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Detecting the effect of Aflatoxin B_1 polluted rations on freshwater fish, *Lates niloticus* reared in fish culture ponds

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ABSTRACT

In the current investigation, chemical analysis of ration samples obtained from a fish farm in Ismailia governorate revealed high aflatoxin B_1 concentration. Chemical analysis of the tissues of *Lates niloticus* fish collected from the farm showed elevated aflatoxin B_1 levels in the liver, kidney and musculature. The fish collected from the farm showed sings of lethargy, sluggish movement, loss of appetite, increased mucous over the skin, protrusive eye and yellowish coloured body. The postmortem examination exhibited that the liver and viscera were deformed and the gall bladder was enlarged and filled with bile. Biochemical examination of the blood sera revealed significant increase in the alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities, creatinine, urea, uric acid, cholesterol concentrations and albumin/ globulin (A/G) ratio. On the other hand, serum total protein (TP), albumin, globulin, sodium, potassium, calcium and inorganic phosphorus concentrations were significantly decreased. From the present investigation it is advisable to control the feeding of fish and supplying the fish with healthy aflatoxin free rations.

Key words: Aflatoxin B_1, pollutions, freshwater fish, *Lates niloticus*

Introduction

Aflatoxins are secondary metabolites produced in specific ecological conditions by some strains of molds belonging to the groups of *Aspergillus flavus*, *A. parasiticus* and *A. nomius* (Miliţă *et al.*, 2010). Aflatoxin B_1 is known to be the most significant form that causes serious risk to animals and human health. Aflatoxins B_1 and B_2 adversely affect the growth, feed utilization and results in deleterious impact on the production of fish (Adeniji, *et al*, 2014). The carcinogenic effect of aflatoxin B_1 has been found in fish (Murjani, 2003). Domesticated rainbow trout (*Oncorhynchus mykiss*) that were fed a pellet feed prepared with cottonseed meal contaminated with aflatoxins, developed liver tumors (Ashley, 1970).

The potential for development of aflatoxicosis increases in warm-water fishes, such as tilapia and channel catfish because plant ingredients, formulated in their diets have a higher potential than animal ingredients for contamination with aflatoxins. In tropical and subtropical conditions, this potential is further increased due to storage under humid and hot conditions. International trade in affected commodities and exposure to aflatoxins are worldwide concerns and the economic impact due to animal losses can be enormous (Russo and Yanong, 2010).

The fish, when exposed to the aflatoxin-contaminated feed suffer from a series of adverse complications ranging from developmental abnormalities to reproductive failure. Importantly the ingested aflatoxins are not totally eliminated from their system, instead they accumulate and cause greater threat not only to themselves but also to fish eaters. The subtle effects often go unnoticed and in the long run results in increased incidence of liver cancer (Madhusudhanan *et al.*, 2006). Aflatoxins lower production efficiency of cultured fish by reducing growth rates, impairing immunity, and, in some cases, causing mortality. Storing feed properly (in a cool, dry area on pallets and at least one foot away from any walls) can prevent unnecessary economic losses (Russo and Yanong, 2010). Common effects of aflatoxicosis in finfish include poor growth, pale gills, reduced RBCs, anemia, impaired blood clotting, damage to liver, decreased immune responsiveness and increased mortality (Goldblatt, 1976).

The aim of the present work is to throw light on the harmful effect of aflatoxin B_1 in ration on liver and kidney functions and some biochemical changes in the blood serum of *Lates niloticus* fish.

Material And Methods

1- Fish:

Ten *Lates niloticus* fish weighing 3-5 kg were collected from an affected farm exhibited health problems and abnormal mortality rates in Ismailia Governorate. Another 10 *L. niloticus* fish with the same weight range

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were collected from healthy good hygienic control fish farm in the same Governorate. The absence of pathogenic infective agents was assured in the fish by microbiological and parasitic examinations.

