SOME STUDIES ON USE OF YEAST (*SACCHAROMYCES CREVICE*) TO REDUCE AFLATOXIN B1IN *OREOCHROMIS NILOTICUS*

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Abstract

One hundred samples of fish rations were collected and subjected to mycological and toxicologically examination. The results showed that four genera of mould, the most commonly isolated mould species were *Aspergillus spp* (80%), *Alternaria spp*. (60%), *Penicillium* spp. (50%) and *Cladosporium* spp (30%). Eighty *Aspergillus* isolates belonged to the five species including *A. flavus* (75%), *A. ochraceus* (50%), *A. niger* (37.5%), *A. clavatus* (25%) and *A. fumigatus* (12.5%). Moulds of *A. flavus* that isolated from fish rations were able to produce aflatoxins on crushed corn media. 30 isolates of *A. flavus* out of sixty isolates (50%) were AFB1 producers in the range of (10 to 400 ppb). AFB1 intoxicated fish showed sluggish swimming, off food, loss of reflexes, increased opercular movements, darkness of the skin and the presence of excessive amounts of mucus on gills. Internally, liver displayed pale coloration with patches of congestion and pin point hemorrhages. The gall bladder was distended with brownish bile. The spleen and the Kidneys appeared enlarged, congested and dark in color.

Effect of the *Saccharomyces cerevisiae (S. cerevisiae*) on survivability, total weight gain and some hemato-biochemical parameters as well as organ residues of AFB1 in *Nile tilapia* fish fed on AFB1-contaminated diet were investigated. The fish were fed diets containing 0 or 200 ppb AFB1 and 0, 1, or 2 gm of

S.cerevisiae/ kg diet in a 2 × 3 factorial arrangement of treatments. After 12 weeks; blood, liver and muscle samples were collected from all fish. The result showed that the total weight gain and survival rate were significantly decreased in fish fed AFB1- contaminated diet ($p<0.05$), while fish fed diets supplemented with *S. cerevisiae* and contaminated with AFB1 showed no significant change in the total weight gain and survival rate compared with negative control group ($p>0.05$). Also, there was a significant decrease in red blood cell (RBCs) count, packed cell volume (PCV), hemoglobin (Hb), and white blood cell (WBC) count in fish fed AFB1 contaminated diet ($p<0.05$). However, fish fed diet supplemented with *S. cerevisiae* and contaminated with AFB1 showed non significant decrease in RBCs, PCV, Hb and WBCs count compared to those fed standard basal diet only. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were significantly increased in fish fed AFB1-contaminated diet compared to those fed standard basal diet only ($p<0.05$). But, fish fed diet supplemented with *S. cerevisiae* at a rate of 1 gm/kg diet and contaminated with AFB1 showed a significant decrease in AST and ALT activities compared to those fed diet contaminated with AFB1 only. Total protein, albumin, and globulin concentrations were significantly decreased in fish fed AFB1-contaminated diet ($p<0.05$). However, *S. cerevisiae* supplementations minimize this negative effect of AFB1 on their concentrations. Also, Urea and creatinine concentrations were significantly increased in fish fed the AFB1-contaminated diet ($p<0.05$). But, *S. cerevisiae* supplementation counteracted the negative effect of AFB1 on their concentrations. Furthermore, AFB1 residues were not detected in liver and muscle of fish supplemented with *S. cerevisiae* at rate of 1 gm/kg diet in comparison with fish fed AFB1-contaminated diet only.

In conclusion, dietary supplementation of *S. cerevisiae* may protect against the toxic effect of AFB1 in *Nile tilapia.*

Key words: *Saccharomyces cerevisiae*, Aflatoxin B1, Toxicity, *Nile tilapia*.

