A trial for Induction of saprolegniosis in *Mugel cephalus* with special reference to biological control

Hussien, A.M. Osman1; Ahmed, I.E. Noor El Deen1; Waled, S.E. Solman1
Omima,A.Aboud2

1-Hydrobiology Department, National Research Center Dokki,Egypt
2-Animal Health Research Institute, Dokki,Egypt, **dr.hussien_osman@yahoo.com**

Abstract: A method was developed to experimentally induce saprolegniasis in *Mugel cephalus* fish exposed to physical stress, experimental descaling and descaling with wounding in addition of sudden and graduall drop of water temperature. Fish which descaled and wounded were mostly affected with saprolegniasis than the other group . Thus combination of descaling with wounding and sudden drop of water temperature were more effective in inducing saprolegniasis in *Mugel cephalus* Present study also investigate biological treatment of *Mugel cephalus* natural infected with saprolegniasis using intestinal non pathogenic aeromonas strain for control saprolegniasis in vitro (plate) and in vivo (treatment tank) as a bath of aeromonas suspension 2 times for 3 days. [Journal of American Science 2010;6(6):203-209]. (ISSN: 1545-1003).

Keywords: Saprolgniasis; Mugel cephalus ; temperature ; biological treatment

1. Introduction

Saprolegniasis is a serious mycotic winter freshwater fish disease, often affects wild and cultured fishes. Its presence is correlated to stress factors such as abrasions, cutenous wounds sexual maturity, poor water quality, crowdness, malnutrition, handling and bacterial and /or parasitic infections (Noga,1993;Pickering 1994). Several authors have carried out experimental infections with various species of saprolegnia using some predisposing factors to increase susceptibility of fish to infection as coetaneous scarification (Howe and Stehly 1998), modification of water temperature (Howe *et al.*1998;Van West 2006), combination of scarification and drop of water temperature (Howe and Stehly1998). Saprolegniosis usually starts as a cotton wool-like white to dark grayish or brownish growth on the head region and dorsal fin then spread allover the body in the form of focal patches (Abdel-Aziz *et al.*, 2002;Bangyakhun *et al.*, 2003;Osman *et.al.*,2008).

Saprolegniosis causes high economic losses in intensive fish farming (Bly et al 1996;Delgado *et al.*,2003). Treatment of saprolegniosis using anti fungal agents *are vital for the maintenance of healthy fishes and their eggs (Bly et al.*,1997;Fornerisa *et al.*,2003). Although, the disadvantages of using chemical fungicides (malachite green and formalin) reprsented as low withdrawal affinity and high carcinogenic activity on human and fish, yet, they used by many veterinarians for the control of saprolegniasis. Biological control of saprolegniosis has received little attention in Egypt, therefore present study was aimed to induce experimental saprolegniosis and investigate potential biological agent for control of saprolegniosis in *Oreochromis niloticus* by using of intestinal non pathogenic aeromonas strain and to confirm the hypothesis that it could be used in treatment of saprolegniosis in field.

2. Material and Methods

2.1 Fish:

A. natural infected fish :

400 natural infected *Mugel cephalus* fingerlings fish with saprolegniasis were obtained from private fish farm from kafr El-sheikh Governorate.100 were used inisolation of spors and 300 were used in biological treatment

B. Experimental Fish :

Apparantly healthy alive sixty *Mugel cephalus* fish of (50±10g) body weight collected from private cement fish farm for experimental induction of saprolgniosis. Fish transported in plastic tanks aerated with battery air pumps.

subdivided into 6 groups of ten fish each in 6 glass aquaria of (50 x 50 x 100 cm^3) dimensions, supplied with the natural water of the farm, fishes were fed with commercial feed pellets daily 5% of body weight.

2.2.Induction of saprolegniosis : Fishes were acclimated at water temperature (22 ± 1^oC) using thermostatically adjusted heater for 7 days . the first three groups (1,2,3) were descaled only while the other groups (4,5,6) were descaled and wounded on the sides and peduncle of the tail using sharp scalple .

First and fourth groups were subjected to sharp drop of water temperture ($5^oC\pm1^oC$) within 5 h using ice pieces placed around the aquaria from outside to avoid direct contact of fish with ice .

