Controlling of Mold Contamination and Ochratoxin A of Corn Snakes and Corn Flakes in Egypt

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Abstract

In this study mycological analysis was carried out to isolate and identify the molds associated with corn-based snacks and corn flakes products and determine the level of Ochratoxin A (OTA) contamination in these products. Fifty samples of corn – based snacks and fifty samples of corn flakes collected from local markets of two Egyptian governorates (Cairo and Beni-Suif), were used for fungal isolation, identification and analyzed for the presence of OTA using High Performance Liquid Chromatography (HPLC). Three species of *Aspergillus* (*Asp. flavus* , *Asp ochraceus* and *Asp. niger*); one species of *Fusarium* (*F. verticilliodes*); one species of *Penicillium* (*P. chrysogenum*); one species of Yeast(*Rhodotorula mucilaginosa*) and one *Alternaria sp.* were the most frequent fungus in the two governorates. OTA was found in 11 of 25

(44%) corn-based snacks samples obtained from Cairo. While, the numbers of contaminated corn – based snacks samples collected from Beni-suif was13 of 25(52%). Moreover, OTA was detected in 8 of 25 (32%) corn-flakes samples obtained from Cairo, While OTA, was detected in nine (36%) of the Beni-suif samples. The results revealed that all corn – based snacks samples that were contaminated with OTA (24samples) with a range of 0.75 to 13.47 µg / kg and all corn flakes samples (17samples) that were contaminated with OTA with a range of 0.65 to 7.92 µg / kg over passed the maximum allowable limits (0.5 µg / kg) for processed cereal-based foods consumed by young children. While, in case of adults and adolescents consumers the established maximum allowable limit was (3 µg/kg) for processed cereal-based foods consumed by adults and adolescents so that, only ten samples of corn– based snacks that were contaminated with OTA with a range of 3.05 to 13.47 µg/ kg exceeded the established limit (3 µg/kg) and eight corn flakes samples that were contaminated with OTA with a range of 3.07 to 7.92 µg / kg over passed this established limit. The other aim of this study was to assess the effectiveness of employing the essentials oils from (garlic, cinnamon, clove, marjoram, fennel, cumin, lemon grass, peppermint, rosemary and thyme) in combating the growth of Ochratoxigenic mold associated with food deterioration, namely *Asp. ochraceus* and its OTA production. The antifungal activity was determined using the Agar well diffusion technique. The ten essential oils had a notable inhibitory effect on the development of *Asp. ochraceus*. However, garlic and cinnamon essential oils had the greatest antifungal potential against *Asp. ochraceus* growth and the production of OTA, followed by clove, and marjoram essential oils.

Introduction

Corn, especially in developing countries, are highly susceptible to fungal attacks while in the field and during storage, resulting in food spoilage. Because of the tropical and humid environment, fungal contamination of stored grains is a severe chronic problem in the storage system. This fungal attack may result in mycotoxin contamination of the crop. These toxins are stable chemicals that don't degrade totally at high temperatures **(Kabak, 2009);** As a result, mycotoxins can contaminate processed food and insert the human food chain via cereals - based food product. Cereals and other crops are an essential resource for humanity's survival as well as a country's economic lifeline. Food, on the other hand, may become contaminated and inedible during storage, resulting in significant financial losses and representing a risk to human life. **Saadia M. Easa, (2010)** reported that Microorganisms found n fast and traditional fast foods.

Ochratoxin A , is one of the most common mycotoxins due to its toxicity and abundance. Any mycotoxin contamination in foods and feeds can result in financial losses as well as a threat to human health, which is a serious issue that arises from period to period in our country. OTA is a carcinogenic fungal secondary metabolite that may be found in a wide range of foods, including cereals, grapes, coffee, nuts, and spices, and has a global distribution **(Malir *et al.*, 2016: Wang *et al.*, 2018).** Because of the threat it presents to human health, OTA has attracted the scientific community's interest in food. OTA is the most common naturally occurring mycotoxin that can contaminate food commodities prior to

harvest or, more commonly, during storage, and is produced primarily by *Penicillium verrucosum* in temperate climates and *Aspergillus ochraceus* and the rare *Aspergillus carbonarius* in warm and tropical climates **(EFSA, 2004 : Gil-Serna *et al.*, 2018).** Some studies on the biological control of wilt and stem – canker of potato were reported by **Saadia M. Easa and Youssef K.A (2011).**

OTA is a well-known nephrotoxic agent that has been linked to a serious and deadly kidney disease known as Balkan Endemic Nephropathy, as well as an increased prevalence of upper urinary tract tumours **(JECFA, 2001: Clark and Snedeker 2006).** Ochratoxin A (OTA) is mainly produced by toxigenic *Aspergillus* and *Penicillium spp*. It has primarily a nephrotoxic agent, with a range of toxicities including teratogenic, immunosuppressive, growth retardation and possible carcinogen to humans **(Alsalabi, *et al* 2023)**. Because of the high toxicity of OTA and potential to accumulate in human and animal organs, it is closely regulated in many countries and subjected to severe legal regulations for particular agricultural products. **Saadia M. Easa and Mallik A.U (2001), Saadia M. Easa (2002)** reported the effect of Garlic (*Allium Sativum*) on lipid pattern and nitrogenous copmounds and growth of selected dermatoohytes.

The Scientific Commission of the European Community have been set the maximum allowed limits for unprocessed cereals (5 µg/ kg), all cereal-derived products (3µ g kg), and processed cereal-based food and baby foods for infants and young children (0.5 µg kg) **(European Commission, 2006).** Contaminated foods constitute a health hazard to human consumption. These foods, especially those for children, must therefore be examined at regular intervals in order to assess their hygienic quality.

