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Reprotoxic Effect of Malachite Green on African Catfish *Clarias gariepinus* (Burchell 1822)

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ABSTRACT

The reprotoxic effects of malachite green on *Clarias gariepinus* was investigated in this study. Male and female broodstock were exposed to malachite green preparation at 20 mg mL^{-1} stock solution dissolved in 20 L of well-aerated water for 45 min. After which fish were removed and placed in fresh water. Treatment was repeated every other day for three treatments; after which spawning was artificially induced. Malachite green had a significantly detrimental effect on reproduction outcome, including egg and milt quality and hatchability. Histological assessments revealed distorted and necrotic eggs, disrupted and depleted seminiferous tubules in the testes. Additionally, in other organs harvested from sacrificed male broodstock, there was local degeneration of the skin. The use of malachite green should be discouraged in Nigerian aquaculture industry by imposing and enforcing a ban on its usage. This is both necessary for sustainable aquaculture production and public health protection. Furthermore, the potential of alternative parasiticides, like humic acid and chlorine dioxide, should be explored to replace malachite green.

Key words: Endocrine disruptors, *Clarias gariepinus,* malachite green, stock solution, reprotoxic effects

INTRODUCTION

The African catfish (*Clarias gariepinus*) is an ecologically important and commercially valued fish in Nigeria. They are frequently and widely cultured in ponds and they also occur freely in Nigerian natural freshwater (Samuel *et al*., 2008). The reproductive development and functions of *Clarias gariepinus* is widely affected by environmental chemicals, including herbicides, fungicides, insecticides and their metabolites. In the fish industry, chemicals used as fungicides include malachite green, formalin and sodium chloride. They can be used together or separately as anti-parasite treatments against ectoparasites such as *Gyrodactylus*, *Dactylogyrus*, *Ichthyobodo*, *Trichodina*, *Chilodonella* and *Ichthyophithirus*. However, malachite green has become a highly controversial compound due to the risks it poses to the consumers of treated fish (Alderman and Clifton-Hadley, 1993), including its effect on the immune system, reproductive system and its genotoxic and carcinogenic properties (Fernandes *et al*., 1991; Rao, 1995; Gouranchat, 2000).

Though, the use of this dye has been banned in several countries and not approved by United States Food and Drug Administration (USFDA), it is still being used in many parts of the world due to its low cost, ready availability and efficacy (Chang *et al*., 2001; Srivastava *et al*., 2004). The USFDA has nominated malachite green as a priority chemical for carcinogenicity testing (Culp and Beland, 1996). There is concern about the fate of malachite green and the reduced form, leucomalachite green in aquatic and terrestrial ecosystems since they occur as contaminants (Burchmore and Wilkinson, 1993; Saglam *et al*., 2003) and are potentially human health hazards. Nigerian aquaculture industries have been using malachite green extensively as a topical treatment for broodstock and egg disinfection without paying any attention to the fact that topically applied therapeutants might also be absorbed systemically and produce significant internal effects. Additionally, fish is considered as an important tool in aquatic toxicology, to assess alterations in physiological and biochemical processes from exposure to pollutants and toxic compounds (Venkataraman *et al*., 2007). This study was therefore, designed to determine the reprotoxic effect of Malachite green, a widely used paraciticide in African catfish.

MATERIALS AND METHODS

Sampling and exposure of broodstock to malachite green: This study was carried out in the month of July 2010. Two each, male and female broodstocks weighing 0.93±0.25 kg and total length 19.2±0.87 cm, were purchased from a private fish farm in Ibadan, Nigeria. Fish were acclimatized for two weeks after which they are subjected to treatment. During the period of acclimation, fish were fed commercially prepared pellets at 3% body weight. Stock preparation of Malachite green was done by adding 20 g of malachite green to one litre of distilled water (20 mg of malachite green per mL of stock solution).