2_{nd} and 5^{th} groups were subjected to graduall drop of water temperature to (5 ± 1^oC) within 10 days.

the 3^{rd} and 6^{th} groups subjected to ($22^oC \pm 1$) during the time of the experiment (control). Fish groups were observed for behavioral, clinical signs of infection and morbidity /mortality rate. spores of saprolegnia were placed in each tank with each group of fish (Willoughby1994;Hatai and Hoshiai 1994) . The spores according to (Bly et al.,1993;Howe and Stehly1998) counted to determine the mean number of spores / ml of holding water .

2.3. Identification of the involved saprolegnia: Wet mount preparations of fungal skin lesions were microscopically examined according to Hussein and Hatai (2001). materials from fungal skin lesions of naturally infected fish were cultured on Sabaroud's dextrose agar(SDA,Difco)

With adding chloramphenicol at the rate of 25mg/L, plates were incubated at 22^oC (temperature resembled to that of the experimental aquaria) and periodically examined and reisolation and cultivation of saprolegnia sp. on plates of Sabaroud's dextrose agar enriched with crushed hempseed for flourishing saprolegnian hyphae. .

Identification of recovered saprolegnia spp. Was carried out using cultural morphological and microscopic characteristics recorded by **(**Hatai 1990) .

2.4.Isolation of saprolagnia spores :

In test tubes containing sterilzed distilled water, one sterilize pierced hemp seeds in each tube with the cotton wool like hyphae and incubated for 24 at room temperature then the water centrifuged (3.000rpm/for 10 mint the spores setteled down discard supernatant) and the spores counted on the haemocytometer and used later in induction of saprolegniasis

2.5. Preparation of Non Pathogenic Aeromonas Strain (NPAS) :

Under complete aseptic condition intestinal swabs were taken from apparently healthy *Mugel cephalus* fish and cultured in tryptone soy broth (TSB $_{CM129Oxid}$) and incubated for 24 h at 27^oC subcultured of these samplas onto TSA for examination of their growth and colony character. Microscopical examination of such bacteria indicates gram negative bacteria, short bacilli. Confermatory biochemical identification of these bacteria was done. *Aeromonas* colonies were taken from the plates and subcultured into TSB for 24 h at 27^oC.(Mayer-Harting *et al.*,1972) .

2.6 .Experimental Checking the virulence of NPAS on healthy *O. niloticus*:

Alive healthy 15 *Mugel cephalus* fish were injected I/P with 0.2 ml of $1x10^7$cells/ml (NPAS)/fish for determination of the pathogensity of the bacterial strain to the fish and observed for 14 days for recording the clinical signs and the PM lesions were recorded.

2.7. Prepration of fungal material and inoculating technique (vitro) :

For testing (NPAS) in vitro, hyphal tips obtained from a culture of saprolegnia grown on sabroud's dextrose agar at 25 ^oC were inoculated onto the prepared (NPAS) plates. In the first half of the plate hyphal tips were inoculated onto the area containing (NPAS) while inoculation in the second half of the plate served as a control to observe the saprolegnian hyphae gowth.This

for confirmatory testing of the antagonistic activity of (NPAS) to saprolegnia in vitro (Fig 5).

2.8.Preparation of NPAS bath for controlling of saprolegnosis (vivo) :

20 natural infected fish with saprolegnosis subjected for treatment using 4 tanks provided with The prepared (NPAS) which grown in Tryptone Soy Broth (TSB) overnight and diluted in the tank water to give approximately 10^6-10^8 cells/mL in 10L of dechlorinated water (provided with air pumps) The suspension was added to the tanks , which contained natural infected fish with saprolegnosis, Fish were observed for behaviour and clinical signs of saprolegnosis. Tankwater was partialy replaced by 2.5L from each tank daily with addition of (NPAS) at conc.10^{3-} 10^4cell / mL (for presrvation the concentration. of NPAS in the Water of the treatment tank)

3.Results and Discussion

Saprolegniosis is an acute infection affecting *Mugel cephalus*, the natural infected fish revealed focal greyish white patches on the head regions as well as skin, fins and occasionally gills. In advanced stages of infection, saprolegniasis spread out to cover the whole body (Fig B). Identification of recovered saprolegnia spp. was carried out using cultural morphological and microscopic characteristics (Fig C)

In regard to experimental induction of saprolegniasis the results showed in (table 1) the 1st group (subjected to sudden drop of water temperature) 30% of the fish were infected with soprolegniasis (Fig A) the 2nd group (subjected to gradual drop of water temperature) 10% of the fish were infected on the other hand 4th group (subjected to sudden drop of water temperature) 70% of the fish were infected, the 5th group of fish (subjected to gradual drop of water temperature) 40% of fish infected with saprolegnia. The mortality rate in the 1st group was 10% while the 4th group was 60% on the other hand the mortality rate in the group was 0% while in the 5th group was 30% .

