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PHOMA HERBARUM AS A MYCOTIC FISH-PATHOGEN IN CLARIAS LAZERA " ARMOUT CATFISH "

By

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INTRODUCTION

In the U.S.A., Wood (1968) recorded an infection of chinook Salmon (Oncorhynch tschowscha) with a fungus belonging to the order Sphaeropsidales. Ribelin and Migaki (1975) mentioned that the fungus causing infection in coho and Chicook fry was identified as Phoma herbarum. They suggested that the original site of infection was the gas bladder, from which the mould may extend to the mesentry, pancreas, kidneys and eventually into the adjacent vertebral skeletal muscles causing acute or chronic granulomatous reaction.

Ross et al. (1975) isolated Phoma herbarum from fry and fingerlings of Salmon and studied the pathogenicity of the fungus in chinook and Salmon fry. Gas bladder was the constant organ of isolation of the fungus. Skeletal muscles, intestines and visceral organs contained large masses of mycelia.

In the U.S.S.R., the disease was investigated by Marchinka (1978) who found a fungus belonging to order Sclerophoma in the gas bladder of Chinook Salmon in the fish ponds of the Altlantic areas. These ponds contain high organic matter and high fish population. Experimentally, the isolated fungus induced mortalities of 25 %, multiplied in gas bladder and induced lesions in visceral organs and abdominal wall.

In Egypt, Easa (1979) isolated Phoma herbarum from gills of diseased mirror carp (Cyprinus carpio L.) from El-Abbasa and El-Manzalah fish farms. The histopathological

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picture, as well as the results of the re-isolation trials suggested that the gills might be the primary organ affected with Phoma herbarum in carp fish

The aim of this work is to determine the pathogenicity and the subsequent tissue reactions of Phoma herbarum in Nile catfish. This type of fish was chosen as a representative of our native fish-breeds.

MATERIAL AND METHODS

Fish :

50 apparently healthy Armout catfish (Clarias lazera), caught from the Nile, were subdivided into five groups each of 10 fish. The weight of each fish was 150-200 g.

Organism:

A strain of Phoma herbarum isolated from gills of diseased carp (Cyprinus carpio L.) in El-Abbasa fish farm (Easa, 1979) was used. The isolated fungus was matched with a standard strain of Phoma herbarum No. PD 79/2157, identified by boerema (1964) in plantenziek-tendige dienst Wageningen-Holland. The Egyptian strain, which was identical morphologically to the standard strain of Phoma herbarum, was cultured and maintained on Sabouraud's dextrose agar medium without the addition of antibiotics. This fungus is characterized by its ability to develop great number of thin-walled pycnidia (fruiting bodies) with only a small amount of aerial mycelia. Only hyaline one-celled pycnidiospores are produced and no chlamydospores exsist. Cultures of Phoma herbarum showed pigment formation. The colour of the pigment is mostly pink, sometimes orange red or red violet. The pH of the medium influences the pigmentation. In acid medium the colour is darker (Boerema, 1964).

Fish were held in glass tanks each of 130 liter capacity; The temperature of water was 22 $\overset{\circ}{C}$ ± 2 $\overset{\circ}{C}$ and an ordinary fish food was fed to them twice daily.

A suspension of Phoma herbarum was prepared for inoculation by emulsification of 7-days old colonies grown

on Sabouraud's dextrose agar in a suitable amount of sterile saline.

The first group of fish was used as control. Fish in group II were inoculated intraperitoneally with 2 ml of the fungal suspension. Group III was adminstered the same dose orally by the aid of a stomach tube. The fish in group IV were subctaneously inoculated each with 2 ml of Phoma herbarum suspension. Moreover, individuals of group V received the fungus by swabing of the emulsified suspension on the scarified gills as shown in Table 1.

All fish were examined daily for symptoms, external lesions or mortalities. Post-mortum, histopathological and mycological examinations were carried out on every fish just after death.

The experiment was terminated 45 days after the induction of the experimental infection. Fish that survived till the end of the experiment were sacrificed and examined pathologically and mycologically.

After P.M. examination and for histopathological studies, tissues from lesions were taken just after death, fixed in formol-saline and embeded in paraffin. Sections of 5-7 um were cut and stained with H & E, as well as PAS (Periodic Acid Shiff) stains.

For mycological examination, direct smears prepared from the different organs were examined microscopically. Cultures from each organ of experimentally inoculated fish were done by plating on Sabouraud's dextrose agar. Plates were kept at room temperature (about 25 C), and were examined daily for growth.