One male and female broodstock were each exposed to malachite green preparation at 1 mL of stock solution per 20 L of well aerated water for 45 min. After which fishes were removed and placed in fresh water. This procedure was repeated every other day for three treatments. The other male and female broodstocks were not exposed to any chemical and were regarded as the control for the experiment.

Assessment of water quality: Water quality assessments were carried out for both the treatment and the control (i.e., at the start and towards the end of the experiment). The water quality parameters determined are: alkalinity, ammonia, carbondioxide, chloride, dissolved oxygen, nitrite, pH and hardness. Water quality parameters were determined using Hach® water quality test kits.

Artificial spawning of broodstocks: Spawning was induced in the females (both treatment and control) using Ovupin® according to recommended manufacturer's dosage rate of 0.5 mL kg^{-1}. Twenty-four hours later, fish were stripped of egg into a dry sterile petri dish. Egg samples were obtained for histological assessment. The remaining eggs were mixed with the milt from corresponding male broodstock and fertilization was activated with distilled water. Fertilized eggs were spread on carcaban in two separate flow-through hatching system for the treatment and control at a constant flow-rate of 3.5 L per min. The set-up was allowed to run for twenty-four hours to allow for hatching of the fertilized eggs. Newly hatched frys swam into fresh water, while the unhatched and dead eggs were siphoned out. The flow-through system was allowed to run for four days, while regression of yolk sac, growth rate and abnormalities in hatchlings were monitored daily using camera mounted light microscope.

Histological assessment: After sacrificing the male broodstocks to obtain milt; necropsy was done and samples of skin and testes was harvested and preserve in Bouin's fluid for 24 h, after which tissues were fixed in 10% phosphate-buffered formalin until processing. Processing involves dehydrating tissues, putting them into a xylene phase and impregnating them with paraffin wax under vacuum. Following this process, the tissues were embedded in wax and sectioned on a microtome into 5 μm sections. Selected sections were floated and stretched on a hot-water bath, mounted on clean glass slides and placed on a warming tray to dry and adhere. Following staining with haematoxylin and eosin; sections were covered with a coverslip and mounted on a light-microscope for evaluation by the pathologist (Kiernan, 1990). Abnormalities were documented using a digital camera.

RESULTS AND DISCUSSION

In order to establish aquaculture as a successful and efficient agricultural activity, there is a need to control reproductive processes in fish, in order to obtain high-quality seed and produce juveniles for grow-out without the need to obtain them from the wild. Throughout the exposure of the catfish to malachite green-treated water, the skin of fish was stained lightly. Fish were calm with no observable discomfort. Water quality was monitored in the fish throughout the experimental period and the results are presented as Fig. 1. Water quality assessment indices are also used to identify the relative health of cultured fish specie (Adeyemo and Babalobi, 2008). Malachite green administered at therapeutic level did not significantly affect the quality of water compared with control (Fig. 1). The no-effect of malachite green on water quality agrees with the report of Omitoyin (2007) following exposure of *Clarias gariepinus* juveniles to 0.15 ppm malachite green. However, malachite green significantly affected reproductive success as observed in Fig. 2-4. When viewed under microscope, the eggs collected from control sample were ovoid (Fig. 2) while that of the malachite treated fish had irregular shape with focal and necrotic lesions (Fig. 3). In the male, the milt collected was watery, brownish in colour and reduced in quantity. Twenty four hours later; normal larvae were hatched in the control (Fig. 4), while the treatments were un-hatched.

