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Effect of Bitter Kola Seed Extract on Water Quality and Short Term Growth Performance of *Clarias gariepinus* Hatchlings from Exposed Broodstock

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ABSTRACT
The bitter kola, *Garcina kola* is an important plant distributed in West Africa and its efficacy in handling fish hatchery challenges have been tested and documented. This study was carried out to evaluate the effect of the *G. kola* on the hatchability of *Clarias gariepinus* broodfish and short-term survival of fry. *C. gariepinus* broodfish were exposed to the same concentration of bitter kola powder. After fertilization, products were incubated in medium containing 1.0, 1.5, and 2g bitter kola seed extract. The control had no bitter kola seed extract. 2 weeks after hatching, the fry were fed with the commercial feed Coppens in which 0.00, 1.00, 1.5 and 2.0gms of bitter kola powder was incorporated for 56 days. The water quality parameters varied following increasing level of bitter kola seed extract but non-significant ($p>0.05$). The ammonia level also followed the same trend approaching intolerable levels for fish production. Hatchability, growth and survival were significantly higher in the control ($p<0.05$) relative the treatments. The elevated levels of ammonia may be responsible for the difference between the control and the treatment. This study is useful to scientists, hatchery managers and fish growers and it emphasizes the need to optimize protocols and conditions for the utilization of phyto resources for improved husbandry practices.

INTRODUCTION
One of the greatest challenges in the aquaculture industry in Nigeria is sustainable growth. The industry grew above global average of 8% per annum to 12% based on catfish culture. Despite the rapid growth, its contribution to total fish food supply is still less than 50%. Over the past several years, aquaculture had contributed only 30% to national fish food supply leaving the balance to capture fisheries and importation.The production of Clariid catfish is boosting food security in Africa and particularly Nigeria. This is due to many favorable zootechnical characteristics including excellent feed conversion ratio (FCR), high

growth potential, and tolerant of adverse environmental conditions where other species cannot survive or reproduce., such as low oxygen or elevated nitrogen compounds in the water [Roques *et al.,* 2015 , Păpuc *et al.,* 2019]. However, the catfish industry in Nigeria is highly challenged. Such challenges include deterioration of water quality during production, promotion of growth, improvement of fecundity, hatchability, survival, feed conversion and disease resistance (Awom and Eyo, 2016).. The need to intensify culture of fish to meet the ever increasing demand has made it essential for fish farmers to develop suitable alternatives towards promoting fish growth and performance (Gabriel *et al.*, 2007). In this regards, the search for alternatives to chemical formulations has continued to attract attention, with emphasis being placed on natural plant products (Dada and Ikuerowo, 2009), Plant products possess attributes which recommends them for safe food-fish production including non-accumulation of residues in fish tissues and water, anti-microbial active ingredients (Bulfon *et al.,* 2013; Wunderlich *et al.*, 2019), minimal or no side effects on consumers, biodegradable and in general eco-friendly. Thus, the properties of many plants have been investigated to ascertain their potency in dealing with some aquaculture challenges. Therefore, the focus of this study is to evaluate the efficacy of bitter kola (*Garcinia kola*) seed on the management of brood catfish, *Clarias gariepinus* to improve hatchability and fingerlings survival.

MATERIALS AND METHODS

Location of the Experiment

The experiment was carried out in the Demonstration Farm of the Department of Fisheries, University of Port Harcourt, Rivers State, Nigeria.

Source and Preparation of Bitter Kola Seeds

The *G. kola* seeds were obtained from Choba market in Obio-Akpor local government Area of River State, Nigeria. The outer coat was removed from each seed and the seeds were ground into powder and sundried for a week. About 1.0 g, 1.5 g and 2.0 g of ground seed were soaked in about 500 mL of water heated to 100°C and allowed to stand for 24 h with shakings at intervals. Afterwards each were filtered using Whatman No. 1 filter paper to obtain the filtrate used to treat the water. The equivalent of 1.0 g, 1.5 g and 2.0 g of the seed powdered were mixed with 1.00 kg of the commercial fish feed fed to the fingerlings.