RESULTS

Mortalities were sporadic in all groups of infected fish during the course of experiments (Table 1). Four fish died in group II (intraperitoneal inoculation), where the first fish died 3 days postinoculation with symptoms of abdominal distention and dark discolouration of the skin. The abdominal cavity contained serofibrinous

exudate and the liver, kidneys and spleen showed pale colour. Another fish died 4 days post-exposure to Phoma herbarum infection. This fish exhibited similar symptoms and lesions with necrotic foci of 2-4 mm in diameter on the liver. Greyish white masses were superficially located on it. The spleen showed pale patches covered with greyish masses. Two fish died 18 days post infection showed white firm nodules beside the spleen. These nodules contained blackish spots. The portal vessels appeared firm and dilated containing greyish white clumps about 1 mm in diameter in one case from which the fungus was isolated.

In group III, that received the fungus orally, 4 fish died in the 4th (2 fish), 11th and 18th day post-inoculation. All of these fish showed congestion of gills. On P.M. examination of dead fish, as well as of those survived till the end of the experiment, the intestine appeared congested and the gall bladder was distended with bile. No pathological alterations could be seen in other organs in all fish of this group. However, the case that died on the 18th day showed congestion at the base of the pectoral and dorsal fins as well as congestion in the ventral portion of the head. The spleen of this fish was highly congested.

Subcutaneous inoculation of Phoma herbarum in Armout catfish (Group IV) resulted in death of 3 fish. On the 4th day post-inoculation, the site of injection appeared bleached, surrounded with hypraemic zone and bulged out with soft consistancy. Sloughing of most superficial cutes was noticed with congestion of the pectoral and dorsal fins. Gills appeared pale in colour, oedematous and necrotized. On P.M. examination, the liver and spleen were congested in most fish. Histopathological examination of the skin and underlying muscles of these fish revealed congestion of the dermal and hypodermal vessels accompanied with oedema of skin layers. In some cases severe haemorrhages were seen in dermal layers with sloughing of the cutis and the superficial layer of the epidermis. The remaining epidermal cells showed necrobiotic changes with irregular shaped and dark-stained nuclei . Granulomatus reactions were seen in the muscular layer. These reactions were formed of macrophages, lymphocytes,

epitheloid cells, as well as giant cells (Fig. 1). Muscle bundles showed myolysis with migration of sarcolemmal nuclei. Fragmentation of muscle fasiculi was also seen. The blood vessels exsisting between muscle fasiculi had severe dilatation with oedema and haemorrhages. Phoma herbarum hyphae were seen having a strong PAS positive reaction (Fig. 2). Such hyphae were seen surrounded with necrobiotic muscle bundles infiltrated with great numbers of macrophages and lymphocytes. In long standing cases, the destroyed muscles were replaced with granulation tissue. Moreover, a great number of regenerating muscle fibers showing reduplication of sarcolemmal nuclei, forming muscle giant cells had irregular-shaped areas of sarcoplasm containing several nuclei.

Fish in group V (Gill-scarification) had only one case of mortality in the 11th day post-infection. Gills appeared pale in colour with necrosis of the free portion of gill fillaments. Dark discolouration of the skin was observed. Internally, the spleen appeared congested. No pathological alterations in other organs could be noticed.

Histopathological findings of gills of these fish were hypertrophy and hyperplastic proliferations of their epithelial linning. Severe congestion of the broncheal vessels with thrombosis in some of them was observed. Fungal hyphae were seen invading the gill lamellae and gill arches (Fig. 4).

The liver, particularly of fish given the fungus intraperitoneally, showed necrobiotic changes with presence of tufts mycelia within the portal vessels. The spleen of I/P inoculated fish showed hyperactivities of the haemopiotic tissue. however, no fungal elements could be detected in this organ. Kidneys of these fish had subcapsular haemorrhages with congestion of renal vessels. Hyperactivity of the renal haematopiotic tissue together with excessive accumulation of siderocytes were also detected. No hyphae could be demonstrated in the renal tissues.

Nither mortalities nor pathological or histopathological alteration were recorded in all fish in the control group.