Natural plant extracts are great alternatives to protect food against fungal infection due to health risks associated with exposure to hazardous toxins and economic considerations. **Saadia *et al.*, 2019** found that using *Moringa olifera* extract of leaves can inhibit *Staphylococcus pasteuri*.

Essential oils are plant extracts with antimicrobial qualities, and some of them are utilized as antispasmodic treatments, food preservatives, and flavour enhancers **(Panizzi *et al.*, 1993).** Some studies reported that some medicinal plants are used as antibacterial on certain human pathogenic bacteria **(Saadia M. E and EL-Beih F.M 2002).**

Essential oils inhibited mould mycelial growth (fungistatic or fungicidal effect) in solid or liquid medium over a wide range of concentrations, according to **Atanda *et al.* (2007),** with concomitant disease or total inhibition of mycotoxins production (e.g. by *Aspergillus parasiticus, A. ochraceus, Fusarium graminearum, F.proliferatum*).

Soliman and Badeaa (2002) discovered that the essential oils of thyme, cinnamon, marigold, spearmint and basil inhibited the development of *Aspergillus flavus, Aspergillus parasiticus, Aspergillus ochraceus,* and *Fusarium verticillioides.*

Material and Methods

1. Samples collection:

A total of 100 samples of corn – based snacks and corn purchased from different retailers in two Egyptian governorates (Cairo and Beni- Suif), Including25 samples of corn – based snacks and 25 samples of corn flakes collected from each governorate.

All samples were kept in their original containers and stored in a dark, cold, and dry environment until they were divided into two portions, one

for fungal isolation and the other for OTA detection.

2. Mycological analysis:

The dilution-plate method (ISO 21527-2 /2008) was used for enumeration and isolation of fungi from the corn- based food samples.

In a laboratory sterile mill, random samples (200g) for each were ground. On a horizontal shaker, portions of 10 g of each sample were homogenized for 20 minutes with 90 mL Peptone medium. Using a sterile pipette, further serial dilutions to 10^{-3} were generated by transferring 1 mL of the original dilution into dilution tubes containing 9 mL of sterile Peptone media. These dilutions were placed in sterilized Petri plates in one milliliter quantities. To avoid killing the fungus in the samples, Difco potato dextrose agar medium was melted on a water bath and warmed to (40-42ºC). The melted and tempered potato dextrose agar medium about (10:15 ml) was placed into each Petri-dish, mixed immediately by rotating the plate, and the Petri-dishes were incubated at 22 - 27 ºC for 5 days following solidification of the media. All of the previous steps were carried out in an aseptic condition using triplicate Petri-dishes for each dilution. The counts of fungi were expressed as CFU/g (colony forming units per gram food sample). According to **Marassas *et al.* (1988)** and **Roige *et al.* (2009),** the frequencies of isolation (F %) of the isolated fungi was estimated as follows:

$$F\ (\%) = \frac{\text{No. of samples of occurrence of a species}}{\text{Total No. of samples}} \times 100$$

Then pure cultures were identified, all mycological analysis was done at Food Safety Department, Microbiology Unit, National Nutrition Institute, Ministry of Health, Cairo, Egypt.

3. Determination of OTA concentration in the collected samples by HPLC:

The concentrations of OTA in the collected samples were determined by using High Performance Liquid Chromatography (HPLC) in Food Toxins and Contaminants Department, National Research Centre, Dokki, Cairo, Egypt according to the AOAC Official Method 2000.03 **(Entwisle *et al.*, 2000)** with some modifications.

a) Preparation of food samples:

The samples (corn-based snacks and corn flakes) were prepared in accordance with **Guidelines of CAC/GL 40-1993 and A.O.A.C (2003).** Each sample was thoroughly mixed and ground to a fine powder. Two duplicates were obtained, one for extraction and the other as a reserve sample in the freezer at -20ºC.

b) OTA extraction:

A 25 gram of each homogenized samples was extracted with acetonitrile / water (80:20, v/v) containing1% NaHCO3by blending at high speed for 3 min and filtered using Whatman filter paper. 4 ml of the filtrate were diluted with phosphate buffered saline (PBS) containing 0.01% tween (pH, 7.4).The mixture was mixed before being passed through an OchraStar (IAC) column at a flow rate of about 2–3 ml per minute. OTA was eluted with 2 ml of methanol/acetic acid (98:2, v/v) through the column. The extracts were carefully evaporated until dry, then re-dissolved in acetonitrile / water (1:1, v/v) and filtered into a Silanized HPLC vial. All of the tests were carried out in triplicate. Chromatographic equipment (HPLC) was used for OTA analysis

4. Antifungal activity assay

Agar well diffusion method was used for screening essential oils for their antifungal activity, to identify the most effective essential oils. It was performed with the help of ten essential oils (cinnamon, cumin, clove, fennel, garlic, lemon grass, marjoram, peppermint, rosemary and thyme) against *A. ochraceus* according to **(Navarro Garcia *et al.*, 2003; Burt, 2004)**.

Aliquots (100 µL) of the prepared spore suspension (10^6spores / ml) were swabbed on the surface of potato dextrose agar plates. Then, using a sterile cork-borer, 6 mm-diameter wells were created in each inoculated plate. Essential oils at different concentrations (10 and 20 µl/ ml) of each were injected into these wells. After that, the plates were kept at room temperature for 30 minutes to allow for oil diffusion before being incubated for three days at 22- 27ºC. The antifungal activity was assayed after the incubation period by measuring the diameter (mm) of the growth inhibition zone surrounding the wells. The experiment was conducted in triplicates for each treatment, with the mean value calculated. The control sets were made with sterilized distilled water instead of the oil.

