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STUDIES ON HEMATOLOGICAL AND HISTOLOGICAL MANIFESTATIONS OF *CHANNA MARULIUS* (Ham.) FOUND INFECTED WITH FUNGI, *ASPERGILLUS SPP.*

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ABSTRACT

Present study deals with the hematological and histopathological alterations in *Channa marulius* found infected with fungi *Aspergillus spp.* Fishes have been collected from local water bodies and fish markets of Bhopal . Isolated fungi from infected specimens of fishes were identified as Aspergillus spp. Considerable changes have been observed in the mean values of blood parameters. Hemoglobin content, RBCs ,Percentage of Eosinophils and Monocytes were found significantly decreased with (30.6%) , (44.3%) , (14.5%) and (19.7%) respectively. WBCs , Neutrophils and Lymphocytes were found significantly increased to (48.8%) , (38.1%) and (20.9%) respectively. Histopathologically various types of destruction were observed in skin and muscles of infected fish . Penetrating fungal hyphae were observed in muscles and complete necrotization of muscles was observed with formation of mycotic fibrillar granulomas.

Key words :- *Channa marulius, Aspergillus spp.,* hematology, histopathology and granulomas.

INTRODUCTION

Channa marulius (Ham.) is a fresh water ornamental fish found susceptible to mycotic infection . *Aspergillus* spp. is a pathogenic group of fungi causing infection in fishes . Pathogenesis of *Aspergillus spp.* have been reported in fresh water fishes by various workers like, [15,16,18,19, 22 and 6].Mycotic infection leads to changes in hematological parameters of fish have been reported earlier by [9,10,26,2,1and 17] Histopathological studies reveals the parasitic ability of fungi to infect the fish and it also helps to find out the extent of infection. Reports on histology of mycotic infected tissue are rare , only few workers [7, 8,20 ,5 and 6] reported histopathological manifestations in the tissue of mycotic infected fresh water fishes. Studies about parasitism of *Aspergillus* on fresh water fishes and especially the reports of hematology and histology of infected fish are not well documented. In present study an attempt has been made to find out the physiology of *Aspergillus* infected *C.marulius* by studying various hematological parameters as well as to find out the changes in skin and underlying musculature with the help of histological studies.

MATERIALS AND METHOD

Collection of fishes:

For this study a total number of 46 mycotic infected *Channa marulius* were collected from different water bodies and fish markets of Bhopal. Fishes were brought to the laboratory for further examination.

Preparation of fungal cultures:
Fungal cultures were prepared by taking small innocula from different infected portions of fish body. Cultures were prepared on Sabourad Dextrose Agar (SDA) and Corn Meal Agar (CMA). Growth was observed by incubating them at temperature 28-30°C.From all the studied isolates 27 were recorded as *Aspergillus spp.*.Cultures were identified with the help of keys of [18 and 24].

Hematological Examination:
For hematological examination six healthy and six infected fishes were used. Blood was drawn from caudal peduncle into Di Potassium EDTA containing tube by the process as described by (Hrubc & smith2000). RBCs and WBCs were counted by haemocytometer and values were calculated as 10^6/mm^3 and 10^3/mm^3 (Wintrobe,1967). Hemoglobin content was determined by using hemoglobin test kit (DIAGNOVA, Ranbaxy, India). The blood film was prepared and stained with Giemsa stain for morphology, micrometry and differential count of leucocytes. All the values of healthy and infected fishes were analyzed by students't' test.

Histological Examination:
For histological examination, tissues of five infected fishes were examined. Tissues were fixed in aqueous Bouin's fluid for 48-72 hours .The tissues were then processed routinely and prepared into paraffin blocks. The blocks then cut into 4-6µm thickness and stained with haematoxylin and eosin. Slides were observed under microscope to study the changes. Standard histological methods of [21] were followed for investigations.

RESULT AND DISCUSSION

A total number of twenty seven specimens of *Channa marulius* were found infected with fungi *Aspergillus spp.* (Fig.1-4). Hematological examination of infected fishes showed variation in parameters.

Figure-1 & 2. Infected *C.maurilus* with *Aspergillus spp.* hyphae on its head region