Re-isolation trials of the fungus from the different

Table : 1 Experimental infection of Armout catfish with *Phoma herbarum*

Group	Route of infection	Mortalities		Reisolation of *Phoma herbarum*							
				Gills		Skin		liver		Fin	
		No.	%	No.	%	No.	%	No.	%	No.	%
I	Control	-	-	-	-	-	-	-	-	-	-
II	Intraperitoneal inoculation	4/10	40	8	80	2	20	10	100	-	-
III	Oral administration	4/10	40	9	90	5	50	-	-	1	10
IV	Subcutaneous inoculation	3/10	30	7	70	10	100	-	-	5	50
V	Gill scarification	1/10	10	10	100	6	-	-	-	-	-

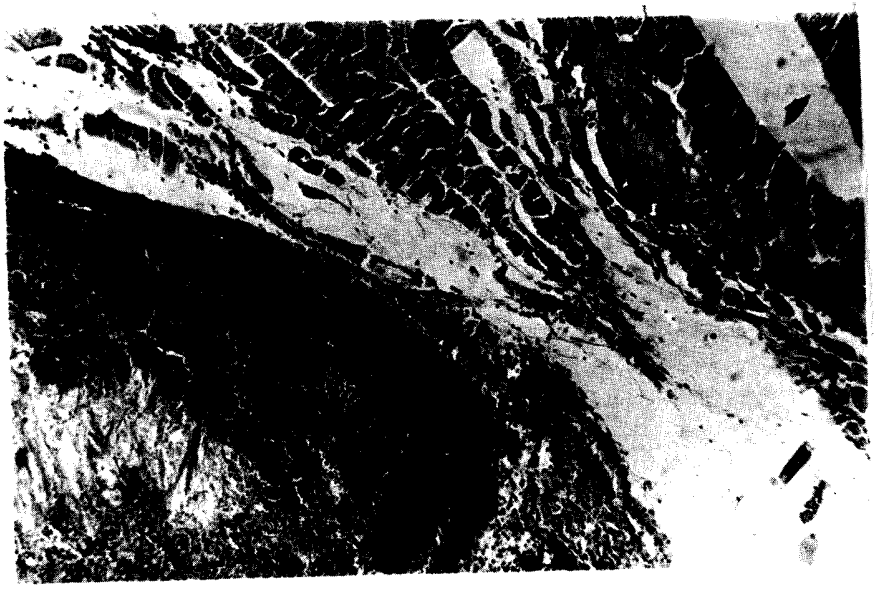

Fig. 1: Skletal muscle of armout catfish showing inflammatory granulomatous reaction formed of Lymphocytes histocytes as well as giant cells (arrow) has destroyed skeletal muscle. Stain: H&E (Obj. 10 p.3.2)

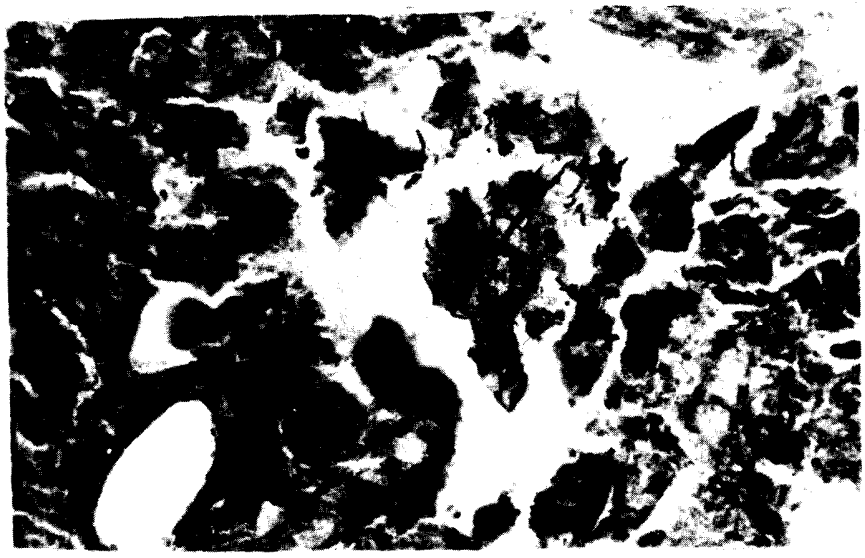

Fig. 2: Skeletal muscle showing: Septate hyphae that invaded and destroyed muscle bundles.
Stain: PAS (Obj. 20 p. 3.2)

