Effect of *Rhizopus Stolonifer* On Morphology and Haematology of Catfish, *Clarias Batrachus* (Linn.)

ASHOK KUMAR[1], SADGURU PRAKASH[2]
Department of Zoology, M.L.K. College, Balrampur U.P.

Abstract- ***The present investigation has been designed to study the effect of Rhizopus stolonifer fungus causes on the behavior and haematological parameters of infected Clarias batrachus. After 60 days of infestation, infected fish show bulging eyes, loss of color, ulcers and cysts in internal organs, and abnormal circular movements i.e. loses of orientation. haematological parameters were measured for healthy and fungal infected Clarias batrachus. Haematological parameters such as WBC, neutrophile, and eosinophile were increased significantly (P < 0.05) in Rhizopus stolonifer infected fishes than healthy ones. The other haematological parameters like RBC, hemoglobin (Hb), hematocrit (HCT), lymphocyte, and monocyte were decreased in healthy fishes.***

Indexed Terms- ***Rhizopus stolonifer, morphology, behavior, Blood indices, Clarias batrachus***

I. INTRODUCTION

Fungal contamination of fish is considered the main cause of signs of spoilage as off flavor and unpalatable taste and it may constitute a public health hazard as well as economic losses also. The processed fish either salted or smoked may be exposed to contamination by moulds and yeasts derived from subsequent handling of fish and/or from the salt or brine used in the processing which undergoes fungal spoilage through utilization of protein and lipids. Fungi are known to attack fish eggs, fry, fingerlings and adult fish. Water molds infections cause losses of freshwater fishes and their eggs in both natural and commercial fish farms (Bangyeekhun and Sylvie, 2001). The fungal diseases occur in brood stock and all life stages of fish and eggs. Fungal infection cause low productivity of fry and low production in fish culture (Kwanprasert *et al.*, 2007). The mortality rate due to fungal &. According to Akande and Tobor (1992) post-harvest handling of fishes may also result in infection with microorganisms such as bacteria and fungi. The ubiquitous fungi are part of the normal mycoflora of fresh and estuarine ecosystems and have a worldwide distribution. However, there are certain fungi which cause fish diseases. *Rhizopus stolonifer* is commonly known as black bread mold. It is a member of Zygomycota and considered the most important species of the genus *Rhizopus*. It is worldwide in distribution and commonly found in tropical and subtropical regions. It grows rapidly, mostly in indoor environments. In the present study, an attempt was made to examine the effects of *Rhizopus stolonifer* on the haematological parameters of freshwater catfish, *Clarias batrachus* which are nutritional and popular edible fish of the study area.

II. MATERIALS AND METHODS

The healthy fish, *Clarias batrachus* ranging from 8.5-9.5 cm in length and 9.0-10.0 g in weight were collected from local fish ponds of Balrampur and washed with 1% solution of $KMnO_4$ for five minute and were transported immediately to laboratory in sterile aerated polyethylene bags then transferred to the plastic jar containing 50L dechlorinated tap water for acclimatization. The healthy fishes were kept separate in glass aquariums with continuous air supply at ambient temperature and acclimatize for 1 week. After 60 days of infestation, blood samples for hematological analysis were taken from the caudal vein and collected in a heparinized tube and then stored in a polystyrene cool bag until used. Hematocrit (HCT) was determined by spinning blood samples in heparinized capillary tubes in micro-hematocrit centrifuge (13,500 g, 5 min). Hemoglobin (Hb) was measured with spectrophotometer at 540 nm absorbance using cyanmethemoglobin method. For counting red blood cells (RBC) and white blood cells (WBC) a neubauer chamber following the method of Blaxhall and Daisley (1973) with Dacies' solution as a diluting fluid were used. The erythrocyte indices

including mean cell volume (MCV), mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) was calculated according to Haney *et al*. (1992). All data were analyzed using independent sample t-test at ($P < 0.05$)

