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Reproductive impact of aqueous extract of *Mangifera indica* leaves on female African catfish *Clarias gariepinus* Broodstock

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Abstract
This study was designed to investigate the efficacy of aqueous extract of *Mangifera indica* on the reproductive performance of female *Clarias gariepinus* broodstock. 60 female fish of mean weight ±460.56g were stocked in triplicates into 15 concrete tanks ($1\times2\times1.5m^3$) at density four fish per tank. Five diets with crude protein of 40% were formulated with inclusion level of 0,0.5, 1.0, 1.5, 1.0g of *Mangifera indica*. The fish were fed twice daily (0900 and 1400) hours for 56 days. The extract significantly improved the reproductive indices compared to the control diet. The results revealed improved gonadosomatic index, fecundity, egg size, percentage fertilization, hatchability, survival, estrogen level and histology of the gonads of female *Clarias gariepinus* broodstock. Aqueous extract of *Mangifera indica* can therefore be used as a feed additive to enhance reproductive performance of the female *Clarias gariepinus*.

Keywords: *Mangifera indica*, histology. estrogen, reproductive performance

1. Introduction
Fish and fishery products represent a very valuable source of protein and essential micronutrients for balanced nutrition and good health. Consumption of fish provides important nutrients to a large number of people worldwide and thus makes a very significant contribution to nutrition [1, 2]. A decline in fish availability will have a detrimental effect on the nutritional status in places where fish contributes significantly to the protein intake of the people [3]. Fishery sub-sector provides employment opportunities for young and old people due to the low capital outlay required to take-off. It can also serve as a source of foreign exchange while also serving as a viable alternative remedy to the already depleted capture fisheries. According to Olaoye and Oloruntoba (2011) [4], Nigerians consume a lot of fish and offer the largest market for fish and fisheries products in Africa. Fish demand in Nigeria is put at about 1.2 million metric tons per annum, and the total domestic fish production is only 511,700 metric tons [5]. The high demand for fishery products has arisen from the awareness of its significance in the local diet and its low-price compared to its substitutes [6].
The African catfish, genus *Clarias* has very high commercial value in Nigeria and most parts of the world owing to its flavour and taste. The attributes that make this species a farmer's choice include faster growth rate and its bigger maturity size, easy to cultivate, accepts artificial feeds tolerates high stocking density, high fecundity and high palatability [7]. The good quality coupled with its ability to feed on virtually anything makes the fish a highly recommended species for aquaculture development in Nigeria [8]. The availability of gametes throughout the year is important to ensure a constant supply of the fish [9]. This availability is dependent on gonadal development and fecundity, which are subsequently affected by dietary nutrients [10]. The ability to produce large numbers of high quality eggs on demand is an important issue for the development of aquaculture. The use of ethnobotanicals seems to be an attractive alternative to enhance growth and fertility.
Medicinal plants have a long folkoric reputation in improving fertility in man and animals. Some medicinal plants have been investigated, evaluated and developed into drugs with little or no side effects [11]. They have unique and valuable properties such as fertility enhancing properties and aphrodisiac qualities.

Using plants immunostimulants seems to be attractive alternative to enhance growth and fertility. Attempts to use the natural materials such as medicinal plants could be widely accepted as feed additives to enhance efficiency of feed utilization and animal productive performance [12]. Various plant extracts have reported to improve fertility in man and animals. These plants contain inherent active ingredients (sterols, flavonoids, saponins) and nutrients for their actions [13] and they also have antioxidant, antibacterial and anthelminthic properties. Dada and Adeparusi (2012) [14] reported that *Sesamum indicum* and *Croton zambesicus* seed powder improved the growth performance and reproductive indices over the control treatment in female *Clarias gariepinus*. *Garcinia kola* was reported to cause dose dependent changes in the sperm and egg characteristics in *Clarias gariepinus* broodstock.

