Toxico-Biological Behaviour Of Toxogenic Fungal Extracellular Products In Freshwater Fish

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ABSTRACT

This study was conducted on 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, Markets and Nawa farm in Kalyoubia Governorate.

One hundred of apparently normal Nile Tilapia fish (*Oreochromis niloticus*) used for experimental infection. Eighteen clinically normal Nile cat fish (*Clarias* species) 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 followed by *Penicillium* species, *Fusarium* species, *Mucor* species, *Alternaria* species and *Scopulariopsis* species. Sixteen strains of *Fusarium* species examined for T-2 toxin production.

Biological methods were done to confirm and study the behaviour of mycotoxin detected. 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 were recorded.

Histopathological findings of examined fish after 3rd weeks of I/P administration of T-2 toxin were recorded.

INTRODUCTION

In tropical and subtropical countries all the surrounding conditions facilitate contamination by fungi and consequently elaboration of their toxic products in food and feed *(1)*.

Fusarium mycotoxins in fish feed is a potential problem because all types of grains are easily contaminated by it. The toxins produced by this group of fungi called trichothecenes. Only four out of approximately fourteen trichothecene derivatives produced under laboratory conditions have been found naturally in food and feed stuff. The four problomatic members are: T-2, nivalenol, deoxynivalenol and diacetoxyscirpenol. They are responsible for a disease of man called Alimentary Toxic Aleukia *(2)*.

The T-2 and vomitoxin have been studied in fish. Hematological effects were observed in rainbow trout fed on diet contaminated by T-2 toxin. Also T-2 and vomitoxin leads to necrosis of skin, mouth, intestine and liver. Beside its effect on the clotting mechanism of blood, it also increases the permeability of small blood vessels leading to extensive haemorrhages *(3)*. The only measures known till now to avoid the dangerous effect of these toxic metabolites are their detection in food and feed before their direct or indirect consumption.

Therefore, the present study was planned to characterize the isolation and identification of toxinogenic fusarium species from fish ration and fish ration components, detection and determination of T-2 in fish feed and feed ingredients, studying the effect of toxinogenic fungal isolates extracts on skin of non scaled fish, as biological indicator and to study the effect of extracts of toxingenic fungal isolates on fresh water fish and studying the histopathological changes in the different organs of the experimentally infected fish.

MATERIALS AND METHODS

Fish Feed Samples

A total number of three hundred and thirty five samples of fish ration and fish ration ingredients of which one hundred and eighty fish meal, eighty yellow corn and seventy five fish ration were collected from Animal Health Research Institute Dokki, local markets and Nawa fish farm in Kalyoubia Governorate. A representative sample (500 gm) was collected from different levels of each of the whole fish feed or its ingredients ' container *(4).*

Fish for experimental purposes

* Fish for experimental infection

Fourty apparently normal Nile Tilapia fish (*Oreochromis niloticus*) of average weights (70 ± 5 g) were obtained from Foky center at Nawa farm in Kalyoubia Governorate. Fish was divided into four equal groups.

* Fish for biological indications

A total of eighteen clinically normal Nile cat fish (*Clarias* species) of an average weights (150-200g) were collected from private fish farms in Sharakia Governorate and divided into six equal groups. For each group, two fish were used for toxin application and the third one remains as control.

All collected fish were transported alive in polyethylene bags to the Dep. Fish Diseases, Animal Health Research Institute, Dokki and grouped in glass aquaria containing an aerated and declorinated freshwater at 25°C. The fish were fed a commercial ration and left for ten days for acclimation.

Media

*Czapek – Dox Agar *(5)* and Potato Dextrose Agar *(6)* were used for fungal isolation and identification .

*Crushed corn medium was used as natural media for toxin production *(7)* .

Strains selected for mycotoxins production

The following *Fusarium* species were selected due to their association with disease conditions in fish, animals and man:

Fusarium poae (5 strains),*Fusarium tricinctum* (4 strains),*Fusarium solani* (3strains),*Fusarium oxysporum* (3 strains), *Fusarium sporotrichioides* (one strain), *Fusarium fusarioides* (one strain).

Standard toxins

Authentic standard of T-2 (Sigma Chemical Company ,U.S.A.).

