Detection of Aflatoxigenic Moulds Isolated From Fish and their Products and its Public Health Significance

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Abstract: A total of one hundred fish samples including; 40 of fresh fish (*Tilapia nilotica)*, 30 each of (smoked fish and salted fish) were randomly collected from different shops and retail markets at different sanitation levels at Giza Governorate. Also, one hundred and fifty samples of fish feeds, worker hands and water surrounding the collected fish (50 of each) were collected. All collected samples were subjected for detection of fungal and aflatoxins contamination. The results showed that 7 genera of mould and 2 genera of yeast were recovered from different types of fish. The most commonly isolated mould species in the examined *Tilapia nilotica* were *Alternaria* spp. (90%), followed by *Penicillium* spp., *Cladosporium* spp. and *Candida* spp. (70.0% for each). Other moulds were recovered in a variable frequency .However, in salted fish samples, *Candida* spp*., Rhodotorula* spp*. and Aspergillus* spp. were the most common isolates (93.3%, 80% and 83.3 %). Of genus *Aspergillus*; *A.flavus* was recovered from (66.6%) of salted fish. On the other hand, in smoked fish samples, members of *Aspergillus* spp. were also the most common isolates (100%), *A.flavus* was recovered from (70%), *A. niger* (36.6%), followed by *Candida* spp.*(`73.3%), Rhodotorula* spp.*(66.6%), Penicillium* spp. (60%), *P. citrinum* and *P. expansum* (33.3% and 26.6%) respectively. Six genera of fungal spp. and one genus of yeast were recovered from fish feeds; worker hands and utilized water with a nearly similar to the incidence of contamination in fish particularly genus *Aspergillus* spp. Where, the *A. flavus* was predominantly recovered from fish feed. Moulds of *A. flavus* that isolated from different types of fish and fish feed were able to produce aflatoxins. Regarding fish feed, ten isolates of *A. flavus* out of 18 (55.5%) were aflatoxins producer strains. On the other hand, smoked fish was highly contaminated with aflatoxins producing strains, followed by the isolated strains from salted fish and *Tilapia nilotica* (53.3, 45 and 40%) respectively. It is interstice to report here that the aflatoxins were detected in fish feeds and different types of fish in significant higher levels. Forty percent of fish feeds and salted fish were contaminated with aflatoxin at mean levels of (105.2±1.3 and 44.1±0.4 ppb) respectively. Accordingly, the safe alternatives methods to conventional chemical antimicrobial therapy are needed due to the emergence of multi-drug resistance. Therefore**,** herbal antifungal oils were evaluated as camphor, clove and rosemary oils. Camphor oil had an inhibitory effect on all tested *C. albicans* isolates, the inhibitory zone in the well or disc-diffusion technique varied between (11±0.71 and 1±0.15 mm) diameter. Whereas, the Inhibitory zones of camphor oil against *A. flavus* were of (9±0.71 and 7±0.52 mm) diameter that were obtained by the well and disc-diffusion technique, respectively. On the other hand, the crud clove oil gave a stronger antifungal effect than other tested oils; the inhibitory zones against *A. flavus* were (15±0.63 and 15±0.25 mm) diameter and in case of *C.albicans* the inhibitory zone (13±0.55 and 9±0.52 mm) in diameter by the well and disc-diffusion technique, respectively. In general the well diffusion test gave a wider zone of inhibition for fungal growth by all tested oils or chemicals antifungal. The quality of fish flesh was preserved after treatment with antifungal included normal taste, odor and palatability of flesh. The continuous investigation is necessary to device drug tested to combat fungal infection.

[Atef, A. Hassan; Manal, A. Hassan; Howayda, M. El Shafei; Rasha, M.H. S. El Ahl and R.H. Abd El-Dayem. Detection of Aflatoxigenic Moulds Isolated From Fish and their Products and its Public Health Significance]. Nature and Science 2011; 9(9):106-114] (ISSN:1545-0740). http://www.sciencepub.net.