INTRODUCTION

Nowadays, aquaculture contributes greatly in global fish production as the world wide consumption of fish is increasing. Fish meat represents one of the most important sources of animal protein for human. Therefore, intensive raising of great numbers of fish has a great

economical important. Furthermore, the use of artificial feeds in aquaculture systems has increased the production and profits considerably (Alceste and Jory, 2000). In many countries, aquaculture is still unindustrialized; pelleted feeds are produced with inappropriate procedures for bagging, transport and storage. These unsuitable procedures, with high levels of temperature and humidity, are probably the causes of fungal growth and the potential for aflatoxin production. Aflatoxins are a group of toxic metabolites produced by some strains of the fungi, *Aspergillus flavus* and *Aspergillus parasiticus.* They are listed as group I carcinogens by the International Agency for Research on Cancer, primarily affecting liver (IARC, 1993). The aflatoxins commonly found are AFB1, AFB2, AFG1, AFG2, and AFM1. AFB1 is the most potent toxic compound of all aflatoxins known to date (Ngethe *et al.*, 1992) and is generally found in the highest concentration in animal feeds (Farr *et al.,* 1989). The biological and toxicological properties of aflatoxin and the impact of this series of mycotoxins on human and animal health have been well documented (Darwish *et al.,* 2011). Mycotoxicosis imposes significant constraints on fish production; adversely affecting the fish industry by reducing growth rate, feed conversion, immune response, and fish meat quality (Royes and Yanong, 2008). Furthermore; they result in liver and kidney degeneration (Amany *et al.,* 2009). This, in turn, can lead to significant losses for the farmer and can increase the potential for the contamination of fish products marketed for human consumption (Jonsyn and Lahai, 1992). Probiotics are live microorganisms that could be successfully used as nutritional tools for promotion of growth, modulation of intestinal microflora and pathogen inhibition, immunomodulation and promoting meat quality (Pooramini *et al.,* 2009). Generally, probiotics are derived bacteria, fungi and yeast.Recently, yeasts have been reported to have high adsorption ability against mycotoxins in aqueous solution; *S. cerevisiae*, one of the most common types of yeast, had the potential to bind AFB1 (Baptista *et*

al., 2008). *S. cerevisiae* was reported to be the most efficient microorganism for aflatoxin B1 adsorption (Bueno *et al.,* 2007). Furthermore, Addition of S. cerevisiae to the common fish diet activates phagocytic activity and phagocytic index (Ortuno *et al.,* 2002). S. cerevisiae has significant radical scavenging and hepato-protective activity (Sujatha *et al.*, 2012). Nile tilapia (Oreochromis niloticus) may represent a model sensitive for mycotoxicosis, since this fish is highly susceptible to nutritional deficits and is extremely vulnerable to toxic insult from various chemicals and poisons including aflatoxin B1 (AFB1). he aim of the present study was to investigate the possible protective effects of *S. cerevisiae* against aflatoxin B1 induced toxicity in *Nile tilapia.*

MATERIALS AND METHODS

Fish Feed Samples.

One hundred samples of fish ration were collected from different fish farms and subjected to mycological examination. A representative sample (500 gm) was collected from different levels of each of the whole fish feed container (APHA, 1992).

Mycological Examination.

The isolation of fungi from fish ration samples was carried out by: dilution plate method according to (Jonson *et al.*, 1960) one ml. of this dilution was spread on the surface of two plates of Czapek –Dox agar medium (Raper and Fennel, 1965) and incubated at 25 ºC for 7days for the appearance of growth.

Identification of fungal isolates.

Examination of incubated plates macroscopically and microscopically. Individual colonies were selected depending upon their morphological characters. A pure culture was prepared from each growing colony on Czapek –Dox agar slants (Pitt and Hocking, 1997).

Mycotoxin production.

Aflatoxins was extracted from isolated strains after their growth on the natural media (crushed corn media) according to (Wyllie and Morehouse, 1978).

Estimation of aflatoxins.

Chromatographically determination.

Chromatography is among the most frequently used techniques and among the most accurate ones. The main chromatographic techniques include thin layer chromatography (TLC) (Schuller and Van Egmond, 1981).

Standard toxins.

Authentic standard of aflatoxins B1, B2, G1, and G2 (Sigma Chemical Company ,St.Louis U.S.A.). Commercial *S. cerevisiae* was purchased from Dosu Maya Mayacilik A. S. Company (Turkey).

Fish and experimental design.

