Contamination of stored crop grains with *Aspergillus niger* fungi and production of the toxin Ochratoxin A and its negative impact when used as a feed for fish in Iraq

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https://doi.org/10.59658/jkas.v11i4.2796

Received:
July 17, 2024

Accepted:
Aug. 18, 2024

Published:
Dec. 15, 2024

Abstract

The study investigated the contamination of stored crop grains used as fish feed in Iraq by fungi capable of producing Ochratoxin A (OTA) and assessed its impact on fish farming and health. A survey revealed that all tested grain samples were contaminated with various fungi, with Aspergillus niger being the most prevalent. Among 1,400 isolates of A. niger, nine were confirmed to produce OTA at varying levels. The highest OTA concentration (80.90 µg/kg) was recorded for isolate ANK1, while the lowest (35.9 µg/kg) was found in isolate ANA1. Molecular and morphological diagnoses of the toxin-producing isolates were consistent, and their genomic sequences were archived in the NCBI database under accession codes OR452868.1 and OR449322.1. Chromatographic analysis detected OTA contamination in five out of nine samples, with the highest contamination level (6.90 µg/kg) found in sample K1F. Contamination impacted fish growth and health, increasing mortality rates to 10.8% compared to 3.5% in the control. Weight loss percentages ranged from 13.7% to 27.2% over 60 to 180 days. Blood analysis showed decreased WBC, RBC, HGB, MCHC, and PLT levels, while HCT levels significantly increased. The findings underscore the risks posed by OTA contamination to fish farming and emphasize the need for improved feed safety measures.

Keywords. Mycotoxins, Ochratoxin A , A. niger , Fish feed , Iraq.

introduction

Many field crop grains, such as wheat, barley, maize, and others, are infected in the field, during harvest, and in the storeroom by many fungi belonging to the genera *Aspergillus spp., Penicillium spp., Fusarium spp., Alternaria, Rhizopus, Rhizoctonia, Trichoderma, Stemphylium*, and other fungi [1]. Many of these fungi raise concerns and risks because they produce toxic compounds called mycotoxins, especially aflatoxins, ochratoxins, fumansins, and others [2]. Recently, the use of field crop grains in the production of animal feed for feeding fish has increased. It is a mixture of products of plant origin in their natural state, fresh or preserved, or products derived from their industrial processing, for oral feeding in the form of complete feed, providing the necessary nutrients

to support the life of the animal. This new trend of using plant-based materials in aquafeeds faces great challenges, the most important of which is the contamination of these components with potentially toxic fungi, especially when they are contaminated with the two fungi *A. flavus* and *A. niger*, which are widely spread in stored crop grains and cause significant contamination with toxins. Fungi such as aflatoxins and ochratoxins, as the mycotoxin ochratoxin A was detected in grains of maize, wheat, and barley intended for the production of fish feed [3] The researchers also recorded particularly high levels of the mycotoxin ochratoxin in soybeans. [4] While in another study, ochratoxin was detected in samples of soybeans, bran, corn bran, and other grains from fish farms [5].

The use of plant crops or grains in fish feed leads to an increased risk of contamination with fungi and mycotoxins and a higher incidence of mycotoxicosis in fish [6]. This may reduce the productivity of aquaculture and thus lead to the occurrence of mycotoxicosis, and as a result there will be a decrease in Body weight, poor growth, and higher rates of disease and mortality in fish. In addition, some mycotoxins in fish may accumulate in the muscular system. These risks are transmitted to humans through the food chain [7].

Materials and research methods

Sample collection

Forty eight different samples of stored field crop grains in their individual forms wheat, barley, yellow corn, and soybeans, mixed in the form of manufactured local fish feed and samples of imported feed, were collected randomly from eight laboratories for the production of animal feed, with two laboratories for each governorate, which included the governorates of Baghdad, Karbala, and Babylon. And Anbar. Three replicates per sample, with weights of 2-4 kg per replicate.

Isolation and identification of the fungus *Aspergillus niger* associated with wheat, barley, maize, soybeans, and fish feed.

The grains were surface sterilized with a 2% sodium hypochlorate solution for one minute, then washed with distilled water, dried with Whatman No.2 filter paper, and planted in Petri dishes containing PDA culture medium, with 100 grains for each sample, and then the dishes were incubated at a temperature of (25 ± 2°C). for a period of 3-5 days), after which the fungi accompanying and contaminating the grains, *A. niger*, were isolated. Toxin-producing isolates were identified and confirmed according to the taxonomic key [8] and the phenotypic characters mentioned by[9,10,11,12,13] Fungi to the species level using molecular methods.

Detection of the ability of fungal isolates to produce the mycotoxin Ochratoxin A

Development of fungal isolates to produce mycotoxins

To detect the ability of fungal isolates to produce mycotoxins, nine fungal isolates of the fungus *A. niger* were grown on sorghum medium. The medium treated with the fungal isolates was incubated for 21 days at a temperature of 25±2 °C for nine isolates belonging to the fungus *A. niger* (Table 1) with stirring. Continuously to ensure the mushroom is distributed throughout all parts of the medium.

Table (1): Coding of fungal isolates grown on sorghum media to detect their ability to produce the mycotoxin Ochratoxin A.

No	Isolated fungi	Symbol of isolation	Samples from which it was isolated
1	***A. niger***	AN A 1	Isolated from the local bush/Al-Anbar 1/Fallujah laboratory
2	***A. niger***	A N A 2	Isolated from the local shrub/Al-Anbar Laboratory 2/Al-Saqlawiyah
3	***A. niger***	A N B 1	Isolated from the local bush / Baghdad 1 laboratory / Yusufiyah
4	***A. niger***	A N B 2	Isolated from the local bush / Baghdad Laboratory 2 / Mahmoudiyah
5	***A. niger***	A N H 1	Isolated from the local bush / Babylon 1 laboratory / Al-Musayyib
6	***A. niger***	A N H 2	Isolated from the local bush / Babylon 2 laboratory / Al-Mahawil
7	***A. niger***	A N K 1	Isolated from the local bush / Karbala 1 laboratory / Hindia
8	***A. niger***	AN K 2	Isolated from the local bush / Karbala 2 laboratory / Al-Hurr
9	***A. niger***	A N F	Isolated from imported bushes / Anbar / Fallujah

Ochratoxin A mycotoxin extraction

Samples of white corn grains on which the fungal isolates were grown were ground using an electric grinder to obtain fine and homogeneous particles. Then 10 grams of powder from each sample was weighed and added to the extraction solution (methanol CH_4O: potassium chloride KCL 4%) at a ratio of (1:9) and in a volume of 60 ml, until it became homogeneous. The mixture was obtained using an electric shaker (45 minutes, 200 rpm, room temperature). The mixture was filtered after 24 hours with Whatman No.2 filter paper (primary filter), from which 30 ml was taken and mixed well with 30 ml ammonium sulphate solution (NH_4). $)_2SO_4$ 30%, then filtered again in the same way[14].

