



Received on 07 February, 2017; received in revised form, 26 July, 2017; accepted, 11 August, 2017; published 01 September, 2017

## THE ASSOCIATION OF DIFFERENT FUNGAL SPECIES CAUSED EPIZOOTIC ULCERATIVE SYNDROME IN FRESH WATER FISH OF CHANNA PUNCTATUS FOUND IN HASANPARTHY LAKE, TELANGANA, INDIA

P. Koteswar Rao \*

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

### Keywords:

*Rhizopus stolonifer*,  
*Penicillium chrysogenum*,  
*Channa punctatus*, EUS

### Correspondence to Author:

**P. Koteswar Rao**

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


**E-mail:** kotesw\_rao37@yahoo.co.in

**ABSTRACT:** Murrels are very often affected by Epizootic Ulcerative Syndrome (EUS) and it causes huge loss to the capture and culture sector. The principal infectious agent of this infestation is a fungal species, *Aphanomyces invadans*. Different fungal species were isolated from EUS infected *Channa punctatus* fish from Hasanparthy Lake of Warangal, Telangana, India. *Aspergillus flavus*, *Fusarium solani*, *Rhizopus stolonifer*, *Aspergillus fumigatus*, *Penicillium chrysogenum*, *Aspergillus niger* and *Trichoderma viridae* were most isolated groups. Isolates have been obtained and ability to induce dermal lesions in infectivity studies make fungi is one of the likely primary aetiological agents that could be triggering the initiation to EUS. Fungi was observed as persistent infections in fishes evidenced by their presence in cell culture also require further investigations for their role in predisposing the freshwater fish of *C. punctatus*. The environmental conditions were also found in fluctuation during the winter season. In the present study isolation of fungal pathogens from infected fish like *Aspergillus flavus*, *Fusarium solani*, *Rhizopus stolonifer*, *Aspergillus fumigatus*, *Penicillium chrysogenum*, *Aspergillus niger* and *Trichoderma viridae* had shown its presence in fungal incidence percentage throughout the year.

**INTRODUCTION:** Fungus has been reported to cause serious diseases in Estuarine and fresh water fishes in Australia, Japan and throughout South Asia <sup>1</sup>. Fungal diseases are the second most serious cause of loss in aquaculture Mayer <sup>2</sup>. Fungal disease in Coho Salmon (*Oncorhynchus kisutch*) caused 50% annual mortality <sup>3, 4</sup>. The effect of these infections on sizes in freshwater ecosystem. In India fishermen is facing serious problems with fungal diseases.

The emergence and spread of aquatic freshwater diseases are the major conservation concern <sup>5</sup>. One of the aquatic diseases implicated in mass mortalities of cultured and wild fish in various countries is epizootic ulcerative syndrome (EUS) <sup>6</sup>. And also called as mycotic granulomatosis, red spot disease and ulcerative mycosis. EUS is caused by the fungus-like oomycete *Aphanomyces invadans* and can cause significant ulceration of the skin, necrosis of muscle with extension to subjacent structures including abdominal cavity and cranium, and leading to mortality in many cases <sup>7, 8</sup>.

Therefore diseases cause high mortality, which results in economic loss <sup>9</sup>. The study of fungal infections in fresh water fish is of great significance as it would be helpful in knowing the diversity and

<p><b>QUICK RESPONSE CODE</b></p> 	<p><b>DOI:</b> 10.13040/IJPSR.0975-8232.8(9).3830-36</p>
<p>Article can be accessed online on: <a href="http://www.ijpsr.com">www.ijpsr.com</a></p>	
<p>DOI link: <a href="http://dx.doi.org/10.13040/IJPSR.0975-8232.8(9).3830-36">http://dx.doi.org/10.13040/IJPSR.0975-8232.8(9).3830-36</a></p>	

life cycle of fungus. The pathogenesis of *Aspergillus fumigatus* and *Aspergillus niger* has been reported in fresh water fishes<sup>10-13</sup>. The aim of present study was to assess the presence of pathogenic fungi on *Channa punctatus* reared in earthen lakes and research fish farms in Warangal district.

**MATERIAL AND METHODS:** In Warangal district of Telangana there are several fish rearing lakes where the farmers are rearing fry and fingerlings of Indian murrels. For the present study,

diseased fish samples were collected from the freshwater lakes during the period *i.e.*, 2012 - 2014. The EUS infected fishes were collected randomly from different rearing lakes of the study area. The diseased fishes exhibit clinical signs of the infections when selected. Fishes were alive when collected from the lake of Hasanparthy. Fish samples of *Channa punctatus* (Plate - I **Fig. 1** and **2**) affected with red spot disease and tail and fin rot were collected in two years both in 2012- 2013 and 2013- 2014.