Source of Brood Stock and Acclimatization

Healthy brood stock of the African catfish *Clarias gariepinus* were obtained from two fish farms with the males from one and the females from another to avoid inbreeding. The brood stock were selected following Olubiyi *et al.* (2005) and acclimatized in separate concrete ponds for 2 weeks during which they were fed with a compounded commercial feed of about 40% crude protein twice daily. After the acclimation period, brood fish were fed twice daily with Coppens thoroughly mixed with 1.0 g of bitter kola powder for 2 weeks until breeding. Bitter kola powder was excluded from the feed administered to the second set of broodfish (Control).

Experiment Design

The experiment was conducted in two stages:

i. Fertilized eggs were distributed in three replicates into the incubated in medium containing 0.0, 1.0, 1.5 and 2.0 grams of bitter kola seed powder liquid extract. The extract was mixed with 15L of water to form the incubating medium. Thus, fertilized eggs were exposed to 1.0, 1.5, 2.0 g of bitter kola powder for the incubation period.
ii. After hatching, the hatchlings were nurtured for 2 weeks. Subsequently 20 individuals (previous treatment) were weighed and distributed in three replicates into tanks and fed 0.0 g, 1.0 g, 1.5 g and 2.0 g of bitter kola powder blended together into Coppens starter feed for fry (0.8mm – 1.5mm) for 56 days. .

The water quality indices that were measured included temperature (°C), pH, Dissolved oxygen (mg^{-}L), and ammonia (mg^{-}L) using multipurpose water test kit. Every week the 20 individuals from each treatment consisting T1, T2, T3 and TC were weighed and measured for total length (TL), standard length (SL), head length (HL) and, pelvic fin length (PvL).

Statistical Analysis

Data analyses was implemented in Past4.1 and excel. Statistical analysis of the means was performed using Analysis of Varience (ANOVA) and where significant differences is indicated, means was tested using Fishers Least Significant Difference (LSD) test at 5% probability ($p<0.05$).

RESULTS

Effect of bitter kola on water quality parameters

Table1 shows the effect of bitter kola on water quality. The water quality monitored in the incubation medium were not significantly different in temperature and Dissolved oxygen among the treatments ($p>0.05$) except temperature at 2g inclusion of bitter kola in incubating water (p<.0.05). However, the pH increased as the quantity of bitter kola increased from 1 grams per kg of feed ($p<0.05$). The ammonia concentration in the control was significantly lower when compared to treatments ($p<0.05$).

Table 1: Effect of bitter kola on water quality in which incubation was carried out

Parameter	TC (Control	T1 (1g)	T2 (1.5g)	T3 (2g)
pH	6.6 ± 0.32^{c}	7.1 ± 0.06^{a}	7.5 ± 0.05^{a}	8.7 ± 0.43^{b}
Temperature (^{O}C	25.8 ± 0.43^{a}	26.1 ± 0.11^{a}	26.7 ± 0.12^{a}	28.5 ± 0.51^{b}
Dissolved Oxygen (mg/L)	5.8 ± 0.01^{a}	5.0 ± 0.14^{a}	4.8 ± 0.31^{a}	4.7 ± 0.32^{a}
Ammonia (NH_3) (mg/L)	0.07 ± 0.11^{c}	0.44 ± 0.411^{a}	0.46 ± 0.51^{a}	0.57 ± 0.36^{a}

Values are Mean±SE with the same superscript values are not significantly different at $p>0.05$,

In Table 2, the water quality parameter were statistically the same for temperature and dissolved oxygen ($p>0.05$) but pH of the control (TC) was significantly lower ($p<0.05$). The effect of bitter kola on water temperature followed a discernible increasing pattern. The dissolved oxygen was not significantly ($p>0.05$) affected by bitter kola. But NH_3 concentration differed significantly between the control and treatments ($p<0.05$).

