

Medicinal herbs against aflatoxicosis in Nile tilapia (*Oreochromis niloticus*): Clinical signs, postmortem lesions and liver histopathological changes

Ahmed Ismail Mehrim¹ and Mahmoud F. Salem²

- 1- Animal Production Department, Faculty of Agriculture, AL-Mansoura University, PO. 35516, Egypt, amehrim2002@yahoo.com
- 2- Central Laboratory of Fish Research Abbasa, Sakha Unit of Fish Research, Kafr El-Sheikh, Egypt, salem_fouad2000@yahoo.com

Abstract

Aflatoxins are known as a hepatocarcinogen in various animal species, including fish. The present study was designed to evaluate the ability of some medicinal herbs against aflatoxicosis regarding clinical signs, postmortem examination and microscopic findings in liver of all male mono-sex *Oreochromis niloticus*. A total of 360 fish (10.00 ± 0.38 g) were randomly distributed into eight groups (three replicates for each), stocked at 15 fish per aquarium, fish group (G₁) being the control and fed a basal ration (BR) free from aflatoxin B₁ (AFB₁) and G₂ fed aflatoxicated BR (150 ppb AFB₁). Other fish groups fed aflatoxicated BR (150 ppb AFB₁) supplemented with 1% of each tested medicine herbs, black seed (*Nigella sativa*) (G₃); liquorice (*Glycyrrhiza glabra*) (G₄); onion (*Allium cepa*) (G₅); garlic (*Allium sativum*) (G₆); fenugreek (*Trigonella foenum-graecum*) (G₇) and ceylon cinnamon (*Cinnamomum verum*) (G₈). Results revealed severe toxic effects of AFB₁ on fish besides the hepatotoxic effects on the liver compared with the control group. Meanwhile, dietary medicinal herbs led to eliminate the toxic effects of AFB₁ on fish, especially black seed or ceylon cinnamon. So, it could conclude the safety and useful dietary addition of 1% black seed (*Nigella sativa*, G₃) or ceylon cinnamon (*Cinnamomum verum*, G₈) to alleviate the toxic effects of AFB₁ on *O. niloticus* fingerlings.

Keywords: Aflatoxin, Nile tilapia, Medicinal plants, Liver poisoning.

Introduction

Many feed ingredients used in aquaculture have been found to be frequently contaminated (Cagauan et al., 2004). At the present time, increased use of ingredients of plant origin in aquafeed formulations for fish culture has intensified the potential onset for aflatoxicosis in farming systems due to the carryover of high loads of aflatoxin contamination by vegetable sources (Murjani, 2003). Aflatoxin B₁ (AFB₁) is produced by *Aspergillus* species on agricultural commodities (Leontopoulos et al., 2003). AFB₁ (C₁₇ H₁₂ O₆) is an

abundant and toxic mycotoxin, where animals that consume aflatoxins (AFs) contaminated feed develop various health problems including growth retardation, reduction of feed efficiency and liver and kidney damage (Bintvihok, 2002). AFs are known as a hepatocarcinogens in various animal species, including fish (Allameh et al., 2005). It is also a suspect human carcinogen and has been shown to play a role in human hepatocarcinoma (Wang et al., 2001). Liver is the target organ of AFs and hepatobiliary damages are associated with pathological changes and alterations in liver function enzymes

(Sur and Celik, 2005). Moreover, Santacroce et al. (2008) reported that AFB₁ is the most biologically active toxin known and has been found to produce hepatotoxic, carcinogenic, mutagenic, teratogenic and immunosuppressant effects in aquatic animals.

Herbal medicinal products are widely used around the world (Jordan et al., 2010). A growing interest has emerged in using herbs in animal feeds by both researchers and feed companies (Logambal and Michael, 2000). The herbal biomedicinal active principles in the aquaculture antimicrobial capability and antistress characteristics due to the active principle natures such as alkaloids, flavanoids, pigments, phenolics, terpenoids, starch, steroids and essential oils. This practice will reduce the side effects of applying the synthetic compounds and the cost and also make it eco-friendly (Cowan, 1999). Hence, the alternative herbal biomedicines prove to be very effective in aquacultural operations (Citarasu, 2010). So, extracts from many oriental spice plants and herbs such as cinnamon, clove, garlic, sage, oregano, thyme, rosemary, mint, and vanilla have been known to possess antimicrobial effects (Tassou et al., 2000; Yildirim et al., 2000 and Abdelhamid et al., 2002 c).

Aflatoxicosis in farm animals is underestimated because of the lack of specific clinical signs that would be useful to establish an early diagnosis. Information regarding the impact of AFs on farm animals is mostly available for mammalian and terrestrial vertebrates. In contrast, there are very limited data on aquacultured species, thus the lack of information regarding the incidence of aflatoxicosis in farm-reared aquatic species may be in part due to the difficulty in accurately diagnosing aflatoxicosis in fish (Santacroce et al.,

2008). So, the present work was designed to evaluate the ability of some medicinal herbs as black seed, liquorices, onion, garlic, fenugreek and cinnamon ceylon (at a level of 1%) to protect fish from dietary AFB₁ (150 ppb). Also clinical signs, postmortem lesions and histopathological alterations in liver of mono-sex Nile tilapia (*O. niloticus*) fingerlings were evaluated.

