GLOBAL JOURNAL OF BIO-SCIENCE AND BIOTECHNOLOGY

www.scienceandnature.org

ISOLATION AND IDENTIFICATION OF PATHOGENIC FUNGI FROM CARP FISH IN SULIAMANIA PROVINCE

Hanan Hasan Ali
Microbiology Department of Immunology, College of veterinary Medicine, Suliamania University, Iraq.

ABSTRACT
Aim of this study was to investigate fungal infections in Different species of carpsincluding common carp (*Cyprinus carpio*); silver carp, Hypophthalmichthys (H.) molitrix; *Carpinus carpio* regularis (Mirror carp) . Thirty specimens were collected randomly and studied for the presence of fungal infections. Infected fishes showed clinical signs such as fungal growth on skin, fins, eyes, Oral cavity , eroded fins and scales, hemorrhages on body surface and abdominal distension .The specimens from infected organs of fish were inoculated on each, malt extract , Sabouraud dextrose and potato dextrose agars.The fungal colonies of white, black, green, grey and brown colors were observed in the forty agar plates. Slides were prepared and stained with 0.05% Trypan blue in lactophenol. The incidence of fungal infection according to different types of carps recorded that Cyprinus carpio showed the highest infection rate (55 %) followed by *H. molitrix* and *L. rohita* (25.5 % each respectively). The five fungal genera of Aspergillus spp. (32.5%), Blastomyces sp. (7.5%), Penicillium sp. (20%) Rhizopus sp. (25%) and Candida sp. (15%) were isolated from the fish. Eyes (25 %) and gills (20 %) were most affected areas followed by skin (17.5%), buccal cavity (15 %) and operculum (12.5 1%) and Head (10 %) respectively. This study showed that most of the pathogenic fungi isolated from carps fishes which are produce many types of Mycotocine that are cases many dangerous for animals and human health.

KEYWORDS: fungal infection, *Cyprinus carpio*, *Carpinus carpio* Aspergillus, Penicillium, Blastomyces.

INTRODUCTION

Fish are one of the most important groups of vertebrat es which give benefits to human beings in several ways . Fish is considered the best animal protein for human consumption. Fish proteins have high biological values. It contains variable quantities of calcium, phosphorus, fat its generous supply of ß- complex vitamins and other nutrients important for human health and growth (Khalil, 2010). Fungal contamination of fish is considered the main cause of signs of spoilage as off flavor and unpalatable taste and it may constitute a public health hazard as well as many of economic losses (Hassan *et al*., 2007 & El Ahl, 2010). As with all common molds, it feeds by secreting digestive enzymes onto its surrounding area. These enzymes break down the cells and tissues of the host, Moulds were recorded to constitute a public health hazard due to mycotoxin production such as aflatoxin, ochratoxin, patulin and zearalenone. These compounds cause some degree of acute toxicity when given in high amounts and are potential carcinogen, where in developing countries, it appears that there is a direct correlation between dietary aflatoxin intake and the incidence of liver cancer (Groopman *et al*., 1988 & Hassan *et al*., 2009). Many of the fungi that affect fishes are considered opportunists, attacking the fishes when they are stressed or immuno compromised because of unfavorable environmental conditions, or secondary to bacterial or viral infections, or when they have lost their mucus protection because of tr auma or excessive handling (Roberts 1989 and Quiniou *et al*.,1998).Oomycetes are classical saprophytic opportunities, multiplying on fishes that are physically injured,stressed or infected (Pickering and Willoughby, 1982). Members of this group are generally considered agents of secondary infection arising from condition s such as bacterial infections, poor husbandry, and infestation by parasite and social interaction. However, there are several reports of Oomycetes as primary infectious agents of fishes (Pickering and Christie, 1980) a nd their eggs (Walser and Phelps, 1993) . Moreover, there are other fungi that have been implicated in fish diseases. Some of the genera involved include *Aspergillus* (Salem *et al*., 1989), *Fusarium* (Bisht *et al*., 2000), *Ichthyophonus* (Faisal *et al*., 1985), *Branchiomyces* (Easa 1984), *Phoma* (Hatai *et al*., 1986), *Paecilomyces* (Lightner *et al*., 1988), *Exophialia* (Langdon and MacDonald 1987), *Phialophora* (Ellis *et al*., 1983), *Rhizomucor* (Wolf and Smith 1999) and *Candida*, Most of these are multiple case reports or single and causing systemic disease with high mortality rates in fishes. (Neish and Hug hes1980) . Certain molds are known to be capable of producing mycotoxins at low temperature as low as- (Hassan and El-Sharnouby, 1997 and Hassan and Aziz, 1998). Moulds were recorded to constitute a public health hazard due to mycotoxin production such asaflatoxin ochratoxin, patulin and zearalenone. These compounds cause some degree of acute toxicity when given in high amounts and are potential carcinogen, where in developing countries, it appears that there is a direct correlation between dietary aflatoxin intake and the incidence of liver cancer (Groopman *et al*., 1988 and Hassan *et al*., 2009).Therefore, new, safe antimicrobialagents are

