DICTARY EXPOSURE AND RISK ASSESSMENT OF MYCOTOXINS IN THYME AND THYME-BASED PRODUCTS MARKETED IN LEBANON

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Table of Contents

List of Tables

List of Figures

Abstract

Thyme is a Mediterranean herb widely used worldwide. However, it is produced in areas of high humidity and temperature which is favorable conditions for mold growth and mycotoxins production which pose public health risk. In Lebanon, no studies have been done to investigate aflatoxin B_1 (AFB_1) and ochratoxin A (OTA) contamination in thyme and thyme-based products. This study aimed at evaluating the incidence of AFB_1 and OTA in those products, related dietary exposure and cancer risk for regular and high consumption. A total of 160 samples of thyme and thyme-based products were collected and 32 composite samples were analyzed. Results showed that AFB_1 and OTA were, respectively, found in 84% (27/32) and 38% (12/32) of the samples. AFB_1 exceeded the limits in 41% (13/32) and 25% (8/32) of the samples according to the Lebanese and European standards, respectively. OTA was unacceptable in only 6% (2/32) and 3% (1/32) of the samples according to the Lebanese and European standards, respectively. AFB_1 was shown to be associated with 0.41 and 0.35 additional cancer cases per 100,000 persons per year for regular consumption of thyme and thyme-based products, respectively. While for high consumption an increase of 0.911 and 0.639 cancer cases per 100,000 person per year was noted for thyme and thyme-based products, respectively. Based on the calculated MOE, the dietary exposure to AFB_1 through the consumption of thyme and thyme-based products in Lebanon poses a potential risk to consumer health. OTA daily exposure from thyme-based products was shown to be 1.345 ng/kg bw/day. MOE for OTA was >10,000 for non-neoplastic effects and >200 for neoplastic effects, representing no toxicological concerns for consumers in both cases. This study's findings showed that the Lebanese

population are exposed to AFB_1 and OTA through thyme and thyme products and call for routine monitoring to ensure the safety of thyme and thyme-based products in the Lebanese market.

Chapter 1

1. Uses of thyme herb

The Mediterranean diet is rich in wild edible plants and herbs. They are nutrient dense, grow independently of human action, and are adapted to various habitats and environments. Thyme is a perennial shrub with greenish-grey aromatic leaves. It is native to the Western Mediterranean and considered as one of the most important spice crops in the world trade (Kabak & Dobson, 2017). In 2008, the world thyme production was 14000 ton and most of is produced by Mediterranean countries and Mexico (Aslan & Gül, 2017). In Lebanon what is collectively known as Zaatar refers to several species from the genus *Origanum, Thymus, Satureja* and *Thymbra*. Thyme is used in its fresh or dried forms for seasoning in baked goods, stews, meats, vegetables and in marinades (Bower et al., 2016; Singletary, 2016). In Lebanon thyme is widely consumed in various ways in recipes such as thyme salads, thyme pizza (Mankooshe) as breakfast or snack and Fatayer Zaatar (Batal & Hunter, 2007; Benelli et al., 2019). It was shown that the average consumption of wild thyme is around five times weekly in Lebanon (Jeambey et al., 2009). In addition to its culinary uses thyme has many health benefits (Sa et al., 2017). Thymol and carvacrol are two principal constituents of thyme essential oil and are shown to have antioxidant, anti-inflammatory, respiratory and neurological benefits (Singletary, 2016). Thyme has economic potential also. In 2014, Lebanese exported 548 tons of dried oregano and zaatar mixes for a value of approximately US$ 1.9 million (Hamade, 2016).

However, thyme is produced in areas of high humidity and high temperature which is favorable conditions for mold growth and mycotoxins production. Herbs can be contaminated with mycotoxins at any time of the process under favorable conditions, from field, to harvesting, washing, sun drying, processing, packaging and storage (Peter, 2012). Moreover, thyme is considered as a good niche for fungus to invade because of its high moisture content (Mandeel, 2005).

2. Economic impact of mycotoxins

Because of mycotoxins' stability in food processing, it is difficult to completely eliminate them from food products (Kabak, 2009). This is leading, in addition to the adverse health effects on humans and animals, to enormous economic losses and to trade problems between countries. Mycotoxins affects economy in many ways, by health care and veterinary care costs, loss of livestock production, regulatory costs and costs on research on mycotoxins (Zain, 2011). It was estimated that 25-50% of the world's food crops may be contaminated by mycotoxins. This is a major concern in both developed and developing countries. A study showed that in the United states about $932 million are lost annually due to crops (corn, wheat and peanuts) contaminated by mycotoxins, in addition to $466 million are lost annually from regulatory enforcement, testing, and quality control measures (Buzby et al., 2003) In 2017, mycotoxins were the main hazard in border rejection notification in the European Union according to the annual report of the Rapid Alert System for Food and Feed (RASFF) (Table. 1). The opportunity for export is a major problem in developing countries, 70% of the food exports in the Middle East and Africa are to the developed countries with high income which have more stringent mycotoxins limitation (Buzby et al., 2003).

Table 1 2017 Notifications by hazard category in the EU (RASFF, 2017).

Hazard Category	Alert	Border rejection	Information for attention	Information for follow-up
Allergens	116	5	20	3
Bio contaminants	34	3	21	2
Food additives and flavorings	22	73	29	55
Genetically modified food or feed	1	10	4	1
Heavy metals	118	57	88	21
Industrial contaminants	38	13	8	11
Mycotoxins	**70**	**464**	**45**	**2**
Parasitic infestation	1	3	11	26
Pathogenic micro-organisms	300	452	198	123
Pesticide residues	30	133	131	44
Radiation		6	4	1

3. Occurrence and dietary exposure of mycotoxins from food

Several studies have reported the occurrence of aflatoxins and ochratoxins in spices (Abdulkadar et al., 2004, Kong et al., 2014 & Ok et al., 2015). A study was done by (Ghali et al., 2008), to determine the incidence of mycotoxins in Tunisian food showed that spices are the most contaminated foods with AFs with incidence of 85.7% of the samples tested with contamination mean of 28.5 ± 26.4 ng/g. ZON was found in highest levels in spices with an average concentration of 21.0 ng/g (Ghali et al., 2008). Similarly, in Morocco it was shown that AFB1 is present in spices with average contamination of 0.09, 0.63, 2.88 and 0.03 µg/kg for black pepper, ginger, red paprika and cumin, respectively. The highest level of contamination was 9.68 µg/kg in red paprika which is about double the European Union (EU) maximum levels (ML) for AFB1 in spices 5 µg/kg (Zinedine et al., 2006). In Sri Lanka AFB1 was found in 77% of the dry chili tested with a mean of 15.6 ± 11.6 µg/kg and 67% exceeding the EU maximum level (ML) of 5 µg/kg for AFB1. OTA was found in 41% of the samples. In Belgium, 9 of the 11 positive chilli samples exceeded the EU ML for AFB1 of which 7 samples were products from the Lebanese market. Two of the Lebanese chillis had AFB1 concentration higher than the ML permitted for total AFs (10 µg/kg) (Yogendrarajah P. et al., 2014). In Iran, among 120 tested samples of spices and herbs, 37 (30.8%) were contaminated with AFs (range: 0.2-57.5 µg/kg) and all these samples were contaminated by AFB1 (0.7-57.5 µg/kg). In 16 (13.3%) and 12 (10%) samples, AFB1 and total AFs levels surpassed the EU limits of 5 and 10 µg/kg, respectively. Only 1 thyme sample out of 10 samples tested was contaminated with AFB1 (2.2 µg/kg) (Khazaeli et al., 2017). In another study, mycotoxins traces were found in 5 oregano samples (10%) and in 15 thyme samples (30%). ZON and DON were found in 9 (18%) and seven (14%) of the analyzed thyme samples, respectively, and the levels of both mycotoxins were distributed over the ranges of 40-209 µg/kg and 10-142 µg/kg, respectively (Reinholds et al., 2017).

Mycotoxins are present in diverse food commodities. Tea was shown to be contaminated with aflatoxins and ochratoxin A. In Iran all the tea samples tested were contaminated with detectable amount of AFs ranging from 1.5-16.5 ng/g. 9.5 and 14.3 % of domestic and imported tea samples, respectively, contained AFs higher than the EU standards (10 ng/g) (Mohamadi Sani et al., 2012). OTA is also detected in tea as shown by Carraturo et al., that 22 of 32 tea samples analyzed were contaminated with 50% of the positive samples having concentrations higher than the limits (Carraturo et al., 2018).

Cheese can also be contaminated by mycotoxins. Sürk a traditional Turkish dairy product was shown to be contaminated with AFB1 with a mean value 0.61 ± 0.20 µg/kg, only two exceeded the maximum limit of 2 µg/kg set up by the European Commission in cereals and cereal products. Health risk assessment for AFB1 in Sürk cheese resulted in MOE of 2982 which indicates health risk for consumers since any MOE lower than 10000 is considered unsafe. OTA was found in 73.3 % of the tested cheese samples with mean of 0.615 ± 0.228 µg/kg. However, these results could be due to herbs and spices found in the Sürk cheese which could be contaminated with mycotoxins (Sakin et al., 2018). Other studies have reported the presence of OTA in blue cheese with a range of (0.25-3.0 µg/kg) (Dall'asta et al., 2007; 2008).