Purification of the extract of Ochratoxin A

The initial extract of the toxins was purified using a chromatographic column 50 cm × 12 mm prepared according to the method used by [15] by activating the silica gel by heating it in an electric oven (130 °C, 45 minutes) with a wool ball placed at its base. Then add 0.5 g of anhydrous sodium sulfate (Na_2SO_4) to give an equal base for the silica gel. After that, add $CHCL_3$ until the column is approximately half full, then slowly add 2.0 g of silica gel while washing the sides of the column with chloroform $CHCL_3$. Stir the mixture with a glass rod to remove the formed air bubbles. To compact the silica, the $CHCL_3$ was pulled to the bottom, leaving a distance of 3 cm above the silica gel to avoid

drying out of the column. After that, 0.5 g of anhydrous sodium sulfate (Na_2SO_4) was added, and thus the column was ready for use**.**

The previously prepared extract was added and placed slowly in the column. Then the final filtrate was collected in opaque vials (25 mL). Separation was done by adding 50 mL of chloroform ($CHCL_3$). The mixture was placed in a separating funnel and shaken well, taking care to open the valve from time to time to empty the formed gases. It was left on the stand for separation. It was neglected. The upper layer and the process was repeated twice, then the filtrate was passed over 10 g of anhydrous sodium sulphate (Na_2SO_4). The solvent was evaporated at a temperature of 50 °C and the filtrate was kept in an opaque Vilas (2.0 mL, at a temperature of -20 °C) until qualitative and quantitative detection was performed [14] .

Equip standard poison

The standard material for the toxin, Ochratoxin A purity>98.9% (2mg), was prepared from Segma-Aldrich (Germany) and stored at a temperature of -20°C until analysis was performed**.**

Qualitative detection and quantitative determination of Ochratoxin A using HPLC technology for fungal isolates

Chromatographic analysis was conducted at the Ministry of Science and Technology/Department of Environmental and Water Research and Technology using a high performance liquid chromatographic (HPLC) system (SYKAMN, Germany) coupled to an RF-10A XL fluorescence detector. OTA toxin separation was performed using a C18 reversed phase column. (150mm × 4.6 mm, 3.5 µm), fluorescent reagent: (FLD, exc=333 nm, em=460 nm; gain=100) The sample was filtered at a flow rate of 1 ml/min and the injection volume was 10 µl. The mobile phase was a mixture of acetonitrile/water/acetic acid (99:99:2, v/v/v) and the column temperature was 30 °C [16].

Detection of the mycotoxin Ochratoxin A in fish feed samples

This test was carried out to detect contamination of animal feed manufactured for feeding fish with mycotoxins, regardless of its contamination with associated fungi. The mycotoxin Ochratoxin A was extracted from these studied samples: 9 main samples 8 local pellet samples made from collecting stored grains for each laboratory and one feed sample. Imported Babel / Al-Musayyab (detection and quantification using a high-performance liquid chromatography (HPLC) system.

Molecular diagnosis of fungal isolates of Ochratoxin A

Two isolates of the fungus Aspergillus niger were targeted according to their ability to produce mycotoxins. The highest producing isolate of ochratoxin and the lowest producing isolate of the fungus *A. niger* were selected to study the genetic and hereditary structure between the ability of these isolates to produce mycotoxins, which were determined by analyzing the sequence of DNA bases. And compared to the genomes of previously diagnosed isolates, the PCR products were sent to Macrogen Company in South Korea for the purpose of determining the nucleotide sequence of the genetic region Internal Transcribed Spacer ITS. After receiving the nucleotide sequences of the fungal

isolates, the sequences were analyzed using the Basic Local Aligment Search Tool (BLAST) program to compare them with the data. Available at the National Center for Biotecnology Information (NCBI) within the electronic gene bank that belongs to the same fungal isolates that have been diagnosed globally. [17]

The fungal isolates that did not match any of the nucleotide sequences were registered 100% at NCBI. Nucleotide analyzes were also conducted using the MEGA Neighbor joining, which was built from the nucleotide molecular sequence of the ITS region belonging to each of the isolates [18] .

Biotests of the effect of feed contaminated with mycotoxins on fish growth.

This experiment was carried out in early June 2022 in one of the areas of Al-Musayyib District (Abu Luka), where three floating cages were used in the Euphrates River. Small fish (hatches) were placed at a rate of 100 grams and 500 hatchlings per cage, with a total of 1,500 carp hatchlings. Using the imported diet remaining from the previous experiment (testing storage periods) in feeding the fish. Which was contaminated with each of the fungal isolates, ANK 2, the highest ochratoxin A-producing isolate from the fungus *A. niger*. Adding this feed to the fish and feeding them for six months [19]

The results were taken periodically, as the weights and deaths of each cage were checked in three stages. Every two months from the beginning of feeding and comparing the results. The examination was carried out at three times for each cage using a plastic box. It calculates the total weight and number of fish, and the total number of fish for each stage.

Average weight of one fish = (total weights)/(fish to total number) x 100%

After the end of each stage of the experiment every 2 months, the final results of weights and deaths were taken, as well as an analysis of the blood parameters of these fish along with some anatomical characteristics of the fish to study the effect of mycotoxins on them.

Results and Discussion

Isolation and diagnosis of the fungus *Aspergillus niger* associated with study samples.

The results of the microbial survey showed that all stored grain samples were contaminated with various fungal isolates. The survey included stored field crop grains, including wheat, barley, yellow corn, and soybeans in their individual forms and mixed in the form of local and imported animal feed, from eight laboratories for the production of animal feed, with two laboratories for each governorate, which included the governorates of Baghdad. Karbala, Babylon, and Anbar. The survey showed that all of these samples were contaminated with many fungal isolates, which are characterized by their great danger due to their high ability to produce mycotoxins.

The most abundant and frequent fungal genera was *A. niger* (Table 2 and Figure 1), with the highest occurrence rates reaching 100% in stored grains and local feed, and 50% in imported feed, with a total of 1,400 isolates. Yellow corn topped the list with the highest

number of isolates, reaching 333 isolates from all samples, followed by wheat, with 311 isolates, and third place was 276 isolates, represented by soybean grains, while the least contamination was in imported fish feed samples, which amounted to only 8 isolates.

Table (2): Level of contamination of study samples with the fungus *Aspergillus niger*

Source	Baghdad Governorate		Karbala Governorate		Babylon Governorate		Anbar Governorate		the total
	The first laboratory	The second laboratory	The first laboratory	The second laboratory	The first laboratory	The second laboratory	The first laboratory	The second laboratory	
Wheat grains	42	39	37	36	39	42	38	38	311
Barley grains	34	29	31	41	31	32	34	32	264
Yellow corn grains	44	39	42	39	45	42	40	42	333
Soybean beans	31	38	36	38	31	36	34	32	276
Local bush	24	28	26	24	29	28	26	23	208
Imported blackberries	0	0	2	1	0	0	3	2	8
The total	175	173	174	179	175	180	175	169	1400