Regarding to checking of the virulence of NPAS on healthy *Mugel cephalus*, the investigated bacterial strain was I/P injected in apparently healthy fish and observed for 2 weeks, no clinical signs produced nor pathological signs was found on the fish.

In regard to antagonistic action of NPAS on saprolegneasis in (vitro). The top half of the plate (Fig D) which contains NPAS had not grown the hyphae of saprolegnia while the bottom half lacked NPAS and served as a control to monitor vegetative growth of saprolegnia after 72 h incubation at room temperature.

In regard to treatment of saprolegniasis with NPAS in (vivo) the study involved 15 *Mugel cephalus* fish naturally infected with saprolegniosis, fish was initially immersed in bath containing NPAS after which normal water of the bath changed (50%) daily. Hyphen masses were observed floating on the water column after overnight exposure to NPAS. The fish appeared to be recovered as judged by absence of saprolegnia growth although the wounds remain unhealed, three days after treatment however the fish began showing clinical signs of saprolegniasis in the inflamed wounds at this stage NPAS could not be isolated from the tank water after 3 days another treatment bath was applied using NPAS at the same concentration. Although the wound was free from saprolegnian growth, the wounds began to heal and the fish recovered from the infection.

Saprolegniasis is an acute infection affecting fishes it is world wide mycotic freshwater disease affects wild and cultured species the clinical signs of saprolegniasis on *M.cephalus* resembled the recorded sings and lesions which were recorded by (Shaheen 1986;Badran 1989;Marzouk *et al* 1990;Kamoun 2003;Van West *et al* 2003;Birch *et al* 2006;Osman et al., 2008).Regarding the experimental induction of saprolegniasis, from the results it is clear that the group of fish which descaled only, the rate of infection and the mortality rate were less than that of the other group which desalted and wounded, also water temperature play on important role in susceptibility to various

infections especially saprolegnia. Several authors induce saprolegniasis in fishes (Howe and Stehly 1998). in rainbow trout (Howe et al 1998). and catfish but the present study was aimed to investigate, the induction of saprolegniasis in *O.niloticus* using some physical predisposing factors (descaling, wounding, sudden and gradual drop of water temperature) saprolegniasis is disease promoted by physical stressors like, poor water quality, malnutrition, injuries during handling, and transportation also overcrowding, temperature shock, spawning or external parasitism (Yanong 2003;Gieseker et al 2006) .

Scales and skin act as physialbarrier against external pathogens especially mycotic agents. The stressors predisposed fishes to saprolegniasis in the present investigation were represented as descaling and/or wounding combined with gradual or sudden drop of water temperature (Howe and Stehly1998). who demonstrated that, handling, rough surfaces of tanks or cages, overcrowding, parasitic infestation, damage skin, fins and gills increasing infections susceptibility causing osmotic stress and mortality Several authors induce saprolegniasis in fishes (Howe and Stehly1998) in Rainbow trout, (Howe et al.,1998) in catfish and (Osman *et al*., 2008) in *Oreochromis niloticus* but the present study was aimed to investigate the induction of saprolegniasis in *Mugel cephalus* using some physical predisposing factors.

In the present study, the prevalence of saprolegniasis hence mortality rate in the group of fishes predisposed to saprolegniasis by (descaling) were lower than that of the other group (descaled and wounded) this indicate that the importance of the scales and skin as physical barrier this may be owed to disturbance of osmoregultion as infection of saprolegniasis generally occur in the epidermis and dermis and occasionally in the superficial musculature so the destruction of skin can disturbs the fish's osmoregulatory system and cause a lethal dilution of body fluids (Pickering and Willoughly 1988;Hatai and Hoshiai 1993;Willoughby 1994). Skin of a fish is the envelope for the body and the first line of defense against diseases it also affords protection from the environmental factors.

