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Studies on the Isolation, Identification and Pathogenecity of Five Species of Genus *Aspergillus* on Some Fishes of River Narmada

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Abstract

Genus *Aspergillus* is known as the causative agent of large number of diseases in human beings, animals and plants. It is also known to cause different types of diseases in fishes as Aspergellomycosis, so pose different problems to the growing aquaculture all over the world. Only a little amount of work has been done in India on conidial fungi in relation to fish diseases. So the present piece of work was carried out to isolate, identify and to test the pathogenecity of some species of *Aspergillus* on some fresh water fishes of River Narmada. The isolation of the conidial fungi (*Aspergillus*) from infected fishes was done by inoculating various infected parts of fishes on two media Potato Dextrose Agar (PDA) and Corn Meal Agar (CMA) in aseptic conditions. Pathogenecity of isolated species was done by injecting them to healthy fishes from which they were isolated. During the present study 33 isolates of *Aspergillus* were isolated from 76 naturally infected fishes. Fishes belonged to 13 species and were collected from river Narmada. In total five species of *Aspergillus* were isolated as *A. fumigates, A. niger, A. sydowii, A. flavus and A. terreus*. *A. fumigates* contributed maximum (29%) of isolates isolated from infected fishes while as minimum were of *A. niger* (9%). *Mystus seenghala* (17%) was most infected fish and least infected was *Labeo calbasu* (3%). After conducting the pathogenecity tests it was found that all five species of *Aspergillus* were pathogenic to fishes. *A. fumigates, A. flavus* and *A. terreus* showed 100% mortality; *A. sydowii* showed 75% mortality while as *A. niger* showed minimum 33% mortality of the fishes.

Key Words: *Aspergillus*, Aspergellomycosis, Histology, Pathogenecity,

Introduction

Fish culture is now quite old in our country, more than 50% fish seed perishes before reaching stackable size. Large scale mortality of fishes is mostly due to microbes, even though with the growing aquaculture no serious attempts have been made to investigate the diseases in fishes in our country. Fresh water fishes are exposed to at least one species of fungus during its life time Neish, 1997. Fungal diseases have

caused great losses in aquaculture being only second to Bacterial diseases Meyer, 1991. There has been lot of work done in our country on Saprolegneaces in relation to fish diseases, but only a little work has been done on conidial fungi in relation to fish diseases. *Aspergillus* (Salem *et al.,* 1989) and *Fusarium* (Bisht *et al.,* 2000) are the two most important genra which are being reported to be associated with fish diseases. Olufemi *et al.,* 1983, and 1985; Salem *et al.,* 1989b; Bhattacharya *et al.,* 1988 have reported *Aspergillus niger* and *A. terreus* as fish pathogens. Shrivastava, 1996 reported *A, terreus* from fresh water fishes and tested its pathogenecty on some species of fishes. Species of *Aspergillus* have also been isolated by many other works from fresh water fishes all over the world some of the notable contributions are Shabazain *et al.,* 2010; Junaid *et al.,* 2010; Fadarfard *et al.,* 2011; Chauhan 2013 and 2014.

Material and Methods

Collection of fish samples: A total number of 76 infected fishes belonging to 13 species were collected from Narmada River with the help of local fisherman and brought to the departmental laboratory of department of zoology and applied aquaculture Barkatullah University Bhopal in polythene bags for further examination.

Isolation of fungi from infected fishes: Infected fishes were washed carefully with tap water and fishes with external infection signs on different parts of body were selected for further study. Potato Dextrose Agar (PDA) and Corn Meal Agar (CMA) were used for culture purpose. All the glass wares, instruments and media were sterilized in autoclave and also streptomycin and streptopenciline were used with media to avoid bacterial contamination. Innocula were taken from different parts of infected fishes, and inoculated on already prepared above mentioned two media in Laminar Flow Chamber in aseptic condition. After inoculation the agar plates were incubated at $28^{O}C$ in BOD incubator for growth of cultures and were observed continuously. Normally all colonies showed full growth in 10-15 days after inoculation. For identification small piece of mycelia were taken from the fully grown colonies and stained with lactophenol blue on slides and observed under microscope. Identification of the fungi was done by using the keys of (Refai *et al.,* 1987) and fishes were identified by using the keys of (Qureshi and Qureshi, 1983).