needed to prevent and overcome severe fungal infectio ns.The objective of this study was to determine the typ es opportunistic of fungal pathogens specially those causing high mortality rates, elucidation of the incidence of Mycotoxin and distribution of such pathogen *Aspergillus* spp, *Penicillum* spp., *Rhizopes* spp. , *Blastomyces* and *Candida* spp. affecting on common carp fish types in Suliamani province / Kurdistan region / Iraq. The main objective of this studies are to nvestigate the main fungal infection in common carps species in Suliamani Provinces & to Isolation and identification most pathogenic opportunistic fungi in suspected case in fish shop in Suliamani provinces.

MATERIALS & METHODS

Sample collection

Thirty samples were collected from different species of c arps from fish shop in Suliamania market, and five specimens each of common carp (C . *carpio*); silver carp (H. *molitrix*) ; Mirror carp (*Carpinus carpio regularis*) were obtained from Fish Farms (Fig 1), The sterile swaps were collected directly The fish body was divided into two parts; Anterior part (head, eyes and gills) and posterior part (all fins and rest of the body) for culturing of fungal specimens.

FIGURE 1: Show several types of carps fish (A) common carp *Cyprinus carpio* (B) Silver carp *Hypophthalmichthys molitrix* and (C) Mirror carp *Cyprinus carpio regularis*.

Preparation of media culturing

The media preperating according to the leaflet of the company, which measuring by sensitive balance then, he glassware (containing media and distilled water covere d with aluminum foil), vials and test tubes (cotton plugged) were autoclaved at 121°C at 1.054 kg/cm^2 for 15 min. Antibiotic streptomycin sulphate 250 mg was added to each preparation of media to reduce bacterial contamination Cycloheximid (Inhibits the non pathogenic fungi growth).

Isolation and culturing

Three different types of media including malt extract agar (A), Sabouraud dextrose agar SDA) and potato dextrose agar (PDA) were culturing by sterile swab that collected from fish lesions.The body surfaces of all the fishes under study were disinfected by dipping each fish in 1% formaldehyde for 1 to 5 minutes followed by 70% alco hol and finally in sterile water in which it was thoroughly rinsed. The fungal isolates were collected from infected or gans of fish with sterile swab and inoculated on malt extract (Oxoid, UK), Sabouraud dextrose (Oxoid, UK) and potato dextrose (Oxoid, UK) agars. The agar plates were divided for two groups mould and yeast culturing which incubated at 28-30°C and fungal growth was observed after 4-7 days for mould growth and 30-37°C for yeast growth. The fungal colonies of various colors were observed in the agar plates. For microscopic examination, slides were prepared from each colony and stained with 0.05%. Trypan blue in lactophenol. The slides were observed under Olympus microscope Under power 4X & 40X. The fungi were identified with the help of available fungal identification keys and literature (Willoughby *et al*., 1994).

Statistical Analysis

The fish body was divided into two parts; Anterior pa rt (including head, eyes and gills) and posterior part (including all fins, abdomen and lateral sides of the fish) to note the infected site. Chi-square test was applied to compare the infection according to site of infection in part of the body of the infected carps sp.

RESULTS & DISCUSSION

Thirty different species of carps specimens were examined for fungal infection Forty out of thirty fishes were infected thus showing 76.66% infection. From 2 infected fishes, four fish showed clinical signs of fungal infection

such as; ruptured dorsal fins, lesions on skin and caudal peduncle; and granuloma on anal fin. The material from va rious parts (eyes, gills, head, buccal cavity, operculum and skin) of 23 infected fishes was taken and inoculated on three types of agarplates. A total of 40 agar plates were inoculated from 23 fish specimens and one plate from pond water. Fife types of fungal colonies appeared on agar plates; whitish–orange colonies, black, yellowish -green, greenish, mucoid colonies. A total of thirty fishes of Three species were examined (Table1). Fungal infection was observed in *C. carpio*, *H. molitrix* and Mirror. The clinical picture of infected C. carpino showed fungal growth on head, gills, eyes and fins. Additionally, infected fishes had eroded scales and hemorrhages over body surface and moderate body distension with infection percent (55%). While *H. molitrix*, C. carpino regularis showed fungal infection on site of infection Head, Abdomen, Caudal fin 25.5 % respectively (Table (1). The infection on head, gills and eyes of fish may lead to serious pathological conditions as extensive growth of fungal hyphae in eyes may cause complete blindness and from eyes may pe netrate into brain and in such condition the treatment is impossible and eventually the fishes die (Srivastava, 2009). Fin infection is considered less pathogenic as such fishes survive but this infection may lead to complete damageb of the fins. Five fungal genera *viz.* Aspergillus spp. showed the highest percentage for fungal infection (32.5%); while *Rhizopus* spp. (25%), *Penicillium* spp. (20%); *Blastomyses* spp, (7.5%), candida (15%) respectively (Table 2), were isolated from Different speci es of carps. Two genera Aspergillus spp. was isolates AspergillusNiger (Black colonies) , Aspegillus Flavus (G reenish colony) From Common carps C. carpio, H. molitrix and C. carpio regularis. Mixed fungal infection was also observed in C. auratus, in Fife combinations as Candida Blastomyces spp., Aspergillus spp., spp., Rhizopus spp. Penicillium spp. The most prevalent genus was Aspergillus spp. It was isolated from C. carpio .

