play a major role in the production of some types of cheese and to develop their taste and odor (3). Raw milk and other components, as well as surfaces, the air, and the equipment used by the operators, can all be sources of contamination in a dairy facility (4). Additionally, certain fungal species are opportunistic pathogens that might pose a threat to certain consumer groups (5). Penicillium,Candida, Cladosporium, Galactomyces and Mucor for yeasts and molds,

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respectively are the principal genera responsible for the spoilage of dairy products (6). The majority of these pollutants come from the dairy environment, and a number of mold species, including Penicillium species & Aspergillus species are capable of producing mycotoxins (7). When it comes to undesired species, their existence within dairy products can cause a variety of food spoilage, including observable fungus growth on the product's surface as well as generation of metabolites that give off unpleasant aromas and tastes and noticeable changes in texture and/or color (8). Additionally, a number of mold spoilage species, including Penicillium & Aspergillus species, have the capacity to create mycotoxins that may be hazardous to human health (9). For potentially toxic effects, such as nephrotoxic,neurotoxic, carcinogenic & mutagenic impacts, the presence of toxic metabolites in food & feed poses a serious global concern for human health (10). The only mycotoxin in milk & dairy products for which maximum limits have been specified (0.05 ppb in the EU) is {aflatoxin M1 -AFM1}, that is produced via several species of Aspergillus. AFM1 in milk is produced by dairy cows given {aflatoxin B1 -AFB1} contaminated foodstuffs converting (AFB1 to AFM1), that passes into their milk & urine. Further mycotoxins found in cheeses include citrinin, ochratoxin A, mycophenolic, cyclopiazonic acids and roquefortin C in varying amounts (11). Controlling fungal spoilage is a key challenge for industrialists & scientists searching for effective ways to inhibit and/or restrict development or fungal growth in dairy products. To control of that kind of contaminations, various conventional means, also known as traditional hurdle techniques, are used, such as treatment of air, disinfection procedures &cleaning, treatment of heat, water activity reduction via refrigeration, brining & modified atmosphere packaging (12), in addition to utilize chemical preservatives that are considered food additives. The use of one or more of these treatments can minimize or decrease the frequency and rate of spoiling in various dairy products. Consequently, fungal spoilage remains a problem for dairy producers. Actually, increasing fungus resistance to chemical preservatives or heat treatments (13) as well as customer desire for more "natural" products, as well as legislation evolution, have prompted industrial dairy producers to develop complementary control strategies (14).

Aim of the research is to determine filamentous fungi responsible of contamination of dairy products and to detect their toxicity.

2. Materials and methods

2.1. Samples collection

Many dairy products were collected from markets for isolation of fungi and these products were from different origins (Iraq,Iran,Turkey,Saudi and Hungary) and these products were packed and unpacked ,then bring to the laboratory.

2.2. Culture media

2.2.1. (SDA) Sabouraud’s – dextrose agar

Dissolve 65.0 grams in (1) L of distilled water. To completely dissolve the medium, heat it until it boiling.sterilize via autoclaving for 15 minutes at 121°C and 15 lbs of pressure, cool to 45–50 °C. Mix carefully, then place to sterilized Petri dishes (15).

2.2.2. (PDA) Potato Dextrose Agar

Dissolve 42 g of a powder in (1) L of distilled water, Mix thoroughly. Bring to a boil, shaking constantly, until totally dissolved. Sterilize for 15 minutes in an autoclave at 121°C. PDA is a medium used to identify, cultivate, and count of yeasts & molds isolated from foods as well as other substances (16).

2.3. Fungi Isolation

Every specimen being immediately placed into sterile petri dishes containing (SDA), and it was incubated at (28-30)°C for (3-5) days (17). Then, a portion of each fungus colony was transferred with a needle to another set of sterile petri dishes with a fresh PDA (18). Three times were needed to complete this process before the colonies in each petri dish became pure.

2.4. Fungal identification

The isolates were characterized based on their morphological characteristics once growing on PDA. The recognition is achieved at the genus level by using the classification keys described in (19, 20). The isolates were examined according to the morphological test of fungal colonies and the microscopic test using lacto phenol solution with blue cotton dye (21).

2.5. Detection of toxicity

The ability of isolates to produce aflatoxins was conducted through the use of ammonia solution with a concentration of 20%. Filter papers moistened with ammonia were placed in the cover of the dish containing the growing fungi, then the dishes were incubated upside down for 7-14 days at a temperature of 28°C. The occurrence of a change in the color of the colony bases from transparent to red indicates that the developing isolates is capable of producing aflatoxins, and the degree of red color indicates the efficiency of the isolates in producing these toxins (22).

