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Prevalence of Toxigenic Fungi in Egyptian Smoke-Dried Herring Fish: I- Antifungal Activity of Basil (*Ocimum basilicum* L.) Essential oil

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A SURVEY was carried out on malt salt agar media to isolate and evaluate mycoflora occurring in samples of smoke-dried herring fish (*Clupea harengus* L.) that are ready for human consumption in Egypt. *Aspergillus* species were the most abundant fungi followed by *Penicillium* species then *Fusarium* species. Fungal genera of *Cladosporium*, *Epicoccum*, *Mucor*, and *Rhizopus* species were also isolated. *A. flavus* and *A. fumigatus* were the most frequently isolated aspergilli. *P. griseofulvum* was the most species common among penicillia, and *F. solani* was the predominant *Fusarium* species. *C. cladosporioides* was recovered in a high incidence. Findings of mycotoxins formation by the isolated mycoflora showed that some mycotoxins (aflatoxins, cyclopiazonic acid and kojic acid) were produced by some isolates of *A. flavus*. Some isolates of *A. niger* and *A. fumigatus* were able to produce kojic acid. *A. ochraceus* isolates produced penicillic acid but were not able to produce ochratoxin A. Two isolates of *A. parasiticus* were aflatoxin B_1-producing and one isolate of *A. versicolor* produced sterigmatocystin. Concerning *Fusarium* species, some isolates of *F. oxysporum* and *F. solani* had the ability to produce cyclosporin A and zearalenone. The antibiotic, griseofulvin, citrinin and patulin were the characteristics metabolites produced by the toxigenic isolates of *Penicillium* species. This study was also extended to evaluate the efficacy of different concentrations of *Ocimum basilicum* L. essential oil on *in vitro* activity against the toxigenic isolated moulds. The recorded minimum inhibitory concentration (MIC) of the essential oil was 15% (v/v) with all the tested mould strains except *A. niger*, *A. ochraceus* (the MIC value was 10%, v/v) and *Penicillium* species (the MIC value was 20%, v/v). This oil was found to be fungicidal at all the tested concentrations. These findings indicate the possibility to use *O. basilicum* essential oil as a substitute for chemical additives to control fungal growth and toxins production

Keywords: Mycoflora, Mycotoxins, Smoke-dried herrings, *Ocimum basilicum* L., Essential oil, Antifungal.

Microscopic filamentous fungi are ubiquitous microorganisms with a great capacity to colonize many kinds of substrates and to proliferate under extreme environmental conditions (Nguefack *et al*., 2009). *Aspergillus*, *Penicillium* and *Fusarium* are the most important causing spoilage of food stuffs **(**Singh *et al*., 1991). Mould contamination is often associated with unpleasant appearance, odor

and changes in taste and nutritional value of foods (Filtenborg *et al*., 1996). Moulds produce mycotoxins that can be teratogenic, carcinogenic or cause feed refusal and emesis (Yu *et al*., 2003 and Magan *et al*., 2004). Toxic syndromes caused by mycotoxin ingestion by humans and animals are indicated as mycotoxicosis (Galvano *et al*., 2001).

Fish is an important commodity in the diet of most people in Egypt. Nutritionally, fish proteins are highly digestible and at least as good as red meat with respect to content of essential amino acids (Norman & Joseph, 1996). Because of the great tendency of fish to spoil, a number of methods of preservation have been developed over the years. The most basic methods are smoking and salting with subsequent drying. This is effective but such preserved fish are not accepted in all cultures. Other societies have found that such preserved fish is highly desirable (Oyewole *et al*., 2006).

Little information has been published on toxigenic mycoflora of smoke-dried fish. The most important studies to date are those of Jonsyn & Lahai (1992) which demonstrated the presence of *A. flavus* and *A. ochraceus* in smoke-dried fish of *Ethmolosa* sp. that are potential for production of aflatoxins and ochratoxin A, respectively. A related study on smoked shark fish (Essien *et al*., 2005) revealed the predominance of *Aspergillus*, *Penicillium*, *Eurotium*, *Fusarium* and *Cladosporium* species in this product, although the potential production of mycotoxins types by these mycoflora, were not detected in their study.

Plant extracts, especially volatile essential oils from medicinal plants, have been reported to possess antimicrobial activity against a variety of food-borne, human and plant pathogens and pests (Burt, 2004 and Soylu *et al*., 2007). The antimicrobial properties of basil essential oils have also draw a great attention from many researchers (Suppakul *et al*., 2003; Piyo *et al*., 2009 and Saggiorato *et al*., 2009). The major components of basil (*Ocimum basilicum* L.) responsible for its antimicrobial properties had been analyzed previously (Oplachenova & Obreshkova, 2003; Suppakul *et al*., 2003 and Viyoch *et al*., 2006).