Iqbal et al., 2014 & Alim et al., 2018 have documented similar results in evaluating mycotoxins in cereal derived products. 53% and 41% of the samples were positive for AFB1 and AFs, with some samples showing values higher than the maximum level of AFB1 (2 µg/kg) and AFs (4 µg/kg) established by the European Commission. 48% and 50% of the samples were positive for OTA, with 30% were found to be higher than the maximum level of OTA (3 µg/kg). 53% and 57% were positive for ZON with up to 30% were found to be higher than the maximum level (50 µg/kg). The estimated average exposure dose for AFB1 was 3.50 ng/kg bw/day with a calculate excess risk of liver cancer incidence of 1.66 per 100,000 adults per year. The average exposure for OTA and ZON were 3.85 and 29 ng/kg bw/ day, respectively as compared to the estimated exposure for OTA (14 ng/kg bw/day) and ZON (500 ng/kg bw/ day) established by JACFA.

In Lebanon, 80% of the analyzed spices and herbs samples were contaminated by 1-11 mycotoxins. Total AFs were detected in spices with a mean of 168.1 µg/kg (19%) which is about 17 times higher than the limit permitted for AFs in spices by the European commission regulation (10 µg/kg). The mean level of AFB1 in the positive spice samples is 193.4 µg/kg which is about forty times higher than the European limit permitted for AFB1 in spices (5 µg/kg). OTA was detected in 30% of the spices with a mean of 7.1 µg/kg which was below the limits (15 µg/kg). Total AFs and OTA were detected in herbs with a mean of 36.1 µg/kg (8% of the samples) and 7.0 µg/kg (11% of the samples), respectively. DON and ZON were also detected in spices and herbs (El Darra et al., 2019).

In a total dietary study done in Lebanon by Raad et al., 2014, the average level of exposure to AFB1 was (0.63-0.66 ng/kg bw/day) and (1.40-1.46 ng/kg bw/day) for excessive consumers. Knowing that AFB1 is classified as a human carcinogen, the ALARA (As Low As Reasonable Achievable) approach is recommended. Thus, cancer risk was estimated at 0.0527-0.0545 cases/100,000 persons/year. The mean and excessive consumers' exposure levels to OTA represented respectively 29.9% and 95.1% of the PTWI provisional tolerable weekly intake set by the JECFA (100 ng/kg/week). For DON both the mean and excessive consumers' exposure levels to DON exceeded the PMTDI provisional maximum tolerable daily intake (1 µg/kg bw/day) set by JECFA by156.8% and 355.8%, respectively. These findings showed that Lebanese population is exposed to mycotoxins through diet (Raad et al., 2014). Moreover, OTA was detected in the human plasma of Lebanese population in 33% of tested samples with a concentration ranging from 0.1 to 0.87 ng/ml and a mean of 0.17 ± 0.01 ng/ml. Food analysis of the most consumed food in Lebanon showed that wheat, burghul and beer were contaminated with mean value of 0.15 ± 0.03 µg/kg, 0.21 ± 0.04 µg/kg and 0.19 ± 0.12 ng/ml. This study suggests that the Lebanese population is exposed to OTA through food ingestion (Assaf et al., 2004).

Another total dietary study done in Viet Nam showed that the dietary exposure to AFB1 (39.4 ng/kg bw/day) mainly due to cereals and meats, is imposing a risk of liver cancer of 2.7 cases/100,000 person/year. The dietary exposure to OTA (18.7 ng/kg bw/day) is higher than the provisional tolerable daily intake (PTDI) of 14 ng/kg bw/day set by JECFA indicating risk levels of public health concern (Huong et al., 2016).

The findings of these studies showed that consumers are exposed to mycotoxins from the diet. Dietary exposure estimations and risk assessments are alarming since some mycotoxin's values are near or exceeding the health guidance values for set for mycotoxins. These results validate the need for more research about dietary exposure and risk assessments for mycotoxins from varying food commodities to fill the gap in the data.

4. Regulations and standards related to mycotoxins in spices and herbs

Mycotoxins are shown to be carcinogenic, teratogenic and immunosuppressive. To ensure food safety maximum limits for mycotoxins are set for various food commodities (Table. 2). Tolerable weekly intake (TWI)/ Tolerable daily intake (TDI) are the levels that can be taken weekly/daily over lifetime without a health risk. The European Food Safety Authority (EFSA) and the Joint FAO/WHO Expert Committee in Food Additives (JECFA) have set a TWI/ TDI for mycotoxins (Table 3). No limits for dietary intake have been set for aflatoxins because they are classified as carcinogenic contaminants, the ALARA approach (As Low As Reasonable Achievable) is recommended (JECFA, 2001).

Table 2 Mycotoxins limits in food products.

Mycotoxin	In Zaatar according to LIBNOR (µg/kg)	In spices according to European commission regulation (µg/kg) (EU, 2015 & EC, 2006).	In Cereals & cereal products according to European commission regulation (µg/kg) (EU, 2015 & EC, 2006).

Total AFs	4	10	4
AFB1	2	5	2
OTA	3	15	3

Table 3 Health-based guidance values for OTA.

Mycotoxin	Health-based guidance value	Reference
OTA	PTWI: 120 ng/kg bw/wk= 17 ng/kg bw/d	(EFSA, 2010)
	PTWI: 100 ng/kg bw/ wk =14 ng/kg bw/d	(JECFA, 2010; JECFA 2007)

5. Objective

In order to evaluate the risk of exposure by consumers, it is important to assess not only the contamination of thyme but also its related products as it is a common ingredient in several recipes. Few studies have been done to evaluate AFB1 and OTA in food products in Lebanon (Raad et al. 2014, Daou et al. 2021, Al Ayoubi et al. 2021, El Darra et al. 2019), however, no studies targeted thyme herbs and its related products specifically. The objective of the present study was to determine the occurrence of AFB1 and OTA, evaluate the dietary exposure and assess the risk posed from AFB1 and OTA in thyme and thyme-based products marketed in Lebanon.

Chapter 2

1. Introduction

Mycotoxin word comes from the Greek terms “mykes” and “toxicum” meaning fungus and toxin, respectively (Thanushree et al., 2019 ; Costa et al., 2019). Mycotoxins are low-molecular weight secondary metabolites of filamentous fungi mainly produced by *Alternaria*, *Aspergillus*, *Fusarium*, and *Penicillium* genera. They contaminate various agricultural commodities like cereals and cereal products, nuts, dried fruits, spices, herbs, coffee, wine and beer (Teheur et al. 2019; Franco et al., 2019; Anfossi et al., 2016). Mycotoxins can contaminate food and agricultural products at any stage of the food chain from pre-harvest, harvest, drying and storage stages and their production increases in areas of high temperature and humidity (Marin et al., 2013; Ismail et al, 2018). Moreover, they are chemically and thermally stable making them more persistent in the food chain and during food preparation and transformation (Raad et al., 2014; Al-Jaal et al., 2019).

Aflatoxin B1 (AFB1) is the most common in foods and considered the most potent metabolite because of its genotoxic and carcinogenic characteristics (IARC, 2002). Chronic exposure to AFB1 increases the risk of liver cancer especially when associated with hepatitis B. The International Agency for research on Cancer classified AFB1 as Group 1 known human carcinogen (IARC, 2002). Ochratoxin A (OTA), another major mycotoxin present in many foods, also known for its nephrotoxicity, hepatotoxicity, teratogenicity and immunotoxicity (EFSA, 2020) and was classified as a possible carcinogen to humans (group 2B) (IARC, 2002).

Thyme is an edible wild plant native to the Mediterranean region and considered as one of the most important spice crops in the world trade (Kabak et al. 2017). In the Eastern Mediterranean, what is collectively known thyme or "Zaatar" in Arabic refers to several species from the *Origanum*, *Thymus*, *Satureja* and *Thymbra* genera (Bower et al., 2016 & Singletary, 2016). Thyme is widely used in its fresh or dried forms for seasoning in baked goods, stews, meats, vegetables, salads and in marinades (Bower et al., 2016 & Singletary, 2016). Moreover, thyme pizza in different sizes and shapes (Mankooshe and Fatayer Zaatar) are very famous breakfast or snacks (Batal & Hunter, 2007). In addition to its culinary uses, thyme has many health benefits. Thymol and carvacrol are two principal constituents of the thyme essential oil and are shown to have antioxidant, anti-inflammatory, respiratory and neurological benefits (Singletary, 2016). Thyme is beneficial not only for culinary and medicinal uses but also for the economy since for example, thyme mix and oregano exports from Lebanon reached 548 tons and a value of 1.9 U.S $ million in 2014 (Hamade, 2016).