A total number of 90 apparently healthy *Nile tilapia* fish (mono sex females) with an average body weight of 70-100 gram were obtained from the earthen nursing pond of a private local farm. Fish were randomly distributed through a total of six fully prepared glass aquaria measuring70 x 30 x 40 cm3. Fish were maintained in the aquaria for two weeks before the beginning of the experiment for acclimation. Fish were fed an artificial basal ration (28% crude protein) at a rate of 3% of the body weight for twice daily as described by Abdelhamid *et al.,* (2002). The diet formulated in small pellets. Water was partially changed every day, using fresh de-chlorinated water. Eighteen fish were randomly selected for bacteriological and parasitological examinations to insure that they were free from natural infection. Only 72 of cultured *Nile tilapia* fish were used and divided into six groups (each 12 fish). Group I

was fed basal diet only. Group II was fed basal diet supplemented with *S. cerevisiae* at rate of 1 gm/kg diet. Group III was fed basal diet supplemented with *S. cerevisiae* at rate of 2 gm/kg diet. Group IV was fed basal diet contaminated with AFB1 (200 ppb). Group V was fed basal diet supplemented with *S. cerevisiae* at rate of 1 gm/kg diet and contaminated with AFB1 (200 ppb). Group VI was fed basal diet supplemented with *S. cerevisiae* at rate of 2 gm/kg diet and contaminated with AFB1 (200 ppb). Body weight gain and survival rate (SR %) were measured weekly according to (Castell and Tiews, 1980).

Sampling.

At the end of the experimental period, blood samples were collected from six fish (randomly collected from each group) from the caudal vein. Approximately 2 ml of blood were collected per one fish. 0.5 ml of this blood was mixed immediately in Eppendorf tubes with EDTA (Anticoagulant) and used for hematological analysis. The rest of blood was kept in sterilized Eppendorf tubes overnight at 4°C. Serum was separated from clotted blood by centrifugation at 3,000 x g for 20 minutes and stored at -80ºC until use. Also, liver and muscle tissues of three fish were taken from all groups and immediately stored at -80ºC until use.

Hematological analysis.

The blood samples were used for determinations of the RBCs count after dilution with Natt and Herrick solution (Natt and Herrick, 1952) and counted in aNeubauer chamber; PCVwas determined using a micro-haematocrit centrifugation (10,500 ×g for 5 min) and a micro-capillary reader (Seiverd, 1983); Hb level by the cyanomethemoglobin method (Blaxhall and Daisley, 1973); WBCs count were similarly enumerated in an improved Neubauer Haemocytometer using Natt and Herrick solution (Natt and Herrick, 1952). Blood films were stained by Giemsa stain for differential leukocytic count (Feldman *et al.*, 2000).

Biochemical analysis.

Total protein content of serum was determined colorimetrically (Henry, 1964). Serum albumin was also estimated by a colorimetric method (Dumas and Biggs, 1972) using commercial kit. Globulin was calculated by mathematical subtraction of albumin value from total protein value. Activities of Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT), Urea and Creatinine were measured calorimetrically usingcommercial diagnostic kit according to Reitman and Frankel (1957).

Measurement of aflatoxin residues.

Residue of aflatoxin B1 was estimated in liver and muscle tissues by Thin Layer Chromatography method as described by (Abdelhamid, 1981).

Statistical analysis.

The obtained numerical data were statistically analyzed using SPSS, (1997) for one-way analysis of variance. When F- test was significant, least significant difference was calculated according to Duncan (1955).

RESULTS AND DISCUSSION

As shown in Table (1), four genera of mould were isolated from fish rations. The most commonly isolated mould species were *Aspergillus spp* (80%), *Alternaria spp.* (60%), *Penicillium spp.(*50%) and *Cladosporium spp* (30%). Similar finding reported by (Samson *et al.*, 2000) who isolated the same genera and mentioned that the genus Aspergillus was the predominant isolates (79%) followed by Penicillium (40%) and Fusarium species (33.4%).These genera were isolated from fish ration. These results are also in agreement with (Alinezhad *et al.*, 2011) that isolated the same genera from trout feed with incidence of

occurrence for *Aspergillus* (57%) *Penicillium* (12.84%) and *Absidia* (11.01%).

Table 1. Incidence of fungal genera isolated from fish ration.

