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ORIGINAL ARTICLE

Two-year survey on the seasonal incidence of aflatoxin M1 in traditional dairy products in Egypt

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Abstract The most popular and economically important traditional dairy products in Egypt are raw milk, Karish cheese (an Arabian dairy product made from defatted cow milk) and Zabady (an Arabian yoghurt made from buffalo and cow milk). In this study, 302 traditional dairy samples including raw milk (120), white Karish cheese (118), and Zabady (64) were analyzed for aflatoxin M1 (AFM1) during different seasons in 2016 and 2017. Contamination of raw milk samples with AFM1 was 21.6% and 18.3% in samples collected in the two respective years with percentages of 100% and 90.9% exceeding the legal European limit (0.05 μg L^{-1}). In Karish cheese samples, the contamination level was 33.9% and 44.6%, in the 2 years examined with percentages of 90.47% and 80% that were above the European limit (0.25 μg kg^{-1}). In the case of Zabady, the AFM1-positive samples were 12.5% and 18.75%, and all of them were above the European limit (0.25 μg kg^{-1}). However, average toxin concentration in Zabady was lower than that detected in milk and cheese. Despite the seasonal variations influencing the occurrence of AFM1 in the three dairy products, the AFM1 levels in samples collected in winter were significantly ($P \leq 0.001$) greater than those collected in summer. The contamination levels of AFM1 in the traditional dairy products consumed in Egypt; represent a serious health risk. It is urgent to inspect dairy farms for contamination with aflatoxins in a regular manner.

Keywords Aflatoxin M1 (AFM1) · Incidence · Raw milk · Karish cheese · Zabady · Egypt

Introduction

Aflatoxins are secondary toxic metabolites which are difuranocoumarin derivatives. They are produced mainly by *Aspergillus flavus* and *Aspergillus parasiticus*. These fungal species commonly contaminate cereals, vegetables, and cattle feed (Ismaiel and Papenbrock 2015). Among different types of aflatoxins, aflatoxin B1 (AFB1) is the most carcinogenic, teratogenic and mutagenic type (Ismaiel and Papenbrock 2015) and is listed as group 1 human carcinogen by the International Agency for Research on Cancer (IARC 2002). AFB1 converts to another hydroxylated toxic metabolite, aflatoxin M1 (AFM1) by hepatic cytochrome p450 from feeding of lactating animals with AFB1-contaminated forages or feeds (Assaf et al. 2019; Iqbal et al. 2015). A strong correlation has been found between the contamination level of excreted AFM1 in milk and consumption of AFB1 in feedstuffs. About 0.3–6.2% of AFB1 is metabolized to AFM1 and released in milk. This variability range depends on some factors such as seasonal variation, level of AFB1 intake, lactation process, genetics of the animals, and environmental conditions (Unusan 2006).

AFM1 showed cytotoxic, genotoxic, and carcinogenic effects, but its toxicity is 10% less than AFB1, and the IARC has shifted it from group 2b to group 1 human carcinogen (Assaf et al. 2019; IARC 2002). The presence

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of AFM1 in milk of breast-feeding mothers is a clear indicator for contamination of mother's diet with AFB1 (Elzupir et al. 2012). The dairy products manufactured from milk could be amenable for AFM1 contamination. Moreover, the main issue is the stability of AFM1 in dairy products during processing and storage stages. The stability of this toxin in traditional white pickled cheese (a soft type Turkish cheese produced traditionally from heat treated milk) for over 60 and 90 days was reported (Oruc et al. 2006). Additionally, thermal processing (pasteurization and ultra-high temperature) could not inactivate it in milk (Fallah 2010b).

The regulations of AFM1 in dairy products were reviewed by Iqbal et al. (2015). The European Union has set a maximum limit of AFM1 at 0.05 μg kg^{-1} for milk, yoghurt and butter, while for cheese it is at 0.25 μg kg^{-1}. Similarly in USA, the legal limit of AFM1 should not exceed than 0.5 μg kg^{-1}. The Codex Alimentarius has set 0.05 μg kg^{-1} and 0.25 μg kg^{-1} as the regulatory limits in butter and cheese, respectively (Codex Alimentarius Commission, 2001).