Mycological Examination

The recovery of *Fusarium* species from fish feed samples was carried out by:

*Direct grain method *(7).*

The grains were cultured directly on Czapek-Dox agar medium.Ten grains were distributed apart from each other on the surface of the medium, then incubated at 25°C, and routinely examined after 2 days till fungal growth was completed.

* Dilution plate method *(8).*

Ten grams of samples (fish meal and fish ration) were placed into a flask containing 90 ml sterile distilled water, the flask was shacked gently to guarantee a complete homogenization. A serial dilution was done in a trial to select the dilution of choice which gave the best density of colonies to be calculated. In this study the best dilution was 1 /1000. One ml from this dilution was spreader on the surface of two plates of Czapek -Dox agar medium and gently rotated by hand in every direction to get even distribution of the diluents.The plates were incubated at 25°C long wave and examined daily for the

appearance of growth after the second day of incubation.

The identification of fungal isolates is based upon the macroscopic and microscopic examinations of incubated plates. Individual colonies were selected depending upon their morphological characters. A pure culture was prepared from each growing colony on Czapek –Dox agar slants. In case of the Genus Fusarium identification was done *(6).*

Mycotoxin production

T-2 toxin was extracted from isolated strains after their growth on the natural media (crushed corn media) *(9).*

Estimation of T-2

Qualitative Estimation

T-2 toxin was qualitatively estimated *(7).*

Quantitative estimation of T-2

The qualitatively positive samples were further studied to estimate the quantities of mycotoxins using the CB-method (one dimensional) *(10).*

Biological method for detection of mycotoxins (Fish skin test)

One of the biological properties of T-2 is its dermonecrotic effect on Nile Catfish *(11)* .The extracts of selected six strains of *Fusarium* related to four species namely *Fusarium tricinctum* (No.8,12), *Fusarium solani* (No.3,7), *Fusarium poae* (No.5) and *Fusarium oxysporum* (No.9) were used to perform this test . A total of eighteen fish were divided into six equal groups. Two fish in each group were used for toxin application and the third one was left as control. The extract of each strain was mixed with corn oil to allow a homogenous distribution of the toxin and a long persistence of it on the fish skin. Each extract was applied to the middle area on skin of fish. 20 and 40 ug of mycotoxin were applied on the skin of the first and second fish respectively, while 20 ul of corn oil was applied on the skin of the control fish. Judgement was monitored by the registration of the changes on the local area of mycotoxin application as well as on the general condition of fish *(7).*

Experimental infection

Preparation of crude T-2 toxin doses for I/P injection *(11)

The extract of T-2 toxin was dissolved in chloroform at level of 1 mg T-2 toxin to 1ml chloroform . A final of calculated level of corn per injection was 0.1mg /injection. Two doses 0.4 and 0.8 mg / kg body weight were experimentally tested.

*** I/P injection of T-2 toxin**

Fish in first and third experimental groups were injected I/P by 0.4 mg and 0.8 mg T-2 toxin /Kg body weight respectively. However, fish in the second and fourth groups were I/P injected with 0.4 ml, 0.8 ml sterile corn oil respectively, and left as negative control. All experimental fish groups were observed for 30 days post injection, during which the morbidity and mortalities were recorded, the clinical sings, post mortum lesions and histopathological changes were also studied *(11).*

RESULTS

Fungi isolated from fish ration and fish ration ingredients

Regarding fungi recovered from fish ration and fish ration ingredients, the results (Table 1) revealed the recovery of 335, 175 and 140 isolates from fish meal, fish ration and yellow corn specimens respectively.

Table 1. Fungi isolated from fish ration, fish meal and yellow corn

Types of examined samples	No. of examined samples	No. of isolates
Fish meal	180	335
Fish ration	75	175
Yellow corn	80	140
Total	335	650

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 followed by *Penicillium* species ,*Fusarium* species, *Mucor* species, *Alternaria* species and *Scopulariopsis* species.

The incidence of recovery of *Fusarium* species from the examined fish meal, fish ration and yellow corn specimens shown in Table (2).

Table 2. The frequency of recovery of *Fusarium* species in the total examined samples (fish meal, fish ration, and yellow corn).