RESULTS AND DISCUSSION

To investigate the changes in haematological parameters, blood of healthy and after 60 days *Rhizopus stolonifer* infestation fishes were measured and the data presented in Table1. WBC, neutrophil, and eosinophil have been significantly ($P < 0.05$) increased in fungal infected fishes than healthy fishes (Table 1). TLC and DLC are generally used in the determination of immune reactions and diseases (Cagirgan, 1990). Changes in WBC (TLC) and differential leucocytes counts (DLC) have been reported to play important roles in the assessment of the state of health of fishes (Gabriel *et al.,* 2004). It is known that leukocyte cells are normally lower in healthy fishes and could be used as a significant indicator for infectious diseases. In this study, leucocytes count (mean ± SD) in healthy fishes was 5207 ± 1660 and in fungal infected ones was10565 ± 3946. The increases in leucocytes count and neutrophil percentage in *Rhizopus stolonifer* infected fishes was the indication of active immune response of fish against fungal infection. The elevation of leucocytes count and neutrophil percentage was reported in fish *Anguilla anguila* as a consequence of parasite infection (Sahan *et al*., 2001). Palikova and Navratil (2001) concluded that immune system of fish displays similar responses to unfavorable conditions.

The other haematological parameters like RBC count, Hb, HCT (PVC), lymphocyte, and monocyte percentages were significantly ($P < 0.05$) reduced in *Rhizopus stolonifer* infected fishes in comparison to healthy fishes (Table 1). Similarly decrease in the RBC count and lymphocyte percentage with an increase in the granulocyte percentage (neutrophile and eosinophile) were observed in European eel *A. anguilla* infected with the parasite (Sahan *et al.,* 2007). Genc *et al.* (2005) and Boon *et al.* (1989) pointed out that erythrocyte count in parasite infected fish was significantly decreased in comparison to those in non-infected. Boon *et al*. (1989) reported there was no significant difference between the fish infected with the parasite and non-infected ones in terms of HCT level, but it was reported that after 7 weeks following infection, HCT and plasma protein levels decreased, WBC quantities reached the highest level in fishes infected with parasite, and there was an adverse relation between the percentages of lymphocyte and granulocyte (neutrophile and eosinophile). Van Der Heijden *et al.* (1996) determined an increase in the numbers of lymphocyte and granulocyte cells of fish infected with *A. crassus* and they claimed that a cellular response to this parasite from specific antibodies in eels infected could be produced in time.

Table 1. Hematology of healthy (n = 20) and infected (n = 20) fishes.

Factor	Healthy	Infected	*t*	*P*-value
Hb (g/dL)	11.38±1.2	9.53±1.6	4.41	0.000
HCT or PVC (%)	45.25±4.3	36.45±7.2	5.18	0.000
RBC (× 10^6/µL)	1.34±0.2	1.05±0.2	4.07	0.000
WBC (/µL)	5207±1660	10565±3946	-6.35	0.000
MCV (fL)	347.39±65.3	348.25±62.1	-0.04	0.964
MCH (pg)	86.98±17.2	92.26±17.8	-1.02	0.312
MCHC (g/dL)	25.18±2.1	26.52±2.4	-1.99	0.052
Lymphocyte (%)	73.37±5.0	61.35±7.7	6.43	0.000
Monocyte (%)	4.37±1.3	1.65±0.8	7.80	0.000
Neutrophile (%)	16.59±4.7	27.35±9.9	-4.91	0.000
Eosinophile (%)	5.29±1.4	9.75±3.3	-6.19	0.000

Data are expressed as means ± SD.

The effect of age-length-weight variables on blood parameters such as enhanced formation of lymphocytes is an essential component of immune system in the early stages of growth period (Aldrin *et al.,* 1982). In a research for *Clarias batracus*, there was an effect of fish size (between small and big fishes) on all the parameters ($P < 0.05$) except for

MCV (Tran-Duy *et al*., 2008). In a study on *Mugil platanus* it was verified that mean values for HCT, Hb, MCV, MCH and MCHC showed a slight increasing tendency as individuals got larger (Ranzani-Paiva, 1995). Also, larger individuals present higher mean values for MCV, MCH and MCHC for dourado *Salminus maxillosus* (Ranzani-Paiva *et al*., 1995). The other parameters including Hb, HCT, RBC, lymphocyte, and monocyte were greater in healthy Caspian salmon (Table 1, $P < 0.05$). The lower value of RBC in fungal infected *Clarias batrachus* was in accordance with European eel *A. anguilla*. Genc *et al*. (2005) and Boon *et al.* (1989) pointed out the erythrocyte amount of fish infected with parasite was significantly lower in compare- son to those in non-infected. Boon et al. (1989) reported there was no significant difference between the fish infected with the parasite and non-infected ones in terms of HCT level, but it was reported that after 60 days following infection, HCT and plasma protein levels decreased, WBC quantities reached the highest level in fishes infected with parasite, and there was an adverse relation between the percentages of lymphocyte and granulocyte (neutrophile and eosinophile).