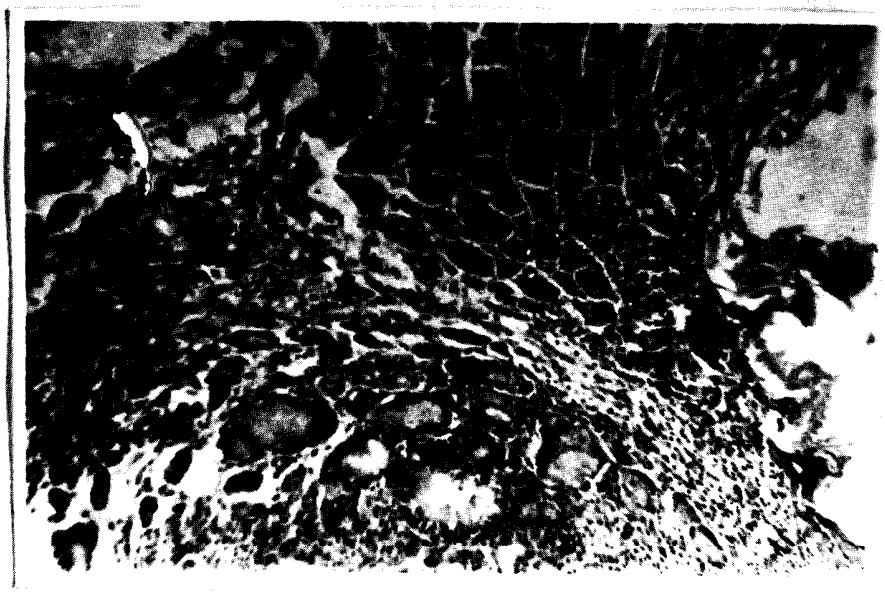

Fig. 3: Regenerated muscle fiber showing reduplication of sarcolemmal nuclei in masses of sarcoplasm (Muscle Giant Cell). Great numbers of inflammatory cells are also seen.
Stain H & E (Obj. 10 p. 3.2)

Fig. 4: Gill of armout catfish. Notice destruction of primary and secondary gill lamellae, oedema in gill arch together with inflammatory cells surrounding the fungal elements (Arrow).
Stain: PAS (Obj. 10 p. 3.2)

organs of experimentally inoculated fish, showed that the fungus was present in the gills of all fish infected by gill scarification , as well as most of fish infected via the three other routes (Table 1). Phoma herbarum was isolated only from the liver of fish inoculated intraperitoneally, while no isolate could be obtained from the liver of fish from any other group. Re-isolation of the fungus was achieved from skin lesions of all subcutaneously inoculated fish, as well as from skin lesions present in fish from all other groups except the control one.

DISCUSSION

The success of experimental infection of Armout catfish with Phoma herbarum, as well as the re-isolation of this fungus from the different organs of this fish, particularly the gills, indicate that this species is susceptible to Phoma herbarum infection. However, the clinical picture, P.M. examination, histopathological findings and the reisolation results, support the idea that this fungus is a facultative pathogen, This goes in harmony with the findings of Ross et al. (1975) in salmonids. It is apparrent that the fungus could localize, grow and induce its lesions in the site of inoculation. It seems uncapable of invading the vascular system and spreading to other organs. The presence of hyphae in the portal vessels of one case inoculated I/P may be just an extension of the fungal elements from the adjacent peritoneal cavity.

The presence of the fungus in the liver of fish infected intraperitoneally and its complete abscence in the internal organs of fish given the fungus orally may give an idea that the digestive tract of Armout cat fish is not the natural route of infection with Phoma herbarum in this type of fish, although Marchinka (1978) isolated the fungus from the intestinal tract of salmonids.

The constant isolation of the fungus from the gills of fish inoculated via the different routes indicates the high susceptibility of Phoma herbarum to gills of catfish. This could support the conclusion that gills are the primary organ affected. Similar finding was achieved in carp

fish by Easa (1979) who noted that gills were the primary organ affected with Phoma herbarum.

Skin lesions were very severe with granulomatous reaction. Re-isolation of the fungus from such skin lesions was constant uptil the end of the experiment. This indicates the susceptibility of skin tissue to infection. However, the predisposition by broken skin due to traumatic or other causes seems to play an important role. This idea was supported by the finding that wounds or abrasions in all experimentally infected groups, had granulomatous lesions from which Phoma herbarum was isolated.

The congestion of the internal organs and fins and the activation of hemopiotic tissues as well as the necrobiotic changes in the liver may be the result of toxic products of the fungus.

SUMMARY

Armout catfish (Clarias lazera) were experimentally infected with a strain of Phoma herbarum isolated from diseased carp fish (Cyprinus carpio L.) obtained from an Egyptian fish farm. This strain was administered to fish via different routs i.e. intraperitoneal injection, oral administration, subcutaneous inoculation and swabing on scarified gills. Re-isolation of the fungus was tried 1, 2, 3 and 4 weeks post-inoculation. Phoma herbarum was constantly isolated from gills and skin of fish receiving the fungus through gill scarification and subcutaneous inoculation, respectively. however, the fungus was less frequently recovered from the liver of intraperitoneally inoculated fish. No isolates could be obtained from the visceral organs or skin of fish given the fungus orally.

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