EFFECTS OF CHICKEN MANURE AND MACROLESIONS ON THE INFECTIVITY OF SAPROLEGNIASIS (SAPROLEGNIALES SAPROLEGNIACEAE) ON NILE TILAPIA *OREOCHROMIS NILOTICUS NILOTICUS FINGERLINGS*

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ABSTRACT

An investigation was carried out to determine the mycotic infectivity of *Saprolegnia ferax* an aquatic Oomycetes on the nile tilapia, *Orochromis niloticus niloticus* with macrolesions using chicken droppings as fertilizer. The development of saprologniasis and the outcome on histological sections of the gills, skin and kidneys on experimental fish were also evaluated. Physiochemical parameter of the water were investigated. Fifty (4 week old) fingerlings of the test fish *O. niloticus nilotcus* with average weight of 13.40 $\pm$ 1.90g and average length of 8.80 $\pm$ 0.91cm were distributed into 5 replicated tanks containing 5 fingerlings each. *Saprolegnia ferax* that was cultured and identified using cultural characteristics from hatchery pond water was introduced into the experimental tanks equally. Experimental fish was disinfected with Ippm malachite green solution by immersion following standard methods. Chicken manure was washed with distilled water to remove dirt and grit before using as fertilizer employing standard application rates. There was no significant difference in all the tanks in respect to temperature, pH, and unionized ammonia values ($P < 0.05$) using One Way Analysis of Variance (ANOVA) and Duncans Multiple Range test. However in the tanks treated with chicken manure there was depletion of oxygen, increase in values of both free carbon *(*iv*)* oxide and dissolved organic content. Mortality of fish fingerlings was lowest (10%) in the control tank and higher (95%) in tanks containing fish with macrolesioins by the 4^{th} day of experiments respectively. Histological examinations of organs of the gill, kidney and skin revealed that the fingerlings in the control tank retained their gill filaments and lamellae while the treated fingerlings showed broken gill filaments and eroded gill lamellae. The mucus lining of the skin section was sloughed off and the lining underneath the mucus was thinnest in the control fingerlings and thickest in the fish treated with chicken manure. Kidney section of fish in the control tank showed fewer circular thyroid follicles as compared with treated tanks. The study has revealed that physico - chemical parameters especially the increase in free carbon (iv) oxide and dissolved organic content might have influenced the high rate of secondary pathogenicty of *Saprolegnia ferax.* Infection due to *S. ferax* greatly affected the mortality rates of experimental fish and caused pathological changes to the gill, skin and kidney of the Nile tilapia, Oreochromis *niolticus niloticus.*

INTRODUCTION

Fish farming has been in existence no sooner than when the early man began to develop their behavioral quest for certain food types. It has over the years become a very important economic activity in both advanced and developing nations.

In Nigeria there is an increased awareness of the importance of fish and fish farming for food and sport. The increasing commercial interests in pisciculture demands an understanding of the commensal flora of healthy fish which tend to be distinct from those of diseased fish. Higher stocking densities of fish call for introduction of large quantities of concentrated feeds and mineral fertilizers. The crowded conditions cause stress that weaken the immune system; affect epidermal integrity and lower resistance of fish to infectious disease Ugwuzor el *al* (1989).

The Nile tilapia, *Oreochromis niloticus niloticus are* fresh water fin fish commercially cultured in Nigeria. They accept artificial feed, convert food efficiently and are prolific breeders (Ofojekwu, 1989). These fish have the whole of their integument covered by a layer of mucus, which inhibits the colonization of micro–organism by entrapping and sloughing them. The rate of mucus production can be increased in response to infection or by physical or chemical irritants (Roberts 1989).

Numerous researches have attributed coincidence of stress, less optimal rearing conditions and outbreaks of infectious disease as the cause of fish mortalities. The pathogen and host have to be present in a favorable environment for disease outbreak to occur Robert (1989), Maston (2008).