Contamination of dairy products with AFM1 is a worldwide issue (Fallah 2010a; Iqbal and Asi 2013; Martins et al. 2005; Öztürk Yilmaz and Altinci 2019). Several studies have analyzed the occurrence of AFM1 in dairy samples in different countries of Africa (Elgerbi et al. 2004; Kuboka et al. 2019; Langat et al. 2016; Oluwafemi et al. 2014). In literature, investigations conducted on the occurrence of AFM1 in dairy products in Egypt are scarce. Only two studies investigated the presence of AFM1 in milk, dairy products and human milk in Egypt (El-Sayed et al. 2000; Motawee et al. 2009). In Egypt, dairy products (milk, cheese and yoghurt) are popular sources of dietary energy for infants, children, convalescents and old people. The demand for dairy consumption in Egypt considerably increases; for instance the national consumption per capita of milk in Egypt in 2012 was 77.9 kg and it is expected to increase to about 91.7 kg in 2020 with growth rate of about 1.8% (Abd El-Latif 2012). Moreover, Egypt is the largest cheese producer in the Middle East with an estimated production of 720 thousand tons in 2012 that is expected to increase to 980 thousand tons in 2020 (Mikkelsen 2014). Milk and dairy products are consumed by all age groups due to the bioavailability of calcium and proteins in these sources. Toxicosis results from consumption of dairy products contaminated with AFM1, is a major health risk for children more than adults (Assaf et al. 2019; Fallah 2010a). Thus, contamination of the dairy products with AFM1 is of a great concern. Therefore, this study was undertaken to determine the incidence of AFM1in various traditional dairy samples that are locally consumed in Egypt during the four seasons of two consecutive years (2016 and 2017).

Materials and methods

Materials

Chemicals

AFM1 standard was obtained from Sigma-Aldrich (Taufkirchen, Germany). Methanol, acetonitrile, chloroform and *n*-hexane were all of HPLC grade. Stock solution of AFM1 was prepared in acetonitrile at a concentration of 1 μg mL^{-1} and kept at − 20 °C.

Thin layer chromatographic (TLC) plates

Silica gel F-254 plates (20 × 20 cm, 0.25 mm thickness) obtained from Merck, Darmstadt, Germany was used for separation of AFM1 from dairy samples, as described later.

Equipments and instruments

UV lamp (Desaga, Heidelberg, Germany) with wavelength of 254 and 364 nm was used for detection of AFM1 spots on TLC.

UV/Vis spectrophotometer (JENWAY-305 spectrophotometer, UK) was used for determination of AFM1 absorption in methanol.

High performance liquid chromatography (HPLC, Agilent 1100, Agilent Corporation, USA) was used for confirmation of the AFM1 identity in the most contaminated dairy sample.

Methods

Dairy samples collection

Traditional dairy product samples were randomly collected from different retail shops and local markets of three big Great Cairo Provinces in Egypt (Cairo, Giza and Qalyubia) during four seasons in 2016 and 2017. A total of 302 samples composed of raw (unpasteurized) milk (n = 120), white Karish cheese (n = 118) and yoghurt (Zabady, n = 64) were placed in sterile plastic sheath, kept in an icebox with ice packs and frozen at − 20 °C until analyzed.

Extraction of AFM1 from milk samples

The method of AFM1 preparation from milk was adopted according to Stubblefield (1979) with sight modification. Briefly, the samples were filtered through a filter paper (Whatman No. 4). In a separating funnel, the sample filtrate was extracted with 225 mL of 4% sodium chloride solution

followed by defatting with 100 mL *n*-hexane, and re-extracting with 100 mL chloroform. The extract was then centrifuged at 3500×*g* for 10 min and combined, washed with 300 ml of 4% sodium chloride solution, filtered over anhydrous sodium sulfate and dried using rotary evaporator (IKA, RV10, Germany).

Extraction of AFM1 from cheese and Zabady samples

AFM1 was extracted either from Karish cheese or Zabady samples according to the method reported by Fallah (2010b) with slight modification. Briefly, 100 g of the homogenized sample was mixed with 10 mL saturated aqueous sodium chloride and 120 mL chloroform, after which the mixture was centrifuged at 3500×*g* for 10 min. The chloroform layer was taken and passed through a Whatman No. 4 filter paper containing anhydrous sodium sulfate then evaporated until dryness.