However, thyme can be contaminated with AFB1 and OTA throughout the food chain, especially that the climate in the Mediterranean area is known for its high temperature and high humidity. Especially in summer, which promote their production. Additionally, when stored improperly, thyme can be considered a good niche for fungal growth and mycotoxins contamination because of its high moisture content (Mandeel, 2005). Previous studies reported mycotoxins occurrence in thyme (Pallarés et al. 2019; Reinholds et al. 2017; Gamnacorta et al. 2019). Therefore, "The Lebanese Standards Institution- LIBNOR" has set maximum limits (ML) for thyme and thyme mixes of 2 μg/kg for AFB1 and 3 μg/kg for OTA (NL:677, 2017). The EU has no specific control measure for thyme. Instead, ML are set for spices of 5 and 15 µg/kg for AFB1 and OTA, respectively (EFSA, 2006). The determination of those levels of contaminants is primordial to assess dietary exposure and the related adverse health effects on the population. Dietary exposure takes into consideration the concentration of the chemical in the examined food like AFB1 and OTA and the daily intake of the food item. Since, ingestion of 1 ng/kg bw/day of AFB1 would induce 0.083 liver cancer cases per 100,000 persons per year according to the Joint FAO/WHO Expert Committee on Food Additives (JECFA 1999) the As Low As Reasonably achievable (ALARA) approach is recommended. For OTA, the European Food Safety Authority (EFSA) considered the previously used Provisional Tolerable Weekly Intake values (PTWI) no longer valid and recommended the use of margin of exposure (MOE) approach for the risk assessment of neoplastic and non-neoplastic effects (EFSA, 2020). As for JECA, the PTWI of 100 ng/kg bw/week is still valid (JECFA, 2007).

In order to evaluate the risk of exposure by consumers, it is important to assess not only the contamination of thyme but also its related products as it is a common ingredient in several recipes. Few studies have been done to evaluate AFB1 and OTA in food products in Lebanon (Raad et al. 2014, Daou et al. 2021, Al Ayoubi et al. 2021, El Darra et al. 2019); however, no studies targeted thyme herbs and its related products specifically. The objective of the present study was to determine the occurrence of AFB1 and OTA, evaluate the dietary exposure and assess the risk posed from AFB1 and OTA in thyme and thyme-based products marketed in Lebanon.

2. Materials and methods

2.1 Food selection and sampling

Thyme herbs can be used in several homemade recipes and sprinkled over a variety of foods and dishes. For this purpose, a screening of the Lebanese market was carried out and a list of commercialized thyme and related thyme-based products were selected to be both assessed in this study (Table 4). This list was used in a food frequency questionnaire to estimate the consumption of thyme and thyme-based products by the Lebanese population and the obtained data was used to estimate the dietary exposure to AFB_1 and OTA and related risk assessment.

Table 4 Daily intake (g day^{-1}) of thyme-based products and dried thyme herbs for regular and high consumption obtained from a FFQ study in Lebanon.

Food items		Daily intake (g/day)		Thyme content % (w/w)
		Regular consumption	High consumption	
Thyme based products	Thyme pie	72.5	141.9	14
	Cheese and thyme pie	55.2	167.3	6
	Fresh thyme salad	68.2	115.8	4
	Thyme regular mix sandwich	16.8	34	31
	Thyme mix with nuts and seeds sandwich	11.2	29.2	35
	Pizza and pasta with thyme sauces	27.3	28.8	1
	Bread sticks with thyme (crunchy)	5.0	13.4	12
	Sesame thick bread with thyme (soft)	13.9	40.8	6
	Crackers with thyme	3.7	7.7	5
	Toast/Bread with thyme	6.6	9.8	3
	Thyme croissant	9.6	23	9

	Cheese with thyme	20.9	38.8	1
Dried thyme herbs [a]	Thyme regular mix [a]	20.5	45.1	
	Thyme mix with nuts and seeds [b]	3.9	7.1	
	Thyme herbs [c]	3.9	10.1	
	Tea thyme	0.9	2	

The daily intake of:

[a] Thyme regular mix was estimated from thyme contribution to thyme pie, cheese and thyme pie, thyme regular mix sandwich, sesame thick bread with thyme (soft) and thyme croissant.

[b] Thyme mix with nuts and seeds was estimated from thyme contribution to thyme mix with nuts and seeds sandwich.

[c] Thyme herbs was estimated from fresh thyme salad, pizza and pasta with thyme sauces, bread sticks with thyme (crunchy), crackers with thyme, toast/bread with thyme and traditional molded aged cheese with thyme & traditional strained yogurt balls with thyme

A total of 160 samples of thyme and thyme-based products were collected at two complete sets of different production dates at six-month interval (September, 2019-March, 2020). Composite sampling approach was applied for each food sample. Five different brands of the same item were purchased and combined together with a 20% weight contribution for each brand to give a homogenized representative sample (Raad et al., 2014). A total of 40 dried thyme herbs samples were collected (4 dried thyme herb types x 5 different brands x 2 collections) and combined into 8 composite samples for analysis. A total of 120 thyme-based product samples were collected (12 thyme-based products types x 5 different brands x 2 collections) and combined into 24 composite samples for analysis. Thus, a total of 32 composite samples were prepared. Sampling was performed from representative and common retail markets in Lebanon. Cooked dishes (pasta, pizza, sandwiches) were prepared using traditional references while the remaining food items were tested as ready to eat without any cooking or preparation. Samples were collected and stored at -18 °C prior to analysis. The equipment used for preparing and homogenizing the composite samples were thoroughly washed between each preparation to avoid the risk of cross-contamination. Although this composite method may dilute the contamination concentration in each individual sample, it has the advantage of screening the overall market of thyme and thyme-based products by collecting a large number of samples (Raad et al. 2014).

2.2 Chemicals

Standard of AFB_1 (2 μg/ml in acetonitrile) was purchased from Trilogy (Washington, USA), standard of OTA (50 μg/ml in Benzene: Acetic Acid 99:1) was purchased from Supelco (Pennsylvania, USA), acetonitrile and methanol (HPLC grade) were purchased from Sigma-Aldrich (Steinheim, Germany), and Aflaochra prep immunoaffinity columns specific to aflatoxins and OTA were purchased from RBiopharm Rhone Ltd. (Glasgow, Scotland, UK). Distilled water was used for the analysis and all other chemicals and reagents were of analytical grade.

2.3 Sample preparation

First, 25 g of the grounded sample were blended with 5g of sodium chloride and 100 ml of 80% methanol at a high speed for 2 minutes. Then, the samples were centrifuged at 4,000 rpm for 10 minutes. Next, 2ml of the filtrate were diluted with 18 ml of phosphate buffered saline (PBS) and filtered through glass microfiber filter paper. After that, 10 ml of the filtrate were passed through the immunoaffinity columns at a slow steady flow rate to purify the toxins. The columns were then washed with 20 ml PBS to get rid of any sample residues. Finally, the toxins were eluted by passing 1 ml of methanol followed by 1 ml of distilled water to attain a final volume of 2 ml. The final eluted volumes were collected and stored in sealed vials at proper temperatures until the time of HPLC analysis.

2.4 HPLC conditions

Reverse phase High Performance Liquid Chromatography (HPLC) (Waters 2690®, Waters Corp., MA, USA) coupled with a fluorescence detector (Waters 2475®) and a Supelco Discovery® HS C18 column (250 mm × 4.6 mm I.D., 5 µm particle diameter) fitted with a C18 guard column (Supelco Supelguard®, Sigma-Aldrich Co., MO, USA) at 25 °C temperature was used for analysis. The method in Aflaochra Prep immunoaffinity column from supplier R-Biopharm Rhone Ltd. was followed for HPLC analysis after optimizing it. Two mobile phases, which were prepared on the same day of HPLC analysis, were used for analysis after filtering; solution A was composed of water: methanol (55: 45 v/v), and solution B was composed of water: methanol (20: 80 v/v). For each 1 L, 119 mg potassium bromide and 350 µl of 4 M nitric acid were added for both solutions. The mobile phases were passed at a flow rate of 0.8 ml/min. The sample injection volume was 100 µL and the wavelengths for excitation and emission were 365 nm and 442 nm, respectively, from the start of the sample analysis till 17 minutes. Then, the excitation and emission were changed to 333 nm and 463 nm, respectively. A calibration curve was illustrated by using aflatoxin B1 standard and ochratoxin A standard at concentrations ranging from 0 to 10 ppb. The performance of the method was tested using different parameters including LOD (0.027 and 0.0034 µg/kg for AFB1 and OTA, respectively), LOQ (0.027 and 0.015 µg/kg for AFB_1 and OTA, respectively), and recovery analysis (88% and 94% for AFB_1 and OTA, respectively).