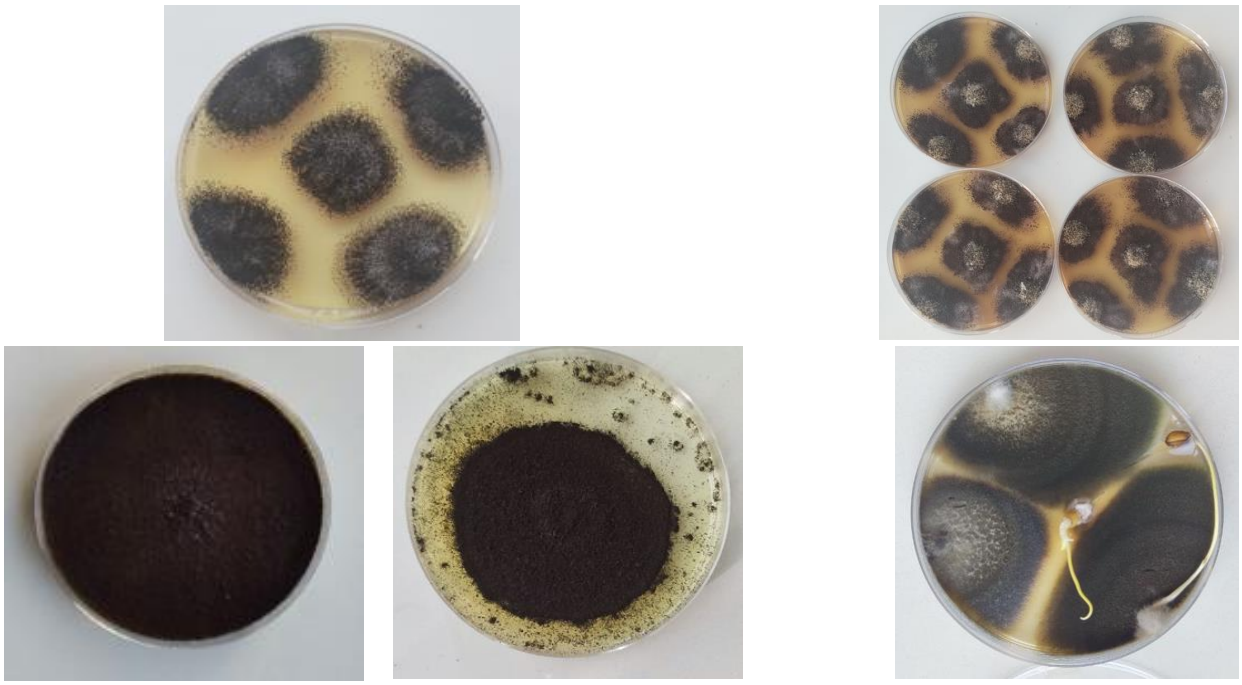


Figure (1): Models of contamination of study samples with isolates of *A. niger*

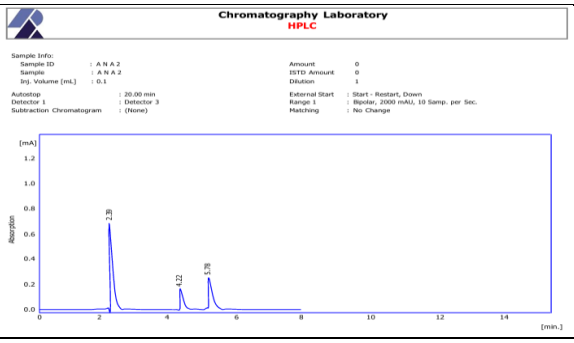
Detecting the ability of *Aspergillus niger* isolates to produce Ochratoxin A

The results of chromatographic analysis using HPLC technology showed that all nine fungal isolates of the fungus *A. niger* showed a great ability to produce the toxin Ochratoxin A in varying proportions as well (Table 3 and Figure 2), as the highest production concentrations reached 80.90 µg/kg for the fungal isolate ANK1, followed by the fungal isolate ANK2 at a rate Ochrotoxin production reached 74.50 micrograms/kg. Then came the two isolates from Baghdad Governorate, where the ability of isolate ANB2 to produce ochratoxin reached 72.60 micrograms/kg. Isolate ANB1 reached 68.90 micrograms/kg. While the ANF fungal isolate isolated from the imported forage and the Fallujah group, Anbar Governorate, recorded the lowest level of mycotoxin ochratoxin production, amounting to 22.90 micrograms.

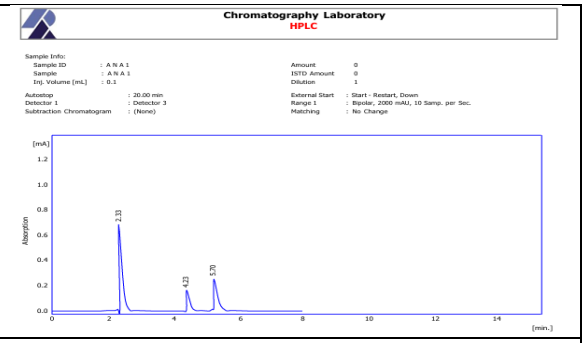
These results are consistent with many previous studies, which indicate that the main producer of ochratoxin is the fungus *A. niger*, as in the study of It has also been proven that Ochratoxin A is produced primarily by *A.niger* [20] While the variation of isolates in producing mycotoxins is attributed to the genetic ability of the fungal isolate, and the preferential conditions suitable for each isolate to produce a specific toxin, mainly humidity and temperature during storage [21] .

Table (3): Ability of *Aspergillus niger* isolates to produce Ochratoxin A

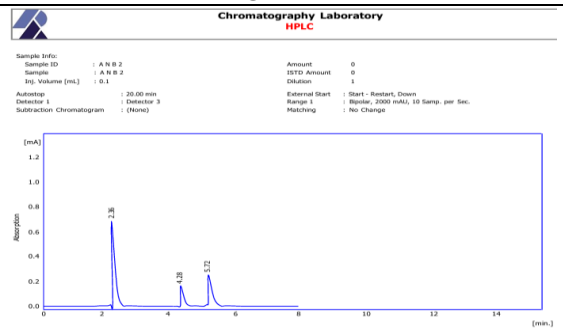
No	Fungal isolation	Symbol of	Isolation place	OTA(ppb)
1	*A. niger1*	AN A 1	Local fish feed / Laboratory 1 /	142.5
2	*A. niger 2*	ANF A 2	Local fish feed / Laboratory 2 /	155.9
3	*A. niger 3*	AN B 1	Local fish feed / Laboratory 1 /	142.8
4	*A. niger4*	AN B 2	Local fish feed / Laboratory 2 /	134.9
5	*A. niger 5*	A N H 1	Local fish feed / Laboratory 1 /	174.9
6	*A. niger 6*	AN H 2	Local fish feed / Lab 2 /	170.8
7	*A. niger 7*	AN K 1	Local fish feed / laboratory 1 /	214.5
8	*A. niger 8*	AN K 2	Local fish feed / laboratory 2 /	226.9
9	*A. niger 9*	A NF	Imported fish	42.65



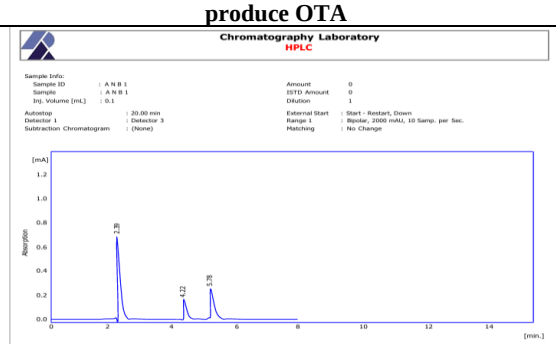
Ability of the fungal isolate *A.niger* (ANA2) to produce OTA



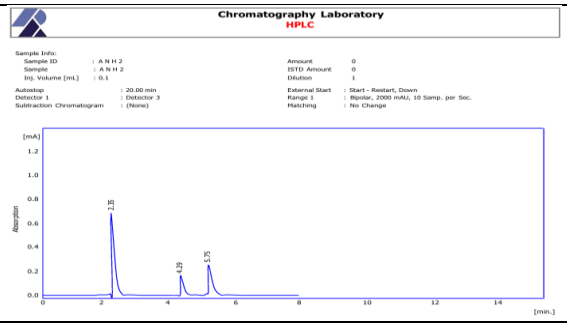
Ability of the fungal isolate *A.niger* (ANA1) to produce OTA



