

## Original Article

## Isolation of aspergillus fumigatus associated with skin lesions in channa striatus

Podeti Koteswar Rao\*

Department of Zoology, Kakatiya University, Warangal-506009, Telangana, India

## ARTICLE INFO

Received: 17 April 2024

Revised: 15 May 2024

Available Online: 31 June 2024

\*Corresponding author:

Podeti Koteswar Rao,  
Department of Zoology, Kakatiya  
University, Warangal - 506009,  
Telangana, India

## ABSTRACT

**Purpose:** The objective of this work was to isolate and identify *Aspergillus fumigatus* from naturally infected *Channa striatus*. A total of 300 cultivated *Channa striatus* were gathered from Dharmasagar Lake in Hanamkonda. The study found that 7.5% of the studied fish were infected, with the highest occurrence of the disease observed between the winter and spring seasons.

**Methods:** The fish that were infected displayed distinct ulcerative sores and fin rot. The oomycetes *Aspergillus fumigatus*, which resemble fungi, thrive on glucose peptone yeast (GPY) agar as colonies that are opaque and have an irregular white transparent velvet-like surface when kept at room temperature.

**Results:** The microscopic analysis revealed the presence of fungal growth, which showed elongated, branched hyphae with a tapered end. These hyphae were thin and lacked septa and contained cytoplasmic organelles. The fungal growth was stained with lactophenol cotton green. Rectangular spores were observed within the hyphae on sporulating media, joined by thin filaments.

**Conclusions:** In conclusions, EUS is an invasive disease of *Channa striatus* and care should be taken with low temperature in managing fish's lake.

**Keywords:** *Aspergillus fumigatus*; *Channa striatus*; Sporulation.

## Introduction

Recently, Epizootic Ulcerative Syndrome (EUS) has been reported as a very threatening disease that leads to significant losses in freshwater aquaculture systems in Telangana. The discovery of epizootic ulcerative syndrome (EUS) dates back to 1971 when it was initially identified in farmed freshwater ayu (*Plecoglossus altivelis*) in Japan [1]. The disorder known as EUS had a significant impact on estuarine fish, specifically grey mullet (*Mugil cephalus*), in Australia in 1972 [2]. The disease gradually propagated over the Asia-Pacific area and North America. Papua New Guinea saw an outbreak between 1975 and 1976 [3]. A similar illness to Eosinophilic Unidentified Syndrome (EUS) was documented on the East coast of the United States in 1980 [4]. *Aphanomyces invadans* was found in naturally

infected striped and thin lip grey mullet and African catfish *Clarias gariepinus* in Egypt [5] [6]. In April 2007, the ailment was officially identified as an epidemic of epizootic ulcerative syndrome (EUS) [7]. Certain fish species, such as common carp (*Cyprinus carpio*) and Nile tilapia (*Oreochromis niloticus*), are known to be resistant to EUS. This resistance is based on the fact that these fish have not been shown to be infected with the disease either in natural or experimental settings [8], [9]. The occurrence of EUS in freshwater fishes in Bangladesh has been linked to low temperature and is frequently observed following periods of severe rainfall [10]. The objective of this study was to isolate and identify *Aspergillus fumigatus* from *Channa striatus* and assess the seasonal prevalence of the resulting illness. The Ectoparasites

frequently infested the skin, fins, and gills of fish. Fish that are infested with ectoparasites typically exhibit distinct alterations, such as the presence of both minor and major skin lesions, as well as variations in skin pigmentation [12]. Ectoparasites commonly infect the gills, skin, and fins of fish, which are their primary body parts. *Trichodina* sp., *Dactylogyrus* sp., *Oodinium* sp., and *Epistylis* sp are several forms of ectoparasites that infect the gills, skin, and fins of snakehead fish [13]. An infection by an ectoparasite leads to changes in the gill filaments, which in turn disrupts the breathing process [14]. This fish's susceptibility to parasitic infection, including both ectoparasites and endoparasites, is due to its capacity to adjust to changing environmental conditions [15].