Materials and Methods

The present work was carried out at the Department of Animal Production, Faculty of Agriculture, Kafr EL-Sheikh University, Egypt. A basal diet (28.66% crude protein, 18.82% ether extract, 7.51% crude fiber, 6.44% ash, 38.57% nitrogen free extract, 496.93 Kcal / 100g DM gross energy (GE) and 57.67 mg CP / Kcal GE, P / E ratio), where $GE (Kcal / 100 g DM) = CP \times 5.64 + EE \times 9.44 + \text{Total carbohydrates} \times 4.11$ (Macdonald et al., 1973). The diet was formulated from the commercial ingredients (fish meal 10%, soybean meal 40%, yellow corn 34%, wheat bran 11.5%, sunflower oil 4% and vitamin & mineral mixture 0.5%). The ingredients and supplemented medicinal herbs were bought from the local market. These ingredients were milled and AFB₁ at level of 150 ppb besides the tested medicinal herbs (as an anti-toxin, were added at a level of 1%) were mixed well all together and pressed by manufacturing machine (pellets size 1mm). The experimental groups were described in Table (1). AFB₁ was produced through pellets fermentation using toxigenic fungal strain of *Aspergillus parasiticus* NRRL 2999 (which was obtained from Mycotoxins Lab., National Research Center, Cairo, Egypt). Identification of AFB₁ was done by thin layer chromatography and spectrophotometric [Spectronic® 20⁺ series spectrophotometers, Spectronic Unicam, 820 Linden Avenue, Rochester,

NY 14625, USA] and quantification according to **Karunaratne et al. (1990)**.

Concentration of the produced AFB₁ was

Table 1: Description of the experimental groups

calculated and incorporated into the experimental diets at a level of 150 ppb.

Experimental group	Details
G ₁	Basal ration (BR) free from AFB ₁ (as a negative control)
G ₂	BR + 150 ppb AFB ₁ (as a positive control)
G ₃	BR + 150 ppb AFB ₁ +1% black seed (<i>Nigella sativa</i>)
G ₄	BR + 150 ppb AFB ₁ +1% liquorice (<i>Glycyrrhiza glabra</i>)
G ₅	BR + 150 ppb AFB ₁ +1% onion (<i>Allium cepa</i>)
G ₆	BR + 150 ppb AFB ₁ +1% garlic (<i>Allium sativum</i>)
G ₇	BR + 150 ppb AFB ₁ +1% fenugreek (<i>Trigonella foenum-graecum</i>)
G ₈	BR + 150 ppb AFB ₁ +1% ceylon cinnamon (<i>Cinnamomum verum</i>)

A group of 360 mono-sex Nile tilapia *O. niloticus* fingerlings with an average initial body weight of 10 ± 0.38 g was used in this study. Fish were obtained from a private hatchery in Al-Reiad, Kafr El-Sheikh governorate, Egypt and transported to the wet lab., Faculty of Agriculture, Kafr El-Sheikh University, Egypt. They were maintained in aquaria for one month before the beginning of the experiment for acclimatization purpose. The fish were fed during the acclimatization period on artificial basal diet at a rate of 3% of the body weight. Mono-sex *O. niloticus* were randomly distributed into aquaria at a stocking rate of 15 fish per aquarium. Fish were assigned into three aquaria for each treatment (as replicates). Water of each glass aquarium (60 × 35 × 40 cm) was continuously supplied with a compressed air from an electric compressor. Dechlorinated tap water was used to change one third of the water in each aquarium daily. During the experimental period (15 week), the fish were fed the experimental diets at a rate of 3% of the live body weight daily. The diet was introduced twice daily, at 8.0 a.m. and 2.0

p.m. The amount of feed was adjusted weekly based on the actual body weight changes. Samples of water were taken daily before and after adding the diets from each aquarium to determine water quality parameters. Since, water temperature degree ranged between 23.0 and 25.0°C, pH values 6.5 – 8.0 and dissolved oxygen 6.0 – 7.0 mg / L, which were suitable for rearing *O. niloticus* according to **Lim and Webster (2006)**. Light was controlled by a timer to provide a 14h light: 10h dark as a daily photoperiod.

Through all the intervals of the experimental period, the clinical signs and postmortem examination of the aflatoxicated fish were recorded by a digital camera, CASIO, Exilim optical 3x, 6.0 Mega pixels, 2.5" LCD, Anti-shake DSP, CASIO Computer Co., LTD., Tokyo, Japan. Also, at the end of the experiment, three fishes from each treatment were sacrificed and the target organ (liver) was sampled. Samples were fixed in 10% neutralized formalin solution followed by washing with tap water, then dehydrated by different grades of alcohol (70%, 85%, 96% and 99%).

Samples were cleared by xylene and embedded in paraffin wax. The wax blocks were sectioned to six microns. The sections were stained by hematoxylin (H) and eosin (E) and then subjected to a microscopic examination according to **Roberts (2001)**.

Results and Discussion

Clinical signs and postmortem finding:

Through all the interval periods of the present study, the clinical signs and postmortem changes of the aflatoxicated fish ($G_2 - G_8$) were recorded. These signs included protrusive eye (Fig. 4), lethargy, less feed acceptability, skin covered by thick mucus, severe hemorrhage on dorsal, anal and caudal fins, discarded scales and caudal fin erosion and loss construction of the body of aflatoxicated fish (Fig. 2), as compared to a normal appearance of the fish in the control group (G_1 , Fig. 1). Anatomically, there were some postmortem lesions included uncharacterized liver, viscera and ascetic. Damaged liver and enlargement of gall bladder of aflatoxicated fish (Figs. 4 & 5) were recorded. While, the liver of fish fed the control diet (G_1) appeared with normal structure (Fig. 3).