The objective of this study was to identify and evaluate the mycoflora occurring in different samples of smoke-dried herring fish that are ready for human consumption in Egypt. In addition, the potential mycotoxins production of isolated mould strains and the *in vitro* evaluation of *O. basilicum* L. essential oil along with its fungicidal activities against the isolated mould strains were investigated.

Material and Methods

Samples collection

Twenty-eight samples of herring fish (*Clupea harengus* L.) were randomly purchased from seven Egyptian fish processing centers namely; Abou El-Dahab, El-Bahreen, El-Gamal, El-Malekha, El-Mohamadia, El-Warda El-Baieda and Egypt (four samples each) at Sharkia Governorate. The salt concentration in the samples was in the range of 3.5 to 15%. The samples were handpicked with

sterilized glove hands and taken to the laboratory in a separate sterilized polyethylene bags to avoid contamination from handling.

Isolation and identification of fungi

Serial dilution technique was adopted for isolation of fungi according to Fafioye *et al.* (2008). Ten grams of tissue portion of each fish sample was cut from the abdominal region with a sterile forceps, grinded aseptically in porcelain mortar and blended for 10 min in a high-speed blender with 90 ml of sterile 0.1 % peptone-water. Serial dilutions were carried out in the same diluent and 1 ml of each dilution was spread on malt-salt agar plate containing 20 g of malt extract (Difco), 50 g of NaCl, 400 µg chlorotetracycline and 13 g of agar (Difco) per liter. The chlorotetracycline was added just prior to pouring the plates. An additional portion of 10 g tissue portion from each sample were surface disinfected for 1 min in 5 % NaOCl, rinsed three times with sterile distilled water and dried aseptically in a laminar flow cabinet for 2 hr before blending and plating as described above. All plates (triplicate for each sample) were incubated at 28± 2ºC for 7 days. The results were expressed as CFU per gram of fish sample. Colonies representative for each type were transferred for sub-culturing on to plates with malt extract autolysate (MEA) agar.

Taxonomic identification of the different genera and species was made according macroscopic and microscopic morphological criteria in accordance with appropriate keys. *Aspergillus* species were identified according to Raper & Fennel (1965) and Samson *et al.* (2007 a, b). For the species belonging to the *Penicillium* genus, growth was also observed on three selective media; Czapek yeast autolysate (CYA) agar, malt extract autolysate (MEA) agar and yeast extract sucrose (YES) agar (Frisvad & Samson, 2004). *Fusarium* species were cultured on potato dextrose agar and identified with other fungal species according to Domsch *et al.* (1980) and Gilman (1957).

In vitro production of mycotoxins by fungal isolates

An agar disc (6 mm, diameter) of each pure fungal isolate growing on to MEA agar plate for 7 days, was transferred aseptically to 250 ml Erlenmeyer flasks (triplicate for each fungal isolate), each containing 50 ml of freshly prepared, sterilized broth medium (Aziz & Moussa, 2002). The medium contained 5 g of Difco mycological peptone, 20 g of yeast extract, 40 g of sucrose and 1L of distilled water (pH 5.5). Flasks were stationary incubated in the dark for 14 days at 28 ºC.

Mycotoxins analysis

Extraction of mycotoxins from fungal cultures

At the end of the incubation period, the contents of each flask (medium + mycelium) were homogenized with either chloroform (for extraction of aflatoxins, citrinin, cyclopiazonic acid, griseofulvin, ochratoxin A and sterigmatocystin) or ethyl acetate (for extraction of cyclosporin A, patulin, penicillic acid and zearalenone) by a rotary shaker (200 rpm) overnight. The

extraction procedure was repeated three times. The extracts were sequentially filtered through anhydrous sodium sulfate then concentrated to near dryness.

Simple screening methods were used to detect the formation of some mycotoxins by the isolated fungal species. For aflatoxin-producing isolates, the presence of aflatoxins was determined by examining the reverse side of Petridishes cultivated with the tested isolate on coconut agar medium under UV-light (366 nm) (Lin & Dianese, 1976). For screening of kojic acid-producing isolates, the presence of kojic acid in their water dissolved extract was determined by the color test of Bentley (1957) using $FeCl_3$ as a reagent. Detection of penicillic acid in water dissolved extract of the screened fungal isolates was done by adding excess of concentrated ammonia as described in the color test of Bentley & Keil (1962). These tests were used as rapid screening methods to detect aflatoxins, kojic acid and penicillic acid produced by the tested fungal isolates. However when positive, the confirmation of their formation was achieved by thin-layer chromatographic technique as shown, as follows.