Key words: fresh fish, fish products (salted and smoked), fish feeds, moulds, yeasts, aflatoxins, herbal antifungal**,** chemical antifungal

1. Introduction

Fish and fish product considered as preferable source of high nutritional values and highly desirable food due to its high quality animal protein content as its exceptional richness in calcium and phosphorus and its generous supply of ß- complex vitamins. Fungal contamination of fish is considered the main cause of signs of spoilage as off flavor and unpalatable taste and it may constitute a public health hazard as well as many of economic losses (Hassan *et al*., 2007 and El Ahl, 2010).The processed fish either salted or smoked may be exposed to contamination by moulds and yeasts derived from subsequent handling of fish and/or from the salt or brine used in the processing which undergoes fungal spoilage through utilization of protein and lipids. Food manufacturers and processors

usually enumerate these organisms only when a problem occurred, due to off flavors, sliminess, lipolysis and unpalatable taste; that render the product of inferior quality unmarketable or even unfit for human consumption (Abd El–Fattah, 2002 and Hassan and Abdel Dayem, 2004). Many mould growth on foods stored at low temperature is common and recurring problem. Certain molds are known to be capable of producing mycotoxins at low temperature as low as (-2 to 10°C). Aflatoxin production is favored by temperature of (20 to 25°C) but has been reported to occur as low as (7 to 12°C) (Hassan and El-Sharnouby, 1997 and Hassan and Aziz, 1998). Moulds were recorded to constitute a public health hazard due to mycotoxin production such as aflatoxin, ochratoxin, patulin and zearalenone. These compounds cause some degree of acute toxicity when given in high amounts and are potential carcinogen, where in developing countries, it appears that there is a direct correlation between dietary aflatoxin intake and the incidence of liver cancer (Groopman *et al*., 1988 and Hassan *et al*., 2009). Therefore, new, safe antimicrobial agents are needed to prevent and overcome severe fungal infections. Some previous investigation as (Preuss *et al*., 2005 and Hassan *et al*., 2009) postulated that herbal essential oils, such as those of origanum, monolaurin and dimethyl 4, 4-dimethoxy 5, 6, 5, 6- dimethylenedioxy-biphenyl - dicarbxylate (D.D.B.) offer such possibilities .Many essential oils as clove (Eugenia caryophyllata) are known to possess an antioxidant activity and antifungal properties and therefore they potentially act as antimycotic agents (Khan *et al*., 2009 and Saleh, 2011).

Therefore, the present study was carried out to study the mycological quality of fresh (*Tilapia nilotica*), salted, smoked fish, fish feed and water, detection of the mycotoxigenicity of the isolated *A. flavus* and investigation of the most suitable methods for reduction of the fungal contamination and degradation of mycotoxins by natural product .

2. Materials and Methods

Materials

Fish samples: A total of 100 fish samples including; 40 samples of fresh fish (*Tilapia nilotica*), 30 samples of each of (smoked fish and salted fish) were randomly collected from different shops and retail markets at different sanitation levels at Giza Governorate. The collected samples were directly identified and transferred to the laboratory in ice box, without delay. Fish were subjected for thorough inspection for the assessment of the general appearance, the odor, the texture and the conditions of the eyes and gills.

Samples of feed, worker hands and water:

One hundred and fifty samples of fish feeds, worker hands and water surrounding the collected fish (50 of each) were collected. The water samples were collected in sterile glass bottles from tanks used in carcasses washing. The samples of worker hands wer taken by sterile swabs. The samples were transferred to laboratory in ice box.

Mycotoxins standard solution for TLC and fluorometric methods:

Aflatoxins standard B_1, B_2, G_1, G_2 and their immunoaffinity column were purchased from (Sigma Chemical Company, St. Louis U.S.A).

Herbal antifungal:

The following oils were purchased from markets of herbs in crude forms ready for use as camphor, clove and rosemary, oils (El Khahira Pharm. Company-Cairo–Egypt). The chemical antifungal as acetic and sorbic acids were used for comparison with herbs.

Methods

Isolation and identification of fungi from fish: Fish, fish feeds and water samples were prepared and examined for mycological contamination according to the technique recommended by (APHA,1992).The identification of isolated mould and yeast genera and species was carried out according to (Pitt and Hocking ,1997).