Regarding water temperature, fish are cold blooded animals primarily dependent upon water as a medium in which to live. Fish can tolerate wide range of water temperature they can distinguish a rise in temperature from a fall but the physiological mechanism for such discrimination is not known (Hatai and Hoshiai 1993;Grandes *et al*.,2001). Temperature stress, particularly cold temperatures can completely halt the activity of immune system eliminating this defense against invading disease organisms (Knights and Lasee 1996).

Furthermore, decreasing of water temperature especially the sudden drop compromise the immune system of the fish, increasing the susceptibility to pathogens especially mycotic agents. Temperature stress particularly rapid changes severely affect the ability of fishes to release antibodies, giving the invaders the chance to produce the disease to fish (Neish and Hughes 1980) .

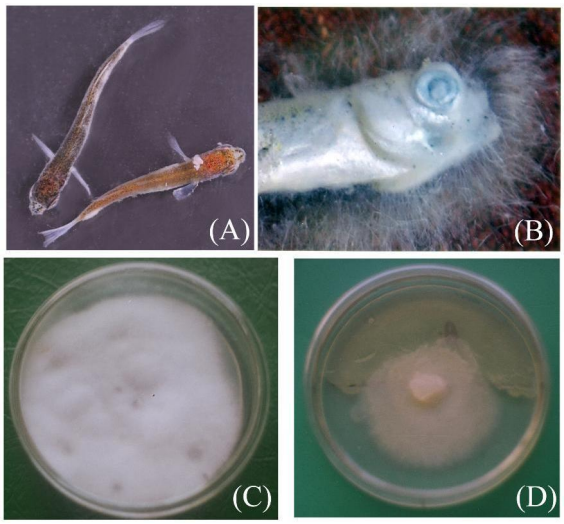
Regarding the antagonism of NPAS as biological control of saprolegniasis could play a significant role in the management of saprolegnia while the in vitro results demonstrated that NPAS was active antagonistic agent against saprolegnia. We can speculate that the presence of viable NPAS created conditions unfavorable for growth of saprolegnia after initial over night exposure to NPAS. It was clear that the growth of the saprolegnia has been retarded. Hyphal masses were also observed floating in the water after the first and second NPAS treatment baths. (3days each). The observations suggest that in these conditions, the pathogen detaches from the mucus and epidermal layer of the fish and released into the water. The ability of NPAS to inhibit saprolegnia appeared related to its ability to liquefy gelatin of such fungi. However the direct effect of gelatin hydrolase on saprolegnia growth. NPAS is considered as gelatinase positive (Holt et al.,1993). Parenthetically another candidate for the inhibitory activity for saprolegnia

is cellulase, an enzyme produced by NPAS (Hussein and Hatai 2001). The saprolegniacae have cellulose rather than chitin in their cell wall (Mullins 1973;Dick 1990). Using live bacteria for biological control may cause disease in fish. The investigated bacterial strain was non-pathogenic and safe for fish confirmed by I/p injection of this strain in apparently healthy fish and observed for 2 weeks. the result was no clinical signs produced nor pathological signs were found . There were reports discussed the in vitro inhibition of saprolegnia sp. by a gram negative rod, *Pseudo m onas fluorescens* by (Hatai and Willoughby 1988;B1y et al 1996;Delgado et al 2003). Reported that inhibition of saprolegnia by bacteria not related to the secretoray substance but rather the result of competition. (Hussein and Hatai 2001). showed in vitro antifungal activity by a number of Gram negative bacteria inclusive of the genus aeromonas, against pathogenic strains of saprolegnia parasitica. The discovery of existence of both in vitro and potential in vivo antifungal activity of NPAS increases its suitability as a probiotic and presents a possible approach to the management of saprolegniasis in M *cephalus.* In conclusion, *M.cephalus* were unable to withstand sharp or sudden drop of water temperature, accompanied with (physical stress) wounding or descaling. Such factors exclusively were the critical points for experimental induction of saprolegniosis in *Mugel cephalus* fish.

