A Risk Assessment Of Fungal Infection With *Aspergillus flavus* In *Oreochromis niloticus* Through A Laboratory-Acquired Infection

Nadia A Abd El Ghany and Nashwa S Elias
Fish Diseases Department, Animal Health Institute, Dokki, Giza

ABSTRACT

In the current research, *Oreochromis niloticus* fish were subjected to experimental infection by *Aspergillus flavus* and treated by the immunostimulants , garlic powder in the ration. The infected fish exhibited signs of protrusive eye, slow-motion, lethargy, skin covered by thick mucus, severe hemorrhage on dorsal, anal and caudal fins, discarded scales and caudal fin erosion. Postmortem examination revealed that accumulative of bloody fluids in viscera and the abdominal cavity, damaged liver and enlargement of gall bladder which was filled with bile. Treatment trials exerted zero mortality with *Allium sativum* (garlic) but group treated by *Aspergillus flavus* with *Allium sativum* the mortality rate was 20% while the mortality rate was 100% with *Aspergillus flavus* group . Hematological picture showed normocytic normochromic anemia in both *Aspergillus flavus* and *Aspergillus flavus* with *Allium sativum* treated groups . Leucopenia and lymphopenia observed in *Aspergillus flavus* group while lymphocytosis was reported in *Allium sativum* treatment group .

There were significant increase in liver transaminases enzymes Aspartate Aminotransferase and Alanine Aminotransferase (AST & ALT) and decrease in total protein and albumin level in *Aspergillus flavus* but in *Aspergillus flavus* with *Allium sativum* significantly reversed the biochemical changes in the blood serum AST, ALT , total protein and albumin level. Histopathological findings of gills in fish group that was infected with *Aspergillus flavus* were severe in forms of proliferation of the filamentary epithelium leading to lamellar fusion of the secondary lamellae and desquamation, particularly at the tips and the pathological changes in the liver showed severe blood congestion and hemolytic with clear diffusion of melanomacrophage (MMC), thrombosis in blood vessels and severe congestion in pancreatic acini. These lesions may become less severe in fish group of *Aspergillus flavus* with *Allium sativum* . It could be concluded that the safety and useful dietary addition of *Allium sativum* (garlic) to alleviate the effects of *Aspergillus flavus* on *Oreochromis niloticus* fish .

INTRODUCTION

Freshwater fish are an important protein source for people of many countries *(1)*. Fish farming in various parts of the world has increased many folds in the last decade. As a result, fish culture has now become commercially an important industry worldwide.

Fungal infections cause losses of freshwater fish and their eggs both in wild and commercial fish farms *(2)*. All life stages of fish might be affected by the fungi. Fungal infections result in low production of fry and fish in farms *(3)*. Many fungi cause fish diseases such as; *Aspergillus (4)*. *Aspergillomycosis* is principally a disease of tilapia spp. *Aspergillus* species as *Aspergillus flavus*, *Aspergillus japonicas* and *Aspergillus terreus* cause aspergillomycosis. These fungal species are presumably infectious through

contamination of fish feed. The distribution of these fungi, results from the production of numerous air born conidia which are easily dispersed by air movement. Factors which enhance the threat of fungal infection through feeds include environmental temperature 27 °C, humidity level greater than 62% and moisture level in the feed above 14%. Poor water quality is one of the most important factors favoring the growth of fungus. *Aspergillus* spp. may have more indirect effect in tropical conditions. Ecological differences play an important role in species diversity of fungi that develop on both fish and eggs as expressed by ***(5,6)***.

Herbal medicinal products are widely used around the world ***(7)***. A growing interest has emerged in using herbs in animal feeds by both researchers and feed companies ***(8)***. Garlic used as immunostimulants in aquaculture it enhancing the activity of the non-specific defense mechanisms and increasing disease resistance, mainly through facilitating the function of phagocytic cells, increasing their fungicidal activities, and stimulating the natural killer cells, complement system, lysozyme activity ***(9)***. Also garlic can help in the control of pathogens, especially bacteria and fungi, and increase the welfare of fish ***(10)***.

The aim of the present study is to throw light on the *Aspergillus flavus* infection in *Oreochromis niloticus* fish and its effect on the functions of internal organs. We also aimed to investigate the effect of garlic as a safe and economic antifungal agent for and treatment of this fungal infection.

MATERIALS AND METHODS

Fish Collection

A total number of 60 *Oreochromus niloticus* fish with an average body weight 90 ± 10 g. transferred from a private fish farm at Borg-El-Arab, Alexandria Governorate to Fish Diseases Department at the Animal Health Research Institute and acclimatized in glass aquaria with re-circulating water at 25^0c ± 0.5 for one week with oxygen and ammonia adjustment daily. At the end of acclimation period, 20 fish were randomly collected and subjected to bacteriological and parasitological examination to confirm that these fish were free from infection.

Preparation of fungal cultures

Fungus Isolation

Isolated fungi of *Aspergillus flavus* from *Oreochromus niloticus* was obtained from Microbiological Unit of Fish Diseases Department, Animal Health Institute, Dokki, Giza.