Screening of isolated strains of *A. flavus* for aflatoxins production using synthetic medium (Gabal *et al*., 1994): The spore suspension of some isolated strains of *A. flavus* from fish feeds and fish samples were prepared and adjusted to be approximately (5 x 10^6 spore/ml) by haemocytometer. One ml spore suspension was inoculated into each flask having (25 ml) of sterile Y.E.S. (2% yeast extract, 20% sucrose) and incubated at room temperature (20-22°C) in the dark for 20 days.

Quantitative estimation of aflatoxins by a fluorometeric method according to (Hansen, 1993): After calibration of the fluorometer by using specific FGis Afla test standards. At the end of the inoculation period, (25 ml) of fungal culture filtrate were extracted three times with (50 ml) chloroform. The chloroform extract was collected and evaporated in a rotator flash evaporator. The residue of chloroform extract was dissolved in (100 ml) methanol:water (80:20 V/V) and filter through fluted filter paper. Then, (10 ml) of filtrate were diluted with (40 ml) distilled water. The diluted extract was filtered through microfiber filter paper. (4 ml) of filtered diluted extract were completely passed through Afla-T-affinity column at a rate of (2 drops/second) until air come through column.

The column was washed twice with (10 ml) distilled water at a rate of (2 drops/second).The affinity column was eluted by passing (1 ml) HPLC grade methanol through column at a rate of (1-2 drops/second) and the sample elute was collected in a glass cuvette. One ml of afla test developer was added to elute in the cuvette and mixed will. The cuvette was placed in a calibrated fluorometer and aflatoxin B_1 concentration was read after (60 seconds).

Evaluation the effect of oils on mould growth production using well and disc diffusion technique (Hili *et al.* , 1997): Herbal oil as camphor, clove and rosemary oil were used.All the tested herbal oils were sterilized by filtration using millipore cellulose filter membrane (0.45 ul pore diameter) and were mixed with the sterilized saline in concentrations of(1%, 2%, 3% and 4%) of each herbal oils .The spore suspension (10^5/ml) of tested isolated mould was either streaked on the surface of SDA medium, or it was incorporated in the medium. The oil was poured in holes made on medium or absorbed by filter paper disc. Wells of(5 mm in Φ) were made on the SDA surface and filled by the oils.On the other hand, in the disc diffusion technique, filter paper discs of (5 mm Φ) soaked with the oils and dried, then placed on the surface of the SDA plates. The plates in both techniques were incubated at (35 ◌̊C for 24 hrs) and the plates were tested for inhibitory zones around the discs.

Organoleptic examination to evaluate the quality of fish before and after addition of chemical or herbal antifungal (FAO, 1995): The inspection of the smoked fish were tested for fitness for human consumption before and after addition of the previous tested herbs or chemicals involved the assessment of the general appearance, odor, texture and the conditions of the eyes and gills. The whole smoked fish were evaluated by a single number of expertise persons according to the scheme provided by the European Economic Community, then the whole fish were dipped in tanks containing the spore suspension (10^6/ml) of tested fungus for (10 minutes) and the previous tested herbs or chemicals were added at MIC which previously determined .Then, after (3-5 days) of incubation at room temperature the organoleptic examination was repeated on the treated fish.

Statistical analysis

The obtained data were computerized and analyzed for significance, calculation of standard error and variance according to (SPSS 14, 2006).