TABLE 1: Fungal infection percent in different three types of carps

Species	Symptoms	Fish Infection	Site of infection in fish
C. carpio	Ulcer, gill rot	55%	fins, abdomen Head, eyes, gills,
H. molitrix	Ulcer	22.5%	Head, Abdomen, Caudal fin
Carpinus carpio regularis	Ulcer, gill rot	22.5%	Caudal fin

TABLE 2: Tissue wise infection and fungal colonies in Carps

S. No	Tissue	No. of plates	% infection	Aspergillus spp.	Blastom yces sp.	Penicillium sp.	Rhizopus	Candida
1	Eyes	10	25	4	-	2	-	2
2	Gills	8	20	1	3	1	-	-
3	B.C	6	15	4	-	1	2	1
4	Head	4	10	-	-	2	1	-
5	OP	5	12.5	3	-	1	3	1
6	Skin	7	17.5	1	-	1	4	2
Total		40		13	3	8	10	6
				32.5%	7.5	20%	25%	15%

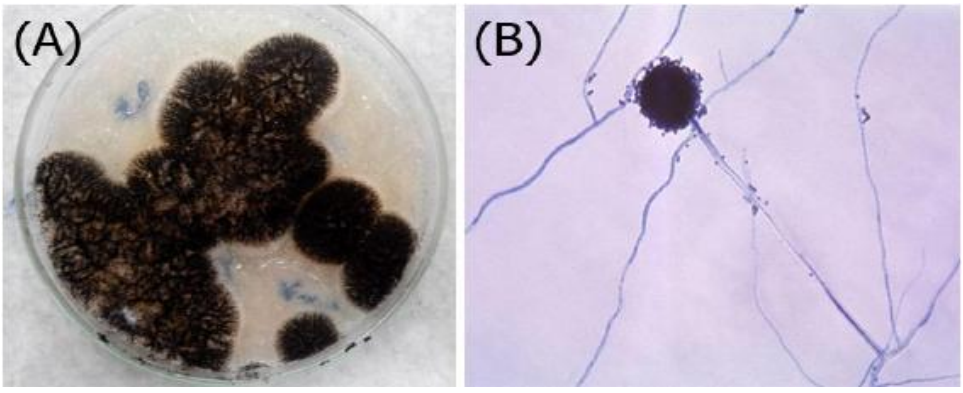

FIGURE 1: A. Colony of *Aspergillus niger* (Black colony) on SDA and PDA from Site of infection, B. *Aspergillus niger* isolated from A, reproductive head on hyphae very clear

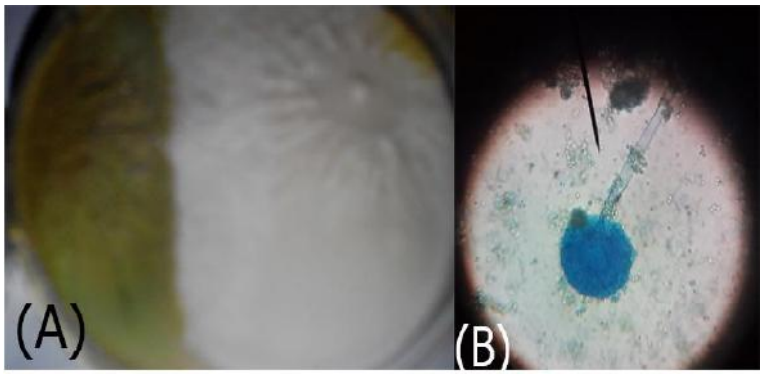

FIGURE 2: A. Aspergillus flavus colonies greenish - yellow color B , reproductive head on Conidiophores very clear.

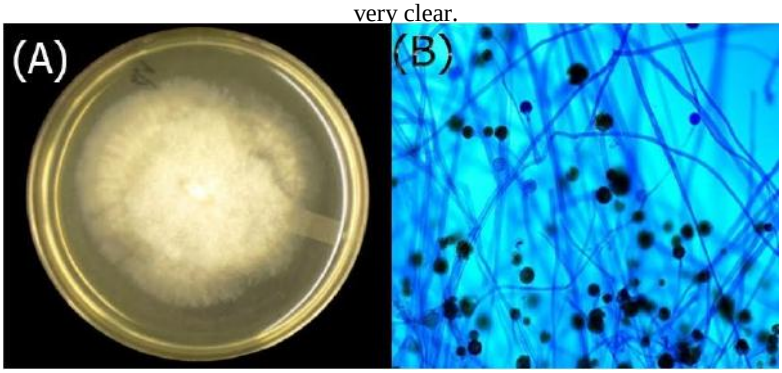

FIGURE 3: A, Colonies of Rhizopus on MEA, (fish 4,C.auratus). B, Rhizopus (from plate–A) showing long branched sporangophore with sporangium bearing spores.