2- Water analysis:

Ten water samples were collected from the affected fish farm and kept in closed glass bottles according to Tucker (1992). Each water sample was subjected to chemical analysis for pH, hardness, phosphorus, nitrate, nitrite and ammonia according to Tucker (1992) and Clescerl *et al.* (1999), lead, cadmium and iron according to Jackson (1973).

3- Fish ration analysis:

Ten samples of fish ration were collected from the affected fish farm and from the control fish farm. The samples were chemically analyzed for the detection of aflatoxins, oxidative rancidity and acid number according to according to AOAC (1980).

4- Clinical examination:

The fish were clinically examined using the methods described by Lucky (1977).

5- Postmortem examination:

Postmortem examination of gills, abdominal cavity and internal organs was carried out according to Amalacher (1970).

6- Blood samples:

Blood samples were collected from the caudal vein of each fish in either group according to Stoscopf (1992). Serum was separated from the blood for biochemical analysis. Each blood sample was kept in sloped glass tube at 4°C over night. Serum was then separated by centrifugation of blood at 3000 rpm for 20 minutes for biochemical analysis.

7- Biochemical analysis:

Serum alkaline phosphatase (ALP) activity was determined according to Marsh *et al.* (1959) by *Spectrum Diagnostics, Egypt* kits. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities in the blood serum were determined according to Reitman and Frankel (1957) using kits of *Coral Co., India*. Serum total protein (TP) was measured by the method described by Cannon *et al.* (1974); *Spectrum Diagnostics, Egypt* kits. Serum albumin was measured after Doumas *et al.* (1971) by *Vitro Scient, Egypt* kits. Blood serum globulin level and albumin/globulin ratio (A/G ratio) were calculated mathematically according to Coles (1986). Serum creatinine concentration was determined by the method adopted by Bowers and Wong (1980); *Spectrum Diagnostics, Egypt* kits. Serum urea level was determined according to Fawcett and Scott (1960) by *Bio-Diagnostic Kits, Egypt.* Determining serum uric acid concentration was performed by the assay of Fossati *et al*. (1980) *Spectrum Diagnostics, Egypt* kits. Cholesterol level was determined according to Young (1990) by *Vitro Scient, Egypt* kits. Sodium concentration in serum was measured according to Henry *et al* (1974); *Vitro Scient, Egypt* kits. Serum potassium concentration was determined as described by Hillman *et al.* (1967); *Spectrum Diagnostics, Egypt* kits. Serum calcium concentration was determined according to Barnett (1965) by *Spectrum Diagnostics, Egypt* kits. Serum inorganic phosphorus concentration was determined according to Daly and Ertingshausen (1972) by *Spectrum Diagnostics, Egypt* kits.

Statistical analysis:

Data were presented as mean ± standard error (SE) and the significance of differences was evaluated using analysis of variance (ANOVA) (SPSS 14, 2006).

Results:

Water chemical analysis:

Table (1) shows the chemical criteria of the water of affected fish and control fish cultures.

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Aflatoxin B_1 in ration:

Chemical analysis showed that aflatoxin B_1 in the ration of affected *Lates niloticus* fish culture was significantly elevated comparing with that in the control fish culture ration (table, 2).

Aflatoxin B_1 in fish tissues:

Table (3) represents that aflatoxin B_1 residues appeared in the liver, kidney and musculature of affected culture fish. No detectable levels of aflatoxin B_1 in the tissues of control fish.

Clinical signs:

Clinical signs in *Lates niloticus* fed ration containing aflatoxin B_1 included lethargy, sluggish movement, loss of appetite, increased mucous over the skin and protrusive eye yellowish coloured body.

Postmortem findings:

The liver and viscera were deformed in *L. niloticus* fed ration containing aflatoxin B_1. The gall bladder in the affected fish was enlarged and filled with bile.