Aquatic Phycomycetes especially Saprolegniales are common in natural water supplies of fish hatcheries and can cause serious disease problems. They attack fish eggs, fry, fingerlings and adult fishes when they get injured mechanically or as a result of infections. These fungi are saprophytic and opportunistic facultative parasites. They may become pathogenic due to environmental degradation caused by changes such as pollution, dietary or hormonal deficiency. Therefore under high population densities necessary for intensive fish husbandry Saprolegniasis is ubiquitous. Saprolegniasis in fish is facilitated by poor water quality, malnutrition, temperature, shock and injuries from handling, crowding, spawning or external parasitism. Jelf, et al., (2005). A knowledge of the common fungal disease of fish is essential to obtain and evaluate the efficiency of drugs or fungicides to control the disease. To this end the effect of different manure levels and macrolesions as predisposing factors to *Saprolegniaisis* on O*reochrmis niloticus niloticus f*ingerlings was investigated.

MATERIALS AND METHODS.

Collection and Acclimation of Experimental Fish

Nile tilapia, *Oreochromis niloticus niloticus* fingerlings with an average weight of 13. 40 ± 1.90g, and an average length of 8.80 ± 0.9cm were purchased from Rock Water Fish Farm Rayfield Jos and transported to the laboratory in plastic bags. Identification was done by experts of Fisheries and Hydrobiology Unit, Department of Zoology University of Jos and confirmed at the Federal Fisheries Department Jos. The fish were acclimated for 1 week in large plastic basins. They were fed formulated fish diet constituted as follows: fish meal 32.80%, soya bean meal 32.80%, cassava 24.50% , groundnut oil 5.00%, vitamin premix 2.50% and minerals premix 2.50% following standard procedure. The fish were measured using Mettler P. 1020 weighing balance and the length measured by using ruler and thread. Two thirds of the water was siphoned out daily and replaced with dochlorinated water to keep the water free of waste products. Dead fish were removed daily to prevent pollution. Five fingerlings each were used in each of the five replicated tanks . Lesions were infected on the experimental fish with sterile instruments.

Isolation of Saprolegnia Species

The direct water – baiting method as described by Alexopoulos and Mims (1988) was employed. Water samples collected from Rock Water Fish farm hatchery Rayfield Jos were distributed into petri – dishes. Five pre – boiled hemp seeds were added to each petri – dish and then incubated at 26^{o}C . They were observed daily for fungal growth and colonization around the seeds. Growth was observed on the 4th day. Identification was done with reference to Rebel and Taplin., 1970, Campbell and Stewart, 1980, Webster and Roland (2007). The organisms were then compared with existing stock cultures obtained from National Veterinary Research Institute Jos. Potato Dextrose agar supplemented with 0.5mg/l chloramphenicol was then used to grow a pure culture of *Saprolegnia ferax* for further work (Agina and Kpu 1988).

Procurement of Chicken Droppings

Fresh chicken droppings were collected from a healthy chicken pen in the University of Jos. The balls of chicken manure were than washed in distilled water for 30 seconds to removed dirt and grit before introduction into tanks B and C. Tank B had standard application rate while tanks C had double standard rates.

The experimental fish were first disinfected against infection by immersing in 1 ppm malachite green solution for 60mins (Robert 1978 Omoregie et al 1998). The fish were then put into 40 litre capacity experiment tanks filled to 35 litres capacity with dechlorinated water. Block of *Saprolegnia ferax* cultures grown on

potato dextrose agar was cut with flamed needles and forceps and introduced into tanks B,C,D and E with tank A as control. The solid potato dextrose agar were than removed from the experimental tanks.

Determination of Physcio Chemical

The physic- chemical parameters of the water namely temperature , pH, dissolved oxygen, free carbon IV oxide, dissolved organic matter and unionized ammonia were determined . The water temperature was recorded using a dry thermeometer 7- 10cm below surface of water. The pH was determined using the pH meter, Corning Model 7. Buffer solution of pH 7 was used to rinse the electrode before each reading. To calculate the dissolved oxygen the modified Winkler method was employed. 2.0mls $MNSO_4$, Alkaline iodide and sodium azide reagents were added to 2.50mls of water sample and mixed thoroughly. 2.0mls of Sulphuric acid was added to the mixture and titrated against 0.0125N Sodium thiosulphate solution with 1% starch solution as indicator. The average of the titre value was the dissolved oxygen content.