Mangoes belong to the family *Mangifera*. The genus plant consisting of numerous species of tropical fruiting tree in the flowering plant family Anacardiaceae. It is one of the most recommended fruits which have medicinal importance to fight beriberi, heal bronchial diseases and cure brain fatigue, mental depression, wrestle heart burn and insomnia [15]. It is the most widely exploited fruits for food, juice, flavor and fragrance a common ingredient in new functional foods often called super fruits [16]. Antioxidants and enzymes present in the mango fruits and leaves are believed to play an important role in the prevention and in the protection of cancer (colon, breast, leukemia and prostate) and heart disease. Presence of fibre and enzymes makes mangoes favorite for healthy digestion. The phytochemical analysis of *Mangifera indica* leaf extract revealed the presence of steroids, saponin, tannin, alkaloid, flavonoid, anthraquinone, cardiac glycosides and phlobatanin in the aqueous extract. There is no documented report on the reproductive effect of *M. indica* on female *C. gariepinus*. Hence, this research is intended to evaluate possible effects of *M. indica* on the reproductive performance of *Clarias gariepinus* broodstock.

2. Materials and Methods

2.1 Experimental site

The experiment was carried out in the Teaching and Research Farm of the Department of Fisheries and Aquaculture Technology, Federal University of Technology, Akure, Ondo State.

2.2 Collection, processing and authentication of plant material

The leaves of *Mangifera indica* were obtained from Government Residential Area, Aule, Akure, Ondo State. The leaves of the plants were identified and authenticated at the Department of Crops, Soil and Pest Management, Federal University of Technology, Akure, Ondo State. The leaves of *M. indica* were sun dried for three weeks and the dried leaves were pulverized to a powdery form by grinding with a commercial grinder. 500g of the powder was soaked in one litre of distilled water for 48 hours after which it was filtered with a muslin bag. The filtrate was air dried for three days to obtain a solid extract.

2.3 Experimental Fish

The fish used for the experiment were obtained from a fish farm in Akure, Ondo State. The fish were kept in outdoor concrete tanks (1×2×1.5m³) for two weeks for acclimation and they were fed with 40% crude protein diet at the Department of Fisheries and Aquaculture Technology, Teaching and Research farm.

2.4 Experimental Diet

Five isonitrogenous diets with 40% crude protein were formulated from practical ingredients (Table 1) where the control basal diet was without *M. indica* and the other diets were supplemented at varying quantities. All dietary ingredients were weighed with a weighing balance (Digital Electronic balance JS10-01). The ingredients were milled to a 6mm particle size. Ingredients were thoroughly mixed in a Hobbart A- 2007 pelleting (Hobart Ltd, London, UK) to obtain a homogenous mass, cassava starch was used as a binder. The resultant mash was pressed without steam through a mixer. The pellets were dried for two days at less than 60°C and kept in a dry place until ready for use.

Table 1: Ingredients composition of experimental diet fed to experimental fish

Ingredients	Experimental Diets				
	Diet1	Diet 2	Diet3	Diet 4	Diet 5
Fish meal (65% CP)	26	26	26	26	26
Soy bean (45% CP)	35	35	35	35	35
Groundnut cake (48% CP)	12.5	12.5	12.5	12.5	12.5
Yellow maize	15	15	15	15	15
Fish oil	4	4	4	4	4
Vegetable oil	3	3	3	3	3
Vitamin premix	2.5	2.5	2.5	2.5	2.5
Starch	2	2	2	2	2
Extract of *M. indica* (g/100g feed)	0	0.5	1.00	1.50	2.00

*Vitamin premix- A Pfizer livestock product containing the following per kg of feed: A = 4500 I, U, D = 11252 I.U, E = 71I.U, K3=2mg, B12=0.015mg, pantothenic acid = 5mg, nicotinic acid = 14 mg, folic acid = 0.4mg, biotin = 0.04 mg. choline = 150mg, cobalt = 0.2 mg, copper = 4.5 mg, iron = 21 mg, manganese = 20mg, iodine = 0.6 mg, selenium = 2.2 mg, zinc = 20 mg, antioxidant = 2 mg

2.5 Procedure for Experiment

The experiment is a completely randomized design in which five treatments (diets) were applied in three replicates. 60 female fish weighing ±460.56g were stocked into the concrete tanks (1x2x1.5m³) in triplicate. The fish were fed the test diets containing 0, 0.5, 1.00, 1.50 and 2.00 (g/100g feed) *M. indica* (as in Table 1) twice a day (0900 and 1600 h) for 56 days. The fish in each tank were collectively weighed fortnightly using a weighing balance (Digital Electronic balance JS10-01) and their average weights recorded. After 56 days, the reproductive indices of the fish were evaluated.