Biochemical changes:

As shown in table (4), ALP, AST and ALT activities, creatinine, urea uric acid, cholesterol concentrations and A/G ratio in the serum of *Lates niloticus* fed ration contained aflatoxin B_1 were significantly higher than those of control fish. On the other hand the table showed that serum TP, albumin, globulin, sodium, potassium, calcium and inorganic phosphorus concentrations in the *L. niloticus* fed ration containing aflatixin B_1 were significantly decreased comparing with control fish.

Table 1: Chemical parameters of control and affected fish culture water (n=10)

Parameter	Control	Affected farm	Standard value*
pH	7.5	8.5	6.5-9.0
Hardness (mg/L as $CaCO_3$)	90.0	125.0	10-400
Phosphorus (mg/L)	1.3	1.5	≤ 3.0
Nitrate (mg/L)	2.5	3.0	≤ 3.0
Nitrite (mg/L)	0.1	0.1	≤ 0.2
Ammonia (un-ionized; mg/L)	0.01	0.01	≤ 0.0125
Lead (mg/L)	0.01	0.02	≤ 0.03
Cadmium (mg/L)	0.0005	0.0005	≤ 0.003
Iron (mg/L)	0.01	0.01	≤ 0. 15

* Standard water parameters related to Swann (1997)

Table 2: Chemical analysis of ration offered to affected *Lates niloticus*.

Group / Parameter	Control	Affected culture
Aflatoxin B_1 (ppb)	20.50 ± 1.20	168.65 ± 9.33**
Rancidity	18.42 ± 1.63	22.00 ± 1.54
Peroxide value	25.74 ± 1.85	30.50 ± 2.26

Each value represents Mean ± SE; n=10.

** Significant difference against control at $p \leq 0.01$.

Table 3: Aflatoxin B_1 level in *Lates niloticus* tissues (ppb).

Group / Organ	Control	Affected fish
Liver	n.d.	5.68 ± 0.24
Kidney	n.d.	5.20 ± 0.31
Musculature	n.d.	4.26 ± 0.21

Each value represents mean ± SE; n=10.

n.d. non detectable.

Table 4: Blood serum biochemical parameters in *Lates niloticus* fed aflatoxin B_1 contaminated ration

Group / Parameter	Control	Affected fish
ALP (U/L)	80.30 ± 3.59	103.64 ± 4.22**
AST (U/L)	38.35 ± 1.65	64.4 ± 2.15**
ALT (U/L)	20.58 ± 1.75	55.46 ± 2.19**
TP (g/dl)	5.61 ± 0.37	4.11 ± 0.31**
Albumin (g/dl)	3.25 ± 0.19	2.53 ± 0.10**
Globulin (g/dl)	2.46 ± 0.11	1.66 ± 0.07**
A/G ratio	1.30 ± 0.06	1.53 ± 0.07*

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Creatinine (mg/dl)	1.20 ± 0.08	1.62 ± 0.09**
Urea (mg/dl)	8.74 ± 0.34	15.62 ± 0.68**
Uric acid (mg/dl)	4.85 ± 0.23	5.83 ± 0.21**
Cholesterol (mg/dl)	185.62 ± 5.11	224.50 ± 2.11**
Na (mM/L)	125.50 ± 5.23	108.10 ± 4.42*
K (mM/L)	5.43 ± 0.25	4.56 ± 0.18*
Ca (mg/dl)	11.27 ± 0.65	7.86 ± 0.26**
P (mg/dl)	4.86 ± 0.22	4.02 ± 0.20*

Each value represents mean ± SE; n=10.
* Significant difference against control at $p \leq 0.05$.
** Significant difference against control at $p \leq 0.01$.

Discussion:

The chemical properties of the affected *Lates niloticus* culture water were within the safe limits for fish according to Swann (1997).

The aflatoxin B_1 concentration in the rations of affected fish was significantly high comparing with those of control. In addition, aflatoxin B_1 residues were present in the affected *L. niloticus* liver, kidney and musculature.

