



International Journal of Fisheries and Aquatic Studies

ISSN: 2347-5129

(ICV-Poland) Impact Value: 5.62

(GIF) Impact Factor: 0.352

IJFAS 2016; 4(4): 315-318

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www.fisheriesjournal.com

Received: 10-05-2016

Accepted: 11-06-2016

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Histological assessment of organs of *Tilapia zillii* (Gervais 1852) Fingerlings subjected to malachite green (Triarylmethane dye) toxicity

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Abstract

A static bioassay was conducted to determine the 96h median lethal level (LC₅₀) of malachite green on *Tilapia zillii* fingerlings (6.35 ± 0.4 g and 6.23 ± 0.8 cm respectively) and to establish histological changes in the gill, liver and heart. LC₅₀ of *T. zillii* fingerlings was determined graphically as 1.65g MG /L of water. Fish displayed the following behaviours gasping for oxygen, erratic swimming, loss reflex, discolouration, irregular operculum and tail beat frequencies during 96 h exposure. Histological examination revealed alteration in the gill architecture vis congestion, degeneration and erosion of gill filaments, gill ray and lamellae. The liver showed vacuolation of the liver cell, hydropic degeneration of the hepatocellular parenchyma, chronic inflammation of hepatocyte and cellular infiltration of the periportal region. Lastly the heart showed evidence of mild lesion, slight disintegration in the heart cell arrangement, degeneration of the adipose tissue surrounding the sinus venosus, severe coagulative necrosis of the atrioventricular and ventriculobular valves at high concentrations of malachite green.

Keywords: Histological assessment, *Tilapia zillii*, malachite green, toxicity

1. Introduction

Tilapia has tremendous economic potential and plays significant role in environmental biodiversity in a number of countries around the world (Fortes 2005) [13]. It is among the easiest, one of the most productive/profitable and internationally traded food fish in the world (Modadugu and Belen 2004) [21]. It is a major protein source in many of the developing countries. They are native to Africa (El Sayed 2006) [7], and Nigeria is the second largest producer of farm-raised tilapia in Africa after Egypt (Fagbenro *et al.*, 2010) [9]. The commodity is not only the 3rd most important farmed fish globally next to carp and salmon but also described as the most important aquaculture species of the 21st century (Shelton 2002) [25]. They are very palatable and have high protein content (FAO 2012) [10]. The most important tilapias in aquaculture amongst others are the red belly tilapia, *Tilapia zillii* and Nile tilapia, *Oreochromis niloticus* (FAO 2002) [12] and these species account for 99.5% of global tilapia production.

Its species have since been introduced in different parts of the world to improve fisheries or to develop aquaculture (Lèvesque 2002) [19] because of their high protein content, large size, rapid growth (6 to 7 months to grow to harvest size) and palatability (FAO 2006) [11].

The realization of the above attributes, coupled with increase in the level of advancement in aquaculture has necessitated fish seeds production. However, fish seed is still at the minimal production level in Sub-saharan Africa, as a result of parasite infestation causing considerable loss to the producer. Fish seeds are often destroyed by treatable diseases because of lack of adequate knowledge of appropriate chemical and chemical dosage.

Malachite green (MG) is an organic compound (C₂₃H₅N₂) traditionally used as dye for materials such as silk, leather, and paper (Gresser and Mayer 2002) [15]. In aquaculture, it is used as bath for treatment protozoal ecto-parasites (ichthyophthirius, oomycete, Saprolegnia, e.t.c), which infects fish and fish eggs in fresh water aquaculture (Alvarez-Pellitero, 2004; Srivastava 2004) [1, 27]. However, malachite green has been reported to be a controversial agent in aquaculture and will persist in aquatic environment for a long time and may pass via food chain from water to untreated fish intended for human consumption, consuming fish contaminated with malachite green has been said to pose a significant health risk and is

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Considered to be carcinogenic, mutagenic and teratogenic (Andersen 2006; Sudova *et al.*, 2007) [2, 28]. In 2000, the use of malachite green for food fish was banned in the EU (Sudova *et al.*, 2007) [28].