Types of examined samples	No. of positive samples	Percentage
Fish meal	55	30.56 %
Fish ration	30	40 %
Yellow corn	20	25 %

Regarding the detection of T-2 toxin production on crushed corn, out of sixteen examined *Fusarium* species for T-2 toxin production, six *Fusarium* strains were positive namely: *Fusarium solani* (2 strains) *Fusarium tricinctum* (2 strains), *Fusarium poae* (one strain) and *Fusarium oxysporum*(one strain).

Regarding the biological toxicity of T-2 on the skin of Nile Catfish, two doses of T-2 toxin 20 and 40 ppb were applied on the fish skin at the region of middle part of the tail .The T-2 toxins produced by the strains of *Fusarium* species number (3,5,7,8,9 and 12) gave different degrees of responses at the end of observation period (2 to 4 hours). Application of 20 ppb T-2 toxins produced by the strains number (5 and 9) were judged as highly toxic (4^{th} grade of toxicity), while the application of its higher dose 40 ppb resulted in a sudden death of the fish (Table 4). The T-2 toxins produced by the strains number (3 and 7) when applied with the dose 20 ppb were judged as toxic (3^{rd} grade of toxicity), but with the dose 40 ppb death occurs after one hour. On the other hand, the T-2 toxins produced by the strains number (8 and 12) were judged as weak toxic after the use of the two doses (Table 4 and photo1-2).

Table 3. The frequency of recovery of members of genus *Fusarium* from examined samples .

Fusarium species	No. of cases of isolation from examined samples	Percentage
Fusarium poae	33	9.85
Fusarium tricinctum	23	6.86
Fusarium fusarioides	15	4.47
Fusarium solani	25	7.46
Fusarium oxysporum	6	1.79
Fusarium sporotrichioides	2	0.6
Fusarium flocciferum	1	0.02

Table 4. Toxicity of T-2 produced by *Fusarium* species on the skin of Nile Catfish.

Code number of isolates	*Fusarium* species	doses		Grades of toxicity			
		20 ppb	40 ppb	1st	2nd	3rd	4th
3	*Fusarium solani*	Reaction	Death after 1 hour	-	-	+	-
5	*Fusarium poae*	Reaction	Sudden death	-	-	-	+
7	*Fusarium solani*	Reaction	Death after 1 hour	-	-	+	-
8	*Fusarium tricinctum*	No Reaction	No death	-	-	-	-
9	*Fusarium oxysporum*	Reaction	Death	-	-	-	+
12	*Fusarium tricinctum*	No Reaction	No death	-	-	-	-

N.B.

Grade 1: Hyperemia, skin sensitive, bran like materials scattered on the skin (very weak toxic)
Grad 2: Hyperemia ,thickness of skin, pain in the area appearance small vesicles (weak toxic)
Grad 3: Hyperemia, skin highly thickened and painful followed by dry necroses (toxic).
Grad 4 : Great thickness of skin with oedema appearance, the area oozing a purulent exudate (highly toxic).

The histopathological changes after skin exposure of Nil Catfish to different doses of T-2 toxin could be classified into two categories according to the time of exposure:

Category I : fish examined after 2 hours exposure

The skin showed focal extravasation of red blood cells as well as pigmentation associated with the appearance of fibrin threads in the from of network in which leucocytic cells entangled in it. The skeletal muscle showed focal extravasation of red blood cells noticed in-between the muscle bundles (photo 3).

Category II : fish examined after four hours exposure

The epidermal layer of skin showed focal extravasation red blood cells while the underlying tissue had pigmented cells. The skeletal muscle showed focal extravasation of red blood cells in-between the muscles bundles (photo 4).

Pathogenicity of T –2 toxins

The results of experimental pathogenicity Performed on Nile Tilapia fish (*Oreochromis niloticus*) with T-2 toxin at doses of 0.4 ppm and 0.8 ppm/ kg weight are shown in Table 5 .