3. Results and Discussion

As shown in Table (1), 7 genera of mould and 2 genera of yeast were recovered from different types of fish. The most commonly isolated mould species in the examined *Tilapia nilotica* were *Alternaria* spp., followed by *Penicillium* spp. and *Cladosporium* spp. and *Candida* spp., it is clear that the most common *Aspergillus* spp. isolates from *Tilapia nilotica* was *A. flavus* and *A. niger*, while the most common recovered *Penicillium* spp. was *P. citrinum and P. expansum*, *Rhizopus* spp. and *Mucor* spp. Other moulds were rarely isolated as *Fusarium* spp. However, in salted fish samples, *Candida* spp., Rhodotorula spp and *Aspergillus* spp. were the most common isolates, followed by *Penicillium* spp., where *P.citrinum* and *P. expansum* were recovered. Of genus *Aspergillus, A.flavus* was recovered of salted fish. Whereas, *Fusarium* spp., *Mucor* spp. *and Rhizopus* spp. were comparatively rarely isolated, respectively. On the other hand, in smoked fish samples, members of *Aspergillus* spp. were also the most common isolates, *A. flavus* was recovered, *A. niger*, followed by *Candida* spp., *Rhodotorula spp, Penicillium* spp., *P. citrinum* and *P. expansum* respectively. Nearly similar results were recorded by (Ammar, 2001; and El- Ahl, 2010).The fungal contamination of fish could be attributed to improper sanitation during catching, handling, manufacturing, storage, transportation and marketing of fish. These findings were supported by the view reported by (Ward and Baji,1988 and Hassan, 2003). The contaminated feeds, water supply and worker hands used fish breeding play the essential role on the health status of fish (Hassan and Abdel Dayem, 2004 and Hassan *et al*., 2007).The current results in (Table, 2) showed that 6 genera of mould species and one genus of yeast were recovered from fish feeds; worker hands and utilized water with a nearly similar to the incidence of contamination in fish particularly genus *Aspergillus* spp. Where the *A. flavus* was predominantly recovered from fish feed. The obtained results came in agreement with those recorded by (Ibrahim, 2000 and Nasser, 2002) who isolated *A. flavus* from most of all samples of fish examined in their studies. Fish are more liable to contamination with moulds and yeasts from animal and human reservoirs which may contaminate the water in the fishing area. Furthermore, contamination during handling and processing may occurred. The contamination was increased in cases of fish caught from polluted areas (Hassan and Abdel Dayem, 2004and Hassan et al., 2007). Many members of the isolated fungi were incriminated in cases of pulmonary infections, urinary tract infection , arthritis , osteomylitis , dermatitis, endocarditis, meningitis and eye infection (Mossel and Shennan, 1976). However, yeast species were spoilage organisms of smoked fish with undesirable changes during prolonged storage and frozen storage (Phaff *et al.*, 1966). However, (Comi *et al.*, 1984) reported that psychotropic yeast may contribute to certain deleterious changes in the

Table (1): incidence of fungal species in examined samples.

Identified mould spp.	*Tilapia nilotica* (40)		Salted fish (30)		Smoked fish (30)	
	No of +ve samples	%	No of +ve samples	%	No of +ve samples	%
Aspergillus spp.	20	50	25	83.3	30	100
A. flavus	20	50	20	66.6	21	70
A. niger	16	40	22	73.3	11	36.6
A. terreus	0	0	4	13.3	-	0
Penicillium spp.	28	70	12	40.0	18	60
P. citrinum	8	20	10	33.3	10	33.3
P. expansum	6	15	10	33.3	8	26.6
Alternaria spp.	36	90	0	0.00	5	16.6
Cladosporium spp.	28	70	6	20.0	14	46.6
Rhizopus spp.	8	20	2	6.6	8	26.6
Fusarium spp.	2	5	4	13.3	3	13.3
Mucor spp.	8	20	4	13.3	6	20.0
Candida spp.	28	70	28	93.3	22	73.3
Rhodotorula spp.	28	70	24	80	20	66.6

All tables expressed as the percentage was calculated in relation to the number of examined sample

Table (2): Incidence of mould in fish feeds and surrounding water

Isolated mould spp.	Fish feeds(50)		Worker hands (50)		Water(50)	
	No.	%	No.	%	No.	%
Aspergillus spp.	28	56	20	40	20	40
A. flavus	28	56	10	20	5	10
A. niger	2	4	20	40	13	26
A. ochraceus	1	2	0	0	2	4
A. candidus	4	8	2	4	3	6
Penicillium spp.	11	22	2	4	4	8
Cladosporium spp	1	2	0	0	0	0
Mucor spp.	25	50	0	0	0	0
Rhizopus spp.	2	4	14	28	10	20
Rhodotorula spp	1	2	10	20	4	8