Thin-layer chromatography (TLC) of mycotoxins

Mycotoxin detection was performed using TLC on silica gel plates (20x 20 cm, 0.25 mm thick, GF-254), at room temperature in a non-saturated chamber. The concentrated extracts were examined on the TLC plates together with the following standards: Aflatoxins (B_1, B_2, G_1 and G_2), citrinin, kojic acid, ochratoxin A, patulin, penicillic acid, sterigmatocystin, zearalenone (Sigma Co., Louis St., USA), cyclopiazonic acid (Wako, Pure Chemical Industries, Ltd., Japan), cyclosporin A (Sandimmun, Novartis Pharma AG, Basle, Switzerland), griseofulvin (Kahira Pharm.& Chem. Ind. Co., Cairo, Egypt). For some acidic metabolites like cyclopiazonic acid, citrinin, penicillic acid, the plate was impregnated in an 8 % solution of oxalic acid in methanol (El-Shanawany *et al*., 2005). Toluene-ethyl acetate-90% formic acid (6:3:1; v/v/v) eluting mixture was used to develop the TLC plates. The mycotoxins were visualized in UV light at 254 and 366 nm (UV-254 and UV-366) before and after treatment as listed in Table 1.

In vitro antifungal testing

Essential oil

Ocimum basilicum L., commonly named basil, was kindly obtained from Sekem Company, Hikstep region, Cairo, Egypt. This essential oil was selected on the basis of its highest broad spectrum and activity against the tested fungi during a screening test was done on a total of 9 types of essential oils.

Preparation of different concentrations of essential oil

Different volumes of the basil essential oil were diluted in different volumes of an aqueous solution of tween-80 to give the following concentrations: 5, 10, 15, 20, 25and 30 %.

TABLE 1. Visualization techniques used for detection of mycotoxins on TLC plates .

Mycotoxin	Fluorescence colour		Visualization	Ref.
	UV-254	UV-366		
Aflatoxins				Scott *et al*. (1970)
Aflatoxin B_1	Faint blue	Blue		
Aflatoxin B_2	Faint blue	Blue		
Aflatoxin G_1	Faint green	Green		
Aflatoxin G_2	Faint green	Green		
Citrinin	Yellow	Yellow	I_2[a], orange	(Scott *et al*. 1970; Grost-Allman & Steyn, 1979)
Cyclopiazonic acid	Purple		EH reagent, bluish-purple	Mphande *et al*. (2004)
Cyclosporin A	Violet		I_2[a], brown	Dreyfuss *et al.*(1976)
Griseofulvin	Blue	Bright blue		Cole *et al*. (1970)
Kojic acid		Pale green	$FeCl_3$+UV(366), dark (F)	Ďuračková *et al.* (1976)
Ochratoxin A	Blue	Blue-green	$FeCl_3$, purple brown	Grost-Allman & Steyn (1979)
Patulin		Yellow	PH+heating, yellow	López-Diáz *et al*. (1996)
Penicillic acid		Dark blue	Amm.+heating+UV (366), blue (F)	Ciegler & Kurtzman (1970)
Sterigmatocystin		Red brown	$AlCl_3$ +heating+ UV(366), yellow (F)	López-Diáz *et al*. (1996)
Zearalenone	Blue-green	Faint blue	$AlCl_3$ +heating+ UV(366), dark blue (F)	Aziz *et al*. (1998)

[a] Plate kept in iodine vapor for 15 min.; EH, Ehrlich's reagent (1g of dimethylaminobenzaldehyde dissolved in a mixture of 75 ml of ethanol and 25 ml of concentrated HCl); $FeCl_3$, 1% solution of Iron (III) chloride in ethanol; PH, phenylhydrazine 4% in water; Amm., ammonia vapour treatment for 10 min.; $AlCl_3$, 20% solution (w/v) in ethanol; heating, 105-110ºC/5-10 min; (F), Fluorescence.