Preparation of Spores Suspension

Fungal strains of *Aspergillus flavus* cultured on Sabourauds dextrose agar (SDA, Difco). Media were supplemented by penicillin at 100 IU/ml and streptomycin at 100 ug/ml. The cultured plates were incubated at 25 °C for 7 days, and then conidial mass was harvested by adding 20 ml. sterile distilled water into each culture plate, followed by collection of the suspension in 30 ml sterile autoclave tubes. Suspensions were filtered through two layers of sterile medical gauze to ensure that filtrate contain fungal conidia, concentrations of which were calculated using a heymocytometer and adjusted to 4×10^3 spore ml^{-1} in sterile distilled water ***(11)***. Isolation of fungi was done in Laminar flow air cabinet Level 2 (CL2), the use of high-efficiency particulate air (HEPA) filters, frequent air exchanges; wear an N95 mask and positive pressure ventilation were used to avoid contamination for pathogenic molds and recommended to limit nosocomial exposure.

Experimental Design

A total number of 40 *Oreochromis niloticus* were divided into 4 equal groups (10 fish). The first group was injected I/ P with 0.2 ml. saline and fed on normal ration as a control. The fish of the second groups were injected I/ P with 0.2 ml. of concentrations 4×10^3 of *Aspergillus flavus* conidial suspension and fed on normal ration. The fish of the third groups

were injected I/ P with 0.2 ml. of concentrations 4×10^3 of *Aspergillus flavus* conidial suspension and fed on ration contain on garlic (MediaVet,Garlien,Egypt) powder 3 g / Kg. ration . The forth group fish were fed on ration containing 3 g of garlic powder/kg ration, all fish were fed twice daily for 4 successive weeks at the rate of 2% of their body weight. Clinical abnormalities, postmortem lesions and mortality rate were recorded ***(12)***.

At the end of experimental trial, blood samples were taken for hematological examination . The hemogram (erythrocytes count, total leukocytic counts and hemoglobin concentration) by using an Ao Bright–Line Haemo-cytometer (Neubauer improved, Precicolor HBG, Germany) were estimated according to the methods described by ***(13)***, PCV value, blood indices as MCV, MCH and MCHC and differential leukocytic counts according to ***(14)***. Serum biochemical parameters, ALT, AST, total protein and albumin were determined spectrophotometrically using commercial kits (Spinreact, Spain) according to the methods of ***(15-17)***.

Fungal reisolation

The glassware (containing media and distilled water covered with aluminum foil), test tubes and vials (cotton plugged) were autoclaved at 121 °C at 15 psi for 15 minutes. Antibiotic streptomycin sulphate 250 mg was added to each preparation of media to reduce bacterial contamination. Specimens from gills, muscle and liver at injection site from each living or dead fish with sterile needle were microscopically examined and inoculated into PDA plates to assure reisolation and identification of fungus ***(11)***.

Histopathological Examination

Tissue specimens from suspected examined organs were fixed in 10%buffered formalin, embedded in paraffin, sectioned and stained with Haematoxylin and Eosine (H&E) as well as periodic Acid Schiff methods (PAS) according to ***(18)***.

Statistical analysis

Data were statistically analyzed by ANOVA test with posthock LSD multiple comparison test using statistical software program ***(19)***. Differences were considered significant at $P < 0.05$.

RESULTS

Clinically the experimentally infected fish with *Aspergillus flavus* was protrusive eye , slow-motion, lethargy, less feed acceptability, skin covered by thick mucus, severe hemorrhage on dorsal, anal and caudal fins, discarded scales and caudal fin erosion. **Fig (1).**

The postmortem lesions showed enlarged and congested visceral organs (liver, spleen and kidney) and the abdominal cavity were filled with heavy amounts of bloody fluids, damaged liver and enlargement of gall bladder which was filled with bile. **Fig (2).**

Treatments effects

Treatment trials exerted zero mortality in *Allium sativum* treated group but in group treated by *Aspergillus flavus* with *Allium sativum* (garlic) the mortality rate 20% and *Aspergillus flavus* group was 100% (Table, 1).

The effects of dietary of *Allium sativum* (garlic) on hematological and some serum biochemical parameters are tabulated in tables (2, 3, &4). The erythrogram results showed normocytic normochromic anemia in both *Aspergillus flavus* (2^{nd} gp.) and *Aspergillus flavus* & *Allium sativum* (garlic) (3rd Gp.) in comparison with the control group. Leucopenia and lymphopenia observed in (2^{nd} gp.) compare to control. Lymphocytosis is reported in *Allium sativum* (garlic) treatment group (4^{th} gps) in comparison to other treated groups.

Regarding serum biochemical parameters, elevation in liver transaminases

(ALT & AST), total protein and albumin level in (2nd gp.) and (3rd Gp.) in comparison with control group.

No fungus was reisolated after treatment with garlic.

Histopathological findings

Gills of the control fish (G1) have normal histological architecture (Fig. 3). Gills of fish group that was infected with *Aspergillus flavus* (2nd gp.) were severe in forms of proliferation of the filamentary epithelium leading to lamellar fusion that could be seen (Fig. 4) with fusion of the secondary lamellae and desquamation, particularly at the tips (Fig. 5). Gills of fish group that were *Aspergillus flavus* & *Allium sativum* (garlic) (3rd Gp.) showed slight congestion of primary gill blood vessels with slight dilation of the vessels at the tips of secondary lamella (Fig. 6).

The pathological changes in the liver of the fish groups G2 showed severe lesions in liver in form of severe blood congestion and hemolysis with clear diffusion of melanomacrophage (MMC) (Fig.7), thrombosis in blood vessels (Fig. 8) and severe congestion in pancreatic acini beside hemosiderin accumulation (Fig. 9). Group 3 *Aspergillus flavus* & *Allium sativum* (garlic) showed congestion in pancreatic acini with slight hemorrhage of hepatic blood vessels. (Fig. 10).