Lates niloticus fed on ration containing aflatoxin B_1 exhibited signs of lethargy, sluggish movement, loss of appetite, increased mucous over the skin and protrusive eye. The affected fish body had yellowish colour. The postmortem lesions included deformed liver and viscera. The gall bladder was enlarged and filled with bile. These clinical and anatomical findings nearly confirm those of El-Barbary and Mehrim (2009) and Mehrim *et al.* (2006) aflatoxicated *Oreochromis niloticus* fish.

Respecting the blood serum biochemical findings, the enzymes, ALP, AST and ALT activities were significantly elevated in the serum of *Lates niloticus* fed on ration containing aflatoxin B_1 comparing with control. Nearly similar results where stated by El Sayed and Khalil (2009) who recorded increase in serum transaminases and alkaline phosphatase activities in sea bass exposed to prolonged oral administration of aflatoxins and Selim et al. (2014) who observed increases in serum AST and ALT in *Oreochromis niloticus* fed aflatoxin B_1 containing ration. However our result nearly disagreed with that of Huang *et al.* (2014) who stated that the exposure of gibel carp fish to aflatoxin B_1 in diet had no significant effect on serum AST and ALT enzymes activities. The increase in the liver enzymes activities may be due to hepatic cell injury or increased synthesis of the enzymes by the liver as clarified by Yang and Chen (2003).

Serum TP, albumin and globulin concentrations were significantly lowered while A/G ratio was increased in *Lates niloticus* fed on aflatoxin B_1 containing rations comparing with control. Nearly similar results were recorded by Sahoo and Mukherjee (2001) who recorded reductions of total protein, globulin levels and enhanced albumin-globulin ratio in Indian major carp (*Laboe rohita*) fish exposed to aflatoxin B_1 and Selim et al. (2014) who recorded decrease in plasma proteins and globulin in *Oreochromis niloticus* fed aflatoxin B_1 containing ration. Blood proteins are used for energy production during toxicity and the protein catabolism is induced by stress (Pfeifer and Weber 1979). Moreover, aflatoxin hepatotoxicity leads to alterations in protein synthesis and cellular integrity of the liver (Jindal et al. 1994). Serum globulin reduction may be related to hemopoietic toxicity (anterior kidney and spleen) and lymphocytolysis (Sahoo et al. 2001). aflatoxin B_1 also proved to be immunosuppressive in fish (Sahoo and Mukherjee, 2001). So, the increase of A/G ratio in *L. niloticus* fed on aflatoxin B_1 containing ration in the present study may be due the immunosuppressive effect of the aflatoxin.

Serum creatinine, urea and uric acid concentrations were significantly increased in *Lates niloticus* fed on aflatoxin B_1 containing ration comparing with control. Our findings are likely in agreement with those of Zaki *et al.* (2010) who found that aflatoxicosis increased urea and creatinine in *Tilapia Zilli* fish and Selim *et al.* (2014) who stated that aflatoxin B_1 increased serum creatinine level in *Oreochromis niloticus* fish. The increase of serum creatinine concentration fish fed on aflatoxin B_1 containing ration may be attributed to renal damage (El-Boshy *et al.,* 2008). The elevation of urea in the present study may be due to gill dysfunction (Lockhart and Metner, 1984). The increase of uric acid levels in aflatoxin B_1 affected fish could be resulted from liver damage induced by aflatoxin (Carlson *et al.,* 2001 and Ottinger and Kaattari, 2000).

Serum cholesterol level was significantly elevated in aflatoxin affected *L. niloticus*. Nearly similar result was concluded by Zaki *et al.* (2010) in *Tilapia zilli* fish supplied with aflatoxin contaminated ration. In the current study, the hypercholesterolaemia in aflatoxin B_1 affected fish may be due to liver injury.