Antifungal assay of O. basilicum essential oil

The effectiveness of each concentration was evaluated using cut plate diffusion method in which, a hole of 1 cm diameter was cut in each plate using a sterilized cork borer. The plates containing 25 ml Czapek-Dox's agar was seeded with 0.1 ml fungal spore suspension (10^5 spores/ml) from 7 days-old culture. Drops of water dissolved agar (2%, w/v) were put in holes and 70 µl of the tested essential oil was pipetted into each hole. The plates were refrigerated for 2hr and incubated at 28± 2ºC for 5 days. Control plates without oil (with aqueous solution of tween-80 only) were also prepared in the same way. Diameter of inhibition zones was measured and the minimum inhibitory concentration (MIC) for each fungal strain was determined as lowest concentration of the essential oil that resulted in a complete inhibition of visible growth. Meanwhile, the fungicidal/fungistatic nature of *O. basilicum* essential oil was detected by cutting discs from the inhibition zones with sterilized cork borer and reinoculating on to fresh Czapek-Dox's agar and the revival of each fungal growth is being recorded (Thompson, 1989).

All tests were carried out in triplicate. The results of antifungal activity tests of basil essential oil were analyzed by ANOVA test for equality of means.

Results and Discussion

Smoke-dried herring fish (*Clupea harengus* L.) is a popular food product in Egypt due to its taste with both rich and poor, relatively cheap and is available all year-round. As might be expected, the surface disinfected herring samples showed a lower fungal contamination than that recorded in non-surface disinfected samples (Table 2). The highest total fungal count either in surface disinfected or non-surface disinfected herring samples was detected in the samples of El-Gamal, El-Malekha, El-Warda El-Baieda and El-Mohamadia, where as Abou El-Dahab, El-Bahreen and Egypt herrings had the lowest fungal counts. Fafioye *et al.* (2008) reported that the contamination of smoke-dried and stored fish with fungal species was as a result of hot climate that provide favorable condition for mould spores to germinate and grow on dried fish.

TABLE 2. Fungal propagules counts in colony forming units (CFU/g) for different samples of smoke-dried herring fish (*Clupea harengus* L.) on malt-salt agar.

Smoked herring fish samples	Total count per gram	
	NSD	SD
Abou El-Dahab	$3.00 X10^2$	$1.66 X10^2$
El-Bahreen	$4.66 X10^2$	$3.00 X10^2$
El-Gamal	$6.66 X10^4$	$6.00 X10^4$
El-Malekha	$4.70 X10^4$	$4.30 X10^4$
El-Mohamadia	$1.10 X10^3$	$1.03 X10^3$
El-Warda El-Baieda	$7.00 X10^3$	$4.30 X10^3$
Egypt	$8.33 X10^2$	$5.66 X10^2$

NSD, non surface disinfected.
SD, surface disinfected.

Results presented in Table 3 indicate that almost all smoked herring fish samples examined in this study were contaminated with moulds. *Aspergillus* species (20-57.5 %) were the most abundant fungi. *Penicillium* species (7.1-36.4 %) were found in most examined samples except Egypt herrings. *Fusarium* species were also detected in most samples (3.90- 44.4 %) except El-Mohamadia and El-Warda El-Baieda herrings. The mycological analysis of the smoked herring samples showed that the isolated fungal genera other than *Aspergillus*, *Penicillium* and *Fusarium* species, were *Cladosporium*, *Epicoccum*, *Mucor* and *Rhizopus* species. Similarly, Essien *et al.* (2005) found that *Aspergillus* and *Penicillium* species were the predominant moulds among *Eurotium*, *Fusarium* and *Cladosporium* species in smoked shark fish (*Chlamydoselachus angunicus*). Also, Fafioye *et al.* (2008) showed that the isolated fungi from various types of smoke-dried fish were *Aspergillus*, *Penicillium*, *Fusarium*, *Mucor* and *Rhizopus* species.

TABLE 3. Total fungal, *Aspergillus, Penicillium,* and *Fusarium* counts in different samples of smoked herring fishes .

Smoked herring fish samples	Total count per gram	*Aspergillus* sp.		*Penicillium* sp.		*Fusarium* sp.		Other genera	
		Total count per gram	%	Total count per gram	%	Total count per gram	%	Total count per gram	%
Abou El-Dahab	$3.00X10^2$	$0.67X10^2$	22.3	$0.33X10^2$	11.0	$1.33X10^2$	44.4	$0.67X10^2$	22.3
El-Bahreen	$4.66X10^2$	$2.33X10^2$	50.0	$0.33X10^2$	7.10	$1.67X10^2$	35.8	$0.33X10^2$	7.10
El-Gamal	$6.66X10^4$	$1.33X10^4$	20.0	$2.00X10^4$	30.0	$1.00X10^4$	15.1	$2.33X10^4$	34.9
El-Malekha	$4.70X10^4$	$2.70X10^4$	57.5	$1.34X10^4$	28.5	$0.33X10^4$	7.00	$0.33X10^4$	7.00
El-Mohamadia	$1.10X10^3$	$5.00X10^2$	45.4	$4.00X10^2$	36.4	0.00	0.00	$2.00X10^2$	18.2
El-Warda El-Baieda	$7.00X10^3$	$4.00X10^3$	57.1	$1.00X10^3$	14.3	0.00	0.00	$2.00X10^3$	28.6
Egypt	$8.33X10^2$	$1.67X10^2$	20.1	0.00	0.00	$0.33X10^2$	3.90	$6.33X10^2$	76.0