Free carbon IV oxide was calculated using standard methods with phenolphthalein as indicator. The solution of water and indicator was titrated with N/44 sodium hydroxoide till a pink colour which remained for at least 30 seconds was observed . Total free carbon IV oxide in ppm = 10 x ml of N/44 NaoH. Dissolved organic matter was calculated by boiling 100mls of water and 9.5mls of N /100 KMNO4 for 10mins. To this was added 10mls ammonium oxalate. The resulting colorless product was titrated against N/100 $KNMO_4$ until the solution become pinksh in colour. The end point was noted, the procedure was repeated using distilled water to get the blank titration. Dissolved organic matter = (Titre Value – value of blank) x 3.15

Following Procedure of Jatau and Wade, (1995) the unionized ammonia was calculated as follows:

$$\% \text{ unionized ammonia} = \frac{100}{1 + \text{antilog (Pka-ph)}}$$

Where PKA =

$$0.09018 + \frac{2729.92}{273.16 + t}$$

PKA is the negative logarithm on ionization constant which depends on temperature (t). Unionized ammonia is toxic and depends on the pH and temperature of the water at time of sampling.

Histological Examination

Histological examinations was done by employing routine histological techniques Baker et al (1980), and Bancroft (1990). The gills, kidney and skin of the fish were dissected out and fixed in normal saline for 48 hours. Automatic tissue duplex processor (Standon and Southern model C. 35H) was used to process the organs. Staining was the Hematoxylin – Eosin method.

Data Analysis

Results obtained from the investigations was analyzed using both One Way Analysis of Variance (ANOVA) and Duncan Multiple Range Test (Kelly and Onyeka 1992).

RESULTS

The mean values obtained for the physico- chemical parameters determined at 2 days intervals are shown in table 1. The highest means temperatures was recorded in tank A with 23. 88 + 0.43 ^{O}C while the lowest was recorded in tank B with 23 .27 $\pm$ 0.63^{O}C.

The highest mean value for pH was recorded in tank C with 6.77 $\pm$ 0.52 and lowest in tank E with 6.47 $\pm$ 0.05 respectively.

There was significant differences between the control tank A and, treatment tanks in the content of dissolved oxygen and free carbon iv oxide. The value obtained were 6.54 $\pm$ 0.11 ppm in control tank, and 1.045 $\pm$ 2.223 ppm in tank B for dissolved oxygen ; while for free carbon iv oxide 0.49 $\pm$ 0.04pp was obtained in tank A and 3.81 $\pm$ 2.57ppm in tank B respectively. The highest value for unionized ammonia was recorded in tank C with double manure rate at 0.55$\pm$ 0.78mg/l.

Generally the development of Sprolegniasis among experimental fish followed a similar trend. Fish fed and swam actively at first. Thereafter whitish blotches were seen on the skin, gills, fins, head and on lesioned sites. The experimental fish thereafter fed less actively and swam weakly and opercula counts reduced, ranging from 101/min to 115 /min in the control tank to 74/min in tanks with chicken manure.

There was erosion of the gill lamellae and broken filaments were observed in fish exposed to chicken manure and macrolesions.

There was increased sloughing off of topmost mucus lining of skin in treatment tanks than the control. Kidney sections revealed fewer number of circular patches of thyroid follicles in control tank than treatment tanks.

Death of fish was preceded by frequent gasping for air, followed by abnormal swimming and jerking and eventual death. Mortality rate were highest;

(95%), in the treatment tanks, 30% in tank with lesioned fish and 10% in the control.

Table 1: Overall values and standard error of the physico-chemical parameters observed during the experimental period.