2.6 Determination of Egg Quality

2.6.1 Fecundity estimation

Ten females were randomly selected which were dissected and the ovaries were removed and weighed. Fecundity estimation was done using gravimetric method. 1g of egg was taken and counted.

2.6.2 Determination of egg diameter

The diameter of the eggs was determined using micrometer viewed at 100× microscope (at 0.01mm sensitivity).

2.6.3 Determination of Gonadosomatic Index (GSI)

The Gonadosomatic Index was determined as described by Kings (1995) [17] as;

GSI = (gonad weight (g) /fish weight(g)) ×100

2.6.4 Breeding and fertilization of *C. gariepinus*

Fifteen female fish were collected from the experimental tanks for breeding. Ovulation was induced in the female using ovaprim at 0.5ml/kg (0.02 mg salmon gonadotropin-releasing hormone-sGnRHa+10 mg domperidone-Dom) of body weight. Stripping of the females was done after 12 hours of injecting the fish; this was done by pressing the abdomen of the fish with a thumb from the pectoral fin towards the papilla. The female was stripped and 1g fresh eggs were measured into fifteen circular plastic bowls of 2L capacity labeled according to treatments. Male spawners were sacrificed to remove the testes. 4 drops of milt from each selected male fish were added to the eggs in the bowls to fertilize the eggs. The milt was squeezed out on the egg batches and dry fertilization was carried out. Fertilization experiment was carried out in triplicate.

The translucent eggs containing embryonic eyes at the time of polar cap formation (about 20minutes after fertilization prior to the 2-cell stage of first cleavage) were considered fertilized and counted to calculate percentage fertilization. Opaque eggs were considered unfertilized. The number of fertilized and unfertilized eggs was counted under a microscope (40x magnifications). Percentage fertilization, percentage hatchability and percentage survival were calculated as described by Ayinla and Akande (1998) [18] as:

$$\% \text{ Fertilization} = \frac{\text{Number of fertilized eggs}}{\text{Total number of eggs counted}} \times 100$$

$$\% \text{ Hatchability} = \frac{\text{Number of eggs hatched}}{\text{Total number of eggs in a batch}} \times 100$$

$$\% \text{ Survival} = \frac{\text{Total No. of hatchlings}}{\text{Total No. of eggs counted}} \text{ x } 100$$

2.6.5 Determination of Estrogen level

Blood samples were taken from the caudal vein of experimental fish, using a sterile syringe containing one drop of heparin. The blood collected was put into clean and dry centrifuge tubes. The tubes were centrifuged at 2500 rpm for 45 min using Uniscope Laboratory Centrifuge (model SM800B, Surgi friend Medicals, England). The serum was thereafter aspirated using Pasteur pipettes into clean and dry sample bottles as described by Kings (1995) [17]. The serum was obtained after centrifuging for 15 minutes, it was then analyzed for quantitative determination of estrogen concentration using Enzyme Linked Immunoassay (ELISA) commercial kit.

2.7 Water quality parameters

The water quality parameters were monitored weekly during the period of study. Temperature, pH and dissolved oxygen concentration were monitored using mercury-in-glass thermometer, pH meter (Hanna H198106 model) and dissolved oxygen meter (JPP-607 model), respectively.