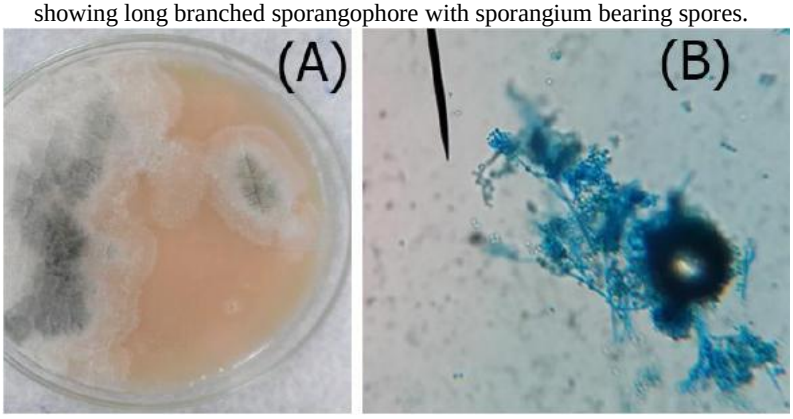

FIGURE 4: A. Colonies of Penicillium on SDA, (fish 1, C. Carpio). B, Penicillium sp. showing brush like arrangement of fruiting head Microscopically .

FIGURE 5: ABranchiomyces spp. culture after 3 days of cultivation, (B) After 7 dayes , the colonies shows as folded heaped, glabrous and velvety, white in color and with white –yellowish in reveries

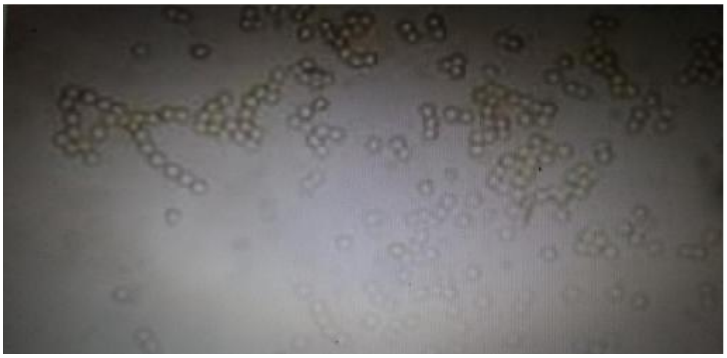

FIGURE 6: Spores of Branchiomyces spp Lactophenol cotton Blue

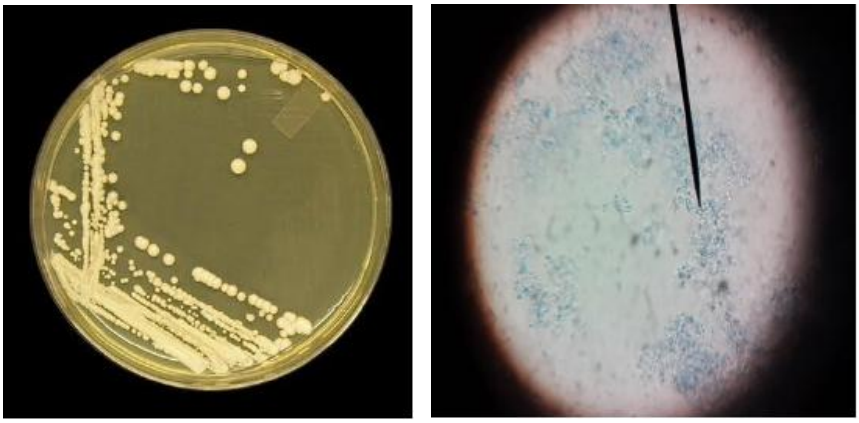

FIGURE 7: A Candida colonies creamy muciod appear on SDA and Malt extract A, B, Unicellular cell of Candida stained by Lacto phenol cotton blue under X 400