2.5 Exposure to AFB_1 and OTA

In order to assess the exposure in Lebanese population to AFB_1 and OTA, a food frequency questionnaire (FFQ) was developed to estimate the consumption of thyme and thyme products in Lebanon. A random representative sample of 1555 people was selected proportionally from 5 different Lebanese governorates (Beirut, Mount Lebanon, North, South and Beqaa) to participate in this study. The FFQ assessed the regular and high consumption related to factors such as thyme cultivation season, lent meals, school snacks, picnic meals, weight loss diet and sickness remedies. After obtaining the consumption data, the exposure to AFB_1 and OTA from thyme and thyme products was evaluated accordingly.

Daily dietary exposure to AFB_1/OTA from thyme-based product or dried thyme herbs (ng/kg body weight/day) was calculated as: [(Daily intake (kg/day) x AFB_1/OTA concentration (ng/kg)]/ body weight. To estimate the daily dietary exposure, the average body weight of 72.4 kg obtained from the FFQ was used. The average daily exposure to AFB_1 and OTA from each item was summed to get the total daily dietary exposure (ng/kg bw/day) from thyme-based products and thyme dried herbs in Lebanon.

According to recent studies highlighting the uncertainty of the mode of action of OTA for kidney carcinogenicity, comparing the dietary exposure to the health-based guidance values (HBGV) is no longer valid (EFSA, 2020). According to the aforementioned, the provisional tolerable weekly intake (PTWI) of 120 ng/kg bw/week previously set by EFSA is no longer valid. The Margin of exposure (MOE) approach is used for risk assessment for neoplastic effects and non-neoplastic effects of OTA. MOE is calculated as a ratio between benchmark dose level ($BMDL_{10}$) which causes 10% increase in cancer incidence in animals and the daily dietary exposure. A $BMDL_{10}$ of 4.73 µg/kg bw/day was used for non-neoplastic effects calculated from kidney lesions and a MOE below 200 indicates a health risk. For neoplastic effects a $BMDL_{10}$ of 14.50 µg/kg bw/day was used calculated from kidney tumors in rats and MOE below 10,000 would indicate a public health concern (EFSA, 2020). However, according to the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2007) the PTWI of 100 ng/kg bw/day is still considered.

For AFB_1, it is estimated that for non-European countries, the ingestion of 1 ng per kg of body weight per day of AFB_1 would induce 0.083 liver cancer cases per 100,000 persons per year according to the Joint FAO/WHO Expert Committee on Food Additives (JECFA 1999). Thus, liver cancer risk based on total daily exposure to AFB_1 (ng/kg body weight/day) from thyme and thyme-based products can be calculated as: Liver cancer risk= Daily exposure to AFB_1 (ng/kg bw/day) x 0.083 cancer cases per 100,000 persons. MOE approach is also used to assess the risk from AFB_1 using a $BMDL_{10}$ of 0.40 µg/kg bw/day for cancer incidence based on rodent data as set by the CONTAM Panel. A MOE below 10,000 indicates a public health concern (EFSA, 2020).

3. Results & Discussion

3.1 Occurrence of AFB_1

The incidence of AFB_1 and OTA in 32 samples of thyme and thyme-based products are shown in Tables 5 and 6. AFB_1 was found in 27 (87%) out of 32 samples with a mean of 4.6 µg/kg and a concentration ranging between 0.08-25.8 µg/kg. The maximum limit (ML) for thyme and thyme mixes according to “The Lebanese Standards Institution-LIBNOR” is 2 µg/kg, compared to 5 µg/kg for spices as set by the European Commission (NL

677:2017; European Commission, 2006). The stricter limit by LIBNOR for thyme and thyme mixes can be related to the different consumption habits and to the higher amounts of thyme consumed and added to food formulations in the Mediterranean region and in Lebanon especially. While thyme dried herbs can be contributed to a high proportion of the product (1 to 35%) (Table 4), spices are generally added at much lower concentrations (10.4 g/portion) as flavoring agents to food (Siruguri et al. 2015). Thyme is added to many dishes in the Near Eastern cuisine and contributes to many food products like thyme pies, thyme salads, croissant and many other breakfast and snacks (Table 4) (Batal & Hunter, 2007). In addition, thyme is the most frequently eaten plant in Lebanon at frequency of five times per week (Jeambey et al., 2009). However, according to the European regulations, thyme is considered within the spice category and follows its limit (5 µg/kg), knowing that the estimated consumption of spices and herbs in Europe (0.5 g/person/day) is lower than that in the Middle East (2.6 g/ person/ day) (Vázquez-Fresno et al. 2019). In addition, the daily intake of thyme dried herbs in the current study reported higher results reaching 20.5 g/person/day (Table 4). For cereals, the ML set by LIBNOR is 2 µg/kg (NL 240: 2011) same as the European commission, (2006). The AFB_1 concentration exceeded the ML in 41% (13/32) and 25% (8/32) of the samples according to the Lebanese and European standards, respectively. Only 5 out of 24 thyme-based products were contaminated with AFB_1 levels above LIBNOR standards (NL 240:2010; NL 677:2017). All dried thyme herbs samples (8/8) exceeded the ML according to LIBNOR, compared to (6/8) samples exceeded the European regulations. From thyme-based products, thyme regular mix sandwich (2.55 µg/kg) and pizza and pasta (3.35 µg/kg) exceeded the ML according to both standards while cheese with thyme was only unacceptable according to the Lebanese standards. The contamination range of cereal-based products in the current study (0.25-3.35 µg/kg) was higher than that found in the same category in Lebanon (0.005-0.827 µg/kg) (Daou et al., 2021), and in Turkey (0.013-0.178 µg/kg) (Kabak B. 2021). The contamination range was also lower in a total diet study in Lebanon (0.010-0.260 µg/kg) (Raad et al., 2014). In Saudi Arabia, all food items from bakery were free from aflatoxin contamination (Serdar et al. 2020). In contrast to the current results that showed the highest AFB_1 concentration in pizza and pasta samples (3.35 µg/kg), all 27 pasta samples in Italy were free from AFB_1 (Raiola et al. 2012).

Table 5 Incidence of AFB_1 and OTA contamination in 32 thyme-based products and dried thyme herbs (µg/kg). [a]

Mycotoxin type		Contamination status N (%)
AFB_1	No. of contaminated samples (%)	27 (84)
	0.08-2	14 (44)
	2-5	7 (22)
	>5	6 (19)
	Mean ± SD	4.6 ± 6.5
	Range	0.08-25.8
	No. of unacceptable samples (%)	
	Total	13/32 (41)
	Thyme-based products	5/24 (21) [b]
	Dried thyme herbs	8/8 (100) [b] / 6 (75) [c]
OTA	No. of contaminated samples (%)	12 (38)
	0.04-3	10 (31)
	3-15	2 (6)
	>15	0 (0)
	Mean ± SD	1.4 ± 2.3
	Range	0.04-8.0
	No. of unacceptable samples (%)	
	Total	2/32 (6)
	Thyme-based products	1/24 (4) [d]
	Dried thyme herbs	1/8 (13) [d]

[a] Calculation of contamination mean and range was based on positive samples. All values are expressed in µg/kg.
Unacceptable samples according to the following limits:
[b] AFB1 maximum limits for Lebanese bread and thyme and thyme mixes (2 µg/kg) (NL 240:2010; NL 677:2017)
[c] AFB1 maximum limit for spices (5 µg/kg) (European Commission, 2015)
[d] OTA maximum limits for Lebanese bread and thyme and thyme mixes (3 µg/kg) (NL 240:2010; NL 677:2017)

Table 6 Mean concentration (µg/kg) and ±SD of mycotoxins in thyme-based products and dried thyme herbs. [a]

[a] Mean was calculated as average of two collections.
Mean was compared to:
[b] AFB1 ML for Lebanese bread and for cereals and cereal products (2 µg/kg) (NL 240:2010; European Commission, 2006)

Food items		AFB_1	OTA
Thyme based products	Thyme pie	0.26±0.36 [b]	0.41±0.58[d]
	Cheese and thyme pie	0.40±0.57 [b]	0.23±0.33 [d]
	Fresh thyme salad	0.52±0.74 [c]	0.00±0.00 [e]
	Thyme regular mix sandwich	**2.55±2.08 [b]**	0.60±0.85 [d]
	Thyme mix with nuts and seeds sandwich	1.85±0.07 [b]	0.00±0.00 [d]
	Pizza and pasta with thyme sauces	**3.35±0.99 [b]**	1.58±2.03 [d]
	Bread sticks with thyme (crunchy)	0.92±1.30[b]	0.00±0.00 [d]
	Sesame thick bread with thyme (soft)	0.25±0.35[b]	0.00±0.00 [d]
	Crackers with thyme	0.90±0.79[b]	0.00±0.00 [d]
	Toast/Bread with thyme	0.69±0.87[b]	0.02±0.03 [d]
	Thyme croissant	1.03±0.33[b]	0.00±0.00 [d]
	Cheese with thyme	2.49±0.50 [c]	0.06±0.09 [e]
	Total	1.27±0.75	0.24±0.33
Dried thyme herbs			
	Thyme regular mix	**11.58[a]±5.92 [c]**	4.83±4.44[e]
	Thyme mix with nuts and seeds	2.78±0.06 [c]	0.09±0.13[e]
	Thyme dry herb	**15.38[a]±0.30 [c]**	0.35±0.49[e]
	Tea thyme	**16.79[a]±12.80 [c]**	0.07±0.10[e]
	Total	**11.63±7.02**	1.34±1.29