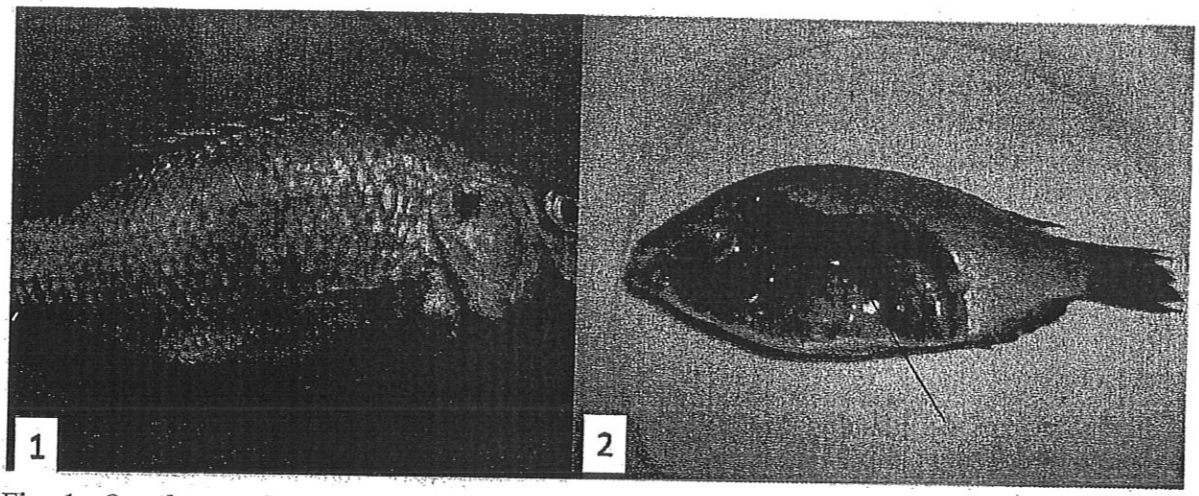


Fig. 1. *O. niloticus* fish experimentally infected with *Aspergillus flavus* showing discarded scales , caudal fin erosion and ulceration of skin (Arrows).

Fig. 2. *O. niloticus* fish experimentally infected with *Aspergillus flavus* showing congestion of gills, enlarged and congested liver, enlargement of gall bladder which was filled with bile ,spleen and kidney (Arrows).

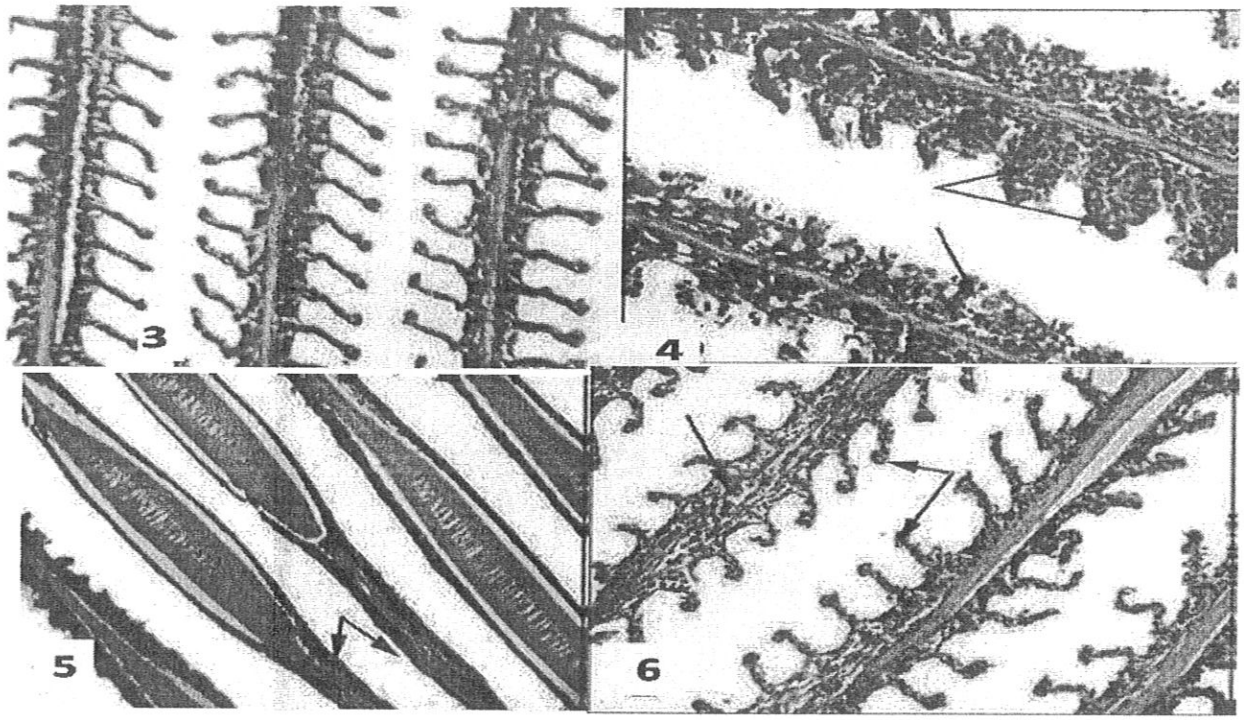
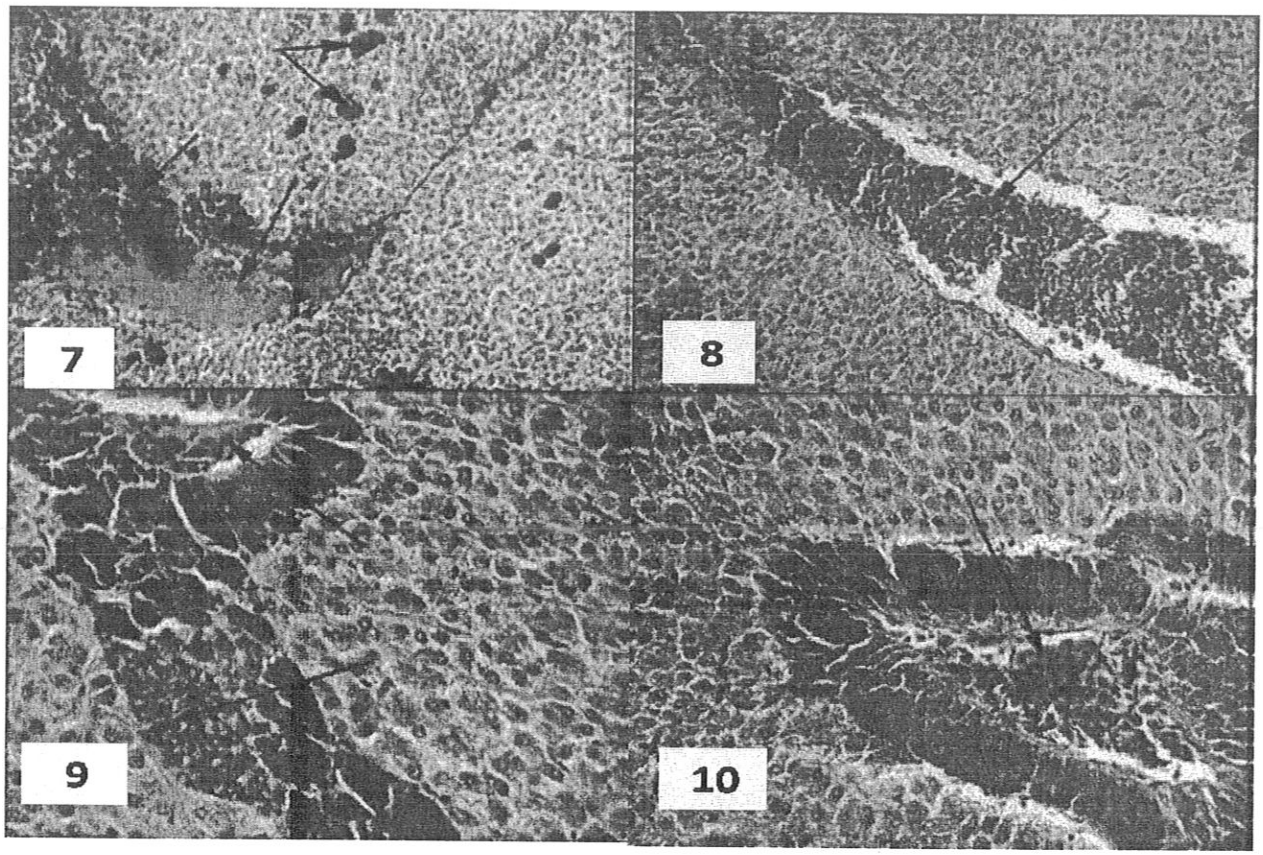


