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Effects of Bitter Leaf *(V. Amygdalina)* Extract On Egg Hatchability And Larval Survival of Catfish *(Clarias Gariepinus)*

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Abstract

This study evaluated the effects of *Vernonia amygdalina* (bitter leaf) extract on the hatchability of *Clarias gariepinus* eggs and its potential as a water disinfectant during larval rearing. The experiment was conducted in a wet laboratory using three feed treatments: Black Soldier fly larva meal (T1), *Artemia* (T2), and zooplankton (T3). Bitter leaf extract was prepared in cold water at concentrations of 5 g/L, 10 g/L, and 20 g/L, with borehole water as the control. Fertilized eggs (1 g per replicate) were incubated in 20 L of treated water. After hatching, larvae were reared for 10 days and fed the different diets. Culture water was disinfected once using 5 g/L extract for two hours. Parameters assessed using standard methods included water quality, hatchability, survival, the weight gain, the specific growth rate (SGR), the condition factor (K), the thermal growth coefficient (TGC), and the daily mortality. Temperature (22.43–23.86°C) was the only water parameter with a significant difference ($P<0.05$). SGR ranged from 39.52g ±10.34 (T1) to 138.93g ±69.34 (T3); survival rate from 1.06±0.53% (T2) to 3.17±2.42% (T3); and weight gain from 1,825.33g ±594.94 to 19,650g ±7,150. T3 (zooplankton) showed the best growth and survival. Bitter leaf extract did not enhance egg hatchability at the tested concentrations but showed potential as a larval disinfectant. Therefore, the use of *V. amygdalina* as a disinfectant for *Clarias gariepinus* eggs is not recommended, it proved ineffective in promoting hatchability and may be toxic to embryos. Further studies with lower concentrations are recommended to determine optimal safe dosages for hatchery application.

Key words: Bitter leaf extract, Clarias gariepinus, Growth parameters, Hatchability, Larval rearing, Water disinfection,

1. Introduction

The African mudfish (*Clarias gariepinus*) is a highly valued fish as food in Nigeria and globally due to its fast growth, omnivorous feeding habits, tolerance to poor water quality, and ability to thrive in high-density systems. It naturally inhabits lakes, rivers, swamps, floodplains, and even urban sewage systems (Shrestha and Niraula, 2020). These traits, along with ease of reproduction and live marketability, make it ideal for smallholder aquaculture (Musa et al., 2012). In Nigeria, Catfish is widely consumed because it is always available, affordable, and has nutritional benefits (Eseoghene and Ekelemu, 2016; FAO, 2003). However, aquaculture faces significant constraints, particularly the inadequate supply of quality fish seed (Otubusin, 1998). Despite advances in breeding techniques, fish seed supply is still heavily dependent on wild stocks and natural spawning cycles (George *et al*., 2010). Effective hatchery systems aim to maximize fingerling production from broodstock, but high mortality at egg and larval stages remains a challenge, often due to microbial infections (FAO, 1987). This highlights the need for natural, eco-friendly disinfectants to improve hatchability and survival without introducing harmful residues. *Vernonia amygdalina* (bitter leaf), a widely cultivated West African plant from the Asteraceae family, is traditionally used to treat a variety of ailments (Abdullahi et al., 2019). It has shown promise in poultry as an antimicrobial, antioxidant, growth promoter, and immune booster (Okukpe et al., 2018). Its leaves, rich in bioactive compounds, could offer similar benefits in aquaculture. Given its antimicrobial properties, bitter leaf extract may serve as an effective natural disinfectant in catfish hatcheries.

Clarias gariepinus is particularly amenable to the farming practices of smallholders, who comprise the majority of farmers in developing countries (Musa et al., 2012). It is like animal protein food source.

Otubusin (1998) in his study opined that inadequate fish seed supply is a major constraint to the development of aquaculture in Nigeria. In as much as domestication of species and advances in artificial insemination have enhanced the breeding of fish indoors and extended the spawning season to a limited degree, it has not yet allowed high seed production outside the normal spawning season for many species (George *et al*., 2010). According to (George *et al*., 2010), presently, a large percentage of the total seed requirements for aquaculture are obtained from the wild, where supply is completely dictated by the natural breeding cycle of the target species.

According to (FAO, 1987) Farming of fish begins with the stocking of fry, which can come from the wild or be produced on the farm. No matter where they come from as their origin, they are indispensable and the means of obtaining them influences directly farm production .