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Table 2: Effect of bitter kola on water quality in which hatchlings were raised for 8 weeks

Parameter	TC (control)	T1 (1g)	T2 (1.5g)	T3 (2g)
pH	6.9±0.51[b]	7.3±1.34[a]	7.5±0.53[a]	7.9±0.35[a]
Temperature (°C)	27.6±0.86[a]	28.2±0.11[a]	29.3±0.54[a]	29.4±0.46[a]
Dissolved oxygen (mg/L)	5.8±1.42[a]	4.7±0.43[a]	4.6±0.23[a]	4.9±0.13[a]
Ammonia (NH_3) (mg/L)	0.6±1.76[a]	0.61±1.43[a]	0.82±0.34[b]	0.95±0.64[b]

Values are Mean±SE with the same superscript values are not significantly different at $p>0.05$,

It can be seen from Table 2 that all the water quality parameters were insignificantly different in all treatments ($p>0.05$) except Ammonia in which Control and T1 were significantly lower than T2 and T3 ($p<0.05$). However, temperature was elevated as the concentration of bitter increased. The temperature rose from 28.2°C in T1 to 29.4°C in T3 ($p>0.05$). NH_3 concentration also increased from 0.61 in T1 to 0.95mg/l ($p<0.05$)

Effect of bitter kola on hatchability and survival of hatchlings

Table 3: Effect of Bitter kola extract on hatchability and survival of Clarias gariepinus.

Parameter	TC (Control)	T1 (1g)	T2 (1.5g)	T3 (2g)
Hatchability (%)	98±1.11[b]	88±0.23[a]	87±3.21[a]	75±3.45[a]
Survival (%)$	95±0.23[b]	86±1.23[a]	80±3.22[a]	77±2.33[a]

Values are Mean±SE with the same superscript values are not significantly different at $p>0.05$, and $ is fry survival after hatching to 2 weeks.

The highest level of hatchability was achieved in the control (about 98%). Hatchability decreased as the concentration of bitter kola increased from 88% in T1 to 75% in T3. Two weeks survival rate after hatching followed the same pattern. Despite the observed pattern, there was no significant difference among or between the different concentrations ($p>0.05$).

Effect of bitter kola on growth performance

The total weight and growth in total length, standard length, and head length was better in the control relative to the treatments, following previously observed trend. (Table 4) Thus, growth decreased relative to the concentration of bitter kola (Figs. 1-5). Despite observed differences, there variations were not significant differences (p>0.05) in total length, standard length and head length with respect to concentration of bitter kola. The pelvic fin length leveled off at the end of 8 weeks (Fig. 4)

Table 4: Effect of Bitter kola on Growth performance of *Clarias garipeinus* fry

	Level of inclusion of bitter kola (g)			
Parameter	T1	T2	T3	T4
Initial weight (g)	2.1±1.23[a]	1.8±5.43[a]	1.7±1.23[a]	1.8±1.23[a]
Final weight (g)	3.8±3.55[a]	3.4±9.86[a]	3.2±4.53[a]	5.4±1.35[b]
Weight gain (g)	1.7±2.32[a]	1.6±4.43[a]	1.5±3.21[a]	3.6±0.12[b]
Initial length (cm)	0.78±0.2[a]	0.81±0.8[a]	0.79± 1.21[a]	0.83±3.21[a]
Final length (cm)	5.38±20[a]	5.51±2.5[a]	6.49±1.12[b]	7.73±2.0
Length gain (cm)	4.6±3.22[a]	4.7±2.12[a]	5.7±2.32[a]	6.9±0.51[b]
SGR	5.71.23[a]	5.4±6.42[a]	6.6±1.11[b]	6.5±0.32[b]
FCR	0.61.11[a]	0.4±1.23[a]	0.4±0.32[a]	0.8±0.21[b]
Condition Factor	0.4±0.32[a]	0.7±0.34[b]	0.7±0.23[b]	0.5±1.92[a]
Survival (%)$	75±0.54[a]	76±0.87[a]	88±0.21[b]	92±0.32[b]

Values are Mean±SE with the same superscript values are not significantly different at p>0.05, and $ represents survival of fry after 2weeks until end of experiment.