Total no. of exam.	*Aspergillus* spp		*Alternaria* spp		*Penicillium* spp		*Cladosporium* spp	
Fish ration	No.	%	No.	%	No.	%	No.	%
100	80	80	60	60	50	50	30	30

Table (2) shows the frequency and distribution of *Aspergillus spp* in fish ration. A number of 80 *Aspergillus* isolates belonged to the 5 species including *A. flavus* (75%), *A. ochraceus* (50%), A. niger (37.5%), *A. clavatus* (25%) and *A. fumigatus* (12.5%).More than half of all the fungi isolated from fish ration in our study belonged to the genus *Aspergillus* of which around 75% were identified as *A. flavus.* The results obtained from same similar studies carried out in other countries are in line with the outcome of this study (Diaz *et al.,* 2009). Among other *Aspergillus species* isolated in the present study, *A. ochraceus* is mycotoxin producer (i.e. ochratoxin A), *A. niger* is human pathogen and an environmental contaminant, *A. clavatus* is involved in the etiology of mycoses and fungal allergies, and A. fumigatus is one of the most important agents of fungal infections in the world. So, they must be considered as potential public health hazards affecting both human and animals (Alinezhad *et al.,* 2011).

Table 2. Frequency of members of the genus *Aspergillus spp in* fish ration.

Total no. of *Aspergillus* spp	*A. flavus*		*A. ochraceus*		*A. niger*		*A. clavatus*		A. *fumigatus*	
	No.	%	No.	%	No.	%	No.	%	No.	%
80	60	75	40	50	30	37.5	20	25	10	12.5

Table (3). The results of AFB1 producing ability of the *A. flavus* isolates showed that only 30 out of 60 isolates produced blue fluorescence under UV light (360 nm) on TLC. Approximately 50% of *A. flavus* strains isolated from fish rations were able to produce AFB1 in the range of (10 to 400 ppb). The percentage of aflatoxigenic fungi among *A. flavus* isolated from fish rations is depend on several factors including the type of feed, environmental conditions, culture conditions and detection method. These results, agree with that obtained by (Alinezhad *et al.,* 2011) who found that the genotype of the isolated fungi determines the type and amount of the aflatoxin produced and they stated that if a strain has the genetic potential to produce aflatoxin , the level of production will depend upon the interaction among the fungi, the host and the environment , also this result agree with (Motallebi *et al.,* 2008) who detected aflatoxins B1 from *Aspergillus flavus* which isolated from fish ration .

Table 3. Aflatoxins producer strains of *Aspergillus flavus*.

No. of tested isolates	Toxigenic strains		Levels of aflatoxins ppb	
	No.	%	Max.	Min
60	30	50	400	10

In this work the quantity of aflatoxins produced ranged from 10-400ppb. These detected levels exceed the maximum permissible limit recommended by the (FDA) in U.S.A for aflatoxins in fish ration, the level which considered as a high risk for aquaculture as well as for the human health trough indirect exposure from fish meat consumption to public health. It worth to mention that the safe guideline given by FDA in all feed was 20 ppb.

Firstly, fish fed basal diet and contaminated with AFB1 were off food and showed sluggish swimming, loss of reflexes, increased

opercular movements, darkness of the skin and excessive amounts of mucus on gills. In addition to; Peticheal hemorrhages were seen on fin, tail and head region; loss of scales; sloughing of tail and sever abdominal distention. The moribund fish was dropped to the bottom of aquarium then dead. Internally, liver displayed pale coloration with patches of congestion and pin point hemorrhages. The gall bladder was distended with brownish bile. The spleen and the Kidneys appeared enlarged, congested and dark in color. Similar pictures were previously reported by Cagauan *et al.* (2004).

The obtained data revealed significant increase of body weight gain of fish in all groups supplemented with *S. cerevisiae* compared to those fed basal diet only. Also, survival rate of fish in all groups supplemented with *S. cerevisiae* showed no significant change compared to those fed basal diet only (Table 4). This result agree with that of Pooramini *et al.* (2009) who stated that addition of yeast as a feed additive in the fish diet at a rate of 5% will improve the growth performance and decrease the mortality rate. On the other hand, Merrifield *et al.* (2011) reported that probiotics has no significant effect on growth performance of on-growing red tilapia *(Oreochromis niloticus)* after a trial extend for 32 days, while the survival rate was significantly high.

