ISSN 110-2047 ALEX. J. VET. Med. SCIENCE 2009 VOL. 28 NO. 1: (81-92)

ISOLATION AND IDENTIFICATION OF ICHTHIOPHONUS HOFERI FROM CULTURED FISH

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ABSTRACT

The *Icthyophonosis* is a dangerous disease, the consequences of which are difficult to assess for wild populations of fish. This study aimed to throw the light on the importance of *I. hoferi* on fresh water fish, it dangerious effect in addition to its identification of isolation, characterization and the most important symptoms showed on naturally infected *Oreochromis niloticus* with *I. hoferi* showing deformity of vertebral column, darkening in color, sand paper appearance of skin, poor appetite listlessness . The young culture of *I. hoferi* on SDA t 10% bovine serum showing rupture of multinucleated bodies and release of spores through extra material discharge after 9 days. At PH 3.5 showing starting of hyphal growth after shours post-culturing the lyphae produce many branches, extending of the hyphae to grow and increase in length after 24 hours, migration of cytoplasm to the apexes of hypae after 3 days, rounding up of the apics of the hyphae after 7 days, finally all the hyphae rounding up to form sphericael hyphae terminal bodies after 10 days .Rounding up of the apics of the hyphae after 7 days, finally all the hyphae rounding up to form sphericael hyphae terminal bodies after 10 days and in old culture showing chlamydo-spores formation around the multinucleated bodies and extend to the test of sticked hyphae at 3 weeks .

INTRODUCTION

The goal of many countries allover the world is the production of fish from natural water resources or from fish farms as a source of high biological value protein. ***(Marzouk et al., 2001).***

Fish diseases considered as the main cause of reduction of fish production from farms and its profitability . These diseases either bacterial, viral, parasitic or mycotic. ***(Ramaiah 2006)*** .

Icthyophoniasis is a cosmopolitan system granulomatus fungal disease of economic significance because epizootics have resulted in mass mortalities among a wide range of freshwater and marine fishes, ***(Nadia and Hoda 2008)*** . **This study was planed to** isolated and identify of ***I. hofri.***

MATERIALS AND METHODS

Materials :

A. Naturally infected fish :

A total number of 300 ***Oreochromis niloticus*** (250 cultured and 50 wild

fish) collected from different localities at Alexandria, Kafr El-sheik and El-Behoira. The fish were obtained from their natural sources and fish farms. The samples were collected during the period from June 2007 to May 2008 with the body weight ranged from 40-150 g. The fish were transported alive in polyethylene bags contained about 30% of its volume water and the remaining volume pumped with pure oxygen. While the freshly dead fish were labelled and packed in a clean plastic bags put in an ice boxer and carried to the laboratory of Faculty of Vet, Med, Alexandria University.

Media used for isolation and identification of I. hoferi :

A. Sabouraud's dextrose agar :

It was supplemented with 10 % bovine serum ***(Leaner and Carey 1978)***

Bovine serum was obtained from Serum and Vaccine Laboratories El-Agoza, Egypt. This medium was sterilized by autoclaving at 121C for 15 mints, containing which the bovine serum was added .

B. Minimum Essential Medium (MEM) + 10% fetal calf serum:

- It was prepared according to ***Spangaard et al., (1994)*** .
- BSA was added at a concentration of 10% .

The medium was sterilized by filtration through a biological filter [Millipore corporation, cat. No. XX 6700P05/ Pore diameter 22 jam] and stored at 4C till used.

Candida albicans **strain :** were kindely provided by Dep. Of Poultry and Fish Diseases; Fac, Vet. Med. Alex. Uni.

A. Stain for mycological examination:

a. Lactophenol Cotton Blue (LPCB) ***(Leaner and Carey 1978)***

Methods :

1. Clinical examination :

The collected fish were examined clinically according to the methods described by ***McVicar (1982)*** to detect any clinical abnormalities .

2. Postmortem examination :

Postmortem examination of the internal organs was carried out on sacrified and freshly dead fish according to ***McVicar, (1982)***.

3. Mycological examination :

Isolation of the fungus :

Mycological examination was done according to ***McVicar (1982).*** The outer surface of the examined fish was cleaned, thoroughly rubbed with piece of cotton moisted with ethyl alcohol (70%) then the abdominal cavity was opened by sterile scissors and the internal organs were exposed. The outer surface of these organs were sterilized by hot metal spatula. Samples were taken by using sterile dissecting needle from the internal organs (liver, kidney, spleen, heart, intestine, ovary and eyes) and inoculated onto the MEM- 10, and Sabouraud's dextrose agar with l% bovine serum. The inoculated plates and tubes were incubated at room temperature for 15 days. Plate cultures were examined continously at the first day of incubation followed by daily examination till the end of incubation period.