Fig. 3. Histological sections in gills of *O. niloticus* fish stained with H&E; (1) control fish showing normal structure of gill lamellae. (x1300).

Fig. 4. Gills of fish exposed to *Aspergillus flavus* showing severe proliferation of the filament epithelium (x400).

Fig.5. Histological sections in gills of *O. niloticus* fish stained with H &E; fish exposed to *Aspergillus flavus* showing fusion and desquamation of secondary lamellae (x150).

Fig.6. Histological sections in gills of *O. niloticus* fish stained with H &E; fish exposed to *Aspergillus flavus* & *Allium sativum* showing dilation in blood vessel of secondary lamellar (x150),



Figs.7-9.Histological sections in liver of *O. niloticus* fish stained with H &E; fish exposed to *Aspergillus flavus* showing severe thrombosis in blood vessels , necrosis, Vaculation , slight congestion in pancreatic, thrombosis in blood vessels and hemosiderin accumulation . (x400).

Fig. 10. Histological sections in liver of *O. niloticus* fish stained with H&E. fish exposed to *Aspergillus flavus* & *Allium sativum* showing slight congestion in pancreatic acini (x600)

Table 1. The mortality rate among the experimentally infected fish after the application of treatment

Fish groups		No. of fish	No. of dead fish	Mortality rate
Control (group I)		10	0	0
Infected group	(2nd gps.)*Aspergillus flavus*	10	10	100%
Treated groups	(3rd gps.) *A. flavus* & (garlic)	10	2	20
	(4th gps) (garlic) treatment	10	0	0

Table 2. Effect of *Aspergillus flavus* and *Allium sativum* (garlic) on erythrogram Picture (Mean ± S.E) in *Oreochromis niloticus*

Groups	RBCs Mill/µL	Hb gm/dl	PCV %	MCV FL	MCH Pg	MCHC %
1st gp. Control	1.95 ± 0.14a	7.98 ± 0.41a	27.10 ± 1.15a	138.5 ± 3.82	40.1 ± 1.85	29.4 ± 1.12
(2nd gp.)*Aspergillus flavus*	1.62 ± 0.12b	6.01 ± 0.24b	21.05 ± 1.05b	129.8 ± 4.95	37.2 ± 1.98	29.1 ± 1.21
(3rd gp.)*A.flavus* & garlic	1.65 ± 0.10b	6.32 ± 0.28b	22.01 ± 1.21b	133.3 ± 4.28	38.2 ± 2.02	28.7 ± 2.31
(4th gp) garlic treatment	2.12 ± 0.18a	8.14 ± 0.34a	29.04 ± 1.86a	136.8 ± 5.06	38.3 ± 2.74	28.3 ± 1.64