5. Application of the essential oils in the preservation of corn grains from OTA contamination

Infected corn grains with *A. ochraceus* were treated with different concentrations of (garlic, cinnamon, clove and marjoram) essential oils in order to estimate the efficacy of these oils as a natural preservative in inhibiting Ochratoxin A production.

Various flasks containing 50 gram of healthy sterilized yellow corn

were prepared, then each flask was infected with 1ml of *A. ochraceus* spore suspension (10^6/ml), and then concentrations of (10, 20,40,60,80 µl) of each of the tested essential oils were added to these flasks individually. The flasks were cultured for 15 days at 25-28ºC.For each treatment, triplicates were made. The control was made in the same way, but without the essential oils. Following the incubation period, Ochratoxin A was extracted and quantified using the HPLC technique of analysis, according to the AOAC Official Method 2000.03 **(Entwisle *et al.*, 2000).**

Results and Discussion

1. Mycological isolation of the molds associated with corn-based snacks and corn flakes products:

In the current study, **Table (1)'s** survey results revealed that five genera of filamentous fungi were isolated and from 100 samples of corn-based foods, including 50 samples of corn snacks and 50 samples of corn flakes that were purchased from local markets in two Egyptian governorates (Cairo and Beni-Suif). Including three *Aspergillus* species (*A. flavus*, *A. ochraceus*, and *A. niger*), one *Fusarium* species (*F. verticilliodes*), one *Penicillium* species (*P. chrysogenum*), one species of yeast (*Rhodotorula mucilaginosa*), and one *Alternaria* species were identified as shown in **Figure (1).**

These results were consistent with those of **Ismail *et al.* (2012),** who found that the most prevalent genera in breakfast cereal and corn flakes were *Aspergillus*, *Fusarium*, and *Penicillium*.

Data in **Table (1)** revealed that *A. flavus* and *A. ochraceus* had the highest incidence in the corn snacks and corn flakes samples obtained

from Cairo governorate.Where,40% of the corn snacks and 28% of the corn flakes samples were contaminated with *A. flavus*. While *A. ochraceus* was detected in 36 % of the corn snacks samples and in28% of the corn flakes samples. Moreover, the findings of this investigation agree with other studies on cereal grains conducted in Uganda, where *Aspergillus* species was the most often isolated fungus from corn-based foods **(Ismail *et al.*, 2003; Taligoola *et al.*, 2004).**

Fusarium verticilliodes was the third common species detected in the corn snacks and corn flakes samples collected from Cairo governorate. It contaminated 32 % of both corn snacks and corn flakes samples. It is in line with previous research on corn that *Aspergillus spp*. and *F. Verticilliodes* are highly prevalent on corn snacks and cornflakes **(Zohri *et al.*, 1995and Ismail *et al.*, 2003).** *A. niger* was found to contaminate 28 % of the corn snacks samples and 20 % of the corn flakes samples. Yeasts were isolated in moderate frequencies, the most common yeasts was *Rhodotorula mucilaginosa* represented by 28% and 12%forcorn snacks and corn flakes samples, respectively. *Penicillium chrysogenum* was found to contaminate 24% of corn snakes samples and 16% of corn flakes samples. The findings of this investigation were consistent with those of **Almeida *et al.* (2000)**, who said that the majority of the fungal species found in corn grains taken from three different locations of Brazil were *Fusarium, Penicillium*, and *Aspergillus* species.

However, *Alternaria spp.* was less frequent contaminating only (4%) of corn snakes samples. This result is consistent with the observation that species of the genus *Alternaria* are common field fungi that contaminate cereal grains and that their number gradually declines during storage, being replaced by storage fungi of the genera *Aspergillus*

and *Penicillium* **(Piotrowska *et al.*, 2013; Bensassi *et al.*, 2011; Saberi-Riseh *et al.*, 2004).**

The results of the fungal survey in Beni Suef governorate samples showed that the same fungi were isolated, but with slightly higher frequencies as shown in **Table (1)**.The results illustrated that *A. ochraceus* contaminating 40 % of the corn snacks samples and 32% of the corn flakes samples. Also, *A. flavus* was detected in 40% of corn snacks samples and in 28% of corn flakes samples, followed by *Fusarium verticilliodes* contaminating36%of the corn snacks samples and 28%of corn flakes samples. *A. niger* was detected in32%of corn snacks and in 24%of corn flakes samples. Moreover, Yeasts (*Rhodotorula mucilaginosa*) were isolated in moderate frequency of 24% from corn snakes samples and 20% of the corn flakes samples. *Penicillium chrysogenum* was detected in 20%of corn snakes samples and 16% of corn flakes. While *Alternaria spp.* was rare in both corn snakes and corn flakes samples its frequency of isolation was 8% for each.

The two most important elements that affect fungal development and the production of mycotoxins are temperature and water availability. Grain stored in Egypt that has been exposed to high temperatures (>20° C) and/or excessive moisture/humidity (>14%) may become infected. In these conditions, grains that have been kept may develop mould **(Richard, 2007).** Additionally, the initial fungus growth in grains can produce enough moisture through metabolism to support subsequent growth and mycotoxin synthesis.

The current survey used in this study showed that corn snacks and corn flakes products had a higher risk of contamination with *A. ochraceus*

and its production of OTA. These products are commonly sold in Egypt in super markets and small shops, and youngsters in particular consume them. Therefore, it is more important to safeguard their safety.

