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Pathogenic Effects of Three Species of Fungi (*Aphanomyces laevis*, *Aspergillus niger* and *Saprolegnia parasitica*) on Gold Fish (*Carrasius auratus* L.)

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ABSTRACT: Present study was conducted to find out the pathogenicity of three species of fungi,viz. *Aspergillus niger*, *Aphanomyces laevis* and *Saprolegnia parasitica* isolated from Gold fish (*Carrasius auratus*) collected from pet shops. Experiment was conducted for 10 days period with concentration of $8x10^3$conidia/ml *(A.niger*), 125 zoospores/ml (*A.laevis)* and $2x10^3$zoospores /ml (*S.parasitica*). All the tested fungi were found pathogenic to fish. Among the three species of fungi tested, *A.laevis* and *S.parasitica* showed 100% mortality in fish while *A.niger* showed 75% mortality. *S.parasitica* was found most virulent causing mortality within six days of experiment. Histopathological examination of fishes showed inflammation of epidermis , loss of epidermis and necrotized hypodermis. Degenerative changes were observed in musculature. Ulcerated skin showed mycotic granulommas.

KEYWORDS: Gold Fish; Pathogenicity; Histopathology; Fungi.

INTRODUCTION

In India variety of fresh water and marine ornamental fishes are available. According to the reports of Central Marine Fisheries Research Institute about 600 species have been identified as potential fishes with ornamental value. The trade in India fetches about 50 crores/year. Fungal infection is very common in ornamental fishes including gold fish (*Carrassius auratus)* which is most domesticated fish. Mostly fungal infection in fish is called Saprolegniasis caused by members of family Saprolegniaceae (*Saprolegnia* and *Achlya*). In aquariums gold fish is mostly attacked by fungus due to change in temperature and filthy conditions of water which allow excessive zoospores to grow and the ammonia which is formed by rottening of fish waste wears away the mucus that protects the skin. Isolation of *Saprolegnia, Aphanomyces* and *Aspergillus* have been reported from ornamental fish by numbers of workers like (Khulbe,1983: Salem *et al.*,1989; Hatai and Hoshiai,1992b; Hatai *et al.*,1994; Shrivastava,1996;Qureshi *et al*.,2002; Sosa *et al.*,2007; Siddiqi *et al.*,and Shabzan *et al*.,2009; Refai *et al.*,2010;Das *et al*., and Chauhan *et al.*,2012.)

(Chauhan,2012) also reported *Saprolegnia, Aphanomyces* and *Aspergillus* from ornamental fishes. Three species of *Aspergillus* have been reported from ornamental fishes by Chauhan,2013; Iqbal and Mumtaz,2013) reported *Aspergillus* from gold fish. Studies on pathogenicity of fungi on gold fish and histological alterations in the infected tissue have been very rare and not been well documented. Present investigation have been designed to find out the fungi involved in mycotic infection in gold fish. The study also describes the experimental exposure of gold fish to isolated species of fungi and histopathological changes in tissue of infected area.

MATERIALS & METHODS

A total number of 37 gold fishes (*Carrasius auratus*) were collected from different pet shops of Bhopal in the period of four months, September,2013 to December 2013. Infected fish were brought to the laboratory in sterilized polythene bags for isolation of fungi. Temperature, pH and D.O of aquarium

water was observed at the same time with digital meters (Hach,India).

The fishes were kept in aquaria with continuous aeration. The fishes were observed to note external symptoms. To avoid bacterial contamination all the glass wares, instruments and media were sterilized, along with all aseptic conditions, Streptomycine sulphate 100mg/ml were used in media. Innoculation was done in Laminar flow in sterilized conditions. The agar plates were incubated at 18±2˚C for the growth of cultures of zoosporic fungi and for conidial fungi cultures were kept at 28+2°C .Full growth of colony was observed in 6-8 days. Media used in the study were Sabourauds Dextrose Agar and Corn Meal Agar. Pure cultures were prepared by single hypha method. Identification of fungi was carried out on the basis of keys of (Raper, 1965; Refai *et al*.,1987; Willoughby, 1994; Khulbe, 2001 and Shrivastava,2009).

Experimental infection trials

To determine the pathogenicity of isolated species of fungi pure cultures were prepared on CMA and SDA and maintained at required temperature. Zoospores concentration was prepared as wet cultures by using baits, Glycine seeds (Soyabean) for Saprolegnia and Sorghumseeds (Jowar) for Aphanomyces. Conidial suspensions was prepared on media. Concentrations were prepared by using haemocytometer.

