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SUB-LETHAL EFFECT OF PAWPAW LEAVES (*Carica papaya*) EXTRACTS ON GROWTH, SURVIVAL AND HAEMATO-BIOCHEMICAL CHANGES OF *Clarias gariepinus*

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Abstract

The effects of *Carica papaya* (pawpaw) leaf extract on *Clarias gariepinus* juveniles (mean weight 22.50±0.02 g) were assessed under laboratory conditions, focusing on growth and haematological parameters. The 48-hour lethal concentration (LC_{50}) was determined as 8.0 ml/L. Fish were exposed to sub-lethal concentrations of 0.00 (control), 0.04, 0.08, 0.15, 0.30, and 0.60 ml/L for 28 days, with 10 fish per tank in triplicate. Data were analysed using ANOVA at P = 0.05. Growth parameters showed no significant differences (P>0.05), but weight gain and specific growth rate declined with increasing extract concentration. Survival was highest in PL5 (95.00±5.00%), with significant differences among treatments (P<0.05). Haematological responses varied with concentration. Packed Cell Volume (PCV) increased slightly in treated groups, except PL2 (42.00±2.83%), PL3 (41.00±2.00%), and PL6 (40.00±2.00%). Haemoglobin (Hb) peaked in PL4 and PL5 (15.00±2.83 g/dl) compared to control (14.3±2.83 g/dl). Red Blood Cell (RBC) counts declined in most groups except PL3 and PL4 ($5.10±2.83×10^6$/l). White Blood Cell (WBC) values dropped overall but increased in PL4 ($17.6±2.83×10^6$/l) and PL5 ($18.00±2.83×10^6$/l). Significant differences (P<0.05) were observed in Total Protein (TP) and Albumin (ALB), while Globulin (GLO) showed marginal increases, except in PL3 (35.00±2.83 g/dl). These findings indicate that sub-lethal concentrations of *C. papaya* leaf extract affect the growth and blood parameters of *C. gariepinus*, and may have potential as a natural piscicide for pond management.

Keywords: *Clarias gariepinus*, *Carica papaya*, Haematology, Growth.

Introduction

Over the last few decades, there has been an increased use of plant-based ichthyotoxins (piscicidal plants) for commercial fishing activities in many parts of the world including Nigeria. Plant-based ichthyotoxins are fish poisons derived from plant and active ingredients commonly detected in such plants include alkaloids, tannins, saponins, flavonoids, resin and phenolics. They are perceived to be less harmful to the aquatic environment than synthetic ichthyotoxins. Favourable properties of plant-based ichthyotoxins

include availability, rapid biodegradability, less bioaccumulation potential, and reduced toxicity to non-target organisms. Many studies including that of Fafioye *et al.* (2004) assessing the toxicity of plant-based ichthyotoxins to target and non-target aquatic species have focused mainly on acute toxic effects basically, mortality.

Fish Farming requires some form of interference in the breeding process to increase yield, such as daily feeding, protection from predators, etc. which also implies individual or corporate ownership of the stock being cultivated (Billard *et al.*, 2017). *Clarias gariepinus* which is the most cultured or commonly cultured species in Africa, known for its robustness and economic value exists in the wild but it is also cultivated in ponds, cages, and pens and is of great commercial importance. The eradication of wild fishes in the culture ponds before the stocking of desired species is an important step in pond management as the former compete and/or prey upon the latter. However, people tend to use synthetic piscicides (rotenone, antimycin, etc.) which along the line destroy both the targeted and non-targeted fish and also have a residual effect on the environment. Then, there is a need to look for organic piscicides that will not affect the non-targeted organism. The use of plant origin ichthyotoxicant as a fisheries management tool during pond preparation to get rid of wild fishes before fish stocking is a recommended practice to obtain good yield (Agbon *et al.*, 2004). Previous studies have shown that extracts from *Carica papaya* leaves exhibit toxicity to aquatic organisms due to their bioactive constituents. Therefore, this study aimed to evaluate the effect of acute and sublethal concentration of this plant on *Clarias gariepinus* juveniles