The incidence of fungal flora isolated from smoke-dried herring fish samples is presented in Table 4. A total of 16 species of fungi belonging to 7 genera were isolated and identified. Six species of *Aspergillus* were isolated; the most prevalent were *A. flavus* (100 %), *A. fumigatus* (92.9 %) and *A. niger* (78.6%). *Cladosporium cladosporioides* was isolated from 92.9 % of samples. Three species of *Penicillium* were isolated. The samples contained high levels of *P. griseofulvum* (78.6 %). By contrast, *P. dipodomyicola* and *P. expansum* were isolated at low frequency as well as that of *Epicoccum* sp. (14.3 %). With respect to *Fusarium* species, *F. solani* (42.9 %) was isolated in a higher contamination frequency than that of *F. oxysporum* (28.6 %). The data also showed that the most prevalent mucorales was *Mucor lamprosporus* which detected in 64.3 % of samples. The degree of analysis on the most frequently encountered fungi on all the smoke-dried herring samples can be arranged, as follows: *A. flavus* > *A. fumigatus* = *C. cladosporioides* > *A. niger* = *P. griseofulvum* > *M. lamprosporus*. These results were in accordance with those reported for smoke-dried fish (Fafioye *et al.*, 2002; 2008). Domsch *et al.* (1981) postulated that the contamination of feed stuffs with fungal species was as a result of natural extraneous contamination by dust following storage in humid conditions.

The number of toxigenic isolates and the mycotoxins produced are shown in Table 4. In this study, all *A. flavus* isolates were found to be aflatoxin-producing (6 of them produced aflatoxins B_1 and G_1; 4 isolates produced aflatoxins B_1 and B_2, while only 2 isolates produced aflatoxins B_1, B_2, G_1 and G_2). These results were in agreement with the finding observed by several authors (Jiménez *et al.*, 1991; El-Shanawany *et al.*, 2005 and Cary & Ehrlich, 2006). Jiménez *et al.* (1991) found that 29 isolates of *A. flavus* were aflatoxin-producing (6 isolates produced aflatoxins B_1, B_2, G_1 and G_2; 8 isolates produced aflatoxin B_1; 9 isolates yielded aflatoxins B_1 and B_2 and the remaining produced aflatoxins B_1 and G_1). Eleven of the twelve *A. flavus* isolates produced cyclopiazonic acid, while kojic acid was produced by eight isolates. *A. flavus* was reported to produce cyclopiazonic acid (Bamba & Sumbali, 2005) and kojic acid (Doster *et al.*, 1996).

Eleven isolates of *A. fumigatus* were examined for kojic acid production. The results revealed that 2 isolates were kojic acid-producing. It is also clear that 7 of 10 isolates of *A. niger*, had the ability to produce kojic acid (Table 4). In a previous study, El-Shanawany *et al.* (2005) found that 4 of 12 isolates of *A. fumigatus* and all tested isolates (four) of *A. niger* had the ability to produce kojic acid. Kojic acid was previously recorded as a neurotoxin for human but also has antimicrobial properties (Wyllie & Morehouse, 1977).

Aspergillus ochraceus was represented by 2 isolates and their extracts proved that the two isolates had the ability to produce penicillic acid but could not able to produce ochratoxin A. Penicillic acid had been produced by *A. ochraceus* isolated from red kidney beans (Sanchis *et al.*, 1988) and has been proven to have carcinogenic nature, hazardous effects and antimicrobial properties (Macri *et al.*, 2002 and Ezzat *et al*., 2007).

TABLE 4. Incidence of fungal species on smoke-dried *Clupea harengus* L. and mycotoxins production by toxigenic isolates.