Identification of I.hoferi :

From the positive culture, a part from the growing fungus was taken by sterile loop, placed on clean slide with drop of water and covered by clean cover slide. Identification of the isolates was done according to the morphological characters including (the hyphal growth and multinucleated spores) through the microscopical examination of wet mount and stained preparation .

Squash preparations were prepared according to ***Amlacher (1970)***, from the affected organs. The suspected nodules were taken and compressed between two clean slides and crushed, then examined under the microscope to detect multinucleated spores and their structures.

Samples from freshly dead fish were taken for examination and kept at room temperature for 4-5 hours to detect postmortem germination. Bacterial and parasitological examination were done to exclude the role of them in the occured lesions.

RESULTS

Prevalence of Icthyophoniasis among the examined *O. Niloticus* :

Mycological examination of the collected fish revealed the prevalence of infection with ***I. hoferi*** among the examined fish more than any other type of fungi. The prevalence of infection in cultured fish was higher than in wild fish. With respect to the localities. The fish collected from Kafr-El-shaikh showed higher infection rate than that obtained from Alexandria and El- behera. (table, 1)

Seasonal prevalence of Ichthyophoniasis in the examined *O. niloticus* in relation to localities :

Regarding to the seasonal prevalence of ***I. hoferi*** infection in the examined ***O. niloticus.*** In the three Governorate the infection was recorded with higher prevalence during autumn followed by winter and in a lower prevalence during summer in the examined fish. (table, 2)

Distribution of the Ichthyophoniasis among different organs of the examined *O. niloticus* :

Concerning the site of isolation of ***I. hoferi*** from different tissues and organs of the examined ***O. niloticus***, revealed that, the fungus was isolated in a higher prevalence from liver followed by kidneys, spleen and intestines respectively. The cultured fish showed high level of isolation rate from liver, followed by kidneys and spleen while those collected from natural resources revealed maximum isolation rate from liver followed by intestine and spleen. (table, 3)

The clinical signs of naturally infected *O. Niloticus* :

The clinical signs were in the form of excessive mucous and dark coloration of the skin, haemorrhage and congesion of the external body surface, emaciation and swelling of musculature, exophthalmia in some fish and detatched scales.

The P.M. lesions of naturally and experimentally infected fish were more or less the same in the form of paleness and enlargement of the liver, congested kidneys, white nodules with enlarged spleen. Inflamation with gray white nodules of the intestine, ascitis with haemorrhagic fluid in the abdomen and congestion of the heart and soft oedematous muscles were

observed. Deformties were recorded in some naturally infected ***Oreochromis niloticus*** (Fig. 1, 2 and 3), Moreover, in experimentally infected ***Oreochromis niloticus*** in case Ich.+ MEM PH 3.5, and Oral intubation with Cortisone (Fig. 4) showed deformities of vertebral column after 4 weeks post infection and in 20% of infected fish the X- ray of the same infected fish (Fig. 5) .

The young culture of ***I. hoferi*** on SDA + 10% bovin serum showing Rupture of multinucleated bodies and release of spores through extra material discharge after 9 days post infection.While the culture of ***I. hoferi*** on MFM. 10 at PH 7.0 showed hyphae with different sizes and formation of multinucleated bodies after 8 days , localization and fixation of multinucleated bodies (ameabo blast) at the end of each hyphae with rupture of some ameoboblasts. At PH 3.5 showed starting of hyphal growth after 5 hours post culturing. (Fig. 7). The lyphae produce many branches, extending of the hyphae to grow and increase in length after 24 hours. Migration of cytoplasm to the apex of hypae was noticed after 3 days. Rounding up of the apix of the hyphae was observed after 7 days. Finally all the hyphae rounding up to form sphericae hyphae terminal bodies after 10 days . The culture of fungal growth on MEM- 10 at PH 3.5 showed starting of hyphal growth after 5 hrs post culturing. The hyphae produce many branches, extending of the hyphae to grow and increased in length after 24 hrs. Migration of cytoplasm to the apex of hypae were noticed after 3 days, Rounding up of the apics of the hyphae were recorded after 7 days and finally all the hyphae rounding up to form sphericae hyphae terminal bodies after 10 days. In case of old culture (6 days) showed chlamydo spores formation arround the multinucleated bodies and extend to the test of sticked hyphae after 3 weeks (Fig. 6 to 12).