The mycological status of these collected samples can be thought of as the first step in this study to know and identify the fungi causing OTA contamination and the level of this contamination in the investigated samples since the carcinogenicity and acute toxicity of OTA have been accurately documented.

Table (1): Frequency of isolated fungi from corn-based snacks and corn flakes samples from Cairo and Beni-suif

Fungi	Cairo				Beni-Suif			
	Corn Snacks (n = 25)		Corn Flakes (n = 25)		Corn Snacks (n = 25)		Corn Flakes (n = 25)	
	N	F%	N	F%	N	F%	N	F%
A. ochraceus	9	36	7	28	10	40	8	32
A. flavus	10	40	7	28	10	40	7	28
A. neiger	7	28	5	20	8	32	6	24
F. verticilliodes	8	32	8	32	9	36	7	28
Rhodotorula mucilaginosa	7	28	3	12	6	24	5	20
P. chrysogenum	6	24	4	16	5	20	4	16
Alternaria spp.	1	4	-	-	2	8	2	8

(F %): frequency, measured as percentage.
(N): Number of samples of occurrence.
(-): no fungal species detected.

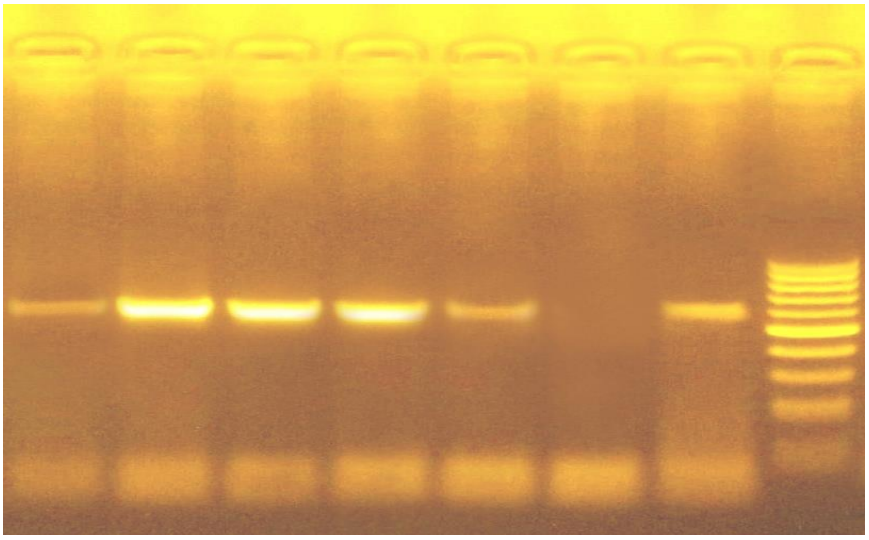


Figure (1): PCR results for the different fungal spp. genes (Gene ruler 100 bp DNA ladder).

2. Determination of OTA contamination in corn-based snacks and corn flakes samples collected from Cairo and Beni-suif:

The majority of people in the globe use cereals as their main source of food and energy, including corn, wheat, and barley. Mold contamination of crops, particularly cereals, during the pre-harvest and post-harvest phases can result in the generation of secondary hazardous compounds known as mycotoxins **(Silva et al., 2022; Lima *et al.*, 2022; Khorshidi *et al.*, 2022)**.To avoid harmful health impacts, OTA levels must be closely monitored. OTA is thought to be a powerful nephrotoxic pollutant with a variety of toxic effects that may contribute to the development of the irreversible kidney disease known as Balkan Endemic Nephropathy.

According to the current survey conducted for this investigation, corn products are contaminated with OTA (corn- based snacks and corn flakes samples). According to the recent data in **Table (2),** there were detectable amounts of OTA contamination in the samples of corn snacks and corn flakes that were under investigation, ranging from 0.65 to 13.47 µg/kg.

Results in **Table (2)** and **Figure (2)** showed that in 25 corn-based snacks samples collected from Cairo,11samples (44%)were positive to OTA in concentration ranging from 0.95 to 8.49 µg/ kg, with a mean value of 3.41µg/kg. On the other hand, in Beni-suif governorate, there were13samples (52%) of corn – based snacks were contaminated with OTA with concentrations ranging from 0.75 to 13.47 µg/ kg, with a mean value of 3.87 µg / kg.