Table 3.Effect of *Aspergillus flavus* and *Allium sativum* (garlic) on leukogram Picture (Mean ± S.E) in *Oreochromis niloticus*

Groups	TLC 10^3/µL	Neutro 10^3/µL	Esinoph 10^3/µL	Basoph 10^3/µL	Lymph 10^3/µL	Monocy 10^3/µL
1st gp. Control	35.76 ± 2.15a	7.81 ± 0.58	0.92 ± 0.09	0.00 ± 0.00	25.4 ± 1.10a	1.63 ± 0.14
(2nd gp.)*Aspergillus flavus*	29.52 ± 2.12b	7.16 ± 0.41	0.84 ± 0.11	0.00	19.98 ± 1.05b	1.56 ± 0.21
(3rd gp.)*A.flavus* & garlic	34.08 ± 2.96ab	7.28 ± 0.38	1.01 ± 0.10	0.14 ± 0.10	23.94 ± 1.34a	1.72 ± 0.22
(4th gp) garlic treatment	41.24 ± 3.75a	7.74 ± 0.50	0.98 ± 0.12	0.00	29.91 ± 1.21c	1.61 ± 0.18

Significant at P > 0.05

Table 4. Effect of *Aspergillus flavus* and *Allium sativum* (garlic) on some biochemical parameters (Mean ± S.E) in *Oreochromis niloticus*.

Group	ALT U/ml	AST U/ml	T. Protein gm/dl	Albumin gm/dl
1st gp. Control	37.1±3.41a	79.50±9.4a	3.18± 0.21a	1.42± 0.11a
(2nd gp.) *Aspergillus flavus*	65.1±6.15b	134± 8.65b	2.81± 0.18b	1.09± 0.10b
(3rd gp.) *A. flavus* & garlic	59.4± 5.6b	92.6± 9.12b	2.95± 0.16ab	1.01± 0.14b
(4th gp) garlic treatment	35.1± 2.8a	124± 7.24a	3.25± 0.24a	1.36± 0.16a

DISCUSSION

Aspergillomycosis is the disease of African fish tilapia (*Oreochromis spp*) and the causative agents of this disease are species of *Aspergillus flavus, Aspergillus terreus* and *Aspergillus japonicas.* These fungal species are reported to be infectious through contamination of fish feed *(20)* Fungal load increases significantly during storage period of feed at high moisture levels in ground and tree nuts *(21)*. Fish feed stored under tropical conditions is contaminated with *Aspergillus flavus*, hence the toxins produced by the fungus may be deposited on feed pallets. If such contaminated feed is consumed by the fish, it may cause acute deleterious effects, which may lead to mass mortalities *(22)* .The source of fungal infection may be the consumption of contaminated feed present in the pond. Moreover, the decomposition of this feed also added to increase infection. There might be certain other conditions in the pond which increased the possibility of fungal infection including: poor pond management, injured fish or fish having other diseases, or large amounts of decomposing organic matter in pond *(23)*.

Clinically the experimentally infected fish with *Aspergillus flavus* was protrusive eye, slow-motion, lethargy, less feed acceptability, skin covered by thick mucus, severe hemorrhage on dorsal, anal and caudal fins, discarded scales, caudal fin erosion and loss construction of fish body. Anatomically, there were some postmortem lesions included uncharacterized liver, viscera of fish and the abdominal cavity were filled with heavy amounts of mucus, damaged liver and enlargement of gall bladder which was filled with bile. Such findings were nearly similar to that previously obtained **(*24* ,*25*).**

The treatment of *Aspergillus flavus* infected fish with garlic(*Allium sativum*) from the beginning of experimental infection reduced the clinical signs and postmortem findings also decreased the mortality rate in infected fish to 20%. This enhancement may indicate the protective role of garlic(*Allium sativum*)on the infection of *Aspergillus flavus* in *Oreochromis niloticus* fish.

Our study showed normocytic normochromic anemia in *Aspergillus flavus* treated group at the end of the experiment. The main hemopiotic tissue in *O. niloticus* is anterior kidney *(26)*. Renal damage of *Aspergillus flavus* treated channel catfish was reported *(27)* as well as in common carp *(28)*. Similarly, *(29)* recorded anemia in *O. niloticus* exposed to *Aspergillus flavus* for 10 days.

The treated groups with *Aspergillus flavus* showed leukocytosis, neutrophilia and lymphopenia. The main leukocytic picture of acute stress in teleost fish is neutrophilia, and lymphopenia *(18)*. The lymphocytolysis was observed in haemopoietic organs (kidney and spleen) during *Aspergillus flavus* contaminated

ration, in Labeo rohita under electron microscopic observations ***(30)***. Leukocytosis had been reported by ***(27)*** in channel catfish *Ictularus punctatus* fed *Aspergillus flavus* contaminated ration. They concluded that leukocytosis was probably as a result of a response to the necrosis of gastric glands.

Interestingly, the fish treated with garlic (Allium sativum)exhibited a significant increase in the total leucocytic count and lymphocytes., these results were supported by ***(31)*** who reported that treatment with garlic(Allium sativum) resulted in the maximum number of leucocytes and macrophages exhibiting enhanced phagocytic activity. Furthermore ***(9)*** found that stimulated phagocytic cells of tilapia *Oreochromis sp* fed on ration contains 1 and 2 gm of garlic. These results agreed with ***(32)*** who revealed that there was a significant increase in leucocytic count and main percent of neutrophils decrease significantly in fish feed in diet containing 0.45 and 0.6 gm of garlin /kg ration.