Significant decrease in the blood serum minerals, sodium, potassium, calcium and inorganic phosphorus levels occurred in the aflatoxin B_1 affected *Lates niloticus* comparing with control. The gills are the major sites of osmotic and ionic regulation in fish, any changes in gill morphology may result in perturbed osmotic and ionic status (Al-Attar, 2007). In the present study, Hyponatraemia in the aflatoxin B_1 affected fish might reflect a decrease in the sodium influx rate through the gills as showed by Martinez *et al.* (2004). The hypokalaemia in the affected fish might result from impaired active reabsorption of potassium in renal tubules (Gill *et al.,* l989). The hypocalcaemia observed in the aflatoxin B_1 fed *L. niloticus* may be due to diffusional losses caused by increased permeability of gill epithelium to water and ions (Giles, 1984), defective intestinal calcium absorption or im-

paired calcium reabsorption in the renal tubules (Gill *et al.*, 1988). The decreased serum inorganic phosphorus may be attributed to disturbance in metabolism resulted from gastrointestinal malfunction (Glavind and Tryding, 1960).

Conclusion: In the present study, aflatoxin B_1 residues appeared in the tissues of *Lates niloticus* fish fed on rations polluted with that toxin. The aflatoxin B_1 had bad impacts on the organs function and blood serum parameters. So, it is advisable to control the feeding of fish and supplying the fish with healthy aflatoxin free rations.

References

Adeniji, C.A., P.A. Okiki, A.M. Rasheed and R. Bolaji, 2014. Mouldy groundnut cake and hydrated sodium calcium aluminosilicate in practical diet for African catfish *Clarias gariepinus* (Burchell, 1822). Fish. Aquac. J., 4: 1.

Al-Attar, A.M., 2007. The influences of nickel exposure on selected physiological parameters and gill structure in the teleost fish, *Oreochromis niloticus.* J. Biol. Sci., 7(1): 77-85.

Amalacher, E., 1970. Textbook of fish disease. Tropical Fish Hobbyist, pp: 143-146.

AOAC-Association of Official Analytical Chemists, 1980. Methods of analysis Washington, D.C. USA.

Ashley, L.M., 1970. Pathology of fish fed aflatoxins and other antimetabolites In: A symposium on diseases of fishes and shellfishes. American Fisheries Society Special Publication, 5: 366-379.

Barnett, R.N., 1965. A scheme for the comparison of quantitative methods. Am. J. Clin. Pathol., 43: 562.

Bowers, L.D. and E.T. Wong, 1980. Kinetic serum creatinine assays. II. A critical evaluation and review. Clin. Chem., 26: 555-561.

Cannon, E.C., I. Olitzky and J.A. Inkpen, 1974. Proteins. In: Clinical chemistry principles and technics, 2nd ed. R.J. Henery, D.C. Cannon, J.W. Winkelman, editors, Harper & Row, New York, pp: 407-421.

Carlson, D.B., D.E. Williams, J.M. Spitsbergen, P.F. Ross, C.W. Bacon, F.I. Meredith and R.T. Riley, 2001. Fumonisin B_1 promotes aflatoxin B_1 and N-methyl-N'-nitro-nitrosoguanidine-initiated liver tumors in rainbow trout. Toxicol Appl Pharmacol., 1, 172 (1): 29-36.

Clescerl, L., A. Greenberg and A. Eaton, eds., 1999. Standard methods for examination of water and wastewaters, 20th edn. Association, pp: 1325.

Coles, E.H., 1986. Veterinary Clinical Pathology, 4th Edition W.B. Sanders Company, Pliladelphia, pp: 22-23.

Daly, J.A. and G. Ertingshausen, 1972. Direct method for determining inorganic phosphate in serum with the "CentrifiChem". Clin Chem., 18(3): 263-265.

Doumas, B.T., W.A. Watson and H.C. Biggs, 1971. Albumin standards and Management of serum albumin with bromocresol green. Chin. Chim. Acta., 31: 87-96.