Experimental infection trails: To determine the pathogenecity of isolated species of conidial fungi, pure cultures of them were prepared on CMA and PDA. Conadial suspensions were prepared on media and concentrations were prepared by using haemocytometer.

Healthy fishes were collected and kept in aquaria of 10 Litre capacities under observation with continuous aeration and fed with artificial feed. For experimental purpose fishes were injected intramuscularly with 0.1 ml of concentration of each species of conidial fungi (8×10^8 conidia/ml), and observed for 7 days.

Histopathological studies:

For histological examination infected tissue of skin and muscles were fixed in aqueous Bouin's fluid for 48 -72 hours. The tissue was then processed routinely and prepared into paraffin blocks. The blocks of the tissues were cut at 4-6 μm thickness and stained with Haematoxylin and Eosin (H-E).Standard histological procedures (Roberts, 2001) were followed for histopathological investigations.

Results and Discussion

Isolation and Identification: During the present study a total number of 76 infected fishes were examined, which showed signs of fungal infection on various parts of their body as fins, eyes and tail (**Fig.1 and Fig. 2**). 13 species of fishes were found infected in total as *Channa punctatus, C.striatus ,Cirrhinus mrigala, Clarias batrachus, Labeo rohita, Labeo calbasu, Macrognathus aculeatus, Mastacembalus armatus, Mystus cavasius, M.seenghala, Puntius sarana, P.ticto and Trichogaster fasciatus* as shown in (**Table-1**). Conidial fungi are known to form different types of their colonies, varying in color and size and form different sexual structures, which were used for identification purpose during the present study (**Fig.3, Fig.4 and Fig.5**). Among the 33 isolates of Genus *Aspergillus* isolated from infected fishes. Isolates were as *A. fumigates* (29%), *A. niger* (9%), *A. sydowii* (19%), *A. flavus* (24%) and *A. terreus* (19%) (**Fig.7**). Isolation of these *Aspergillus* species are supported by the findings of (Iqbal and Mumtaz, 2013). *A. fumigates* (29%) showed maximum number of isolates, which is supported by the findings of (Willoughby, 1994) and (Chauhan *et al.,* 2014 c). Minimum isolates were of *A. niger* (9%) which is in agreement with the findings of (Chauhan *et al.,* 2014 c). *Mystus seenghala* (17%) was the most affected fish and *Labeo calbasu* (3%) showed least infection (**Fig.8**) during the present study, these findings are supported by the findings of (Iqbal and Mumtaz, 2013).

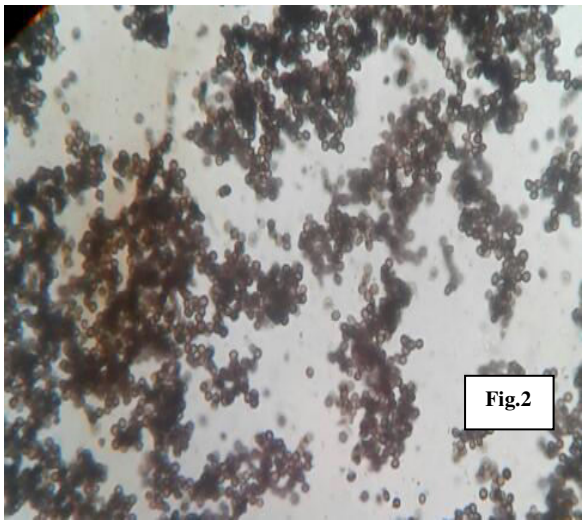
Pathogenecity : to determine the pathogenecity of the isolated species of genus *Aspergillus*, these species were tested/inoculated in the healthy fishes from which they were originally isolated. All the experiment was done in 10 L capacity aquarium. 3 numbers of fishes were taken of each species and kept in aquaria for 7 days and were fed with artificial feed. It was found that all the five species of *Aspergillus* were pathogenic to fishes in which they were injected and showed 100% mortality within 7 days except *A. sydowii* and *A. niger* which showed 75% and 33%