3. Results and discussion

Table No. 1 shows the dairy products collected from the markets, which were of different manufacture origins, as (Spring Flower, Al-asbat, Local Milk, Local Kaymer, Local cheese, Local butter, Local yogurt and Kidy) were of Iraqi origin, while (Amal cheese, Pinka, Cream cheese, Sabah, Raw cream, Kibi, Kaleh, Fresh Dairy, and Kiri) are of Iranian origin, Rawan cheese is of Turkish origin, Anchor is of Saudi origin, and VONK is of Hungarian origin. 15 of them were packaged and 5 were not.

Table 1. Dairy product and their Manufacture

	dairy products	Package	Manufacture
1	Amal cheese	Packaged	Iran
2	Pinka	Packaged	Iran
3	Cream cheese	Packaged	Iran
4	Spring Flower	Packaged	Iraq
5	Fresh Dairy	Packaged	Iran
6	Al-asbat	Packaged	Iraq
7	Local Milk	Unpackaged	Iraq
8	Local Kaymer	Unpackaged	Iraq
9	Local cheese	Unpackaged	Iraq
10	Local butter	Unpackaged	Iraq
11	Local yogurt	Unpackaged	Iraq
12	Sabah	Packaged	Iran
13	Raw cream	Packaged	Iran
14	Kibi	Packaged	Iran
15	Kalleh	Packaged	Iran
16	Rawan cheese	Packaged	Turkey
17	Anchor	Packaged	Saudi
18	Kiri	Packaged	Iran
19	Kidy	Packaged	Iraq
20	VONK	Packaged	Hungary

Table No. (2) shows the contamination of some dairy products under study, among the 20 samples of dairy products, it was found that 12 of them were contaminated with fungi and 8 samples were not contaminated.as it appears to us that Kalleh, an Iranian product, was the most contaminated with fungi, as three genera were found in it: *Penicillium expansum*, *Aspergillus niger*, Cladosporium sp. Also, the local yogurt product, which is Iraqi with the presence of *Aspergillus niger*, *Penicillium expansum Fusarium oxysporum*, then the product Cream cheese, which is of Iranian origin, with two genera: *Alternaria alternata*, *Penicillium expansum*, and then the Al-asbat product, which is of Iraqi origin, with two genera, *Penicillium expansum* and *Aspergillus flavus* .The Local Kaymer also contained Aspergillus niger and *Aspergillus flavus*. The following samples contained only one genus: Raw cream, an Iraqi product (*Penicillium expansum*), Rawan cheese, which contained (*Penicillium expansum*), and Sabah, an Iranian product, contained (Cladosporium sp.). The sample Local cheese, which is of Iraqi origin, contained the genus (*Aspergillus niger*), as well as the product, which is also Iraqi, Local Milk, contained the genus (*Penicillium expansum*), and the product, which was Iranian made Fresh Dairy, contained the genus (*Aspergillus flavus*), and finally the Local butter, which was an Iraqi industry, contained the genus (Fusarium oxysporum). As for the other eight models that were not contaminated, they are Amal cheese, Pinka, Kiri, Kibi (Iranian-made), Spring Flower, Kidy (Iraqi-made), Anchor (Saudi-made), and VONK (Hungarian-made).

Penicillium genus was detected in the most of the types of products analyzed, which were indicative of local manufacture, and was especially prevalent in hard cheeses. P. brevicompactum was already identified as a substantial dairy product contamination throughout southern Italy (23).

Elbagory et al. (24) as well as Seddek et al. (25) demonstrated that Aspergillus was the most prevalent isolated mould from Ras cheese, with *Aspergillus niger*,*Aspergillus flavus*, *Aspergillus fumigatus* ,*Aspergillus ustus*.

Table 2. Filamentous fungi diversity isolated from contaminated dairy products

	Product Types	Contamination	Fungi	Control	No of Appearance
1	Amal cheese	-	-	-	-
2	Pinka	-	-	-	-
3	Cream cheese	+	*Alternaria alternata* *Penicillium expansum*	-	1 2
4	Spring Flower	-	-	-	-
5	Fresh Dairy	+	*Aspergillus flavus*	-	1
6	Al-asbat	+	*Penicillium expansum* *Aspergillus flavus*	-	1 1
7	Local Milk	+	*Penicillium expansum*	-	1
8	Local Kaymer	+	*Aspergillus niger* *Aspergillus flavus*	-	1 1
9	Local cheese	+	*Aspergillus niger*	-	2
10	Local butter	+	*Fusarium oxysporum*	-	1
11	Local yogurt	+	*Aspergillus niger* *Penicillium expansum* *Fusarium oxysporum*	-	1 2 1
12	Sabah	+	*Cladosporium sp.*	-	2
13	Raw cream	+	*Penicillium expansum*	-	3
14	Kibi	-	-	-	-
15	Kalleh	+	*Penicillium expansum* *Aspergillus niger* *Cladosporium sp.*	-	1 2 2
16	Rawan cheese	+	*Penicillium expansum*	-	2
17	Anchor	-	-	-	-
18	Kiri	-	-	-	-
19	Kidy	-	-	-	-
20	VONK	-	-	-	-

Figure (1) shows the fungal isolates percentage that contaminated dairy products, as it was found that Penicillium was dominant with a percentage of 42.85%, followed by Aspergillus with a percentage of 32.14%, then with Cladsporium 14.30%, Fusarium 7.14%, and finally Alternaria 3.57%.

