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STUDIES ON HISTOLOGICAL AND HISTOPATHOLOGICAL MYCOSIS VARIATIONS OF *CHANNA STRIATUS* (BLOCH) FOUND INFECTED WITH *ASPERGILLUS FUMIGATUS* AND *ASPERGILLUS NIGER* SPP. CAUSED EUS CHARECTERSTICS.

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ABSTRACT: The present investigation deals with the histological and histopathological alterations in *Channa striatus* infected with fungi *Aspergillus fumigatus* and *Aspergillus niger*. Fresh water murrels were collected from Dharmasagar and Hasanparthy lakes and Local fish markets of Warangal district. This is due to the nature of their habitats as many native species of fish suffer from living beneath turbid water. Among the recent emerging infection diseases responsible for severe population declines in plant and animal taxa, fungal and fungi-like microbes have emerged as significant contributors. Isolated fungi from infected fish bodies were identified as *Aspergillus fumigatus* and *Aspergillus niger*. Histopathologically different kinds of destructions were observed in Skin, Muscles, Gills and Liver of the infected fish. Histopathological observations of stained sections of infected fishes revealed pathological lesions of varying severity in studied organs including skin (exhibits completely lost epidermis, destiended cells, many of cells lost their cytoplasm and nuclei. Necrotized muscular layer with the formation of granulomas and encysted conidiophores), gills (hyperplasia and hyperplasia of lamellar epithelium, fusion of lamellae, rod like structures of secondary gill lamellae, cystic- like lesions) and liver (hypermia and haemorrhage, bile duct hyperplasia, dialated sinuses, interstitial oedema, monocellular necrosis, nuclear degeneration and hypertrophy in hepatocytes). The present study is aimed to assess the histopathological impact of fungal infection of an air breathing freshwater teleost *C. striatus*. Penetrating fungal hyphae were observed in skin and complete muscles were observed with formation of mycotic fibrillar granulomas. This is concluded that the mycosis variations in certain histological and histopathologically, exerted destructive effects on skin, gills and liver of *Channa striatus*.

INTRODUCTION: *Channa striatus* (Bloch) the air breathing fishes are one of the most important groups of vertebrates which provide free economic services to human beings in multipule ways ¹. In the world fish production, India ranks third ², in particular fish is a rich source of animal protein and its culture is an efficient protein food production system from aquatic environment.

With increasing freshwater fish production movement around the world for farming, releases into the wild for sport fishing and human-driven habitat changes and this is expected, along with improved environmental monitoring of fungal and fungal-like infections that are full extent of the impact of these pathogens on wild fish populations will soon emerge as a major threat to freshwater biodiversity ³. In India the fisher men is facing serious problems with fungal diseases. Fungi is mostly attacked by due to change in temperature and filthy conditions of water which allow excessive zoospores to grow and the ammonia which is formed by rotting of fish waste wears away the mucus that protects the skin ⁴. It is fresh water edible fish found susceptiable to fungal

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infection *Aspergillus fumigatus* and *Aspergillus niger* is a pathogenic group of fungi causing EUS infection in fresh water fishes. The pathogenesis of *Aspergillus fumigatus* and *Aspergillus niger* have been reported in fresh water fishes by ⁵. The effect of these infections on fishes in fresh water ecosystems. Some other workers isolated *Aspergillus sp* from fresh water fishes are ^{5, 6}. Stressed environmental condition and low temperature will increase fungal pathogens in the fresh water and marine water fishes. This kind of pollution has been developed to causes the EUS infection is serious diseases in India and through out of South Asia.

The commercially important fish populations are declining due to over fishing, pollution in water and low aeration in lakes. Infection of *L. Rohita* and silver carp, *Hypophthalmichthys Molitrix* with *Aspergillus sp* and *Alternaria sp* has also been reported by ⁷. The present study is an experiment to diagnose the pathogenicity of the isolated species of *Aspergillus fumigatus* and *Aspergillus niger*, infects on some fresh water murrel fishes. And in the histological alterations in various tissues such as Skin, Muscle, Gills and Liver due to the fungal infection.