Table 1: showing the number of experimentally inducing saprolegniasis to *Mugel cephalus*

time Of exp												
	1st gp control *		dccatdMaphalu~rd 2nd gp gradual **		9P sudden drop ***		1st gp control *		2nd gp gradual **		3rd 9P sudden drop ***	
	no. of inf	no. of died	no. of inf	no. of died	no. of inf	no. of died	no. of inf	no. of died	no. of inf	no. of died	no. of inf	no. of died
1st day	0	0	0	0	0	0	0	0	1	0	1	1
5th day	0	0	1	0	1	1	0	0	3	3	3	0
10 day	0	0	1	0	2	0	0	1	0	3	3	2
total	0	0	10	0	30	10	0	10	40	60	70	30

* ISt group+4th group= sudden drop of water temperature (22-5oc within5hours)
**2nd group+5th group= graduall drop of water temperature(22-5oc within 10 days)
*** 3rd group+6th group= room temperature (22+loc control)



A- *Mugel cephalus* **experimentally infected with saprolegniosis .**
B -*Mugel cephalus* fingerlings natural infected with saprolegniosis .
C- Saprolegnia growth on sabaroud's dextrose agar .
D-The upper half of plate with NPAS while lower have without NPAS showing growth of sapralegnia hyphae .

References

1. Noga E.J. (1993): Water mold infections of freshwater fish: recent advances. Annual Review of Fish Diseases 3, 291–304 .
2. Pickering, A.D. 1994. Factors influencing the susceptibility of salmonid fish to saprolegniasis. In: Sabman Saprolegniasis (ed. by C.J. Mueller), pp. 67 – 86. Bonneville Power Administration, U.S. Department of Energy, Portland, OR, USA.
3. Howe, G., G. Stehly, 1998. Experimintal infection of rainbow trout with Saprolegnia parasitica. J. Aquat. Anim. Health 10, 397 – 404.
4. Howe, G.E., J.J.Rach, and J.J. Olson. 1998. Method for inducing saprolegniasis in channel catfish. Journal of Aquatic Animal Health 10: 62 – 68.
5. Van West, P. 2006. Saprolegnia parasitica, an oumycete pothogen with a fishy appetite new challenges for an old problem the British my cological Socaty 20: 99 – 104 .
6. Abdel Aziz, E.S., A.A. Yonis and M.N.M. Ali 2002. Effect of water temperature upon the response of cultured Clarias Lazera to Saprolegnia infection and the consequent haematologecal changes Egyptian Journal of compartive & clinical Pathology Vol 15 No. 2pp 108-125.
7. Bangyakhun, E., P. Pylkko, P. Vennerstrom, H. Kuronen and L. Cerenius 2003. Prevalence of osingla fish pothogenic saprolegnia sp. Clone in Finland and sweden diseases of Aquatic Organisms vol 53: 47 – 53.
8. Bly, J. E., S.M. A. Quiniou, L.A. Lawson and L.W. Clen 1996. Therapeutic and prophylactic measures for winter saprolegniosis in channel catfish. Diseases of Aquatic Organisms 24, 25-33.
9. Osman H.A .M., W.E. Solman, A.E. Noor El Deen and Lada A. Mohamed (2008) Induction of Saprolegniosis in Oreochromis niloticus with Special Reference to its Biological . Control. Global Veterinaria 2 (1):pp 32-37.
10. Delgado, C.L., N. Wada, Mw. Rosegrant, S. Meijer and M. Ahmed 2003. Outlook for to 2020 : Meeting Global Demand. Report By the International food poliay Reaserch Inistitute.
11. Bly, J.E., S.M. Quiniou, L.A. Lawson and L.W. Clem 1997. Inhibition of Saprolegnia pathogenic for fish by Pseudomonas fluorescenso, Journal Fish Diseases 20(1),35-40 .
12. Fornerisa, G., S. Bellardib, C.B. Palmegianac, M. Sarogliad, B. Sicuroa, I. Gascol and I. Zoccarato. The use of ozone in trout hatchery to reduce saprolegniasis incidence Aquaculture, 2003. 221: 157 – 166 .
13. Willoughby, L.G. Fungi and Fish Diseases Pisces press, Stirling Stirling Scotlond, 1994.PP 57 .
14. Hatai, K., C.I. Hoshiai. Pathogenieity of saprolegnia parasitica coker. In: Maeller GI (ed), Salmon saprolegniasis. U.S. Department of Energy, Bonneville power Adminstration, Portland, Oregon, 1994. PP. 87 – 98.
15. Bly, J.E., L.A. Lawson, D.J. Dale, A.J. Szalai, R.M. Durborow and L.W. Clem. Environmental factors affecting outbreaks of winter saprolegniosis in channel catfish, Ictalurus punctatus. Journal Fish Diseases, 1993 16, 541– 549.
16. Hussein, M.M.A. and K. Hatai. In vitro inhibition of Saprolegnia by bacteria isolated from lesions of salmonids with saprolegniosis. Fish Pathol. 2001.36(2), 73-78.
17. Hatai, K., L.G. Willoughby and G.W. Beakes. Some characteristis of Saprolagnia obtained from fish hatcheries in Japan Myeol Resesrch 1990. 94: 182 – 190.