### **Materials and Methods**

**Fish samples:** A total of 300 cultured *Channa striatus* with an average body weight of  $300\pm 10$ g. Specimens were obtained at Dharmasagar lake in Hanamkonda. The specimens were gathered between May 2022 and April 2023. The captured fish were carried in big tanks equipped with an oxygen pump to maintain their vitality. The live fish were housed in meticulously prepared glass aquariums filled with dechlorinated tap water and aerated with oxygen. The recently deceased fish were labeled, packaged, and transported in refrigerated containers [16]. The samples were transferred to the wet lab of fish disease and management. The fish that were naturally infected were examined using microscopy, following the method described in reference [16].

**Culture Characters:** The fish samples were processed, and mycological investigation samples were collected from the skin and underlying musculature using strict aseptic techniques. These samples were then cultured in a glucose peptone yeast extract broth (GPY) medium. Employing the modified 5-stage culture technique described in reference [17] to isolate the probable fungus. The morphological characteristics of the hyphal growth on (GPY) agar, such as the overall appearance of the cultures, growth rates, texture, and surface color, were observed and documented in the text. The hyphal development is studied microscopically in a wet mount preparation to identify the putative fungus [18]. The hyphae of *Aspergillus fumigatus* were stained using lactophenol cotton green and observed under a microscope.

### **Detection of asexual characters of *Aspergillus fumigatus* according to (Hatai and Egusa, 1979)-**

An uncontaminated strain of *Aspergillus* species was obtained from *C. striatus* and cultivated at ambient temperature for 8 days on glucose yeast (GY) agar.

Subsequently, the hyphae were fragmented and transferred into a 1000 ml Erlenmeyer flask containing 500 ml of GY broth. The mycelia were gathered and rinsed two times with sterile tap water. The sterilized tap water, which contained sterilized punctured hemp seed, was used as a medium for spore formation. Washed mycelia were added to the medium and cultured at room temperature for 2 days. The process involves examining sporulating material and observing the expanding hyphae under a microscope to detect the presence of mature zoospores. Over a span of 6 weeks after sporulation, samples were collected every 48 hours to conduct microscopic examinations and study the growth of reproductive organs.

### **Result**

In this study, the infected fishes exhibited several observable symptoms, including redness on the skin surface, erosions, small and large ulcers, excessive mucous production, damaged fins, fin rot, congested and protruding anal aperture, and darkening of the body surface. Fishes with positive *Aspergillomycosis* exhibit clinical symptoms of damaged skin and superficial ulcers on their fins. In some cases, they may also have significant ulcers and bleeding on their fins and body surface (refer to Fig. A and Fig. B). Among the 300 *C. striatus* fish that were investigated, the overall infection rate was 7.6%. In regard to the health condition of the examined fish, approximately 10.25% of the 130 clinically diseased fish were found to have the disease. On the other hand, the apparently healthy fish showed no fungal isolation. In terms of seasonal prevalence, the disease was only observed in winter (25%) and spring (15%) (Fig. C). The cultural characteristics of *Aspergillus fumigatus*, isolated on glucose peptone yeast agar, exhibited opaque mycelia with an irregular white transparent velvety surface (Fig1. D). The hyphae initiate development on the agar plate on the third day after being cultured, and their size progressively increases until they completely cover the plate by the seventh day. Wet mount preparations of the growing cultures on GPY agar and broth showed the existence of elongated hyphae with tapered ends that were branching and lacked septa. The hyphae possessed a cytoplasmic organelle. The hyphae of *A. fumigatus* were stained with lacto phenol cotton green, resulting in some of them exhibiting a -y- shape appearance. After an extended period of incubation, the hyphae collected from the central region of the expanding colonies were thicker and had a wavy shape, while those acquired from the outside edges were thinner in the wet mount preparations from the media that promotes spore formation after 48 hours (Figures A, B, C & D).