MATERIAL AND METHODS:

Collection of fresh water fish samples:

The total number of 60 mycotic infected fishes showing external symptoms were collected fresh water fish *Channa striatus* from Dharmasagar and Hasanparthy lakes at Warangal district, Telangana, India. The present study was during the period of October 2013- February 2014. Fishes were brought to the laboratory for further clinical examination. The infected fishes were identified from red spots on their body, excess mucus secretions, damaged and sluggishness. The Microbial investigation was carried out in different infected parts were separated from fish of *Channa striatus*, namely, Skin, Muscle, Gills and Liver. The Microorganisms such as *Aspergillus fumigatus* and *Aspergillus niger* were determined in the various organs.

Preparation Fungal Culture and Isolation of Fungi: Small pieces of organs was taken from the lesion and culture media for the isolation of the

fungus. Potato Dextrose Agar (PDA); Corn Meal Agar (CMA) were used as media in to the Petri dishes the tissue pieces were transferred in to other set of Petri dishes. Petri dishes were placed inverted in incubate at 28°C- 30°C for three days, until a circular fungal mat developed, which were used for sub culture of fungus. Identification of fungi was carried out on the basis of keys of ⁸. These fungal culture results are obtained from NCFT (National Center OF Fungal Taxonomy) New Delhi, followed *A. fumigatus* (6627.15), *A. niger* (6629.15).

Histological Examinations:

For the histological study, Skin, Muscle, Gills and Liver tissues control and infected were examined. Tissues were fixed in Aqueous Bouin's solution for 48- 72 hours. The fixed tissues were then processed routinely and with Azan stain. Prepared in to paraffin blocks then sectioning in to 3- 6µm thickness and stained with Azan and Aniline Blue. Slides were observed under the Microscope to study the variations. Standard histological methods of ⁹ were followed for histo- pathological investigations.

RESULTS AND DISCUSSION: A total number of 60 fresh water fishes were observed during the investigation for fungal identification and 30 fishes were observed to have infection of conidial fungi showing the symptoms like eroded scales, skin darkening, damaged caudal, pectoral and pelvic fins. In some fishes caudal fin was found completely lost, black patches on abdominal region and ulceration in skin. Total infection was observed mostly on head and tail region of *Channa striatus* fishes. One of the key risks associated with this new aquaculture environment is the stress caused by intensive production.

Some of fungal pathogens such as *Saprolegnia* for example are more prevalent and virulent in host (salmonids in particular) that are raised under stressful conditions ^{10, 11}. In the examination of laboratory isolation of fungi reveals that the presence of Mycelia growth in form of small cottony tufts on the external body surface. The fungal diseases in aquaculture represents the most significant economic losses and in particular fungal infections, which in terms of economic impact are

second only to bacterial diseases^{12, 13, 14, 15, 16, 17}. One of the examples it has been reported in Japan some annual losses of 50% in the production of coho salmon *Oncorhynchus kisutch* and elvers of eel *Anguilla Anguilla* due to outbreaks of *S. Parasitica*^{18, 19}. In some fishes hyphae, wound and

necrotic lesions were observed on the Skin, Muscle, Gills and Fins. (Fig. 1-3). Cultures grown from inocula takes from different regions of the body showed the presence of two species of *Aspergillus fumigatus* and *Aspergillus niger* (Fig. 4- 8).