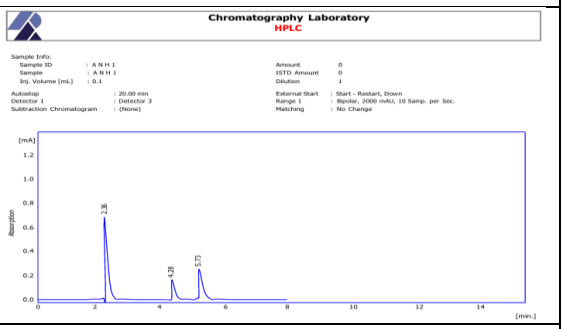
Ability of the fungal isolate *A.niger* (ANB2) to produce OTA



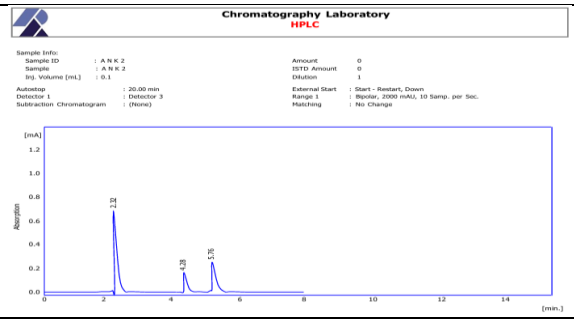
Ability of the fungal isolate *A.niger* (ANB1) to produce OTA



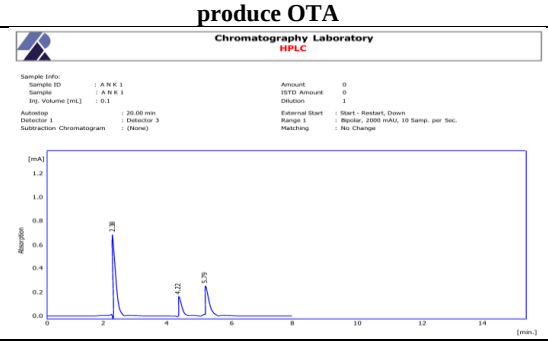
Ability of the fungal isolate *A.niger* (ANH2) to produce OTA



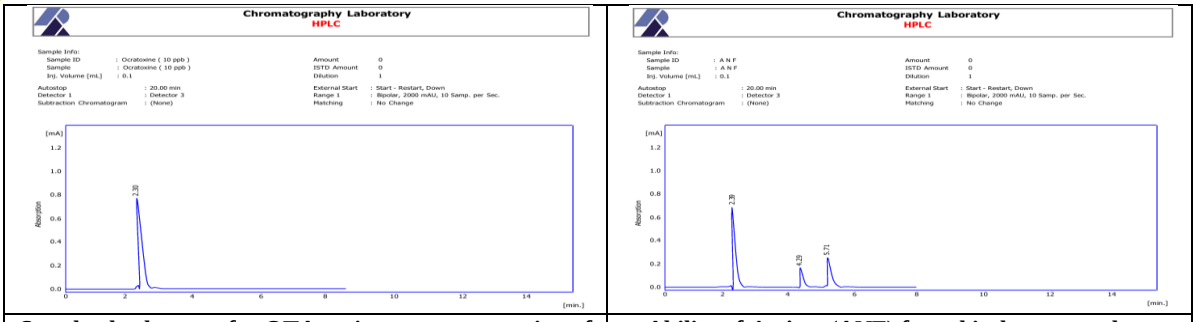
Ability of the fungal isolate *A.niger* (ANH1) to produce OTA



Ability of the fungal isolate *A.niger* (ANK2) to produce OTA



Ability of the fungal isolate *A.niger* (ANK1) to produce OTA



Standard substance for OTA toxin at a concentration of (10 ppb	Ability of *A.niger* (ANF) fungal isolate to produce OTA

Figure (2): Quantitative and qualitative estimation of OTA toxin produced from isolates of the fungus *Aspergillus niger*

Detection of mycotoxin Ochratoxin A in fish feed samples

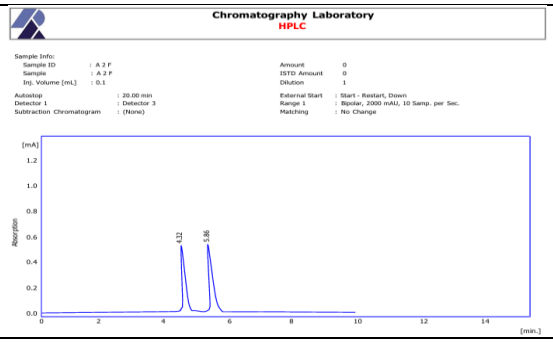
Chromatographic analysis to detect contamination with ochratoxin A showed that five out of nine samples were free of contamination with the mycotoxin ochratoxin, which are (the local Anbar samples (A1F and A2F), the local Babylon samples (H1F and H2F), and the imported Babylon sample (H1FF)) (Table 4, Figure 3) While four samples appeared to be contaminated with ochratoxin in varying proportions, with the highest percentage of ochratoxin A contamination recorded, reaching 6.90 micrograms/kg for sample K1F, followed by B2F, K2F, and B1F at concentrations (6.20, 5.70, and 5.50 micrograms/kg, respectively).

The absence of contamination with mycotoxins in some samples, despite the fact that they are accompanied by some fungi known for their ability to produce these toxins, may be due to good storage conditions, or they will not be exposed to sufficient storage periods for the fungi to be active and produce mycotoxins in them [22] The discrepancy between the concentration of Mycotoxins in samples contaminated with mycotoxins are mainly due to climatic conditions and water activity during storage that favor fungal growth and mycotoxin production [23] Although the samples were contaminated with small levels of the mycotoxin ochratoxin, they exceed the maximum limits permitted by the European Union for all samples, which are 5 micrograms/kg.

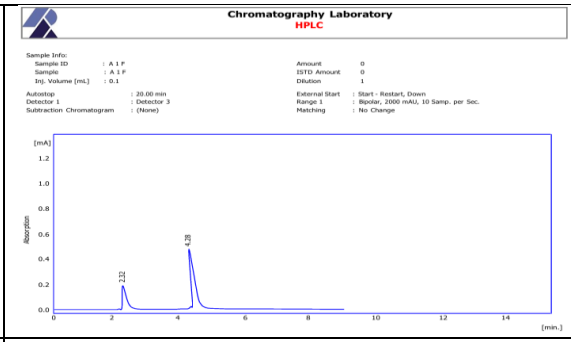
Table (4): Contamination of local and imported fish feed samples with Ochratoxin A

No	Sample type	Sample code	(OTA (ppb
1	**Local fish feed / Laboratory 1 / Anbar**	A 1 F	UDL
2	**Local fish feed / laboratory 2 / Anbar**	A 2 F	UDL
3	**Local fish feed / Laboratory 1 / Baghdad**	B 1 F	5.5
4	**Local fish feed / Laboratory 2 / Baghdad**	B 2 F	6.2
5	**Local fish feed / Laboratory 1 / Babylon**	H 1 F	UDL
6	**Local fish feed / Laboratory 2 / Babylon**	H 2 F	UDL
7	**Local fish feed / laboratory 1 / Karbala**	K 1 F	6.9
8	**Local fish feed / laboratory 2 / Karbala**	K 2 F	5.7
9	**Imported fish feed/Babylon/Al-Musayyab**	H1FF	UDL