Material and Methods

Mature leaves of *Carica papaya* were collected from a cultivated tree in Olusegun Agagu University of Science and Technology community and identified by the Department of Biological Sciences (Botany programme) of Olusegun Agagu University of Science and Technology, Okitipupa. One hundred and twenty (120) juveniles of *Clarias gariepinus* of average weight 22.50 ± 0.02g were obtained from a fish farm in Akure. They were transported to the Fisheries and Aquaculture Technology Laboratory, Olusegun Agagu University of Science and Technology, Okitipupa and acclimatized for 7 days and fed twice daily with pelleted fish feed (Coppens 2mm) containing 40% crude protein. One-tenth value of the resulting 48 hours' LC50 value (8ml/L) was considered as sublethal concentration. Ten (10) fish per treatment were exposed to sub-lethal concentrations for a period of 28 days. The five groups each received, 0.04, 0.08, 0.15, 0.30 and 0.60ml/L of *Carica papaya* respectively with 35 litres of water. The test plant was changed every 72 hours and the experiment lasted for 28 days. The fish were fed at 5% body weight and the following biological evaluation were measured:

Weight gained = Final Body Weight – Initial Body Weight.

$$\text{Percentage weight gain} = \frac{(\text{Final Body Weight} - \text{Initial Body Weight})}{\text{Initial body weight}} \times 100$$

$$\text{Specific Growth Rate (SGR)} = \frac{100(\log_e \text{Final Body Weight} - \text{Initial BodyWeight})}{\text{Experimental days}}$$

$$\text{Feed Conversion Ratio (FCR)} = \frac{\text{Dry weight of the feed}}{\text{Fish weight gained}}$$

$$\text{Production Performance Index} = \frac{\text{Survival rate} \times \text{Weight gained}}{\text{Experimental day}}$$

$$\text{Survival Rate (SR)} = \frac{\text{Initial number of fish stocked} - \text{Mortality}}{\text{initial number of fish stocked}} \times 100$$

Nitrogen metabolism = $\frac{(0.549)(a+b)h}{}$

Where;

a = Initial mean weight of fish

b = Final mean weight of fish

h = Experimental periods in days.

Table 1: Behavioural changes and biological responses in *Clarias gariepinus* exposed to different concentrations of *Carica papaya*

EXP/ BEH	24 HOURS						48 HOURS						72HOURS					
CONC (ml/L)	0.00	2.00	4.00	6.00	8.00	10.00	0.00	2.00	4.00	6.00	8.00	10.00	0.00	2.00	4.00	6.00	8.00	10.00
E. S	–	–	–	–	+	+	–	–	–	+	+	N/A	–	–	+	+	N/A	N/A
A. G	–	–	+	+	+	+	–	–	+	+	+	N/A	–	+	+	+	N/A	N/A
V.P	–	–	–	–	+	+	–	–	–	–	+	N/A	–	–	–	+	N/A	N/A
L.R	–	–	–	–	+	+	–	–	–	+	+	N/A	–	–	–	+	N/A	N/A
LTG	–	–	+	+	+	+	–	+	+	+	+	N/A	–	+	+	+	N/A	N/A
L.E	–	–	–	–	+	+	–	–	–	+	+	N/A	–	–	+	+	N/A	N/A
M.S	–	–	–	–	+	+	–	–	–	–	+	N/A	–	–	–	+	N/A	N/A

- = No change in behaviour found, + = change in behaviour found; E.S =Erratic swimming, A.G = Air gulp, V.P = Vertical positioning, L.R = Loss of reflex, LTG= Lethargy, L. E= Loss of equilibrium, M.S = Mucus secretion, N/A= Not applicable (dead).

Table 2: Growth Performance and Nutrient Utilisation of *Clarias gariepinus* Juveniles Exposed to Different Concentrations of Pawpaw Extract

Parameters	Control (0.00ml/l)	PL_2 (0.04ml/l)	PL_3 (0.08ml/l)	PL_4 (0.15ml/l)	PL_5 (0.30ml/l)	PL_6 (0.60ml/l)
FBW	31.38±0.53^a	30.44±5.47^a	28.97±3.49^a	29.00±0.47^a	28.78±1.73^a	25.34±1.89^a
WG	8.85±0.53^a	7.94±5.47^a	6.47±3.49^a	6.50±0.47^a	6.28±1.73^a	2.84±1.89^a
PWG	39.45±3.33^a	35.27±17.18^a	28.76±10.98^a	28.89±1.47^a	27.91±5.42^a	12.60±5.93^a
SGR	0.52±0.02^a	0.46±0.28^a	0.39±0.19^a	0.40±0.21^a	0.39±0.91^a	0.18±0.11^a
NM	410.13±0.00^a	406.86±42.02^a	395.60±26.85^a	395.83±3.59^a	394.14±13.27^a	367.66±14.51^a
SR	85.00±7.07^b	85.00±21.21^b	85.00±7.07^b	90.00±0.00^b	95.00±7.07^b	45.00±21.21^a
PPI	26.88±0.63^a	26.16±22.60^a	20.09±12.24^a	20.89±1.50^a	21.53±7.45^a	3.84±0.89^a
FCR	1.10±0.07^a	1.25±0.59^a	0.91±0.35^a	1.16±0.06^a	1.24±0.24^a	1.14±0.54^a

