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Original Article

CHANGES IN HAEMATOLOGICAL PARAMETERS OF CLARIAS GARIEPINUS EXPOSED TO CENTURY PLANT (*Agave americana*) LEAF DUST

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ABSTRACT

The effect of sub-lethal concentrations (0.1250, 0.0625, 0.0313, 0.0156, 0.0078 mg/L and Control) of *A. americana* leaf dust on haematological parameters of *C. gariepinus* was investigated, using static renewable bioassay system for 28-day period. There was significant difference ($p<0.05$) in the monitored physico-chemical parameters (hydrogen ion concentration (pH), total alkalinity, dissolved oxygen (DO) and free carbon (iv) oxide (CO_2)), however, temperature was not significantly different ($p>0.05$) in aquaria with *A. americana* compared to the Control. The packed cell volume, red blood cell counts, haemoglobin, and mean corpuscular haemoglobin concentration were significantly depleted ($p<0.05$) whilst, white blood cell counts significant increased ($p<0.05$) as the concentration of *A. americana* leaf dust increased. The implication of these findings revealed that the leaf dust of *A. americana* have negative effects on the test fish which may help explain the decline in wild fishery resources, especially, in areas where the plant is used for obnoxious fishing practices.

Keywords: African catfish,Blood Parameters, *Clarias gariepinus*, Medicinal plant, Sub-lethal effect,

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INTRODUCTION

The aquatic ecosystem like the terrestrial environment, is continuously subjected to changes in quality that are due to the introduction of substances of diverse characteristics arising from man's cultural activities (Oluah, 2001). The accumulation of toxicants in an aquatic environment can result in reduced reproductive capabilities, alter growth rates and reduce ability to withstand variations in pH, temperature and dissolved oxygen (Adamu *et al.,* 2008).

Majority of herbal plants and their products have been used as natural alternatives for treatment and management of various diseases including hepatic disorders (Stickel *et al*., 2005), as pesticides (Maikai *et al*., 2008), as molluscides (Azare *et al*., 2007) and piscicides (Tiwari and Singh, 2004). According to FAO (1991), more than 60,000 plant species are used for various purposes all over the world. Istvan (2000) reported that plants are virtually an inexhaustible source of biological active substances. The usefulness of plants for piscicidal and medicinal purposes has been reported (Adewole *et al*., 2002; Adamu, 2009; Akobundu, 1987) and the use of toxic plants to harvest fish is common practices worldwide (Ugwemorubong *et al*., 2009). Kawazu,(1972) reported that piscicidal plants have been used by traditional societies all over the world as a means of catching fish in small bodies of water. Piscicidal plants like *Blighia sapida, Kigelia africana, Tetrapleura tetraptera, Raphnia vinifera, and Parkia biglobosa* are commonly used by fisher folks to harvest fishes (Fafioye *et al*., 2004).

The eradication of wild fishes in the culture ponds before stocking of desired species is an important step in pond management as the former compete and/or prey upon the latter. Consequently, the control and eradication of unwanted fishes in the pond requires effective piscicides which are usually not easily accessible; farmers use synthetic compounds including malachite green (Ayotunde *et al.,* 2011). Different species of plants employed as piscicides have different effects, depending on the species of fish targeted. According to Yadav (2000), *A. americana* has been used by fishermen in catching fish. It contains different active ingredients such as nicotine, pyrethrum, rotenone, resin, tannins and saponins (Wang and Huffman, 1991).

*Agave americana*commonly known as century plant, maguey or American aloe belongs to the Family Asparagaceae. The plant is originally native to Mexico, Arizona and Texas. They are cultivated worldwide as an ornamental plant. The plant has become naturalized in many regions including the West Indies, parts of Africa and South America.