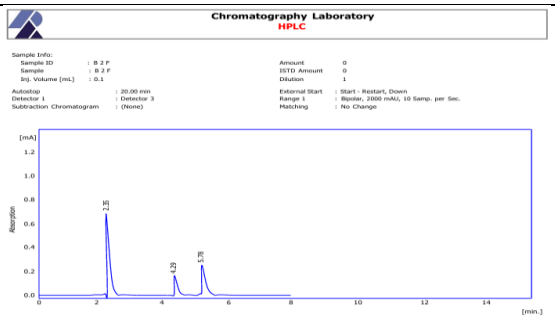
The discrepancy between the concentration of ochratoxins in the samples may be mainly attributed to the intensity of fungal growth of the fungus *A. niger*, as well as the moisture content. If the environmental conditions that accompanied the manufacture of the feed, the moisture content, and the temperature and humidity of the store were suitable for the growth of the fungus, this would lead to the presence of ochratoxins to very high levels[24,15] .



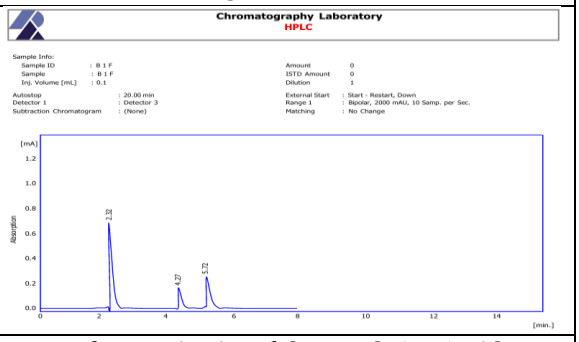
Amount of sample contamination (A2F) with mycotoxin OTA



Amount of sample contamination (A1F) with mycotoxin OTA



Amount of sample contamination (B2F) with mycotoxin OTA



Amount of contamination of the sample (B1F) with mycotoxin OTA

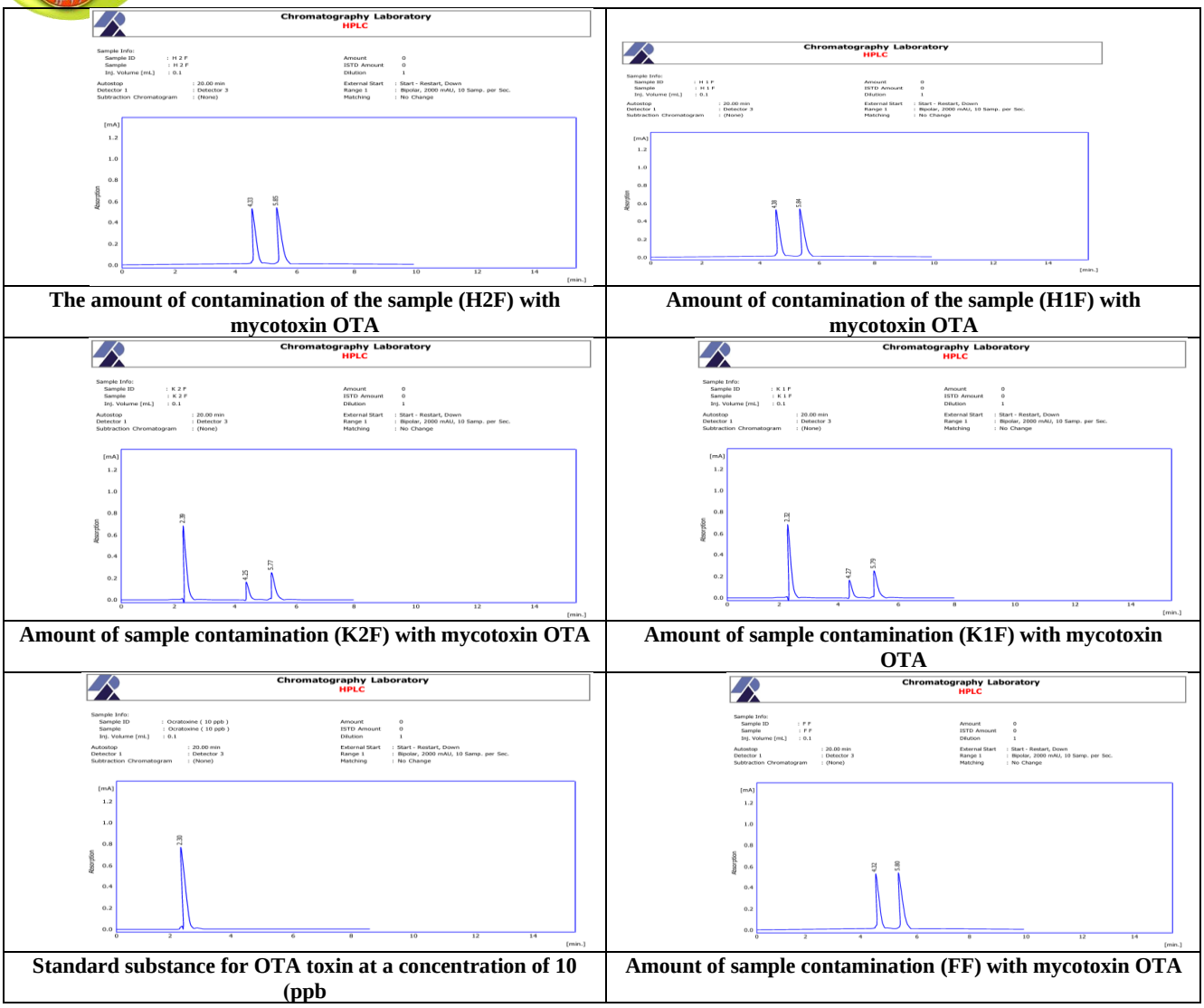


Figure (3): Quantitative and qualitative estimation of OTA toxin as a natural contaminant of the study samples

Nucletide sequence analysis of *Aspergillus spp* isolates

The results of the nucleotide sequence analysis of the two isolates of the fungus *Aspergillus spp* that were isolated from the local fish feed (pelt) confirmed that the first of the two isolates was characterized by the highest production of the fungal toxin Ochratoxin A, and the other isolate produced the least of it in samples of the local fish feed. The results of the nucleotide sequence analysis showed that the two isolates belong to For the fungus *A. niger***.**

The two fungal isolates were registered at the National Center for Biotechnology Information (NCBI) under the special codes (OR452868.1 and OR449322.1). The molecular nucleotide sequences achieved the highest percentage of identity, ranging between 99.68-98.77%, with the ITS genetic region when compared with the equivalent nucleotide sequences retrieved from the gene bank at the National Center for

Biotechnology Information (NCBI) using the (BLAST) program for each fungal isolate individually**.**

Nucleotide analyzes were also conducted using the (MEGA) program to analyze the isolates and draw a kinship tree between each of these isolates and similar isolates registered at the NCBI Center, which was built from the nucleotide molecular sequence of the ITS region belonging to each of the isolates.

Analysis of the nucleotide sequence of the isolate *A. niger* isolate y.n.174.Khafori and comparison of the similarity percentages of the nucleotide base sequences of the ITS gene region with global fungal isolates of the same fungus.