Keys: FBW= Final Body Weight, WG= Weight Gain, PWG = Percentage Weight Gain, SGR = Specific Growth Rate, NM = Nitrogen Metabolism, SR = Survival Rate, PPI = Production Performance Index, FCR = Feed Conversion Ratio. Mean of duplicate data, mean value in each row with similar superscripts are not significantly different(P>0.05)

Table 3: Mean haematological parameters of *Clarias gariepinus* juveniles before and after exposure to different concentrations of *C. papaya*

Parameters	Before	Control (0.00ml/L)	PL2 (0.04ml/L)	PL3 (0.08ml/L)	PL4 (0.15ml/L)	PL5 (0.30ml/L)	PL6 (0.60ml/L)
PCV (%)	41.00 ±2.83^{a}	43.00 ±2.82^{a}	42.00 ±2.83^{a}	41.00±2.00^{a}	44.00±2.83^{a}	45.00±2.83^{a}	40.00±2.83^{a}
HB (g/dl)	13.70±2.83^{a}	14.30±2.83^{a}	14.00±2.83^{a}	13.70±2.83^{a}	15.00±2.83^{a}	15.00±2.83^{a}	13.40±2.83^{a}
RBC × 10a/l	4.50 ±2.83^{a}	4.90±2.83^{a}	4.10±2.83^{a}	5.10±2.83^{a}	5.10±2.83^{a}	4.60±2.83^{a}	4.30±2.83^{a}
WBC x 10a/l	12.60±2.83^{a}	17.10±2.83^{a}	13.50±2.83^{a}	16.90±2.83^{a}	17.60±2.83^{a}	18.00±2.83^{a}	11.30±2.83^{a}
Platelets(m/µl)	33.40±2.83^{a}	34.00±2.83^{a}	35.00±2.83^{a}	33.80±2.83^{a}	30.90±2.83^{a}	28.10±2.83^{a}	29.40±2.83^{a}
MCV (F1)	91.00±2.83^{bc}	87.80±2.83^{b}	102.40±2.83^{d}	80.40±2.83^{a}	86.30±2.83^{ab}	97.80±2.83^{cd}	93.00±2.83^{bc}
MCH (pg/cal)	30.40±2.83^{ab}	29.20±2.83^{ab}	34.20±2.83^{b}	26.90±2.83^{a}	28.80±2.83^{ab}	32.60±2.83^{ab}	31.20±2.83^{ab}
MCHC (g/dl)	33.40±2.83^{a}	33.30±2.83^{a}	32.30±2.83^{a}	33.40±2.83^{a}	33.40±2.83^{a}	33.30±2.83^{a}	33.5±2.83^{a}
NEU (%)	69.00±2.83^{a}	70.00±2.83^{a}	71.00±2.83^{a}	70.00±2.83^{a}	69.00±2.83^{a}	73.00±2.83^{a}	69.00±2.83^{a}
LYMP (%)	28.00±2.83^{a}	27.00±2.83^{a}	29.00±2.83^{a}	28.00±2.83^{a}	31.00±2.83^{a}	32.00±2.83^{a}	30.00±2.83^{a}
MONO (%)	2.00±2.83^{a}	2.00±2.83^{a}	0.00±0.00^{a}	1.00±0.71^{a}	0.00±0.00^{a}	0.00±0.00^{a}	1.00±0.71^{a}
EOS (%)	1.00±0.71^{a}	1.00±0.71^{a}	0.00±0.00^{a}	1.00±0.71^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}

Keys: PL= Pawpaw Leaves, PCV = Packed Cell Volume, HB = Haemoglobin, RBC = Red Blood Cell, WBC = White Blood Cell, MCV = Mean Corpuscular Volume, MCH = Mean Corpuscular Haemoglobin, MCHC = Mean Corpuscular Haemoglobin Concentration, NEU = Neutrophils, LYMP = Lymphocytes, MONO = Monocytes, EOS = Eosinophil. Mean of duplicate data, mean value in each row with similar superscripts are not significantly different (P>0.05).

