# SOME REPRODUCTIVE CHARACTERISTICS OF FEMALE *Clarias gariepinus* EXPOSED TO DIETS CONTAINING Bryophyllum *pinnatum* LEAF

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**ABSTRACT**
*The utilization of medicinal plants as alternatives to synthetic materials as pro-fertility has been highly harnessed. Phytochemical analysis of Bryophyllum pinnatum leaf (BL) and its nutritional effects on some reproductive characteristics of female Clarias gariepinus was determined. Fifty-six C. gariepinus sub-adults (482.48±18.27 g/fish) were fed seven iso-nitrogenous diets (30% CP) containing B. pinnatum leaf at varying levels: T1 (1gBL/100g), T2 (2gBL/100g), T3 (3gBL/100g), T4 (4gBL/100g), T5 (5gBL/100g), T6 (6gBL/100g) and Control (0gBL/100g). The results of the phytochemical analysis of B. pinnatum revealed the presence of medicinally active constituents with the highest concentration (µg/mL) in Steroid (116.85±0.03), then Saponin (37.00±0.58), Glycoside (10.30±0.12), Flavonoids (4.50±0.06), Tannin (2.35±0.03) and least in Phenol (0.18±0.00). These confirmed the possible use of B. pinnatum as a pro-fertility agent in fish. Reproductive indices of fish were evaluated after 28 weeks of feeding and spawning. Diets containing BL improved ($p<0.05$) egg weight (492.95±50.32g), fecundity (320417.50±32705.45), pseudo-gonadosomatic index (0.23±0.02) and % survivals (41.13±0.3) in T6, fertilization and hatchability % (67.45±1.64 and 47.50±1.44) in T4 compared to the Control. Conclusively, the study confirmed the presence of bio-active compounds of reproductive importance in B. pinnatum leaf and it can be included in C. gariepinus sub-adult diets up to 6% to enhance its reproductive characteristics.*

**Keywords:** Bio-active compounds, *Bryophylum pinnatum*, Reproduction, Female, *Clarias gariepinus.*

## INTRODUCTION

The continuous growth of aquaculture is hinged on the production of fish seeds from parent stocks with high fecundity, fertilization, and hatchability rates; seeds of high growth rate, survival and great resistance to diseases among others (Alimi, 2019). Artificial breeding especially induced breeding is a panacea to adequate production of widely acceptable and culturable fish species especially *Clarias gariepinus* (Charo and Oirere, 2000). *Clarias gariepinus* is very important to the sustainability of the aquaculture industry in the country. The high demand for its fingerlings and juveniles in the growing aquaculture industry has stimulated the need for its artificial propagation (De Graaf and Janssen, 1996). According to Dada and Ajilore (2009), the rapid growth of fish farming should consider the need to focus on improved broodstock for an increase in fish seed production. Effective fish seed production demands a thorough understanding of the special husbandry and in particular nutritional requirements of broodstock, which greatly affect fecundity, survival, egg size and egg and larval quality. Given this, medicinal plants that can enhance the fertility of broodstock fit exactly into this.

Medicinal plants have been reported to enhance fertility and modern scientific research has confirmed pro-fertility effects in some plants tested in animals (Salman *et al.,* 2008). However, there is little information on the use of plant materials as a fertility enhancer in fish. Although the toxicity profile of most medicinal plants has not been thoroughly evaluated, it is generally accepted that medicinal plant products are safer than their synthetic counterparts (Oluyemi, *et al*., 2007). Many studies have shown that antioxidants present in plants can enhance fertility either directly or indirectly and that most plants rich in antioxidants have the tendency to increase sperm count, its motility, and enhance sperm morphology (Oluyemi *et al*., 2007; Adesanya *et al*., 2007).