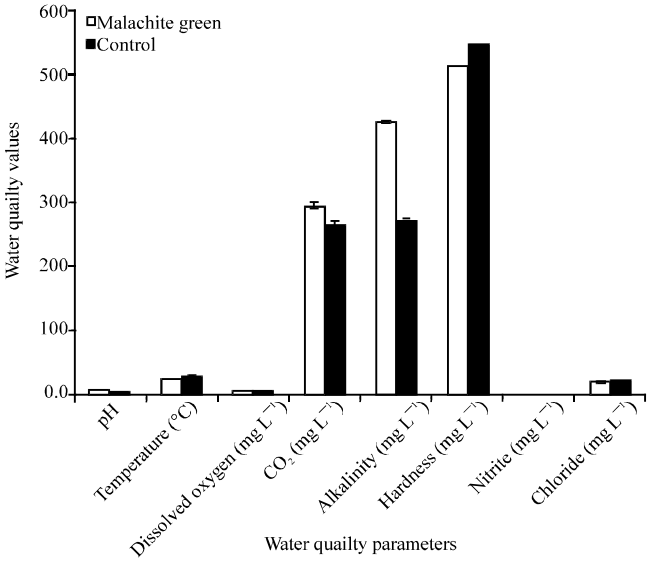

Fig. 1: Comparative values of water quality parameters of malachite green-treated tank and the non-treated tank (control)

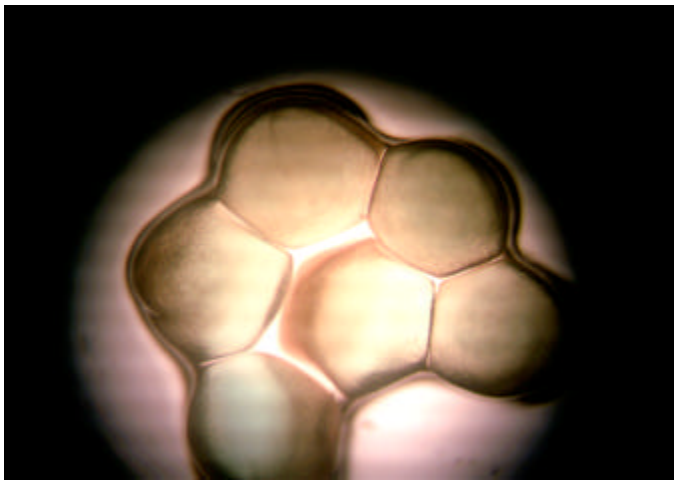

Fig. 2: Normal eggs from the control sample (x 400)

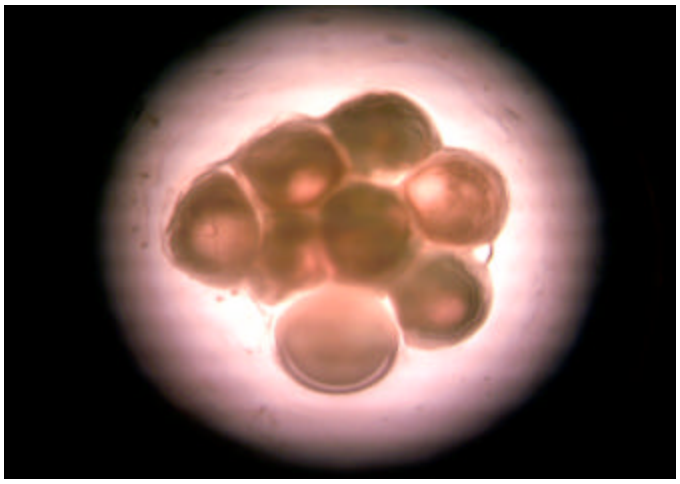

Fig. 3: Distorted and necrotic eggs collected from malachite treated Broodstock (x 400)

Significant developmental abnormalities in eggs, predominantly chromosome breaks, have been reported in rainbow trout, *Oncorhynchus mykiss* after long-term exposure to malachite green (Meyer and Jorgenson, 1983).