MG absorbed by fish tissue is metabolically reduced to leucomalachite green (LMG), which is lipophilic and can be stored in edible fish tissues for an extended period of time (Mitrowska and Posyniak, 2004; Plakas 1999) [20, 30]. Though not approved for use in many countries (El-Newehy and Abou Srag 2011) [6], it is considered one of the most effective treatments for some fish diseases and it is still in use by most fish farmers in Nigeria.

Hence this study is aimed at determining the median lethal concentration (LC₅₀) of malachite green on *Tilapia zillii* fingerlings and to determine the effects of MG on the histology of gills, liver and heart tissues of fingerlings of *T. zillii*.

2. Materials and Methods

Two hundred apparently healthy *Tilapia zillii* fingerlings of mixed sex and the same genetic stock, mean weight and length of (6.35 ± 0.4 g) and (6.23 ± 0.8 cm) respectively were procured from State Ministry of Agriculture fish farm Ado Ekiti, Ekiti State, Nigeria. They were transported alive to Fisheries Management laboratory of Ekiti State University, Ado Ekiti, Ekiti State in 45 liter capacity plastic containers, half filled with pond water between 1700-1730 h. They were later stocked in rectangular plastic tank (75 cm x 40 cm x 40 cm) of 50-liter capacity where they are allowed to acclimatize for 7 days. Ten *T. zillii* fingerlings (6.35 ± 0.4 g) were stocked into each rectangular plastic tank (75 cm x 40 cm x 40 cm), with three replicates per treatment. Malachite green was obtained from Ware Laboratory Nigerian Limited, Taiwo Rd., Ilorin, Kwara State, Nigeria and kept in a dry, clean, air-tight well labelled transparent plastic container. Malachite Green was weighed by using a Metler top-loading balance (Model P13 8001). Dilution technique was used in dissolving Malachite Green salt, in water prior to toxicity test. The treatments were: Treatment 1, 1.0g MG /L of water; Treatment 2, 1.6g MG/L of water; Treatment 3, 2.2g MG /L of water,

Treatment 4, 2.8g MG/ L of water and Control, 0 g MG/L of water. Standard methods (APHA, 1998) [3] were employed in carrying out the experiment. Prior to the commencement of the experiment, the fish were starved for 2 days to minimize the amount of waste in the test media and to prevent organic decomposition and oxygen depletion. The experiment was conducted under standard static bioassay conditions. Temperature, pH, dissolved oxygen, and conductivity level were determined using standard methods and readings were taken at 24 h interval for 96 h.

At the end of the treatment period, dead fish were recorded and removed from the tanks. Fish were regarded as dead when all opercular movements stopped and eyes (pupils) got fixed. Two fish from each treatment tank were removed, weighed, killed by decapitation and vital organs such as the gill, liver and kidney were removed, fixed for 24 h in formalin-saline solution made of equal volumes of 10% formalin and 0.9% NaCl solution. Histological sections of 8 μ thickness were prepared following standard procedures (Chieli *et al.*, 1995) [4]. Photomicrographs were taken with Leitz (Ortholux) microscope and camera.

3. Results

The following behaviors were exhibited during the definitive test; gasping for oxygen, erratic swimming, loss reflex, discoloration, irregular operculum and tail beat frequencies. Fish subjected to the control, survived the 96 hours duration of the experimental period. There were significant losses of fish with increase in MG concentration ($P < 0.05$).

The LC₅₀ was determined graphically and recorded to be 1.65g MG /L of water (Fig.1).