El-Barbary, M.I. and A.I. Mehrim, 2009. Protective effect of antioxidant medicinal herbs, rosemary and parsley, on subacute aflatoxicosis in *Oreochromis niloticus*. J. Fish. Aquat. Sci., 4(4): 178-190.

El-Boshy, M.E., A.M.M. El-Ashram and N.A. Abd El-Ghany, 2008. Effect of dietary beta-1, 3 glucan on immunomodulation on diseased *Oreochromis niloticus* experimentally infected with aflatoxin B_1. 8th International Symposium on Tilapia in Aquaculture.

Fawcett, J.K. and J.E. Scott, 1960. A rapid and precise method for the determination of urea. J. Clin. Path., 13(2): 156-159.

Fossati, P., L. Prencipe and G. Berti, 1980. Use of 3,5-dichloro-2-hydroxybenzene-sulfonic acid/4-aminophenazone chromogenic system in direct enzymatic assay of uric acid in serum and urine. Clin. Chem., 62: 227-231.

Giles, M., 1984. Electrolyte and water balance in plasma and urine of rainbow trout Salmo gairdneri during chronic exposure to cadmium. Can. J. Fish Aquat. Sci., 41: 1678-1685.

Gill, T.S., C.P. Jagdish, And P. Jaishree, 1988. Gill, liver and kidney lesions associated with experimental exposure to carbaryl and dimethoate in fish, Puntius conchonius (HAM.). Bull. Environ. Contam. Toxicol., 41: 71-78.

Gill, T.S., J.C. Pant and H. Tiwari, 1989. Cadmium nephropathy in freshwater fish Puntius conchonius (HAM.). Ecotox. Environ. Safe,. 18: 165-172.

Glavind, J. And N. Tryding, 1960. Acta physiologicl. Scand. 49 (97) cited by Artman, B.R.(1969).

Goldblatt, L.A., 1976. Significance of aflatoxin in foods. Proc. 80th Annual Conference of the Association of Food and Drug Officials, Atlanta, Georgia, 22: 191-201.

Henry, R.F., D.C. Cannon and J.W. Winkelman, 1974. Clinical Chemistry Principles and Techniques. 2nd Ed. Harper and Roe, Hagerstown, M.D.

Hillman, G., G. Beyer and Z. Klin, 1967. Determination of potassium concentration. Chem. Clinic. Biochem., 5: 93.

Huang, Y., D. Han, X. Xiao, Y. Yang, J. Jin, Y. Chen and S. Xie, 2014. Effect of dietary aflatoxin B_1 on growth, fecundity and tissue accumulation in gibel carp during the stage of gonad development. Aquaculture, 429: 236-242.

Jackson, M.L., 1973. Textbook of Soil Chemical Analysis, Prentice Hall of Indian Private Ltd.

Jindal, N., S.K. Manipal and N.K. Mahajan, 1994. Toxicity of aflatoxin B1 in roiler chicks and its reduction by activated charcoal. Res. Vet. Sci., 56: 37-40.

Lockhart, W.L. and D.A. Metner, 1984. Fish chemistry as a pathology tool, pp. 73-86. In: Contaminant Effects on Fisheries. Vol. 16 (Cairns, V.W., P.V. Hodson and J.O. Nriagu eds.). New York: Wiley Interscience.

Lucky, Z., 1977. Textbook for the diagnosis of fish diseases, Amerind Publishing Co. PVT. Ltd., New Delhi.

Madhusudhanan, N., S.N. Kavithalakshmi, E.R.B. Shanmugasundaram and K.R. Shanmugasundaram, 2006. Aflatoxin B_1-induced DNA damage in *Labeo rohita*: protective effect of an antioxidant supplement, *Amrita Bindu*. Basic & Clinical Pharmacology & Toxicology, 98: 473-479.