Qualitative and quantitative analysis of AFM1

The dried sample extract was dissolved in 200 µL of methanol and spotted with the AFM1 standard working solution on TLC plates based on the method of Stubblefield (1979) as described by Fallah (2010b) with slight modifications. In which, the TLC plate was first developed in diethyl ether: methanol: water (94:4.5:1.5, v/v/v). The plate was then removed from the first developing system, dried, turned 90° and developed in chloroform: acetone: methanol (87:10: 3, v/v/v) as a second system. Blue influorescence of AFM1 spots were visualized under 360 nm UV lamp, scrapped off, eluted with methanol and quantified using UV spectrophotometer after monitoring the absorbance against a standard curve. The AFM1 detection limit of this method is 0.012 µg L^{-1}.

AFM1 identity in the most contaminated sample (Karish cheese, KCA 50) was also confirmed by HPLC equipped with fluorescence detector (FLD) with excitation at 360 nm and emission at 440 nm. The chromatographic separation was carried out on C18 HPLC column (4.6 × 250 mm, 5 µm). The mobile phase consisted of water: acetonitrile: methanol at a ratio of 680:240:80 (v:v:v) pumped at a flow rate of 1 mL min^{-1}. The injection volume of the standard and sample used was 20 µL. Data of HPLC chromatograms (Supplementary Fig. S1) showed that the retention time (Rt) of AFM1 in both standard and sample was 8.70 min and no interfering peaks were detected near the Rt. Also, the concentration of AFM1 determined by HPLC method (2.19 µg kg^{-1}) was found comparable with that determined by TLC method (2.23 µg kg^{-1}).

Statistical analyses

Data were expressed as the mean ± standard error (SE) and were subjected to analysis of variance using SAS software (version 9.2; SAS Institute 2004). Differences were considered significant if $P \leq 0.001$.

Results

Milk samples

Table 1 presents the incidence and concentration of AFM1 in raw milk samples collected during four seasons in 2016 and 2017. Of a total of 60 samples examined per year, 13 (21.6%) in 2016 and 11 (18.3%) in 2017 were positive with AFM1. The highest incidence in the first year was equally detected in winter and autumn with 4 (25%) of 16 samples being positive. In the second year, the highest incidence was detected in autumn with 5.0 (31.2%) samples being positive. In particular, the total concentration of AFM1 in examined samples was greater in 2016 than in 2017. The statistical analysis indicated that the average concentration of AFM1 in both spring and autumn of 2016 was significantly higher than in winter and summer. In 2017, the average concentration of AFM1 in spring was the highest significant when compared with other seasons. The average concentration of AFM1 was significantly higher in winter than in summer of the 2 years. The total range of AFM1 contamination was 0.05–0.66 µg L^{-1} in 2016 and 0.05–0.51 µg L^{-1} in 2017 (Table 1). The statistical analysis further showed that the average concentration of AFM1 in summer and autumn of 2016 was significantly higher than the two respective seasons of 2017. However, no significant difference ($P \geq 0.001$) was found when winter and spring of 2016 were compared with those of 2017.

Karish cheese samples

A total of 62 Karish cheese samples were examined for the presence of AFM1 in 2016 (Table 2). The highest incidence was found in winter with 9 (42.9%) of 21 samples being positive. The highest concentration and level was found significantly in this season, recording 1.34 µg kg^{-1} and 0.31–2.07 µg kg^{-1}, respectively. Seasonal variation in AFM1 incidence and level was detected in 2017 (Table 2), whereas the highest incidence was found in autumn with 6.0 (50%) of 12 samples being positive. Though, the incidence was lower in winter (45.4% of sample being positive), however the highest concentration and level was significantly found in this season recording 0.855 µg kg^{-1} and 0.2–2.12 µg kg^{-1}, respectively. Comparing the

Table 1 Incidence and level of AFM1 analyzed in Egyptian raw milk samples during different seasons of 2016 and 2017

Season	2016				2017			
	Samples analyzed (*n*)	Positive (%)	Mean ± SE (μg L^{-1})	Range of AFM1	Samples analyzed (*n*)	Positive (%)	Mean ± SE (μg L^{-1})	Range of AFM1
Winter	16	4.0 (25)	0.28 ± 0.12b	0.1–0.64	16	3.0 (18.8)	0.27 ± 0.02b	0.23–0.29
Spring	18	4.0 (22.2)	0.41 ± 0.101a	0.6–0.66	18	2.0 (11.1)	0.41 ± 0.1a	0.31–0.51
Summer	10	1.0 (10)	0.18c	0.18	10	1.0 (10)	0.09d	0.09
Autumn	16	4.0 (25)	0.41 ± 0.112a	0.05–0.33	16	5.0 (31.2)	0.186 ± 0.05c	0.05–0.33
Total	60	13 (21.6)	0.351 ± 0.058	0.05–0.66	60	11 (18.3)	0.240 ± 0.04	0.05–0.51