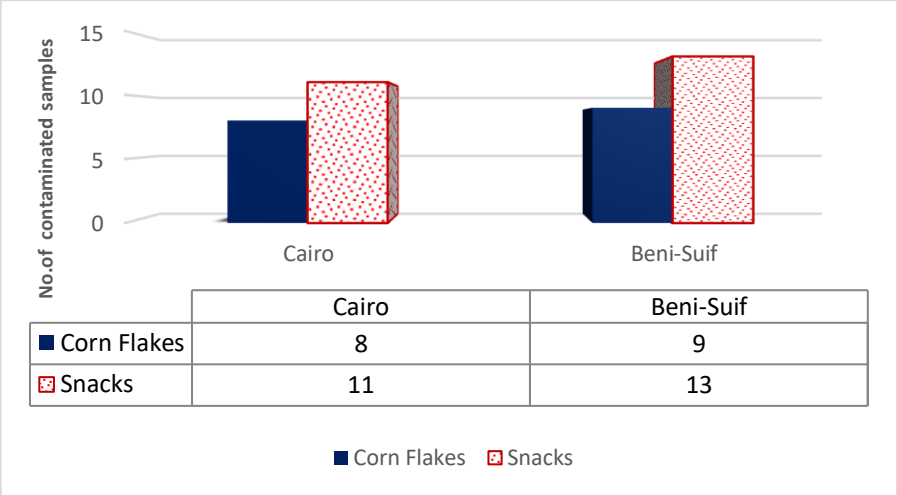
Moreover, in case of corn flakes samples in the present study, OTA was detected in 8 samples (32%) of corn-flakes samples obtained from Cairo, its concentration ranged from0.85 to 5.26µg /kg with a mean value of 2.71µ g/kg. While the corn flakes samples obtained from Beni-suif OTA, was detected in 9 samples (36%) with concentrations ranging from 0.65 to 7.92 µg/ kg and a mean value of 2.93µg/kg **Table (2) and Figure (1). Adebajo *et al* (1994)** his assessment of the OTA contamination in maize cake and maize roll snack samples collected from southern Nigeria at mean concentrations of 5.38 and 10 µg/kg provides good support for our findings. Additionally, they discovered that OTA concentrations in cereal products ranged from 0.11 to 33.9 µg/kg, and OTA concentrations in corn ranged from 22.3 to 28µg/kg. In this regard, **Toffa *et al.* (2013)** discovered that positive corn product samples had contamination levels ranging from 0.1 to 5.0 µg/ kg. Our results differ from those of the study conducted by **Majeed *et al.* (2018),** who found the OTA contamination in cornflakes samples varied from 5.1 to 15.7 µg/ kg, while contamination in corn-based goods ranged from 0 to 139.2 µg/ kg.

Table (2): Ochratoxin A concentrations by (µg/kg) in contaminated corn-based snacks and corn flakes samples from Cairo and Beni-suif:

No. of samples	OTA concentrations in positive samples (µg/Kg)			
	Cairo (n = 50)		Beni-Suif (n = 50)	
	Corn snakes (n = 25)	Corn flakes (n = 25)	Corn snakes (n = 25)	Corn flakes (n = 25)
1.	0.95±0.02	0.85±0.04	0.75±0.1	0.65±0.09
2.	1.25±0.06	0.87±0.01	0.75±0.10	0.95±0.05
3.	2.03±0.34	2.05±0.31	1.26±0.07	1.09±0.01
4.	2.46±0.10	2.25±0.10	1.96±0.07	1.37±0.22
5.	2.68±0.20	3.07±0.20	2.08±0.30	2.27±0.01
6.	2.95±0.07	3.25±0.11	2.37±0.10	3.08±0.17
7.	3.05±0.16	4.05±0.30	2.54±0.30	3.25±0.12
8.	3.53±0.20	5.26±0.30	2.79±0.30	5.79±0.07
9.	4.06±0.88	-	3.52±0.40	7.92±0.06
10.	6.05±0.23	-	4.55±0.15	-
11.	8.49±0.32	-	5.83±0.50	-
12.	-	-	8.42±0.53	-
13.	-	-	13.47±0.50	-

The results were expressed as (mean ±SD).

According to **Figure (2),** samples of corn-based snacks had a higher incidence of contamination than samples of corn flakes. This variation in frequency may be attributable to how each product is processed, how it is stored, and how it is marketed. High humidity and temperatures seen in tropical and subtropical climates make them perfect for toxin generation. Inadequate conditions during processing, marketing, and storage could encourage fungus development and raise the probability of mycotoxin generation **(Sherif *et al.*, 2009).**



	Cairo	Beni-Suif
Corn Flakes	8	9
Snacks	11	13

Figure (2): Number of contaminated samples of corn-based snacks and corn flakes by OTA from two governorates.

The maximum permitted levels of OTA for unprocessed cereals (5 µg/kg), all products derived from cereals (3 µg/kg), processed cereal-based foods, and baby foods for infants and young children (0.5 µg/kg) have been set by the Scientific Commission of the European Community **(European Commission, 2006).** As a results in **Table (3),** all samples of corn-based snacks (24 samples) that were contaminated with OTA with a range of 0.75 to 13.47 µg/kg and all samples of corn flakes (17 samples) that were contaminated with OTA with a range of 0.65 to 7.92 µg/kg are regarded to have violated the maximum allowable limits (0.5 µg/kg) for processed cereal-based foods consumed by young children. While the established maximum allowable limit (3 µg/kg) for processed cereal-based foods consumed by people of all ages was 3 µg/kg for consumers who were adults and adolescents, only ten samples of corn-based snacks that were contaminated with OTA with a range of 3.05 to 13.47 µg/kg exceeded the established limit (3 µg/kg) with a violation percentage of 20% and eight samples of corn flakes that were contaminated with OTA

with a range of 3.07 to 7.92 µg / kg over passed the established limit(3 µg/kg) with violation percentage of 16 % as shown in **Table (3).**

The acute toxicity and carcinogenicity of OTA have both received extensive research. Consuming grains infected with mycotoxin can have a range of biological impacts, including genotoxic and carcinogenic ones **(EFSA 2020: Tangni *et al.*, 2021).** Even among young people and children, corn snacks and corn flakes are highly popular and beloved snacks; yet, contamination of this type of food may provide a risk to those at young ages. Therefore, continual monitoring is required, and it is beneficial to utilise average contamination levels found in multiyear surveillance studies to more precisely estimate exposure.

Table (3): Number of contaminated samples with Ochratoxin A, mean, minimum, maximum, number of violated samples from the maximum allowable limits and percentages of violation in the fifty samples of corn-based snacks and fifty samples of Corn flakes.

