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Effect of Copper Sulphate on Spawning Success in African Catfish (*Clarias gariepinus*, Burchell 1822)

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ABSTRACT

Copper sulphate is a naturally-occurring inorganic salt used as herbicide and algaecide in irrigation and treatment of municipal water, also, as a molluscicide in destroying snails and slugs. Its toxicity to fish varies with the species and the physical and chemical characteristics of the water. When used at recommended doses, it may be poisonous to trout and other fish, especially in soft or acid waters. The effect of copper sulphate on spawning success in *Clarias gariepinus* was determined using a pair of adult Catfish (Male and female broodstock) and exposed to copper sulphate at the recommended treatment dose of 0.26 mg L^{-1} of copper sulphate for 10 days. The control pair was exposed to copper-free water for the same period after which both sets were subjected to artificial spawning. Water quality test was monitored pre and post treatment. Blood sample was collected for haematological assessment while organs and tissues were collected for histological assessment. Copper sulphate had no significant effect on both haematology and water quality parameters. However, significant histological changes observed in copper-exposed broodstock were multifocal severe degenerative necrosis of the testes resulting in the production of watery and brownish milt, necrosis of the ovaries producing shrunken, discoloured and cloudy eggs and severe matting of the gills lamina. Caution should be applied in the use of copper as a therapy for external parasites in catfish broodstock due to its effect on reproductive performance as observed in the present study.

Key words: Copper sulphate, *Clarias gariepinus*, artificial spawning, aquaculture

INTRODUCTION

The demand-supply gap for fish in Nigeria is about one million tonnes per annum which was as a result of emergence of commercial catfish farming industry. Catfish, (*Clarias* spp., *Heterobranchus* spp. and their hybrids) covers more than over (80%) of cultured fish in Nigeria followed by tilapias. Though, Nigeria depends on fish for (40%) of her animal protein requirement, still, the required quality and quantity of fish seed are yet to be met (Atanda, 2007).

Copper compounds are both effective algaecide and parasiticide and are prophylactically used to control fish diseases and parasites. Copper as a chemical has been used in sulphate forms for years in freshwater ponds and aquaculture operations (Moore *et al.*, 1984). According to Siddiqui *et al.* (2009), toxicity of copper can lead to metabolic processes disturbances and imbalanced physiological activities such as bone formation, reproduction, respiration and some nutrients metabolism. The main problem identified with the use of copper is its low safety margin which is lethal to fish (Watson and Yanong, 2006).

Additionally, Copper is a common pollutant in surface waters and its toxicity is largely attributable to its cupric (Cu^{2+}) form, which is the species commonly found or readily complexed by inorganic and organic substances (Alabastar and Lloyd, 1982). Copper has received considerable attention in biodata and fish toxicity level (Javed, 2004). According to Patel and Bahadur (2011), toxicity biomarkers in specific lesions present in fish organelles exposed to toxicants can be identified under laboratory condition. This study was aimed at determining the effect of therapeutic dose of copper sulphate on haematology, histology and spawning success in *Clarias gariepinus* broodstock.

MATERIALS AND METHODS

Sampling and exposure: Two each, male and female broodstocks were purchased from a private fish farm in Ibadan, Nigeria. Fishes were acclimatized for two weeks after which they are subjected to treatment. During acclimatization, fish were fed with commercially prepared pellets at 3% body weight. One male and female broodstock were each exposed to Copper Sulphate preparation at 0.26 mg L^{-1} for one week. The experimental set-up was renewal. Water was changed and fresh copper sulphate made every day and with constant aeration. Another male and female broodstocks were not exposed to any chemical and were regarded as the control.

Water quality parameters: Water quality assessments were carried out for both the treatment and the control (at the start and towards the end of the experiment). The parameters determined using Hach water quality test kit and Hanna® photometer were: Alkalinity, Ammonia, Chloride, Carbondioxide (CO_2), Dissolved Oxygen (D.O), Nitrite, pH and Total Hardness.

Artificial spawning: Spawning was induced after acclimatization of the *Clarias gariepinus* and subsequent treatment with copper sulphate. The broodstock were spawned (both treatment and control) following administration of Ovupin® according to manufacturer's recommendation (Dose of 0.5 mL kg^{-1}). The fishes were stripped the following morning and 3 g of egg each were collected from the stripped fishes into a dry sterile petri dish and then mixed with the milt from the corresponding male catfish. Fertilized eggs were incubated in plastic containers in two separate flow-through hatching system at a constant flow-rate of 3.5 L min^{-1}. The set-up was allowed to run for 24 h to allow for possible hatching of the fertilized eggs.

Histopathological assessment: The male broodstock was sacrificed to obtain the milt after which samples of organs and tissues were harvested and preserved in Bouin's fluid for 24 h. The collected tissues were processed for embedding and sectioning for slide preparation after which they were stained with haematoxylin and eosin and mounted on a light microscope for evaluation.

Statistical analysis: The statistical analysis used for this experiment was standard deviation which was used to measure the dispersion of obtained set of data from its mean.