Table 4: Mean plasma biochemistry parameters of *C. gariepinus* exposed to sub-lethal concentrations of *C. papaya* leaves extract

Parameters	Before	Control (0.00ml/L)	PL2 (0.04ml/L)	PL3 (0.08ml/L)	PL4 (0.15ml/L)	PL5 (0.30ml/L)	PL6 (0.60ml/L)
TP (g/dl)	78.00±2.83^{b}	74.00±2.83^{ab}	69.000±2.83^{a}	75.00±2.83^{ab}	74.00±2.83^{ab}	80.00±2.83^{b}	77.00±2.83^{b}
ALB(g/dl)	38.00±2.83^{ab}	38.00±2.83^{ab}	31.00±2.83^{a}	40.00±2.83^{b}	35.00±2.83^{ab}	41.00±2.83^{b}	38.00±2.83^{ab}
GLO(g/dl)	31.00 ±9.90^{a}	36.00±2.83^{a}	38.00±2.83^{a}	35.00±2.83^{a}	39.00±2.83^{a}	39.00±2.83^{a}	39.00±2.83^{a}
ALB/GLO	1.00 ±0.71^{a}	1.10±0.71^{a}	0.80±0.71^{a}	1.10±0.71^{a}	0.90±0.71^{a}	1.10±0.71^{a}	1.00±0.71^{a}

KEYS: PL= Pawpaw Leaves, T P= Total Protein, ALB = Albumin, GLO = Globulin, ALB/GLO = Albumin-Globulin ratio. Mean of duplicate data, mean value in each row with similar superscripts are not significantly different (P>0.05).

Discussion

The fish exposed to the toxicant (*Carica papaya* leaves extract) displayed abnormal swimming behaviour (erratic swimming, gasping for air, loss of reflex, lethargy) in respect to the increase in the concentration of the toxicant. These behavioural alterations were in accordance with the reports of Edet *et al.* (2008) who made similar observation when *Clarias gariepinus* was exposed to *Tetrapleura tetraptera* (commonly known as Aridan) and Ayebidun *et al.* (2024) when *Clarias gariepinus* was exposed to *Manihot esculenta crantz.* The result of this experiment showed that there were no significant differences (P>0.05) in the FBW, WG, PWG and SGR among the treatments, but the values decreased as the concentration of Carica papaya increased. This is in accordance with the report of Olusola S.E *et al.*, (2021), who recorded decrease in mean weight gain of *C. gariepinus* exposed to different concentration of *Datura stramonium* (Thorn Apple). The packed cell volume (PCV), haemoglobin (Hb), White Blood cell (WBC) and Red blood cell (RBC) of the fish in the control and exposed groups showed no significant differences (P>0.05) while there were significant differences (P <0.05) in the MCV and MCH with the highest values recorded as 102.40±2.83 and 34.20±2.83

respectively at PL2.This agrees with Odioko *et al.* (2016) who reported an increase in MCV of *Clarias gariepinus* exposed to *Luffa cylindrical* (Sponge gourd) leaves extract. The increase in MCV may be attributed to the swelling of the erythrocytes as a result of hypoxic conditions (low oxygen conditions) or impaired water balance (osmotic stress), resulting in macrocytic anaemia in fishes exposed to toxicants (Larsson *et al.*, 1985). There were significant differences ($P<0.05$) in total protein and albumin among the control and exposed group where PL5 (0.30ml/L) recorded the highest values in albumin as 41.00±2.83g/dl which aligns with the finding of Cimanga (2018) who reported an increase in TP and ALB of *C. gariepinus* exposed to subacute toxicity of *Crytolepis sanguinolenta* (Ghana quinine) root.

Conclusion

This study revealed that *C. papaya* leaves extract of higher concentrations induced greater impact on *C. gariepinus* than lower concentrations, considering the growth performance, haematology and plasma biochemistry of *Clarias gariepinus*. Hence, *Carica papaya* could be a potential plant-derived fish poison to be used for catching fish or eradicating unwanted fish from nursery and rearing ponds before stocking.

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