mortality of fishes respectively (**Table 2**). This result is in the agreement with the findings of (Chauhan *et al.,* 2014, Chauhan *et al.,* 2014 b.)

Histology of skin: Histological investigations of skin of Mystus cavasius showed epithelial desquamation which displayed erosion leads to ulceration in the infected region. Varying degree of destruction had taken place in the dermis and hypodermis. Epidermal and dermal cells showed vacuolar degeneration and focal necrosis (**Fig.6**).

Fig.1: *Showing Macrognathus aculeatus infected with fungus.*

Fig.2: *Showing infected Mystus sp with severe infection on tail.*



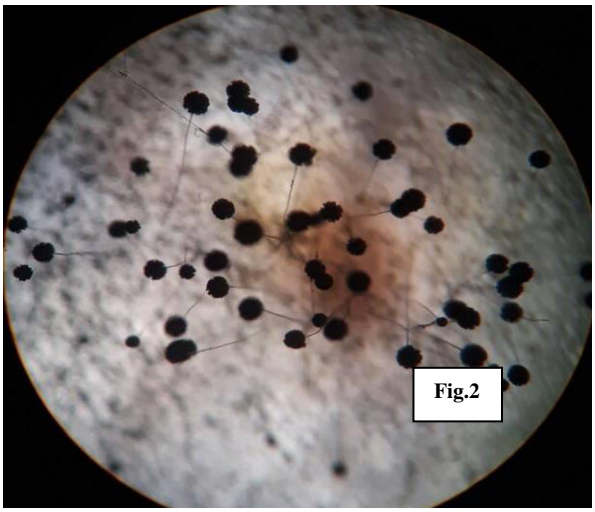


Fig.3

Fig.3: *Showing microphotograph of coniodiospores of Aspergillus species.*

Fig.4

Fig.4: *Showing microphotograph of Aspergillus niger at 10X*

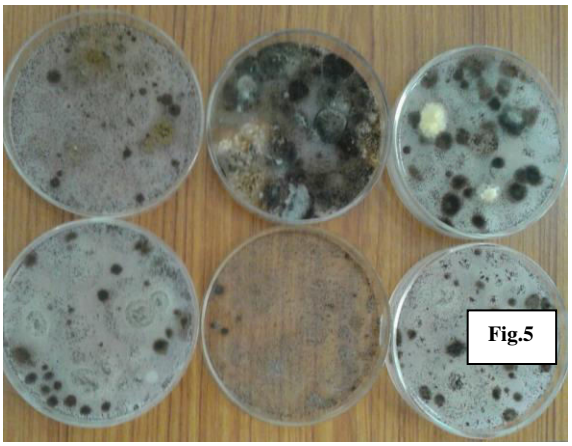


Fig.5*:* Showing colonies of different species of Aspergillus on PDA and CMA

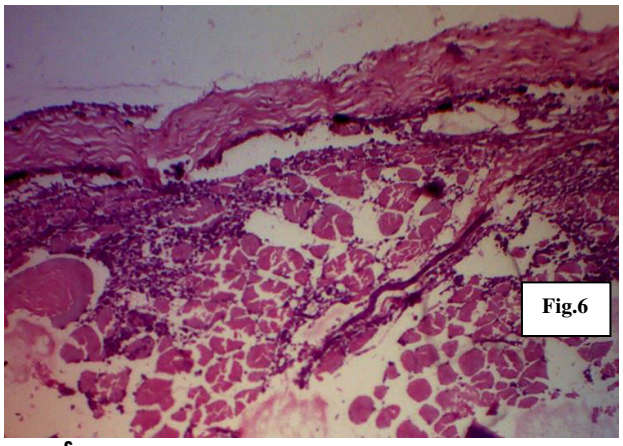


Fig.6: Showing the underling dermis was necrotized, also showing fungal hyphae with focal aggregation of melanomacrophages cells. (Skin of *Myustus seenghala*)