Fungal sp.	Frequency of occurrence [a] (%)	Number of isolates		Mycotoxin detected
		Examined	Positives	
Aspergillus flavus	100	12	12	Aflatoxins[b]
		12	11	Cyclopiazonic acid
		12	8	Kojic acid
A. fumigatus	92.9	11	2	Kojic acid
A. niger	78.6	10	7	Kojic acid
A. ochraceus	14.3	2	2	Penicillic acid
A. parasiticus	21.4	3	2	Aflatoxin B_1
A. versicolor	28.6	4	1	Sterigmatocystin
Cladosporium cladosporioides	92.9	-	-	N.D.
Epicoccum sp.	14.3	-	-	N.D.
Fusarium oxysporum	28.6	4	1	Cyclosporin A
		4	2	Zearalenone
F. solani	42.9	6	1	Cyclosporin A
		6	4	Zearalenone
Mucor lamprosporus	64.3	-	-	N.D.
M. petrinsularis	28.6	-	-	N.D.
Penicillium dipodomyicola	14.3	2	2	Griseofulvin
P. expansum	14.3	2	2	Citrinin
		2	2	Patulin
P. griseofulvum	78.6	8	8	Griseofulvin
		8	8	Patulin
Rhizopus stolonifer	21.4	-	-	N.D.

[a] Number of samples from which mould were isolated / Total number of samples (%).
[b] two isolates yielded aflatoxins B_1, B_2, G_1& G_2 ; 4 isolates yielded aflatoxins B_1& B_2 and 6 isolates yielded aflatoxins B_1& G_1.
N.D., not detected

Three isolates of *A. parasiticus* were screened in this study for aflatoxin-production; the extracts showed that two isolates were aflatoxin B_1-producing. Aziz *et al.* (1998) isolated *A. parasiticus* with a high tendency for aflatoxin-production from some medicinal plant samples and spices.

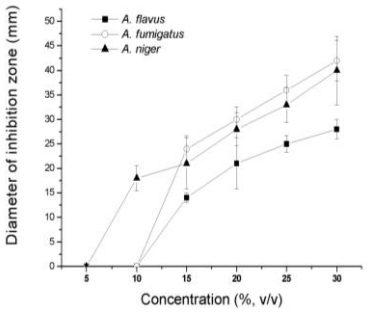
The extracts of 4 isolates of *A. versicolor* were screened for sterigmatocystin production. Only one isolate produced sterigmatocystin (Table 4). This finding is consistent with results of others. Jiménez *et al.* (1991) found 2 of 6 *A. versicolor* isolated from sunflower seeds produced sterigmatocystin. Sterigmatocystin is a carcinogenic compound that had been shown to affect various species of experimental animals (Van der Watt, 1974).

A total of 10 *Fusarium* isolates (represented by 4 isolates of *F. oxysporum* and 6 isolates of *F. solani*) were examined for production of their respective toxins. Only one isolate among the tested isolates of both species had the ability to produce cyclosporin A. Production of cyclosporin A by *F. oxysporum* and *F. solani* have been reported (Sawai *et al.*, 1981 and Sallam *et al.*, 2003). Cyclosporin A improved the

diminish of transplant rejection as immunosuppressive compound (Borel, 1986), however it was found to be nephrotoxic (Mihatsch *et al*., 1995). With regard to the screening of zearalenone production, 2 isolates of *F. oxysporum* and 4 isolates of *F. solani* were positive. Zearalenone was reported to be produced by *F. oxysporum* isolated from red kidney beans and nuts and sunflower seeds (Sanchis *et al*., 1988 and Jiménez *et al*., 1991) and *F. solani* (Richardson *et al*., 1985). It was demonstrated to have estrogenic properties (Meronuck *et al*., 1970).

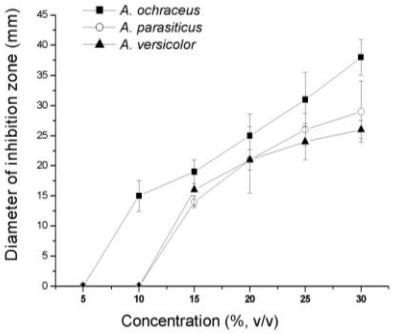
Two isolates of *Penicillium dipodomyicola* were examined for production of the antibiotic, griseofulvin and patulin and the results showed that griseofulvin was detected in the extract of the two isolates but patulin was not detected. The mycotoxicosis of *P. dipodomyicola* and its ability to produce griseofulvin was reported (Frisvad & Samson, 2004). All the tested isolates (eight) of *P. griseofulvum* were found to have the ability to produce griseofulvin and patulin. Also, Jiménez *et al.* (1991) found that 6 of 11 isolates of *P. griseofulvum* yielded griseofulvin and patulin. The data (Table 4) revealed that citrinin and patulin were detected in the extracts of 2 tested isolates of *P. expansum*. Production, teratogenicity and toxicity of patulin and citrinin from *P. expansum* were previously recorded (Ciegler *et al*., 1977).