Similar findings were recorded by **Hussein et al. (2000)**; **Abdelhamid et al. (2002 a & b)** and **Tuan et al. (2002)**. In addition, evidence of morphological



Fig. (1): Shows normal appearance of *O. niloticus* fed the control diet (G_1).

alterations in *O. niloticus* fed aflatoxin-contaminated feeds include ocular opacities leading to cataracts and blindness; lesions on the body surface such as fin and tail rot; yellowing of the body surface, termed “yellowing tilapia”; abnormal swimming patterns and anorexia were recorded also by **Cagauan et al. (2004)**. Similarly, **Mehrim et al. (2006)** observed the same external symptoms and postmortem signs of aflatoxicated *O. niloticus*. Moreover, **El-Barbary and Mehrim (2009)** confirmed that the most common clinical signs observed on aflatoxicated *O. niloticus* were lethargy, loss of appetite, sluggish movement, dark discoloration of the skin and respiratory manifestations. Also, they added that the common lesions noticed by necropsy of aflatoxicated fish were accumulation of fluid in abdomen, congested gills and dark liver.

Generally, it is difficult to recognise or diagnose this condition because of its slow, subclinical trend. The majority of clinical signs is related to chronic, impaired liver function, such as reduced feed efficiency, weight loss, increased susceptibility to secondary infectious diseases, necrosis and tumor development in the liver and other organs and increased mortality (**Santacroce et al., 2008**).

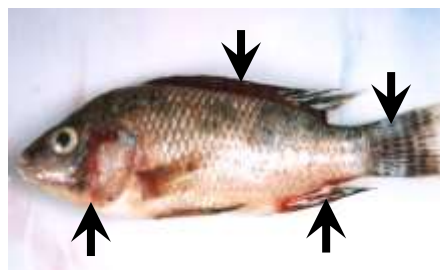


Fig. (2): Shows *O. niloticus* fed a diet containing 150 ppb AFB_1 (G_2), with severely hemorrhages on dorsal, anal and caudal fins (arrows), discarded scales and caudal fin erosion (arrow).



Fig. (3): Shows normal liver of *O. niloticus* fed on the control diet (G₁).



Fig. (4): Shows aflatoxicated *O. niloticus* with 150 ppb AFB₁ (G₂), revealing uncharacterized liver and viscera of fish and the abdomen cavity was filled with ascetic fluid and protrusive eye (arrow).



Fig. (5): Shows aflatoxicated *O. niloticus* with 150 ppb AFB₁ (G₂), congested liver (arrow) and enlargement of gall bladder (arrow), which was filled with bile.

Histopathological changes:

Liver is the most susceptible organ to AFB₁ injury in fishes (Hendricks, 1994). The present microscopical changes in liver of *O. niloticus* fed BR free from AFB₁ (G₁) or aflatoxicated diets without (G₂) or with tested medicine herbs (G₃ – G₈) are shown in Figures 6 a – h.

AFB₁ is hepatotoxic and hepatocarcinogenic in several animal species at a very low concentration (Sharma et al., 2011). Hence, the microscopic finding in the present study indicated that AFB₁ induced severe histopathological changes in the hepatic tissues. Similar histological changes in the liver of *O. niloticus* have been documented previously (Abdelhamid et al., 2004 and Mehrim et al., 2006). In addition, El-Barbary and Mehrim (2009) found that AFB₁ presented histopathological changes in

hepatopancrease of *O. niloticus*, which increased in severity with increasing AFB₁ level. Furthermore, Deng et al. (2010) reported that the liver of tilapia fed with 638 and 793 µg AFB₁ / kg diet were infiltrated by many inflammatory cells. In this respect, the pathological changes of liver observed in the present investigation may be due to primary site of metabolism of ingested aflatoxins as well as the primary laceration of residues and lesions (Newperne, 1999).

Histopathological alterations like necrosis, fibrosis and lymphocytolysis in liver and other organs had been observed due to aflatoxin exposure in different fish species such *Labeo rohita* (Mohapatra et al., 2011); *Clarius lazara* (Zaki et al., 2002); channel catfish (Jantrarotai and Lovell, 1990) and hybrid sturgeon *Acipenser ruthenus* ♂ x *A. baeri* ♀ (Raghavan et al., 2011). In contrary, no

histopathological lesions were identified in the liver of gibel carp (*Carassius auratus gibelio*) treated with AFB₁ (Huang et al., 2011), as well as they indicated that gibel carp is a less susceptible species to AFB₁ exposure up to approximately 1000 ppb, at least for 12 weeks. Furthermore, experimental studies on various species of rodents, birds and fishes indicated that aflatoxins are capable to induce oxidative damage in the cells and produce reactive oxygen species (ROS), such as superoxide radicals, hydroxyl radicals and hydrogen peroxides in hepatocytes (Abdel-Wahhab et al., 2007 & 2010). Additionally, Alpsoy and Yalvac (2011) reported that elevation of ROS levels causes inhibitory effects on biological processes including DNA synthesis, DNA-dependent RNA synthesis, DNA repair, and protein synthesis. Thus, the extent of the damage produced by aflatoxins depends on the toxin concentration present in foods or feeds and also the period of exposure, as well as animal species susceptibility (Stewart and Larson, 2002).