Mean ± SE with different letters in the column of AFM1 concentration are considered statistically different among the seasons ($P \leq 0.001$)

Table 2 Incidence and level of AFM1 analyzed in Egyptian Karish cheese samples during different seasons of 2016 and 2017

Season	2016				2017			
	Samples analyzed (*n*)	Positive (%)	Mean ± SE (μg kg^{-1})	Range of AFM1	Samples analyzed (*n*)	Positive (%)	Mean ± SE (μg kg^{-1})	Range of AFM1
Winter	21	9.0 (42.9)	1.34 ± 0.24a	0.31–2.07	22	10 (45.4)	0.855 ± 0.2a	0.2–2.12
Spring	16	5.0 (31.2)	1.19 ± 0.34b	0.15–2.23	10	4.0 (40)	0.305 ± 0.04c	0.19–0.42
Summer	17	7.0 (41.2)	0.915 ± 0.22b	0.05–1.71	12	5.0 (41.6)	0.597 ± 0.11b	0.33–1.02
Autumn	8.0	0.0 (0)	0.0c	0.0	12	6.0 (50)	0.515 ± 0.11b	0.2–0.83
Total	62	21 (33.9)	1.11 ± 0.15	0.05–2.07	56	25 (44.6)	0.632 ± 0.09	0.19–2.12

Mean ± SE with different letters in the column of AFM1 concentration are considered statistically different among the seasons ($P \leq 0.001$)

average concentration of AFM1 in 2016 and in 2017 along the different seasons, data clearly indicated that AFM1 concentrations in winter, summer, and spring of 2016 were significantly higher than those detected in the respective seasons of 2017. It could be observed that the total concentration of AFM1 in samples examined in 2016 was 1.75-fold of that in 2017.

Yoghurt (Zabady) samples

The frequency distribution and concentration of AFM1 at the four seasons of 2016 and 2017 in the yoghurt samples is given in Table 3. Out of 32 samples examined per year, 4.0 (12.5%) in 2016 and 6 (18.75%) in 2017 were positive with AFM1. The positive samples were found in winter and spring of the 2 years. In 2016, the AFM1 incidence in winter and spring was equal (2 samples being positive, 25%) with contamination level of 0.185 μg kg^{-1}. In 2017, the AFM1 contamination level was lower recording 0.144 μg kg^{-1} and 0.105 μg kg^{-1} in winter and spring, respectively. The frequency of positive samples detected in winter was twice of that detected in spring of this year and the average concentration of AFM1 was significantly higher in winter than in spring. Data further showed that

Table 3 Incidence and level of AFM1 analyzed in Egyptian Zabady samples during different seasons of 2016 and 2017

Season	2016				2017			
	Samples analyzed (*n*)	Positive (%)	Mean ± SE (μg kg^{-1})	Range of AFM1	Samples analyzed (*n*)	Positive (%)	Mean ± SE (μg kg^{-1})	Range of AFM1
Winter	8	2.0 (25)	0.185 ± 0.015a	0.17–0.20	8	4.0 (50)	0.144 ± 0.01a	0.13–0.17
Spring	8	2.0 (25)	0.185 ± 0.05a	0.13–0.24	8	2.0 (25)	0.105 ± 0.01b	0.10–0.12
Summer	8	0.0 (0)	0.0b	0.0	8	0.0 (0)	0.0c	0.0
Autumn	8	0.0 (0)	0.0b	0.0	8	0.0 (0)	0.0c	0.0
Total	32	4.0 (12.5)	0.185 ± 0.02	0.13–0.24	32	6 (18.75)	0.130 ± 0.01	0.1–0.17

Mean ± SE with different letters in the column of AFM1 concentration are considered statistically different among the seasons ($P \leq 0.001$)

the total AFM1 concentration in samples of 2016 was 1.4-fold higher than that of 2017. Interestingly, none of the positive samples were detected in summer and autumn of the two studied years.