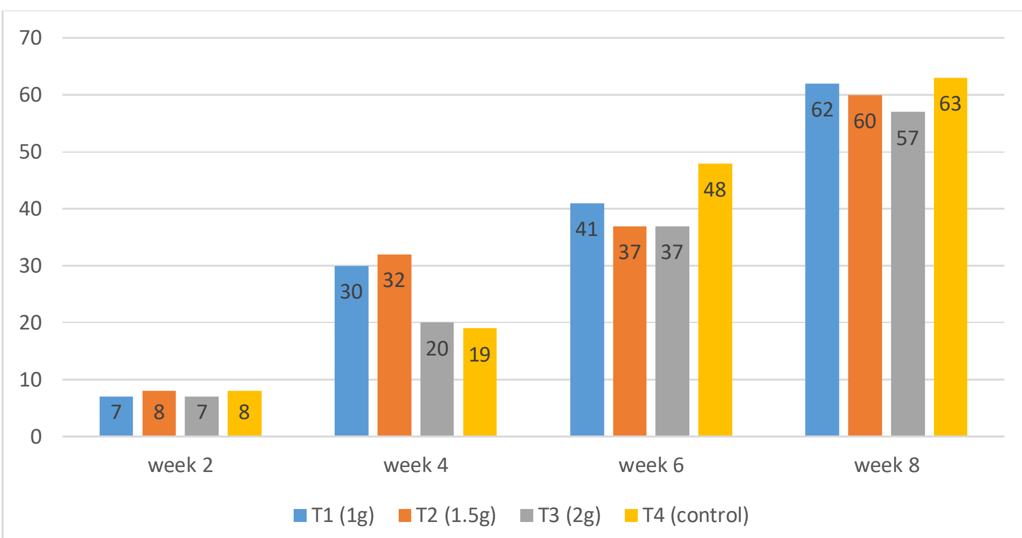

Fig. 1: Variations in the standard length (mm) of the fingerlings exposed to different concentrations of G. kola for 8 weeks

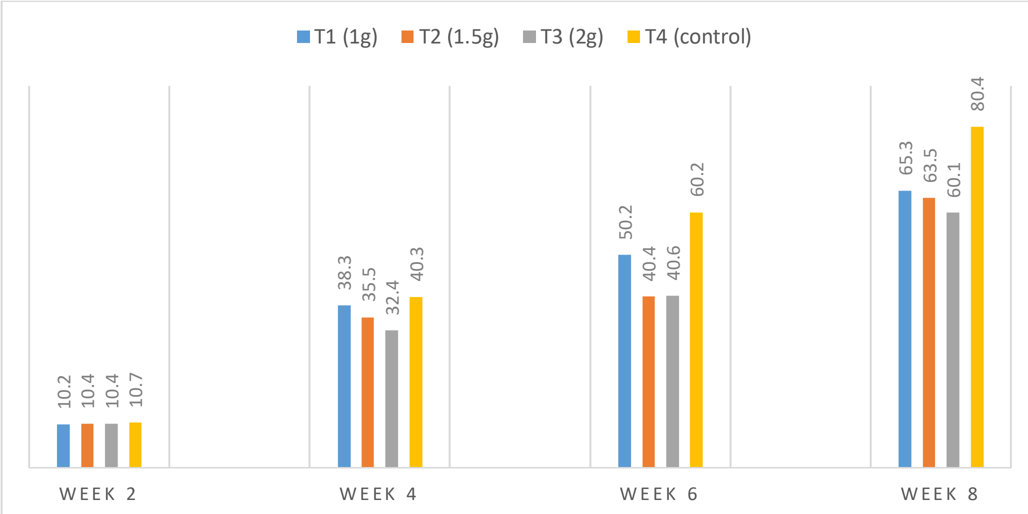

Fig. 2: Variations in the Total length (mm) of the fingerlings within the culture period of eight weeks