**FIG. 1: CONTROL C. PUNCTATUS**

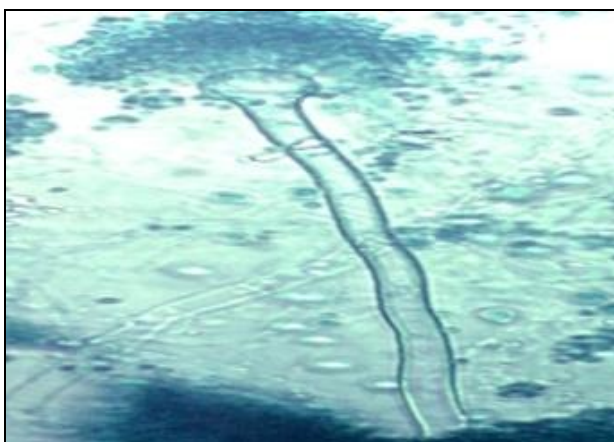


**FIG. 2: INFECTED C. PUNCTATUS**

**Preparation of Fungal Culture and Isolation:**

Small pieces were taken from the lesions (Skin, Liver, Gill and Pancreas) and cultured for the isolation of fungus. Potato Dextrose Agar (PDA); Corn Meal Agar (CMA) were used as media. The tissues were transferred in to set of Petri dishes. Petri dishes were incubated at 28 °C - 30 °C for three days in inverted form until a circular fungal mat developed, which was used for sub culture of fungus.

Identification of fungi was carried out according to<sup>14, 15</sup>. The following fungal culture results were obtained from NCFT (National Centre of Fungal Taxonomy) New Delhi. The fungal species found are *Aspergillus flavus* (6630.15), *Fusarium solani* (6633.15), *Rhizopus stolonifer* (6631.15), *Aspergillus fumigatus* (6627.15), *Penicillium chrysogenum* (6632.15), *Aspergillus niger* (6629.15) and *Trichoderma viridae* (6628.15) (Plate II. **Fig. 3 - 16**).