2.8 Histological examination of ovaries of *Clarias gariepinus*

A total of ten female fish were randomly taken from all the five treatments (2 from each treatment) after 56 days of feeding trial. They were dissected and the ovaries removed for sectioning and histological examination. The gonads, that is the ovaries were put in formalin solution made of equal volumes of 10% formalin and 0.9% sodium chloride solution for 24 hours after which they were dehydrated in graded levels of alcohol prepared at 50% for 30 minutes, 70% for 90 minutes, 95% alcohol for two hours and 100% for 2 hours. The one of the absolute alcohol i.e. 100% alcohol was repeated twice. Xylene was poured into the test bottles containing the test organs, this process lasted for 1 hour. The later process was repeated three consecutive times each lasting for an hour. Embedding was done by dispensing enough paraffin into an embedding block mould to cover the bottom. Mounting was done by cutting the solidified wax containing the test organs with a hot knife in a square form and mounted on a wooden block. This was followed by staining the sectioned tissues in haematoxylin-eosin for 10 minutes. The stained slides were observed under light microscope at varying magnifications. Photomicrographs were taken with Leitz (Ortholux) microscope and camera.

2.9 Statistical analysis

All values were recorded as mean and subjected to a one-way ANOVA test comparison of each of the test groups and the control using the SPSS for window software package. Duncan's multiple range tests was used to compare differences among means [19].

3. Results and Discussion

3.1 Effect of aqueous extract of *Mangifera indica* on egg quality and estrogen level of *C. gariepinus* fed dietary supplementation

Table 2 shows reproductive performance of female *C. gariepinus* fed *M. indica* extract. There was increase in the weight of all fish fed with *M. indica* extract although there was no significant difference among the diets. The observation on fecundity, percentage fertilization, gonad weight and G.S.I revealed that there was no significant difference (P>0.05) among the diets but the values increased in the fish fed with the *M. indica* extract. The highest mean fecundity was observed in fish fed Diet 4 (42760.17) while the least was observed in fish fed Diet 1(39636.67). Fecundity of fish fed experimental diet revealed that fish fed Diets 3, 4 and 5 increased greatly compared to the fish fed control diet, this could also be attributed to the presence of *M. indica* extract which has active ingredients (saponins, alkaloid, flavonoids). This in agreement with the findings of Ejete-Iroh *et al*. (2018) [20] who revealed that supplemented diets with *P. guineense* fruit aqueous extract improved gonadosomatic index, fecundity, percentage fertilization, hatchability and survival, egg size and estrogen level of female *C. gariepinus* broodstocks.

The egg size observed was not significantly different among the fish fed experimental diets. The highest mean egg size was observed in fish fed Diet 5 while the least was observed in fish fed Diet 1. The mean egg size increased with increasing level of inclusion of the extract. Larger egg size shows a higher percentage fertilization which was observed in this study. This agrees with the findings of Bichi (2005) [21] who revealed that the size of egg has a significant effect on the rate of fertilization. The large egg sizes which ranged between 1.30 mm to 1.52mm in the treated group could have contributed to the enhancement of larval and fry viability. Thus, large egg size in *C. gariepinus* may be an indication of better larval viability. Adams (2016) [22] also revealed that the bigger the size of eggs, the higher the fertilization, hatchability and survival rates in *C. gariepinus* larvae.

Table 2: Reproductive indices of *Clarias gariepinus* as affected by the dietary supplementation of aqueous extract of *Mangifera indica* extract for 56 days (mean ± S.E)