According to Stentiford *et al.* (2003) and Zimmerli *et al.* (2007), histological analysis represents a useful tool to assess the degree of pollution, particularly for sub-lethal and chronic effects. The histological changes observed in this experiment revealed that malachite green causes detrimental effects to the skin and testes of *Clarias gariepinus*. The pathological lesions in the testes of treatments compared to control (Fig. 5) was necrosis, disruption and depletion of the seminiferous tubules (Fig. 6). Additionally, compared with the skin of the control (Fig. 7); treatment had focal localized degeneration of the skin (Fig. 8). This is in agreement with the degenerative changes, reported to have occurred in the gonads of catfish following acute and chronic exposures to

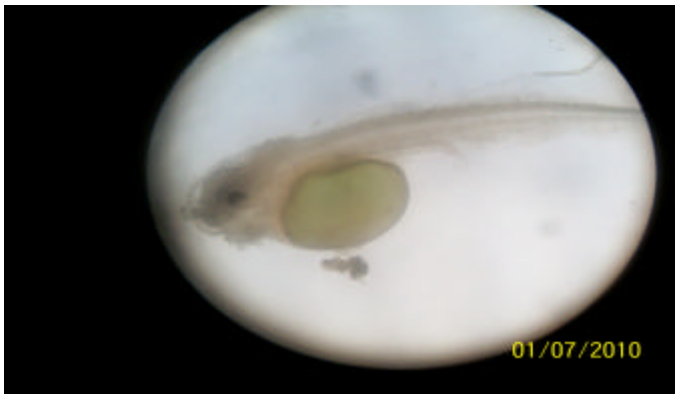

Fig. 4: Larvae within twenty-four hours of hatching in the control group (x 400)

Fig. 5: Normal histology of the testes, observed in control broodstock (H and E x100)

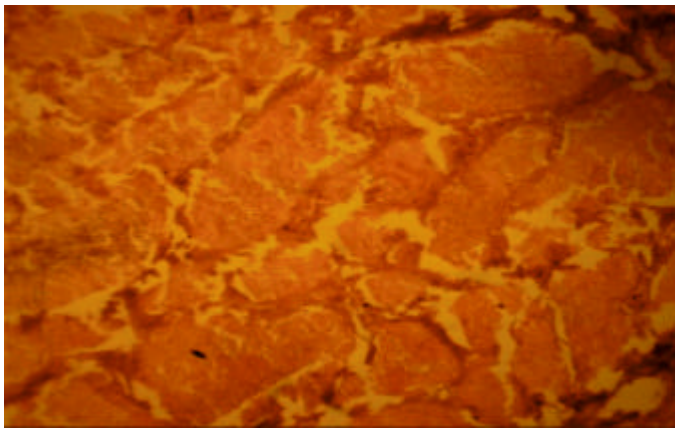

Fig. 6: Necrotic, disrupted and depleted seminiferous tubules observed in malachite treated broodstock (H and E x100)

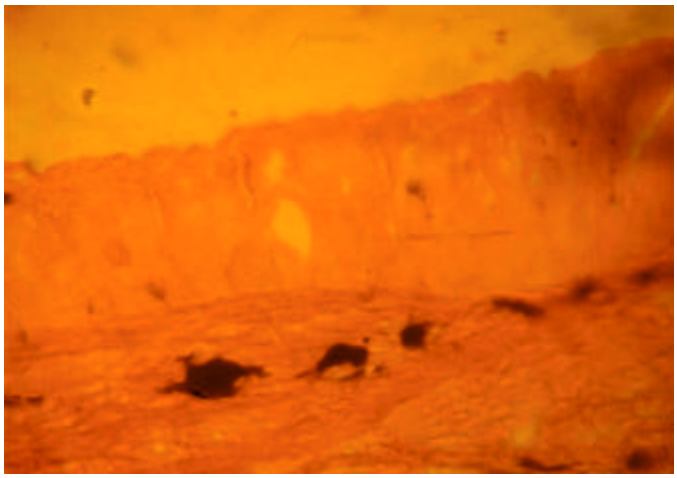

Fig. 7: Normal histology of skin as observed in the control (H and E, x 400)