Discussion

In this study, 60 raw milk samples were collected in 2016 and 2017 and analyzed for contamination with AFM1. Data indicated that AFM1 was detected in 13 samples (21.6%) in 2016 with a contamination level ranging from 0.05 to 0.66 μg L^{-1}. In 2017, AFM1 was detected in 11 samples (18.3%) with a contamination level ranging from 0.05 to 0.51 μg L^{-1}. Contamination of milk with AFM1 is a world-wide health problem. The contamination level of AFM1 obtained in this study is within the range found in many countries. In Turkey, Tekinşen and Eken (2008) analyzed 100 milk samples for contamination with AFM1 and found that 67% of the samples were positive with levels ranging from 0.010 to 0.630 μg L^{-1}. In another study done by Öztürk Yilmaz and Altinci (2019) for analysis of AFM1 in Turkish milk samples, they found that AFM1 was detected in 61.54% (16/26) with a positive mean value of 0.0382 μg L^{-1}. In Pakistan, Hussain and Anwar (2008) found that AFM1 was detected in all of the examined raw milk samples (168 samples) that obtained from 14 districts of the Punjab province. They further showed that 162 samples (96.4%) were with a contamination level less than 0.5 μg L^{-1}, one sample (0.6%) was contaminated with 0.5 μg L^{-1} and the remaining five samples (3%) were with contamination level more than 0.5 μg L^{-1}. In Libya, Elgerbi et al. (2004) detected a higher incidence of AFM1 in raw milk samples and found that 71% of 49 samples collected during July–August, 2002 were positive with levels ranging from 0.03 to 3.13 μg L^{-1}. In Sudan, Elzupir et al. (2012) found a high concentration and incidence of AFM1 in milk samples recording 2.07 μg L^{-1} and 42 (95.5%) out of 44 samples were positive.

This study showed that the highest incidence of AFM1 in milk samples in 2016 was recorded in winter and autumn in equal percent (25%) and in 2017 the highest incidence was recorded in autumn (31.2%). Furthermore, the highest contamination level (0.41 μg L^{-1}) was recorded in the spring of the two studied years when compared with the other seasons. However, it is worthy to mention that AFM1 concentrations in the winter were significantly higher than in the summer along the two analyzed years. Several previous studies reported higher concentration of AFM1 in cold seasons than in hot seasons (Hussain and Anwar 2008; Ruangwises and Ruangwises 2009; Fallah 2010a). This could be related to the amount of mixed feed ingested in each season. However, Venâncio et al. (2019) showed that there was no difference in the concentrations of AFM1 occurred in milk from farms between the summer and winter months in subtropical and temperate climates. The highest AFM1 concentration detected in our milk samples in spring indicates that the compound rations or silage fed by lactating animals is higher in this season compared to the other seasons. Consequently, the environmental conditions may be more favorable in this season for contamination with toxigenic *Aspergillus* fungi and formation of aflatoxins. In Thailand, Mahosotanand (2002) screened the AFB1 contamination of the mixed feed collected from 24 different dairy farms and found that the highest AFB1 concentration was detected in winter (126 μg L^{-1}), compared with rainy season (41 μg L^{-1}) and summer (30 μg L^{-1}).

Our findings showed that 13 (100%) milk samples in 2016 and 10 (90.9%) in 2017 were contaminated with AFM1 above the maximum permitted level (> 0.050 μg L^{-1}) of the European Commission (EC 2001). According to the US regulatory limit established by the Food and Drug Administration (FDA 1996), 3 (23%) samples in 2016 and 1 (9.1%) sample in 2017 exceeded 0.5 μg L^{-1}. When comparing our data regarding concentrations and level of AFM1 in milk samples with some European countries, they are higher than those found in Portugal (Martins et al. 2005), Spain (Rodriguez Velasco et al. 2003), and in Italy (Capei and Neri 2002). This may be attributed to the strict regulations on this mycotoxin in milk and good agricultural handling and good storage practices applied in European countries that minimize the contamination of milk and food products with toxigenic fungi and mycotoxins (Fallah 2010a; Iqbal et al. 2015). In a similar study, Kuboka et al. (2019) have determined the occurrence of AFM1 in 96 samples of raw milk traded in peri-urban Nairobi, Kenya, and they found that all the samples examined had AFM1 with a mean of 0.290 μg kg^{-1} and the minimum level detected was 0.0154 μg kg^{-1} and the maximum was 4.563 μg kg^{-1}. They further showed that 66.4% of samples analyzed were above the EC detection limit (0.05 μg kg^{-1}) and 7.5% of the samples exceeded the legal limit of FDA (0.5 μg kg^{-1}).