ALT and AST activities were significantly increased in the treated fish groups with *Aspergillus flavus* in comparison with control one. This elevation could be attributed to hepatic injury. Several authors concluded that the increase of such enzymes to the hepatic effect of *Aspergillus flavus* ***(33,34)***. Our result in accordance with ***(28)*** who observed elevation in liver transaminase enzymes in *Cyprinus carpio* fed on moldy ration for 42 days. Garlic had reduced liver enzymes through enhancing activity of non specific defense mechanism, ***(35,36)*** which elucidated the highly significant decrease of liver enzymes in this research.

Total protein and albumin concentrations were decreased in *Aspergillus flavus* group. This decline in serum protein concentration may due to impaired protein synthesis and/or liver disorder caused by fungal infection ***(37)***. The reduction in total protein level may be due to the fungal infection in *Labeo rohita* ***(30)***. Several authors also reported declines in plasma protein concentration in fresh water fishes (*O. niloticus* and rainbow trout) exposed to *Aspergillus flavus* (***25,28***).

Treatments trials were held out using medicinal plant(garlic) owing to their immunostimulants properity and antifungal activities, (***9,38***) .Garlic was defined by ***(39)*** as the promising treatment of fungal – associated diseases and proved its' ability to inhibit growth of fungi.

Regarding the histopathological examination of Gills of fish infected with *Aspergillus flavus* (2^{nd} gp.) were severe in forms of proliferation of the filamentary epithelium leading to lamellar fusion with fusion of the secondary lamellae and desquamation, particularly at the tips. These gill alterations might harm the changes in gills of fish ***(40)***. Similar gill changes were observed by ***(41)***. Gills of fish group that were *Aspergillus flavus & Allium sativum* (garlic) (3rd Gp.) showed slight congestion of primary gill blood vessels with slight dilation of the vessels at the tips of secondary lamella .

The pathological changes in the liver of the fish groups G2 showed severe lesions in liver in form of severe blood congestion and hemolysis with clear diffusion of melanomacrophage (MMC) ,thrombosis in blood vessels and severe congestion in pancreatic acini beside hemosiderin accumulation similar results were recorded by ***(42)***. Group 3 *Aspergillus flavus & Allium sativum* (garlic) showed congestion in pancreatic acini with slight hemorrhage of hepatic blood vessels because garlic contain antifungal and antioxidant compounds ***(43)***. Our results also agreed with that obtained by ***(36*** and ***44)*** who found that garlic has the ability for the control of *Aspergillus flavus* infection in Rainbow trout.

We concluded that the use of the garlic in the laboratory conditions reduced many harmful effects of *Aspergillus flavus* infection in *O. niloticus* fish and decreased the mortality rate.

REFERENCES

1.Rubbani B, M Afzal, MS Mubarik, M Salim and SM Hussain, (2011): Estimation of apparent digestibility coefficients of soybean meal based diets with different protein levels for Labeo rohita. Pak J Agric Sci, 48: 231-235.

2.Roberts, R. J. (1995): Aquatic animal health towards. 2000 In: Diseases in Asian Aquaculture II. Shariff, M., T. R. Arthur, R. P. Subasinghe (eds.). Fish Health Section, Asian Fisheries Society, Manila. pp. 3-7.

3.Kwanprasert. P, Hangavant C and Kitancharoen (2007): Characteristics of Achyla bisexualis Isolated from Eggs of Nile Tilapia (Oreochromis niloticus Linn.). KKU Res J., 12(3), 195-202.

4.Saleem A A, Refai M, Eissa I A, Mmarzouk M, Bakir A, Mustafa M, Mandmanal A (1989): Some studies on aspergillomycosis in Tilapia nilotica. Zagazig Vet. J., 17(3), 315-328.

5.Hussein M M, Hatai K and Nomura T (2001): Saprolegniasis in salmonid and their eggs in Japan. J. Wildl. Dis., 37, 204-20

6.Robert A Weinstein (2009): Laboratory-Acquired Infections. Cline Infect Dis. 49 (1):142-147.

7.Jordan SA, Cunningham DG and Marles RJ (2010): Assessment of herbal medicinal products: challenges, and opportunities to increase the knowledge base for safety assessment Toxicology and Applied Pharmacology, 243 , pp. 198–216

8.Logambal SM, Venkatalakshmi S, and Dinakaran Michael R (2000): Immunostimulatory effect of leaf extract of Ocimum sanctum Linn. in Oreochromis mossambicus . Hydrobiologia Volume 430, Issue 1-3, pp 113-120

9.Khalil1.R, El-Hofy H, Yehya M M and Selim KM (2014): Some Biochemical and Immunological Changes Associated with Use of Garlic Extract (allicin) in Combating Some Fish Pathogens. Alexandria Journal of Veterinary Sciences 41:1-18

10.Corzo-Martinez, M, Corzo N, Mar Villamiel (2007): Biological properties of onions and garlic, Trends in Food Science Technol.18: 609-625.

11.Willoughby LG (1994): Fungi and Fish Diseases. Pisces Press, Stirling. UK.pp 57.

12.Elias NS and Abd El Ghany NA (2008): Monitoring the Effect of Garlic (Allium sativum) and Black Seed (Nigella sativa) on Fusarium moniliform Infection in Fish with Emphasis on Fecundity. Global Veterinaria 2 (5): 242-249.