Table 5. The mortality rate among Tilapia fish injected I/P with different doses of cured T-2 during one month

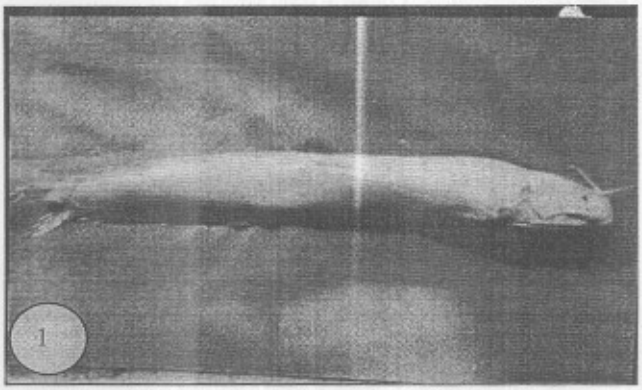
Group No.,	Dose of crude T-2 mg/kg	No., of fish group	No.,of dead fish during one month				Total No. of dead fish	Mortality %
			1st	2nd	3rd	4th		
1st	0.4	10	+	++	+++	+++	10	100
2nd	0	10	-	-	-	-	-	-
control	0.8	10	++	+	++	+++-	10	100
3rd	0	10	-	-	-		-	-
4th								
control								

Clinically, 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, lower jaw and finally loss of the superficial layer of skin. 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, dark spleen and enlarged also autolysis of muscles (Photo 5,6).

The histopathological finding of fish examined 3rd weeks after I/P administration of T-2 toxin revealed the following changes.

* 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. (Photo 7).

* In the spleen, a severe congestion of spleenic ellipsoids with depletion of haemopiotic cells with the appearance of local melanin pigmented cells. The skeletal muscle showed local mononuclear leucocytic inflammatory cells infiltration which clearly observed in-between the muscle bundles. (Photo 8).



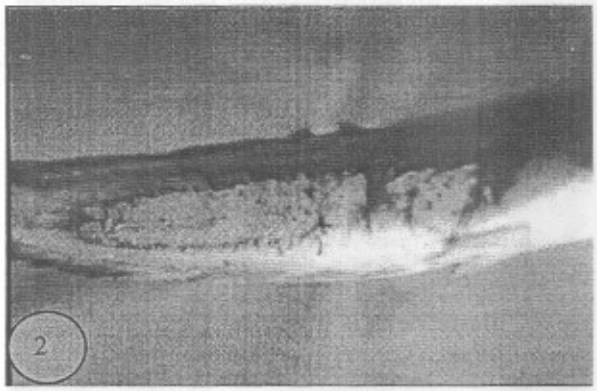
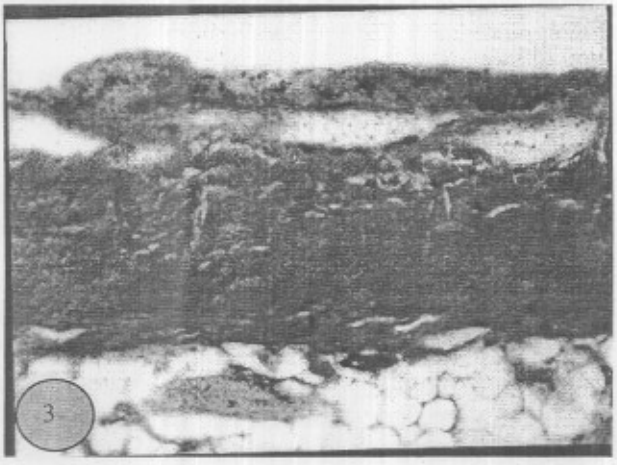


Photo. 1 .Nile cat fish after application of 20 ug of corn oil on skin as control

Photo.2. Nile cat fish locally 20 ug of T-2 on skin Petical hemorrhage, great thickness of skin with appearance of eczema and the area oozing blood (highly toxic).