Fish have been the most popular choice as test organisms because they are presumably the best understood organisms in the aquatic environment

(Buikema *et al*., 1982) and also due to their importance to man as a protein source (Kime *et al*., 1996). The African catfish (*Clarias gariepinus*) are freshwater fishes found in the tropical regions of West African; therefore this fish may be one of the non targeted organisms that may be affected by the use of *A. americana.* In order to ascertain the effect of the plant piscicide on the health status of the test fish before death, haematological studies have been recommended (Kori-Siakpere *et al*., 2005). Such studies have generally been used as an effective and sensitive index to monitor physiological and pathological changes in fishes (Iwama *et al*., 1976). According to Babatunde *et al.,* (1992), changes in the constituents' component of blood sample when compared to the blood profile could be used to interpret the metabolic state of fish and its state of health. This present study investigated the sub-lethal effect of *A. americana* on the haematological indices (packed cell volume, red blood cell counts, haemoglobin, mean corpuscular haemoglobin, and mean corpuscular haemoglobin concentrati-on and white blood cell counts) of the African catfish (*Clarias gariepinus*) after 28-day exposure period.

MATERIALS AND METHODS

The juveniles of *Clarias gariepinus* of mean weight and length of 30±0.20mgand 17±0.40cm respectively of mixed sex were purchased from Renajj Fish Farms, Rayfield, Jos, Plateau State, Nigeria. The juveniles were acclimatized for 14 days in 60L capacity circular tanks in the Departmental Laboratory, Department of Zoology, University of Jos. During the acclimatization and trial periods, the fish were fed with coppens feed. Similarly, during the acclimatization and trial periods, 50% of water was siphoned daily in order to remove leftover feed and faecal matter. The water was thereof replaced with fresh 50% dechlorinated municipal tap water that was stored in laboratory condition.

The leaves of *A. americana* were collected from the University main Campus. Identification of the plant was aided by the technical staff of the Department of Botany. The leaves were air dried before being ground into powder and sieved with 30µm mesh size. The concentrations of *A. americana* leaf dust used was achieved after series of preliminary and acute toxicity tests. 0.125, 0.0625, 0.0313, 0.0156, 0.0080 and 0.000mg/L were concentrations used for the exposure. During the 28-day experimental period, some physico-chemical parameters such as pH, dissolved oxygen (DO), free carbon (iv) oxide (CO_2), total alkalinity and temperature (^0C) were monitored using standard procedures of APHA, (1998) weekly with the exception of temperature that was determined daily. At the end of the 28-day experiment, blood samples were collected from all the experimental fishes in ethylene diamine tetra acetic acid (EDTA) test tubes for the determination of

haematological indices as described by Blaxhall and Daisley (1973).

The data from the physico-chemical and haematological parameters were subjected to analysis by calculating means, standard error and Analysis of Variance (ANOVA) and student's t-test at 5% probability level.

RESULTS

The mean values of the monitored physico-chemical parameters of the test and control media are presented in Table 1. The pH and dissolved oxygen decreased significantly ($p<0.05$) while free carbon (iv) oxides and total alkalinity increased significantly ($p<0.05$) as the concentrations of the plant leaf dust increased. The haematological indices of *Clarias gariepinus* exposed to sub-lethal concentrations of *A. americana* leaf dust and the Control after the 28 days exposure period are presented in Figures 1 to 6. The mean value of packed cell volume (PCV), haemoglobin (Hb), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and red blood cells (RBC) were found to significantly ($p<0.05$) decrease as the concentration of *A. americana* leaf dust increased while that of white blood cells (WBC) showed significant ($p<0.05$) increase as the concentration of *A. americana* leaf dust increased.

Table1: Mean (standard error) physico-chemical parameters for sub-lethal bioassay of *A. americana* leaf dust on *Clarias gariepinus* for 28 days.