TABLES AND FIGURES

Table 1 : The prevalence of ***I. Hofri*** in ***O. Niloticus*** in relation to governorate :

Location	Cultured *O. niloticus*		Wild *O. niloticus*	
	No. of examined fish	No. of infected	No. of examined	No. of infected
Kafr El-sheikh	120	82	24	15
Alexandria	80	39	15	8
El-behera	50	15	11	2
Total	250	136	50	25

Table 2 : The seasonal prevalence of Ichthyophoniasis in the examined ***O. niloticus*** in relation to seasonality :

Season	Cultured *O. niloticus*		Wild *O. niloticus*	
	No. of examined	No. of infected	No. of examined	No. of infected
Autamn	92	72	18	11
Winter	85	44	15	8
Summer	54	11	10	0
Spring	9	9	7	6
Total	240	136	50	25

Table 3 : Distribution of the Ichthyophoniasis among different organs of the examined ***O. niloticus*** :

Organs	Cultured *O. niloticus*		Wild *O. niloticus*	
	No. of examined	No. of infected	No. of examined	No. of infected
Liver	250	92	50	13
Kidney	250	21	50	7
Spleen	250	13	50	4
Intestine	250	10	50	1

Fig.1

Naturally infected Oreochromis niloticus with I. hoferi showing deformity of vertebral column.

Fig.2

Naturally infected ***Oreochromis niloticus*** with ***I. hoferi*** showing deformity of vertebral column .

Fig.3
Naturally infected Oreochromis niloticus with I. hoferi showing different shape of deformitis of vertibral colomn.

Fig.4
Experimentally infected ***Oreochromis niloticus*** with ***I. hoferi*** .+ MEM PH 3.5, Oral intubation with Cortisone . showing deformities in constricted area of caudal peduncle . (after 1 week post infection)

Fig.5
X-ray of the (fig. 4) showing deformity of Oreochromis niloticus due to I. hoferi .+ MEM PH 3.5, oral intubation with Cortisone .

Fig.6
Showing young culture of I. hoferi on SDA + 10% bovin serum, (creamy white color) .

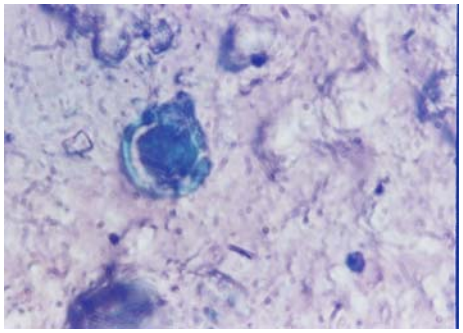

Fig.7
Showing rupture of multinucleated bodies and release of spores through extra material discharge after 9 days post cultivation on SDA (arrow) X 160.

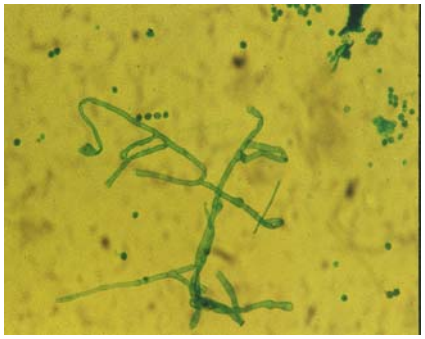

Fig.8
Culture of ***I. hoferi*** on MFM. 10 at PH 7.0 showing : hyphae with different sizes and formation of multinucleated bodies after 9 days (arrows) X 160 .

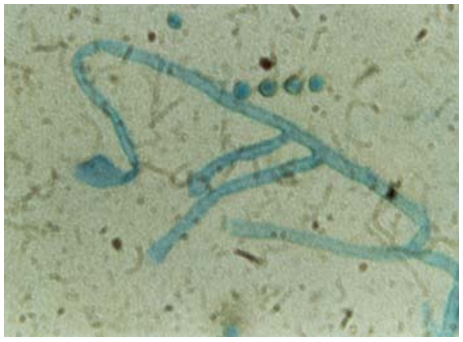

Fig.9
Culture of I. hoferi on MFM. 10 at PH 7.0 showing : hyphae with different sizes and formation of multinucleated bodies after 10 days. (arrows).X 160 (arrows)