Table-1: Showing Species of Genus *Aspergillus* isolated from infected fishes

S.No	Isolated Fungi	Host fish species
1	*Aspergillus fumigates*	*Channa striatus, Labeo rohita, Mystus seenghala, Cirrhinus mrigala, Macrognathus aculeatus, Puntius sarana .*
2	*Aspergillus niger*	*Mystus seenghala, Puntius ticto.*
3	*Aspergillus sydowii*	*Trichogaster fasciatus. Puntius sarana, Channa punctatus , Mystus cavasius*
4	*Aspergillus flavus*	*Channa punctatus , Mystus cavasius, Clarias batrachus, Labeo calbasu, Mystascembalus armates.*
5	*Aspergillus terreus*	*Puntius ticto, Labeo rohita, Labeo calbasu, Channa straitus*

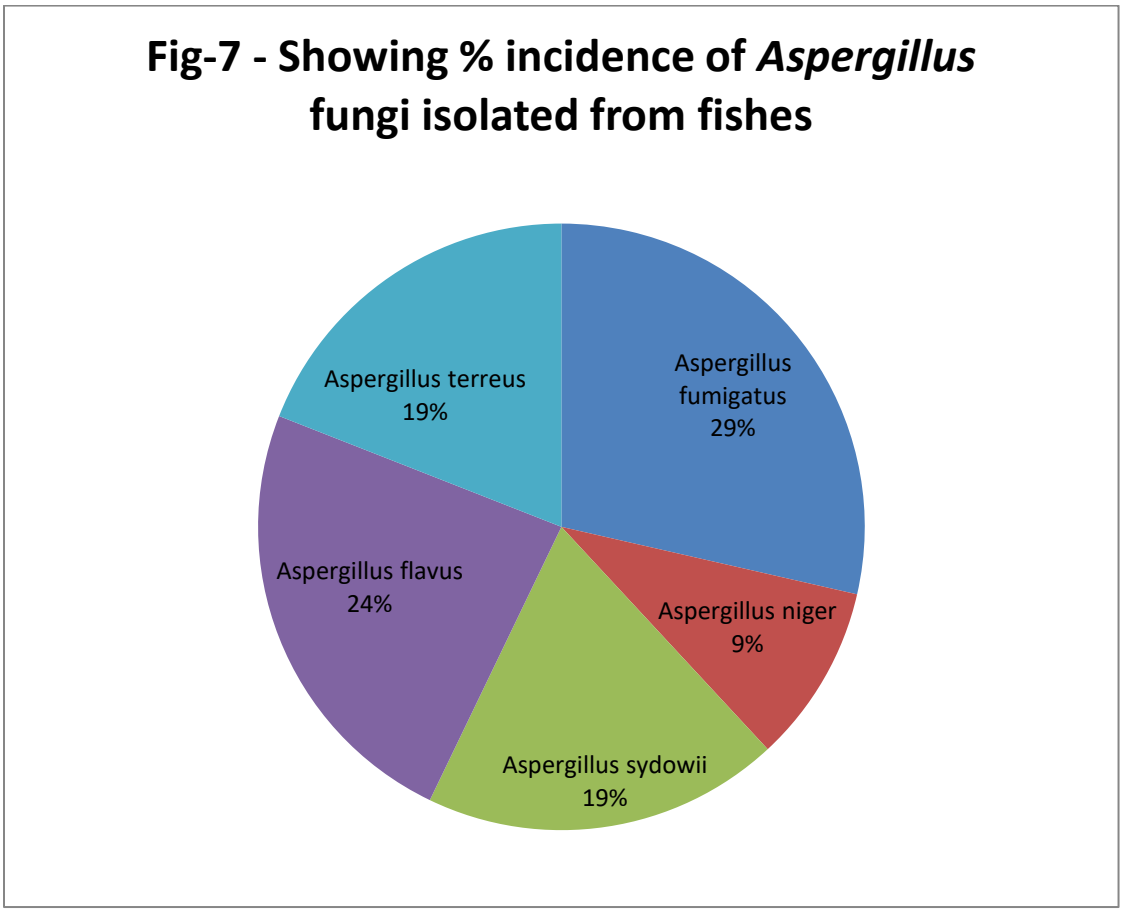


Fig.8- Showing % incidence of infected fishes

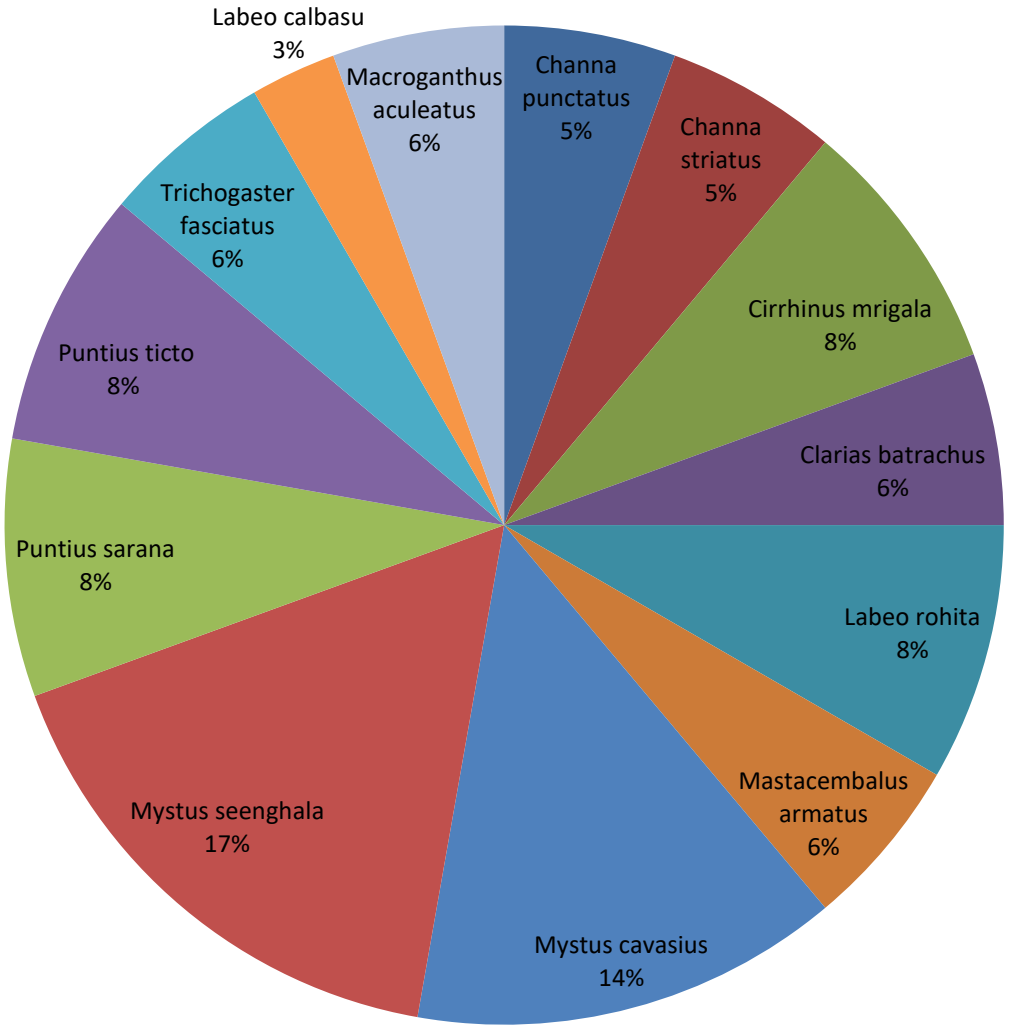


Table-2: Showing results of pathogenecity test of species of Genus Aspergillus on fishes from which they were originally isolated.