[c] AFB1 ML for thyme and thyme mixes (2 µg/kg) (NL 677:2017) & for spices (5 µg/kg) (European Commission, 2015)
[d] OTA ML for Lebanese bread and for cereals and cereal products (3 µg/kg) (NL 240:2010; European Commission, 2006)
[e] OTA ML for thyme and thyme mixes (3 µg/kg) (NL 677:2017) & for spices (15 µg/kg) (European Commission, 2006)
Bold: Exceeding the limits of the Lebanese Standards and the European Commission

Underline: Exceeding the limits of the Lebanese Standards only

In the dried thyme herbs category, all the samples exceeded the limits with tea thyme concentration reaching 6 times the Lebanese standards and 3 times the European standards. Tea thyme showed the highest AFB_1 concentration of 16.79 µg/kg. This high level was also shown in another study done in herbal green tea available in the Moroccan market with a mean of 16.9 µg/kg in some areas (Mannani et al. 2020). In Pakistan and Latvia, AFB_1 was also detected in tea samples with a range of 0.08-8.24 µg/kg (Ismail A. et al. 2020) and 3.40-23.7 µg/kg (Reinholds et al., 2019), respectively. In Italy, none of the herb-tea samples was contaminated with AFB_1 (Romagnoli et al., 2007). The high levels of AFB_1 in thyme dried herbs was also shown in other studies done in Lebanon showing a mean of 193.4 µg/kg and 36.1 µg/kg in spices and herbs (El Darra et al 2019), respectively, and in Al Ayoubi et al. (2021) with a mean of 99.4 µg/kg of AFB_1 in spices. A range of 5.3-17 µg/kg of AFB_1 contamination was shown in oregano in Serbia (Škrinjar et al. 2012) and a mean of 16.8 µg/kg was reported in thyme in Egypt (Migahed et al., 2017) which are comparable to the our results. In Greece, 20 out of 29 spices samples were positive for AFB_1 with a mean of 9.89 µg/kg and a range of 0.40-132.70 µg/kg (Koutsias et al., 2021). Unlike our results, AFB_1 was not detected in any of 6 thyme samples in a study done in Turkey (Tosun & Arslan, 2013) nor in Italy (Romagnoli et al., 2007). In Iran, 1 out of 10 thyme samples was contaminated with AFB_1 with a concentration of 2.2 µg/kg (Khazaeli et al. 2017). In Italy, the mean contamination of AFB_1 in spices was 0.31 µg/kg, which is much lower compared to the current study (Prelle et al. 2014). These findings may be related to high counts of molds reported in thyme samples collected from the Lebanese market (Karam et al., 2021). *Fusarium, Penicillium* and *Aspergillus,* the later which produces aflatoxins were reported. Molds can contaminate spices and herbs due to bad agricultural practices at any stage during growth, harvesting, transportation, processing and storage (Mandeel et al. 2005; Akpo-Djènontin et al., 2018). This, highlights the importance of implementing food safety management systems, monitoring storage conditions and applying good hygienic and manufacturing practices in spices and herbs sector to ensure the microbiological quality of the products and prevent molds contamination (Karam et al., 2021; El Darra et al., 2019).

AFB1 contamination in thyme-based products can be caused by thyme dried herb, wheat, oil, nuts, seeds and any other ingredient or the contribution of all ingredients. While, dried thyme herbs showed high AFB_1 contamination levels (2.78-16.79 μg/kg); the contamination of the thyme-based products can vary depending on the AFB_1 concentration in the different used ingredients and thyme content in the final product (Table 4). Many other studies reported as well AFB_1 contamination in several ingredients used in those products such as wheat (Daou et al.2021, Joubrane et al. 2011), thyme herb (El Darra et al. 2019, Al Ayoubi et al.2021), nuts (Mokhtarian et al. 2020; Eneroth et al. 2017; Emadi et al. 2021) and sesame (Kollia et al. 2016; Heshmati et al. 2021).

3.2 Occurrence of OTA

OTA was found in 38% (12/32) of the samples with a mean of 1.4 µg/kg and a concentration ranging between 0.04-8.00 µg/kg. Only 6% (2/32) and 3% (1/32) samples were contaminated with levels of OTA above the Lebanese and European standards, respectively (Table 5). All thyme-based products were below the limits of OTA for cereals and thyme, similar to a study in the US which showed that breakfast cereals had generally low OTA levels with a mean 1.51 µg/kg the first year and 0.60 µg/kg the second year (Lee et al. 2015). In Spain, the OTA mean concentration in breakfast cereals was 0.265 µg/kg (Araguás et al. 2005). A study done in Lebanon to assess OTA in 24 hours diet in students showed a mean of 0.16 µg/kg. However, OTA was not detected in thyme manakish, nor in wheat products such as toast and tea (Al Ayoubi et al. 2021). Toast, croissant and Kaak samples in a study previously done in Lebanon (Daou et al., 2021) reported OTA concentration of 4.11, 0.74 and 2.03 µg/kg, respectively. While croissant and kaak were free from OTA in our study, pasta and pizza samples in our study showed a concentration of OTA of 1.58 µg/kg which was higher than in Turkey (0.065 µg/kg) and Czech Republic (0.14 µg/kg) (Kulahi & Kabak, 2020; Ostry et al. 2015). In Morocco, 125 pasta samples showed OTA mean concentration of 0.63-0.64 ng/kg which is much lower compared to the current study (Tabarani et al. 2020).

From the dried herbs category, only thyme regular mix exceeded the acceptable limit according to the Lebanese standards with a concentration of 4.83 µg/kg. Other studies done in Lebanon reported higher OTA concentrations of 9.13 µg/kg (Al Ayoubi et al. 2021) and 7.0-7.1 µg/kg (El Darra et al. 2018) in spices and herbs, respectively. A study done in Italy also showed higher OTA concentration in spices with a mean of 6.18 µg/kg (Prelle A. et al. 2014). In Serbia, oregano was contaminated with OTA with a range of 4-22.4 µg/kg which is much higher than the current study (Škrinjar et al. 2012). In Poland, whole oregano and crushed oregano samples showed OTA mean of 0.37 µg/kg and 1.77 µg/kg, respectively (Waśkiewicz et al. 2013). In Belgium, herbs and spices showed a mean of 1.51 µg/kg for OTA (Meerpoel et al. 2021) lower than the current study for thyme dried herbs. OTA mean contamination in tea samples in this study was 0.07 µg/kg with a range of 0.00-0.135 µg/kg, while in Latvia a range of 2.99-30.3 µg/kg was reported (Reinholds et al., 2019). In China, 2 out of 108 tea samples were positive for OTA with an average of 0.66 µg/kg (Z Ye et al. 2020).

OTA incidence in food items in the current study was not only due to thyme but can be due to any of the raw materials in the food item, and can appear in the final product since OTA is thermally stable (Ozden et al., 2012). For example, Pizza and pasta reported OTA concentration of 1.58 µg/kg, which may be contributed to thyme, tomato sauce, wheat or other raw materials and persisted after cooking. This highlights the importance of setting limits for all raw materials used.

3.3 Risk characterization and dietary exposure to AFB_1

Table. 7 shows the dietary exposure to AFB_1 for regular and high consumption of thyme-based products and dried thyme herbs. For thyme-based products, the average daily exposure to AFB_1 were 4.270 and 7.701 ng/kg bw/day for regular and high consumers, respectively. Thyme dried herbs category showed higher exposure values of 4.977 ng/kg bw/day and 10.980 ng/kg bw/day for the same consumption, respectively.

Table 7 Dietary exposure to AFB_1 and the calculated MOE for regular and high consumption of thyme dried herb and thyme-based products marketed in Lebanon.