**FIG. 3, 4: ASPERGILLUS FLAVUS**



FIG. 5, 6: *FASUARIUM SOLANI*

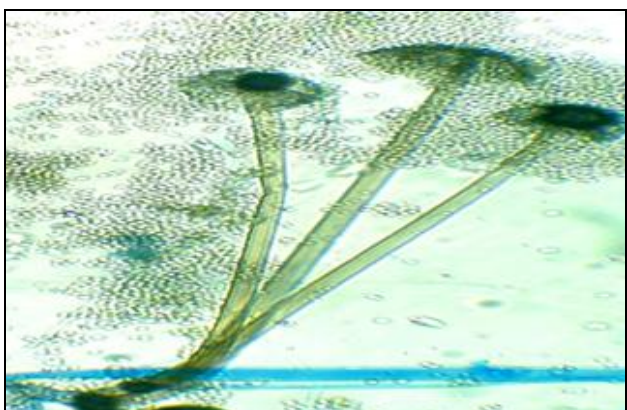


FIG. 7, 8: *RHIZOPUS STOLONIFER*

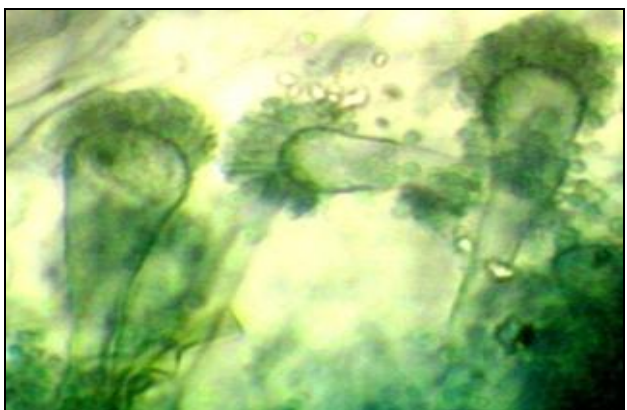


FIG. 9, 10: *ASPERGILLUS FUMIGATUS*

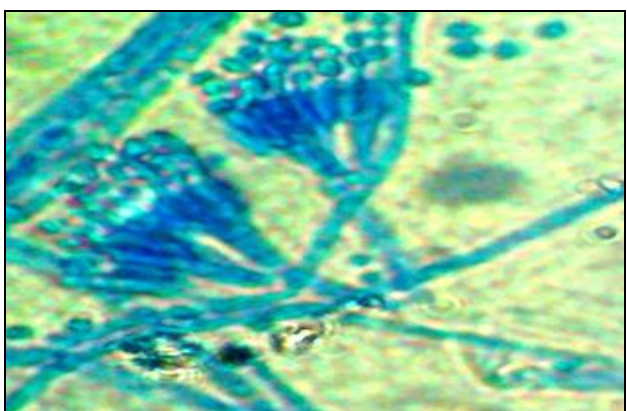
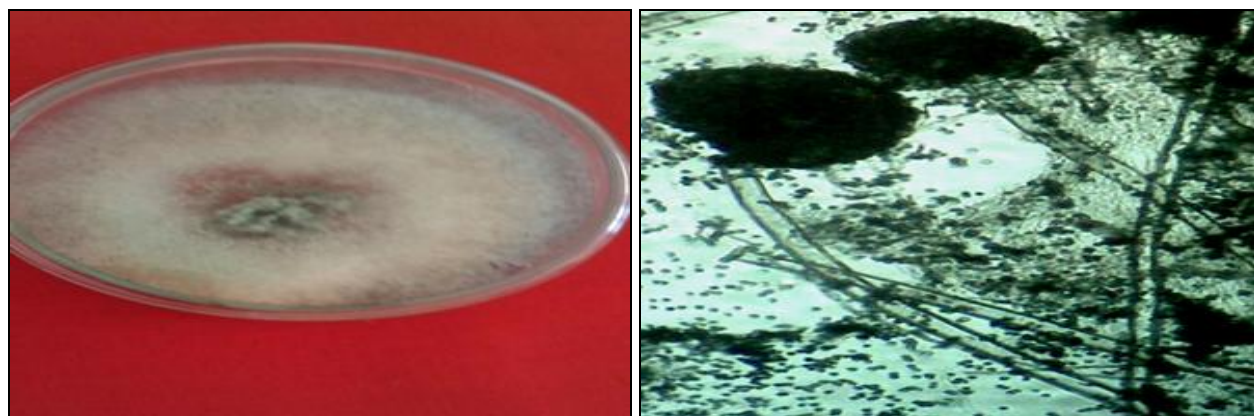
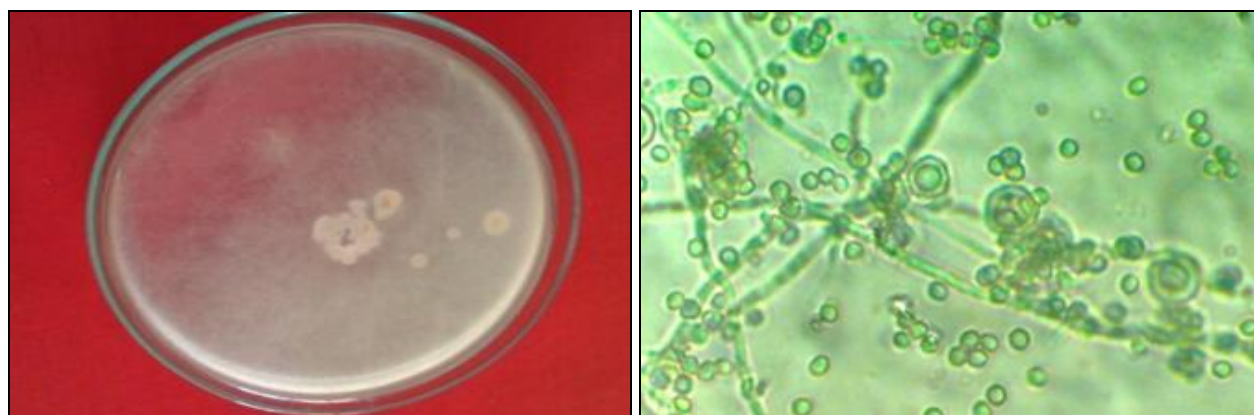


FIG. 11, 12: *PENICILLIUM CHRYSOGENUM*

FIG. 13, 14: *ASPERGILLUS NIGER*FIG. 15, 16: *TRICHODERMA VIRIDAE***RESULTS:**

***Aspergillus flavus*:** The infection of *Aspergillus* sp in the fishes was very common during the present study. Infection of *A. flavus* categorically was more from October to December in *C. punctatus*. However, these fungi were reported even up to February. It was not reported in *C. punctatus* between April and June.

***Fusarium solani*:** The incidence of *Fusarium solani* infection was at the peak (11%) during the month of October in *C. punctatus*. The infection starts to appear from the month of July and it prevails up to January in *C. punctatus*. While no incidence was observed between March to June.