S. No	Fungi Inoculated	Challenged Fish	No. of fish used	Conc. Of conidiospores /ml	Infection in days	Mortality % after 3 days	Survibality after 7 days	Resolution
1	*Aspergillus fumigatus*	*Channa straitus*	3	8×10^8 conidia/ml	3	67	0	+
		Labeo rohita	3	8×10^8 conidia/ml	3	100	0	+
		Mystus seenghala	3	8×10^8 conidia/ml	5	33	0	+
		Cirrhinus mrigala	3	8×10^8 conidia/ml	4	100	0	+
		Macrognathus aculeatus	3	8×10^8 conidia/ml	5	100	0	+
		Puntius sarana	3	8×10^8 conidia/ml	3	67	0	+
2	*Aspergillus niger*	*Mystus seenghala*	3	8×10^8conidia/ml	5	0	67	+
		Puntius ticto	3	8×10^8 conidia/ml	5	0	67	+
3	*Aspergillus sydowii*	*Trichogaster fasciatus*	3	8×10^8 conidia/ml	5	33	67	+
		Puntius sarana	3	8×10^8 conidia/ml	3	67	33	+
		Channa punctatus	3	8×10^8 conidia/ml	3	67	33	+
		Mystus cavasius	3	8×10^8 conidia/ml	3	33	0	+
4	*Aspergillus flavus*	*Channa punctatus*	3	8×10^8 conidia/ml	3	67	0	+
		Mystus cavasius	3	8×10^8 conidia/ml	3	100	0	+
		Clarias batrachus	3	8×10^8 conidia/ml	5	33	0	+
		Labeo calbasu	3	8×10^8 conidia/ml	3	67	0	+

		Mastacembalus armatus	3	8×10^8 conidia/ml	5	33	0	+
5	*Aspergillus terreus*	*Puntius ticto*	3	8×10^8 conidia/ml	3	67	0	+
		Labeo rohita	3	8×10^8 conidia/ml	3	67	0	+
		Labeo calbasu	3	8×10^8 conidia/ml	5	33	0	+
		Channa straitus	3	8×10^8 conidia/ml	5	100	0	+

Conclusion and future Research:

From the present study it is concluded that all the species of genus *Aspergillus* are pathogenic to freshwater fishes and pose great threat to the growing aquaculture. Controlling diseases caused by conidial fungi is necessary to make certain continued development in the aquaculture industry, particularly in Asian steamy aquaculture systems, where over 80% of fish produced by aquaculture come from the area. For an industry that accounts for roughly 30% of the worldwide production of fish for consumption, it is vital to prolong studying the fundamental molecular processes of different conidial fungi and their host fish relations.

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References

1. Bhattacharya, U., Prasad, J., and Dubey, N. K... *Aspergillus terreus* Thom. - A new record as a fish pathogen. 1988, Current Science,. 57 (11): 622-623.
2. Bisht, D., Bisht G.S. and Khulbe R.D. (2000). Fusarium a new threat to fish population in reservoirs of Kumaun India. *Curr. Sci.*, 78 (10), pp.1241- 12.
3. Chauhan, R. (2013). Studies on conidial fungi isolated from some fresh water fishes, *International Journal of Advanced life Sciences (IJALS)*. 6(4), pp. 277-281.
4. Chauhan, R. (2014). Studies on Some Fresh Water Fishes Found Infected with Dermatomycoses, Collected From Different Water Bodies in and Around Bhopal, India*., Indo American Journal of Pharm Research;* 4 (03).