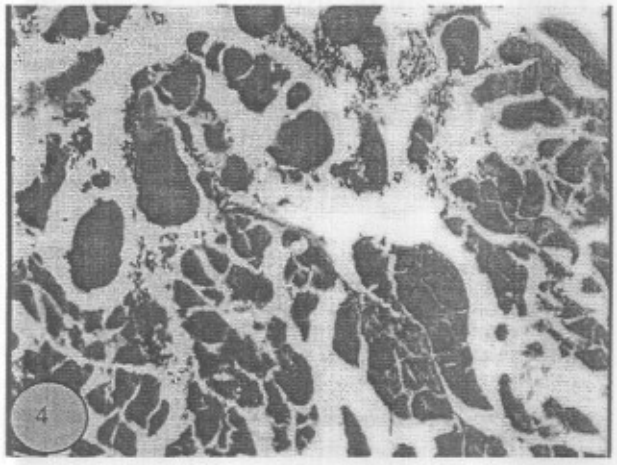
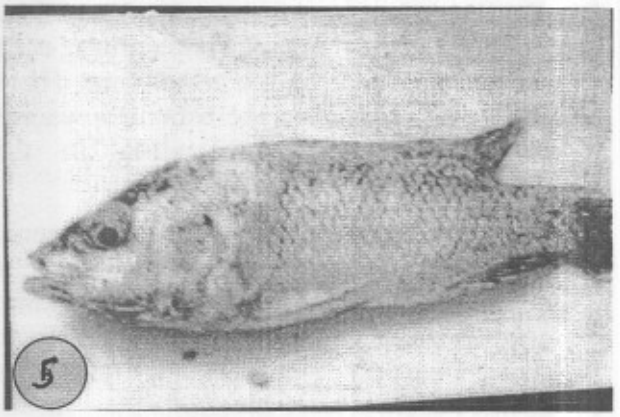


Photo.3. Skeletal muscle of fish after two hours showing local extravasation of red blood cells between the muscle bundles . H& E α 400.

Photo.4. Skin of fish after four hours showing local extravasation of red blood cells in the epidermis with pigmented cells in the underlying area . H& E α 400.



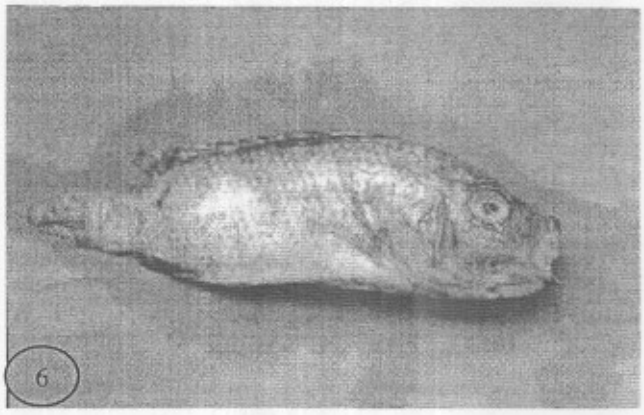
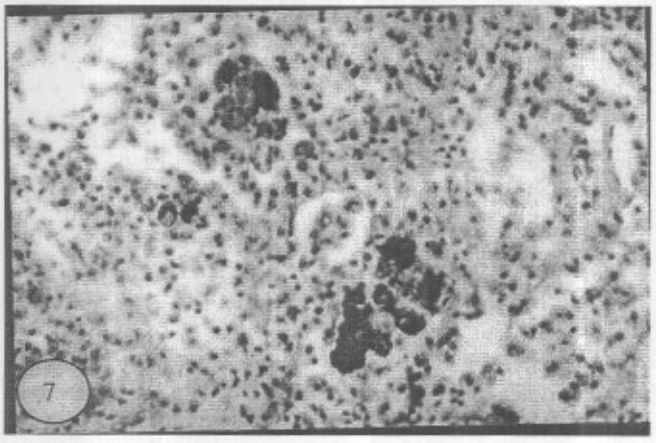


Photo.5.*Oreochromis niloticus* exposed to 0.8 ppm T-2 showing sloughing of lateral caudal fins and erosion of upper and lower jaw .

Photo.6.*Oreochromis niloticus* exposed to 0.8 ppm T-2 toxin showing loss of scales, erosion of tail fin, erythema of caudal fin redding .



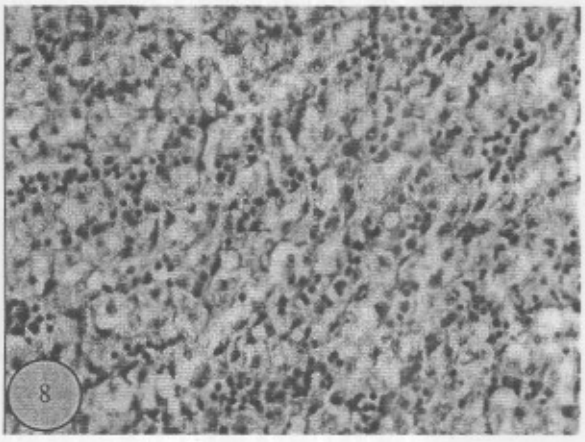


Photo.7. Spleen of fish 3rd weeks after administration of T-2 showing local melanin pigment H& E α400

Photo.8. Liver of fish after 3rd weeks of I/P administration of showing fatty change in the hepatocytes with activation, prcliyuation of hypertrophied Kupffer cells.H& E α400.