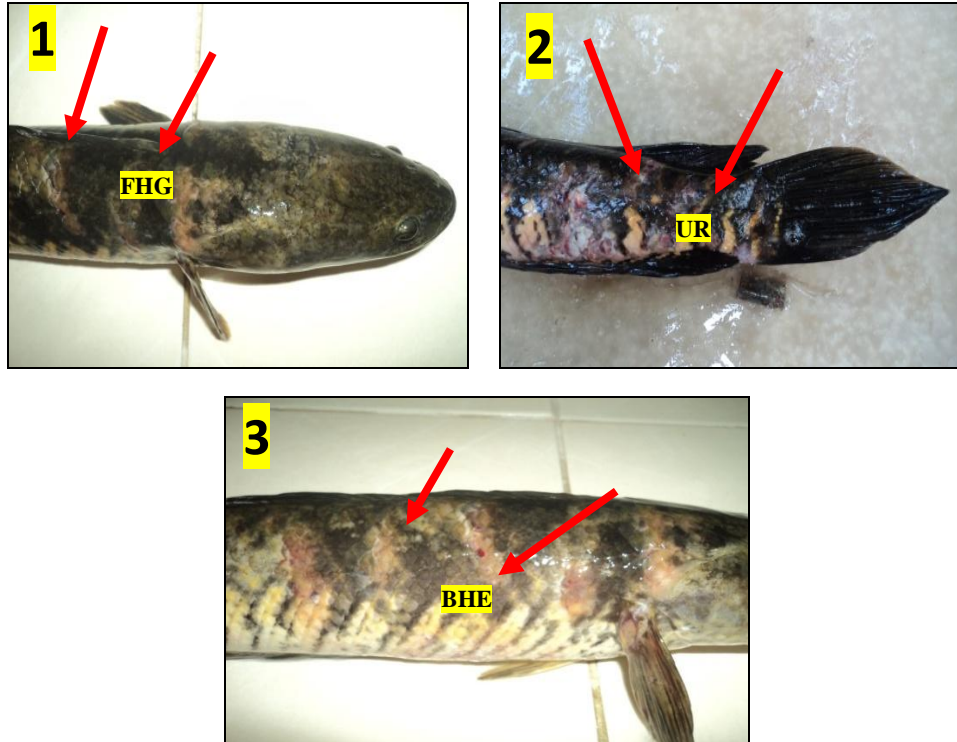


FIG.1AND 3: *CHANNA STRIATUS* INFECTED WITH *ASERGILLUS FUMIGATUS* AND *ASPERGILLUS NIGER* SPP. BLOOD HAEMORRHAGE (BHE), FUNGAL HYPHAE (FHG), ON ITS HEAD AND TAIL WITH ULCER REGIONS (UR).



FIG. 4: *ASPERGILLUS FUMIGATUS* CULTURE ON POTATO DEXTROSE AGAR (PDA) AND CONIDIA RELEASING SPORES.

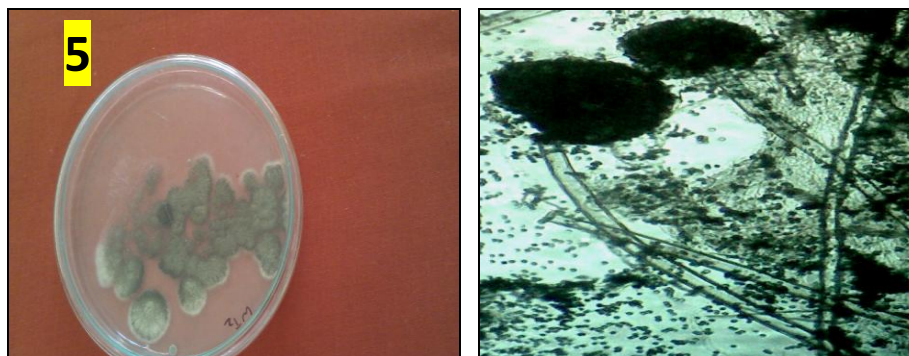


FIG. 5: *ASPERGILLUS NIGER* CULTURE ON POTATO DEXTROSE AGAR (PDA) AND CONIDIA RELEASING SPORES.

Histo- Histopathological Examinations:

Results of inoculation of pure cultures on infected fish showed both species of fungi are pathogenic to *Channa striatus*. Fungal infection was studied in *C. Catla* three types of genera like *Aspergillus sp*, *Blastomyces sp* and *Pencillium sp* were isolated from head, operculum, eye, buccal cavity, abdomen, skin and gills of *C. Catla*. *Aspergillus sp* was the most prevalent fungus infecting all the organs of *C. Catla* followed by *Blastomyces sp* and *Pencillium sp*.^{15, 7} In addition, mycoses spread in freshwater fishes are often seen as a secondary phenomenon. However, due to their virulence, their current emergence in wild fish populations and also the risk of spill back from aquaculture facilities to the wild, routine pathological examination should include (in addition to bacteriological ones) mycological examination²⁰. Dominant fungal pathogens reported in aquaculture are oomycetes including the genera *Achlya*, *Aphanomyces* and *Saprolegnia*^{10, 21, 22}. *A. invadans* for example can cause epizootic ulcerative syndrome in over a hundred of mostly freshwater fish^{23, 24, 25}, but also in some brackish fish species^{26, 27}. In aquaculture conditions, the most appropriate control is through eradication of the stock, quarantine of new stocks and good husbandry^{19, 28}, and as such represent a significant cost to the trade²⁹.