Healthy fishes with average length of 10.5±2cm and average weight of 18±4gmwere collected and kept in aquaria of 10L capacity under observation for three days with continuous aeration and fed with artificially. For experimental purpose fishes were injected intramuscularly with 0.1 ml of concentration of each species of fungi *Aspergillus* ($8x10^8$conidia/ml), *Aphanomyces* (125zoospores/ml)and *Saprolegnia* ($2x10^3$zoospores/ml).Temperature was maintained as 18±2°C and 28±2°C for zoosporic and conidial fungi respectively. Fishes were observed for 10 days and morbid fish was immediately removed from trough for re isolation and histological examination. For histological studies standard methods given by Roberts, 2001 were followed. Tissue was fixed in aqueous Bouin's fluid for 48-72 hours, processed routinely and slides were prepared by using Haemetoxilyn and Eosin stain.

RESULTS & DISCUSSION

A total number of 37 infected fishes were collected for mycotic examination. Among them *S.parasitica* was isolated from 21 specimens, *A.laevis* from 9 fishes and *A. niger* from 7 fishes. The symptoms are white fungoid patches on body, ulcers with hyphal growth and darkening of skin with black patches (Fig-1-6). Average values of water quality parameters were, temperature (18±3°C), pH (7.2-8.0), D.O (6-10.2). *S.parasitica* have been isolated from gold fish by (Tiffney,1939 b; Sati et al.,1982 and Khulbe,1989).Isolation of *A.laevis* have been reported from ornamental fish by (Shrivastava and Shrivastava,1977and 1979b).*Aspergillus.spp*. have been reported from gold fish by (Iqbql *et al*.,2012 and Iqbal and Mumtaz,2013). (Chauhan, 2013) reported *A. niger* from ornamental fish. Present findings are supported by reports of given workers.

Results of artificial infection showed that all the three species of isolated fungi were found to be pathogenic to fish.

S.parasitica:-Artificially challenged fish showed external lesions on skin with 25% mortality on 2nd day, 25% on 4th day and rest 50% died on sixth day with ulcers surrounded by mycelium on body surface. It shows 100% mortality within six days of experiment.

A.laevis:-On the second day only behavioral changes were observed.25% mortality was recorded on 4thday and 37.5% mortality was recorded on 6th and 8th days respectively. Ulcers were observed in all the fishes but size of the ulcers were very small with tiny threads of mycelium. Mortality was 100% .

Table-1.Pathogenicity of three species of fungi (*Aphanomyces laevis, Aspergillus niger* and *Saprolegnia parasitica*)on gold fish (*Carrasius auratus L.*)

S. no.	Fungi injected	No. of fish used	Conc. of spores/ml	Mortality % in days					Total mortality %	Re-isolation
				2	4	6	8	10		
1.	A	8	125/ml	0	25	37.5	37.5	0	100	+
2.	B	8	$8x10^8$/ml	0	25	0	12.5	37.5	75	+
3.	C	8	$2x10^3$/ml	25	25	50	0	0	100	+

****A- Aphanomyces laevis, B- Aspergillus niger, C-Saprolegnia parasitica***

A.niger:-The fish challenged with *A.niger* showed some change in swimming and on fourth day 25% mortality was observed. On sixth day dark patches on skin was observed on three fishes and on 8th day 12.5% mortality was recorded and on 10th day37.5%mortality was observed with dark patches on skin and de scaling in few areas. Ulcers not developed and total mortality was 75%. Re isolation in all the infectd fishes was positive. The work on the pathogenicity of isolated species of fungi on gold fish is very rare and not well documented, however the given species of fungi have been reported as pathogenic on many ornamental fish by (Chauhan and Qureshi,1994; Sosa *et al.*,2007;Hussian *et al.*,2013and Chauhan *et al*.,2014).present work is in agreement with the reports of above workers.