Fish fed standard basal diet and contaminated with AFB1 showed a significant decrease in body weight gain and survival rate compared to those fed basal diet only. But, different groups of fish fed diet supplemented with *S. cerevisiae* at a rate of 1 or 2 gm/kg diet and contaminated with AFB1 showed a significant increase in body weight gain and survival rate compared to those fed diet contaminated with AFB1 only (Table 4). Similar negative effects of AFB1 on growth performance and survival rate of tilapia fish were recorded by Omar *et al.* (1996). Also, it was reported that the percent survival of *Oreochromis*

niloticus fingerlings was significantly decreased to 33% when the level of aflatoxin contamination increased (Chavez-Sanchez *et al.,* 1994). Recently, Salem *et al.* (2009) stated that aflatoxin B1 (AFB1) significantly decreases the growth performance and survival rate of *Oreochromis niloticus* fish.

Table 4. Effect of S.Cerevisiaeon the total weight gain and survival rate of AFB1-treated Nile Tilapia fish.

Item	Treatment					
	C	**CT**	**S1**	**S2**	**ST1**	**ST2**
TWG g/fish	18.75	11.86	30	26.43	30.63	26.4
SR%	100	80	100	100	100	100

Hematological data revealed that fish fed standard basal diet and contaminated with AFB1 showed a significant decrease in RBCs, PCV, Hb, total WBCs count compared to those fed basal diet only. However, fish fed diet supplemented with *S. cerevisiae* at a rate of 1 or 2gm/kg diet and contaminated with AFB1 showed nonsignificant decrease in RBCs, PCV, Hb and WBCs count compared to those fed standard basal diet only (Table 5). These results agree with Salem *et al.* (2010) who stated that contaminated diet with 10 ppm AFB1 had adverse effects on hematocrit values, Hb content and RBCs count after 10 weeks feeding. Furthermore, Yiannikouris *et al.* (2006) stated that *S. cerevisiaeis* able to degrade mycotoxins.

Also, the result of hematology revealed a significant increase in RBCs count, PCV, Hb, WBCs count of fish in all groups supplemented with *S. cerevisiae* compared to those fed basal diet only (Table 5). These results supported by a similar result of Osman *et al.* (2010) who stated that dietary supplementation of *S. cerevisiae* could increase the physiological parameters like RBCs count, Hb concentration and cellular

immune parameters (total leucocytic count) in *Oreochromis niloticus*. The administration of yeast has been recognized as immunostimulant agent (Sakai, 1999). Many reports have showed that *S. cerevisiae* contains various immunostimulating compounds such as β-glucans, nucleic acids as well as mannon oligosaccharides that have the ability to stimulate non-specific defense mechanisms in vivo and vitro and enhance immune responses (Abdel-Tawwab *et al.,* 2008).

Table 5. Hematogram of experimental fish groups.

Item	Treatment						
	C	**CT**	**S1**	**S2**	**ST1**	**ST2**	**ST3**
Hb (g/dl)	6.27 ± 0.46 ^{cd}	4.6 ± 0.21 ^{b}	8.82 ± 0.44 ^{a}	6.57 ± 0.38 ^{cd}	7.33 ± 0.34 ^{c}	6.41 ± 0.67 ^{cd}	5.73 ± 0.45 ^{bd}
RBCs (x10^{6}/cmm)	1.32 ± 0.17 ^{c}	0.61 ± 0.08 ^{b}	2.75 ± 0.40 ^{a}	1.35 ± 0.13 ^{c}	2.18 ± 0.16 ^{a}	1.03 ± 0.03 ^{c}	1.11 ± 0.09 ^{c}
WBCs (x103/cmm)	1.34 ± 0.07^{b}	0.02 ± 0.01^{a}	3.52 ± 0.35^{c}	3.28 ± 0.84^{cd}	3.46 ± 0.57^{c}	2.1 ± 0.06^{bd}	0.04 ± 0.01^{a}

Values are expressed as mean ± standard errors. Means in the same row had different letters significantly differ at ($p<0.05$).