Marsh, W.H., B. Finger hut and E. Kirsch, 1959. Adaptation of an Alkaline phosphatase method for automatic colorimetric analysis. Clin. Chem., 5: 119.

Martinez, C.B.R., M.Y. Nagae, C.T.B.V. Zaia and D.A.M. Zaia, 2004. Acute morphological and physiological effects of lead in the neotropical fish Prochilodus lineatus. Braz. J. Biol., 64: 797-807.

Mehrim, A.I., A.M. Abdelhamid, A. Abou-Shousha, M.F.I. Salem and M.A.M.M. El-Sharawy, 2006. Nutritious attempts to detoxify aflatoxic diets of tilapia fish: 2- Clinical, biochemical and histological parameters. J. Arab. Aquacult. Soc., 1: 69-90.

Miliţă, N.M., G. Mihăescu, C. Chifiriuc, 2010. Aflatoxins-Health risk factors. Bacteriol. Virusol. Parazitol. Epidemiol., 55: 19-24.

Murjani, G., 2003. Chronic aflatoxicosis in fish and its relevance to human health. Central Institute Of Fresh Water Aquaculture. India.

Ottinger, C.A. and S.L. Kaattari, 1998. Sensitivity of rainbow trout leucocytes to aflatoxin B_1. Fish Shell Fish Immunol, 8: 515.

Pfeifer, K.F. and L.J. Weber, 1979. The effect of carbon tetrachloride on the total rotein concentration of rainbow trout, Salmo gairdneri. Comp. Biochem. Physiol., 64(C): 37-42.

Reitman, S. and S. Frankel, 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Amer. J. Clin. Path., 28: 56.

Russo, J.R. and R.P.E. Yanong, 2010. Molds in fish feeds and aflatoxicosis. Fact Sheet FA-95, Institute of Food and Agricultural Sciences, University of Florida.

Sahoo, P.K. and S.C. Mukherjee, 2001. Immunosuppressive effects of aflatoxin B1 in Indian major carp (*Laboe rohita*). Microbiol. Infectious Dis., 24: 143-149.

Sahoo, P.K., S.C. Mukherjee, A.K. Jain and A. Mukherjee, 2001. Histopathological and electron microscopic studies of gills and opisthonephros of Rohu, Labeo rohita to acute and subchronic aflatoxin B1 toxicity. Asian Fish Sci., 16: 257-268.

Selim, K.M., H. El-hofy and R.H. Khalil, 2014. The efficacy of three mycotoxin adsorbents to alleviate aflatoxin B_1-induced toxicity in *Oreochromis niloticus*. Aquacult. Int., 22: 523-540.

SPSS, 14, 2006. Statistical package for social science, SPSS for windows release 14.0.0, Standard version, SPSS Inc.

Stoskopf, M.K., 1992. Clinical Pathology. In: "Fish Medicine", M.K. Stoskopf (ed.), 1st edn., W.B. Saunders Company, Philadelphia.

Swann, L., 1997. A Fish Farmer's Guide to Understanding Water Quality. Aquaculture extension, Illinois-Indiana Sea Grant Program. AS-503, Purdue University, IL-IN, pp: 1-8.

Tucker, C.S., 1992. Water analysis. In: Fish Medicine, M.K. Stoskopf (ed.) 1st edn., W.B. Saunders Company, Philadelphia, pp: 166-197.

Yang, J. and H. Chen, 2003. Serum metabolic enzyme activities and hepatocyte ultra structure of common carp after gallium exposure. Zoological Studies, 42(3): 455-461.

Young, D.S., 1990. Effects of drugs on chlinical laboratory tests. Third edition, 3: 6-12.

Zaki, M.S., O.M. Fawzi, S. Omar, M. Khafagy, M. Fawzy and I. Awad, 2010. Diminution of aflatoxicosis in *Tilapia zilli* fish by dietary supplementation with fix in toxin and *Nigella sativa* oil. Nature and Science, 8(2): 43-49.