Parameters	Treatments*				
	A	**B**	**C**	**D**	**E**
Temperature (°C)	23.88[a] (+0.43)	23.27 (+0.63)	23.32[b] (+0.64)	23.83[a] (+0.54)	23.70[b] (±0.78)
PH	6.54[c] (+0.11)	6.53[c] (+0.18)	6.77 (+0.52)	6.54[c] (0.11)	6.47 (+0.05)
Dissolved oxygen (ppm)	6.10[d] (+0.53)	1.51[e] (+2.23)	1.47e (+2.78)	6.03[d] (+0.64)	3.68 (+2.79)
Free Carbon (IV) Oxide (ppm)	0.49[f] (+0.04)	3.81 (+2.57)	3.31 (+2.63)	0.55[f,g] (+0.03)	0.69[g] (+0.23)
Dissolved organic matter (ppm)	10.50[h] (+1.94)	46.79 (+31.19)	55.88 (+25.77)	10.55[h] (+1.45)	19.41 (+0.15)
Unionized ammonia (mg/L)	0.20 [I,J] (+0.06)	0.18i (+0.08)	0.55 (+0.78)	0.18 j (+0.04)	0.15 (+0.03)

Values in brackets are the standard errors of treat-ments. Treatment with same superscripts means that there is no significant difference between average values.

Treatments*

A = Control tank
B = Chicken manured tank with standard application rate of inclusion.
C = Chicken manured tank with double the standard rate of inclusion.
D = Lessioned fish (water changed daily)
E = Lessioned fish (water not changed)

DISCUSSION AND CONCLUSION

The mean temperature values recorded over the experimental period for the various treatment was optimum 23.58 ± 0.60^{o}C and is in accordance with the desired range required for survival of Nile Tilapia *Oreohromis* n*iloticus*. (Ofojekwu 1989). The mean pH value of 6.27±01 most probably did not have any detrimental effect on the experimental fish as this value also falls within the range of 6.60-6.80 considered safe for the survival fish (Ferguson 1984). With respect to dissolved oxygen the mean value of 6.10 ± 0.53ppm in control tank favors fish survival while the depletion recorded in chicken manure treated tanks of 1.26 ± 2.51pm might have encouraged the virulence of *Sprolegnia ferax* . Fresh water fish did not thrive well whenever dissolved oxygen was continuously between 4ppm – 5ppm . However the study suggests that the Nile Tilapia are tolerable to low dissolved oxygen.

Stirling (1985) recommended 0.50ppm of free carbon iv oxide for the survival of juvenile fish. However the values of free carbon IV oxide geometrically increased in the chicken manure treated tanks. This could have acted as a stressor to the fish rendering them more susceptible to *Saprolegnia* infections. The increase in dissolved organic matter in treated tanks also could have encouraged the secondary pathognecity (Roberts 1989).

Unionized ammonia did not exceed the tolerance range of 0.07 + 0.10mg/ L to 0. 049 + 0.19mg/ l (Stirling 1985)

The low mortality rate in the control tank (10%) could be due to minimal stress as compared to the treated tanks with 95% mortality. Primary Sapolgniasis was observed without prior injury on eels, Robert and Tiffany (1989). They stated that wounds or macorlesions greatly increased secondary infections due to *Saprolegina ferax.* Saprolegniasis on the lesioned fish seem to be the only evidence for the 95% mortality recorded. Literature abound that attribute high mortalities in fish culture system to the presence of pathogens. The high rate of sloughing off of mucus lining agrees with the work of Roberts (1989) who explained that the mucus lining sloughs more repeatedly in the presence of pathogen in order to inhibit the colonization of fish integument. The alteration in the general arrangement and number of reticulo endothelial tissues of the kidney agrees with previous findings that reported similar histological change in *Cyprinus carpio* when they studied the effects of Bangladesh oil seed on sections of its kidney.

In conclusion the study has revealed that physico-chemical parameters did not determine the role of Saprolegnia species on the survival of the experimental fish.

Pathological changes were observed on the gills, skin and kidney of fish as a result of sprolegniasis and lastly that infections due to *saprolegnia ferax* greatly influenced the mortality rates of experiential fish. There is need to investigate the toxicity and role of the enzymes produced by Saprolegnia on fish tissue with a view to producing a cost– effective fungicide of aquatic phycomycetes to assist the profit oriented fish farmer.

REFERENCES

Agina, S.E. and R.S., Kpu (1988): A Survey of Aquatic Phycomycetes of Rock Water Fish Farm in Jos, Plateau State, Nigeria. *Nig. Soc. of App. Fish and Hydro.* ***3:*** *39-44*

Alexopoulos, C.J. and C.W. Mims (1988): *Introductory Mycology Third Edition*. John Wiley and Son Inc. New York London. 991 p.