Biochemical analysis of serum revealed that fish fed standard basal diet and contaminated with AFB1 showed a significant decrease in total protein, albumin and globulin concentrations and a significant increase in AST, ALT, creatinine and urea concentrations compared to those fed basal diet only. These results are agreed with that of Joner, (2000) who reported that the principal target organ for aflatoxins is the liver; their metabolites react negatively with different cell proteins and inhibit carbohydrate, lipid metabolism, and protein synthesis. Mehrim *et al* (2006) reported that aflatoxins contaminated diets lead to pathological alterations in the liver of tilapia that leads to increased hepatic enzymes. But, fish fed diet supplemented with *S. cerevisiae* at a rate of 1 gm/kg diet and contaminated with AFB1 showed a significant increase in total protein, albumin and globulin concentrations and significant decrease in

AST, ALT, creatinine and urea concentrations compared to those fed diet contaminated with AFB1 only (Table 6). Fish fed diet supplemented with *S. cerevisiae* at a rate of 2 gm/kg diet and contaminated with AFB1 showed a significant increase in total protein, albumin concentrations and significant decrease in ALT, creatinine and urea concentrations compared to those fed diet contaminated with AFB1 only (Table 6). Yiannikouris *et al.* (2004) reported that beta-D-glucans isolated from *S. cerevisiae* was able to complex with mycotoxins and limit their bioavailability in the digestive tract and protect animals against its adverse effects.

Table 6. Effect of S. Cerevisiae on serum biochemical parametersof AFB1-treated Nile Tilapia fish.

	Treatment					
Item	C	CT	S1	S2	ST1	ST2
AST(u/l)	44± 1.15^{b}	62.5± 0.28^{c}	24.5 ±0.87^{a}	60± 1.15^{c}	38.33± 0.33^{b}	62 ± 1.15^{c}
ALT(u/l)	9± 0.57^{a}	17.5± 0.28^{b}	3.5 ± 0.28^{c}	15± 0.58^{d}	6 ± 0.57^{e}	14 ± 1.15^{d}
Total protein (g/dl)	3.99± 0.003^{bd}	2.19± 0.23^{a}	3.49± 0.06^{d}	4.29 ± 0.29^{b}	3.29± 0.055^{c}	3.51± 0.34^{d}
Albumin (g/dl)	1.94± 0.03^{cd}	0.81± 0.07^{a}	1.46± 0.017^{bd}	2.07± 0.28^{c}	1.39± 0.05^{b}	1.98± 0.28^{c}
Globulin (g/dl)	2.05± 0.03^{b}	1.38± 0.15^{a}	2.1± 0.003^{b}	2.31± 0.01^{db}	1.83± 0.07^{cb}	1.44 ± 0.14^{a}
Creatinin (mg/dl)	2.16± 0.03^{b}	9.31± 0.69^{a}	1.98± 0.32^{b}	1.26± 0.095^{b}	1.95± 0.39^{b}	2.05± 0.26^{b}
Urea (mg/dl)	13.6± 0.35^{a}	16.16± 1.14^{b}	9.15 ± 0.32 ^{cd}	9.67 ± 0.66^{c}	9.65 ± 0.57 ^{c}	11.07± 0.16^{ce}

Values are expressed as mean ± standard errors. Means in the same row had different letters significantly differ at ($p<0.05$).

Furthermore, serum biochemical analysis showed nonsignificant change in the activity of ALT, total protein, albumin, globulin and creatinine concentrations in fish groups fed diet supplemented with *S. cerevisiae* compared with those of fish fed basal diet only. But, the

activity of AST was significantly decreased in fish supplemented with *S. cerevisiae* at the rate of 1gm/kg diet and urea concentration was significantly decreased in both fish groups supplemented with *S. cerevisiae* at the rate of 1 or 2gm/kg diet (Table 6). These results are partially in agreement with that of Abdel-Tawwab *et al.* (2010) who stated that yeast supplementation has no significant effects on creatinine, AST, and ALT in *Galilee tilapia.*

Aflatoxin residues in liver and muscle.