Karish, Kariesh, or *Kareish* cheese is a popular soft dairy product in Egypt and Arab countries made from defatted milk, as described previously (Abou-Donia 2008). In this study, Karish cheese samples were examined for the occurrence of AFM1. Along the two studied years, 62 samples were analyzed in 2016 and 56 samples were analyzed in 2017. The results showed that the frequency of AFM1 contamination was 33.9% in 2016 and 44.6% in 2017, with a mean contamination level of 1.11 and 0.632 μg kg^{-1}, respectively. Along the analyzed seasons of each year, it was found that the highest AFM1

concentration was significantly found in winter recording 1.34 μg kg^{-1} in 2016 and 0.855 μg kg^{-1} in 2017. In agreement of these results, Fallah (2010a) stated that AFM1 detected in white cheese samples had higher level in winter than in summer. Tekinşen and Eken (2008) have analyzed 132 samples of kashar cheese and found that 82.6% of samples were contaminated with AFM1 in a range from 0.05 to 0.691 μg kg^{-1} with a mean of 0.194 μg kg^{-1}. Bahrami et al. (2016) reported that AFM1 was detected in 65.5% of cheese samples with a mean level of 0.158 μg kg^{-1}. Several studies around the world reported on the high contamination level of various cheese samples with AFM1 (Dashti et al. 2009; Mohajeri et al. 2013; Tekinşen and Eken 2008). Contamination of white cheese with AFM1 in large cities of Iran was found greater than 60% of samples (Fallah 2010a; Mohajeri et al. 2013).

Comparing the concentrations of AFM1 detected in raw milk and Karish cheese samples, it was found that the toxin is higher in cheese than in milk. This result was highly supported with Mohajeri et al. (2013) who stated on the preferential affinity of AFM1 for casein fraction of milk. The association of AFM1 with casein is more frequently manifested in cheese than in milk from which the cheese is manufactured; therefore, cheese curd contained a higher concentration of AFM1. Iqbal and Asi (2013) reported that the increase in AFM1 concentration is associated with the cheese type, processing techniques applied, and the amount of water eliminated during processing. The current study indicated that 90.47% of positive samples of Karish cheese analyzed in 2016 and 80% of positive samples analyzed in 2017 had levels of AFM1 exceeded the maximum permitted limit (> 0.250 μg kg^{-1}) (EU legislation). Based on the US regulatory limit, 16 (76%) samples in 2016 and 14 (56%) samples in 2017 exceeded 0.5 μg kg^{-1}. Furthermore, the high contamination of cheese with AFM1 may be attributed to the ability of *Aspergillus* spp. to grow on cheese under appropriate conditions of storage and subsequently produce aflatoxins B1, B2, G1, and G2 (Dashti et al. 2009).

Zabady or Zabade is popular traditional yoghurt in Egypt that is made from cow's or buffalo's milk or mixture of them in ranches or small dairy shops. It is traditionally prepared by boiling raw milk for few minutes then cooling to 45 °C followed by fermentation with activated yoghurt starter (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) in porcelain pots or plastic cups and incubation at 42 °C until complete coagulation and cooling overnight in the refrigerator (El-baradei et al. 2008). Based on our results, AFM1 was detected in Zabady samples in spring and winter of 2016 in equal incidence (25%) with average concentration of 0.185 μg kg^{-1} and with a contamination level ranging from 0.13 to 0.24 μg kg^{-1}. In 2017, AFM1 was found in Zabady samples collected in spring and winter with a contamination level ranging from 0.1 to 0.17 μg kg^{-1}, meanwhile the incidence and concentration of AFM1 was significantly greater in winter (50%, 0.144 μg kg^{-1}) than spring (25%, 0.105 μg kg^{-1}). These results coincide with those reported with Fallah et al. (2011) and Fallah (2010a) who showed that AFM1 concentration in yoghurt samples collected in winter had higher significant level than those collected in other seasons. In the previous studies, the incidence and contamination level of AFM1 in yoghurt samples were varied. Martins and Martins (2004) found that 18 (18.8%) out of 96 Portuguese samples were contaminated with AFM1 in concentrations between 0.019 and 0.098 μg kg^{-1}. Iqbal and Asi (2013) found that 61% of 96 Pakistani samples were contaminated with AFM1 in concentrations between 0.004 and 0.615 μg kg^{-1}. Bahrami et al. (2016) found AFM1 in 23.8% (10 out of 42) of Iranian samples with a range from 6.3 to 21.3 ng kg^{-1}. Our data further showed that all Zabady samples contaminated with AFM1 either in 2016 (4 samples) or in 2017 (6 samples) were above the European limit of 0.05 μg kg^{-1} and the average concentration of AFM1 found in these samples were lower than those found in milk and cheese samples. This could be attributed to the presence of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* in Zabady which have a role in binding of AFM1 (Iqbal and Asi 2013). Additionally, Mohajeri et al. (2013) stated that AFM1 is bound to bacterial cell wall components and removed. On the other hand, Fallah et al. (2011) reported that lactic acid bacteria of yoghurt convert lactose of milk into lactic acid, consequently the pH is diminished to 4.0–4.5 (acidic) which denaturate or coagulate casein protein affecting the adsorption of AFM1 in yoghurt coagulum.