- Clarias batrachus

Rhizopus stolonifer (black mold)

Microscopic Rhizopus stolonifera

Effect of exposure of Rhizopus stolonifer on Clarias batrachus

Rhizopus stolonifer fungus also causes bulging eyes, loss of color, ulcers and cysts in internal organs, and sometimes causes fish to swim in abnormal circular movements. Fungal infections in fish. *Clarias batrachus* can cause damage to multiple body systems, such as the liver, kidney, and brain, and usually occur when the fish is in a weakened state, either due to injury or trauma. It can also develop if a fish is placed in poor living conditions (i.e., substandard water quality or an overstocked fish tank).

REFRENCES

[1] Akande GR and JG Tobor, 1992. Improved utilization and increased availability of fishing products as an effective control of aggravated animal protein deficiency induced malnutrition in Nigeria. Proc 10th Annual Conference Fisheries Society of Nigeria, pp: 18-31.

[2] Aldrin JF, Messager JL, Laurencin FB (1982). La Biochimie Clinique en Aquaculture. Interet et Perspective. CNEXO, Actes Colloq. 14: 291-326.

[3] Bangyeekhun E and MA Sylvie, 2001. Characterization of Saprolegnia sp. isolates from channel catfish. Dis Aquat Organ, 45: 53-59.

[4] Blaxhall PC, Daisley KW (1973). Routine Haematological Methods for Use Fish with Blood. J. Fish Biol. 5: 771-781.

[5] Boon JH, Lokin CJA, Ceusters R, Ollevier F (1989). Some properties of the blood of European eel (Anguilla anguilla) and the possible relationship with Anguillicola crassus infestations. Aquaculture 76: 203-208.

[6] Cagirgan H (1990). Immune system in teleost and its significance on diagnosis (in Turkish). J. Fisher. Aquat. Sci. 7: 57-68

[7] Gabriel UU, Ezeri GNO, Opabunmi OO (2004). Influence of sex, source, health status and acclimation on the haematology of Clarias gariepinus (Burch, 1822). Afr. J. Biotechnol. 3(9): 463-467.

[8] Genc E, Sahan A, Altun T, Cengizler I, Nevsat E (2005). Occurrence of the swim bladder parasite Anguillicola crassus (Nematoda, Dracunculoidea) in European eels (Anguilla anguilla) in Ceyhan River, Turkey. Turk. J. Vet. Anim. Sci. 29: 661-663.

[9] Haney DC, Hursh DA, Mix MC, Winton JR (1992). Physiological and hematological changes in chum salmon artificially infected with erythrocytic necrosis virus. J. Aqua. Anim. Health 4: 48-57.

[10] Kwanprasert P, C Hangavant and N Kitancharoen, 2007. Characteristics of Achyla bisexualis Isolated from Eggs of Nile Tilapia (Oreochromis niloticus Linn.). KKU Res J, 12: 195-202.

[11] Palikova M, Navratil S (2001). Occurrence of Anguillicola crassus in the water reservoir Korycany (Czech Republic) and its influence on the health condition and haematological indices of eels. Acta Vet. Brno.

[12] Ranzani-Paiva MJT (1995). Características hematológicas de tainha Mugil platanus Günther, 1880 (Osteichthyes, Mugilidae) da regiao estuarino-lagunar de Cananeia – SP (Lat. 25°00'S - Long. 47°55'W). Boletim do Instituto de Pesca 22(1): 1-22.

[13] Sahan A, Altun T, Cevik F, Cengizler I, Nevsat E, Genc E (2007). Comparative Study of some Haematological Parameters in European Eel (Anguilla anguilla L., 1758) Caught from Different Regions of Ceyhan River (Adana, Turkey). J. Fisher. Aquat. Sci. 24: 167-171.

[14] Tran-Duy A, Schrama JW, Van Dam AA, Verreth JAJ (2008). Effects of oxygen concentration and body weight on maximum feed intake, growth and hematological parameters of Nile tilapia, Oreochromis niloticus. Aquaculture 275: 152-162.

[15] Van Der Heijden MHT, Booms GHR, Tanck MWT, Rombout JHWM, Boon JH (1996). Influence of flumequine on invivo mitogen responses of European eel (*Anguilla anguilla* L., 1758) lymphoid cells. Veterin. Immun. Immunopathol. 47: 143-152.