13.Jain NC (1993): Essential of veterinary hematology copyrights by Lea and Febiger Philadelphia,USA.

14.Stoskoph M (1993): Fish Medicine. PP, 116,128,129.W.B. Saunders Company.

15.Henry RL, Cannon DC, Winklemen JW (1974): Clinical chemistry: principles and techniques,2nd(ed) Harper and Row Hagerstown,NewYork,pp726–727

16.Doumas T, Bayso DD, Carter RJ and Peters RS (1981): Determination of total serum protein.Clin Chem 27:1642–1843

17.Coles, EH (1986): Veterinary clinical pathology, WB Saunders, Philadelphia , pp 10–42.

18.Roberts R J (2001): Fish Pathology, 3rd edition, W.B. Saunders.

19.State View (1993): Version 4.01. Abacaus Institute. Berkeley, California.

20.Saleem M J, Hanan A, Nisa A U and Qasir TA (2012): Occurrence of Aflotoxinin Maize seed under different condition.Int. J.Agri. Biol.,14,473-476

21.Jubeen F, A.Bhatti I, Maqbool U and S.Mahboob (2012): Fungal incidence, aflotoxin B1, tocopherols and fatty acids dynamics in ground and tree nuts during storage at two moisture levels. Int. J. Agri. Biol., 14,521-527.

22.Olufemi BE The Aspergilli as Pathogens of cultured fishes (1985): In: Recent advances Aquaculture, (eds. JF Munir and RJ Roberts), pp: 193-218.

23.Firouzbakhsh F, Ebranhim Zadeh morusavi E H A and Khosravi A R (2005): Isolation and Identification of Pathogenic and saprophytic fungi from gill lesion in cultivated cyprinids (common carp, silver carp and grass carp). J. Fac. Vet. Med. Univ. Tehran., 60(1), 15-19.

24.Santacroce MP, Conversano MC, Casalino E, LaiO Zizzadoro C, Centoducati G and Crescenzo G (2008): Aflatoxins in aquatic species :metabolism, toxicity and perspectives .Rev Fish Biology Fish18:99–130

25.Deng SX, Tian LX, Liu FJ, Jin SJ, Liang GY, Yang HJ, Du ZY, LiuY J (2010): Toxic effects and residue of aflatoxin B1in tilapia(Oreochromis niloticus and O.aureus)during long-term dietary exposure.Aquaculture307:233–240.

26.Stoskoph M (1993): Fish Medicine. PP, 116,128,129.W.B. Saunders Company.

27.Jantrarotai W and Lovell RT (1990): Subchronic toxicity of dietary aflatoxin B_1 to channel catfish. J Aquat Anim Health, 2:248–256.

28.Pepeljnjak S, Petrinec Z and Kovacic S, Segvic M (2003): Screening toxicity study in youngcarp (CyprinuscarpioL.) onfeedamendedwithfumonisinB1.Mycop athologia156(2):139–145

29.Hussein SY, Mekkawy IAA, Moktar, ZZ and Mubarak M (2000): Protective effect of *Nigella sativa* seed against aflatoxicosis in *Oreochromis niloticus*. Proc. Conf. Mycotoxins and Dioxins and the Environment, Bydgoszcz, 25 – 27 Sept., pp: 109 – 130.

30.Sahoo P K and Mukherjee S C (2003): Immunomodulation by dietary vitamin C in healthy and aflatoxin B_1-induced immunocompromised rohu (*Labeo rohita*). Comp Immunol Microbiol Infect Dis. 2003 26(1):65-76.

31.Rose P, Whiteman MPK, Moore and Zhu Y Z (2005): Bioactive Salk (en) yl cysteine sulfoxide metabolites in the genus Allium: the chemistry of potential therapeutic agents. Natural Product Rep. 22:351-368.

32.Fazlolahzadeh F, Keramati K, Nazifi S, Shirian S and Seifi S (2011): Effect of garlic (Allium Sativum) on haematological parameters plasma activities of ALT and AST Rainbow, Trout in temperature stress. Aus. J. of Basic and Appl. Sci., 5(9): 84-90 .

33.Jones T C, Hunt R D and King N W (1997): Veterinary Pathology. 6th Ed. Baltimore, MD, Williams and Wilkins.

34.Soliman K M, Ayesh AM, Manal EM and Naguib K (1998): Effect of aflatoxins decontamination by a selective chemisorbent materials on the *Oreochromis niloticus* with considering fish processing efficiency. J. Egypt Ger. Soc. Zool, 25(A): Comparative Physiology: 1-19.

35.Shalaby AM, Khattab YA and Abdel Rahman AM (2006): Effect of Garlic (Allium sativum) and chloramphenicol on growth performance, physiological parameters and survival of Nile tilapia. J.Venom. Anim. Toxins incl. Trop. Dis. Vol. 12 no.2.

36.Sandosskumar R, Karthikeya M, Mathiyazhaga S, Mohankumar M, Chandrasekar G and Velazhahan R (2007): Inhibition of *Aspergillus flavus* growth and detoxification of aflatoxin B by medicinal plant zimnu (*Allium sativum* L. X *Allium cepa* L.). World Journal of Microbiology and Biotechnology, 23:1007-1014.

37.Buhler D R, Miranda C L, Henderson M C, Yang Y H, Lee S J and Wang-Buhler J L (2000): Effects of 17beta-estradiol and testosterone on hepatic mRNA/protein levels and catalytic

activities of CYP2M1, CYP2K1, and CYP3A27 in rainbow trout (*Oncorhynchus mykiss*). Toxico. Appl. Pharmacol.; 168(2):91-101.