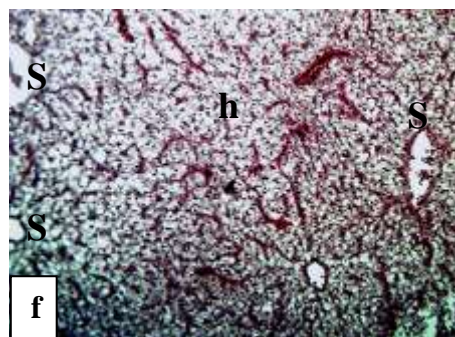
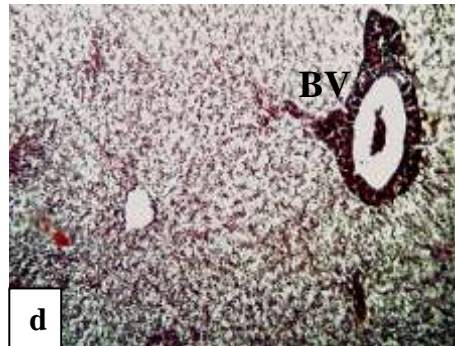
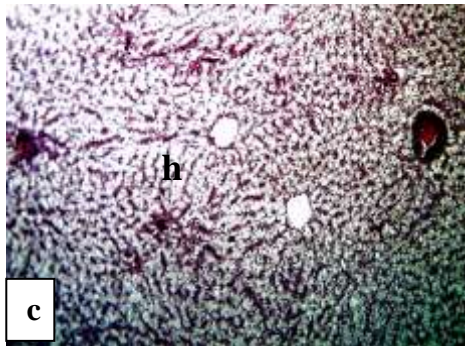
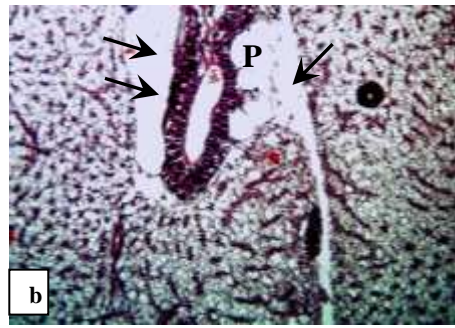
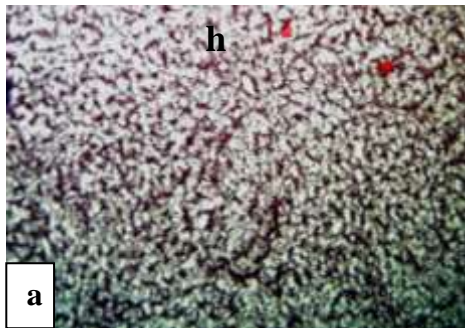
Because of herbal products or medicinal herbs are cheaper source for therapeutics, they have greater accuracy than chemotherapeutic agents, and offer a viable solution for all problems which aquaculture faces today (Citarasu et al., 2002, 2003 a & b); as well as they contain potentially active antioxidant and antimicrobial principles (Sivaram et al., 2004); antifungal, antiaflatoxicogenic and antioxidant compounds (Kumar et al., 2007); so, currently histopathological findings revealed the positive effects of tested medicine herbs, which alleviated the hepatotoxic effects of AFB₁ on the experimental fish. Similarly, numerous scientific efforts were conducted for using medicine herbs against toxic effects of AFB₁ (Madhusudhanan et al., 2006; Mehrim et al., 2006 and El-Barbary, 2008). Yet, the potential of some herbal

plants to degrade AFs has been detected by Sandoskumar et al. (2007), whereas garlic (*Allium sativum* L.) and onion (*A. cepa* L.) roots incubated in water containing 70 mg of AFB₁ for 5 days cause 58.5% reduction in aflatoxin level. Also, the impact of both of medicinal herbs, namely rosemary and parsley, at a low level (2 g / kg B.W.) to eliminate the drastic effects of AFB₁ on *O. niloticus* were reported by El-Barbary and Mehrim (2009).

Moreover, medicinal herbs contain a number of bioactive compounds e.g., glycyrrhizin and its aglycon glycyrrhetic acid, liquiritin, liquiritin apioside, isoliquiritin and glabridin, as well as contain several active components, such as polysaccharides, alkaloids and/or flavonoids (Cinatl et al., 2003). Hence, glycyrrhizin containing one molecule of glycyrrhetic acid, which has anti-inflammatory and anti-tumor activities, mediated by its immunomodulatory activities (Zhang et al., 1990). Yet, flavonoids are typical phenolic compounds has long been recognized to possess anti-inflammatory, antioxidant, antiallergic, hepatoprotective, antithrombotic, antiviral, anticarcinogenic activities and free radical scavengers (Carroll et al., 1998), as well as, Citarasu et al. (1998) found that herbal compounds have the ability to inhibit the generation of oxygen anions and to scavenge free radicals. So, the alternative herbal biomedicines prove to be very effective in aquacultural operations (Citarasu, 2010). Consequently, administration of ginseng and *Nigella sativa* oil via injection 1% of body weight reduced the development of hepatotoxicity by AFB₁ and may protect liver from free radical reactions due to aflatoxin (Zaki et al., 2011). More recently, El-Nagerabi et al. (2012) found that calyx of *Hibiscus sabdariffa* and *Nigella sativa* oil can be effectively used