In the present study Results of histopathological examine showed various types of destructions in the selected tissues of Skin, Muscle, Gills and liver. The Skin of *Channa striatus* exhibits completely lost epidermis, destiended cells, many of cells lost their cytoplasm and nuclei. Necrotized muscular layer with the formation of granulomas and encysted conidiophores. Gill lamallae were observed necrotizes with degraded epithelium and fungal hyphae encapsulated by multiple layer of fusiform hepatocytes found in the gill tissue³⁰.

Conidiophores were observed in between hepatic tissue of Liver, showed vacuolization of liver cells was observed. Similar pattern of histological changes were observed by⁹, in *Tilapia sp* infected with *A. flavas*. Histopathological manifestations due to Mycotic infection fresh water fishes have been reported by^{31, 32, 4}. A pathogenisity of three species of *A. fumigatus*, *A. niger* and *A. sydowiion* some fresh water fishes.

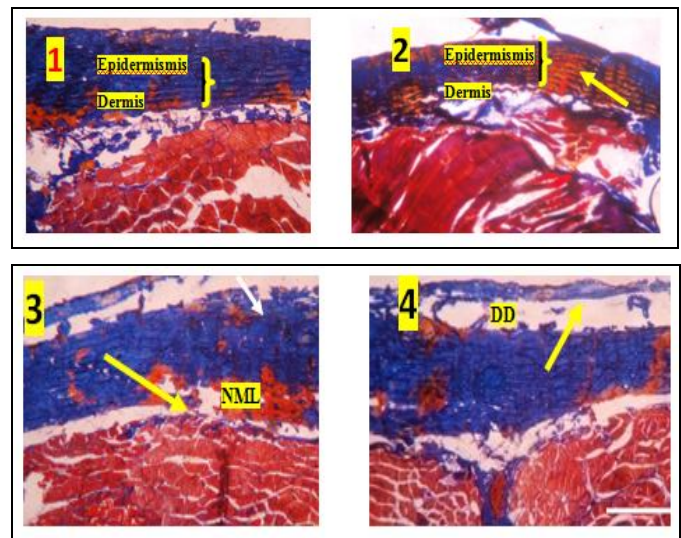


FIG.7: INFECTED SKIN SHOWS THE PATHOLOGICAL CONDITIONS OF EPIDERMIS AND DISTENDED DERMIS (DD) AND NECROTIZED MUSCULAR LAYER (NML).

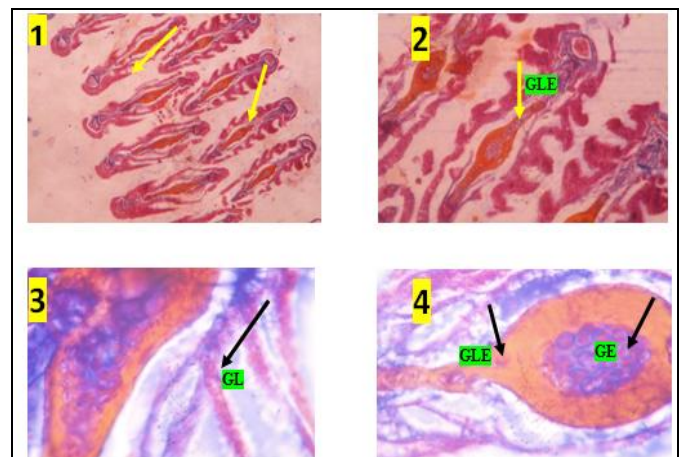


FIG. 8: CONTROL GILL SHOWS WITH GILL LAMELLAE (GL), LAMELLAR EPITHELIUM (GLE) AND GILL EPITHELIUM (GE).

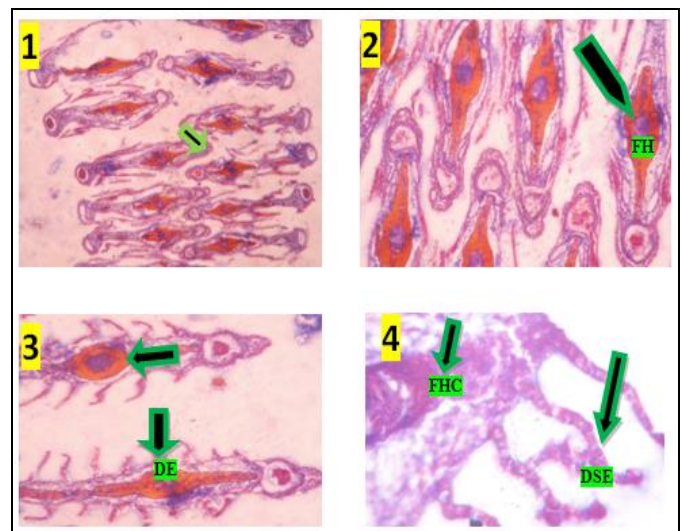


FIG. 9: INFECTED GILL LAMELLAE SHOWS WITH DEGENERATED EPITHELIUM (DE), FUNGAL HYPHAE (FH), ENCAPSULATED BY FUSIFORM HEPATOCYTES AND DISORGANIZATION OF SECONDARY GILL (DSE).