5. Chauhan, R., Ganaie,S.A., and Lone,S.A. (2014a). Studies on hematological and histological manifestations of Channa Marulis (Ham.) found infected with Aspergillus spp. *Biolife.* 2(2), pp. 649-654.
6. Chauhan, R., Nisar,Z., and Baig, A.H. (2014b). Studies on Aspergillomycosis in Labeo calbasu found infected with Aspergillus flavus and A. terreus. *World Journal Of Pharmacy And Pharmaceutical Sciences*. 3(7). pp. 1842-1848.
7. Chauhan, R., Bhatt.M.H., and Lone.S.A. (2014c). Pathogenic effects of three species of fungi (Aphanomyces laevis, Aspergillus niger and Sapolegnia parasitica) on Gold fish (Carrasius auratus L.) *Indo Global Journal of Pharmaeceutical Sciences,* 4(2), pp.41-46.
8. Fadaeifard,F ., Raissay,M ., Bahrami.H.,. Rahim,Ei and Najafipoor,A. Freshwater Fungi Isolated from Eggs and Brood stocks with an Emphasis on *Saprolegnia* in rainbow Trout Farms in West Iran. Afri. J. Microbiol.2011, 4(22) 3647-3651.
9. Junaid, S.A., Olarubofin, F. and Olabode, A.O. 2010. Mycotic contamination of stockfish sold in Jos, Nigeria. *J. Yeast and Fungal Res.,* 1: 136 - 141.
10. Meyer, F. P. (1991). Aquaculture Diseases and Health management*. Anim. Sci.* 69, pp. 4201-4208
11. Neish, G.A. (1997). Observations on Saprolegniasis of adult sockeye salmon, Oncorhynchus nerka (Walbaum). *J. Fish. Biol.,* 10, pp.513-522
12. Olufemi, B.E. 1983. The Aspergilli as pathogens of cultured fishes. In: *Recent advances of Aquaculture*, (Eds. J.F. Munir and R.J. Roberts). pp. 193 - 218.
13. Olufemi, B.E. 1985. The Aspergilli as pathogen of cultured fishes. *In*: *Recent Advances of Aquaculture.* pp. 193–218.
14. Qureshi, T.A. and Qureshi, N.A. (1983). Indian Fishes, *Brij Brothers,* India. pp. 209.
15. Refai, M., Abdel, M.M., Halim Afify, M.M.H., Youssef. H. and Marzou, K.M. (1987). Studies on aspergillomycosis in catfish (*Clarias Lasera*). Allgemeine Pathologic and pathologische Anatomic. Tagung der Deutachen Veterinar – Medizinischen Gesellschaft. *der Europeischen Gesellschaft fur Vet. Pathol.,* 63, pp.1-12.
16. Roberts, R. J. (2001). Fish Pathology. 3rd Ed., W. B Sauders, U. K.pp124.
17. Salem, A., Refai, M., Eissa, I.A., Mmarzouk, M., Bakir, A., Mustafa, M. Mandmanal, (1989). Some studies on aspergillomycosis in *Tilapia nilotica. Zagazig Vet. J.,* 17(3), pp. 315-328.
18. Shahbazain, N., Ebrahimzadeh, M., Soltani, M., A.R Khosravi, A.R., Mirzagai, S. and Sharifpour, I. (2010). Fungal Contamination in Rainbow trout Eggs in Kermanshah Province Propagation with Emphasis on Saprolegniaceae. *Iranian J. Fish. Sci.,* 9(1), pp.151 - 160.

19. Shrivastava, A. K. Record of *Aspergillus terreus* (Thorn.) Fungi as fish pathogen .Indian J of fish .1996, 43, 2,203-204 pp.
20. Willoughby, L.G. 1994. Fungi and Fish Diseases. Pisces Press, Stirling, UK. p. 57.