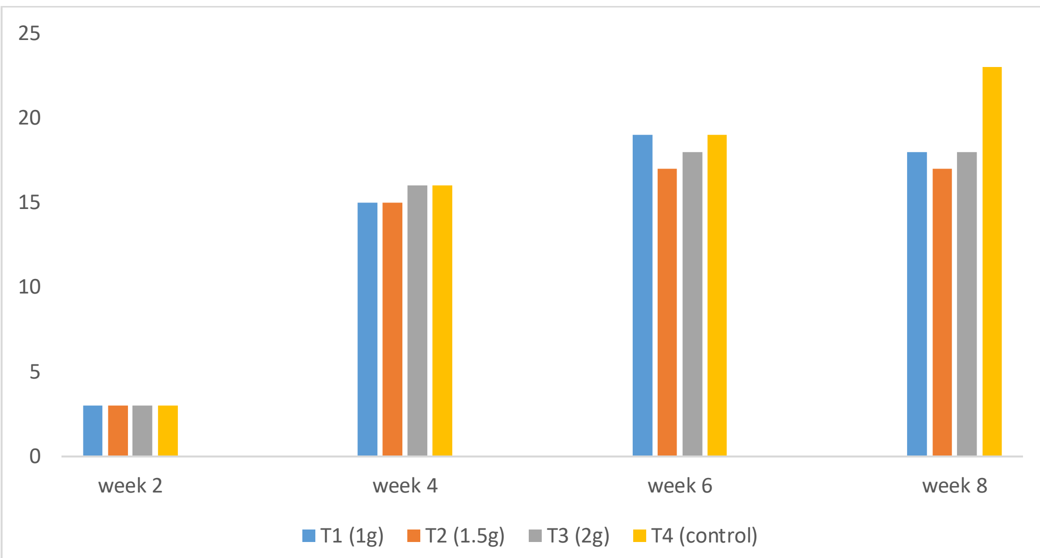

Fig. 3: The head length (mm) of the fingerlings within the culture period of eight weeks