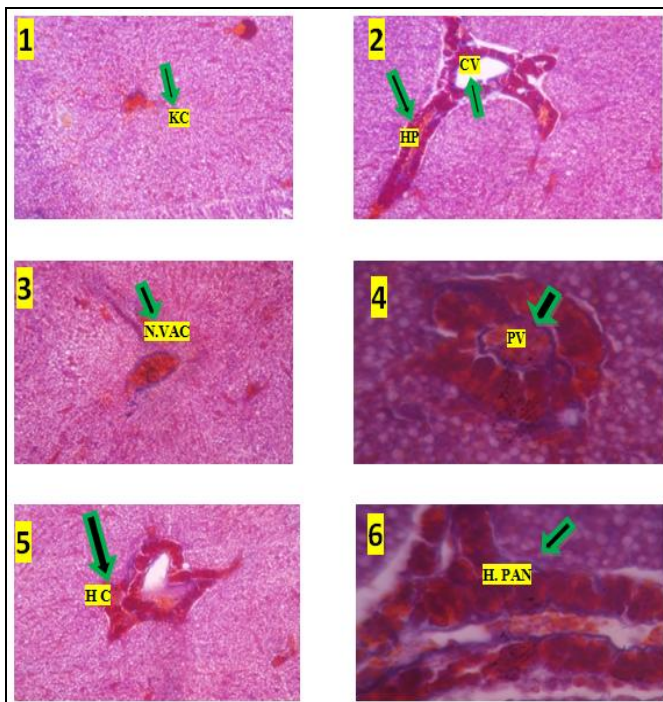


FIG. 10: CONTROL LIVER SHOWS WITH NORMAL HEPATOCYTES (HC), CENTRAL VEIN (CV), PORTAL VEIN (PV), HEPATO PANCREAS (HP), NORMAL VACUOLES (N.VAC) AND KUPFER CELLS (KC).

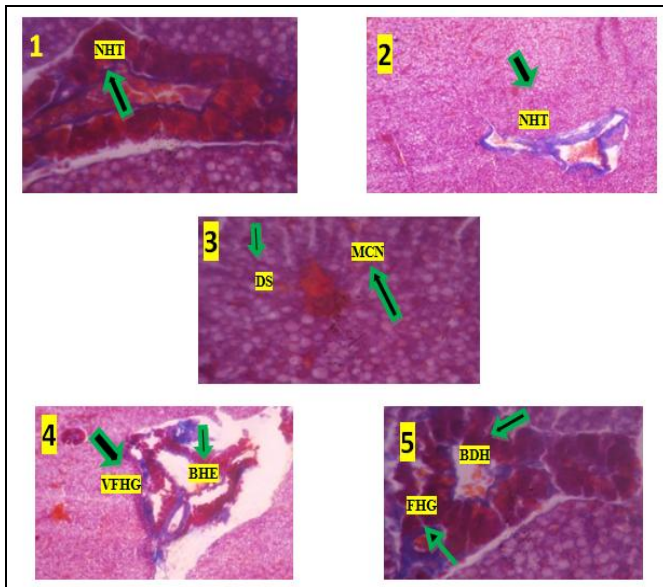


FIG.11: INFECTED LIVER SHOWS NECROTIZED HEPATIC TISSUE (NHT), BLOOD HEMORRHAGE (BHE), BILE DUCT HYPERPLASIA (BDH), DIALATED SINUSES (DS), MONOCELLULAR NECROSIS (MCN) AND VACUOLIZATION OF LIVER CELLS WITH FUNGAL HYPHAL GROWTH (VFHG).