Samples (n=100)	No. of contaminated samples	Mean (µg/kg)	Minimum (µg/kg)	Maximum (µg/kg)	maximum allowable limit (µg/kg)		No. of violated samples from limit		Violation percentage %	
					For Adult	for Children	For Adult limit	For Children limit		
Corn- based snacks (n=50)	24	3.66± 0.59	0.75	13.47	3.0*	0.5**	10	24	41.7	100
Corn flakes (n=50)	17	2.82± 0.45	0.65	7.92	3.0*	0.5**	8	17	47	100

***European maximum allowable limit for OTA in processed cereal products and cereal grains intended for direct human consumption is 3.0 µg / kg.**

**** European maximum allowable limit for OTA in processed cereal-based foods for young children are 0.5 µg / kg.**

3. Antifungal activity assay

Ten essential oils (cinnamon, cumin, clove, fennel, garlic, lemon grass, marjoram, peppermint, rosemary, and thyme) were tested for their antifungal activity against the growth of *A. ochraceus* on potato dextrose agar medium using the agar well diffusion technique. The growth inhibition of *A. ochraceus* was measured in (mm) diameter and expressed as inhibitory zones (Mean) as shown in **Table (4), Figure (3, 4).** Furthermore, at a concentration of (10 µl /ml), the essential oils garlic, cinnamon, clove, and marjoram were highly effective against *A. ochraceus* growth, demonstrating the largest diameters of growth inhibition (72.4, 42.3, 30.7, and 32.6 mm, respectively compared to other essential oils. However, the other essential oils (thyme, cumin, peppermint, lemon grass, fennel, and rosemary) had a moderate to low antifungal activity toward *A. ochraceus* growth, with diameters of growth inhibition of approximately (20.3, 19.2, 13.3, 12.4, 12.2, and 8.5 mm), respectively. The current results are in agreement with **(Mondéjar-López *et al.*, 2022)** who found that the essential oil of *Allium sativum* (garlic) is primarily composed of sulphured compounds that can interact with the cell's hydrogen sulphide groups and create disulfide bonds, exhibiting antifungal activity against different fungus species such as *Aspergillus spp*. Also, our results are in agreement with the report of **Moghadam *e t al.*, (2019**) who found that cinnamon, clove, thyme, cumin and caraway essential oils have fungistatic effect at different concentrations against *A. ochraceus*. However, Cinnamon and Clove essential oil were higher antifungal activity than the others due to two components of cinnamaldehyde and eugenol. Furthermore, **Hu *et al.*, (2019)** investigated The antifungal activity of seven essential oils (cinnamon, anise, clove, citronella, peppermint, pepper, and camphor) against *A. ochraceus* and

found that among all the essential oils, the cinnamon essential oil showed the highest antifungal activity with the largest inhibition zone followed by clove essential oil. The remaining essential oils exerted moderate inhibitory effects this result agree with our results. Also**, Roquia, 2012** found that among the spices and plants that have been shown to reduce toxigenic and food-borne moulds were cloves, cinnamon and garlic.

Table (4):Antifungal activity of different essential oils against *A. ochraceus* growth expressed as inhibition zone diameter.

Essential oils	Inhibition zone diameter (mm)	
	(10 µl EO /ml)	(20µl EO/ ml)
Garlic oil	**72.4*******	**>90.0*******
Cinnamon oil	**42.3*******	**61.5*******
Clove oil	**30.7******	**35.2******
Marjoram oil	**23.6*****	**33.7******
Thyme oil	**20.3*****	**25.4*****
Cumin oil	**19.2*****	**24.5*****
Peppermint oil	**13.3****	**17.6****
Lemon grass oil	**12.4****	**17.5****
Fennel oil	**12.2****	**16.5****
Rosemary oil	**8.5***	**11.3***
Means followed by the same stare* are not significantly different (P ≤ 0.05) according to Duncan' s multiple range tests.		

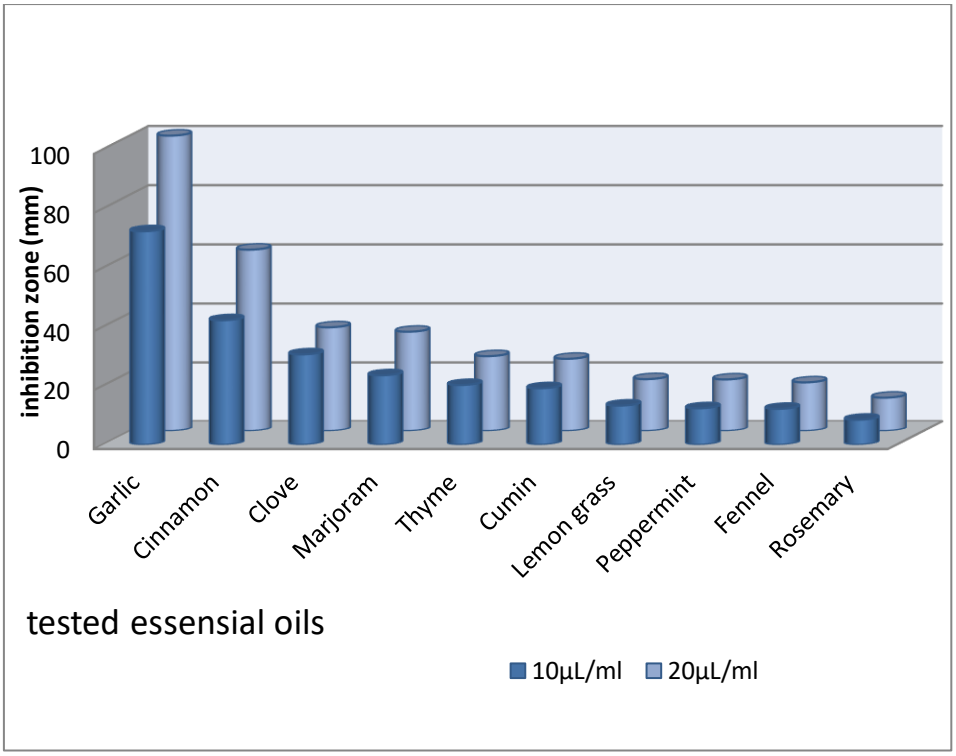


Figure (3): Antifungal activity of different essential oils against *A. ochraceus* growth.