It was observed by comparing the nucleotide sequence of the DNA band of the fungus *A. niger* isolate y.n.174.Khafori isolated from local fish feed with the data available at the Center for Biotechnology Information (NCBI) that the percentage of genetic similarity reached (99.68 - 98.77%) with all isolates of the fungus *A. niger* (Table 5). While Figure (4), represented by the genetic tree, showed that this isolate did not show genetic closeness to any of the recorded isolates (it did not appear with the same branching. clade), while it appeared with separate branches (clades) from the recorded fungal isolates, especially from the two American isolates (KX664401.1). and Kenyan (OP737610.1) due to the large genetic divergence between them.

Table 5: Comparison between the similarity ratios of the nucleotide base sequences of the ITS gene region of the fungal isolate (*A. niger* isolate y.n.174.Khafori) and other fungal isolates of the same fungus registered globally at the National Center for Technical and Biological Information (NCBI).

No	Fungal name	Isolate name	Origin	GenBank Accession Number	Sequence similarity %	Date of registration with NCBI
1	***A. niger***	Y.n.176.Khafori	Iraq	OR452868.1	100%	2023
2	***A. niger***	isolate T8	India	MN180811.1	99.12 %	2019
3	***A. niger***	isolate 596	China	PP070353.1	99.64 %	2024
4	***A. niger***	isolate F43-02	USA	KX664401.1	98.77 %	2016
5	***A. niger***	isolate CA60	Kenya	OP737610.1	98.77 %	2023
6	***A. niger***	strain S5F	Iran	MW897780.1	% 99.11	2021

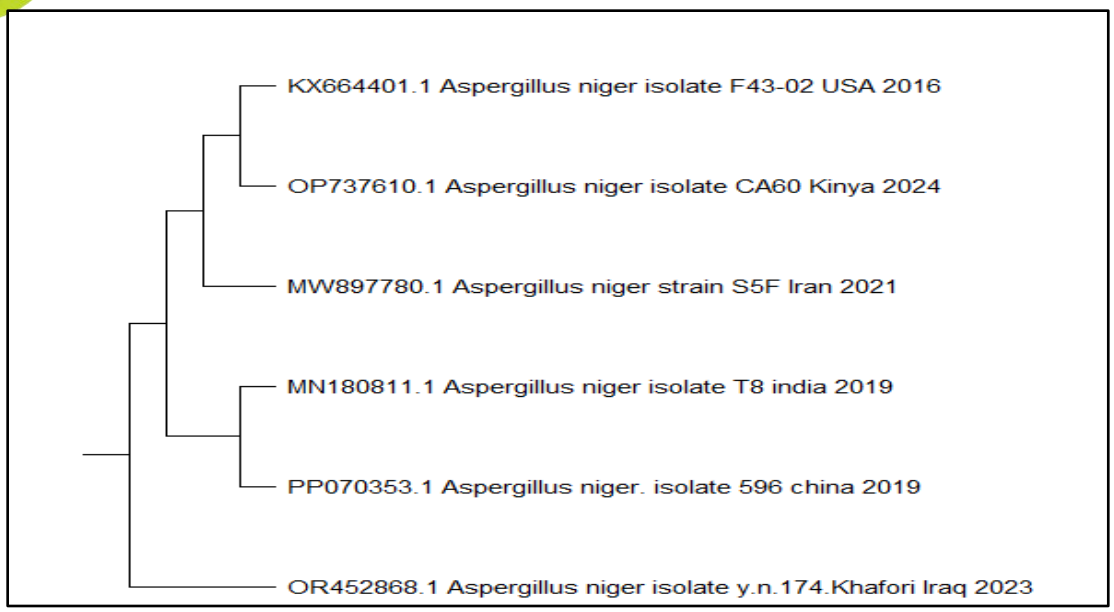


Figure (4): Genetic affinity tree for the fungal isolate *A. niger* isolate y.n.174.Khafori

Analysis of the nucleotide sequence of the isolate *A. niger* isolate y.n.175.Khafori and comparison of the similarity percentages of the nucleotide base sequences of the ITS gene region with global fungal isolates of the same fungus.

It was observed by comparing the nucleotide sequence of the DNA band of the fungus *A. niger* isolate y.n.175.Khafori isolated from local fish feed with the data available at the Center for Biotechnology Information (NCBI) that the percentage of genetic similarity reached (99.68 - 98.77%) with all isolates of the fungus A. niger (Table 6). While Figure (5), represented by the genetic tree, showed that this isolate showed genetic closeness to the Indian isolate (MN180811.1) (it appeared with the same branching. clade), while it appeared with separate branches (clades) from the recorded fungal isolates, especially from the Chinese isolate (PP070353.1).) due to the large genetic divergence between t.

Table (6): Comparison between the similarity ratios of the nucleotide base sequences of the ITS gene region of the fungal isolate (*A. niger* isolate y.n.175.Khafori) and other fungal isolates of the same fungus registered globally at the National Center for Technical and Biological Information (NCBI).

Fungal name	Isolate name	Origin	GenBank Accession Number	Sequence similarity	Date of registration with NCBI
A. niger	Y.n.177.Khafori	Iraq	OR449322.1	100%	2023
A. niger	isolate T8	India	MN180811.1	99.12 %	2019
A. niger	isolate 596	china	PP070353.1	99.64 %	2024
A. niger	isolate F43-02	USA	KX664401.1	98.77 %	2016
A. niger	isolate CA60	Kenya	OP737610.1	98.77 %	2023
A. niger	strain S5F	Iran	MW897780.1	99.11 %	2021

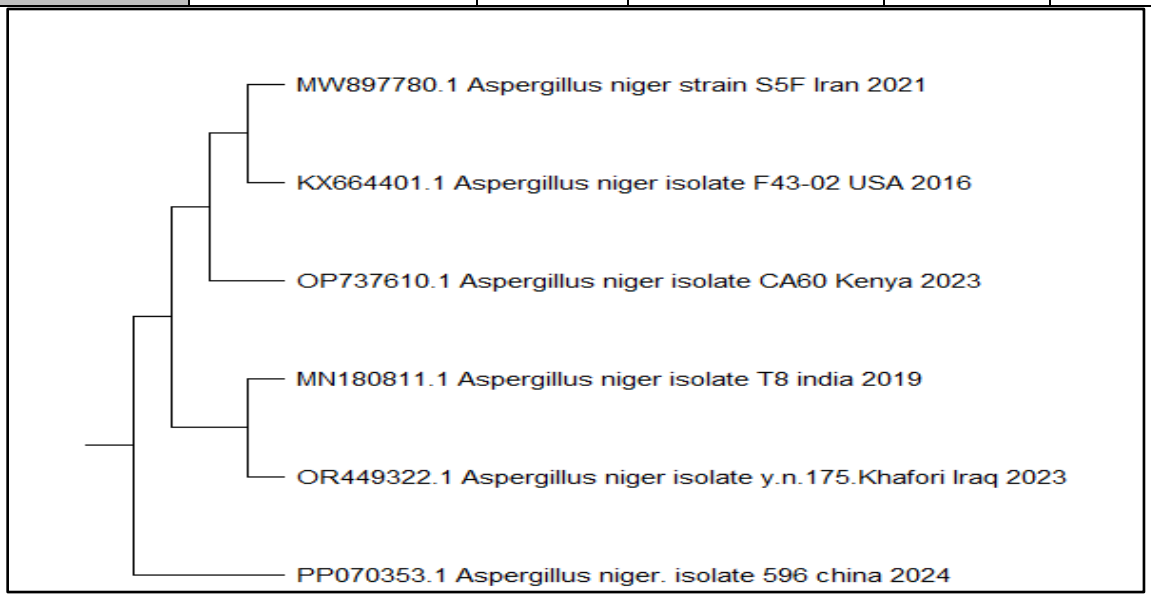


Figure (5): Genetic affinity tree for the fungal isolate *A. niger* isolate y.n.175.Khafori

Biological tests for the effect of feed contaminated with mycotoxins on the growth rates and health of fish.