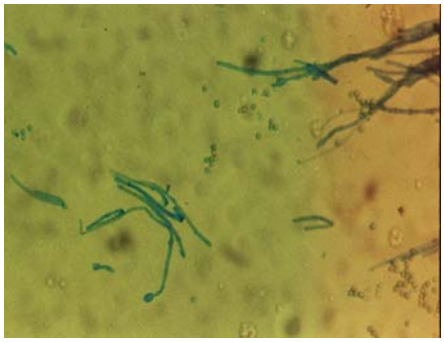

Fig.10
Culture of I. hoferi on MEM. 10 at PH 7.0 showing : localization and fixation of multinucleated bodies (ameaboblast) at the end of each hyphae (A) with rupture of some ameobo blasts (B). X 160.

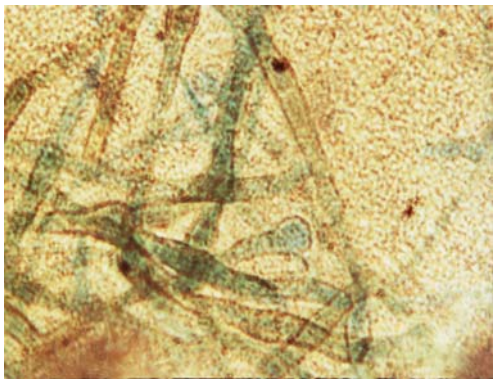

Fig.11

Culture of I. hoferi on MEM- 10 at PH 3.5 showing starting of hyphal growth after 5 hours post culturing. The lyphae produce many branches, extending of the hyphae to grow and increase in length after 24 hours, migration of cytoplasm to the apex of hypae after 3 days, Rounding up of the apics of the hyphae after 7 days, finally all the hyphae rounding up to form sphericae hyphae terminal bodies after 10 days . X 160.

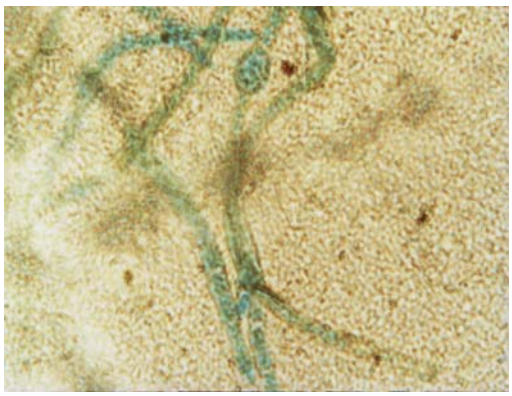

Fig.12

Culture of ***I. hoferi*** on MEM- 10 at PH 3.5 showing starting of hyphal growth after 5hours post culturing the lyphae produce many branches, extending of the hyphae to grow and increase in length after 24 hours, migration of cytoplasm to the apex of hypae after 3 days, Rounding up of the apics of the hyphae after 7 days, finally all the hyphae rounding up to form sphericae hyphae terminal bodies after 10 days. X 160.

DISCUSSION

Ichthyphoniasis is a granulomatous systemic fungal disease, occures in both freshwater and marine fishes. Ichthyophoniasis is considered as important newly recorded disease among cultured tilapia species at different localities in Egypt.***(Manal 2002)***.

The most important symptoms showed on naturally infected ***Oreochromis niloticus*** with ***I.hoferi*** revealed deformity of vertebral column . While in case of experimentally infected ***O. niloticus*** with I. hoferi injected plus cortisone showed deformities in constricted area of caudal peduncle and darkness coloration of the body with abnormal swmming and off-food . This results agreed with those, of ***Nadia and Hoda (2008)*** They indicated that ***I. hoferi*** infection in fish is usually associated with darkening in color, sandpaper appearance of skin, poor appetite listlessness.

The young culture of ***I. hoferi*** on SDA + 10% bovin serum showed rupture of multinucleated bodies and release of spores through extra material discharge after 9 days post inoculation . In case of media with PH 3.5 showed starting of hyphal growth after 5 hours post-culturing. The lyphae produce many branches, extending of the hyphae to grow and increase in length after 24 hrs. Migration of cytoplasm to the apex of hypae after 3 days, Rounding up of the apex of the hyphae after 7 days, finally all the hyphae rounding up to form sphericae hyphae terminal bodies after 10 days and the culture of ***I. hoferi*** on MEM- 10 at PH 3.5 showed starting of hyphal growth after shours post culturing the lyphae produce many branches, extending of the hyphae to grow and increase in length after 24 hours, migration of cytoplasm to the apex of hypae after 3 days, Rounding up of the apics of the hyphae after 7 days.