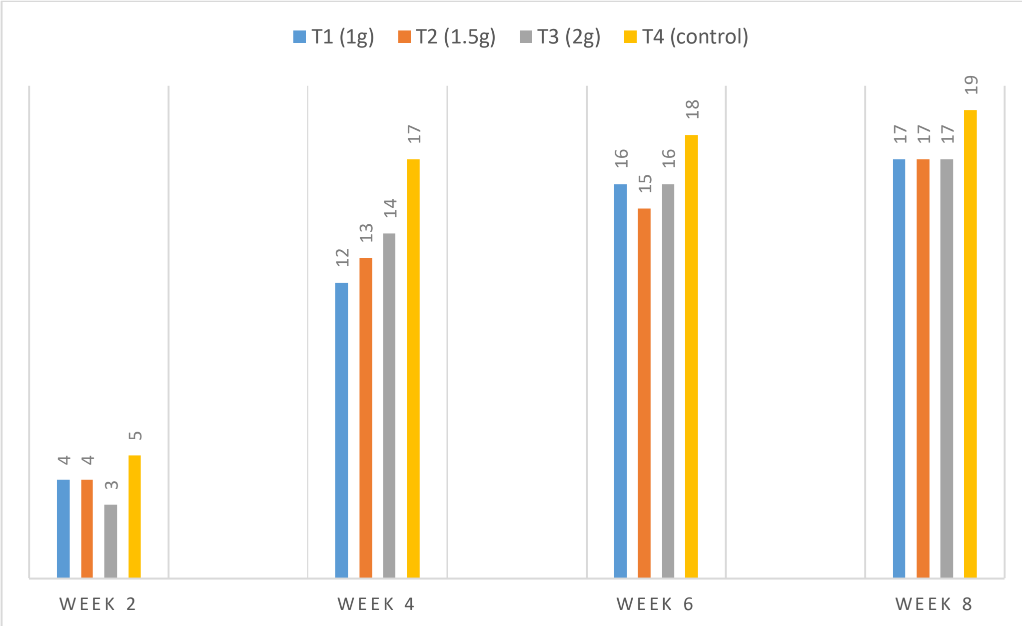

Fig 4: The pelvic fin length (mm) of the fingerlings cultured for eight weeks

DISCUSSION

The quality of water in which fish is raised directly affects growth rates, the health, survival, reproductive performance and feed conversion efficiency. Maintaining the optimum physico-chemical conditions within acceptable limits in aquaculture facilities is not only challenging but essential to obtaining maximum yield (Bhatnagar and Devi, 2013). Environmental parameters influence significantly the maintenance of a healthy aquatic environment and production of natural food organisms (Haque et al., 2003). The water quality parameters measured such as temperature (<30 °C), pH (6.6 – 8.7) and dissolved oxygen (<6 mg/L) were within acceptable range for aquaculture It can, however, be seen that mortality increased with concentration of bitter kola. This may be due to increasing ammonia level. The range of ammonia recorded in this study was .6±1.76 -0.95±0.64mg/L. The sources of ammonia are usually not only from uneaten feed and fecal waste but also result untreated borehole water and stress which alters the blood chemistry (Durborow, *et al.,* 1997, Robert *et al.,* 1997; Hargreaves and Tucker 2004; Ogbonna and Amajuoyi, 2010). Another reason for the high concentration of ammonia is likely due to the fact that the seed powder was not bound to the feed making it to be lost and unavailable to fish through leaching. The toxicity of ammonia increases with increasing temperature and pH. For every pH increase of one unit, the amount of toxic ammonia increases about 10 times (Paley *et al.,* 1993; Brinkman., 2009). Similarly, Shin *et al.* (2016) found that the rockfish, *Sebastes schlegelii* exposed to ammonia ranging from 0.1 to 1.0mg/L experienced significantly reduced growth as concentration increased. Furthermore, El-Greisy *et al.* (2016) reported 100% mortality in *Oreochromis niloticus* exposed to 1.5mg/L of ammonia and fertilization and reduced hatching rate at 0.5 – 0.6 mg/L.

The control showed significantly better hatchability and survival rates compared to the treatments. This result agrees with Akinsaya *et al.*(2010) and Aprioku *et al* (2018) on the Wistar rats but contradicts such as those of Dada (2012) and, Dada and Ikuerowo (2017) on *Clarias gariepinus* Our results actually showed that hatchability decreased though insignificantly with increasing concentration of aqueous extract of bitter kola seed; it interestingly tends to align with Sulem-Yong *et al* (2018) who showed that in *Oreochromis niloticus* increased incorporation level of G. kola decreased fecundity ($p < 0.05$) irrespective of spawn and reduced growth considerably when compared to the control. Studies have shown that bitter kola manifest negative effects that can destroy sperm cells and reduce fecundity due to the presence of epigenen, an aromatase enzyme (Iwu and Igboko, 1982; Braide, 1993; Akpantah *et al.*, 2003; Iranloye and Owokunle, 2008; Dada and Ikuerowo, 2017), all of which validates the results obtained in the present work The method extraction and incorporation differed and may trigger these differences in results. Thus, despite the potency of the plant as growth promoter among other useful remedies in aquaculture systems there is the need for standardization and optimization of processes to exploit the plant maximally.

The FCR is lower in fingerlings exposed to bitter kola. The implication is that the fingerlings were able to more efficiently convert a kilogram of feed into weight gain (Bai et al., 2022). Despite the seeming negative effects of bitter kola on the fingerlings, fish converted feed more efficiently. This could be attributed to medicinal values of bitter kola and ability of the fingerlings to better utilize quantity of feed consumed.The observed discrepancies between the control and the treatments was likely due to the inclusion of bitter kola in the medium of incubation of fertilized eggs and rearing of fingerlings. The inclusion of bitter kola in the incubation and rearing medium increased the ammonia concentration.

Conflict of interests

There is no conflict of interest.

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