CONCLUSION: During the present investigation it was concluded that the both *Aspergillus fumigatus* and *Aspergillus niger* were pathogenic fungi of *Channa striatus* causes for development of EUS charesterstics on fresh water fishes of Dharmasagar and Hasanparthy Lakes. This was

also observed during the study period these species of fungi may develop conidiophores inside the body as well as external surface. And the infection was observed in internal organs and on epidermis of Skin. Although wounds and lesions were also observed on epidermal surface of the body. According to review of literature the fungal and fungi-like pathogens of fish has highlighted present knowledge gaps that need to be rapidly filled if future epizootics are to be control.

It has also indicated that epidemiological elements arising from other non-fish specific fungal pathogens could be used to refine our true understanding of present and future ecological impacts of these types of pathogens on global fish diversity. Existing pathological data arising from fungal pathogen challenges of fish should be used to develop SEIR models (i.e., susceptible-exposed-infectious-recovered) specific to fungal pathogens and fish hosts. This would allow a simulation of the true extent of the ecological risk and provide elements for a better environmental monitoring and understanding of these species of fungal pathogens. These types of *Aspergillus fumigatus* and *Aspergillus niger* may be effects by fish even under the low temperature (20- 25°C) and stressed environmental conditions.

REFERENCES:

1. Pachade GR, Bhatkar NV, and Hande DV. Incidences of Mycotic infections in *Channa punctatus* of Wadali Lake, Amravati, MS, India. *International Research Journal of Biological Sciences*, 2014; 3: 47-50.
2. World Bank, India marine fisheries, issue opportunities and transition for sustainable developments Agriculture and rural development sector unit South Asia region, Report No. 2010; 54259: 109.
3. Rodolphe E. Gozlan, Wyth L. Marshall, Osu Lilje, Casey N. Jessop, Frank H. Gleason and Demetra Andreou. Current ecological understanding of fungal-like pathogens of fish: what lies beneath?. www.Frontiersin.Org; February 2014; (5) 62. 1.
4. Chauhan R, Lone SA and Beigh AH. Pathogenicity of three species of *Aspergillus* (*A. fumigatus*, *A. niger* & *A. sydowii*) on some fresh water fishes. *Life science leaflets*, 2014; 2: 65-72.
5. Chauhan, R. Studies on conidial fungi isolated from some fresh water fishes. *Int. j. of Advanced life sciences*, 2013; 6: 131-135.
6. Chauhan R. Studies on some fresh water fishes found infected with dermatomycoses, collected from different water bodies in and around Bhopal, India. *Indo American J. of Pharmaceutical Research* 2014a; 4 : 1591 -1596.
7. Iqbal and Ramsha.. Some pathogenic fungi parasitizing two exotic tropical ornamental fishes. *Int. J. Agric. Biol.*, 2013; 15: 595 – 598.