Aspergillomycosis has been principally described in African fish, especially the tilapia Oreochromis sp. (Olufemi, 1983). A number of Aspergillus species such as A. flavus, A. japonicus, and A. terreus are involved in this infection. These species presumablycause infection via entry into the fish through contaminated feed. Jalilpoor et al. (2006) reported infection of Aspencer percicus eggs with Penicillium spp., Fusarium spp., Mucor spp. and Saprolegnia spp. which caused 7 and 22% mortality ofthese eggs.Fayioye *et al.* (2008) isolated five different species of fungi including Fusarium, Aspergillus, Rhizopus, Mucor, and Penicillum from 8 edible smoke-dried freshwater fishes Junaid *et al.* (2010) isolated 7 fungal species from stockfish in Nigeria and these included *A.flavus, A.fumigatus, A. niger, Trihophyton verrucosum,* RhizopusMucor and Penicillum spp. and among these Mucorspp. Showed the highest occurrence. In another study, fungi of eight different genera; Saprolegnia, Aspergillus, Fusarium, Mucor, Pencillium, Rhizopus, Scopulariopsis and Curvularia were isolated from two fish species, Oreochromis spp. and Claris gariepinas (Refai *et al*., 2010). Shahbazian *et al*. (2010) isolated Penicillium expansum, Penicillium citrinium; Aspergillus terruse, Aspergillus clivatus; Alternaria spp. and 11 other fungal species from infected eggs of rainbow trout. Moreover, Fadaeifard *et al.* (2011) reported the occurrence of different fungal species of genera including, Penicillium, Acreomonium, Alternaria, Fusarium, Aspergillus, Mucor, Saprolegnia and Cladosporium from the eggs and brood stock of rainbow trout. Findings of the present study are comparable to the findings of Refai *et al*. (2010), Shahbazian *et al.* (2010) and Fadaeifard *et al.* (2011).However, Refai *et al.* (2010) has characterized Aspergillus spp., Penicillium spp. and Rhizopus spp. as opportunistic pathogens(Refai *et al*., 2004) as many of them possess virulence factors which enable them to cause disease (Refai *et al*., 2010), especially under favorable predisposing conditions. Ecological differences play an important role in species diversity of fungi that develop on both fish and eggs (Hussein et al., 2001). Interaction of physiochemical factors generally has influence on the diversity of water molds (Paliwal and Sati, 2009). Lack of good aquarium keeping in pet Shops and fish farms increases the chances of fungal infection in fishes.The basic health management practices may be easily over looked due to dearth of trained personal or resources. This study indicates that although most fungi isolated from fishes are considered as normal mycoflora, yet they can causeinfections. This is confirmed by the presence of fungal hyphae in the lesions on the body of the fish. These findings point our attention to the possible role of fungi in affecting CarpsSpecies in Suliamani.

CONCLUSION

Detection of five pathogenic genera of fungi (yeast & mould) in fish (carps species) in Suliamani Province, classification the types of the fungi according to the organs incidence, Isolation and purification each types of pathogenic fungi on three types of media cultures.

RECOMMENDATION

1. Study the fungal infection in another fish species in Iraq.

2. Molecular detection for pathogenic fungal infection in fishes.
3. Make awide study about the fungal infection in fish in K urdistan region.
4. Differentiate between the pathogenic infection and opport unistic infections fungi in fishes .
5. Detection the titer of Mycotocine in fish Mycotoxins standard solution for TLC andfluorometric methods Aflatoxins standard B1, B2, G1, G2 and their immuno affinity.

REFERENCES

Abdel-Wahhab, M.A. & A.M. Kholif, (2008) Mycotoxins in animal feeds and prevention strategies: Areview. *Asian J. Anim.* Sci., 2: 7-25.

Al-Hamed MI (1960) Carp culture in Iraq. *Iraqi J Agric* Res. 1(2): 14-23.

Bisht, D., Bisht, G.S. & Khulbe, R.D. (2000) Fusarium-a new threat to fish population in reservoirs of Kumaun, India. *Current Science*, *78*(10), 1241-1245.

Cervinka, S., Vitovec, J., Lom, J., Hoska J. and Kubu, F. (1974). Dermocystidiosis-a gill disease of the carp due to *Dermocystidium cyprini*n. sp. *J. Fish Biol*., 6: 689-699.

Chauvier, G. (1979) Mycoseviscerale de poissondulcaquic olestropicaux. Ann. Parasitol. Hum. Comp., 54: 105-111.

Chien, C. (1981) Fungal diseases of fresh water fish in Tai wan. Nation. Sci. Counc. (Republic of China) Symp. Ser., 3:33-45.

Crow, G. L., Brock, J. A. & Kaiser, S. (1995) *Fusarium solani* fungal infection of the lateral line canal system in captive scalloped hammerhead sharks (*Sphyralewini*) in Hawaii. J. Wildl. Dis., 31: 562-565.

Easa, M. EI-S; Hatem, M.E., Sark E.E. & Refai, M. (1984) *Phomaherbarum*as a mycotic fish pathogen in *Clar iaslazera*, Armout catfish. *Vet. Med. J*., 32, 257- 267.

El-Ahl, M.H.S. Rasha (2010): *Studies on fungi in fish and fish products and their control* for the degree of doctor of phylosophy Thesis, Dept. of Microb, Fac. of Vet. Med., Cairo University.

llis, A.E., Waddell, I.F. and Minter, D.W. (1983): A syste matic fungal disease in Atlantic *salmon parr*, *Salmosalar L*., caused by a species of Phialophora. *J. Fish. Dis*. 6:511-523.