Parameters	*A. americana* leaf dust concentration (mg/L)					
	0.0000	0.0080	0.0156	0.0313	0.0625	0.1250
Temperature(˚C)	22.50	22.50	22.50	22.50	22.50	22.50
	(0.10)	(0.10)	(0.10)	(0.10)	(0.10)	(0.10)
pH	7.09	6.77	6.70	6.90	6.66	6.22
	(0.03)	(0.41)	(0.15)	(0.18)	(0.55)	(0.57)*
Dissolved oxygen(mg/l)	5.85	5.30	4.85	4.62	4.45	4.30
	(0.05)	(0.10)	(0.23)	(0.38)	(0.29)*	(0.09)*
Free carbon (iv) oxides (mg/L)	3.57	3.75	4.00	4.15	4.32	4.60
	(2.38)	(0.38)	(3.30)	(2.10)	(0.05)*	(0.04)*
Total alkalinity (mg/L)	6.90	8.62	10.63	11.92	14.40	15.97
	(0.05)	(1.58)	(3.27)*	(4.40)*	(6.16)*	(7.02)*

* significant difference $p<0.05$ compared to the control.

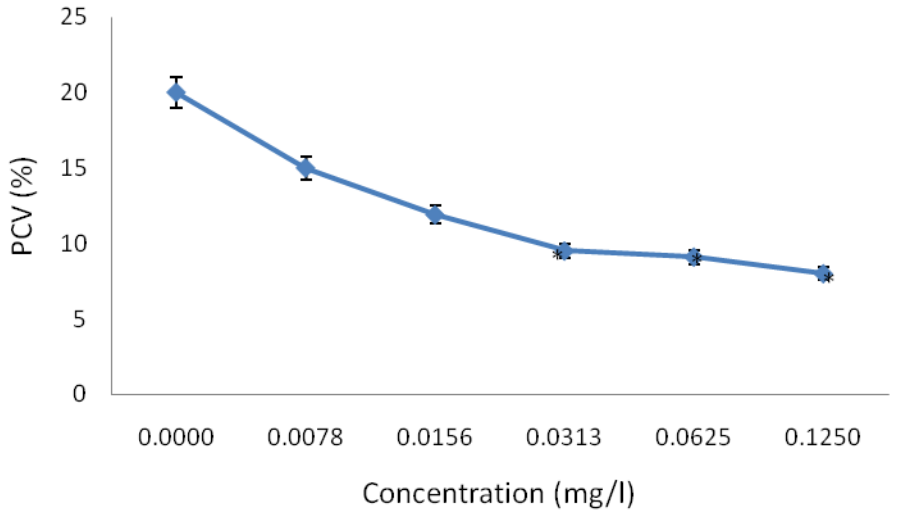

Fig 1: Packed cell volume of *C. gariepinus* exposed to concentrations of *A. americana* leaf dust for 28 days. Vertical bars represents standard error, * = $p<0.05$ compared to control

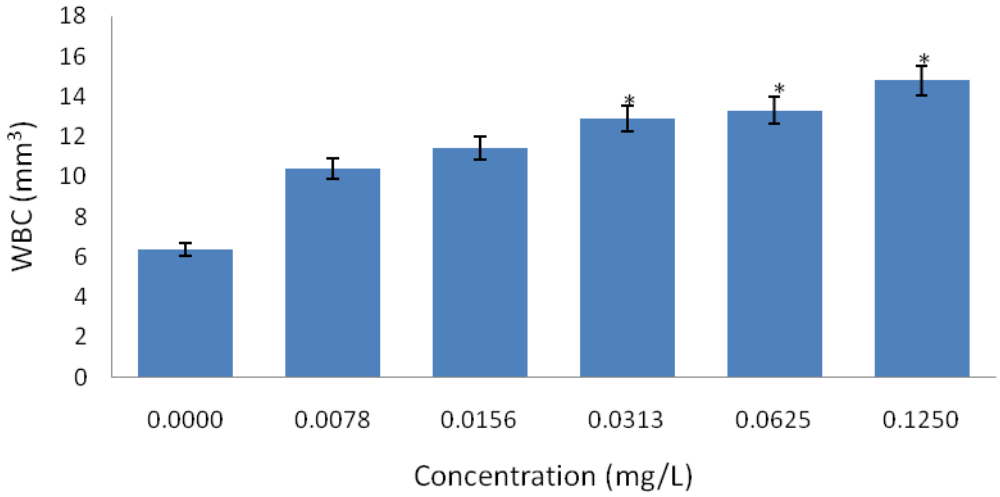

Fig 2: White Blood Cell level of *C. gariepinus* exposed to concentrations of *A. americana* leaf dust for 28 days. Vertical bars represents standard error, * = $p<0.05$ compared to control