***Rhizopus stolonifer*:** The infection of *R. stolonifer* was reported from August to February in *C. punctatus*. The maximum average incidence (11%) was observed in the month of December in *C. punctatus*. Whereas in *C. punctatus* no incidence of fungus was observed between April and June.

***Aspergillus fumigatus*:** The infection of *Aspergillus fumigatus* in the fishes was very common during the present study. *A. fumigatus* was more during

November to January in *C. punctatus*. *A. fumigatus* was more during September to January. However these fungi were reported even up to February. It was not reported in *C. punctatus* during April to June.

***Penicillium chrysogenum*:** The incidence of infection in *C. punctatus* appears from the month of July in *C. punctatus* and lasts up to the month of February. Whereas in *C. punctatus* no infection was observed during the March to June.

***Aspergillus niger*:** Maximum average incidence *i.e.* (19%) of *A. niger* was reported during the month of December in *C. punctatus*. It is noticed that no incidence of *A. niger* was observed during the month of April to June.

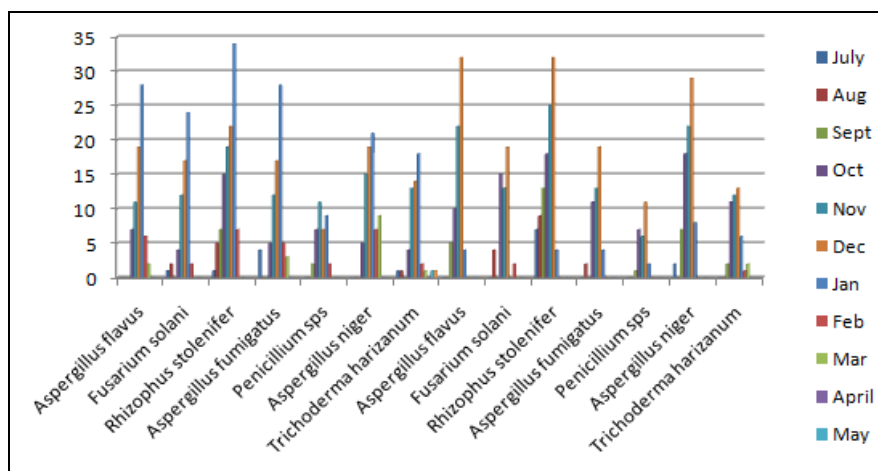
***Trichoderma viridae*:** From the study of fungal incidence it was observed that *T. viridae* prevails throughout the year except in the month of May and June in the case of *C. punctatus*. The maximum incidence (11%) was reported during the month of November. It was more or less equally reported during the month of August, September, October, December and January in infected *C. punctatus*.

**TABLE 1: AVERAGE INCIDENCE PERCENTAGE OF INFECTION BY FUNGAL SPECIES IN CHANNA PUNCTATUS FROM HASANPARTHY LAKE DURING THE YEAR 2012 - 2014**

S. no.	Fungi Isolated	Fungal species incidence % per month 2012 - 13											
		July	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	April	May	June
1	<i>Aspergillus flavus</i>	0	0	0	7	11	19	28	6	2	0	0	0
2	<i>Fusarium solani</i>	1	2	0	4	12	17	24	2	0	0	0	0
3	<i>Rhizophus stollenifer</i>	1	5	7	15	19	22	34	7	0	0	0	0
4	<i>Aspergillus fumigatus</i>	4	0	0	5	12	17	28	5	3	0	0	0
5	<i>Penicillium</i> sps.	0	0	2	7	11	7	9	2	0	0	0	0
6	<i>Aspergillus niger</i>	0	0	0	5	15	19	21	7	9	0	0	0
7	<i>Trichoderma viridae</i>	1	1	0	4	13	14	18	2	1	0	1	1
<b>2013 - 14</b>													
8	<i>Aspergillus flavus</i>	0	0	5	10	22	32	4	0	0	0	0	0
9	<i>Fusarium solani</i>	0	4	0	15	13	19	0	2	0	0	0	0
10	<i>Rhizophus stollenifer</i>	7	9	13	18	25	32	4	0	0	0	0	0
11	<i>Aspergillus fumigatus</i>	0	2	0	11	13	19	4	0	0	0	0	0
12	<i>Penicillium</i> sps.	0	0	1	7	6	11	2	0	0	0	0	0
13	<i>Aspergillus niger</i>	2	0	7	18	22	29	8	0	0	0	0	0
14	<i>Trichoderma viridae</i>	0	0	2	11	12	13	6	1	2	0	0	0

Most of these fungal species were abundant in the month of January in *Channa punctatus* collected from Hasanparthy Lake during the year 2012 - 13. A similar trend was observed during 2013 - 14

years in the Hasanparthy Lake where all the fungal species were dominated in the month of January. The results are tabulated in (Table 1 and Fig. 17).