Fadaeifard, F., Raissay, M. Bahrami, H. Rahimi E. and Najafipoor, A. (2011) Freshwater fungi isolated from eggs and broodstocks with an emphasis on saprolegnia in rainbow trout farms in west Iran. Afr J. Microbiol Res, 4: 3647-3651.

Faisal, M., Torky, H. & Reichenbach-Klinke, H. H. (1985) A note on swimming disease among the Labyrinth catfish (*Clariaslazera*). *J. Egypt. Assiut Vet. Med*., 45: 53-60.

Fayioye, O.O., Fagbohun,T.R .& Olubanjo,O.O. (2008) Fungal infestation and nutrient quality of traditionally smoke-dried freshwater fish. Turk J Fish Aqua Sci, 8: 7-13.

Furhan, T.M., Khalidah S. Al-Niaeem, Ali B. Al-Zubaidy (2012) Literature review on fish parasites of Al-Furat fish farm, Babylon province, Iraq. *Iraqi J. Aquacult*. Vol. (9) No.(1) : 95-122.

Galvano, F., Ritieni, A., Piva G. & Pietri, A. (2005) Mycotoxins in the Human Food Chain. In: The Mycotoxin Blue Book, Diaz, D.E. (Ed.). Nottingham University Press, England, ISBN: 9781904761198,pp: 187-225.

Gottlich, E.W., van der Lubbe, B., Lange, S., Fiedler S. and Melchert, I. (2002). Fungal flora in groundwater-derived public drinking water. Int. J. Hyg. Environ. Health, 205: 269-279.

Groopman, J.D., Cain, L.G. & Kensler, T.W. (1988)Aflato xin exposure in human populations: measurements and relation to cancer". Critical Review in Toxicology CRC, 19: 113.

Hassan A.A.,Hammad, A.M, El Barawy, A.M. & Manal, A.H. (2007) Incidence of aflatoxigenic fungi in frozen and canned fishes and trials to inhibit aflatoxin production by use of some minor elements and *lupinustermis*seeds. Egypt. J. Appl. Sciences, 22 (10B) 351-360.

Hassan, A. A & N.H. Aziz (1998): Influence of moisture c ontent and storage temperature on the production of aflat oxin by*Aspergillus flavus* EA-8 1 in maize after exposure to gamma radiation. J. of Food Safety, 18(3): 159-17 1. (USA).

Hassan, A.A.& El- Sharnouby, R. (1997) Antimicrobial effect of some chemicals and plant extracts on Egyptian food. J. Egypt. Vet. Med. Assoc.,57 (4): 1331- 1349.

Hassan, A.A., Wael, M. Tawakkol,Abdel Aziz A. El Maaz &Howayda M. El Shafei (2009) The hepatoprotective effect of dimethyl 4,4- dimethoxy 5, 6, 5, 6-dimethy lenedioxy -biphenyl – dicarbxylate (D.D.B.) against liver injury induced by aflatoxin B1 in rates. *Egypt. J. of Appl.* Sci., 24 (9), 86-100.

Hatai, K. & Egusa S. (1979). Studies on visceral mycosis of salmonid fry-II. Characteristics of fungi isolated from the abdominal cavity of amago salmon fry. Fish Pathol., 11: 187-193.

Hatai, K., Fujimaki Y. and Egusa, S. (1986) A visceral mycosis in ayu fry, Pleoglossusaltivelis Temminck & Schlegel, caused by a species of Phoma. *J. Fish. Dis*., 9: 111-116.

Hedrick, R.P., Friedman, C.S. & Modin, J. (1989) Systemic infection in Atlantic salmon Salmosalar with a *Dermocystidium*-like species. *Diseases of aquatic organis ms*, *7*(3), 171-177.

Hussain, S.M., Javed, M., Javid, A. Javid, T. and Hussain, N (2011). Growth responses of *Catlacatla*, *Labeorohita*

and *Cirrhinamrigala* during chronic exposure of iron. *Pak J Agric*Sci, 48: 225-230.

Hussein, M.M., Hatai, K. & Nomura, T. (2001) Saprole gniasis in salmonid and their eggs in Japan. J Wildl Dis, 37: 204-207.

Jalilpoor, J., Mosouleh S.A. & Masoumzadeh, M. (2006) Fungal flora in *Acipenserpersicus*eggs with particular emp hasis on *Saprolegnia*sp (*Oomycetes*) and mortality during mass incubation at the Shahid Behesti hatchery . J App Icthyol, 22: 265-268.

Jay, J.M., Loessner, M.J. and Golden, D.A. (2005) Modern Food Microbiology. 7th Edn., Springer, New York, USA., ISBN-13: 9780387231808, pp: 709-726.