Parameter	D1 (Control)	D2	D3	D4	D5
Initial weight (g)	457.37± 5.99^{a}	480.30±2.79^{a}	475.94±2.63^{a}	463.67±6.27^{a}	450.54±2.27^{a}
Final weight (g)	552.47±23.25^{a}	524.03±17.27^{b}	560.17±34.82^{a}	576.83±6.14^{a}	580.17±57.14^{a}
Weight gain (g)	95.10±24.90^{a}	43.73±22.32^{b}	84.24±22.32^{a}	113.69±9.38^{a}	114.62±55.98^{a}
weight of ovaries	51.25±2.62^{a}	50.60±2.13^{a}	52.05±3.43^{a}	52.89±3.19^{a}	55.05±4.21^{a}
GSI (%)	9.30±0.54^{ab}	9.48±0.10^{ab}	9.29±0.21^{ab}	8.81±0.47^{b}	10.67±0.54^{a}
Fecundity/ fish (No)	39636.67±11295.34^{b}	40810.73±6596.14^{a}	45408.01±10479.4^{a}	42760.17±6744.04^{a}	41253.67±8095.24^{a}
Egg diameter (mm)	1.25±0.03^{b}	1.30±0.02^{b}	1.35±0.08^{a}	1.45±0.03^{a}	1.52±0.01^{a}
% Fertilization	76.60±3.70^{b}	81.54±1.49^{ab}	81.82±3.42^{ab}	82.85±1.44^{ab}	89.82±2.40^{a}
% Hatchability	42.76±8.13^{b}	61.71±6.31^{a}	63.60±3.02^{a}	68.33±2.15^{a}	77.72±3.80^{a}
% Survival	33.10±3.22^{d}	51.05±2.44^{bc}	55.59±3.18^{b}	61.04±0.61^{b}	67.12±2.20^{a}
EG level (pg/ml)	1597.90±104.96^{b}	1638.40±116.74^{a}	1604.47±200.60^{a}	1677.07±139.86^{a}	1667.70±27.02^{a}

Mean in a given row with same superscripts are not significantly different at $P>0.05$
EG = Estrogen

Sule and Adikwu (2004) [23] also reported that species of the genus *Clarias* with larger eggs also had a higher viability and endurance to starvation than those with smaller eggs. The highest percentage fertilization was observed in fish fed Diet 5 while the least was observed in Diet 4. The percentage hatchability and survival revealed that there was significant difference ($P< 0.05$) among the diets. The highest percentage hatchability was observed in fish fed Diet 5 which was not significantly different ($P>0.05$) from Diets 4, 3 and 2 but significantly different ($P< 0.05$) from diet 1. For the survival rate, the highest mean was observed in fish fed Diet 5 which was not significantly different from fish fed Diet 4 but significantly different from fish fed Diet 1, 2 and 3. There was significant improvement in the percentage hatchability and survival of fish fed *M. indica* at all levels compared to the fish fed the control diet. This shows that the aqueous extract of *M. indica* is useful as fertility enhancer in *Clarias gariepinus* broodstock. The presence of alkaloids, flavonoids, sterols and saponins in this plant may account for its use as fertility enhancer. Flavonoids present in plant extracts have been shown to possess many pharmacological properties including antioxidant activities and hence flavonoids also may have a contributory effect on its fertility properties [24]. Estrogen level was highest in fish fed Diet 4 and lowest in the fish fed the control diet although there was no significant difference ($P>0.05$) among the treatments, the increase observed could be attributed to the presence of *M. indica* extract in the diet. This agrees with the report of Ngokere *et al.* (2014) [25] that *M. indica* aqueous extract significantly ($P< 0.05$) increased the serum concentration of estradiol in groups of rats treated with the extract compared to the control. The increase in the estrogen level in fish fed Diets (2, 3, 4 and 5) was evident in high fecundity, percentage fertilization and hatchability obtained in the experimental fish. Estrogen is the key hormone involved in the production and maturation of eggs in the ovaries. Magnesium which is present in the *M. indica* leaves is a cofactor for many biochemical reactions in the body which include synthesis of sex hormones such as androgens and estrogens, this could be attributed to the increase of estrogen in the fish fed extract. The water quality parameters measured ranged from 26 to 26.60 °C (temperature), dissolved oxygen; 6.20 - 6.80 mg/l, and hydrogen ion concentration (pH); 9.60 - 9.75.

3.2 Effect of aqueous extract of *Mangifera indica* on histology of ovaries of female *C. gariepinus* fed dietary supplementation

The result of the ovarian histology of *C. gariepinus* (Plate 1) of fish fed control diet and fish fed aqueous *M. indica* extract revealed that the ovaries were at vitellogenic and perinulear stages. These stages are characterized by oocytes formation, formation of small basophilic yolk globules and vacuolated globules. The ovary of the control fish showed early vitellogenic stages while the fish fed Diets 2, 3, 4 and 5 showed late vitellogenic stages which are characterized by abundant yolk granules. Majority of the developing oocytes were of late vitellogenic stage that is stage 4 gonadal developments which is characterized by the presence of a full range of oocyte maturation stages (oogonia, previtellogenic oocytes and mature vitellogenic oocytes). The effect of *M. indica* extract was more on the oocytes formation and maturity. The improvement in the estrogen level of fish fed *M. indica* extract is obvious in the gonadal development of *C. gariepinus.*