RESULTS

Treated catfish were calm throughout their exposure to copper sulphate. There was no significant difference in the water quality parameters (Table 1), for instance, the alkalinity pre-exposure value was 324.9±24.2 while post-exposure was 289.0±19.6, Carbondioxide (150.0±0.00 and 197.5±14.4), Dissolved Oxygen (3.0±0.00 and 1.0±0.00), Hardness (444.6±19.7 and 383.65±11.1). Nitrite was 1.815±0.23 but absent after exposure while pH was 7.0 at both pre and post-exposure. Table 2 shows the haematological values obtained in the course of the experiment the Red Blood Cell (RBC) value of the treated male was lower (1.14×10^3 mm^3) than the reference value ($2.17\pm0.3\times10^3$ mm^3) while that of the treated female (2.53×10^3 mm^3) and control male (2.44×10^3 mm^3) fall within the normal range and that of the control female above. The White Blood Cell (WBC) values obtained in both treatment and control experiment were above the reference value. Haemoglobin concentration and Parked Cell Volume (PCV) of the treated male (5.7 g mL^{-1} and 18%) were within the normal range (6.29±0.46 g dL^{-1} and 18±1.4%), respectively while others were above. The values of the Mean Corpuscular Volume (MCV) of both treated and control experiments were above the reference value while that of the Mean Corpuscular Haemoglobin Concentration (MCHC) of both treated and control experiments fall within the reference value. However, the effects of copper sulphate were significantly observed on the spawning success. When viewed under microscope, the collected egg sample from the control experiment maintained the normal ovoid shape (Fig. 1) while the shape of the treatment were shrunken, cloudy and dicoloured (Fig. 2). The eggs collected from the treated fish remained unhatched at the end of the experiment (Fig. 3). Histopathological observations

Table 1: Water quality parameters pre and post exposure

Parameter	Pre-exposure to copper sulphate	Post-exposure to copper sulphate
Alkalinity (mg L^{-1})	324.9±24.200	289.0±19.60
Carbondioxide (mg L^{-1})	150.0±0.0000	197.5±14.40
Dissolved oxygen (mg L^{-1})	3.0±0.0000	1.0±0.000
Nitrite (mg L^{-1})	1.815±0.23	-
Wide range (pH)	7.0±0.0000	7.0±0.000
Hardness (mg L^{-1})	444.6±19.700	383.65±11.1

Table 2: Comparative haematological parameters of experimental fish with reference values

	Copper sulphate treated		Control		
Parameters	Male	Female	Male	Female	Reference values
RBC (mm^3)	1.14×10^3	2.53×10^3	2.44×10^3	3.12×10^3	$2.17\pm0.3\times10^3$
WBC (μL)	12,300	39,500	19,200	19,700	$56.46\pm13.5\times10^3$
Hb concentration	5.7 g mL^{-1}	9.2 g dL^{-1}	7.8 g dL^{-1}	10 g dL^{-1}	6.29±0.46 g dL^{-1}
PCV (%)	18	28	26	31	18±1.4
MCV (fL)	157	110	106	99	88.2±11.7
MCHC (g dL^{-1})	32	32	30	32	30.7±1.8

Red blood cells (RBC), White blood cells (WBC), Haemoglobin (Hb), Packed cell volume (PCV), Mean corpuscular volume (MCV), Mean corpuscular haemoglobin concentration (MCHC)

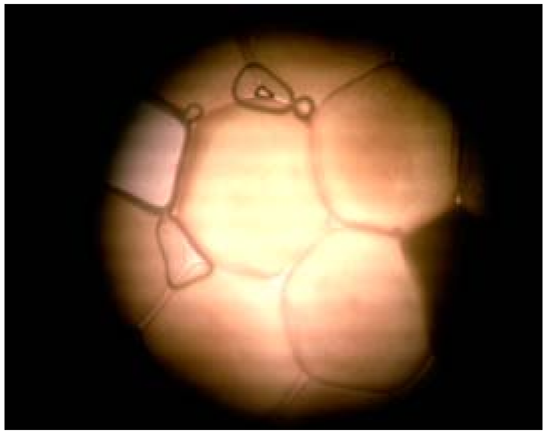

Fig. 1: Normal eggs collected from the control experiment (x100)

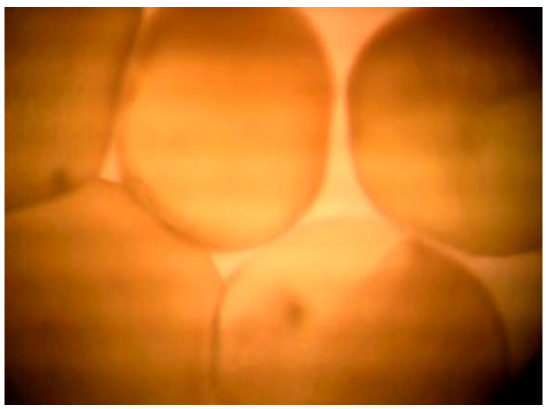

Fig. 2: Discoloured and cloudy eggs collected from the treatment experiment (x100)