Junaid, S.A., Olarubofin F. & Olabode, A.O. (2010) Mycotic contamination of stockfish sold in Jos, Nigeria. J Yeast and Fungal Res, 1: 136–141.

Khan, A.M., Ali, Z., Shelly,S.Y.& Mirza, M.R. (2011). Aliens; a catastrophe for native fresh water fish diversity in Pakistan. J Anim Plant Sci, 21:435-440.

Khalil, B. (2010) *Histopathology of skin of some Fishes of f amily sciaenidae from Karachi coast*. For the degree doctor of phylosophy Thesis. Jinnah University For women.

Khidhir, K. Z. (2011) *Comparative Quality Assessments of Five Local Fresh Fish in Sulaimani City Markets*. For the Degree of Doctor of Philosophy thesis. University of sulaimani.

Kikkiwa, J. & Kosuci, K. (1937) The fungi grown on dried bonito.*Zentralbl. Bakteriol. ll, 97*, 885-885.

Kita, G. (1914) Some Japanese fungi. Zentralbl. Bacterio.I 11: 351-363.

Kovac-Gayer, E., Csaba, G., Ratz, F., Bekesi L. & J. Szakolczai, (1986) Granuloma of common carp (*Cyprinus carpio*L.). Bull. Eur. Ass. Fish Pathol., 6: 72-75.

Landsberg, J.H. & Paperna, I. (1992) Systemic granuloma in goldfish caused by a *Dermocystidium* like aetiological agent. Dis. Aquat. Org., 13: 75-78.

Langdon, J.S. and W.L. MacDonald (1987): Cranial *Exoph iliapiscphilia*infection in *Salmosalar* in Australia. *Bull. Eur. Ass. Fish Pathol.* 7:35-37.

Lightner, D., Redman, R.M., Mohney, L., Dickenson G. & Fitzsimmons, K. (1988) Major Diseases Encountered in Controlled Environment Culture of Tilapias in Fresh- and Brackish water over a three year period in Arizona. In: Pullin, R. S. V., K. Tonguthai and J.L. Maclean, (Ed.) The Second International Symposium on Tilapia in Aquaculture. ICLARM Conf. Proceedings, pp: 111-116.

Lightner, D.; R.M. Redman; L. Mohney; J. Sinski, and D. Priest (1988): A renal mycosis of an adult hybrid red tilapia, *Oreochromismossambicus* and *O.hornorum*, caused by the imperfect fungus, *Paecilomyces marquandii*. *J. Fish. Dis*., 1.1: 437-440.

Limburg, K.E., Hughes, R.M. Jackson, D.C .and Brain, C.Z. (2011) Human Population Increase, Economic Growth and Fish Conservation Collision course or Savvy Steward ship? Fisheries, 36: 27-34.

Lom, J. & Dykova, I. (1992) Protozoan parasites of fishes. Developments in aquaculture and fisheries science.Volume 26: Elsevier Science, New York, New York, USA.

Motalebi, A.A., Ardalani K. & Jamili, S. (2008) Effect of temperature on the produced aflatoxins in the rainbow trout feed in West Azerbaijan province. *J. Fish. Aquatyic Sci*., 3: 392-397.

Nash, G., Southgate, P.,Richards, R.H. & Sochon, E. (1989). A systemic protozoal disease of cultured almonids. J. Fish Dis., 12: 157-173.

Neish, G.A and Van As G.C., (1980). Fungal Diseases of F ishes. In: Snieszko, S.F. and H.R. Axelrod,(Eds.), Diseases of Fishes, Book 6. T.F.H. Publ. Inc. Ltd., Country, pp: 159.

Neish, G.A. & Hughes, G.C. (1980): *Diseases of fishes*, Book 6. Fungal diseases of fish.TFH Publications,Neptune, Ng.1159.

Nitzan, S.(1990)*Dermocystidiumanguillae*in elvers of the E uropean eel *Anguilla anguilla*L. in Israel. Israel J. Aquac. Bamidgeh, 42: 52-55.

Nofiani, R., M. Kalbar and P. Ardiningsih, (2010). Chemic al characteristic and microbiological safety of commercial fermented ale-ale from West Kalimantan, Indonesia, J. Fish. Aquat. Sci., 5: 483-493.

Olson, R.E., Dungan C.F. & Holt, R.A. (1991) Waterborne ransmission of *Dermocystidiumsalmonis* in the laboratory. Dis. Aquat. Org., 12: 41-48.

Olufemi, B.E., Agius C. &Roberts, R. J.(1983) Aspergillo mycosis in intensively cultured tilapia from Kenya. *Vet. Rec*., 112: 203-204.

Olufemi, B.E., C. Agius and R.J. Roberts, (1983).Aspergill omycosis in intensively cultured tilapia from Kenya. Vet. Rec., 112: 203-204.

Pailwal, P. & Sati, S.C.(2009) Distribution of Aquatic fun gi in relation to physiochemical factors of Kosi River in Kumaun Himalaya. Nat Sci, 7: 70-74.