as biologically safe control additives against aflatoxin contamination of human food, animal feed and other agricultural products. Additionally, liver detoxification function it self was discussed by **Apte and Krishnamurthy (2011)**, where detoxification function of the liver is highly complex involving hundreds of different enzymes with

unique substrate specificity, regulation, and activity. Furthermore, **Kumar et al. (2011)** reported that a combination of different herbal extracts/fractions is likely to provide desired activities to cure severe liver diseases. Development of such medicines with standards of safety and efficacy can revitalise treatment of liver disorders and hepatoprotective activity.



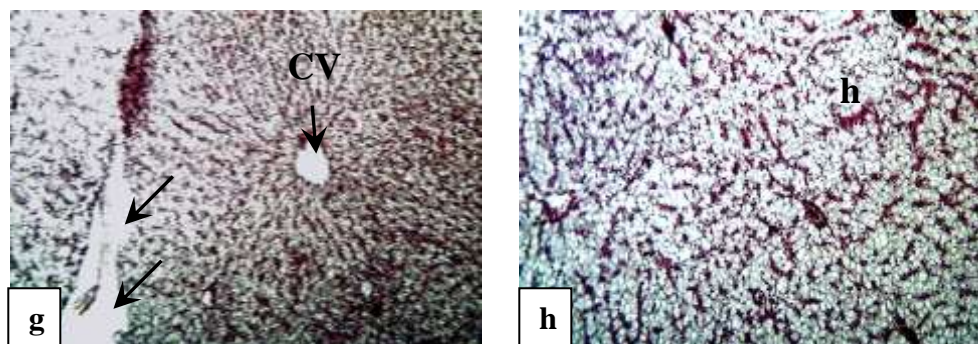


Fig. (6): Sections in liver of *O. niloticus* (a) G₁: showing intact hepatic lobular architecture; (b) G₂: showing severe necrosis of the hepatocytes; enlargement, dilatation and adjacent areas of degenerated hepatocytes (arrows); (c) G₃: showing intact hepatic lobular architecture with slightly necrosis of the hepatocytes; (d) G₄: showing slight congestion and infiltration by inflammatory cells (BV); (e) G₅: showing severe congestion with areas of hemorrhage (arrows) and focal necrotic hepatocytes; (f) G₆: showing focal necrosis of the hepatocytes (g) G₇: showing scattered necrosis of the hepatocytes around the central vein (CV) adjacent large area of degenerated hepatocytes (arrows); (h) G₈: showing intact hepatic lobular architecture with slight degenerative changes of the hepatocytes (x100, H&E stains); h: hepatocytes; PV: portal vessel; BV: blood vessel; S: sinusoid; CV: central vein.

Conclusions

Based on the foregoing results, hepatotoxic effects of AFB₁ were indicated on mono-sex *O. niloticus* fingerlings. Also, it is of interest to conclude the safety and useful addition of tested medicine herbs to eliminate the toxic effects of AFB₁, especially 1% black seed (*Nigella sativa*, G₃) and/or ceylon cinnamon (*Cinnamomum verum*, G₈). So, further studies are needed for studying the effect of the combination of these medicine herbs against the toxic effects of various AFB₁ levels or other mycotoxins on tilapia or other fish species at different ages, physiological stages or nutritional status.

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References

- Abdelhamid, A.M., Abdel- Khalek, A.E., Mehrim, A.I. and Khalil, F.F. (2004). An attempt to alleviate aflatoxicosis on Nile tilapia fish by dietary supplementations with chicken-hatchery by-products (egg shells) and shrimp processing wastes (shrimp shells). 2- On clinical, blood and histological parameters. J. Agric. Sci. Mansoura Univ., 29: 6175 - 6196.
- Abdelhamid, A.M., Khalil, F.F.M., El-Barbary, M.I., Zaki, V.H. and Hussein, H.S. (2002 a). Feeding Nile tilapia on Biogen[®] to detoxify aflatoxic diets. Proc.1st Conf. Animal & Fish Prod., Mansoura, 24 & 25, Sept., 207-230.
- Abdelhamid, A.M., Magouz F.I., Salem M.F.E., Mohamed A.A. and Mohsen M.K. (2002 b). Effect of graded levels of aflatoxin B₁ on growth performance and biochemical, chromosomal and histological behaviour of Nile tilapia *Oreochromis niloticus*. Proc.1st Conf. Animal & Fish Prod., Mansoura, 24 & 25, Sept., 231-250.
- Abdelhamid, A.M., Sallam, A.E., Abd Allah, G.A. and El-Samra, S.H. (2002