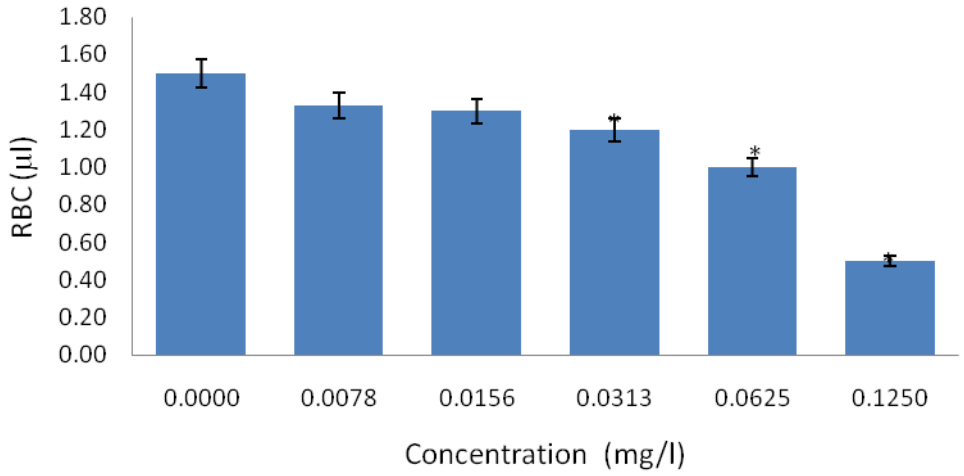

Fig 3: Red Blood Cell level of *C. gariepinus* exposed to concentrations of *A. americana* leaf dust for 28 days. Vertical bars represents standard error, * = $p<0.05$ compared to control

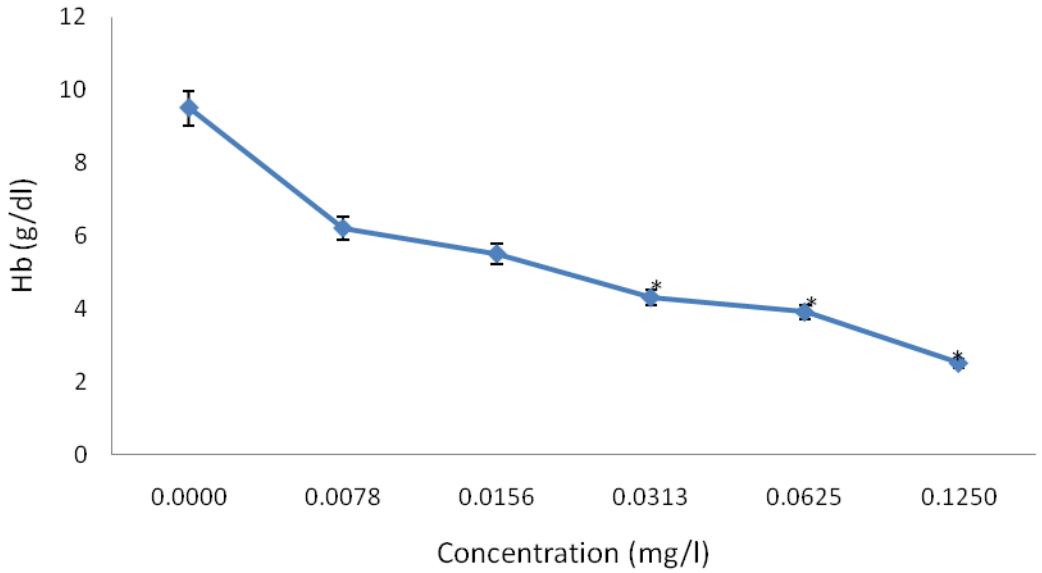

Figure 4: Haemoglobin level of *C. gariepinus* exposed to concentrations of *A. americana* leaf dust for 28 days. Vertical bars represents standard error, * = $p<0.05$ compared to control