Vernonia amygdalina (bitter leaf) is the most extensively cultivated member of the genus *Vernonia*, which comprises approximately 1,000 shrub species (Munaya, 2013). The plant belongs to the family Asteraceae and is commonly propagated through stem cuttings. It is widely distributed across several West and Central African countries, including Nigeria, Cameroon, Gabon, and the Democratic Republic of the Congo (Abdullahi et al., 2019). The genus was named in honour of the English botanist William Vernon, and the plant is also commonly known as ironweed (Abdullahi et al., 2019). Across Africa, *V. amygdalina* is known by various indigenous names, reflecting its broad cultural significance. It is called **Omjunso** in Tanzania and other parts of East

Africa, **Onugbo** among the Igbo of southeastern Nigeria, **Orugbo** by the Itsekiri and Urhobo ethnic groups, **Ewuro** in Yoruba, **Etidot** in Ibibio, **Ityuna** in Tiv, **Oriwo** in Edo, and **Shiwaka** in Hausa communities (Abdullahi et al., 2019).

The leaves are eaten, after crushing and washing thoroughly to remove the bitterness. All parts of the plant are pharmacologically useful. Both the roots and leaves are used in phyto-medicine to treat fever, hiccups, kidney disease and stomach discomfort, among others (Abdullahi et al., 2019). Research has also shown that *V. amygdalina* have some beneficial effects in disease management of poultry, such as anti-coccidiosis, anti-bacterial and anti-parasitic, as an anti-oxidant and as a growth promoter enhancing the gastro intestinal enzymes thus increasing feed conversion efficiency (Okukpe et al., 2018). The major problems in fish production are obtaining a sufficient number of eggs, a good hatching rate, and good survival and growth of the larvae obtained. In nature, there is high mortality of these stages and a lot attention and effort is needed to overcome these difficulties (FAO, 1987). The mortality of this seeds can be as a result of many factors including and not limited to microbial attacks. This therefore sparks the interest for the need of biotic disinfectants that can ward off these microbes, without the bioaccumulations of harmful substances that can be detrimental to human health in the long run. This study therefore seeks to assess the effects of *V. amygdalina* as a biotic disinfectant on egg hatchability and fry survival in *C. gariepinus*, incorporating *Artemia*, zooplankton, and black soldier fly maggot meal in the nursery phase.

2. Materials and Methods

2.1: Materials

The experiment was carried out in the wet laboratory of the Department of Fisheries and Aquaculture Management, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria. Fresh *Vernonia amygdalina* (bitter leaf) was harvested from the university's Crop Science Department and prepared in different concentrations of the following 5 g/L, 10 g/L, and 20 g/L. Leaves were washed, blended, filtered with muslin cloth, and the extracts stored in labeled airtight containers at 4°C (Omoregie, 2019). These extracts were used as treatment media, while plain borehole water served as the control.

2.2 Methods

12 Spawning tanks (20 L capacity) were labeled according to treatments and filled with the respective extract solutions or control. Mature *Clarias gariepinus* broodstock (1.5 kg female and 1.6 kg males) were procured and subjected to artificial propagation. The female was induced with Ovaprim hormone and stripped after 12 hours, while male testes were dissected for milt extraction (FAO, 2007). Eggs (1 g ≈ 650 eggs) were fertilized using the wet method and immersed in treatment media for up to 24 hours to evaluate hatchability.

Experiment 1, water quality parameters, temperature, pH, dissolved oxygen, and conductivity were measured using standard techniques (Boyd, 1981). Each treatment replicate received 1 g of eggs, (1 g of eggs measured 650 eggs using Metler balance, Model: Toledo PB 8001).

Egg masses were exposed to three concentrations of bitter leaf (*Vernonia amygdalina*) extract (5 g, 10 g, and 20 g) and a control treatment consisting of clean water. Each treatment was replicated twice. The eggs were initially immersed in the respective solutions for 6 hours to determine the optimum extract concentration and exposure duration for enhanced hatchability. Following the initial exposure, the treatment was repeated at 24-hour intervals until the completion of the experimental period.

Experiment 2 involved larval feeding trials. Sixty-three larvae per tank were fed with either zooplankton, *Artemia* (INVE Aquaculture, 2021), or black soldier fly larva meal. Zooplankton (mainly rotifers and paramecium) was harvested from on-site ponds, while blackfly larvae were processed by drying, freezing, milling, and sieving (Ovie and Egborge, 2002).

The hatchlings were subjected to a feeding trial using zooplanktons, *artemia* and blackfly maggot. The larvae absorbed their yolk on the third day after hatching and feeding commenced on the fourth day which is the start of the experiment.