*Bryophyllum pinnatum* is one of the important medicinal plants that have pro-fertility potentials. Qualitative phytochemical studies showed that the plant contained alkaloids, steroids, flavonoids, tannins, anthocyanins, glycosides, bufadienolides, saponins, coumarins, sitosterols, quinines, carotenoids, tocopherol and lectins (Hossan and Yemitan, 2009). The need for pro-fertility agents of indigenous plants in fish which are less expensive and readily available informed the dietary use of *B. pinnatum* to improve egg quality in female *C. gariepinus*, especially because of their medicinal purposes. The objective of this research work was to evaluate the dietary effects of *Bryophyllum pinnatum* leaf on some reproductive characteristics of *Clarias gariepinus*.

## MATERIALS AND METHODS

### Collection of medicinal plants and Phytochemical Analyses of the Test Plant

Fresh leaves of *B. pinnatum* were collected from the Forestry garden at the Federal University of Agriculture, Abeokuta, Nigeria (FUNAAB) and later authenticated in the herbarium of Forestry Department, FUNAAB. Phytochemical analyses of

the extract of *B. pinnatum* leaf measured some bio-active constituents that included anthraquinones, alkaloids, steroids, tannins and phenols, flavonoids, cardiac glycoside, terpenoids and saponi**ns** using modified standard methods of Sofowora (2008).

**Collection and Acclimatization of *Clarias gariepinus* Sub-Adult**

Several 64 healthy female *Clarias gariepinus* (mean weight, 600±23.56 g) and 20 healthy male *Clarias gariepinus* (mean weight, 560±21.35 g) were acclimatized for two weeks, feeding them in outdoor concrete holding tanks with commercial diets (Skretting, 35% CP) twice daily at 3 % of their body weight**.**

**Preparation of experimental diets for sub-adult**

Seven (7) experimental diets (Table 1) containing test plant were compounded by adding 0, 10, 20, 30, 40, 50 and 60 g of *B. pinnatum* homogenate into 1000 g of 30% (CP) formulated diet for the fish.

**Proximate Analysis**

Proximate composition of test diets was carried out according to standard procedures (AOAC, 2000**).**

**Experimental Design for *Clarias gariepinus* Sub-adult**

A total number of 42 female and 21 male *C. gariepinus* (482.48 ±18.27 g/fish) were selected (2:1) and distributed into 7 experimental vats (L X B X D, 1 X 1X 0.6 $m^3$) at God's Glory Fish Farm after acclimatization at the rate of 9 fish per tank. Experimental diets were manually fed to the fish at a daily rate of 3% body weight (BW), twice a day (09:00 and 16:00 h) for 7 months. At the end of the feeding trials, the maturity of the female was confirmed by a slight press on the ventral side of the fish for oozing of eggs. Three females randomly selected per dietary treatment were weighed and were artificially induced by intramuscular injection with Ovaprim C (SYNDEL, CANADA) at 0.5 ml/kg body weight and kept for the completion of the latency period. Approximately 10 to 11 hrs after induction, the females were checked for their ovulatory response by the release of eggs through the genital pore. Eggs from the ovulated females were stripped into dry plastic bowls and weighed, the number of eggs in one gram was noted.

**Reproductive Characteristics of Experimental Fish**

Fecundity, egg diameter, % pseudogonadosomatic index (PGSI), egg fertilized as well as the % number of eggs hatched and survived were determined (Ayinla and Akande, 1988).

Fecundity estimation: Wt. of eggs per ovary X no of eggs in 1g …. (i)

$$\% \text{ PGSI} = \frac{\text{Weight of eggs}}{\text{Total body weight}} \times 100 \text{ ……....(ii)}$$

Egg Diameter: Microscopic examination

$$\% \text{ Egg Fertilized} = \frac{\text{No of eggs incubated} - \text{No of opaque eggs}}{\text{Total number of eggs incubated}} \times 100 \text{…. (iii)}$$

$$(\%) \text{ Hatchability} = \frac{\text{No of eggs incubated - No of whitish broken eggs}}{\text{Total no of hatchlings}} \times 100 \text{ …… (iv)}$$

$$\% \text{ Survival} = \frac{\text{Total no of hatchlings survived to larvae stage}}{\text{Total number of hatchlings}} \times 100 \text{ …. (v)}$$

**Statistical Analysis of Experimental Data**

Experimental data were subjected to both descriptive and inferential statistics using SPSS 16.0 statistical package.