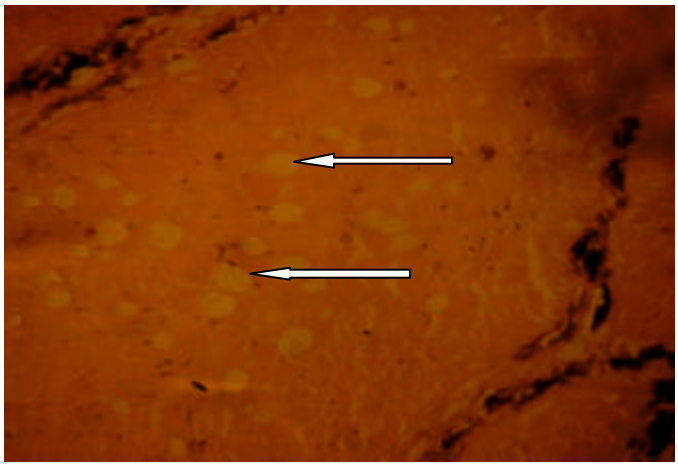

Fig. 8: Focal localized vacuolation of the skin in malachite treated broodstock (H and E, x 400)

subacute and sublethal concentrations of Malachite green (Srivastava *et al*., 1998). As observed in the skin of catfish in the present study, vacular degenerations of the liver was similarly reported in *Oreochromis niloticus* subsequent to exposure to copper sulphate, another fish paraciticide (Osman *et al*., 2009) and in the liver of *Clarias lazera* exposed to untreated dyestuff effluent (Abdel-Moneim *et al*., 2008). According to Desciens and Bablet (1994), malachite green is a multi-organ toxin. A zero tolerance of 0.01 mg kg^{-1} for the sum of malachite green and leucomalachite green in edible fish has been established (Klein *et al*., 1991). A pharmaceutical alternative to malachite green, 'Pyceze' with 'bronopol' as its active ingredient, has been developed in UK. It is being used for the treatment of fish and their ova; and appears to be a safe and effective replacement (Kaijser *et al*., 2001).

CONCLUSION

The present study showed that malachite green has a negative effect on the reproductive outcome of broodstocks. Histological changes were also observed in the testes and skin of the treated fish. Accumulation of malachite green in various tissues of fish was published by Alderman and Clifton-Hadley (1993) and Srivastava *et al.* (2004). It is, therefore, timely to explore further the potential of alternatives for the eventual complete replacement of malachite green in Nigeria's aquaculture industry.

REFERENCES

Abdel-Moneim, A.M., N.M. Abou Shabana, S.E.M. Khadre and H.H. Abdel-Kader, 2008. Physiological and histopathological effects in catfish (*Clarias lazera*) exposed to dyestuff and chemical wastewater. Int. J. Zool. Res., 4: 189-202.

Adeyemo, O.K. and O.O. Babalobi, 2008. Geographical information system (GIS) mapping of spatio-temporal pollution status of rivers in Ibadan, Nigeria. Pak. J. Biol. Sci., 11: 982-988.

Alderman, D.J. and R.S. Clifton-Hadley, 1993. Malachite green: A pharmacokinetic study in rainbow-trout, *Oncorhynchus mykiss* (Walbaum). J. Fish Dis., 16: 297-311.

Burchmore, S. and M. Wilkinson, 1993. Proposed environmental quality standards for malachite green in water (DWE 9026). United Kingdom Department of the Environment, Report No. 3167/2 (November 1993). Water Research Center, Marlow, Buckinghamshire, United Kingdom.

Chang, C.F., C.H. Yang, Y.O. Shu, T.I. Chen, M.S. Shu and I.C. Liao, 2001. Effects of temperature, salinity and chemical drugs on the *in vitro* propagation of the Dinoflagellate parasite, *Amylodinium ocellatum*. Asian Fish Soc., pp: 31.

Culp, S.J. and F.A. Beland, 1996. Malachite green: A toxicological review. Int. J. Toxicol., 15: 219-238.