18. Mayr-Harting, A., A.Y. Hodges and R.C.W. Berkeley. Methods for studying bacteriocins Methods in Micrubiology 1972. 7A, 315-422.
19. Shaheen, A.A.M. "Mycoflora of some freshwater fishes." M.V.Sc. Thesis, Zagazig University. 1986.
20. Badran, R.A. "Studies on fungi associated with Tilapia fish in River Nile water. "Ph. D. Thesis, Faculty Science, Assiut Univeristy in Egypt. 1989.
21. Marzouk, M.S.M., F. El-Far and M. Nawal "Some investigations of moulds and yeasts associated with tail and fin rot in freshwater fish in Egypt." Alexandrian Journal of Veterinary Science 1990. 6 (1): 193 – 203.
22. Kamoun, S. Molecular genetics of pathogenic oomycetes. Eukaryotic Cell 2003.2: 191–199.
23. Van West, P., A.A. Appiah and N.A.R. Gow, Advances in research on root pathogenic oomycetes. Physiological and Molecular Plant Pathology 2003.62: 99–113.
24. Birch, PRJ., AP. Rehmany, L. Pritchard, S. Kamoun and JL. Beynon. Trafficking arms: oomycete effectors enter host plant cells. Trends in Microbiology 2006.14: 8–11.
25. Yanong, P.E. Fungal diseases of fish. Veterinary. Clinical Exotic Animal Prac. 2003.6, 377– 400.
26. Gieseker, C.M, S.G. Serfling and R. Reimschuessel. Formalin treatment to reduce mortality associated with Saprolegnia parasitica in rainbow trout, Oncorhynchus mykiss Aquaculture 2006.253 120– 129.
27. Pickering, A.D. and L.G. Willoughby. Diseases of salmonid fish. 1988,Pages 38 – 48 in U>S. Fish and Wildlife Service, 15th Annual Report, Washington, D.C.
28. Hatai, K. and G. Hoshiai. Characteristics of two Saprolegnia species isolated from coho salmon with saprolegniasis. Journal of Aquatic Animal Health 1993. 5, 115-118 .
29. Grandes, J.M.F., M.F. Diez and J.M.A. Gancedo Experimental pathogenicity in rainbow trout. Oncborbyncbus mykiss (Walbaum), of two distinct morphorypes of longspinned Saprolegnia isolates obtained from wild brown trout. Salmo trutta L, and river water. Journal of Fish Diseases 2001.24, 351-359.
30. Knights, B.C., and B.A. Lasee. Effects of implanted transmitters on adult bluegills at two temperatures, Transactions of American Fisheries Society 1996.125: 440 – 449.
31. Neish, C.A. and G.C. Hughes. Fungal Diseases of Fishes, T.F.H. Publications, Neptune, New Jersey. 1980.
32. Holt, J.G., N.R. Kreig, P.H.A. Sneath, j.t. Staley and S.T. Williams Bergeys Manual of Determinative Bacteriology,.9th edn. Willians and Wilkins, Bastimore, MD 1993.
33. Mullins, J.T. Lateralbranch formation and celulase production in the water molds. Myclolgia 1973.65, 1007-1014.
34. Dick, M.W. Phylum Oomycota. In: Handbook of Protoctista (ed. by L. Margulis, J. Corliss, M. Mclkonian & D.J. Chapman), 1990.pp. 661-685. Jones and Bartlett, Boston.
35. Hatai, K. and L.G. Willoughby Saprolegnia Parasitica from rainbow trout ingibited by the bacterium Pseudomonas fluorescens. Bulletin of the European Association of fish Patbologists ,1988.8, 27

3/15/2010