spoilage of stored fish and shell fish. Most of moulds and yeasts are capable of hydrolyzing a wide range of proteinaceous materials (El-Ahl, 2010 and Awaad *et al*., 2011). Several strains of moulds particularly *Aspergillus flavus* isolated from different types of fish and fish feed were able to produce aflatoxins on Yeast Extract Sucrose medium, under the ideal experiment condition(Table, 3).Regarding fish feed, ten isolates of *A. flavus* out of 18 were aflatoxins producer strains . On the other hand, smoked fish was highly contaminated with aflatoxins producing strains, followed by the isolated strains from salted fish and *Tilapia nilotica* respectively. It is interstice to report here that the aflatoxins were detected in fish feeds and different types of fish in significant higher levels. Forty % of fish feeds and salted fish meat were contaminated with mean levels of (105.2±1.3 ppb and 44.10±0.4) respectively (Table, 4). Where as *Tilapia nilotica* and smoked fish had mean levels of aflatoxins of (49.8±0.02 and 65.1±0.05 ppb) respectively. It is worth while from the recorded results that the most of the isolated moulds are toxigenic types and have the ability to produce mycotoxins whenever the condition are right and become of public health hazards previously reported by (El-Ahl, 2010 and Hassan *et al*.,2010).Most of detected levels of aflatoxins were over the permissible limits in food reported by(WHO ,1979) (who stated that the aflatoxins must be not more than 15 ppb) and (FAO ,1995 and FDA , 2000) (who stated that the levels of aflatoxins must be not more than 20 ppb in food). Hence, most of detected levels were health hazard for consumers where, cases of carcionogenic effects for internal vital organs are resulted particularly for liver and kidney. In

Table (3): Toxigenicity of the isolated *Aspergillus flavus* strains.

Source of *A.flavus*	No. of tested isolates	Toxigenic strains		Levels of aflatoxins (mg/l)		
		No	%	Max. (mg/)l	Min. (mg/ l)	Mean± SE
Fish feeds	18	10	55.5	4.2	0.4	1.8±0.02
Tilapia nilotica	15	6	40.0	6.3	1.5	4.5±0.01
Salted fish	20	9	45.0	7.1	1.8	5.92±0.04
Smoked fish	15	8	53.3	10.0	3.0	7.6±0.13

SE = standard error Mg/l = ppm

Table (4): levels of aflatoxins in fish and fish feed samples.

Types examined samples	Prevalence of aflatoxins		Levels of aflatoxins(ppb)		
	No. of +ve samples	%	Max.	Min.	Mean± SE
Fish feeds (50)	20	40	150	52.5	105.2±1.3
Tilapia nilotica (30)	10	33.3	70.5	22.0	49.8±0.021
Salted fish*(30)*	12	40.0	50.0	18.5	44.10±0.4
Smokedfish*(30)*	8	26.6	96.0	32.0	65.1±0.05

No. = number µg/kg = ppb

addition the consumption of food contaminated with mould and their toxins induced food poisoning, hemorrhages, hepatotoxicity, nephrotoxicity, neurotoxicity, dermatitis, carcinogenic, hormonal and immunosppression effects (Hassan , 2003 and Hassan *et al*., 2008). It was noticed recently that, an increase in the frequency of fungal infections is related with progress in mycology and decreased susceptibility of fungal strains to commonly used antifungal agents as reported by (Nowakowska *et al.*, 2009). Moreover , the changes in treatment strategies and the increased use of antifungal prophylaxis (Lass- Flörl, 2009) and the lack of an effective fungicidal regimen as well as the development of antifungal resistant strains suggest that continued investigation is necessary to devise immuno therapeutic strategies and / or drug targets to combat fungal infection (Wormly and Perfect , 2005) . Accordingly, alternatives to conventional antimicrobial therapy are needed due to the emergence of multi-drug resistance as reported by(Van Vuuren *et al.*,2009).In the present work It is clear from the results presented in Table (5) that the inhibitory zone in the well or disc-diffusion technique varied between (11±0.71 and 1±0.15mm) diameter for C. albicans. Whereas, the Inhibitory zones of camphor oil against *A. flavus* was of (9±0.71 and 7±0.52 mm) diameter were obtained by the well and disc-diffusion technique, respectively. One the other hand, the crud clove oil gave a stronger antifungal effect than other tested oils , the inhibitory zones against *A.flavus* were (15±0.63 and 15±0.25 mm) diameter and in case of *C.albicans,* the inhibitory zone (13±0.55 and 9±0.52 mm) in diameter by the well and disc-diffusion technique, respectively. On the other hand, the rosemary oil yielded the lowest effect than camphor and clove oils, where the inhibitory zone to *A. flavus* and *C.albicanse* were 4±0.71 and 4±0.12 by well diffusion test, respectively. All tested compound were compared to the control antifungal (sorbic acid). The obtained results were confirmed by previous investigators as (Hassan *et al*., 2008and Saleh, 2011), who evaluated active principles of Ramus cathertica leaves, dimethyl 4,4- dimethoxy 5,6,5,6- dimethylenedioxy-biphenyl - dicarbxylate (D.D.B.) DDB, biological compound of streptomyces spp. and many natural herbs oils in control of fungal diseases. In the present work, the inhibitory zones were increased in diameter by elevation the concentration of oils (Tables, 6&7).The diameter of inhibitory zones of camphor oils against growth of *A.flavus* were increased from (0-9±0.71 mm) diameter when the concentration increased up to using well diffusion test. Whereas, in case of *C.albicans* it increased from (0- 12.1±0.25). In general the well diffusion test gave a wider zone of inhibition for fungal growth by all tested oils or chemicals antifungal. Whereas, the variation in the size of zone in both well and disc diffusion tests could be attributed to the differences in diffusion of oils in the contaminated medium with fungi under laboratory condition (Hassan *et al*., 2009 and 2011).