**RESULTS**

**Phytochemical Characteristics of *Bryophyllum pinnatum***

Table 2 summarized the phytochemical characteristics of *B. pinnatum* leaf. The results revealed the presence of medicinally active constituents with the highest concentration (µg/mL) in Steroid (116.85±0.03), then Saponin (37.00±0.58), Glycoside (10.30±0.12), Anthraquinone (8.85±0.03), Flavonoids (4.50±0.06), Tannin (2.35±0.03), Alkaloid (2.05±0.09) and least in Phenol (0.18±0.00).

**Proximate Composition of Experimental Diets for *Clarias gariepinus* Sub-Adult:**

Analyses of the experimental diets showed significant differences ($p<0.05$) in their mean proximate contents of nitrogen-free extract (NFE), crude fiber, fat and moisture contents except the ash and crude protein contents ($p>0.05$) as presented in Table 3. NFE was highest in D2 (33.67±0.33%) and least in D4 (30.00±0.58%). Fat was highest in D3 (7.16±0.23 %) and also least in D4 (5.22±1.20%). On the other hand, D4 has the highest mean values of ash (9.67±0.33%), crude fiber (7.50±0.29), crude protein (30.86±1.28) and moisture (15.75±0.43%) contents.

**Water quality parameters of Experimental Design**

Data analysed varied significantly ($p<0.05$) in the mean values of pH and DO and insignificantly in the mean values of temperature and ammonia.

The mean temperature (°C) ranged from 26.17±0.13 to 28.35±0.10, mean pH ranged from 6.63±0.08 to 7.14±0.06. DO values (mg/L) ranged from 6.13±0.06 to 6.58±0.08 and ammonia (mg/L) ranged from 0.35±0.03 to 0.44±0.03.

**Growth and Reproductive Performance of Female *Clarias gariepinus* fed on Experimental diets**

Table 4 showed the growth and reproductive performances of *C. gariepinus* females. The result showed that all experimental fish fed with *B. pinnatum* included diets have better MWG/PWG than the control fish. However, the highest mean values (MWG, PWG) were observed in T6 (1000.00±28.87g, 87.65±6.94%) followed by T4 (870.00±17.32g, 74.07±4.38%) and least in the control (696.67±43.72g, 59.24±8.12%). Feed intake of experimental fish on the other did not have any significant difference across the experimental groups ($p>0.05$), though mean feed intake was highest in T4 (1577.50±131.35g) and also least in the control (1070.33±17.33g).

The reproduction performance of female *C. gariepinus* female showed significant differences ($p<0.05$) in terms of some indices such as fecundity, egg weight (EW), egg diameter (ED), pseudogonadosomatic index (PGSI), fertilization, hatchability and survival percentages (Table 4).The highest mean values (Fecundity, EW, PGSI) were observed in T6 (320417.50±32705.45, 492.95±50.32g, 0.31±0.02) and least in the control (170332.50±17957.04, 262.05±27.63g, 0.21±0.01). ED was also highest in T6 (1.31±0.01 mm), $p>0.05$ and least in the control (1.27±0.01 mm). Fertilization, hatchability, and survival percentages showed significant ($p<0.05$) differences across the experimental groups. Mean values were highest (Fertilization, hatchability) in T4 (67.45±1.64%, 47.50±1.44%), followed by T6 (67.25±0.87%, 46.50±0.87) and least in control (51.23±1.89%, 32.50±1.44%). Survival was highest in T6 (41.13±0.36%), and least in control (22.40±0.81%).