A well cut diffusion method was employed for screening the antifungal activity of different concentrations of basil (*Ocimum basilicum* L.) essential oil (5%-30%, v/v) against the isolated toxigenic fungi. Basil essential oil revealed the highest antifungal activity against the isolated fungi among nine essential oils tested (data not shown). The obtained results (Fig. 1) indicated that the recorded MIC value of basil essential oil was 15% with all tested fungal strains except with *A. niger*, *A. ochraceus* (the recorded MIC value was 10%) and *Penicillium* species (the recorded MIC value was 20%) but the concentration of the oil at 5% showed no any inhibitory effect on the growth of the tested fungi. Piyo *et al.* (2009) found that basil essential oil at a concentration of 0.6% v/v showed mycelium growth inhibition of *Fusarium moniliforme* (100%), *F. proliferatum* (49.6%), *Pyricularia grisea* (100%). Furthermore, they showed that at 2.0% v/v, *Bipolaris oryzae*, *Alternaria brassicicola* and *Aspergillus flavus* were inhibited by 97.4, 94.6 and 59.2%, respectively. With all tested fungal strains, the diameter of inhibition zone was clearly proportional to the concentration of basil essential oil applied to each well. Among the tested aspergilli, *A. fumigatus* and *A. niger* showed their highest sensitivity to the inhibitory effect of the essential oil at 30% (v/v) and gave rise to inhibition zone of 42 and 40 mm diameter, respectively; however *A. versicolor* and *A. parasiticus* showed the lowest sensitivity to the antifungal activity at the same concentration of the essential oil and gave rise to an inhibition zone of 26 and 29 mm diameter, respectively. The fungicidal activity of basil essential oil against wide range of fungi was reported previously (Suppakul *et al*., 2003 and Saggiorato *et al*., 2009). Resubculturing from the inhibition zone of the tested fungi on Czapek-Dox's agar plates emphasized that this essential oil has a fungicidal activity. The antifungal activity of basil (*Ocimum gratissimum* L.) was attributed to presence of thymol and other phenol derivatives in its composition (Koba *et al*., 2009).



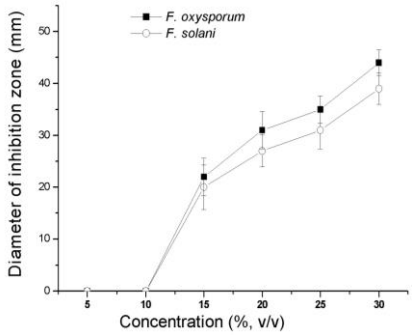
a

P< 0.05 at all the tested concentrations except at 20% (p=0.07). This indicates that the inhibitory effect of the essential oil at 15, 25 and 30 was significant on the tested aspergilli. P< 0.01 at the concentration 25%. This indicates that the inhibitory effect of this concentration on the tested aspergilli was high significant.



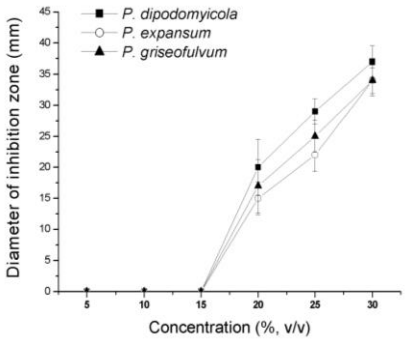
b

P >0.05 at all the tested concentrations except at 30% (P=0.019). This indicates that 30% was the lonely concentration that showed significant inhibitory effect on the tested aspergilli.



c

P< 0.05 at the concentration 30%. This indicates that 30 % is the lonely concentration that showed significant inhibitory effect on the tested *Fusarium* species



d

P < 0.05 at the concentration 25%. This indicates that 25% is the lonely concentration that showed significant inhibitory effect on the tested *Penicillium* species.

Fig.1. Inhibitory effect (represented by inhibition zones diameters, mm) of various concentrations of *Ocimum basilicum* L. essential oil on the growth of the isolated toxigenic fungi.

Conclusion

The present study is the first survey on moulds associated with Egyptian smoke-dried herring fish and capable of mycotoxin production by several species of *Aspergillus*, *Penicillium* and *Fusarium*. This constitutes not only an economic problem, but also a potential health hazard. The results also indicated that the diluted preparations of *O. basilicum* essential oil showed a broad spectrum of antifungal activity against the growth of toxigenic moulds contaminating smoke herring fish. So, *O. basilicum* essential oil appears to have promise as a safe alternative natural food preservative. Further work should focus on identifying the most important contamination sources of smoke-dried herring fish and possible preventive measures in the production and preservation process.