Desciens, R. and J. Bablet, 1994. Recherches sur la toxicite des derives triphenylmethaniques anthelminthiques on the laminthgues. Compt. Rendus. Soc. Biol., 138: 838-839.

Fernandes, C., V.S. Lalitha and K.V. Rao, 1991. Enhancing effects of malachite green on the development of hepatic preneoplastic lesions induced by N-nitrosodiethylamine in rats. Carcinogenesis, 12: 839-845.

Gouranchat, C., 2000. Malachite green in fish culture (state of the art and perspectives). Bibliographic studies. Ecole Natl. Veterinaire ENVT, Nantes, France, pp: 142.

Kaijser, B., B. Torud and M. Sorgaard, 2001. Replacing malachite green. Fish Farm. Int., 28: 25-25.

Kiernan, J.A., 1990. Histological and Histochemical Methods. 2nd Edn., Pergamon Press, New York..

Klein, E., M. Edelhauser and R. Lippold, 1991. Occurrence and determination of residues of malachite green and leucomalachite green in edible fish. Poster Presentation (PE31), EURO Food. Chem. VI.

Meyer, F.P. and T.A. Jorgenson, 1983. Teratological and other effects of malachite green on development of rainbow trout and rabbits. Trans. Am. Fish. Soc., 112: 818-824.

Omitoyin, B.O., 2007. Metabolic effects of malachite green on *Clarias gariepinus* juveniles (Burchell, 1822). J. Fish. Aquatic Sci., 2: 422-426.

Osman, M.M., S.A. El-Fiky, Y.M. Soheir and A.I Abeer, 2009. Impact of water pollution on histopathological and electrophoretic characters of *Oreochromis niloticus* fish. Res. J. Environ. Toxicol., 3: 9-23.

Rao, K.V.K., 1995. Inhibition of DNA synthesis in primary rat hepatocyte by malachite green: A new liver tumor promoter. Toxicol. Lett., 81: 107-113.

Saglam, N., U. Ispir and M.E. Yonar, 2003. The effect of a therapeutic bath in malachite green on some haematological parameters of rainbow trout (*Oncorhynchus mykiss*, Walbaum, 1792). Fresenius Environ. Bull., 12: 1207-1210.

Samuel, O.B., O.Q. Suleiman and W.O. Odiete, 2008. Synergistic evaluation of oil-dispersant and oil-detergent mixtures using African Catfish, *Clarias gariepinus* fry. J. Fish. Aquatic Sci., 3: 280-290.

Srivastava, S., R. Sinha and D. Roya, 2004. Toxicological effects of malachite green. Aquat. Toxicol., 66: 319-329.

Srivastava, S.J., N.D. Singh, R. Sinha and A.K. Srivastava, 1998. Acute and chronic toxicity of malachite green: microscopic changes in the pituitary gonadotropic cells and gonad in a freshwater catfish, *Heteropneustes fossilis* (Bloch). Proc. Natl. Acad. Sci. India, 68: 253-256.

Stentiford, G.S., M. Longshaw, B.P. Lyons, G. Jones, M. Green and S.W. Feist, 2003. Histopathological biomarkers in estuarine fish species for the assessment of biological effects of contaminants. Mar. Environ. Res., 55: 137-159.

Venkataraman, G.V., P.N.S. Rani, N.S. Raju, S.T. Girisha and B.V. Raghavendra, 2007. Physico-chemical characteristics and impact of aquatic pollutants on the vital organs of a freshwater fish *Glossogobius giuris*. Res. J. Environ. Toxicol., 1: 1-15.

Zimmerli, S., D. Bernet, P. Burkhardt-Holm, H. Schmidt-Posthaus, P. Vonlanthen, T. Wahli and H. Segner, 2007. Assessment of fish health status in four swiss rivers showing a decline of brown trout catches. Aquat. Sci., 69: 11-25.