**FIG. 17: AVERAGE INCIDENCE (%) OF INFECTION BY FUNGAL SPECIES IN CHANNA PUNCTATUS FROM HASANPARTHY LAKE DURING THE YEAR (2012 - 2014)**

**DISCUSSION:** The quality of water is the first and most important limiting factor in existence of fish in any ecosystem. The fungi can spread rapidly among fish population and the fungal spores dispersed with water currents<sup>16</sup>. The first time reported on fungal infection in fish during the mid eighteenth century<sup>17</sup>. The fungi have wide range of infection, depending on the management of farm and environment<sup>14</sup>, several pathogenic fungi from different species of fish<sup>18</sup>. This was supported by field level studies with pathology based investigations in Bangladesh. Where it has been reported that the major carps are the most significantly affected farmed fish and once on

outbreak in carp lakes, EUS can damage the entire crop<sup>19</sup>.

Fish feed stored under tropical conditions is contaminated with *Aspergillus flavus* hence the toxins produced by the fungus may be deposited on feed pellets<sup>20</sup>. If such contaminated feed is consumed by the fish, it may cause deleterious effects which may lead to mass mortality<sup>21</sup>. There are some reports on fungal infections in commercial fishes<sup>22</sup>. *A. niger* from common carp its eggs and environment causes to internal and external infection in fishes. *Fasarium* sp., *Pencillium* sp., *Saprolegnia* sp. and *Mucor* sp. were

reported in the eggs of *Aspencer percicus*<sup>23</sup>, according to<sup>24</sup> *Penicillium* sp., *Aspergillus* sp. and *Rhizopus* sp. are the normal Mycoflora present in fresh water fishes. Fungal load increases significantly during storage period of feed at high moisture levels in ground and tree nuts<sup>25</sup>.

Fish diseases caused by the *Aeromonas* and *Aspergillus* are considered to be the major bacterial and fungal problems causing heavy mortality, reduced production and low quality of aquatic organisms. Both *Aeromonas* sp. and *Pseudomonas* sp. has been implicated in severe outbreaks among *Oreochromis niloticus* in fish hatcheries<sup>26</sup>.

Out breaks of fungal diseases such as *Aspergillus flavus*, *Fusarium solani*, *Rhizopus stolonifer*, *Aspergillus fumigatus*, *Penicillium chrysogenum*, *Aspergillus niger* and *Trichoderma viridae* were usually associated with changes in the environmental conditions. Stress factors, including overcrowding, low temperature, transfer of fish, low dissolved oxygen, poor nutritional status and parasitic damage of the epidermis, contribute to physiological changes and high susceptibility to infection.

In the present study, red spot disease is recorded in both first and second cycles of fish rearing, during the year 2012 - 2013 and 2013 - 2014 in Hasanparthy, Lake in Warangal district, Telangana, India. The red spot disease is high during in the winter season than the summer season. Low temperature, poor nutrients and poor oxygen were also noticed in the lakes and this clearly indicates the deterioration of water quality in the lakes. The water parameters might have induced the fungal infections causing red spot disease as well as tail and fin rot in the fry and fingerlings of murrel fishes.

**CONCLUSION:** The present study, showed the Average incidence percentage of infection by fungal species in *Channa punctatus* from Hasanparthy Lake during the year 2012 - 2014. Contribution of different fungal species *Aspergillus flavus*, *Fusarium solani*, *Rhizopus stolonifer*, *Aspergillus fumigatus*, *Penicillium chrysogenum*, *Aspergillus niger* and *Trichoderma viridae* in different tissues of teleost fishes seems to be fluctuating owing to the potential hazard of

*Aspergillus* sp. Aqua farmers should be educated on how to observe clinical signs of fish disease and stress and control/ preventive measures to be taken as a remedial action. Aqua farmers also need to follow good management practices.

**ACKNOWLEDGMENTS:** The authors would like to thank the Department of Zoology, Kakatiya University, Warangal, for providing laboratory facilities.

**CONFLICTS OF INTEREST:** Nil.

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**How to cite this article:**

Rao PK: The association of different fungal species caused epizootic ulcerative syndrome in fresh water fish of *Channa punctatus* found in Hasanparthy lake, Telangana, India. Int J Pharm Sci Res 2017; 8(9): 3830-36. doi: 10.13040/IJPSR.0975-8232.8(9).3830-36.

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