Panasenko, V.T. (1967) Ecology of microfungi. *The botanical review*, *33*(3), 189-215.

Paperna, I., (1986). *Ichthyophonus*infection in grey mullets from Southern Africa: histopathological and ultrastructural study. Dis. Aquat. Org., 1: 89-97.

Peduzzi, R. (1973). Diffusainfezionebranchiale da funghiat tribuiti al genere *Branchiomyces* Plen (Phycomycetes,

Saprolegniales) a caricodell' ittiofauna di laghisituati a nord e a suddelleAlpi. II. Esigenzecolturali, transmission sperimentaleedaffinitatassonomiche del micete. Mem. Ist. i tal. Idrobiol.,30: 81-96.

Pickering, A.D and Willoughby, L.G.(1982) *Microbial Dis eases of Fish* in: Roberts, RJ.(Ed.). Academic Press, London, England. pp: 271-297, 1982.

Pickering, A.D. & Christie, P. (1980) Sexual differences in the incidence and severity of ectoparasitic infestation of the brown trout, *Salmotrutta L. J. Fish Biol*., 16: 669–683.

Population in reservoirs of Kumaun India .*Curr. Sci*., 78 (1 0): 1241-1245.

Quiniou, S.M.A., Bigler, S. and Clem, L.W. (1998): Effects of water temperature on mucous cell distribution in channel catfish epidermis: a factor in winter saprolegniasis. *Fish ShellfImmunol.* 8:1-11.

Refai, M., Attia, S. Salem, R.M. & EM El-Dahsham, (2004). Studies on the pathology of *Aspergillusfumigatus*, *A. flavus* and *A. niger* isolated from chicken and their environment. Egypt J Comp Path Clinic Pathol, 17: 193-205.

Refai, M., Laila, K., Mohamed, A., Kenawy, M. and El-SMS Shimaa (2010) The assessment of Mycotic settlement of freshwater fishes in Egypt. J Am Sci, 6: 595-602.

Roberts ,R.J. (1989) The mycology of teleosts, in Roberts RJ (ed). Fish Pathology, 2nd edition. London, England, Baillere Tindall, pp 320-336.

Rubbani, B., Afzal, M. Mubarik, M.S., Salim, M. & Hussain, S.M. (2011) Estimation of apparent digestibility coefficients of soybean meal based diets with different protein levels for *Labeorohita.* Pak J AgricSci, 48:231-235.

Salem, A.A., Refai, M.K., Eissa, I.A.M., Marzouk, M., Bakir, A., Moustafa, M. & Manal Adel. (1989): Some studies on aspergillomycosis in Tilapia nilotica. *Zagazig Vet. J.*, 17(3): 315-328.

Shahbazain, N., Ebrahimzadeh, M., Soltani, M.,Khosravi, A.R., Mirzagai, S. & Sharifpour,I. (2010) Fungal contami nation in rainbow trout eggs in Kermanshah Province propagation with emphasis on Saprolegniaceae. Iranian J Fish Sci, 9: 151-160.

Srivastava, R.C. (2009) Fish mycopathology/RC Srivastav *Repr. New Delhi: Today & Tomorrow sprinters and publi cation.*

Van Eer, A., Van Schie, T. &Hilbrands, A. (2004). *AD15E Small-scale freshwater fish farming* 2nded.pp 41. Agromisa Foundation. Digigrafi, Wageningen, the Netherlands.

Voelker, F.A., M.R. Anver, A.E. McKee, H.W. Casey, H. W and G.R. Brenniman, (1977). Amebiasis in goldfish. *Vet. Pathol*., 14: 247-255.

Walser, C.A. and R.P. Phelps (1993): The use of formalin a nd iodine to control Saprolegnia infections on channel catfish, *Ictaluruspunctatus*, eggs. *J. Appl. Aquacult*., 3: 269 –278.

Willoughby, L.G. & Roberts, R. J. (1994) Improved metho dology for isolation of the *Aphanomyces* fungal pathogen of Epizotice Ulcerative Syndrome (EUS), in Asian fishes. Proceedings of the ODA Regional Seminar on Epizootic Ulcerative Syndrome. Aquatic Animal Health Research Institute, Bangkok, Thailand. Pp 231-237.

Willoughby, L.G. and A.D. Pickering, (1977). Viable Sapr olegniaceae spores on the epidermis of the salmonid fish *Salmotrutta* and *Salvelinusalpinus*. Trans. Br. Mycol. Soc., 68: 91-95.

Wolf, J.C. and S.A. Smith (1999): Systemic zygomycosis i n farmed tilapia fish. *J. Comp. Pathol*., 121(3):301-306.

Wooten, R. & McVicar, A.H. (1982) *Dermocystidium* from cultured eels *Anguilla anguilla*L. In Scotland.J. Fish Dis., 5: 215 -232.