From the results recorded in table (8), it evident that the organoleptic examination of fish flesh before and after addition of antifungal revealed that the tested normal flesh before contamination and treatment were excellent in characters(Table, 8). On the other hand, the experimental fungal contamination followed by treatment with antifungal included in this study not affected the normal appearance as skin condition, skin

Table (5): Minimal inhibitory zone of natural oils in comparison to sorbic acid against isolated *A. flavus and C.albicans*.

Tested oils	The inhibitory zone in the disc or well-diffusion technique			
	Well diffusion test		Disc diffusion test	
	A. flavus	*C. albicans*	*A. flavus*	*C. albicans*
Camphor oil	9 ±0.71*	11±0.71*	7 ±0.52*	1±0.15*
Clove	15±0.63*	13±0.55*	15±0.25*	9±0.52
Rosemary	4±0.71*	4±0.12 *	9±0.43 *	R
Sorbic Acid (Control)	15±0.52*	12±0.71*	11±0.51*	9±0.23 *

Table (6): The inhibitory zones natural oils in comparison to sorbic acid and acetic acid against isolated *A. flavus* using well and disc diffusion tests. (mm Ø)

Herbal oils	Zone of inhibition of herbal oils at different concentration							
	1%		2%		3%		4%	
	W.D.	D.D.	W.D.	D.D.	W.D.	D.D.	W.D.	D.D.
Camphor	R	R	R	7±0.52*	R	8±0.31	9±0.71*	8.75±0.22
Clove	R	R	R	15±0.25*	R	17±0.11	15±0.63*	17.6±0.21
Rosemary	R	R	R	R	R	R	4±0.71*	9±0.43 *
Sorbic acid	R	R	12±.05	10±0.03	14±0.02	10.5±0.1	18±2.0	18.0±0.2
Acetic acid	R	R	R	R	15±0.52*	11±0.51*	16.2±0.6*	11.7±0.11

Table (7): The inhibitory zones of natural oils in comparison to sorbic acid against isolated *C. albicans* using well and disc diffusion tests (mm Ø)

Herbal oils	Zone of inhibition of herebal oils at different concentration							
	1%		**2%**		**3%**		**4%**	
	W.D.	D.D.	W.D.	D.D.	W.D.	D.D.	W.D.	D.D.
Camphor	R	R	11±0.71*	R	11.3±0.43	R	12.1±0.25	1±0.15**
Clove	R	R	13±0.55*	9±0.52	13.3±0.11	9.5±0.33	13.9±0.35	10.2±0.12
Rosemary	R	R	R	R	R	R	13.9±0.35 *	3.5±0.10
Sorbicacid	11±1.0	9±0.3	13±0.5	10±0.4	13.5±0.7	11.5±0.8	15±1.0	13±1.0
Acetic acid	R	R	12±0.71*	9±0.23 *	12.0±0.55	9.2±0.6	12.5±0.66	9.5±032

Table (8): Organoleptic evaluation of contaminated fish samples before and after treatment.