Figure 1. Fungal isolates percentage

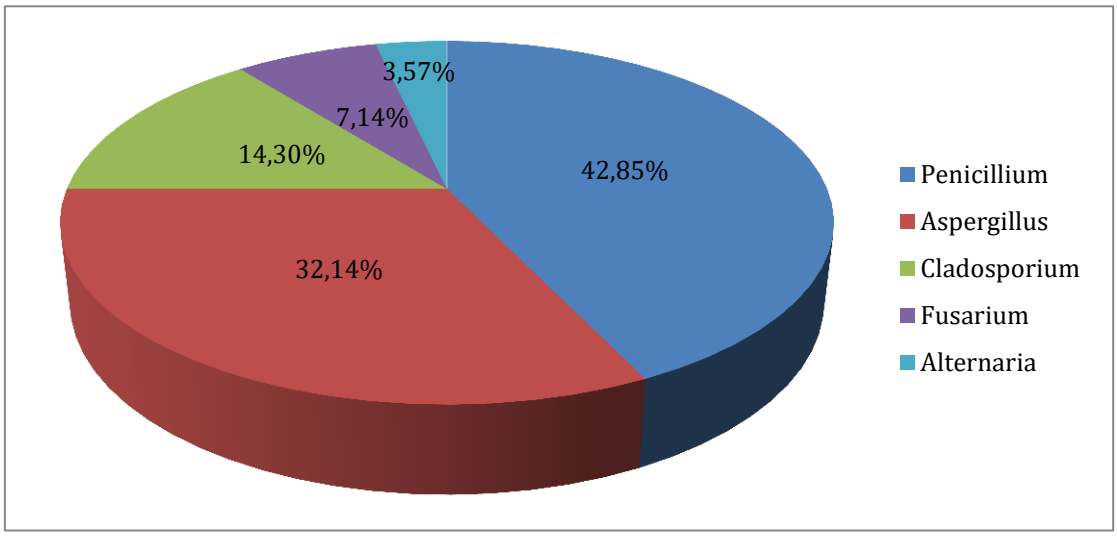


Figure 2. *Aspergillus niger*

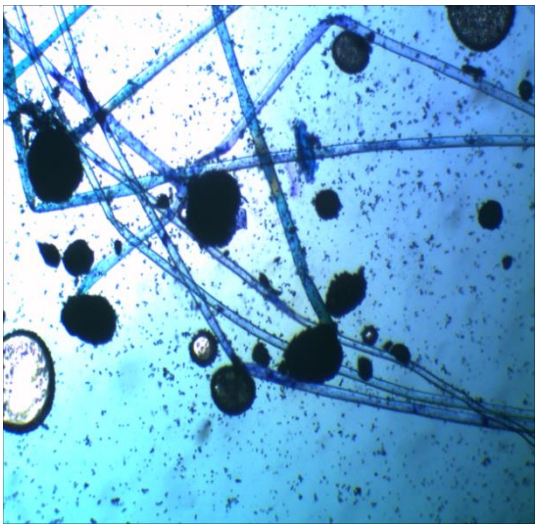
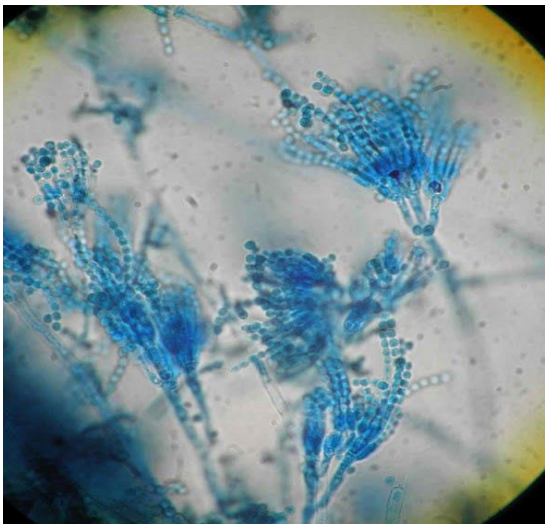


Figure 3. Penicillium sp.