## DISCUSSION

Generally, plants have a diversity of functions because of the presence of various active compounds like alkaloids, flavanoids, pigments, phenolics, terpenoids, steroids and essential oils (Citarasu, 2010). The preliminary study of *B. pinnatum* has been reported to contain a wide range of active compounds, including alkaloids, triterpenes, glycosides, flavonoids, steroids, bufadienolides, lipids and organic acids, have been isolated from this species (Salman *et al*., 2008). The higher quantities of saponins and flavonoids in this study were in line with the study of Kamboj and Saluja (2009) that found out that proteins (73.88±0.28mg/100g), flavonoids (89.13±1.15mg/100g), saponins (40.93±1.15mg/100g), pro-anthocyanidins (46.83±1.44mg/100g) were high in the leaf of this plant. The quantitative measurement of the phytochemicals present in this plant confirmed its possible use as a pro-fertility agent in fish.

Results obtained in this study showed that diets containing *B. pinnatum* leaf enhanced growth and reproductive performance as reflected in the improved mean weight gain, fecundity, egg weight, pseudogonadosomatic index, percentage fertilization, hatchability, and survival. The supplementation of some plants has also been observed to improve reproductive indices in fish. For example, the effect of dietary *Sesamum indicum* (beniseed) and *Croton zambesicus* seed powder have been observed by Dada and Adeparusi (2012) to improve the fecundity, ovary weight, egg size and GSI, growth and reproductive indices of female catfish, *Clarias gariepinus* broodstocks. Ikenweiwe *et al*. (2016) also found out that *Ageratum conyzoid* (leaf) and *Terminalia superba* (leaf and root) can be included in the diets of *C. gariepinus* broodstock for fertility enhancement. The increased fecundity and PGSI of *C. gariepinus* obtained in this study could be as a result of the presence of high levels of steroids and flavonoids in *B. pinnatum*. These compounds are potent antioxidants that are capable of increasing the production of estrogen, the key hormone involved in the production and maturation of eggs in the ovary (Dada and Adeparusi, 2012).

## CONCLUSION

This investigation showed that *B. pinnatum* leaf possesses promising pro-fertility property which can be exploited in fish seeds production. The results of this study provides baseline information and established safe limits of using *B. pinnatum* leaf as a pro-fertility agent to increase seeds production in *C. gariepinus* under hatchery conditions.

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**Table 1: Experimental diets for *Clarias gariepinus* Sub-adult (30% CP)**

| | Experimental Diet | | | | | | |
|---|---|---|---|---|---|---|---|
| **Feed ingredients (g/100g of feed)** | **Control** | **D1** | **D2** | **D3** | **D4** | **D5** | **D6** |
| Fish meal (65% CP) | 14 | 14 | 14 | 14 | 14 | 14 | 14 |
| Soybean meal (48% CP) | 15 | 15 | 15 | 15 | 15 | 15 | 15 |
| Groudnut cake (45% CP) | 22 | 22 | 22 | 22 | 22 | 22 | 22 |
| Maize (10% CP) | 37 | 37 | 37 | 37 | 37 | 37 | 37 |
| Palm oil | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| Chicken oil | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| Vitamin premix | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Dicalcium phosphate | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Methionine | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Lysine | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| NaCl | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Binder | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| *B. pinnatum* homogenate | 0 | 1 | 2 | 3 | 4 | 5 | 6 |

**Table 2: Phytochemical Composition of *Bryophylum pinnatum* Leaf Extract**

| **Chemical Compound** | **Amount µg/mL) (Mean±SE)** |
|---|---|
| Flavonoids | 4.50±0.06 |
| Saponin | 37.00±0.58 |
| Tannin | 2.35±0.03 |

| | |
|---|---|
| Steroid | 116.85±0.03 |
| Anthraquinone | 8.85±0.03 |
| Glycoside | 10.30±0.12 |
| Alkaloid | 2.05±0.09 |
| Phenol | 0.18±0.00 |

