CONTENTS

LIST OF ABBREVIATIONS

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AFB1 : Aflatoxin B1.

AFG1 : Aflatoxin G1 .

B.F. : Best Food .

C.B. : Contaminated Branch.

F.A.O : Food and Agriculture Organization .

I/P : Intraperitoneal injection .

PPb : Part Per billion .

PPm : Part Per million .

R.f : Rate of flow .

TLC : Thin layer chromatographic .

ug : Microgram (micron).

6-SUMMARY

This study was conducted on a total number of three hundred and thirty five samples of fish ration and fish ration ingredients. One hundred and eighty from fish meal, eighty from yellow corn and seventy five from fish ration, all samples were collected from Animal Health Research Institute Dokki, from Markets, from Abbasa Fish Hatchery in Sharkia Governorate and Nawa farm in Kalyoubia Governorate.

One hundred apparently normal Nile Tilapia fish (Oreochromis niloticus) of different weights (60-80g) were obtained from Foky center at Nowa farm in Kalyoubia Governorate for experimental infection.

Eighteen clinically normal Nile cat fish (Clarias species) at weights (150-200g) collected from private fish farms in Sharakia Governorate to be used as a biological indicators (skin test).

The results of mycological studies revealed that from fish meal 335 isolates related to 5 genera and 18 species were recovered, from fish ration 175 isolates related to 5 genera and 19 species were isolated and from yellow corn 140 isolates related to 4 genera and 16 species were isolated .
Five genera of different fungi were isolated from fish meal,fish ration and four genera from yellow corn . The genus *Aspergillus* was the most predominant isolates with occurrence of (88.05 %) , it was isolated from examined samples followed by *Penicillium* species with occurrence of (37.3 %), *Fusarium* species with occurrence of (34.3 %), *Mucor* species with occurrence of (25.3 %) , *Alternaria* species with occurrence of (7.4 %) and *Scopulariopsis* species with occurrence of (4.4%) .

Incidence of fungi isolated from examined fish meals as follow : from the genus *Aspergillus* was the predominant isolates (86.11 %), from the genus *Penicillium* species (33.3 %) , *Fusarium* species (30.5 %) , *Mucor* species (22.2 %), *Alternaria* species (8.3 %) and *Scopulariopsis* species (5.5 %) .In fish ration the genus Aspergillus was the predominant isolates (100%), *Penicillium* species (53.3 %), *Fusarium* species (40 %), *Mucor* species (20 %), *Alternaria* species (13.3 %) and *Scopulariopsis* species (6.6 %).From yellow corn the *Aspergillus* species are (81.3%) *Penicillium* species (31.3 %), *Fusarium* species (25 %) and Mucor specie (37.5%).

Sixty strains of *Aspergillus flavus* were examined for their ability to produce aflatoxins. The yield of aflatoxins produced, the quantities determined varied from 10 ppb up to 1000 ppb .

Sixteen strains of *Fusarium* species examined for T-2 toxin production . six *Fusarium* strains only produced T-2 toxin namely *Fusarium solani* (2 strains) *Fusarium tricinctum* (2 strains) , *Fusarium poae* (one strain) and *Fusarium oxysporum* (one strain) .The yield of T-2 toxin produced was varied from 10 – 500 ppb .

Seven strains of *Aspergillus ochraceus* were tested for their ability to produced ochratoxin A , the strains were isolated from fish meal, fish ration and yellow corn .All the examined strains were failed to produced ochratoxin A .

Biological methods were done to confirm and to study the behaviour of mycotoxin detected . Toxicity of T-2 on skin of Nile Cat fish by the extracts of selected sex strains mentioned above . A total of eighteen fish

were divided into six equal groups, from each group two fish were used for toxin application and the third one remains as control.

The T-2 toxins produced from the strains of *Fusarium* species number (3,5,7,8,9 and 12) gave different degrees of response at end of observation period (2-4) hours. T-2 toxins produced by strains number (5 and 9) with dose 20 ppb were judged as highly toxic , gave the 4th grade of toxicity but the same strain with a dose of 40 ppb gave sudden death.