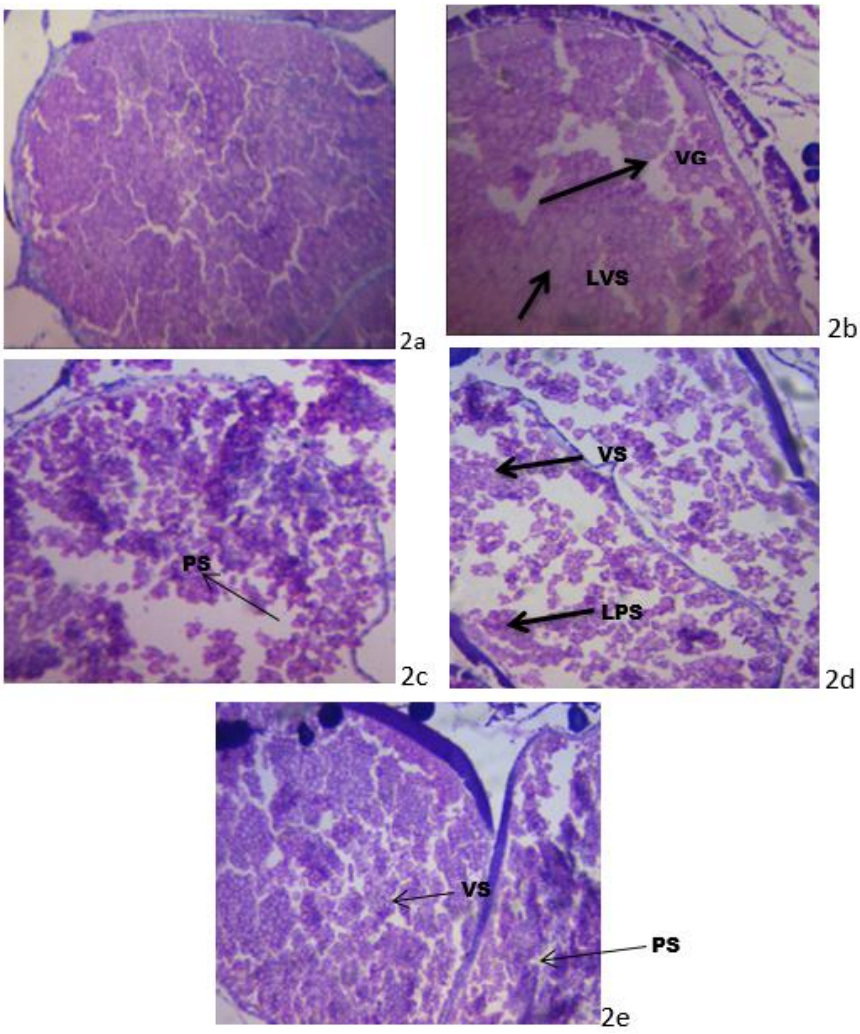

Plate 1a-e: Microphotograph section of ovaries (×100)
a. D1 (0.00) showing mostly vitellogenic stages
b. D2 (0.50) showing perinuclear stages, large vitellogenic stages (LVS) also seen
c. D3 (1.00) showing predominantly vitellogenic stages with fewer number of perinuclear stages (PS)
d. D4 (1.50) showing late perinuclear (LPS) and vitellogenic stages (VS).
e. D5 (2.00) showing vitellogenic (VS) and perinuclear stages (PS)

4. Conclusion

The present study showed that inclusion of *Mangifera indica* extract to the experimental diets improved the egg quality of *Clarias gariepinus* at all levels of inclusion. The inclusion level of 1.50 (Diet 4) gave the best performance for the estrogen level. The histology in this study also showed that *M. indica* extract had a positive effect on the ovaries of *C. gariepinus.*

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