**Table 3: Proximate composition of Experimental Diets**

| | Experimental diet (Mean ±SE) | | | | | | |
|---|---|---|---|---|---|---|---|
| **Parameter (%)** | **Control** | **D1** | **D2** | **D3** | **D4** | **D5** | **D6** |
| Ash | 9.33±0.33$^{a}$ | 9.50±0.29$^{a}$ | 8.67±0.67$^{a}$ | 9.33±0.33$^{a}$ | 9.67±0.33$^{a}$ | 9.22±2.15$^{a}$ | 8.67±0.67$^{a}$ |
| NFE | 33.33±0.17$^{ab}$ | 32.33±0.33$^{bc}$ | 33.67±0.33$^{a}$ | 33.33±0.33$^{ab}$ | 30.00±0.58$^{c}$ | 33.50±0.29$^{a}$ | 33.67±0.32$^{a}$ |
| Fat | 6.08±0.18$^{bc}$ | 6.47±0.20$^{b}$ | 6.22±0.32$^{bc}$ | 7.16±0.23$^{a}$ | 5.22±1.20$^{c}$ | 5.36±0.89$^{c}$ | 6.50±0.18$^{b}$ |
| Crude Fibre | 6.50±0.27$^{b}$ | 6.25±0.14$^{b}$ | 6.67±0.33$^{ab}$ | 6.33±0.33$^{b}$ | 7.50±0.29$^{a}$ | 6.75±0.14$^{ab}$ | 6.97±0.33$^{ab}$ |
| Crude Protein | 29.93±0.18$^{a}$ | 29.95±0.82$^{a}$ | 30.27±0.37$^{a}$ | 30.35±0.52$^{a}$ | 30.86±1.28$^{a}$ | 30.67±3.21$^{a}$ | 30.44±0.29$^{a}$ |
| Moisture | 14.83±0.44$^{abc}$ | 15.50±0.29$^{ab}$ | 14.50±0.29$^{abc}$ | 13.50±0.29$^{c}$ | 15.75±0.43$^{a}$ | 14.50±0.87$^{abc}$ | 14.75±0.58$^{abc}$ |

**Mean values with different superscripts are significantly different (p<0.05) across the row**

D1 –Diet containing 1 g of *B. pinnatum* in 100g of compounded feed, D2 - Diet containing 2 g of *B. pinnatum* in 100g of compounded feed, D3 - Diet containing 3 g of *B. pinnatum* in 100g of compounded feed, D4 - Diet containing 4 g of *B. pinnatum* in 100g of compounded feed, D5 - Diet containing 5 g of *B. pinnatum* in 100g of compounded feed, D6 - Diet containing 6 g of *B. pinnatum* in 100g of compounded feed, NFE – Nitrogen free extract.

**Table 4: Growth and Reproduction Performance of Female *Clarias gariepinus* fed on *Bryophyllum pinnatum* included diets**