Histological alterations in the organs (gill, liver and heart) of *T. zillii* fingerlings were represented in Table 1). Water samples were collected at an interval of seven days at a depth from each fibre tank. Temperature and dissolved oxygen (DO₂) were measured using glass thermometer and digital oxygen meter (YSI model 58, Yellow Spring Instrument Co) respectively. pH was measured with pH meter (Digital Mini-pH Meter, model 55, Fisher Scientific

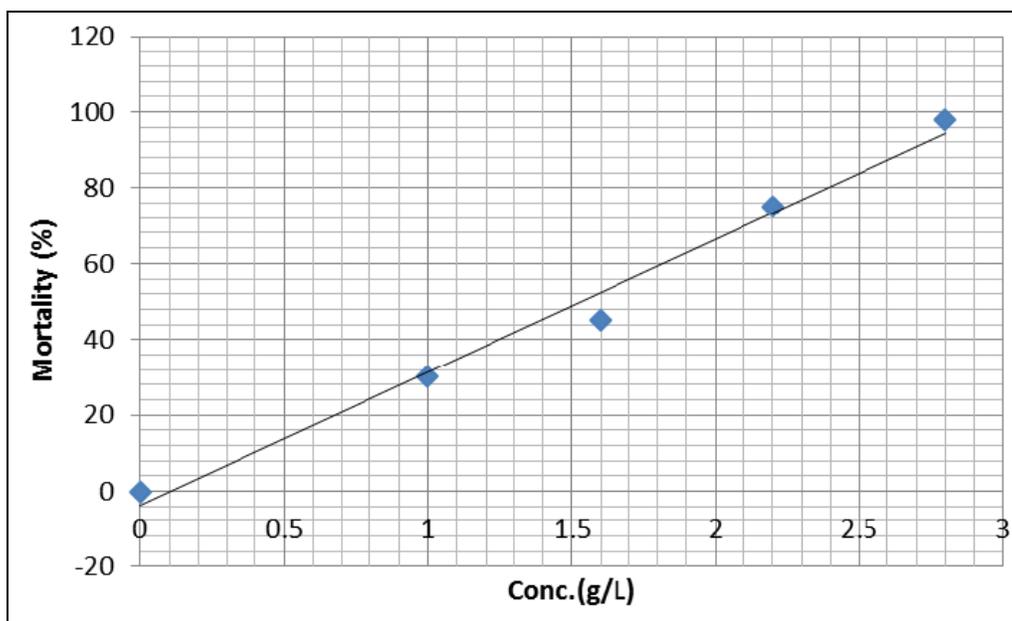


Fig 1: Effect of malachite green on fingerlings of *Tilapia zillii*

Table 1: Histological modifications in organs of *T. zillii* fingerlings subjected to malachite green toxicity

Concentration g/L	Gills	Liver	Heart
0	Normal gill architecture. Gill lamellae and secondary lamellae are visible	Normal hepatocellular architecture.	Normal heart architecture.
1.0	No visible change in the normal gill architecture.	No noticeable lesion.	Evidence of mild lesion.
1.6	Mild fusion/ congestion of gill lamellae.	Vacuolation of the liver cell and mild hepatic damage.	Slight disintegration in the heart cell arrangement.
2.2	Degeneration of the gill lamellae and gill ray	Hydropic degeneration of the hepatocellular parenchyma.	Degeneration of the adipose tissue surrounding the sinus venosus.
2.8	Epithelia proliferation/erosion of gill filaments and lamellae.	Chronic inflammation of liver cells and cellular infiltration of the periportal region.	Severe coagulative necrosis of the atrioventricular and ventriculobular valves.

4. Discussion

This study revealed that *T. zillii* fingerlings exposed to various concentration of malachite green demonstrated some behavioral changes such as jumping out of the tank (at higher concentration),

erratic swimming, discoloration, hyperventilation, irregular operculum beat frequencies, irregular tail beat frequencies, loss of reflex and loss of balance, which are sensitive indicators of physiological stress in the fish. In a related study on *Heterobranchus bidorsalis* fingerlings exposed to various concentrations of copper sulphate, some marked behavioral changes were observed such as jumping out of the tank (at higher concentration), erratic swimming, discoloration, hyperventilation, irregular operculum beat frequencies, irregular tail beat frequencies, loss of reflex and loss of balance, which are sensitive indicators of physiological stress in the fish (Jegade 2013) [16].