- c). Effect of feeding male rats on aflatoxic diets without or with medicinal herbs (thyme, safflower, ginger, black cumin and/or garlic). Proc. 2nd Conf. Foodborne contamination and Egyptian's Health, 23-24 April, El-Mansoura, 99-121.
- Abdel-Wahhab, M.A., Hassan, N.S., El-Kady, A.A., Khadrawy, Y.A., El-Nekeety, A.A., Mohamed, S.R., Sharaf, H.A. and Mannaa, F.A. (2010). Red ginseng extract protects against aflatoxin B₁ and fumonisins-induced hepatic pre-cancerous lesions in rats. Food Chem. Toxicol., 48 (2): 733–742.
- Abdel-Wahhab, M.A., Omara, E.A., Abdel-Galil, M.M., Hassan, N.S., Nada, S.A., Saeed, A. and El-Sayed, M.M. (2007). Zizyphus Spina-Christi extract protects against aflatoxin B₁-inhibited hepatic carcinogenicity. Afr. J. Trad. CAM, 4 (3): 248–256.
- Allameh, A., Safamehr, A., Mirhadi, S.A., Shivazad, M., Razzaghi-Abyaneh, M. and Afshar-Naderi, A. (2005). Evaluation of biochemical and production parameters of broiler chicks fed ammonia treated aflatoxin contaminated maize grains. Anim. Feed Sci. Technol., 122: 289–301.
- Alpsoy, L. and Yalvac, M.E. (2011). Key roles of vitamins A, C, and E in aflatoxin B₁-induced oxidative stress. Vitam. Horm., 86: 287–305.
- Apte, U. and Krishnamurthy, P. (2011). Detoxification Functions of the Liver, Chp, (11), 17p. In: Monga, S.P.S. (ed.), Molecular Pathology of Liver Diseases, Molecular Pathology Library 5, Springer Science+Business Media, LLC.
- Bintvihok, A. (2002). New insights to controlling mycotoxin danger in ducks. Feed Tech., 6: 28–29.
- Cagauan, A.G., Tayaban, R.H., Somga, J. and Bartolome, R.M. (2004). Effect of aflatoxin-contaminated feeds in Nile tilapia (*Oreochromis niloticus* L.). In: Abstract of the 6th International Symposium on Tilapia in Aquaculture (ISTA 6) Section: Health Management and Diseases Manila, Philippines, 12–16 September.
- Carroll, K.K., Guthrie, N., So, F.V. and Chambers, A.F. (1998). Anticancer properties of flavonoids, with emphasis on citrus flavonoids, in Flavonoids in Health and Disease (Rice-Evans CA and Packer L, eds.) pp: 437–446, Marcel Dekker Inc., New York.
- Cinatl, J., Morgenstern, B., Bauer, G., Chandra, P., Rabenau, H. and Doerr, H.W. (2003). Glycyrrhizin, an active component of liquorice roots, and replication of SARS associated corona virus. Lancet, 361: 2045–2046.
- Citarasu, T. (2010). Herbal biomedicines: a new opportunity for aquaculture industry. Aquacult Int., 18: 403 – 414.
- Citarasu, T., Babu, M.M. and Marian, M.P. (1998). Application of biomedical products for improving marine shrimp larval production. Aqua-Terr. Annual Symposium. School of Biological sciences, MK. University, Madurai, India.
- Citarasu, T., Raja Jeya Sekar, R.J., R., Venketramalingam, K., Dhandapani, P.S. and Marian, M.P. (2003a). Effect of wood apple *Aegle marmelos*, Correa (*Dicotyledons*, *Sapindales*, *Rutaceae*) extract as an antibacterial agent on pathogens infecting prawn (*Penaeus indicus*) lariviculture. Indian J. Mar. Sci., 32: 156–161.
- Citarasu, T., Sekar, R.R., Babu, M.M. and Marian, M.P. (2002). Developing Artemia enriched herbal diet for

- producing quality larvae in *Penaeus monodon*. Asian Fish Sci., 15:21–32
- Citarasu, T., Venket Ramalingam, K., Raja Jeya Sekar, R., Micheal Babu, M. and Marian, M. (2003b). Influence of the antibacterial herbs, *Solanum trilobatum*, *Andrographis paniculata* and *Psoralea corylifolia* on the survival, growth and bacterial load of *Penaeus monodon* post larvae. Aquac. Int., 11: 583–595.
- Cowan, M.M. (1999). Plant products as antimicrobial agents. Clinical Microbiology Reviews, 12: 564–582.
- Deng, S.X., Tian, L.X., Liu, F.J., Jin, S.J., Liang, G.Y., Yang, H.J., Du, Z.Y. and Liu, Y.J. (2010). Toxic effects and residue of aflatoxin B₁ in tilapia (*Oreochromis niloticus* × *O. aureus*). Aquaculture, 307: 233 – 240.
- El-Barbary, M.I. (2008). Aflatoxin B₁ induced-changes in protein electrophoretic pattern and DNA in *Oreochromis niloticus* with special emphasis on the protective effect of rosemary and parsley extracts. American-Eurasian J. Agric. & Environ. Sci., 4 (3): 381-390.
- El-Barbary, M.I. and Mehrim, A.I. (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.
- El-Nagerabi, S.A.F., Al-Bahry, S.N., Elshafie, A.E. and AlHilali, S. (2012). Effect of *Hibiscus sabdariffa* extract and *Nigella sativa* oil on the growth and aflatoxin B₁ production of *Aspergillus flavus* and *Aspergillus parasiticus* strains. Food Control, 25: 59-63.
- Hendricks, J.D. (1994). Carcinogenicity of aflatoxins in nonmammalian organisms. In: Eaton, D.L., Groopman, J.D. (Eds.), Toxicology of Aflatoxins: Human Health, Veterinary, and Agricultural Significance. Academic Press, San Diego, pp. 103–136.
- Huang, Y., Han, D., Zhu, X., Yang, Y., Jin, J., Chen, Y. and Xie, S. (2011). Response and recovery of gibel carp from subchronic oral administration of aflatoxin B₁. Aquaculture, 319: 89–97.
- Hussein, S.Y., Mekki, I.A.A., Moktar, Z.Z. 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.
- Jantrarotai, W. and Lovell, R.T. (1990). Subchronic toxicity of dietary aflatoxin B₁ to channel catfish. J Aquat Anim Health, 2:248–256.
- Jordan, S.A., Cunningham, D.G. and Marles, R.J. (2010). Assessment of herbal medicinal products: Challenges, and opportunities to increase the knowledge base for safety assessment. Toxicology and Applied Pharmacology, 243:198–216.
- Karunaratne, A., Wezenberg E. and Bullerman, L.B. (1990). Inhibition of mold growth and aflatoxin production by *Lactobacillus* spp. J. Food Protection, 53: 230 - 236.
- Kumar, C.H., Ramesh, A., Kumar, J.N.S. and Ishaq, B.M. (2011). A Review on hepatoprotective activity of medicinal plants. Int J Pharm Sci Res., 2 (3): 501-515.
- Kumar, R., Mishra, A.K., Dubey, N.K. and Tripathi, Y.B. (2007). Evaluation of *Chenopodium ambrosioides* oil as a potential source of antifungal, antiaflatoxicogenic and antioxidant