This experiment was carried out to evaluate the effect of continuous exposure to fish feed contaminated with the mycotoxin ochratoxin on the feeding of carp fish, and to study the deterioration of their final weights and deaths and some health standards represented by their effects on blood components. The results of the effect of the mycotoxin ochratoxin A on growth rates and the number of fish kills during the rearing periods showed a high level of fish kills, with the percentage of kills reaching 10.80% compared to the control treatment, which amounted to 3.5% (Table 7). At the same time, the

percentage of weight loss in fish increased, as the highest percentage of weight loss was recorded in the 180-day period, amounting to 27.20%, while the 120-day period recorded a weight loss percentage of 20.20%, and the lowest percentage of loss was in the first 60 days, which amounted to 13.70%, compared to the control treatment of 0.00%. . While some negative phenotypic effects were recorded on the experimental fish (Figure 6), such as some cases of spots on the fish's body, cases of gill deformities, cases of eye opacity, and others.

Many previous studies have shown the health and behavioral effects on fish when raised on feed contaminated with the mycotoxin ochratoxin A. The most important of these effects are decreased feed intake, weight loss, poor performance, changes in swimming behavior, the appearance of abdominal skin spots, as well as yellow spots in the thoracic region and cord bending. Spinal fluid accumulation in the ventricle and kidneys. Hepatitis and liver cancer. And hyperinflammation of the gallbladder. [25,26] .

Table (7): Effect of the mycotoxin ochratoxin A on fish weight, growth rates, and number of kills.

NO	Transactions Mycotoxins / Breeding periods		Average weight of the box			Number of fish	Average per fish	% Lose weight	Number of deaths
			First raise	Another lift	Third lift				
1	after 60 day	addition OTA	40	39	42	242	500 g	%13.7	20
		Control	39	38	39	200	580 g	0.00	3
2	After 120 day	addition OTA	39	45	42	87	1487 g	%20.2	21
		Control	40	41	46	67	1865 g	0.00	7
3	After 180 day	addition OTA	40	44	39	63	2000 g	%27.2	13
		Control	45	40	39	45	2750g	0.00	8
LSD at level 0.05			4.12						

A model of the effect of Ochratoxin A on fish gills

A model of the effect of Ochratoxin A on fish eye opacity

A model of the effect of Ochratoxin A on fish mortality

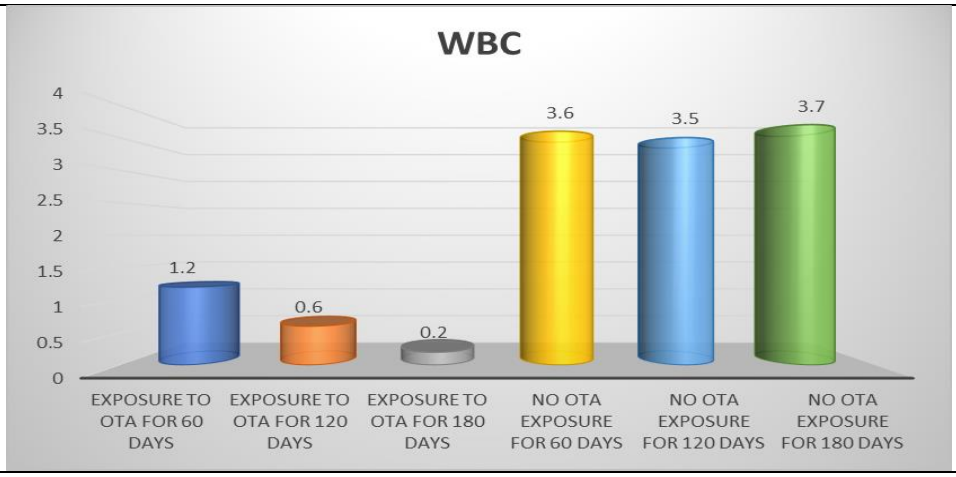
A model of the effect of Ochratoxin A on fish back spotting

Figure (6): Effect of the mycotoxin ochratoxin A on some phenotypic conditions of fish.

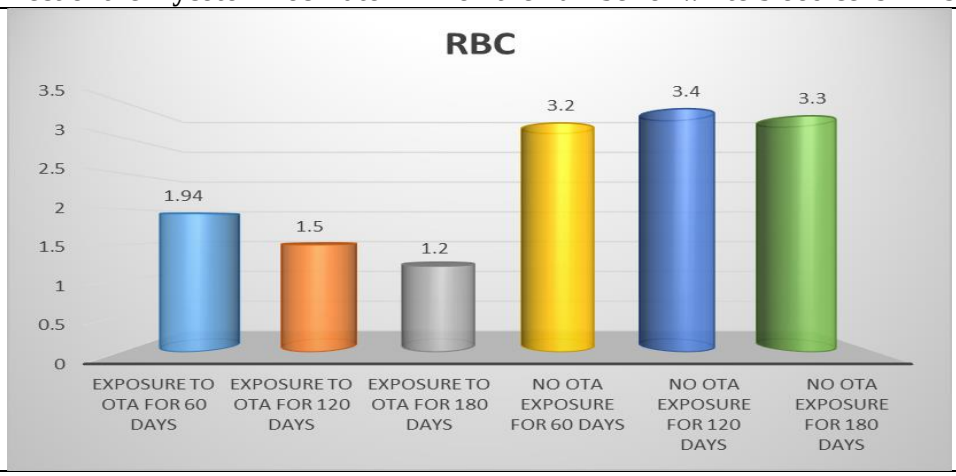
Effect of the mycotoxin ochratoxin A on physiological blood parameters of fish.

The results of physiological blood components showed different effects, as an effect was found in reducing the number of white blood cells (WBC) in the treatment of adding ochratoxin B1, which caused immunosuppression, with the highest effect recorded, resulting in a decrease in the number of white blood cells compared to the control treatment (Figure 7), as well as a significant reduction in Treatment to control the number of red blood cells (RBC), especially during the last stage of fish farming (after 180 days in the soil). The reason may be attributed to low levels of iron in the body, which may cause problems with vital organs such as lung, heart, kidney, and liver diseases, or the destruction of red blood cells before Replace it.