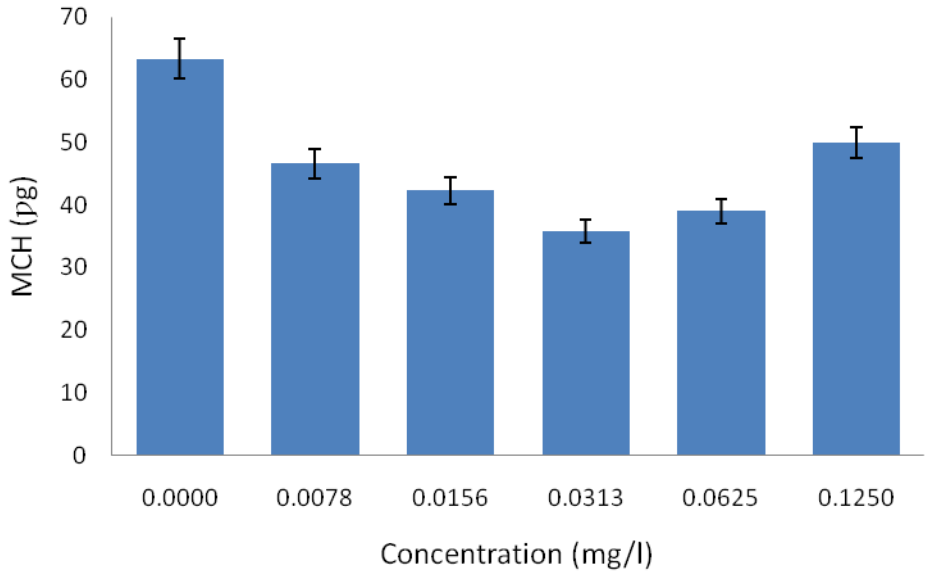

Fig 5: Mean corpuscular haemoglobin level of *C. gariepinus* exposed to concentrations of *A. americana* leaf dust for 28 days. Vertical bar represents standard error

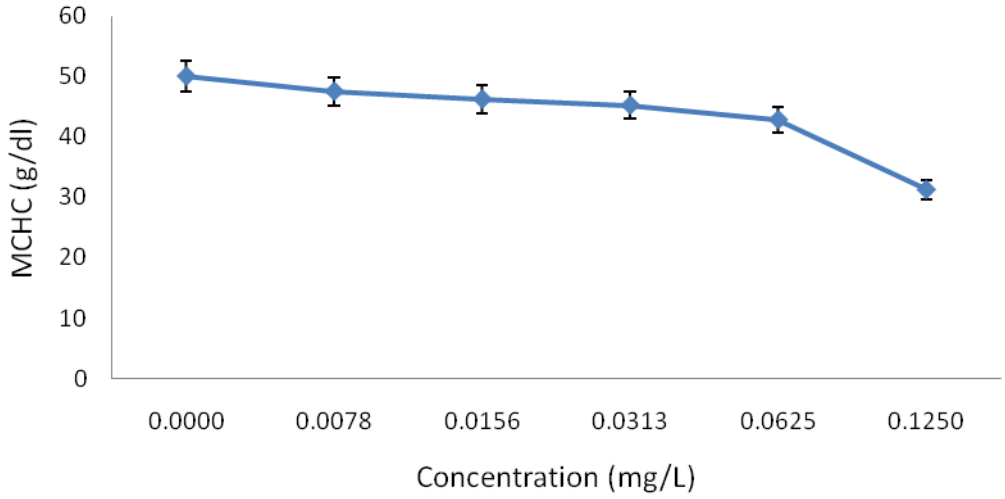

Fig 6: Mean corpuscular haemoglobin concentration level of *C. gariepinus* exposed to concentrations of *A. americana* leaf dust for 28 days. Vertical bar represents standard error