| | Experimental group (Mean ±SE) | | | | | | |
|---|---|---|---|---|---|---|---|
| **Parameter** | **Control** | **T1** | **T2** | **T3** | **T4** | **T5** | **T6** |
| IMW (g) | 503.33±9.55$^{a}$ | 503.33±8.55$^{a}$ | 430.00±9.28$^{a}$ | 500.00±3.51$^{a}$ | 480.00±6.19$^{a}$ | 530.00±3.83$^{a}$ | 430.00±7.74$^{a}$ |
| FMW (g) | 1200.00±7.74$^{b}$ | 1232.00±7.44$^{b}$ | 1200.00±7.74$^{b}$ | 1500.00±7.74$^{ab}$ | 1550.00±6.87$^{ab}$ | 1550.00±6.87$^{ab}$ | 1650.00±6.87$^{a}$ |
| MWG (g) | 696.67±43.72$^{c}$ | 723.67±23.72$^{c}$ | 770.00±11.55$^{bc}$ | 800.00±5.77$^{bc}$ | 870.00±17.32$^{b}$ | 820.00±51.96$^{b}$ | 1000.00±28.87$^{a}$ |
| PWG (%) | 59.24±8.12$^{c}$ | 59.24±8.11$^{c}$ | 68.79±5.27$^{ab}$ | 67.09±4.05$^{bc}$ | 74.07±4.38$^{ab}$ | 67.81±8.74$^{ab}$ | 87.65±6.94$^{a}$ |
| FI (g) | 1070.33± 17.33$^{a}$ | 1268.50± 155.02$^{a}$ | 1307.00± 125.29$^{a}$ | 1475.00± 181.87$^{a}$ | 1577.50± 131.35$^{a}$ | 1565.00±216.51$^{a}$ | 1470.00±271.35$^{a}$ |
| EW (g) | 262.05± 27.63$^{d}$ | 304.35± 26.70$^{cd}$ | 307.70± 18.71$^{bcd}$ | 389.95±10.25$^{b}$ | 363.70±7.77$^{bc}$ | 372.10±1.96$^{bc}$ | 492.95±50.32$^{a}$ |
| Fecundity | 170332.50± 17957.04$^{d}$ | 197827.50± 17356.59$^{cd}$ | 200005.00± 12159$^{bcd}$ | 253467.50± 6661.18$^{b}$ | 236405.00± 5028.72$^{bc}$ | 241865.00± 1275.94$^{bc}$ | 320417.50± 32705.45$^{a}$ |
| ED (mm) | 1.27±0.01$^{a}$ | 1.24±0.01$^{a}$ | 1.27±0.01$^{a}$ | 1.30±0.02$^{a}$ | 1.29±0.02$^{a}$ | 1.31±0.01$^{a}$ | 1.31±0.01$^{a}$ |
| PGSI | 0.21±0.01$^{d}$ | 0.24±0.01$^{cd}$ | 0.24±0.00$^{cd}$ | 0.28±0.00$^{b}$ | 0.26±0.00$^{bc}$ | 0.26±0.00$^{bc}$ | 0.31±0.02$^{a}$ |
| Fert. (%) | 51.23±1.89$^{c}$ | 55.35±2.84$^{c}$ | 61.25±1.44$^{b}$ | 60.28±0.65$^{b}$ | 67.45±1.64$^{a}$ | 62.50±0.99$^{b}$ | 67.25±0.87$^{a}$ |
| Hatch (%) | 32.50±1.44$^{c}$ | 35.25±2.74$^{c}$ | 42.50±1.44$^{ab}$ | 41.50±0.87$^{b}$ | 47.50±1.44$^{a}$ | 43.87±1.99$^{ab}$ | 46.50±0.87$^{ab}$ |
| Survival (%) | 22.40±0.81$^{d}$ | 29.20±2.77$^{c}$ | 36.13±1.23$^{ab}$ | 34.28±1.31$^{bc}$ | 40.50±2.60$^{a}$ | 32.88±0.51$^{bc}$ | 41.13±0.36$^{a}$ |

**Mean values with different superscripts are significantly different (p<0.05) across the row**

T1 – *C. gariepinus* fed with diet containing 1 g of *B. pinnatum* in 100g of compounded feed, T2 – *C. gariepinus* fed with diet containing 2 g of *B. pinnatum* in 100g of compounded feed, T3 – *C. gariepinus* fed with diet containing 3 g of *B. pinnatum* in 100g of compounded feed, T4 – *C. gariepinus* fed with diet containing 4 g of *B. pinnatum* in 100g of compounded feed, T5 – *C. gariepinus* fed with diet containing 5 g of *B. pinnatum* in 100g of compounded feed, T6 – *C. gariepinus* fed with diet containing 6 g of *B. pinnatum* in 100g of compounded feed, IMW – Initial mean weight, FMW – Final mean weight, MWG – Mean weight gain, FI – Feed intake, PWG – Percentage weight gain, ED – Egg diameter, PGSI – PseudoGonadosomatic index, Fert. – Fertilization, Hatch – Hatchability