The T-2 toxins produced by strains number (3 and 7) with dose 20 ppb were judged as considered toxic , as they gave 3rd grade of toxicity but with dose 40 ppb death occurs after one hour. The remaining T-2 toxins produced by strains number (8 and 12) were judged as very weak toxic with both doses of 20 ppb and 40 ppb.

The results of pathogenicity tests performed on Nile Tilapia fish Oreochromis niloticus injected I/P by aflatoxin B1 at the doses 0.2 , 0.4 , 0.6 , 0.8 and 1 mg revealed that, the doses of 1ppm and 0.8 ppm crude aflatoxin / kg body weight injected in groups 5 and 4 were highly toxic resulting in mortality rate 100% and 80 % respectively among the injected fish . While the doses 0.6 , 0.4 and 0.2ppm crude aflatoxin /kg body weight injected in groups 3 , 2 and 1 produced a mortality rate reached 60% , 20 % and 10% respectively within the experimental period .

The most common clinical signs observed were dropping of moribund fish to the bottom of aquarium to death with opening of gill covering , increase opercular beats, sluggish swimming movements and skin darkening .

The post mortum changes in moribund fishes were seen in liver, gall bladder, spleen and kidney liver displayed pale coloration with congested patches and pin point hemorrhages and gall bladder distended with

brownish bile . Spleen appeared enlarged, dark in colour and kidneys were congested and enlarged.

Histopathological finding revealed that no obvious lesions at dose 0.2 ppm aflatoxin B1 injected I/P , while at the dose 0.4 ppm , 0.6 , 0.8 ppm and 1 ppm the histopathological changes were very clear . Concerning the liver of fish exposed to 0.6 ppm after the 2nd week of the experiment , severe hyperemia in the central and portal veins and also sinusoids .In the fish exposed to 0.8 ppm after 3rd week exposure a melanin pigmented cells were replaced the pancreatic cells in focal manner surrounding the portal vein. While the gills of fish exposed to 0.6 ppm AFB_1 after three weeks of experiment, hyperplasia and fusion were observed in the lamellae , while the base of the filaments showed inflammatory cellular infiltration .Fish exposed to 1 ppm AFB1 in 4th week of experiment , showed severe degenerative and necrobiotic alterations in the living epithelium of the renal tubules, an esinophilic round bodies replaced the necrobiotic cells in the tubules . Spleen showing hyperemic red pulps with depletion in white pulps .Concerning the skin of fish exposed to 1 ppm at 4th week of experiment , a massive number of mononuclear leukocyte infiltrating the dermal layer as well as the underlying skeletal muscle were noticed .

The residual analysis of aflatoxin B1 in the fish muscles was performed using TLC .The analysis indicated that only aflatoxin B1 was detected in fish muscles in groups (2-5) which injected I/P aflatoxin B1. The result also revealed that no other aflatoxin metabolites were detected in fish muscles .

The results of pathogenicity tests performed on Nile Tilapia fish (Oreochromis niloticus) using T-2 toxin at doses of 0.4 ppm and 0.8 ppm

T-2 / kg weight I/P injected. Fish exposed to 0.4 ppm of T-2 toxin showed loss of scales at the site of injection , erosions of tail and fins . Fish exposed to 0.8 ppm of T-2 toxin showed erythema at site of injection , redding and hemorrhage at the pectoral fins, destruction of upper jaw .The post mortum changes in moribund fish were obvious in liver, gall bladder and spleen, congestion of all organs was noticed ,in addition to hemorrhages of liver with dark enlarged spleen and autolysis of muscles .

Histopathological finding of fish examined after 3rd weeks for I/P administration of T-2 toxin . In the liver the portal area was infiltrated by mononuclear leucocytic inflammatory cells with severe dilatation in the portal vein . There was severe hyperemia in the hepatic sinusoids with activation and proliferation in the hypertrophied kupffer cells in between the fatty degenerated hepatocytes .

In the spleen severe congestion of splenetic ellipsoids with depletion of haemopiotic cells with appearance of local melanin pigmented cells .

In skeletal muscle local mononuclear leucocytic inflammatory cells infiltration were observed in between the muscle bundles .