DISCUSSION

Physico-chemical parameters such as temperature, dissolved oxygen, free carbon (iv) oxide, pH, alkalinity are paramount to the many factors which affect fish health, growth and reproduction (Camus *et al.*, 1998). In this study, the monitored parameters were noted to be significantly different from the Control except for temperature. The decline in pH with time may be due to the production of acidic metabolites (Delyan *et al.,* 1990) by the plant material in water. Noga (1996) recommended pH range of 6.5

to 8.5 for fresh water fishes, the value ofpH in the two highest concentrations of the plant leaf dust were found to be lower than the recommended value. Prasad *et al.* (1995) reported that the reduction in dissolved oxygen content in a bioassay media as toxicant concentration increased may be due to antioxidant property of the toxicant. Similarly, free carbon (iv) oxide content increased with increase in the concentration of the toxicant.

The sub-lethal toxicity test carried out showed that *A. americana* leaf dust caused significant changes in the hematological indices of *C. gariepinus.* The changes in the values of the haematological indices of the test fish is similar to that reported by Ayotunde *et al.* (2011). The packed cell volume, haemoglobin and erythrocyte counts are good indicators of oxygen transportation capacity of fish thus making it possible to establish relationship with the oxygen concentration available in the habitat and the health status of the fish (Lamas *et al.,* 1994). On the other hand the white blood cells confer protection against infectious agent caused by microbial and chemical factors (Gusmao *et al.,* 2007).

The significant reduction in PCV, Hb, RBC, could be indication of severe anaemia caused by destruction of erythrocytes (Omoniyi *et al*., 2002) or haemo-dilution (Adeyemo, 2005), resulting from impaired osmoregulation across the gill epithelium as there was significant decrease in dissolved oxygen level. PCV is used to determine the ratio of plasma to corpuscles in the blood as well as the oxygen-carrying capacity of the blood (Larsson *et al.,* 1985). Adamu and Audu (2008) reported that the significant decrease in PCV may be attributed to gill damage and/or impaired osmoregulation causing anaemia and haemodilution. Haemoglobin is the oxygen-carrying component in the blood of fish and its concentration can be used as good indicator of anaemia (Blaxhall and Daisley, 1973). The decrease in Hb corresponds with the decrease in dissolved oxygen; an indication that the decrease in haemoglobin resulted in haemodilution. The Hb values fall lower than the range reported for catfish (Iheukwumere *et al.,* 2002; Ayotunde *et al.,* 2011). The reduction may be due to increased rate of breakdown of red blood cells and/or reduction in the rate of formation of red blood cells (Mossa, 2004) which may probably have been caused by the plant leaf dust. Haemoglobin concentration and packed cell volume values are directly correlated to erythrocytes count which may be due to the synergistic linkage of the blood cells. The increase in the white blood cells may have been induced as protection against disease and improving the health mechanism of the fish in the stressed condition.

The MCH and MCHC of the test fish were found to decrease with increasing toxicant concentration. Similar reduction has been observed by

Okomoda *et al.* (2010) in *C. gariepinus* exposed to formalin. The mean corpuscular haemoglobin concentration which is the ratio of the mean haemoglobin concentration is not influenced by blood volume neither by the number of cells in the blood, but can be interpreted incorrectly only when new cells, with a different haemoglobin concentration are released (Tawari-Fufeyin e*t al.,* 2008). Gafaar *et al.*(2010) reported that prolonged reduction in haemoglobin content is deleterious to oxygen transport and degeneration of the erythrocytes could be due to pathological condition in fish exposed to toxicants.

CONCLUSION

The study revealed that sub-lethal concentrations of *A. americana* leaf dust has deleterious and debilitating effects on the haematological indices of *C. gariepinus* juveniles as it also interferes with the water quality. Therefore, the use of the plant leaf dust should be discouraged.

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