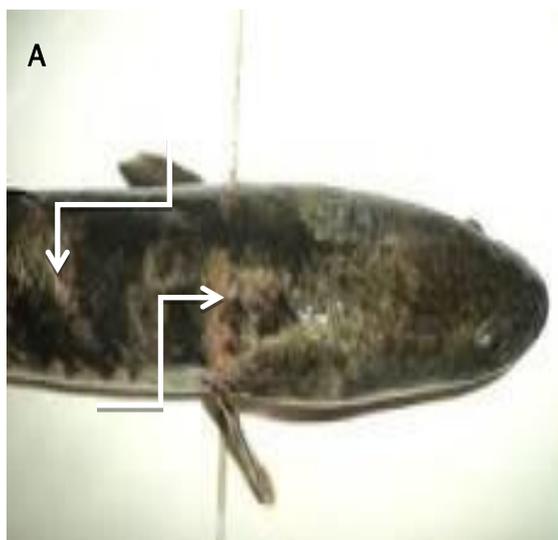


Fig. A

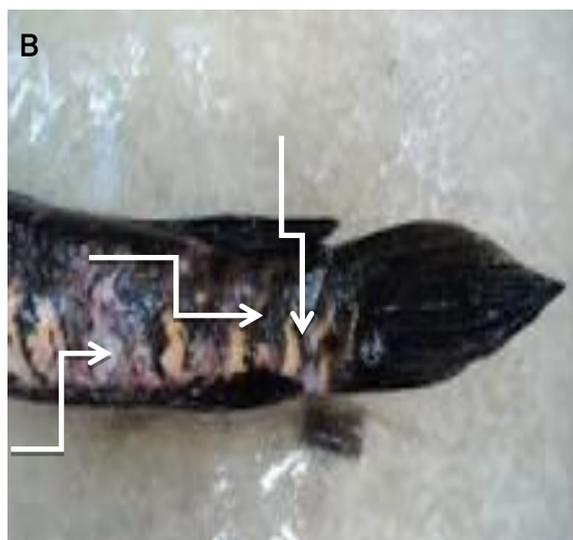
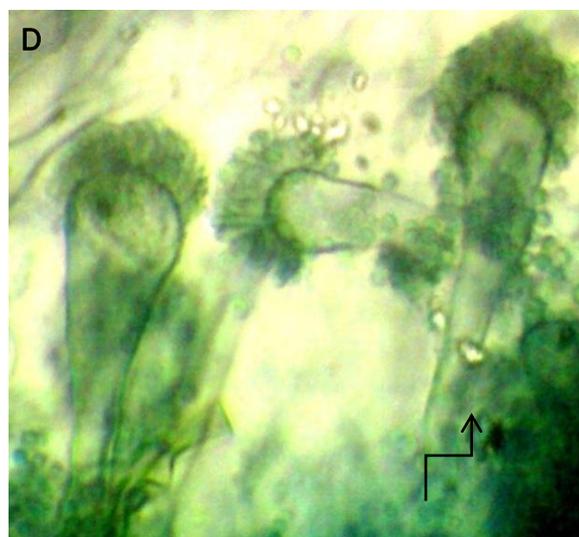


Fig. B



**Figure 1:** (A). naturally infected *C.striatus* with *A. fumigatus* showing multiple small ulcers with hemorrhagic border all over the body. (B). naturally infected *C. striatus* with *A. fumigatus* showing diffuse large ulcer with fin erosion. (C). growth of *A. fumigatus* on GPY agar revealing opaque mycelia with uneven white transparent velvets surface. (D). Mass of mycelia of branched non septated hyphae obtained from periphery of the culture.

### Discussion

The EUS is a term used to describe a seasonal outbreak of disease in freshwater and estuary warm water fishes. It is caused by a complex combination of infectious agents, with the presence of invasive *Aspergillus* spp. being a defining characteristic. A variety of causes have been suggested for the outbreak of EUS, with viruses, bacteria, parasites, and fungus being seen as the main culprits [19]. A novel ulcerative ailment in wild fish was documented for the inaugural time in Africa [7]. Regarding the overall prevalence, the infection rate among the studied *C. striatus* was 8.6%. The results were almost identical to those of [20], who reported a 10% prevalence of infection in *C.striatus*. Furthermore, the disease was documented in naini and

rohu, which are Indian big carps, with a prevalence rate of 97.1% [21]. Out of the fish that were clinically assessed for illness, 15.25% tested positive for *Aspergillus fumigatus*. *Aspergillus fumigatus* is capable of infecting *C. striatus*, however it primarily spreads among fish that are clinically unhealthy rather than those that appear healthy. *Aspergillus fumigatus* requires predisposing factors that impact the integrity of the skin in order to commence the infection. The seasonal occurrence of *Aspergillus* species infection in *C. striatus* was found to be 30% and 15% in winter and spring, respectively. No instances of infection were documented during the autumn and summer seasons. These findings are corroborated by [22], who discovered that the occurrence of the disease (EUS) is frequently associated with a decrease in water