	Regular consumption		High consumption	
Thyme based products	Exposure (ng/kg bw/day) [a]	MOE [b]	Exposure (ng/kg bw/day) [a]	MOE [b]
Thyme pie	0.257	**1554**	0.504	**794**
Cheese and thyme pie	0.306	**1308**	0.927	**432**
Fresh thyme salad	0.490	**817**	0.832	**481**
Thyme regular mix sandwich	0.592	**675**	1.198	**334**
Thyme mix with nuts and seeds sandwich	0.287	**1395**	0.748	**535**
Pizza and pasta with thyme sauces	1.262	**317**	1.332	**300**
Bread sticks with thyme (crunchy)	0.063	**6322**	0.170	**2359**
Sesame thick bread with thyme (soft)	0.047	**8474**	0.139	**2887**
Crackers with thyme	0.046	**8663**	0.096	**4163**
Toast/Bread with thyme	0.063	**6352**	0.094	**4278**
Thyme croissant	0.136	**2932**	0.327	**1224**
Cheese with thyme	0.720	**556**	1.336	**299**
Total	4.270	**94**	7.701	**52**
Dried thyme herbs				
Thyme regular mix	3.280	**122**	7.216	**55**
Thyme mix with nuts and seeds	0.150	**2675**	0.272	**1469**
Thyme dry herb	1.339	**299**	2.146	**186**
Tea thyme	0.209	**1917**	0.464	**863**
Total	4.977	**80**	10.980	**36**

[a] The average body weight of 72.4 kg was used.
[b] BMDL10 of 0.4 µg/kg bw/day was used for MOE calculation of AFB1 (EFSA, 2020)
Bold: MOE below 10,000 indicates a public health concern (EFSA,2020)

The average dietary exposure values to AFB_1 for regular consumption of thyme-based products and thyme dried herbs as documented in the present study (4.270-4.977 ng/kg bw/day) were higher than those reported in Lebanese wheat products (0.92 ng/kg bw/day)

(Daou et al., 2021), in a Lebanese total diet study (0.66 and 1.46 ng/kg bw/day for regular and excessive consumers) (Raad et al., 2014) and in spices used in Lebanese cooking (1.55 ng/kg bw/day) (Al Ayoubi et al., 2021). The exposure levels to AFB_1 in this study were similarly higher than those reported in other countries. They were higher than the exposure from spices in Malaysia (0.59 ng/kg bw/day; Ali & Watt, 2019), from breakfast cereals in Spain (0.01-0.06 ng/kg bw/day; Ibáñez-Vea et al. 2011) and from wheat flours in Iran (0.25 ng/kg bw/day; Jahanbakhsh et al. 2021). In China and Turkey, the dietary exposure to AFB_1 from various food stuff was 0.57 ng/kg bw/day and 0.433 ng/kg bw/day much lower compared to the current study (Kabak, 2021; Zhang et al. 2020). Dietary exposure of AFB_1 from a certain food item, depends on the dietary habits of the specific country population and on the occurrence of AFB_1 in this food item during the analysis period. The high concentration levels of AFB_1 in thyme and thyme-based products and the high consumption of these products by the Lebanese population, contributed to the high dietary exposure levels in the current study.

The main contributor to the dietary exposure to AFB_1 in thyme-based products was pizza and pasta (30%) followed by cheese with thyme, thyme regular mix sandwich and fresh thyme salad (17-12%), then cheese and thyme pie, thyme mix with nuts and seeds sandwich and thyme pie (7-6%), and then the remaining ones were below 3% (Figure 1a). For dried thyme herbs, thyme regular mix was the main highest contributor (66%) followed by thyme dry herb (27%), then tea thyme and thyme mix with nuts and seeds (3-4%) (Figure 1b). The contribution of different food samples to the dietary exposure depends on both the concentration of AFB_1 in the sample and the consumption of this food item by the population. For high consumption, the contribution percentage to the dietary exposure to

AFB_1 changed. The first three main contributors were cheese with thyme, pizza and pasta and thyme regular mix sandwich (17-16%), followed by cheese and thyme pie, fresh thyme salad and thyme mix with nuts and seeds (12-10%). This increases in percentage contribution in thyme regular mix sandwich, cheese and thyme pie and thyme mix with nuts and seeds reflects the increase in thyme consumption during thyme cultivation season, lent meals, school snacks and other meals.

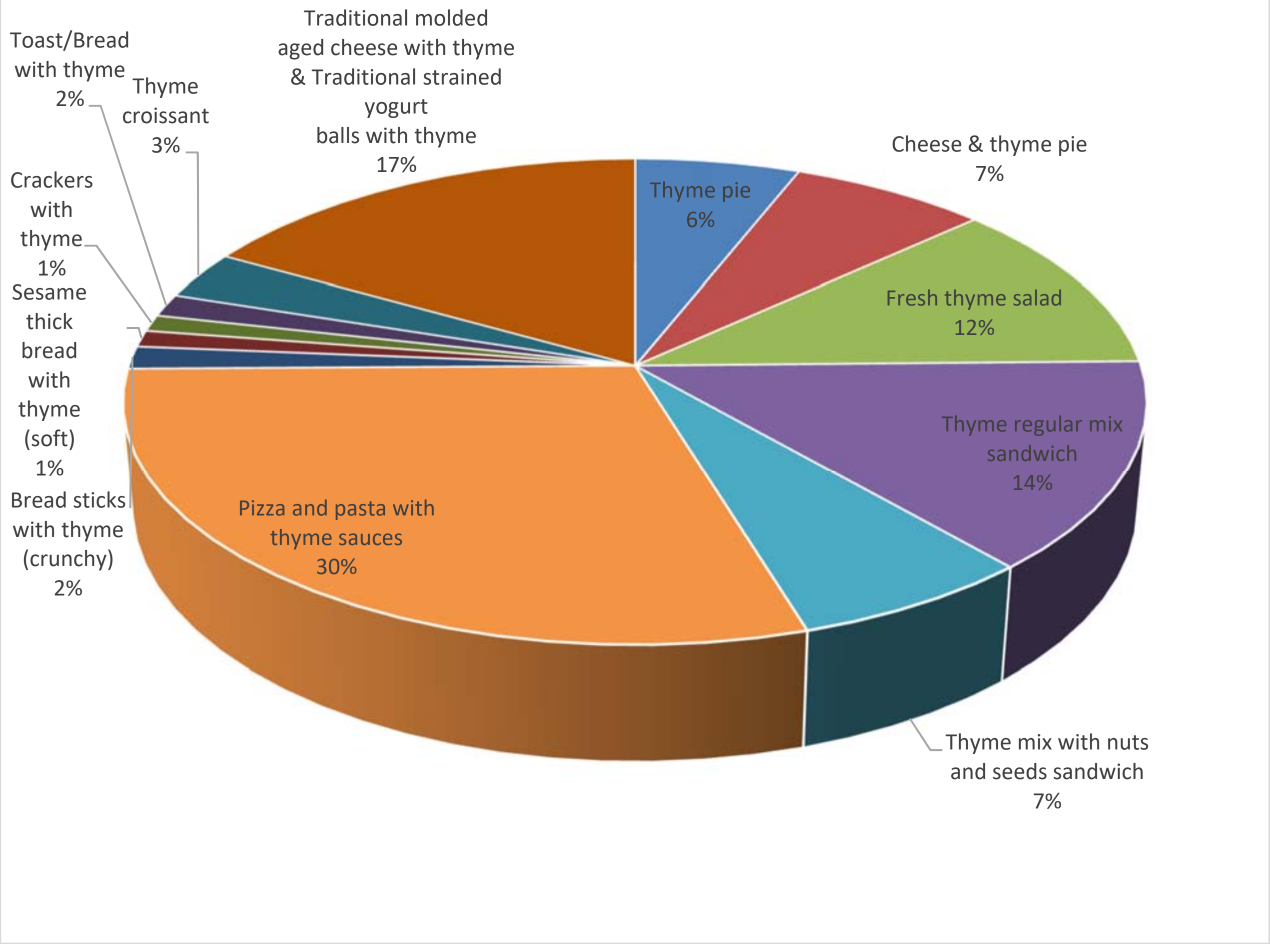

Figure 1 The percentage contribution of thyme-based products to the average dietary exposure to AFB_1

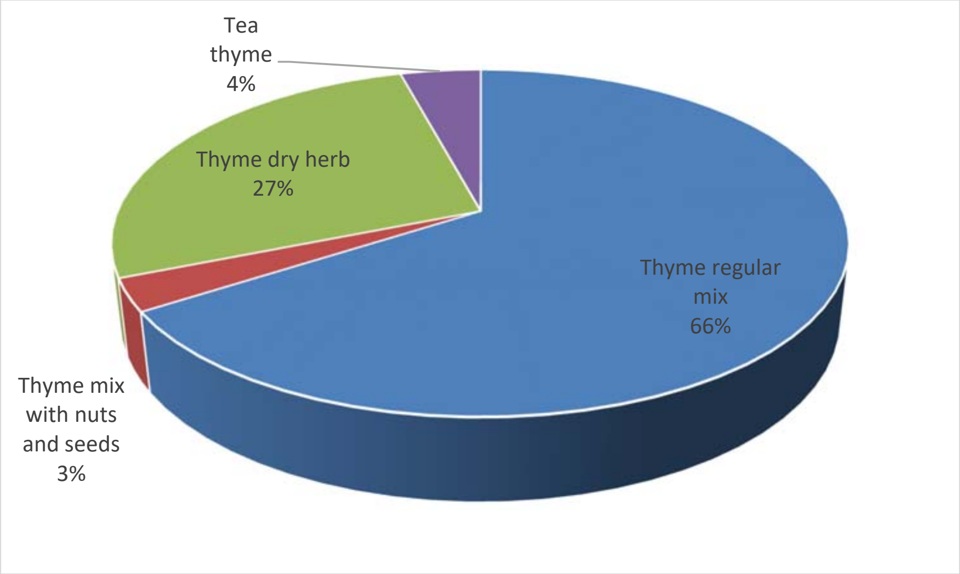

Figure 2 The percentage contribution of dried thyme herbs to the average dietary exposure to AFB_1

In the study of Raad et al. (2014), the main contributor to the average exposure to AFB_1 was "bread and toast" (79.4%-82.2%) while "Pizza and pies" contributed 4.9% to the dietary exposure to AFB_1. The difference in the observed dietary exposure trend can be related to the difference among products tested, variability of contamination levels over time and change of consumer's behavior due to several factors such as globalization, current economic crisis or other socio-demographic aspects. A study in New Zealand has shown that spices were the major contributors to the dietary exposure to AFB_1 followed by nuts, cereal products and snacks (Cressey, P. J. & Reeve, J., 2013).