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تواجد الفطريات السامة في أسماك الرنجة المصرية الجافة-المدخنة. ١- النشاط الضد فطري لزيت الريحان الأساسي

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في هذا البحث ، تم عمل مسح لعزل الفطريات وتقييم تواجدها في عينات مختلفة من أسماك الرنجة الجافة-المدخنة والمعروضة مباشرةً للإستهلاك المحلي الأدمي في مصر. وقد إتضح أن أنواع *الأسبرجلس* يتبعها أنواع *البنسيليوم* ثم أنواع *الفيوزاريوم* على الترتيب ، هي الأنواع الأكثر تواجداً وإنتشارًا. إشتملت نتائج العزل أيضاً علي تواجد بعض الأجناس الفطرية الأخرى مثل *الكلادوسبوريوم* و*الإيبيكوكم* و *الميوكر* و أنواع *الرايزوباس* . ومن بين أنواع *الأسبرجلس* المعزولة فقد وجد أن كلا من *الأسبرجلس فلافس* و *الأسبرجلس فيوماجاتس* هما أكثر الفطريات إنتشاراً. ومن بين أنواع *البنسيليوم* ، وجد أن *فطرة البنسيليوم جرسيوفلفم* هي الأكثر تواجداً بينما كانت فطرة *الفيوزاريوم سولاني* هي الأكثر تواجداً من بين أنواع *الفيوزاريوم* المعزولة. تبين أيضاً من نتائج عزل الفطريات أن فطرة *الكلادوسبوريوم كلادوسبوريوديس* لها تواجد عالي من بين الفطريات المعزولة.

أوضحت نتائج تحاليل السموم الفطرية المنتجة بواسطة الفطريات المعزولة أن بعض عزلات فطرة *الأسبرجلس فلافس* تنتج بعض السموم الفطرية مثل سموم الأفلاتوكسينات وحمض السيكلوبيازونيك وحمض الكوجيك. ولوحظ أيضاً أن بعض عزلات فطريات *الأسبرجلس نيجر* و *الأسبرجلس فيوماجاتس* كانتا لهما القدرة على إنتاج حمض الكوجيك. تبين أن عزلات فطرة *الأسبرجلس أوكراثيوس* تنتج السم الفطري حمض البنسيليك ولا تنتج سم الأوكراتوكسين (أ). وإتضح أن عزلتين فقط من فطرة *الأسبرجلس باراثيتيكاس* كانتا لهما القدرة على إنتاج سم الأفلاتوكسين (ب١) وأن عزلة واحدة فقط من فطرة *الأسبرجلس فيرسيكلور* تنتج السم الفطري ، ستريجماتوسيستين. عندما تم عمل مسح للسموم الفطرية المنتجة بواسطة أنواع *الفيوزاريوم* المعزولة ، فقد تبين أن بعض عزلات من فطرة *الفيوزاريوم أوكسيسبورم و الفيوزاريوم سولاني* كانتا لهما القدرة على إنتاج السيكلوسبورين (أ) والزيرالينون.

أظهرت نتائج تحاليل إنتاج السموم الفطرية لأنواع من *البنسيليوم* أن المضاد الحيوي جرسيوفلفين والسم الفطري السترنين والسم الفطرى الباتيولين هم المواد المميزة والمنتجة بواسطة انواع *البنسيليوم* السامة المعزولة.

إمتد هذا البحث لعمل دراسة تقييمية للنشاط الضد فطري لتركيزات مختلفة من الزيت الأساسي ، الريحان علي نمو الفطريات السامة المعزولة. وإتضح أن أقل تركيز من الزيت مثبط لنمو كل الفطريات هو ١٥٪(حجم/حجم) ما عدا فطريات *الأسبرجلس نيجر* و *الأسبرجلس أوكراثيوس* (فقد كان أقل تركيز مثبط لنموهما هو ١٠٪ ، حجم/حجم) وفطريات *البنسيليوم* (كان أقل تركيز مثبط للنمو هو ٢٠ ٪ ، حجم/حجم). وقد لوحظ أن هذا الزيت له تاثير قاتل علي الفطريات السامة المعزولة. وهذه النتائج تشير إلى إمكانية إستخدام زيت الريحان كمادة حافظة طبيعية كبديل للإضافات الكيمائية.