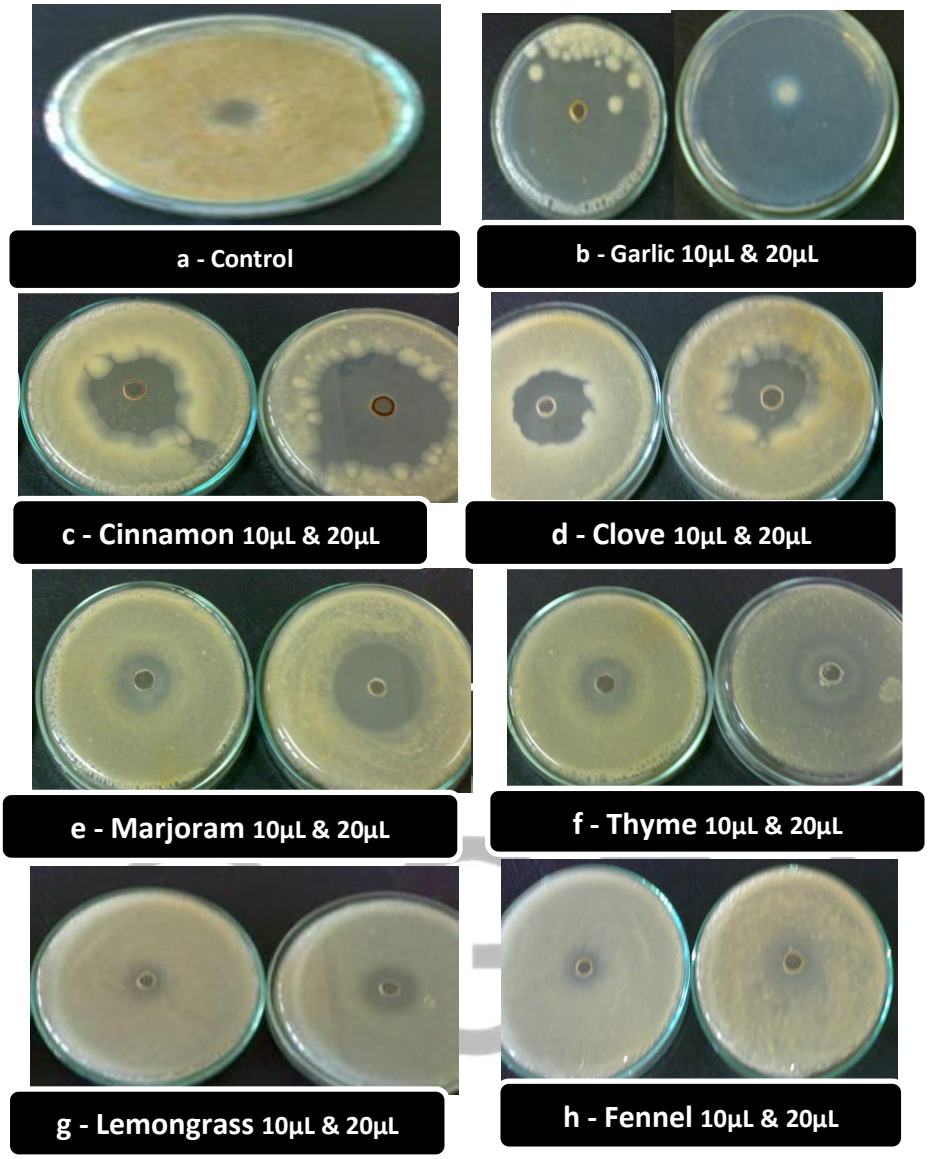


Figure (4):The antifungal activity of different essential oils concentrations (10 and 20 µL/ml) against *A. ochraceus* growth,(a) Control (b) Garlic oil (c) Cinnamon oil (d) Clove oil(e) Marjoram oil(f) Thyme oil(g) Lemongrass oil(h) Fennel oil.

4. Application of essential oils in the preservation of corn grains from OTA contamination

we use corn grains as a food model in order to evaluate the effect of (garlic, cinnamon, clove, and marjoram) essential oils in preventing OTA contamination in corn as an application study. **Table (5), Figure (5,6)** illustrated the inhibition effect of different concentrations of garlic, cinnamon, clove, and marjoram essential oils against OTA production in corn grains infected by10^6*A. ochraceus*. The control sample had the highest content of OTA95.2 µg/ 50 gm grains. The results revealed that there was a corresponding decrease in OTA production with increasing concentration of the essential oil; moreover, by using garlic essential oil at concentration of 10µl EO/50gm exhibited a significant decrease in OTA level by half of the amount that detected in the control sample with 51.9% inhibition percentage. Using the concentrations of (10, 20 and 40µl/50 gm grain) garlic essential oil, the amounts of OTA reduced to 45.7, 29.5 and 8.4 µg / 50 gm grain, respectively. However, the concentrations of 60 and 80µl EO / 50 gm grains showed complete inhibition of OTA production and the inhibition percentage was 100%.