Also, a study by Chinabut *et al.*, (1988) [5]. showed that silver barb, common carp and snakehead exposed to formalin toxicity indicated that at high concentration there was an increase in opercular beat frequency but slower swimming than the controls and after 18 hours of exposure fish swam near the surface. Moribund fish swam up and down rapidly and frequently gulping at the surface. Fish exhibited uncoordinated movements and finally lay on the bottom.

4.1 Water Quality Measurements

Water samples were collected weekly at a depth from each plastic tank.

In all the treatment groups, DO₂ concentrations decrease with the increase in the concentration of MG at a range of 9.3-3.2 mg/ L⁻¹, water temperature average was 25.62 °C and pH value ranged between 6.53 and 8.21. All the water quality parameters were within the acceptable ranges for fish growth (Environment Protection Authority EPA, 2003) [8]. and tilapia culture (Ross 2000) [23]. However, this study corroborate the study by Johnson (2009) [18]. which reported that malachite green is also more toxic at low pH as well as high temperatures.

4.2 Histological Changes in Gill

This study reveals normal gill architecture i.e. secondary filament and gill lamella were noticeable (control group), while the other treatment groups shows alteration in the gill architecture vis congestion, degeneration and erosion of gill filaments, gill ray and lamellae.

In a related study on *Corydoras melanistius* exposed to formalin toxicity, histological examination of the gill revealed hyperplasia and epithelial hyperplasia with filling of interlamellar spaces at 50 and 100 mgL⁻¹ respectively (Santos

et al., 2012) [24]. Also a study by Nouh and Selim (2013) [29]. on the effect of formalin in tilapia as a commonly used disinfectant in aquaculture, histological examination of the gill revealed congestion and hyperplasia in the epithelium of the secondary lamellae.

4.3 Histological Changes in Liver

The liver plays a central role in the breakdown of foreign substances. With relation to liver histology, the control group revealed normal hepato-cellular architecture, which is similar to the normal liver architecture of tilapia as described by Morrison *et al.*, (2006). While other treatment groups showed vacuolation of the liver cell, hydropic degeneration of the hepatocellular parenchyma, chronic inflammation of liver cells and cellular infiltration of the periportal region.

In an akin studies by Chinabut *et al.*, (1988) [5]. and Santos *et al.*, (2012) [24]. silver barb and *Corydoras melanistius* exposed to formalin toxicity revealed hepatocytic fatty degeneration in the liver of silver barb fry and congestion of the hepocyte in *Corydoras melanistius* respectively.

4.4 Histological Changes in the Heart

This study showed histological changes in heart, the control group revealed normal heart histology (Morrison *et al.*, 2006). The treatment groups revealed evidence of mild lesion, slight disintegration in the heart cell arrangement, degeneration of the adipose tissue surrounding the sinus venosus, severe coagulative necrosis of the atrioventricular and ventriculobular valves.

A comparable studies by Jegede (2007) [17] and Jegede (2013) [16]. revealed a hole in the heart and degeneration in the cardiac muscle fiber, focal necrosis, vacuolar degeneration of cardiac muscle fiber and atrophy of the cardiac muscle fiber and edema of the atro-ventricular funnel near the entrance of the ventricle when *Oreochromis niloticus* and *Heterobranchus bidorsalis* are both exposed to high concentrations sodium chloride and copper sulphate toxicity respectively.

5. Conclusion

This present study have showed the median lethal level (LC₅₀) of *Tilapia zillii* fingerlings exposed to malachite green toxicity to be 1.65g MG /L of water. It also revealed the various histological alterations in gill, liver and heart of *T. zillii* fingerlings subjected to varying degrees of malachite green toxicity. Hence the knowledge of this could help in fish health management and the use of malachite green should be done under strict regulation and care to avoid its pathological side effect.

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