Data presented in Table 7 reveal that liver and muscle tissues of fish fed basal diet and contaminated with AFB1 contained a significant amount of AFB1 residues. Ibrahim, (2004) stated that cumulative AFB1 residues in fleshy part of the *O. niloticus* are related to the levels of dietary AFB1 and feeding period. Also, Hussain *et al.* (1993) reported that, aflatoxin B1, G1 and G2 were detected in muscles of treated groups of walleye fish (up to 20 ppb). Soliman *et al.* (1998) mentioned that the significant increase of aflatoxin residues were observed in *O. niloticus* flesh after 6 months of treatment. Salem *et al.* (2010) found residues of AFB1 in the whole body of the aflatoxicated *O. niloticus* fish at the end of the experiment.

Table 7. Effect of S. Cerevisiaeon AFB1 residues in liver and muscle of AFB1-treated Nile Tilapia fish.

	Treatment		
Item	**CT**	**ST1**	**ST2**
LiverAFB1 (ppb)	10	0	**7**
Muscle AFB1 (ppb)	7	0	**5**

AFB1 residues are also detected in the liver and muscle of fish fed on diet supplemented with *S. cerevisiae* at rate of 2gm/kg diet and contaminated with AFB1 but was zero in liver and muscle of those fed

diet supplemented with *S. cerevisiae* at rate of 1gm/kg diet and contaminated with AFB1. Kusumaningtyas *et al.* (2006) reported that *S. cerevisiae* able to degrade mycotoxins. Furthermore, *S. cerevisiae* was able to remove AFB1 from liquid medium (Shetty *et al.*, 2007).

We concluded that *Saccharomyces cerevisiae* has the ability to counteract immunosuppressant induced by AFB_1 in Nile tilapia (*O. niloticus*).

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استخدام الخميرة (سكارواميسس سيرفيسيا) للحد من سموم الأفلاتوكسين ب١ فى الأسماك البلطى

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الملخــص العربــى

أجريت هذه الدراسة علي ١٠٠ عينة من أعلاف الأسماك وذلك من مزارع سمكية مختلفة وفحصت هذه العينات لغرض محاولة عزل الفطريات وذلك باستخدام العزل المباشر على الوسط شابكس أجار.

ولقد أسفرت هذه الدراسة عن النتائج الآتية:

الفطريات المعزولة تنتمي إلي ٤ عائلات من الفطريات وهى: أسبرجيلس، الترناريار، بنسليوم، الكلدوسبوريم.

وقد وجد من الدراسة إن جنس أ سيبرجيلس كان أكثر شيوعا في العينات المختبرة بنسبة ٨٠ %، الترناريار ٦٠%، البنسليوم ٥٠ %، الكلدوسبوريم٣٠%. وكان جنس أ سيبرجيلس ينتمى الى خمس أنواع (أسيبرجيلس فلافس ٧٥%، أسيبرجيلس أوكرشيس ٥٠%، أسيبرجيلس نيجر٥ .٣٧%، أسيبرجيلس كلافتس ٢٥%، أسيبرجيلس فيوميجتس ١٢.٥%. وقد اختبرت هذه العترات لمعرفة قدرتها على إفراز السموم الفطرية وقد أسفرت النتائج عن استخدام ٦٠ عترة من الأسبرجيلس فلافس وتتميتها على الوسط الطبيعي (مجروش الذرة الصفراء المعقمة) ونتج عن ذلك إفراز أفلاتوكسين ب١ من ٣٠ عينة إيجابية بنسبة ٥٠% وكانت كميته تتراوح من ١٠- ٤٠٠ جزء فى البليون.