Baker, F.J., R.E. Silverton (1980): *Introduction to Medical Laboratory Technology*. Fifth ed. Butterworths London. 1310 p.

Bancroft, J.D., A. Stevens (1990): *Theory and Practice* of Histological Technique. Third ed. Churchill Livingstone. Edingburgh, London.1523 p.

Ferguson, H.W. (1984): *Disease in Ontario Hatcheries*. In: Jatau L.E. (1995) Growth Responses of the Mudfish *Clarias Gariepinus* Fry Fed Different Starter Diets *of* Dried brewers yeastmeal and cow livermeal. 109.

H. R Jamlazadeh, A. Keyvan, M. R Ghomi, F. Gheraidi, (2009), Comparison of Blood Indices in Health and Fungal Infected Caspian salmana (*Salma titutta Caspius*). African Journal of Biotechnology **8** (2). 319 – 322.

Ingold, C.T. (1971): Fungal Spores; Their *Liberation and Dispersal*. First ed. Claventon publ. Oxford 591 p.

Ingold, C.T. and H.T. Hudson (1993) : The Biology of Fungi. Sixth ed. Chapman and Hall, London. 228 p.

Jatau, L.E. (1995): Growth response of the mudfish *Clarias gariepinus* fry fed different starter diets of dried brewers yeastmeal and cow livermeal. 149 p.

Jeteux, F. and P. Meyer (1972): Mixture of malachite green and formalin for controlling Ichthyopthirius and other protzoan parasites of fish. *Prog. fish cult.* **34**: 21

Jeff J. Rach, Theresa Schreir, Mark P. Gaikowski and Suscn, M. Schleis. (2005) Efficacy of formalin and Hydrogen Peroxide to increase Survival of Channel Catfish infected with saprolegniasis. *North American Journal of Aqua culture* **67**:312-318.

Kelly, M.G. and J.O.A. Onyeka (1992): Introduction to Statistics for the Life Sciences. First ed. ABIC Enugu Nigeria. 189 p.

Mackereth, F.J.H., J. Hebron and J.F. Tailing (1978): Water analysis; some revised methods for limnologists. Second ed. Biol. Assoc. 201 p.

Maston, *S. A (2008)* , Incidences of Dermatomycosis in fishes in Lampur Reservoir, Bhopal (M. P). *Journal of Herbal Medicine and toxicology* **2** (1) 37 – 40.

Lucky, Z. (1977): *Methods for the diagnosis of fish diseases*. Third ed. Mac. Publ. Lagos. 341 p.

Ofojekwu, P.C. (1989): Feed utilization in a *Tilapia* species *(Oreochroms niloticus niloticus* Trewava), Ph.D. dissertation, University of Jos, Nigeria.1990): Practical hydrobiology manual for fish culturists. Hydro, and fish. University of Jos.

Ogbonna, C.I.C. and R.O. Alabi (1991): Studies on the species of fungi associated with mycotic infection of fish in Nigerian freshwater fish ponds *Hydrobiol.* 220: 131 -135

Omoregin, E., Ofojekwu, P.C, Anosike J. C and Adeleye (1998). Acute Toxicity of Malachite green to the Nile tilapia, *Oreochromis niloticus*. J. Aqua. Trop. **13** (4) 233 – 237 pp.

Rebel, G and Taplin, David (1970)*:* Dermatophytes: Their recognition and identification University of Miani Press Coral Gables, Florida U.S.A.

Roberts, R. .J. (1972): Ulcerative Dermal Necrosis (U.D.N.) of Salmon *(Salmo salar* L.) *Zoo. Soc. On Dis, of Fish.* **30:** 53- 81.

Roberts, R. J (1978): Fish Pathology. Balliere Tindal Publishers London 318 pp.

Roberts R. J. (1989)*:* Fish Pathology. Second Edition Balliere Tindal Publ.

Stirling, H. P. (1985): *Chemical and biological methods of water analysis for aqua ulturists. Inst.* of Aqua. Univ. Publ.119 p.