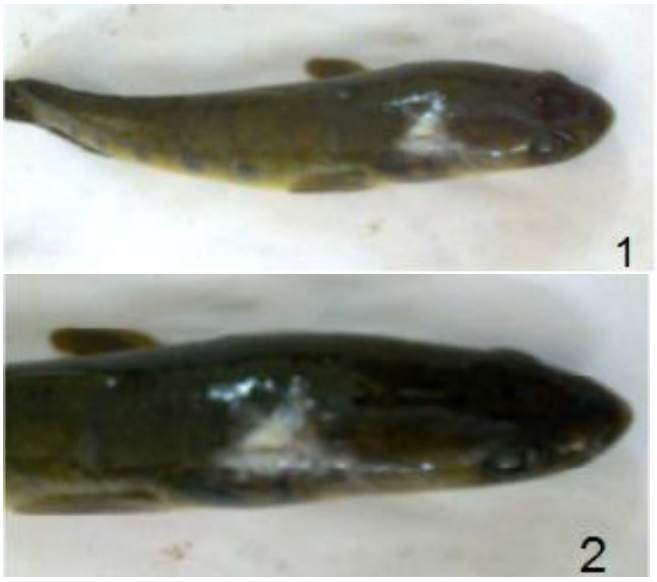


Figure-3 & 4. *Aspergillus spp*. culture on Sabourad Dextrose Agar (SDA) & Conidia releasing spores

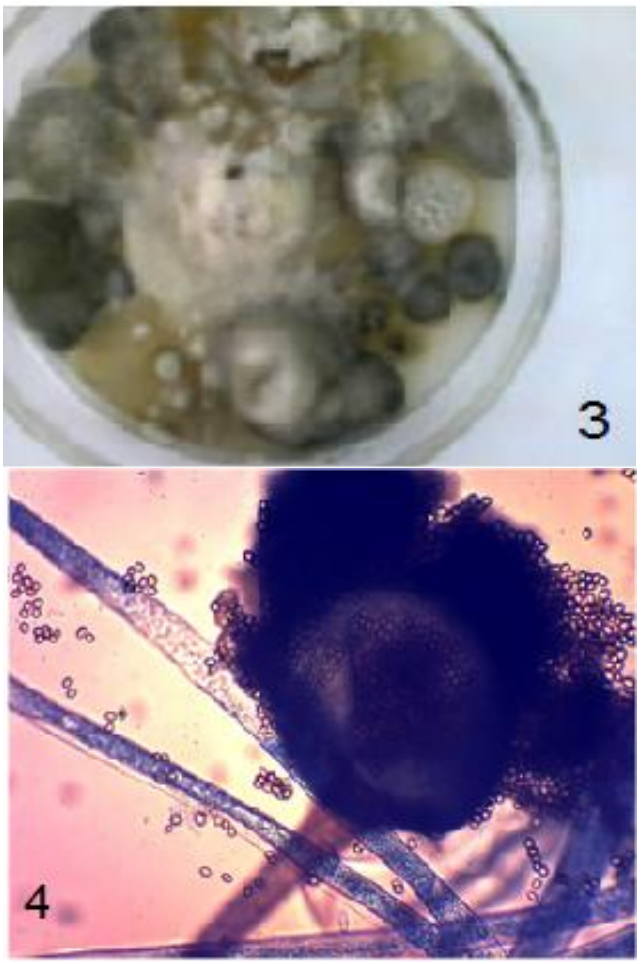


Table-1. Haematological parameters of *Channa marulius* (Ham.) Infected with fungi, *Aspergillus spp.*

S.No.	Parameters	units	Normal fish	*Aspergillus* infected fish	Percentage change	Significance
1.	Haemoglobin	g/100 ml	12.13± 0.12	8.42 ± 0.92	- 30.6 %	P < 0.05
2.	RBC count	10^6/mm^3	4.59 ± 0.16	2.56 ± 0.24	- 44.3 %	P < 0.05
3.	WBC count	10^3/mm^3	3.97 ± 1.04	7.74 ± 0.16	+48.8 %	P < 0.05
	Differential count of leucocytes					
1	Neutrophils	%	18.06± 1.41	29.0 ± 1.4	+ 38.1 %	P < 0.05
2	Eosinophils	%	73.08± 1.15	62.5 ± 0.5	- 14.5 %	P < 0.05
3	Lymphocytes	%	2.65 ± 0.1	3.35 ± 0.16	+ 20.9%	P < 0.05
4	Monocytes	%	1.72 ± 1.2	2.14 ± 0.8	- 19.7 %	P < 0.05

Mean values ± SE , (-) Decrease, (+) Increase.

Hemoglobin content:
In normal fish hemoglobin content was found to be 12.13±0.12gm/100ml while in infected fish there was observed a significant decrease ie.8.42±0.92gm/100ml.

Total RBCs:
The mean values of erythrocytes in healthy fish were found to be 4.59±0.16 10^6/mm^3 which is significantly decreased down to 2.56±0.24$10^6$/mm^{3}in infected fish.

Total WBCs:
In healthy fish the mean values of White blood cells were found to be 3.97±1.0410^3/mm^3 and in infected fish values were significantly increased to 7.74±0.16 10^3/mm^3.