temperatures. This resulted in a suppressed immunological response in the fish [7]. Similarly, in Bangladesh, EUS in freshwater fishes has been linked to cold temperatures and frequently follows periods of intense rainfall (Khan and Lilley, 2002). The variations in occurrence can be ascribed to the specific types of fish, the temperature and salinity of the water, as well as the presence of *Aspergillus fumigatus* fungi in *C. striatus* infected by *Aspergillus fumigatus*, which manifests in clinical symptoms such as excessive mucous secretion, dark gray discoloration of the skin, inflamed fins with hemorrhaging and superficial ulcers. Comparable results were documented in *C. striatus* [23], [24]. Furthermore, snakehead *Channa marulius* [25], Labeo, and Catla [26] exhibit symptoms such as reduced hunger, irregular swimming patterns, and the presence of red spots on their body surface. The *Aspergillus fumigatus* isolated on glucose peptone yeast agar exhibited opaque colonies with an uneven white transparent velvety surface, indicating infection with EUS based on its morphological characteristics. Multiple researchers [27], [28] reported similar findings. Upon microscopic examination of wet mount preparations from actively growing cultures on GPY agar and broth, it was seen that there were non-septated thin branched long hyphae with tapered ends. The hyphae possessed a cytoplasmic organelle. The findings of [29] are consistent with these data, which indicate that the *Aspergillus* spp. isolates had vegetative mycelium with a diameter ranging from 4 to 8 units. The mycelium was aseptate, smooth, slightly wavy, and moderately branching. The wet mount preparations of the sporulating media revealed that the sporangia were predominantly located at the tips of the hyphae, while the diameter of the hyphae remained constant. The spores were arranged in a rectangular fashion within the hyphae, forming chains that were connected to each other by thin filaments. This arrangement differed from that of *Saprolegnia*.

### Conclusion

The current investigation demonstrated that the analyzed *Aspergillus fumigatus* did not generate reproductive structures in the sterilized incubated tap water including hemp seed cultures. Similarly, a study demonstrated that the *Aspergillus* spp. culture had filamentous sporangia that were indistinguishable from hyphae. The main spore release and encystment occurred in a 'achlyoid' way, and no oogonia and antheridia were found. *Aspergillus* formed sporangia containing a single row of primary spores. These primary spores were finally discharged and encysted at the hyphal tip, generating spore-balls. This is a typical feature of the *Aspergillus* genus. The zoosporangia were slender and had the same diameter as the hyphae.

The strains seemed to be sterile and did not exhibit sexual reproduction.

### Funding

No financial assistance was provided for this project.

### Conflict of Interest

None declared.

### References

1. Egusa, S., Masuda, N., 1971. A new fungal disease of *Plecoglossus altivelis*. *Fish Pathol* 6, 41–46.
2. McKenzie, R.A., Hall, W.T., 1976. Dermal ulceration of mullet (*Mugil cephalus*). *Aust Vet J* 52, 230-231. Pradhan, P.K., Mohan, C.V., Shankar, K.M., Kumar, B.M., 2008. Infection Experiments with *Aphanomyces invadans* in Advanced Fingerlings of Four Different Carp species. *Diseases in Asian Aquaculture* 105-114.
3. Haines, A.K., 1983. Fish fauna and ecology. In: Petr T (ed) *The Purari—tropical environment of a high rainfall river basin*. Dr W. Junk Publ, The Hague, pp 367–384.
4. Hargis, W.J.J., 1985. Quantitative effects of marine diseases on fish and shellfish populations. *Trans North Am Wild Nat Resource Conf* 50, 608–640.
5. Shaheen, A.A., El sayed, E., Faisal, M., 1999. Isolation OF *Aphanomyces* SP(P). associated with Skin Lesions and Mortalities in the Striped (*Mugil Cephalus*) and the Thin Lip (*Liza ramada*) grey mullets. *Bull. Eur. Ass. Fish Pathol* 19, 79.
6. Amany, A.A., Shaheen, A.A., Abdel-Latif, A.M., 2004. *Aphanomyces* In African Catfish "*Claris gariepinus*". First Scientific Conference of Fac.Vet. Med.; Moshtohor, Sept. 1-4, Benha-Ras sedr.
7. Huchzermeyer, K.D., van der Waal, B.C., 2012. Epizootic ulcerative syndrome: exotic fish disease threatens Africa's aquatic ecosystems. *J S Afr Vet Asso.* 83, 204.
8. Anon, 2010. Manual of diagnostic tests for aquatic animals. World Organization for Animal Health, Paris, viewed in 2011.
9. Khan, M.H., Lilley, J.H., 2002. Risk factors and socio-economic impacts associated with epizootic ulcerative syndrome (EUS) in Bangladesh. p. 27-39. In: J.R. Arthur, M.J. Phillips, R.P. Subasinghe, M.B. Reantaso and I.H. MacRae.
10. K.A. Yildiz, Kumantas. *J. Vet. Med.*, 57 (2002).
11. B. Salam, D. Hidayat. *J. Sains dan Seni ITS.*, 6 (2017).
12. A. Chaudhary, C. Harenram, S.S. Hridaya SS. *Bioinvasions Rec.*, 6 (2017).
13. Nurliza Zaiyana et.,al 2021. E3S Web of Conferences 339, 01001 (2022)