Fig. 3: Un-hatched fertilized eggs from copper sulphate exposed broodstock

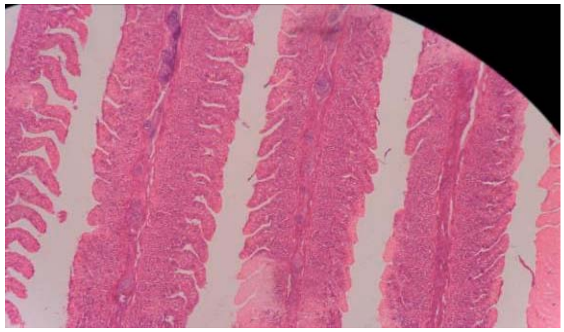

Fig. 4: Normal outlines of gills from the control experiment (H and E, x100)

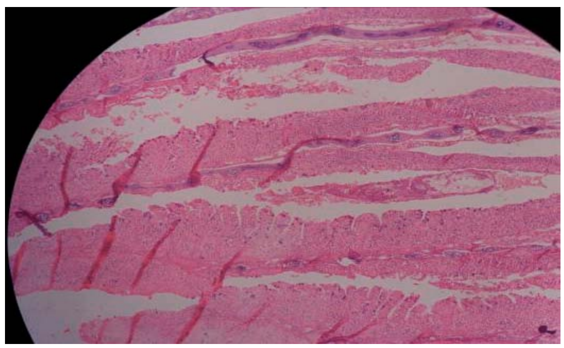

Fig. 5: Gills from copper sulphate with matted laminae (arrows) H and E, x100

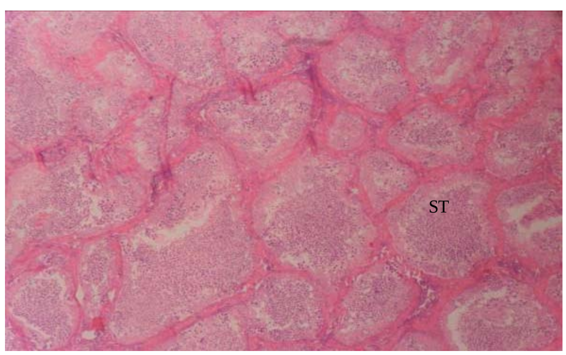

Fig. 6: Testes of the control experiment with well outlined Seminiferous Tubules (ST) with normal varying degree of maturity (H and E, x100)

revealed that copper sulphate had effects on various tissues and organs in the treated fish. Compared with the control (Fig. 4), there are matted laminae of the gills of catfish treated with copper sulphate (Fig. 5). Likewise, in relation to the normal testes from the control (Fig. 6) there was multifocal severe degeneration of the testes in the treated fish (Fig. 7). All other sampled organs were normal.

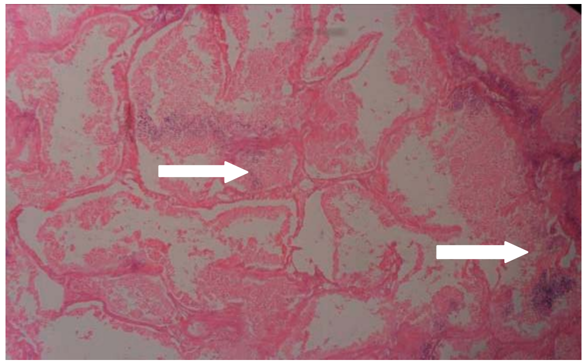

Fig. 7: Arrows showing the testes of the Copper sulphate treated experiment with multifocal severe degeneration (H and E, x100)

DISCUSSION AND CONCLUSION

Copper is widely used as an algaecide and mulluscicide (Paris-Palacios *et al*., 2000) and mostly used in the treatment of fish for pathogenic parasites (Bassleer, 1996; Noga, 2000; Perschbacher, 2005). Although, copper toxicity arising from dietary exposure only occurs at very high levels, low levels exposure of dissolved copper in the holding water can cause toxic effects in aquatic organisms (Grosell *et al*., 2004). This study showed that copper sulphate had a negative effect on spawning success in African catfish broodstocks. Grossly, the collected egg samples were cloudy, shrinken and discoloured and remained unhatched at the end of the experiment. Additionally, the copper sulphate treated male fish had a lower Packed Cell Volume (PCV) value compared to the controls. However, according to Adedeji and Adegbil (2011) the values were all within the normal range (18-37%). According to Wedemeyer (1996), gill is the most important and sensitive organ of fish that can be affected by water born irritant chemicals such as copper sulfate and also, acute copper toxicity in fish has been reported to be caused by direct effects on the gill epithelium (Noga, 2000) causing gill edema and epithelial lifting (Handy, 2003). The use of copper as a therapy for external parasites in these species should be cautioned due to its effect on reproductive performance of catfish broodstock and also its low safety margin which is lethal to fish.

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