Data in **Table (5) and Figure (5,6),** illustrated that comparing with the control sample, the concentrations 10, 20, 40, 60 and 80 µl EO /50 gm grain of cinnamon essential oil gave marked inhibition percentages of OTA by 49.7%, 65.8%, 87.3%, 96.3%and 100% when the amounts of OTA were 47.8,32.5, 12.0, 3.5 and 0 µg/50 gm grain, respectively. Complete suppression of OTA observed at concentration of80µl EO /50 gm grain.

While by using clove essential oil at concentration of 10 µl /50 gm grains induce a moderate reduction in OTA compared with garlic and

cinnamon essential oils, with inhibition percentage of 36.7% at this concentration. Furthermore, at 20 and 40 µl clove EO/50 gm grain, the amount of OTA was decreased to 40.30 and 19.03 µg/ 50 gm grain, respectively, with inhibition percentages of 57.6% and 80% respectively. Clove essential oil had a strong significant inhibitory impact starting at a dose of 60 µl EO / 50 gm grain, with a 94.6% inhibition percentage. Complete inhibition was achieved at 80 µl EO / 50 gm grain, with a 100% inhibition percentage as shown in **Table (5).**

Data in **Table (5)** demonstrated that marjoram essential oil at concentration of 10 µl EO /50 gm grains induce a low reduction in OTA levels compared with the other oils, with inhibition percentage of 31.7%. Furthermore, at doses of 20 and 40 µl marjoram EO/50 gm grain, the amount of OTA was decreased to 50.02 and 22.3µg/ 50 gm grain, respectively, with inhibition percentages of 47.4% and 76.5% respectively. Beginning at a dosage of 60 and 80 µl EO / 50 gm grain, marjoram essential oil exhibited high significant inhibitory effect, with inhibition percentages of 91.5% and 98.4%, respectively.

Table (5): Effect of different concentrations of garlic, cinnamon, clove and marjoram essential oils in controlling OTA production.

Treatments (µl/50 gm)	OTA concentrations (µg/gm)							
	Garlic oil		Cinnamon oil		Clove oil		Marjoram oil	
	Mean ± SD	IP	Mean ± SD	IP	Mean ±SD	IP	Mean ± SD	IP
Control	95.2	0	95.2	0	95.2	0	95.2	0
10	45.7±0.9*	51.9	47.8±0.91*	49.7	60.2±0.8*	36.7	65.01±0.9**	31.7
20	29.5±0.7**	69.0	32.5±0.76**	65.8	40.3±0.7**	57.6	50.2±0.4**	47.4
40	8.4±0.02***	91.1	12.0±0.05***	87.3	19.03±0.1***	80.01	22.3±0.2***	76.5
60	0.00***	100	3.5±0.05***	96.3	5.06±0.05***	94.6	8.03±0.2****	91.5
80	0.00***	100	0.00***	100	0.00***	100	1.44±0.02***	98.4

IP: Inhibition Percentage %.
***** indicate high significant difference of values at P<0.001.**
The Duncans multiple range test at P ≤0.05 was used.

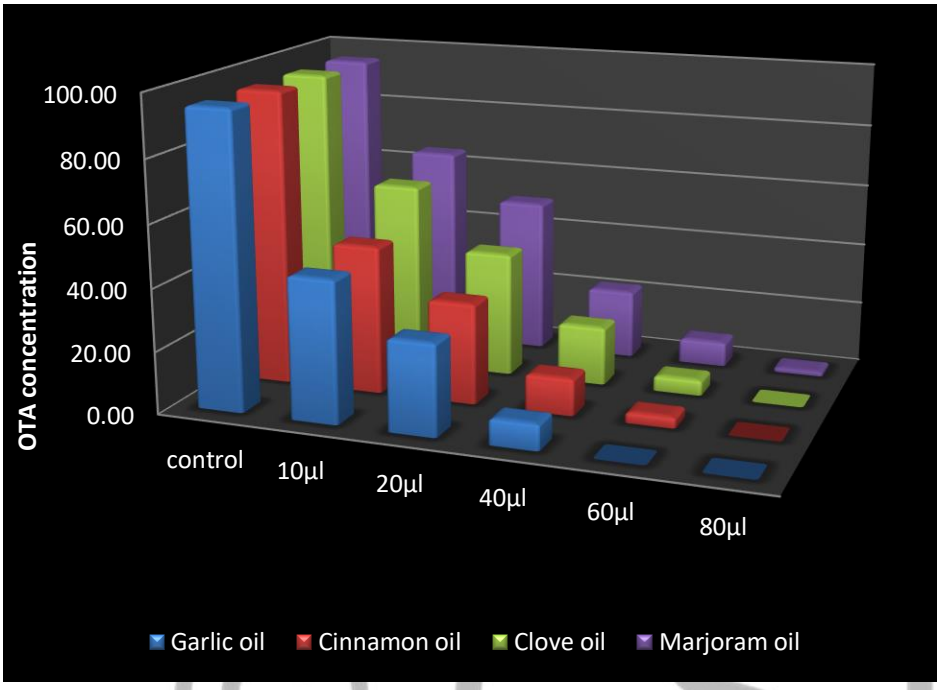


Figure (5): Effect of different concentrations of garlic, cinnamon, clove and marjoram essential oils in controlling OTA production in corn grains.

Figure(6): Effect of different concentrations of (a) garlic,(b) cinnamon, (c)clove and(d) marjoram essential oilsin controlling OTA production in corn grains.

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