Moulds that cause spoilage of dairy product extremely varied of both levels of the species & genus. Within Eurotiomycetes, Penicillium is the most often documented spoilage genus, of 40 species, and the genus Aspergillus sp. came second (10 species) (26). Dalia et al. (27) showed that penicillum spp. was the highest isolated mold for feta cheese with 38.5% followed by *Aspergillus fumigatus* with 30.8% then *Aspergillus niger* with 19.2% and Mucor with 7.7% while Rhizopus scored the lowest percentage 3.8.

Since Penicillium species are tolerant for lower temperatures & some among them are classified as (xerophilic), their presence in spoiled dairy products isn't unusual. (Penicillium spp.) seems to be well adapted to the cheese environment and has long been associated with cheese production (28). Moulds can lead to contamination of heat-treated dairy products .As well, Penicillium,Aspergillus, Fusarium,Cladosporium, and other genera have been isolated from cream cheese,

pasteurized milk& heat treated dairy beverages. Mould in dairy products or heat-treated milk could be caused by post-processing contaminations during bottling or packing (29). (Cladosporium spp.) actually be slow of growth, however they are common airborne fungi that are xerotolerant and psychrotolerant (28).

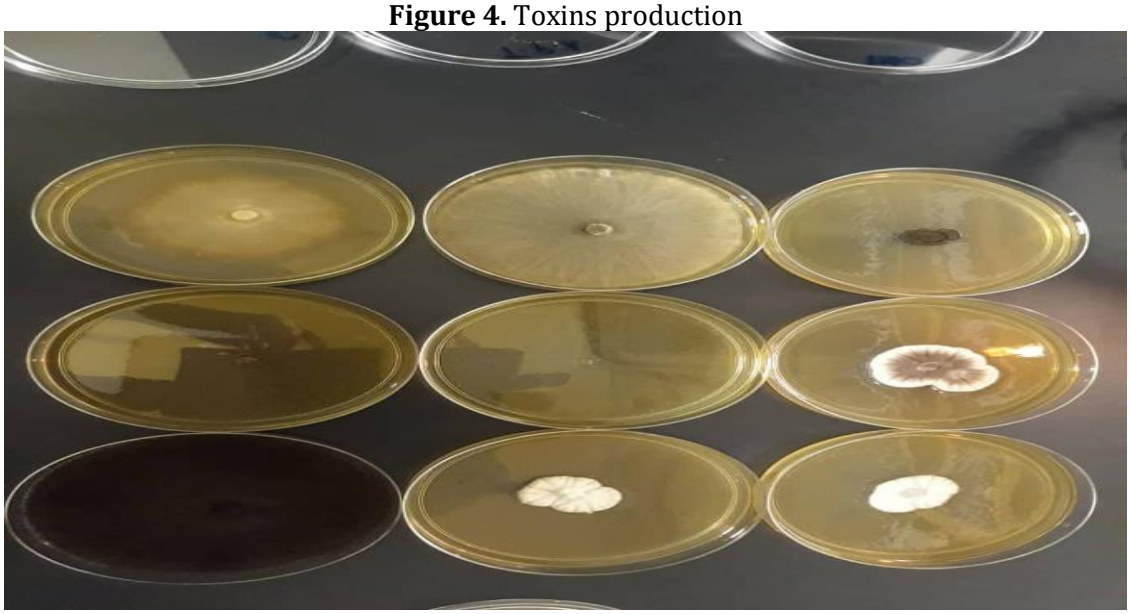
Table (3) shows the ability of fungi to produce toxins. Aspergillus flavus showed great ability to produce toxins, while Aspergillus niger and Fusarium oxysporum showed moderate ability to produce toxins. Penicillium expansum and Alternaria alternate showed low ability to produce toxins while Cladosporium sp did not produce any toxins.

Table 3. Fungal toxicity

Fungal isolates	Toxicity
Aspergillus flavus	+++
Aspergillus niger	++
Penicillium expansum	+
Cladosporium sp.	-
Fusarium oxysporum	++
Alternaria alternata	+

+++ highly toxic ++ moderate toxicity + Low toxic - non-toxic

Figure 4. Toxins production



The level of AFM1 contamination in milk and milk products differs greatly depending on variability in the original milk contamination, techniques of cheese production ,dairy feed quality, type of cheese, extraction, environmental factors, and analytical methods, as well as regulatory limits for AFM1 in animal feeds, milk, and dairy products (30).

The presence of AFB1 in milk and milk products can be attributed to the ingestion of AFB1-containing feedstuffs that the cow liver has not completely metabolized to AFM1, resulting in AFB1 in milk, as well as contamination of cheese with mould

spores that produce AFB1 during processing and storage due to a lack of or inadequate hygienic measures (31).

4. Conclusion

Results showed that high percentages of the examined samples did not comply with standards and most of them are contaminated with fungi, leading to high health risks for consumers. To counter this risk, effective safety measures need to be taken, and educational programs such as HACCP system (Hazard Analysis & Critical Control Point) must be launched for producers of milk and dairy products.

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