The MOE to AFB_1 determined using the dietary intake levels of each sample and the $BMDL_{10}$ of 0.4 µg/kg bw/day (EFSA, 2020) is shown in Table 7.

The MOE for thyme-based products for regular and high consumption were 94 and 52, respectively. These very low MOE (less than 10,000) indicates high risk from consumption of thyme-based products since the risk increases when MOE decreases. On the individual product level, the lowest MOE was for pizza and pasta (317) followed by cheese with thyme (556) then thyme regular mix sandwich (675). For dried thyme herbs, the MOE were 80 and 36 for regular and high consumption, highlighting the high daily intake and contamination of thyme herbs. As all the obtained MOE values were less than 10,000, the consumption of thyme and thyme-based products in Lebanon can put consumers at an exposure risk to AFB_1.

No studies regarding the MOE in thyme or thyme-based products were reported previously. However, MOE calculated for other spices in past studies was similarly high. For example, the MOE to AFB_1 through spices used in Lebanese cooking was reported to range between 108 and 444 (Al Ayoubi et al. 2021). In Malaysia, the MOE derived from mean exposure to AFB_1 contamination in spices was reported to be 520 (Ali & Watt, 2019).

It is estimated that for non-European countries, the ingestion of 1 ng per kg of body weigh per day of AFB_1 would induce 0.083 liver cancer cases per 100,000 persons per year according to the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1999). Thus, liver cancer risk based on total daily exposure to AFB_1 (ng/kg body weight/day) from thyme and thyme-based products can be calculated as: Liver cancer risk= Daily exposure to AFB_1 (ng/kg bw/day) x 0.083 cancer cases per 100,000 persons. Therefore, according to the aforementioned, the Lebanese population would have 0.35 and 0.41 additional cancer cases per 100,000 persons per year for thyme-based products and dried thyme herbs, respectively. These corresponding values in the current study were higher than a study done in Malaysia on spices (0.01) (Ali & Watt, 2019) and in Lebanon on wheat and products

(0.076) (Daou et al. 2021). For high consumption, an additional 0.911 and 0.639 cancer cases per 100,000 person per year for thyme and thyme-based products, respectively.

3.3Risk characterization and dietary exposure to OTA

The dietary exposure to OTA for regular consumption was calculated and shown in Table 8. The average total dietary intake of OTA was 1.345 ng/kg bw/day from thyme-based products, which is similar to another study done in Lebanon testing 24 hours diet of students by Al Ayoubi et al. (2021) showing a mean exposure of 1.4 ng/kg bw/day from 24 hours diet. However other studies in Lebanon reported higher exposure of 4.28 ng/kg bw/day (Raad et al. 2014) and 7.60 ng/kg bw/day (Daou et al. 2021). In Turkey, higher dietary exposure of 2.585 ng/kg bw/day from various food stuff was reported (Kulahi & Kabak, 2020), while, in Czech Republic, it was comparable to the current study with an exposure of 1.8 and 1.2 ng/kg bw/day for adult men and women, respectively (Ostry et al. 2015). Also, in Morocco, the average OTA daily exposure from cereal derived products was 1.93 ng/kg bw/day similar to our results (Tabarani et al., 2020). The exposure to OTA from tea alone in Czech Republic was 0.36 and 0.45 ng/kg bw/day for men and women, respectively, much higher than the current study (0.001 ng/kg bw/day from tea thyme). The dietary exposure to OTA from dried thyme herbs was reported 1.392 ng/kg bw/compared to 0.0165 ng/kg bw/day for adults from herbs and spices in Belgium (Meerpoel et al. 2021).

Table 8 Dietary exposure to OTA, Margin of exposure (MOE) and the percentage contribution to Toxicological Reference Values (TRVs) for thyme-based products and dried thyme herbs during regular consumption.

Thyme based products	Exposure (ng/kg bw/day)	Exposure (ng/kg bw/week)	Dietary exposure expressed as %TRV JECFA [a]	MOE Non-neoplastic effect [b]	MOE Neoplastic effect [b]
Thyme pie	0.410	2.870	3	11,536	35,364
Cheese and thyme pie	0.179	1.250	1	26,478	81,170
Fresh thyme salad	0.000	0.000	0	-	-
Thyme regular mix sandwich	0.140	0.981	1	33,752	103,467
Thyme mix with nuts and seeds sandwich	0.000	0.000	0	-	-
Pizza and pasta with thyme sauces	0.596	4.171	4	7,938	24,333
Bread sticks with thyme (crunchy)	0.000	0.000	0	-	-
Sesame thick bread with thyme (soft)	0.000	0.000	0	-	-
Crackers with thyme	0.000	0.000	0	-	-
Toast/Bread with thyme	0.002	0.013	0	2,607,340	7,992,902
Thyme croissant	0.000	0.000	0	-	-
Cheese with thyme	0.018	0.127	0	260,685	799,140
Total	1.345	9.415	9	3,517	10,781
Dried thyme herbs					
Thyme regular mix	1.368	9.575	10	3,458	10,601
Thyme mix with nuts and seeds	0.005	0.035	0	958357	2,937,880
Thyme dry herb	0.02	0.131	0	252264	773,324
Tea thyme	0.001	0.006	0	5637070	17,280,658
Total	1.392	9.747	10	3,398	10,417

[a] OTA PTWI according to JECFA: 100 ng/kg/week (JECFA, 2007)

[b] BMDL10 of 4.73 µg/kg bw/day for non-neoplastic effects and 14.50 µg/kg bw/day for neoplastic effects were used for MOE calculation of OTA (EFSA, 2020).

MOE below 200 indicates a public health concern for non-neoplastic effects

MOE below 10,000 indicates a public health concern for neoplastic effects

The estimated regular and high consumption weekly exposure levels to OTA from thyme-based products were 9.415 and 16.048 ng/kg bw/week, respectively. regular and high consumption exposure levels to OTA represented 9% and 16 %, respectively, of the provisional tolerable weekly intake (PTWI) set by JECFA (100 ng/kg bw/week).

According to EFSA (2020), the PTWI of 120 ng/kg bw/week for OTA is no longer valid after the recent studied that reported uncertainty regarding the mode of action for kidney carcinogenicity. Thus, Margin of exposure (MOE) approach is applied to assess the risk of OTA for both non-neoplastic and neoplastic. Both MOE were calculated and shown in Tables 8 and 9. The calculated MOE for regular consumption are above 10,000 for neoplastic effects and above 200 for non-neoplastic effects which indicates in both cases, no potential health risk related to OTA from thyme and thyme-based products consumption in Lebanon. Al Ayoubi et al. (2021) reported MOE below 10,000 for 24 hours meals and snacks for neoplastic effect indicating a major health threat, however, for non-neoplastic effect the MOE was above 200 posing no threat. In Belgium, MOE showed a health concern from intake of biscuits, croissant, rice, flour, herbs and spices (Meerpoel et al., 2021). On other hand, the calculated MOE, for high consumption, were above 200 for non-neoplastic effects. For neoplastic effects, all the MOE values were above 10,000 except for thyme regular mix (4,818<10,000) indicating a risk from OTA intake for high consumers.