Histopathological changes:-Tissue infected with *S.parasitica* showed loss of epidermis and necrotized hypodermis. Muscle layer shows inflammation with necrotic tissue debris and formation of number of well developed layered granulomas surrounded by hyphae. Granulomas showed fribrillar structures due to hyphal infection. *A.laevis* injected tissue showed more or less similar symptoms as that with *S.parasitica.* Necrotised epidermis and hypodermis with granulomatous response of musculature. Histological studies of tissue injected with *A.niger* showed no granuloma formation in musculature , no hyphae was seen in deeper layers although epidermis was degenerated and edema was observed in underlying hypodermis and musculature.(Fig-7-12). These histopathological findings are supported by reports of (Miyazaki and Egusa,1972; Hatai,1980; Hatai *et al*.,1994and Hussian *et al*.,2013). Granulomatous response due to *Aphanomyces* infection was also reported by (Qureshi *et al*.,2001;Sosa *et al*., 2007and Chauhan and Qureshi,2012).Persent findings are in support of above reports. Histopathological studies of tissue infected with *A.niger* did not show any granuloma formation, however degenerated musculature was observed with necrotized hypodermis and epidermis.

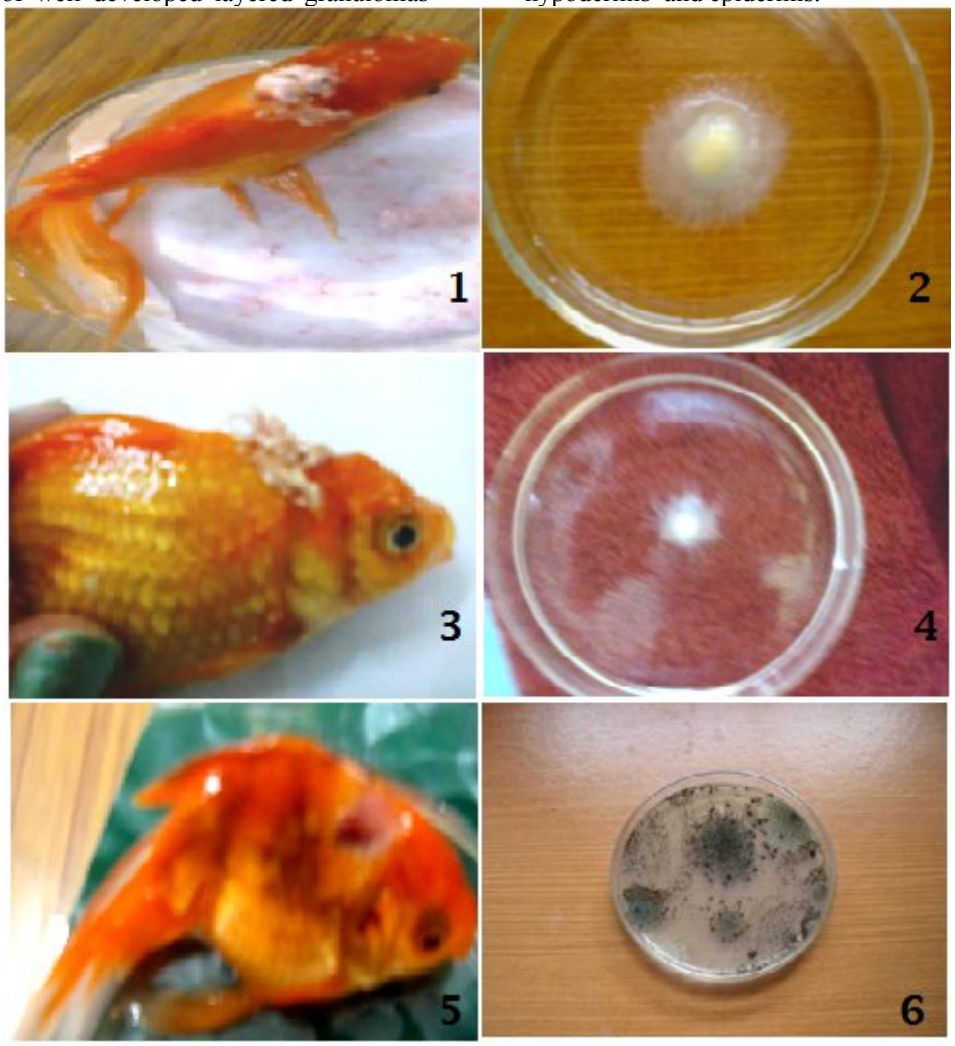

Fig- 1. Showing white fungoid patches of S.parasitica. Fig- 2. Wet colony of *S.parasitica* on Soya bean seed.
Fig-3. Showing hyphal tufts of *A.laevis* on head region. Fig-4. Wet culture of *A.laevis* on Jowar seed.
Fig- 5. Showing black patches on skin due to *A.niger* infection. Fig-6. *A.niger* grown on Corn Meal Agar (CMA)