DISCUSSION

A wide variety of commodities are subjected to contamination by fungi and their metabolites which are produced and elaborated during the normal activity of fungi called mycotoxins, among these fungi certain species of the genus *Fusarium* are certainly producing a health threat by the virtue of the mycotoxins produced. The later were intensively studied by many authors and through many ways; chemically by determining the variety of toxins they elaborate in the contaminated substrate and biologically by studying their effect on selected biological indicators which by itself evoke the same toxic response, which appeared on the original susceptible host.

A total of 335 samples of fish ration and fish ration ingredients were mycologically and toxicobiologically studied. 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 in (Table1). The same fungal genera

from fish meal, fish ration and yellow corn were isolated *(12)*.

Members of genus *Fusarium* are associated with fusariotoxicosis in farm animals *(13)* and a disease of man called Alimentary Toxic Aleukia *(14)* and in fish *(11)*. The *Fusarium* species associated with disease condition in farm animals, poultry and fish and known by their capability to produce toxins are *Fusarium tricinctum, Fusarium sporotrichioides, Fusarium poae* and *Fusarium solani.*

Data shown in Tables (2-3) revealed the isolation of *Fusarium* species isolates from all examined samples. However, the incidence of recovery of *Fusarium* species from fish meal, fish ration and yellow corn reached 30.56% (belonged to 5 species), 40% (belonged to 6 species), and 25% (belonged to 5 species) respectively.

The predominated species in the present study was *Fusarium poae*, its incidence in fish ration was (13.33%), in fish meal (11.11%), and in yellow corn (3.8%). *Fusarium poae* was was isolated from cereal products in North Central United States*(15)*.

Fusarium tricinctum was recovered with an incidence 10% in yellow corn, 6.66% in fish ration and 5.55% in fish meal. The recovery percent was 12% of *Fusarium tricinctum* from yellow corn *(16)*.

Concerning *Fusarium fusarioides* its incidence of recovery in fish ration reached 10.66%, in fish meal reached 2.7% and in yellow corn reached 2.5%. Our results agreed with previous study from feed stuffs *(17)*.

The incidence of *Fusarium solani* recovery from fish meal was 8.33, from fish ration was 5.33 and from yellow corn was 7.6%. *Fusarium solani* was isolated from yellow corn and fish meal *(18)*, *Fusarium solani* was isolated from corn *(19)* from fish meal and fish ration *(16)*.

In this study, the lowest incidences of recovery was for *Fusarium oxysporum.* The percent of recovery from fish meal and yellow corn reached 2.77% and 1.25% respectively. Meanwhile, it was not isolated from fish ration. These results are similar to that cited previously *(18,20)*.

In the present study *Fusarium sporotrichioides* was uncommon, its incidence of recovery reached 2.66% from fish ration and did not isolated from both fish meal and yellow corn. The organism has been isolated from fish ration *(19)* and yellow corn and previously fish meal *(16,21).*

Fusarium flocciferum was isolated only from fish ration with an incidence of 1.33% .

One of the major toxic metabolites attracted the attention of the food and feed central authorities and produced by members of the genus *Fusarium* are the trichothecenes. These group are responsible for many serious farm problems in many countries including Russian, USA, Canada and Japan .

Trichothecenes comprise a closely related group of about 40 biologically active fungal secondary metabolites predominantly although not exclusive, associated with *Fusarium* species.