Table 9 Dietary exposure to OTA, Margin of exposure (MOE) and the percentage contribution to Toxicological Reference Values (TRVs) for thyme-based products and dried thyme herbs in high consumption

Thyme based products	Exposure (ng/kg bw/day) [a]	Exposure (ng/kg bw/week)	Dietary exposure expressed as %TRV JECFA [b]	MOE Non-neoplastic effect [d]	MOE Neoplastic effect [d]
Thyme pie	0.803	5.618	6	5894	18,068
Cheese and thyme pie	0.541	3.790	4	8736	26,782
Fresh thyme salad	0.000	0.000	0	-	-
Thyme regular mix sandwich	0.284	1.985	2	16677	51,125
Thyme mix with nuts and seeds sandwich	0.000	0.000	0	-	-
Pizza and pasta with thyme sauces	0.629	4.400	4	7524	23,066
Bread sticks with thyme (crunchy)	0.000	0.000	0	-	-
Sesame thick bread with thyme (soft)	0.000	0.000	0	-	-
Crackers with thyme	0.000	0.000	0	-	-
Toast/Bread with thyme	0.003	0.019	0	1755964	5,382,975
Thyme croissant	0.000	0.000	0	-	-
Cheese with thyme	0.034	0.236	0	140420	43,046
Total	2.293	16.048	16	2,063	**6,323**
Dried thyme herbs					
Thyme regular mix	3.009	21.065	21	1572	**4,818**
Thyme mix with nuts and seeds	0.009	0.063	0	526421	1,613,765
Thyme dry herb	0.049	0.340		97409	298,610
Tea thyme	0.002	0.013	0	2536681	7,776,296
Total	3.069	21.481	21	1,541	**4,725**

[a] OTA PTWI according to JECFA: 100 ng/kg/week (JECFA, 2007)

[b] BMDL10 of 4.73 µg/kg bw/day for non-neoplastic effects and 14.50 µg/kg bw/day for neoplastic effects were used for MOE calculation of OTA (EFSA, 2020).

Bold: MOE below 200 indicates a public health concern for non-neoplastic effects
MOE below 10,000 indicates a public health concern for neoplastic effects

The percentage contribution of each thyme-based product to the average dietary exposure to OTA was calculated (Figure 2a). Pizza and pasta (44%) were the highest contributor to the dietary exposure to OTA followed by thyme pie (32%) then, cheese and thyme pie and thyme regular mix sandwich (13-11%) while the other products had a contribution below 1%. For high consumers, thyme pie was the first contributor (35%), followed by pizza and pasta and cheese and thyme pie (27-24%), and then thyme regular mix sandwich (12%). Thyme regular mix was the major contributor to the exposure to OTA in the dried herbs category by 98% (Figure 2b). The main contributors to the dietary exposure to OTA in a total diet study previously done in Lebanon (Raad et al., 2014) were caffeinated beverages > biscuits and croissant> alcoholic beverages> bread and toast> rice. Cereal based products were also the main contributors for OTA in Europeans and North Americans areas (Park et al. 2005).

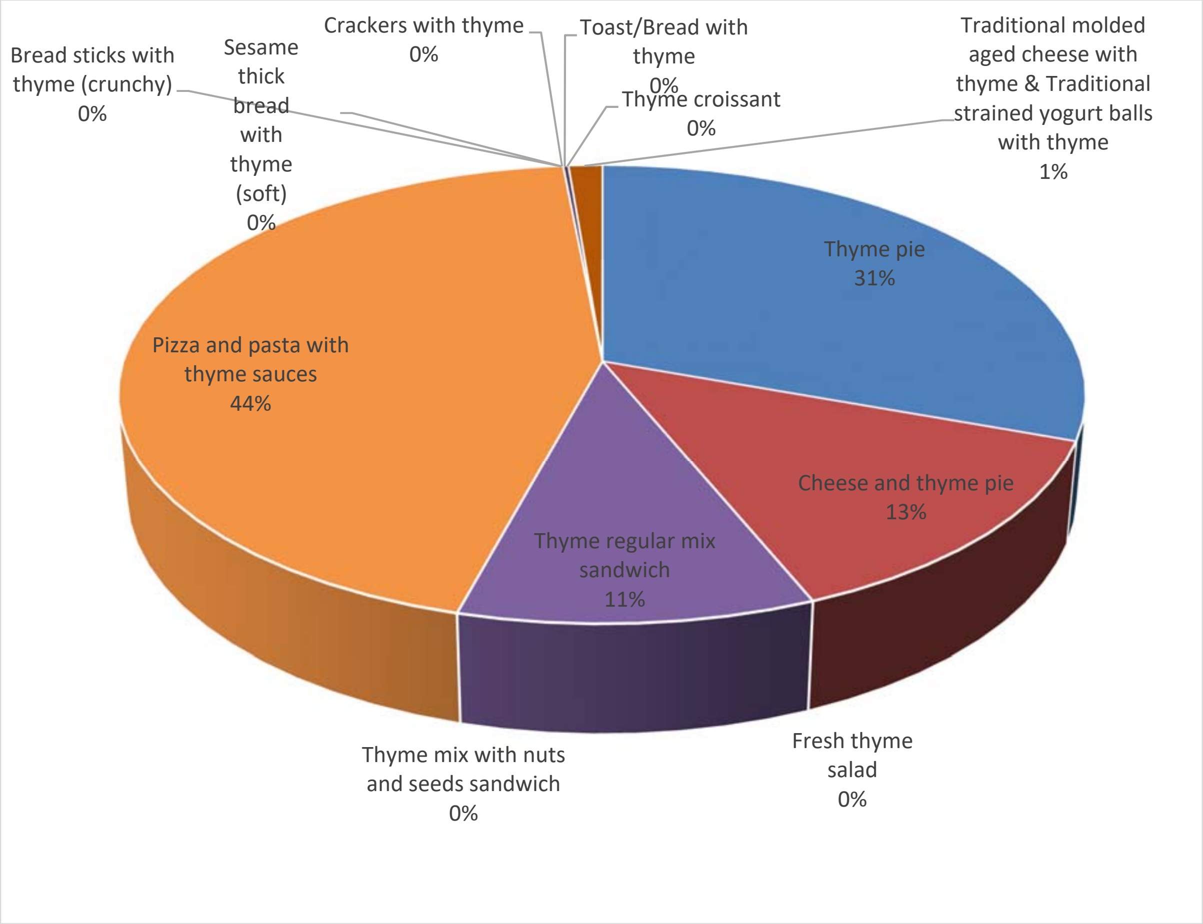

Figure 3 The percentage contribution of thyme-based products to the average dietary exposure to OTA.

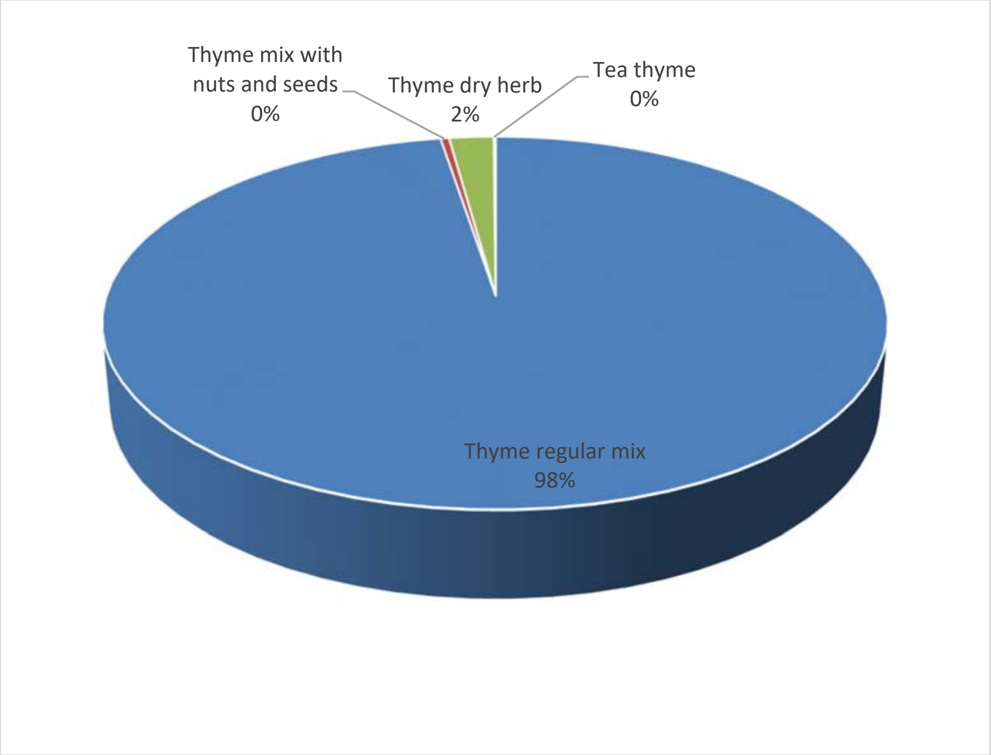

Figure 4 The percentage contribution of dried thyme herbs to the average dietary exposure to OTA

4. Conclusion

In this study, the occurrence of AFB_1 and OTA, dietary exposure and risk assessment related to the consumption of thyme herbs and thyme-based products was investigated. Consumption of thyme and thyme-based products could lead to an increase in health risks associated mainly with AFB_1 while no potential risk was associated with OTA. This is very critical especially that this food group is highly consumed in Lebanon and the Easter Mediterranean area. This study highlights the importance of developing maximum mycotoxins limits specific to each product and country based on risk assessment and consumption patterns. It highlights as well the importance of monitoring and control of herbs for not exceeding the maximum limits to ensure the safety of thyme herb and its products, knowing that mycotoxins have the ability to persist during processing ang cannot be eliminated while cooking or heating.

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