- activity. *Int. J. Food Microbiol.*, 115: 159 - 164.
- Leontopoulos, D., Siafaka, A. and Markaki, P. (2003). Black olives as substrate for *Aspergillus parasiticus* growth and aflatoxin B₁ production. *Food Microbiology*, 20: 119–126.
- Lim, C. E. and Webster, C. D. (2006). Nutrient requirements. In: Lim, C.E. and Webster, C.D. editors. *Tilapia: biology, culture and nutrition*. The Haworth Press, Inc., Binghamton, New York, pp: 469–501.
- Logambal, S.M. and Michael, R.D. (2000). Immunostimulatory effect of Azadirachtin in *Oreochromis mossambicus* (Peters). *Indian J. Exp. Biol.*, 38: 1092–1096.
- Macdonald, P., Edwards, R.A. and Greenhalgh, J.F.D. (1973). *Animal Nutrition*, 2nd Ed., Longman, London.
- Madhusudhanan, N., Kavithalakshmi, S.N. Shanmugasundaram E.R.B. and Shanmugasundaram K.R. (2006). Aflatoxin B₁-Induced DNA Damage in *Labeo rohita*: Protective effect of an antioxidant supplement, *Amrita bindu*. *Basic and Clinical Pharmacology and Toxicology*, 98: 473 – 479.
- Mehrim, A.I., Abdelhamid, A.M., Abou-Shousha, A., Salem, M.F.I. and El-Sharawy, M.A.M.M. (2006). Nutritious attempts to detoxify aflatoxic diets of tilapia fish: 2- Clinical, biochemical and histological parameters. *Journal of the Arabian Aquaculture Society*, 1: 69-90.
- Mohapatra, S., Sahu, N.P., Pal, A.K., Prusty, A.K., Kumar, V. and Kumar, S. (2011). Haemato-immunology and histo-architectural changes in *Labeo rohita* fingerlings: effect of dietary aflatoxin and mould inhibitor. *Fish Physiol Biochem.*, 37:177–186.
- Murjani, G. (2003). Chronic aflatoxicosis in fish and its relevance to human health. Central Institute of Freshwater Aquaculture, India.
- Newperne, P.M. (1999). Chronic aflatoxicosis in animals and poultry. *J. Am. Vet. Med. Assoc.*, 263:1269.
- Raghavan, P.R., Zhu, X., Lei, W., Han, D., Yang, Y. and Xie, S. (2011). Low levels of Aflatoxin B₁ could cause mortalities in juvenile hybrid sturgeon, *Acipenser ruthenus* ♂ × *A. baeri* ♀. *Aquaculture Nutrition*, 17: 39 – 47.
- Roberts, R. J. (2001). *Fish Pathology*, 3rd edition, W.B. Saunders.
- 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.
- Santacroce, M.P., Conversano, M.C., Casalino, E., Lai, O., Zizzadoro, C., Centoducati, G. and Crescenzo, G. (2008). Aflatoxins in aquatic species: metabolism, toxicity and perspectives. *Rev. Fish Biol. Fish.*, 18: 99–130.
- Sharma, V., Gupta R. and Sharma, S. (2011). Effect of oral administration of ethanolic root extract of *Tinospora cardifolia* on aflatoxin B₁- induced toxicity in Swiss albino mice. *Journal of Natural Pharmaceuticals*, 2: 125 - 132.
- Sivaram, V., Babu, M.M., Citarasu, T., Immanuel, G., Murugadass, S. and Marian, M.P. (2004). Growth and immune response of juvenile greasy groupers (*Epinephelus tauvina*) fed with herbal antibacterial active principle supplemented diets against