ويعتبر الافلاتوكسين ب١ تلوث فى الاعلاف مشكلة حيوية فى العديد من البلدان. وقد تم فحص تأثير. سكارواميسس سيرفيس على البقاء، ومجموع زيادة الوزن وبعض قياسات الدم والكيمياء الحيوية وكذلك بقايا الافلاتوكسين ب١ فى الاعضاء فى أسماك البلطى النيلى التى غذيت على عليقة ملوثة. تم استخدام ٧٢ من الاسماك الاصحاء ظاهريا بمتوسط وزن الجسم من ٧٠-١٠٠ جرام. وقد صممت ٦ مجموعات تجريبية، تضم كل منها ١٢ سمكة. وقد تم

تغذية الاسماك على علف يحتوى على صفر، ٢٠٠ جزء فى البليون من الافلاتوكسين ب١ وصفر، ١ أو ٢ جرام من /كجم من العلف فى ٢×٣ ترتيب مضروب من العلاجات. وبعد ١٢ اسبوعا أظهرت النتائج عن الأعراض الظاهرية والصفة التشريحية . ظهرت الإصابة فى أسماك على هيئة تأكل فى الزعانف وانتفاخ أوديمى بالبطن – تقرحات فى الجلد ، أنزفة فى الزعانف الجانبية و الذيلية وسقوط القشور. داخليا ، الكبد شاحب اللون مع بقع نزيفية.

تم جمع عينات الدم والكبد والعضلات من جميع الاسماك. وقد أوضحت النتائج أنة حدث انخفاض مجموع زيادة الوزن ومعدل البقاء بشكل كبير فى الاسماك التى تتغذى على علف ملوث بالافلاتوكسين ب١ ($p\leq0.05$)، فى حين لم تظهر الاسماك التى تتغذى على علف مزود بسكارواميسس سيرفيس وملوث بالافلاتوكسين ب١ ووجد تغير ملموس فى إجمالى زيادة الوزن ومعدل البقاء مقارنة مع مجموعة الضابطة السلبية ($p\geq0.05$). أيضا كان هناك إنخفاض ملحوظ فى عد خلايا الدم الحمراء(كرات الدم الحمراء)، حجم الخلية معبأة (PCV)، الهيموجلوبيين وعد خلايا الدم البيضاء فى الاسماك التى تتغذى على علف ملوث بالافلاتوكسين ب١ ($p\leq0.05$) . ومع ذلك ، الاسماك التى تتغذى على علف مزود بسكارواميسس سيرفيس وملوث بالافلاتوكسين ب١ أظهرت انخفاضا غير معنوى فى كرات الدم الحمراء،PCV ،هيموجلوبيين وعد كرات الدم البيضاء مقارنة بأولئك الذين يتغذون معيار النظام الغذائى القاعدى فقط. وزادت أنشطة ALT، AST بشكل كبير فى الاسماك التى تتغذى على عليقة ملوثة بالافلاتوكسين ب١ مقارنة مع تلك التى تتغذى معيار النظام الغذائى القاعدى فقط ($p\leq0.05$). لكن أظهرت الاسماك التى تتغذى على علف مزود بسكارواميسس سيرفيس بمعدل ١جم /كجم علف وملوث بالافلاتوكسين ب١ انخفاض ملحوظ فى أنشطة ALT ، AST مقارنة بتلك التى تتغذى على علف ملوث بالافلاتوكسين ب١ فقط. وقد انخفضت تركيزات البروتيين الكلى، الالبيومين والجلوبيولين وبشكل كبير فى الاسماك التى تتغذى على علف ملوث بالافلاتوكسين ب١ ($p\leq0.05$). ومع، إضافة سكارواميسس سيرفيس قل الأثر السلبى بالافلاتوكسين ب١ على حسب تركيزها. وأيضا ، تم زيادة تركيز اليوريا والكرياتينين بشكل كبير فى الاسماك التى تتغذى على علف ملوث بالافلاتوكسين ب١ ($p\leq0.05$). ولكن، إضافة *S. Cerevisiae* تتصدى للأثر السلبى بالافلاتوكسين ب١ على حسب تركيزاتها. وعلاوه على ذلك، كانت بقايا افلاتوكسين ب١ غير محسوسة فى الكبد والعضلات فى الاسماك التى تتغذى على علف مزود بسكارواميسس سيرفيس بمعدل ١جم /كجم علف بالمقارنة مع الأسماك التى تتغذى على علف ملوث بالافلاتوكسين ب١ فقط.

فى الختام، إضافة بسكارواميسس سيرفيس على العلف ربما يحمى أو يقلل التأثير السام من افلاتوكسين ب١ فى *البلطى النيلى*.