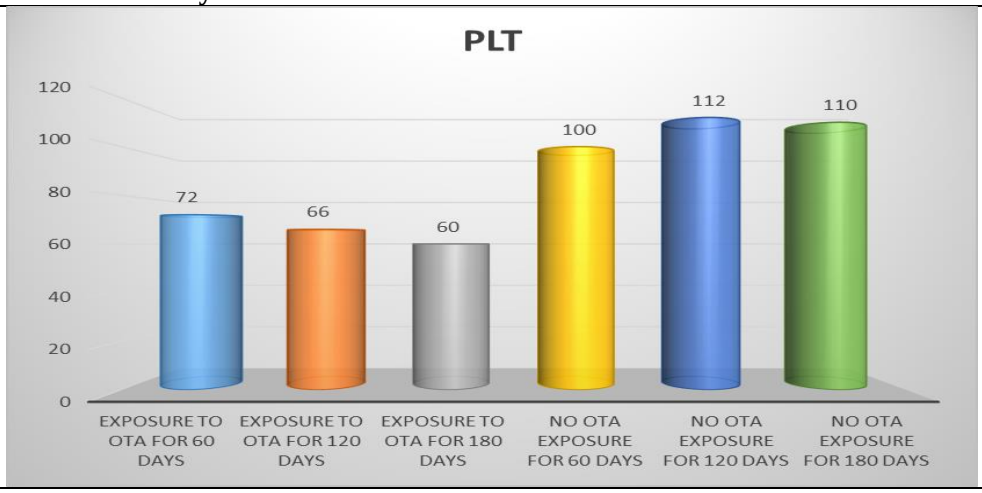
At the same time, exposure to ochratoxin reduced the number of platelets (PLT) in the blood of treated fish. The reason for the decrease in PLT was likely to be a result of a bone marrow disorder or a problem in the immune system, liver and spleen, or cancer due to the direct effect of mycotoxins. These results agreed with what was indicated. [27] indicated that the most important effects of ochratoxin on fish were a decrease in the number of red and white blood cells and platelets, general necrosis of blood components, and the appearance of histological abnormalities in the intestinal mucosa, liver cells, pancreatic cells, and gastric glands, and a decrease in the size of blood cells Red blood, its number, and white blood cells in the spleen.



Effect of the mycotoxin ochratoxin A on the number of white blood cells in fish



Effect of the mycotoxin ochratoxin A on the number of red blood cells in fish

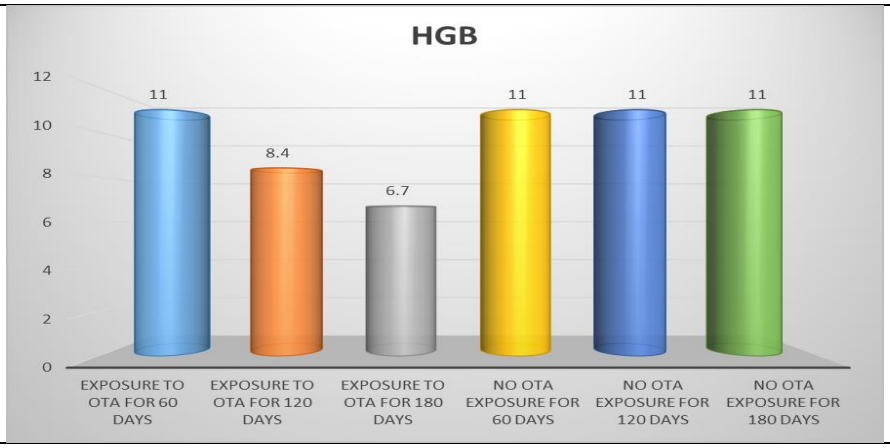


Effect of the mycotoxin ochratoxin A on platelet counts in fish

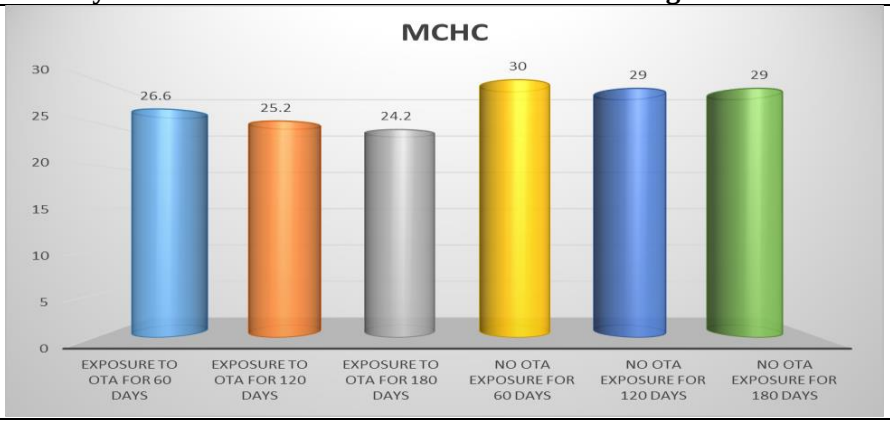
Figure (7): Models of the effect of the mycotoxin ochratoxin A on blood cells in fish

While the results did not record a clear effect in the amount of hemoglobin (HGB) in the ochratoxin treatment over the control treatment, as it reached (11 g/dl) during the first stage of rearing (within the first 60 days), while the amount of hemoglobin appeared to decrease gradually during the period of 120 - 180 days. From breeding (Figure 8), a

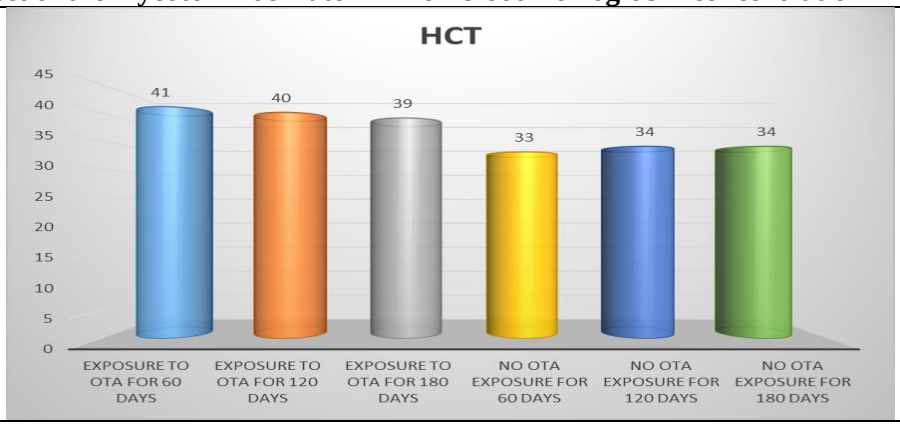
decrease in hemoglobin may cause failure of the respiratory system to transport oxygen and a deterioration in fish nutrition. It may result in kidney cancer or the production of red blood cells in larger quantities to compensate for the decrease in oxygen levels in the blood. While the results showed a decrease in the concentration of hemoglobin in red blood cells (MCHC) amounting to (24 g/dl, 25 g/dl), with a non-significant difference for the ochratoxin treatment compared to the control treatment, which amounted to (29 g/dl).



Effect of the mycotoxin ochratoxin A on the amount of hemoglobin in the blood in fish



Effect of the mycotoxin ochratoxin A on blood hemoglobin concentration in fish



Effect of the mycotoxin ochratoxin A hemacarbit on the blood of fish

Figure (8): Effect of the mycotoxin ochratoxin A on hemoglobin and hematocrit in fish blood

While the results in the ochratoxin treatment showed a clear increase in the percentage of compressed cell volume, reaching 50%. (Blood hemoglobin) at all stages of fish rearing, while in the control treatment it reached (40%). This increases the possibility of blood clotting in the blood vessels and may cause new deaths. Another study showed a decrease in the growth rate and a decrease in the amount of hemoglobin concentration and the number of red blood cells. Upon continuous exposure to ochratoxin, apparent necrosis of liver cells and necrosis of the gastric glands occurs. Increased activity of components of hematopoietic tissues. Iron rust pigments may accumulate in the epithelium of the intestinal mucosa. [28,29]

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