In the present study 16 isolates belonging to genus *Fusarium* were tested for T-2 toxin production these are *Fusarium poae* (5strains),*Fusarium tricinctum*(4strains), *Fusarium solani* (3strains), *Fusarium oxysporum* (3strains)and *Fusarium sporotrichioides* (1strain). The results revealed that 6 isolates identified as *Fusarium solani* (2 strains), *Fusarium tricinctum* (2 strains), *Fusarium poae*(one strain) and *Fusarium oxysporum* (one strain) these strains isolated from fish meal, fish ration and yellow corn were toxins producer. *Fusarium oxysporum* produced T-2 toxin in nature and in culture *(14)*. *Fusarium solani* when grown on synthetic or nature media give T-2 toxin *(22)*. T-2 and HT-2 toxins were isolated from culture of *Fusarium solani.* Also five strains of *Fusarium* yielded T-2 toxin when inoculated on corn kernels *(11)*.

The high production of T-2 toxin by *Fusarium poae* strain was previously reported in Egypt *(23)*. T-2 toxin has been isolated

from *Fusarium sporotrichioides* and *Fusarium poae (16)* .

The biological methods performed using the crude or pure extracts of the mycotoxins require confirmation; also it is of special value in cases where the toxicity of the fungal metabolites not yet detected chemically i.e to detect toxicity of metabolite of unknown previous history of toxicity. The most important condition when using this method is to choose the biological indicator that could produce the same effect of the toxin to be tested i.e., produced a measurable similar response in coincidence with the severity of toxic substance.

Nile Cat fish was selected to detect T-2 toxin which is known to have a mechanical irritating effect *(24)*. Two doses (20 ppb and 40 ppb) were used on two separate Cat fish. The judgment of the reaction was done two and four hours after application.

Table 4 showed that extracts of T-2 toxins produced by the two isolates of *Fusarium solani* (No., 3,1) gave 3rd grade of toxicity. The extracts of *Fusarium solani* caused reddening and crust formation when applied on the skin of rabbit *(24)*.

While the extracts of T-2 toxins produced by *Fusarium poae* (No.5) and *Fusarium oxysporum* (No. 9) gave 4th grade of toxicity. The lesion produced by highly toxic doses of T-2 toxins caused generalized edema of trout and localized edema of treated rat skin *(11)*.

The T-2 toxins produced by the strains of *Fusarium tricinctum* number 8 and 12 were judged as very weakly toxic with both used doses 20 ppb and 40 ppb this may indicate that there is a certain concentration of the toxin below which there no measurable skin response obtained. Since application which resulted in local reaction and even death which were evidence that absorption can occur through intact skin. The T-2 toxin produced by *Fusarium tricinctum* gave no reaction when applied on skin of rabbit *(25)*.

Irritation, inflammation, wrinkling and lastly painful eczema formation are considered the characteristics of some fungal metabolites specially T-2 toxin produced .It is mechanically highly inflammatory action but non carcinogenic substance *(11)*. It would be interesting to mention that the toxic behavior of T-2 toxins was attributed to the presence of epoxoid ring which has a mechanical inflammatory action on the skin and mucous surfaces.

Experimental injection with T-2 toxin I/P into *Oreochromis niloticus* with the different mentioned doses of T-2 toxin during 30 days experimental period revealed that most commonly detected clinical manifestations were erythema at the site of injection, redding and hemorrhage at the pectoral fins and destruction of caudal fins, the same finding which reported that same clinical signs were observed *(26)* in Channel Cat fish. The post mortum findings were the pale liver appearance with several dark red areas, large gall bladder and autolysed muscle

Histopathological findings demonstrated were the erosion of skin loss of superficial cells and hyperplasia of mucus secretion cells. The muscles showed necrosis and replacement of fibrous tissue infiltrated with leukocytes. The mortality rate was high at 3rd weeks post injections; the hydrolyzes of the mucopolysaccardes and the chitin of muscles *(26)*.

It could be cocluded that toxins production is a property of certain strains within the species i.e. certain strains of the same species do not produce toxins and no methods available yet to differentiate the toxin forming from the non toxin forming . There has also been no proof of any reference of non-toxic-forming species to prefer certain food, non for their geographical distribution according to climate. Environmental condition specially temperature and humidity play the major role in growth of fungi and production of toxins, taken for granted uniquitous availability of spores in the environment especially in soil. In addition, the primitive methods used in agriculture, harvestion ,transport, processing and storage of agriculture commodities are responsible for

the most part of mycotoxins problem in developing countries .