8. Srivastava RC. *Fish Mycopathology*. Today and tomorrow's Publishers New Dehli, India. 2009; 106.
9. Refai MK, Laila A, Mohamed M, Amany M Kenawy and Shimaa SMA: The assement of mycotic settlement of fresh water fishes in Egypt. *J. OF American Science* 2010; 6.
10. Willoughby, L. G., and Pickering, A. D. Viable Saprolegniaceae spores on the epidermis of the salmonid fish *Salmo trutta* and *Salvelinus alpinus*. *Trans. Br. Mycol. Soc.* 1977; 68, 91–95.
11. Jeney, Z., and Jeney, G. (1995). Recent achievements in studies on diseases of common carp (*Cyprinus carpio* L.). *Aquaculture* 129, 397–420.
12. Neish, G. A. and Hughes, G. C. *Disease of Fishes, Book6, Fungal Disease of Fishes*. Neptune, N J: T.F.H. Publications. 1980; 159.
13. Noga, E. J. "Fungal diseases of marine and estuarine fishes," in *Pathology of Marine and Estuarine Organisms*, eds J. A. Couch and J. W. Fournie (Boca Raton, FL: CRC Press), 1993; 85–110.
14. Bruno, D. W., Van West, P., and Beakes, G. W. "Saprolegnia and other oomycetes," in *Fish Diseases and Disorders. Viral, Bacterial and Fungal Infections*, Vol. 3, eds P. T. K. Woo and D. W. Bruno (Wallingford; Oxon: CABI Publishing), 2011; 599–659.
15. Ramaiah, N. A review on fungal diseases of algae, marine fishes, shrimps and corals. *Ind. J. Mar. Sci.* 2006; 35, 380–387.
16. Van West, P. *Saprolegnia parasitica*, an oomycete pathogen with a fishy appetite: new challenges for an old problem. *Mycologist*, 2006; 20, 99–104.
17. Gonçalves, A. A., and Gagnon, G. A. Ozone application in recirculating aquaculture system: an overview, ozone: science & engineering. *J. Int. Ozone Assoc.* 2011; 33, 345–367.
18. Hatai, K., and Hoshai, G. I. "Pathogenecity of saprolegnia parasitica coker," in *Salmon Saprolegniasis*, ed G. J. Mueller (Portland, OR: U. S. Department of Energy), 1994; 87–98.
19. Scarfe, A. D. "State, regional, national, and international aquatic animal health policies: focus for future aquaculture biosecurity," in *Biosecurity in Aquaculture Production Systems: Exclusion of Pathogens and Other Undesirables*, eds C. S. Lee and P. J. O'Bryen (Baton Rouge, LA: The World Aquaculture Society), 2003; 233–262.
20. Rehulka, J. "Prevention and therapy of fish diseases: fungal diseases," in *Diagnostics, prevention and therapy of fish diseases and intoxications*, eds J. Tesarèik and Z. Svobodová (Rome: FAO), 1991; 270–307.
21. Blazer, V. S., and Wolke, R. E. An exopholia-like fungus as the cause of a systematic mycosis of marine fish. *J. Fish Dis.* 1979; 2, 145–152.
22. Noga, E. J. "Fungal diseases of marine and estuarine fishes," in *Pathology of Marine and Estuarine Organisms*, eds J. A. Couch and J. W. Fournie (Boca Raton, FL: CRC Press), 1993; 85–110.
23. Vishwanath, T. S., Mohan, C. V., and Shankar, K. M. Epizootic Ulcerative Syndrome (EUS), associated with a fungal pathogen, in Indian fishes: histopathology – "a cause for invasiveness." *Aquaculture*, 1998; 165, 1–9.
24. Blazer, V. S., Volgelbein, W. K., Densmore, C. L., May, E. B., Lilley, J. H., and Zwerner, D. E. Aphanomyces as a cause of Ulcerative Skin Lesions of Menhaden from Chesapeake Bay tributaries. *J. Aquat. Anim. Health.* 2013; 11, 340–349.
25. Nsonga, A., Mfitilodze, W., Samui, K. L., and Sikawa, D. Epidemiology of epizootic ulcerative syndrome in Zambesi River system. A case study for Zambia. *Hum. Vet. Med.* 2013; 5, 1–8.
26. Catap, E. S., and Munday, B. L. Development of a method for reproducing epizootic ulcerative syndrome using controlled doses of *Aphanomyces invadans* in species with different salinity requirements. *Aquaculture*. 2002; 209, 35–47.
27. Sosa, E. R., Landsberg, J. H., Stephenson, C. M., Forstchen, A. B., Vandersea, M. W., and Litaker, R. W. *Aphanomyces invadans* and ulcerative mycosis in estuarine and freshwater fish in Florida. *J. Aquat. Anim. Health*, 2007b; 19, 14–26.
28. Whittington, R. J., and Chong, R. Global trade in ornamental fish from Australian perspective: The case for revised import risk analysis and management strategies. *Prev. Vet. Med.* 2007; 81, 92–116.
29. Forneris, G., Bellardi, S., Palmegiano, G. B., Saroglia, M., Sicuro, B., Gasco, L., et al. The use of ozone in trout hatchery to reduce saprolegniasis incidence. *Aquaculture*, 2003; 221, 157–166.
30. Rekha Chauhan, Zeeshan Nisar and Ashiq Hussian Baig. Studies on aspergillomycosis in *Labeo calbasu* found infected with *Aspergillus flavus* and *A. terreus*, *world journal of pharmacy and pharma. ceutical sciences*, 2014; 3: 1842-1848.
31. Laxmareddy B and Benarjee, G: Intestinal histopathology of trematode infected fish, *Channa striatus*. *Biolife* 2013; 1: 29-31.
32. Hussian MMA, Hassan WH and Mahmood MA. Pathogenicity of *Achlya proliferoids* and *Saprolegniadictina* (Saprolegniaceae) associated with saprolegniasis outbreaks in cultured Nile Tilapia (*Oreochromis niloticus*). *World J. of Fish and Marine Science*. 2013; 5:188-193.

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