38.Diab AS, Sakr SF, Abd El- Hadi YM and Ahmed MH (2006): Evaluation of time and dose related use of Allium sativum (Garlic) and Nigella sativa (Black seeds) mixture as feed additives for Oreochromis niloticus. Egypt. Journal. Agriculture. Rescaech . Vol. 84 Issue 1B.

39.Ghahfarokhi MS, Shokoohamiri M, Rajab NA, Moghadasi B, Ghajari A, Zeini F, Sadeghi G and Abyaneh MR (2006): In vitro antifungal activities of Allium cepa, Allium sativum and ketoconazole against some pathogenic yeasts and dermatophytes. Fitoterapia Vol. 77 Issue 4.

40.Mehrim AI, Abdelhamid AM, Abou-Shousha A, Salem MFI and El-Sharawy MAMM (2006): Nutritious attempts to detoxify aflatoxic diets of tilapia fish: Clinical, biochemical and histological parameters. Journal of the Arabian Aquaculture Society, 1: 69-90.

41.Abdel-Wahhab MA, Hassan NS, El-Kady AA, Khadrawy YA, El- Nekeety AA, Mohamed SR, Sharaf HA and Mannaa FA (2010): Red ginseng extract protects against aflatoxin B_1 and fumonisins-induced hepatic pre-cancerous lesions in rats. Food Chem. Toxicol., 48 (2): 733–742.

42.El-Barbary MI and Mehrim AI (2009): Protective effect of antioxidant medicinal herbs, rosemary and parsley, on subacute aflatoxicosis in *Oreochromis niloticus*. Journal of Fisheries and Aquatic Science, 4 (4): 178-190.

43.Kumar CH, Ramesh A, Kumar JNS and Ishaq BM (2011): A Review on hepatoprotective activity of medicinal plants. Int J Pharm Sci Res., 2 (3): 501-515.

44-Mehrim AI (2013): Medicinal herbs against aflatoxicosis by Nile tilapia (Oreochromis niloticus): Clinical, postmortem signs and liver histological patterns. Egy. J. aquac,Vol. 3, No.1:13-25 ISSN: 2090-7877.

الملخص العربى

مخاطر العدوى بفطر الاسبرجيلس فلافس فى البلطى النيلى من خلال العدوى المكتسبة معمليا

نادية أحمد عبد الغنى ، نشوى سمير الياس
قسم بحوث أمراض الأسماك - معهد بحوث صحة الحيوان –الدقى - جيزة

تم في هذه الدراسة بحث التأثيرات الضارة للعدوى التجريبية بفطر الاسبرجيلس فلافس على أسماك البلطي النيلي، كما تم تجربة علاج الأسماك من تلك الآثار الضارة باستخدام مسحوق الثوم مضافا إلى عليقة الأسماك.

كانت العلامات المرضية على الأسماك المصابة عبارة عن فقدان فى الشهية، عتامة فى العين، زيادة فى كمية المخاط المغطى للجسم ، نزيف حاد في الزعنفة الظهرية والذيلية و وجود سوائل ارتشاحية دموية فى التجويف البطنى. بعد النفوق كشفت دراسة الأحشاء فى الأسماك امتلأء التجويف البطنى بكميات كبيرة من السوائل المدممة، تليف فى الكبد وتضخم فى المرارةحيث كانت ممتلئة بالعصارة الصفراوية. أظهرت تجارب العلاج المبذولة نسبة النفوق صفر في المجموعات المعالجة بمسحوق الثوم ولكن في المجموعة التي عولجت بفطر الاسبرجيلس فلافس ومسحوق الثوم فكان معدل النفوق بها ٢٠٪ وبفطر الاسبرجيلس فلافس ١٠٠٪. صورة الدم أظهرت فقر الدم فى كريات الدم الحمراء في فطر الاسبرجيلس فلافس وزيادة طفيفة فى فطر الاسبرجيلس فلافس ومسحوق الثوم. نقص فى الكريات الدم البيضاء واللمفاويات والتي لوحظت في مجموعة الاسبرجيلس فلافس و زيادة في المجموعة المعالجة بفطر الاسبرجيلس فلافس ومسحوق الثوم .

بالنسبة للتغيرات الكيميائية الحيوية وجدنا أن الإصابة بفطر الاسبرجيلس فلافس أدت إلى زيادة معنوية في نشاط إنزيمات الأسبرتات أمينوترانسفيراز والألانين أمينوترانسفيراز في مصل دم الأسماك وأدت إلى نقص معنوي في نسبة الألبيومن والبروتين الكلى. وقد أدى علاج الأسماك بفطر الاسبرجيلس فلافس ومسحوق الثوم إلى تقليل معنوي في التغيرات الحادثة في نشاط إنزيمات الأسبرتات أمينوترانسفيراز والألانين أمينوترانسفيراز وزيادة فى نسبة الألبيومن والبروتين الكلى في مصل دم الأسماك.

وكانت نتائج الهستوباثولوجى فى الخياشيم والكبد والتي زادت في شدتها مع فطر الاسبرجيلس فلافس قد أصبحت أقل حدة في مجموعة الأسماك المعالجة بفطر الاسبرجيلس فلافس ومسحوق الثوم ويمكن أن نستنتج من النتائج سلامة مسحوق الثوم و ذ لك با ضافتة فى علائق الأسماك للتخفيف من آثار فطر الاسبرجيلس فلافس على أسماك البلطي النيلي.