A high economic losses are resulting from mycotoxin contaminated feeds the losses express itself in : direct illness ,death, reduction of productivity, lowering growth rate and feed efficiency .

Mycotoxins exert both acute and chronic effects, the later after repeated subacute doses. The major danger of food and feed contaminated with mycotoxins is in this later category where as acute toxicosis are comparatively rare. The tolerance level for food and feed products established in several countries should be understood as management tools intended to facilitate the implementation of mycotoxin control programmer, and not as exposure limits that necessarily ensure health production .

It is clear now that a cooperative efforts needed in Egypt between veterinarians, agriculture men, physicians, chemist and feed industrial association to assess the nature and degree of risk and to set-up suitable permissible limits to control contaminated food and feed .

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الملخص العربى

دراسة سمية بيولوجية لسلوك المواد السامة الفطرية الخارجية للفطريات السامة لأسماك المياه العذبة

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تعتبر الثروة السمكية من أهم المصادر التى تساهم فى توفير البروتين الحيوانى للإنسان وقد اهتمت معظم دول العالم بالثروة السمكية سواء الطبيعية منها أو العمل على نشر المزارع السمكية بالنظام المكثف وقد اعتمدت هذه المزارع على تقديم العلائق لزيادة نمو هذه الأسماك فظهرت العديد من المشاكل التى من المشاكل التى تؤثر على الإنتاج ومن أهم هذه المشاكل السموم الفطرية نتيجة استخدام هذه العلائق ولذلك قمنا بهذة الدراسة لكى نوضح بعض تأثير هذة السموم على الأسماك ونسبة إنتاجها . أجريت هذه الدراسة علي ٣٣٥ عينة من أعلاف الأسماك و مكوناتها(مسحوق السمك- الذرة الصفراء)وكانت مقسمة كالتالي :- ٧٥ عينة من أعلاف الأسماك- ١٨٠ عينة من مسحوق السمك و ٨٠عينة من الذرة الصفراء وذلك من مصادر مختلفة(المفرغ السمكي بالعباسة- محافظة الشرقية ، مزرعة نوى- محافظة القليوبية، معهد بحوث صحة الحيوان(قسم أمراض الأسماك)- الدقي ومن الأسواق بمحافظة الشرقية.

وفحصت هذه العينات لغرض محاولة عزل الفطريات وذلك باستخدام العزل المباشر على الوسط شابكس أجار.ولقد أسفرت هذه الدراسة عن النتائج الآتية:-
تم عزل ٦٥٠عترة من الفطريات الموجودة في ٣٣٥ عينة من العينات المستخدمة تنتمي إلي ٦ عائلات من الفطريات وهي: أسبرجيلس، بنسليوم، فيوزاريم، ميوكور، الترناريار و السكبيولوبس وقد اختبرت هذه العترات لمعرفة قدرتها على إفراز السموم الفطرية .
وقد استخدم ١٦ عترة للفيوزاريم لمعرفة قدرتها على افراز ت-٢ واسفرت النتائج عن افراز ٦عينات لذلك.

-استخدم السم الناتج عن العترات الفيوزاريم في اجراء تجربة بيولوجية علي جلد اسماك القرموط وأسفرت النتائج عن أعراض ظاهرية واكلينكية وباثولوجية وكانت هذة التغيرات عبارة عن تقرحات نزيفية فى الجلد بصور مختلفة ومتفاوتة . وكانت التغيرات الباثولوجية تورم فىالجلد وزيادة فى كرات الدم البيضاء وكذلك تهتك فى الأنسجة وطبقات الجلد.
استخدمت هذه السموم الفطرية عن طريق الحقن البريتوني في أسماك البلطي لمعرفة درجة سميتها فقد أظهرت النتائج عن الأعراض الظاهرية والتغيرات البا ثولوجية . ظهرت الإصابة فى أسماك الدراسة على هيئة تأكل فى الزعانف وانتفاخ أوديمى بالبطن - تقرحات فى الجلد ، أنزفة فى الزعانف الجانبية و الذيلية وسقوط القشور. وبالفحص المجهرى للأعضاء والأنسجة المختلفة كانت التغيرات المرضية مختلفة من التهابات وتكسير للخلايا ووجود خلايا فى النسيج العضلى والنسيج الكلوى.