The clinical signs due naturally infected while the mortality in case of infection with ***I. hoferi*** without cortison as immunosupressive was 20%. The same % was in case of pure culture of ***I. hoferi*** + MEM 10% at ph 7. while, at ph 3.5 the mortality was 30%, but in case of infection with ***I. hoferi*** with cortison as immunosupressive was 80%. So, the cortisone play a role in increasing mortality and ***I. hoferi*** infection as astress condition ***(Easa, 1997).*** On the hand fish with fungal infection which previously treated with cortisone showed higher mortalities due to immunosupression and increase succeptability to fungal infection ***(Manal et al., 1996)*** infect fish orally by eating thick old spherical multinucleated bodies and recorded that mortaslity rate was 90-100%).

These results agree with those of Nadia and Hoda (2008) who indicated that, microscopical examination of the ***I. hoferi*** cultures showed branced non-septated hyphae with bulbos hyphal tips and the cytoplasm migrated to be concentrated at the apex contained spherical multinucleate bodies of varying sizes, with peripheral nucleoli, and abundant glycogen granules. The spore's wall consists of parallel arrangements of microfibrills. The spores had mitochondria with scarce tubulovesicular cristae and rough

endoplasmic reticulum. The tickness of the spore wall differed according age and size of spore. Also, the number of the nuclei per spore was extremely differed according to the spore growth.

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Infection with Ichthyophonus hoferi was diagnosed based on the presence of visible white nodules on the surface of the hearts as recommended by ***(Anonymous, 1993).***

In the present study the total prevelance of ***I. hofri*** infection in O. niloticus was 66%. These findings were higher than that recorded by ***Sitja-bobadilla and Alvarez pellitero(1990)*** in seabass ***Shaheen and Easa (1990)*** in tilapia and the variation may be due to the difference in susceptability of fish species to infection or the difference in the virulence of the injected fungus. The data showed that the prevalence of infection in cultured fish was higher than that of wiled fish, the results was supported by the results of ***Sitja-Bobadilla and Alvarez Pellitero (1990),*** they revealed that the prevelance of infection was higher in cultured seabass(24.4%) than the wild on (14%), ***Athanssopoulo(1992)*** who found a higher prevalence of infection in cultured ***Sparus aurata*** (19.8%) than in the wild one (9.7%) and Shaheen and Easa (1996) who recorded that the prevelasnce of infection was higher in cultured tilapia(37.5%) than in wild one (20%). The higher prevalence in cultured fish may be attributed to the overcrowdness which increase the chance of infection between fish as well as thepresence of high number in fish farm which increase the possibility of infection, and other cultured stressors that decrease the resistance of fish to infection.

In concerning to localities, the fish colected from Kafr El-Sheikh governorate showed higher infection rate than that collected from Alexandria and El-behera . The variation in the prevalence of infection in different localities may be due to increase water pollution in Kafr El-Sheik than other localities.

Regarding to the seasonal prevalence of ***I. hofri*** infection in the examined ***O. niloticus*** the higher prevalence was recorded during the autamn followed by winter, summer and spring season, this result proved that the growth of ***I. Hofri*** need low temperature . ***Sindermann and Scattergood (1954)*** reported that growth of this fungus occurs over wide range of temperature (3-20C) with an optemum at 10C, and also, they added that the higher prevalence of infection in herring found in shore waters, during and late winter.

The disteribution of ***I. hofri*** in different organs of infected fish , showed higher prevalence of infection in liver, kidneys, spleen and intestine. This results was

agreed with those of ***Faisal et al.,(1985),*** who recorded a higher prevalence of infection in liver followed by kidneys, spleen and intestine of examined ***Clarias lazera*** in arate of 42%, 36%, 14% and 4%.respectively. ***Ziedan (1999),*** stated that ***I. hofri*** causes systemic infection in fish, which spread via circulatory system . the priciple infected organs are that riched with blood (haematobiotic organs).