In this study, AFM1 was determined in our dairy samples by TLC technique and for confirmation we have selected a Karish cheese sample (KCA 50, the most contaminated sample with AFM1 among dairy samples) and analyzed it by HPLC. Data obtained showed that HPLC analysis confirmed TLC results and the concentrations of AFM1 determined by the two techniques were found comparable. The same TLC trend was previously used by several authors (Fallah 2010a; Fallah et al. 2011; Stubblefield 1979). Other authors determined AFM1 by HPLC (Elzupir et al. 2012; Iqbal and Asi 2013; Oluwafemi et al. 2014) and ELISA (Langat et al. 2016; Motawee et al. 2009; Tekinşen and Eken 2008). In accordance with our results, Shundo and Sabino (2006) analyzed Brazilian milk samples for AFM1 contamination by the TLC method and they demonstrated a satisfactory correlation when compared with HPLC. These authors further showed that no significant difference was found between the two methods compared. Bahrami et al. (2016) analyzed Iranian dairy

samples for AFM1 by using ELISA and HPLC and found high frequency of false positive results in ELISA, hence they reported that ELISA cannot be considered as a very effective screening test when a large number of samples are tested.

Aflatoxins reduce growth, development and performance in lactating animals (Panahi et al. 2011). The occurrence of AFM1 in milk is a carryover from AFB1 contamination of lactating animals' feedstuffs (Iqbal et al. 2015). Van der Fels-Klerx et al. (2019) used various models and datasets to investigate the impacts of climate change on AFB1 production in East European maize and its consequences on AFM1 contamination in cows' milk. These authors suggested an increase (up to 50%) of maximum mean of AFM1 in milk by 2030. Using climate models, they further suggested a similar or slight increase (up to 0.6%) of the chance of AFM1 contamination in milk above the EC limit (0.05 μg L^{-1}) by 2030. Hence, control of aflatoxin contamination (especially AFB1) in animal feed is very important and dairy farmers should be informed about the consequences of aflatoxins.

From this study, it is worthy to state that the variations in AFM1 level in milk and dairy products could be explained on the basis of geographical conditions, climate and seasonal changes, different analytical methods employed in the toxin detection, variety and making procedure of the dairy product (Karish cheese and Zabaday), and practices of feed storage and farm management (Iqbal et al. 2015; Iqbal and Asi 2013). Moreover, the wide variation in contamination of milk with AFM1 was found to be related to different factors including animal species, season, milking time, level of AFB1 intake, and volume of milk produced by the mammal (Assaf et al. 2019). In Egypt, the Ministry of Health recommended that the dairy products should be free from AFM1 (0 μg kg^{-1}) (Iqbal et al. 2015). However, strict legislations need to be applied by special governmental agencies in order to inspect the presence of AFM1 in dairy products. Therefore, the quality assurance of raw milk products in Egypt by monitoring the contamination of AFM1 is an urgent issue.

Conclusion

Overall, this study demonstrated the incidence and contamination level of AFM1 in traditional dairy products consumed in Egypt along two consecutive years (2016 and 2017). A seasonal variation was detected in AFM1 incidence and level in the two studied years and the toxin level in the examined samples was higher than the maximum permitted limit in Europe and other countries. Hence, this appears to be a serious public health hazard and it is very important to find safe and efficient strategies for controlling fungal growth and AFB1 levels in feedstuffs of dairy animals. Based on these results, good agricultural practices (handling and storage) and regular monitoring of aflatoxin, in addition to strict regulatory limits and legislations are necessary to be applied in order to minimize and control the contamination of this toxin in Egyptian dairy farms.

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