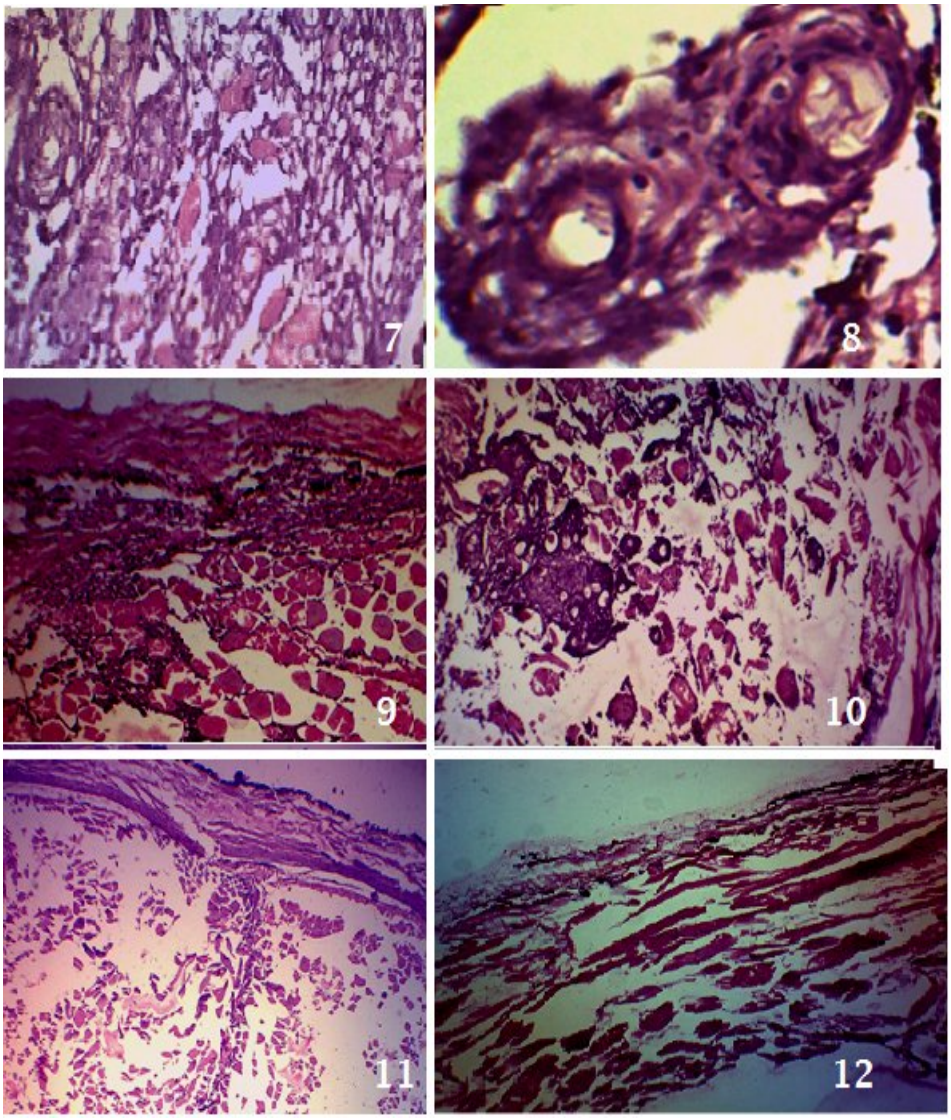

Fig-7 Showing many granulomas formed after penetration of hyphae of *S.parasitica.* Fig-8. Fibrillar granulomas in larger view surrounded by fungal hyphae. Fig-9.Showed lost epidermis, necrotized hypodermis in the tissue infected with *A.laevis.* Fig-10.Degenerated muscle tissue with accumulated cells forming granulomas. Fig-11. Degenerated epidermis with necrotized hypodermis with the invasion of hyphae of *A.niger.* Fig-12.Necrotic changes in hypodermis and muscle fibers.

CONCLUSION

In the present study it was found that all the three species of isolated fungi were found to be pathogenic to gold fish and S.parasitica was most virulent.

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