1. **This study concluded that *I. hoferi*** showing deformity of vertebral column at caudal peduncle, darkening in color, sandpaper appearance of skin.The young culture of ***I. hoferi*** showing rupture of multinucleated bodies and release of spores through extra material discharge after 9 days. While, the culture of ***I. hoferi*** on MFM. 10 at PH 7.0 showed hyphae with different sizes and formation of multinucleated bodies after 8 days.

REFERENCES

Amlacher, E. (1970): Textbook of fish diseases. P. 117 – 135. T.F.H. Publication, Neptune, U.S.A.

Anonymous. (1993): Report of the second special meeting on Ichthyophonus in herring. Comm. Meet. Int. Coun. Explor. Sea CM-ICES 1993/F: 9.

Athanassopoulou, F. (1992): Icthyophoniasis in seabream, Sparus aurtata (L.), and Rainbow trout, Oncorhynchus mykiss (Walbaum), from Greece. J. Fish Dis, 15, 15: 437 – 441.

Easa, A.A.M. (1997): Effect of aspergillus ochraceus mould and its metabolites on some cultured freshwater fish in Egypt. M. V. Sc. Thesis Dept. of Int. Medicine. Enfectious Diseases and Fish Fac. of Vet. Med. Cairo. Univ.

Faisal, M.; Torky, H. and Richenbach-Klinike, H. H. (1985): A note on swinging disease among the labyrinth catfish (clarias lazera). J. Egy. Vet. Ass. 45, 1: 53 – 60.

Leaner, G. and Carey, M. E. (1978): Determination of Myxozoma and I. hoferi in blood. Ann. Clin. Biochem. 1. 10: 25.

Manal Easa (2002): Effect of ImmuPlus on cell mediated immune response in chicks. J. Immunol. Immunopathol. 6 (1): 49 - 53.

Manal, A. A. Essa.; Nahal, R. H. El-Khatib and Easa, M, El-S.: (1996): Some epizootiological aspects of Icthyophonosis in freshwater fish from Egypt. 7th Sci. Cong. Vet. Med. Assiut, Egypt., pp. 290 – 305.

Marzouk, M. S.; Ezz-El-Din, N. M.; Hashem, M. E. and Adawy, R. S. (2001): Studies on digenetic trematodes of some freshwater fishes in Dakahlia Province. The Second Int., Scientific Conference. Fac. of Vet. Mans. Univ. 8 – 9 April, PP. 387-401.

Mc Vicer, A. H. and Mackenzie, K. (1972): A fungus disease of fish. Scottish Fisheries Bulletin. 37: 27 – 28.

Nadia, A. Abd-El-Ghany; Hoda, M. Abd-alla and El-Ashram, M. M. (2008): Diagnosis of Icthyophoniasis in Oreochromis niloticus in Egypt by polymerase chain reaction (PCR). 8th International Symposium on Tilapia in Aquaculture. 1307 -1328.

Nadia, Abd-El-Ghany and Hoda, M. Abd-alla (2008): A trail for treatment

of Icthyophonosis in cultured Oreochromis niloticus using focus and neem plants. 8th International Symposium on Tilapia in aquaculture pp: 1329-1349.

Ramaiah, N. (2006): A review on fungal diseases of algae, marine fishes, shrimps and corals. Indian J. Mar. Sci.: 35(4): 380-387.

Shaheen and Easa; (1990) : Molecular typing of fungal agents isolates. Journal of Clinical Microbiology, 07 1997, 1675-1680, Vol 35, No. 7

Sindermann, C. J. and Scattergood, L. W. (1954): Diseases of fishes of the Western North Atlantic. II. Icthyosporidium disease of the sea herring (Clupea harengus). Research Bulletin of the Department of Sea and Shore Fisheries, Marine 19, 1 – 40.

Sitja-Bobadilla, A. and Alvarez-Pellitero, P. (1990): Diseases of fishes of the Western North Atlantic. II. Icthyosporidium disease of the sea herring (Cluppea harengus). Research Bulletin of the department of sea and shore fisheries. Marine, 19: 1 – 40.

Spanggaard, B.; Gram, L.; Okamoto, N. and Huss, H. H. (1994): Growth of the fish pathogenic fungus, Ichthyophonus hoferi measured by condictimetry and microscopy. J. Fish Dis. 17, 2: 145 – 153.

Ziedan, A. H. H. (1999): Comparative study between hormonal effect and hybridization on the susceptibility of tilapia to infection with Ichthyophonus hoferi fungus. M. V. Sc. Thesis (Fish Medicine & management) fac. Of Vet. Med., Cairo university.