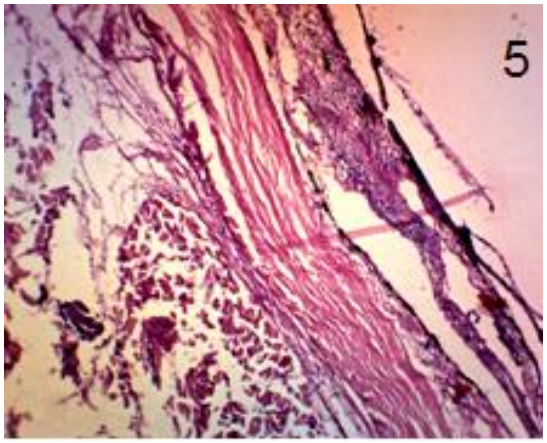
Differential count of leucocytes:
The mean value percentage in healthy fish was found to be 18.06±1.41 which raised to 29±1.4 in infected fish. Eosinophils were 73.08±1.15 which is significantly decreased down to 62.5±0.5.Lymphocytes and Monocytes percentage was 2.65±0.1 and 1.72±1.2 in healthy fish. In infected fish lymphocytes increases to 3.35±0.16 and Monocytes decreased down to 2.14±0.8 (Table-1.)

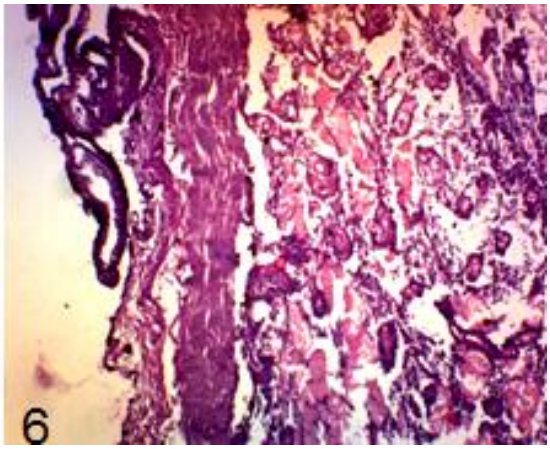
During the study *Aspergillus spp*. have been isolated from *Channa marulius*. This species has a host of *Aspergillus* has not been reported earlier however few workers [16,19,20 and 4-6]. Hematological examination of infected *C.marulius revealed* that there has been considerable decrease (30.6%) in hemoglobin content. Decrease in hemoglobin trend may be a result of swelling of RBC as well as poor mobilization of hemoglobin from spleen [23]. Erythrocytes percentage was considerably decreased to (44.3%). In WBCs (48.8%) increase was observed Neutrophils and lymphocytes showed significant increase of (38.1%) and (20.9%) respectively. An increase in the percentage of granulocytes indicates infection in fishes. The increase in number of granulocytes in infected fish may be due to increase in tissue damage by pathogens or other stress factors.[13,25,3 and 17] also reported similar trend of change in blood profile as reported in present study.

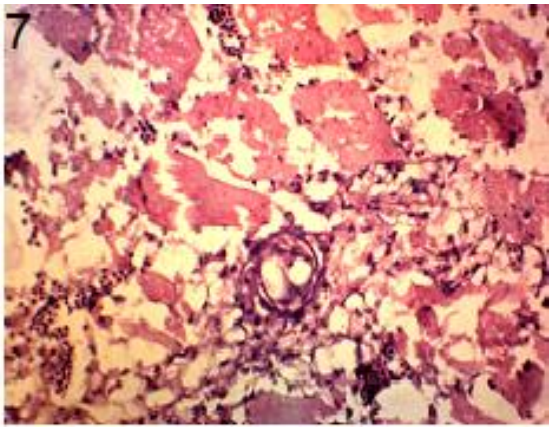
Histological Examination:
Results of histopathological examination of infected skin and muscles of *Channa maurilus* showed various types of destructions in tissues. Loss of epidermal layer with complete necrotization of dermis and hypodermis. Penetrating fungal hyphae were clearly observed in muscular layer. Muscle cells were completely lost their original appearance and hyphal granulomas were observed (Fig-5, 6 and 7). Histopathological manifestations due to mycotic infection in fishes have been reported by [14, 7,

8, 12 and 5] Varying degree of destructions have been observed due to *Aspergillus spp.* Infection observed in present study is supported by reports of above workers.

Fig-5, 6 & 7. Showing loss of epidermis, necrotized hypodermis with fungal hyphae penetrating in muscle layer.degenerated muscular layer and muscles completely lost their appearance and fibrillar granulomas developed.







CONCLUSION

As the fungi are species specific in causing parasitism, it was found from the study that *Aspergillus spp.* is highly pathogenic to fresh water ornamental fish *C.marulius* causing parasitism. There may be certain toxins present in given species of fungi which cause pathogenesis in fish lead to change in hematological parameters and varying degree of destruction in the tissue which leads to mortality of fish.

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