<https://doi.org/10.1051/e3sconf/202233901001>  
10th ICMR-2ndINSAEF 2021.

14. Huet, M., 1986. Textbook of fish culture Breeding and cultivation of fish. 2 editions. Chapter XVI: Harvesting the fish: Section IV: Transport of fish. Blackwell Science Ltd. Combridge. pp 406.
15. Amlacher, E., 1970. Textbook of fish diseases. T.F.S. publication, Jersey, USA, 117-135.
16. Willoughby, L.G., Roberts, R.J., 1994. Improved methodology for isolation of the *Aphanomyces* fungal pathogen of epizootic ulcerative syndrome (EUS) in Asian fish. *J. Fish Dis.* 17, 541-543
17. Dovorak, J., Atecenasek, M., 1969. Mycological diagnosis of animal dermatophytosis. *Academia* 213.
18. Kamilya, D., Baruah, A., 2014. Epizootic ulcerative syndrome (EUS) in fish: history and current status of understanding. *Rev Fish Biol Fisheries* 24, 369–380.
19. Amany, A.A., Shaheen, A.A., Abdel-Latif, A.M., 2004. *Aphanomyces* In African Catfish "*Claris gariepinus*". First Scientific Conference of Fac.Vet. Med.; Moshtohor, Sept. 1-4, Benha-Ras sedr.
20. Baidya, S., Prasad, A., 2013. Prevalence of epizootic ulcerative syndrome (EUS) in carps. *Nepalese Journal of Zoology* 1, 41.
21. Baldock, F.C., Blazer, V., Callinan, R., Hatai, K., Karunasagar, I., Mohan, C.V., BondadReantaso, M.G., 2005. Outcomes of a short expert consultation on epizootic ulcerative syndrome (EUS): Re-examination of casual factors, case definition and nomenclature. pp. 555-585.
22. Abd el-Latif, A., 2003. *Aphanomyces* in freshwater fish and cray fish. MVSs Thesis (Fish diseases and management) Fac. of Vet. Med. Zagazig University (Benha Branch).
23. Saylor, R.K., Miller, D.L., Vandersea, M.W., Bevelhimer, M.S., Schofield, P.J., Bennett, W.A., 2010. Epizootic ulcerative syndrome caused by *Aphanomyces invadans* in captive bulls eye snakehead *Channamarulius* collected from south Florida, USA. *Diseases of Aquatic Organisms* 88, 169–175.
24. Afzali, S.F., Hassan, M.D., Abdul-Rahim, A.M., Sharifpour, I., Sabri, J., 2013. Isolation and Identification of *Aphanomyces* species from natural water bodies and fish farms in Selangor, Malaysia. *Malays. Appl. Biol.* 42, 21-31.

**Copyright:** ©2024 Rao. This article is distributed under the terms of the Creative Commons Attribution 4.0 International License [<http://creativecommons.org/licenses/by/4.0/>], which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author[s] and the source, provide a link to the Creative Commons license, and indicate if changes were made.



**Internationale Pharmaceutica Scientia** is a renowned international journal specialized in publishing research and review articles exclusively of pharmacy and allied health science domain.

We promote research among students and young scientist by providing them with special discounts and assist them in article writing and editing.

**Submit your articles at:**

[editor.ijpp@edwiserinternational.com](mailto:editor.ijpp@edwiserinternational.com) or [edwiser.ijpp@gmail.com](mailto:edwiser.ijpp@gmail.com)