Items	Characters	The tested fish before treatment		The tested fish after treatment	
		No. of samples	%	No. of samples	%
Skin condition	Intact skin	25	100	22	88
Skin colour	Golden brown	24	96	25	100
	Mouldy growth	1	4	0	0
Flesh taste	Fishy flesh taste	18	72	18	72
	Salty	4	16	25	100
	Bitter	3	12	0	0
Flesh odour	Fishy flesh odour	25	100	25	100
	Musty	1	4	0	0
Condition of the belly	Intact	17	68	17	68
	Swellon	2	8	0	0
	Burst	6	24	8	32

color, flesh taste and odor and the condition of belly. It is interstice to report here that up to (100%) of healthy and normal character of fish flesh were preserved after spore contamination and addition of herbs and chemical antifungal (Table, 8). Nearly similar results were obtained by (Ismail, 1999 and Ammar, 2001) who recorded that the organoleptic examination of *Tilapia nilotica* and *Clariaslazera* after antifungal treatment revealed that all samples can be considered fresh. In this respect, (Abd El- Fattah, 2002) recorded that the mean percent of the organoleptic examination of fresh fish was (71.6 %.) On contrary, (Mahmoud , 1990) recorded that the organoleptic examination of *Tilapia nilotica* and *Clariaslazera* revealed that all samples can be considered fresh.

The initial quality of sea foods on board is affected by the species characteristics, the seasonal biological changes in the gonads and muscles, the culture conditions and fishing techniques. Until fish reaches the consumer, its quality attributes are prone to change under the impact of post- harvest handling, standard of hygiene during handling, storage and processing, environmental factors and parameters of applied preservation treatments (Tsukuda and Amano,1968).

Therefore, the food industry utilizes numerous effective measures which inactivate critical microbial contaminants so as to control and limit potential hazards. Extensive quality control programs are implemented to ensure these procedures are effective. However, the new consumer trend towards healthy eating habits places restrictions on the processor in the measures that can be used to maintain a safe food supply are now being altered to suit consumer desires for healthy food. Hence, all hygienic measures must be performed during handling, processing and different stages of preparing of human food to prevent the reach of mould and their toxins to safe the human health.

Fig. (1): Minimal inhibitory zone of natural oils in comparison to sorbic acid against isolated *A. flavus*

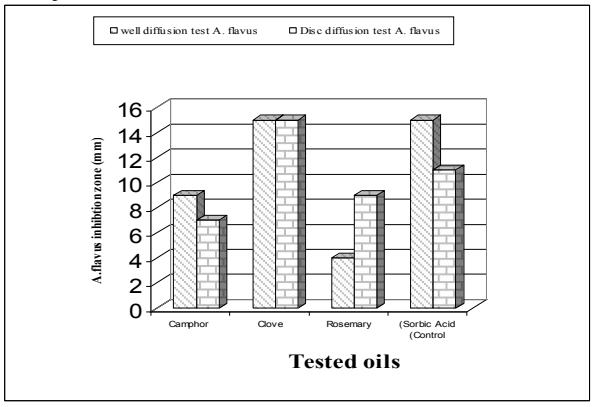
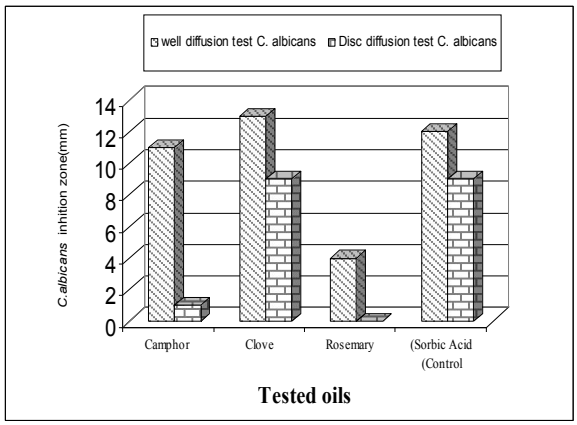


Fig. (2): Minimal inhibitory zone of natural oils in comparison to sorbic acid against isolated *C. albicans*.



All figures expressed as the area of the zone of fungal inhibition (mm Ø)

Significance at *p< 0.05 (ANOVA), R = resistant

W.D.: Well diffusion test D.D: Disc diffusion test

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7/7/2011