- Vibrio harveyi* infections. Aquaculture, 237:9–20.
- Stewart, D. and Larson, E. (2002). Aflatoxicosis in wildlife. Information Sheet 1582. Mississippi State Univ. Extension Service, Cooperating with U.S. Dept. of Agriculture.
- Sur, E. and Celik, I. (2005). Effects of aflatoxin B-1 on the development of chicken thymus and blood lymphocyte alpha-naphthyl acetate esterase activity. Vlaams Dier Geneeskundig Tijdschrift, 74: 432–439.
- Tassou, C., Koutsoumanis, K. and Nychas, G.J.E. (2000). Inhibition of *Salmonella enteritidis* and *Staphylococcus aureus* in nutrient broth by mint essential oil. Food Research International, 33: 273–280.
- Tuan, N.A., Grizzle, J.M., Lovell, R.T., Manning, B.B. and Rottinghaus, G.E. (2002). Growth and hepatic lesions of Nile tilapia (*Oreochromis niloticus*) fed diets containing aflatoxin B₁. Aquaculture, 212: 311–319.
- Wang, J.S., Huang, T., Su, J., Liang, F., Wei, Z., Liang, Y., Luo, H., Kuang, S.Y., Qian, G.S. and Sun, G. (2001). Hepatocellular carcinoma and aflatoxin exposure in Zhuqing Village, Fusui County, People's Republic of China. Cancer Epidemiol. Biomark. Prev., 10: 143–146.
- Yildirim, A., Mavi, A., Oktay, M., Kara, A.A., Algur, O. F., and Bilaloglu, V. (2000). Comparison of antioxidant and antimicrobial activities of tilia (*Tilia argentea* Desf Ex DC), sage (*Salvia triloba* L.), and black tea (*Camellia sinensis*) extracts. Journal of Agricultural and Food Chemistry, 48: 5030–5034.
- Zaki, M.S., Fawzi, O.M. and Zytuun, I.M. (2011). Reduction of alfatoxin in *Clarius lazara* catfish by ginseng extract and *Nigella sativa* oil. Journal of American Science, 7(2): 591-596.
- Zaki, M.S., Osfor, M.H., Tohamy, A. and Zytuun, I.M. (2002). Some Clinicopathological and nutritional studies on reduction of aflatoxin Induced hepatotoxicity in *Clarius lazara* catfish by Fax-Atoxin and *Nigella sativa* oil. Egypt. J. Microbiol., 37(2): 185-195.
- Zhang, Y.H., Yoshida, T., Isobe, K., Rahman, S.M., Nagase, F., Ding, L. and Nakashima, I. (1990). Modulation by glycyrrhizin of the cell-surface expression of H-2 class 1 antigens on murine tumor cell lines and normal cell populations. Immunology, 70: 405–410.

الأعشاب الطبية ضد التسمم الأفلاتوكسينى لأسماك البلطي النيلي: دلالات العلامات الظاهرية ، الصفة التشريحية والنسجية للكبد

أحمد إسماعيل محرم^١ ، محمود فؤاد سالم^٢

١- قسم إنتاج الحيوان -كلية الزراعة - جامعة المنصورة - المنصورة - مصر.

٢- المعمل المركزي لبحوث الأسماك بالعباسة - وحدة بحوث الأسماك بسخا - كفر الشيخ - مصر.

الملخص العربي

تعرف الأفلاتوكسينات كمسرطنات كبدية للأنواع المختلفة من الحيوانات ، تشمل الأسماك. صممت الدراسة الحالية لتقييم مدى قابلية بعض الأعشاب الطبية ضد التسمم الأفلاتوكسينى فيما يتعلق بالعلامات الظاهرية ، اختبار الصفة التشريحية والتغيرات النسيجية لكبد أسماك البلطي النيلي وحيد الجنس. تم التوزيع العشوائى للأسماك (0.38 ± 10 جرام) على ثماني مجموعات (ثلاث مكررات لكل مجموعة)، خزنت ١٥ سمكة لكل حوض بحيث غذيت أسماك المجموعة الأولى (ج١) الضابطة على العليقة الأساسية الخالية من الأفلاتوكسين ب١، غذيت أسماك المجموعة الثانية (ج٢) على العليقة الأساسية الملوثة بالأفلاتوكسين ب١ (١٥٠ جزء فى البليون). غذيت المجموعات الأخرى من الأسماك على العليقة الأساسية الملوثة بالأفلاتوكسين ب١ (١٥٠ جزء فى البليون) مع إضافة مستوى ١% من كل من الأعشاب الطبية المختبرة بذور الحبة السوداء (ج٣)، العرق سوس (ج٤)، البصل (ج٥)، الثوم (ج٦)، الحلبة (ج٧)، القرفة (ج٨). أوضحت النتائج التأثير الحاد السام للأفلاتوكسين ب١ على الاختبارات الظاهرية والصفة التشريحية بجانب التأثيرات النسيجية على الكبد مقارنة بالمجموعة الضابطة. بينما أدت إضافة الأعشاب الطبية للعليقة إلى تخفيف تلك التأثيرات السامة للأفلاتوكسين ب١ على الأسماك خاصة المعاملة بكل من بذور الحبة السوداء والقرفة. لذلك يمكن التوصية بالإضافة الأمانة والمفيدة لكل من الحبة السوداء (ج٣) والقرفة (ج٨) بمستوى ١% للعلائق لتخفيف التأثيرات السامة للأفلاتوكسين ب١ على أصباغيات البلطي النيلي.