Sixty three larvae selected from the hatchlings were kept in each of the fish tank used for the experiment. The fish tanks were divided into 3 treatments of 3 replicates based on zooplankton, *artemia* and blackfly larvae feed. The *Artemia* is a product of INVE Aquaculture Nutrition, INVE Aquaculture, inc, Utah, zooplankton was sourced from the tarpaulin pond. They were harvested by the horizontal trawl method. The zooplankton feed were made of Rotifers and paramecium, while the Black fly larvae was sourced from Emmanuel agro farm in Agulu, it was demolized by freezing after oven drying at 60ºc and milled using a corona hand grinder. It was then sieved using a fine sieve for shaft removal and kept in an air tight container. Each plastic tanks measuring (0.44×0.31×0.15) were filled with 20L of water sourced from the university borehole ifite gate near Faculty of Social science. Feeding wasdon thrice daily at three hour interval

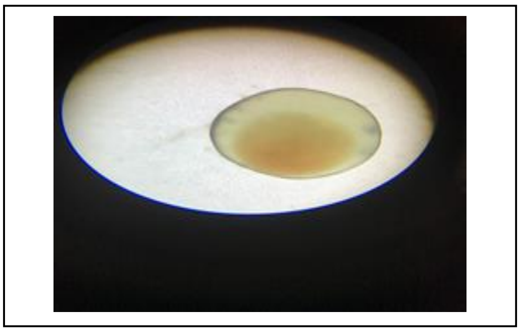


Plate 1 :
View of developing egg treated leaf extract for 30 mins

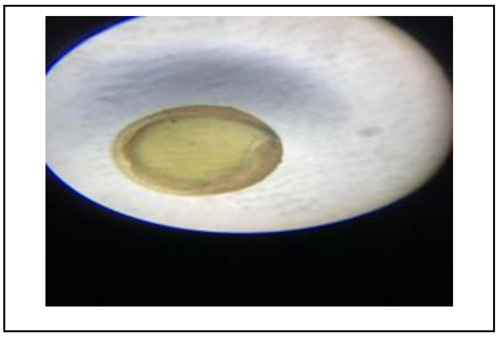
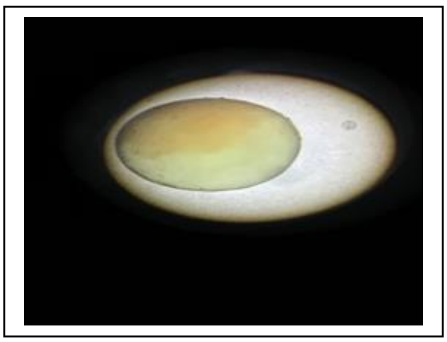


Plate 2 :
View of the developing egg with bitter treated with bitter leaf extract for 6 hrs



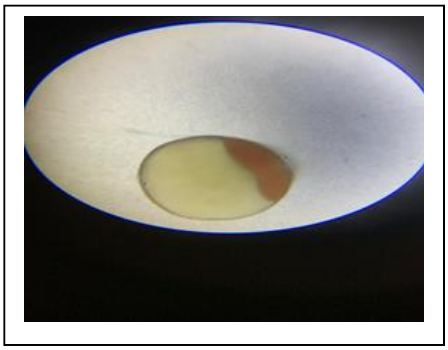


Plate 3:

View of development eggs treated leaf with extract for 12hours

Plate 4:

View of developing egg treated bitter with bitter leaf extract for 18hours

Experiment 3 assessed bitter leaf extract (4 mL/20 L) as a larval water disinfectant, applied for two hours and then drained. Growth and survival parameters such as mean weight gain, specific growth rate (SGR), percentage survival, condition factor (K), and thermal growth coefficient (TGC) were calculated using standard biometric formulas (Bagenal, 1978). The catfish were randomly assigned to six antibiotics/phytobiotics treatments. The 3 treatments and replicates, totally 9 samples, were subjected to disinfection. The 4ml of the bitter leaf extract was injected into each plastic tank measuring 20L of water and then the water solution is drained after 2hrs.

Using the method described by Bagenal, (1978), the growth parameters were calculated. Fish body weight and feed intake were recorded throughout the experimental period. These data were used to determine growth performance indices, including mean weight gain (MWG), percentage weight gain (PWG), specific growth rate (SGR), feed conversion ratio (FCR), survival rate (%), and thermal growth coefficient (TGC).

Mean Weight Gain (MWG) was calculated as:

MWG=W2−W1/n

where:

- W2 = final mean body weight (g),
- W1 = initial mean body weight (g), and
- n = duration of the experiment (days).

% weight gain (PWG) % = mean weight (g) / initial mean weight (g) ×100

Specific growth rate (SGR) = Log W2 – Log W2 / T2 – T1×100

Where W1 = initial weight (g), W2= final weight (g), T2 – T1 = is time interval between initial & final weight calculated in (days). Log = natural log to base.

Survival rate (SR) (%) = No x 100 / Ni

Ni = Number of fishes stocked at the beginning of experiment

No = Number of live fish at end of experiment

Condition factor (k) = W x 100 / L3.

Results and Discussion

The experiment evaluated the effects of *Vernonia amygdalina* (bitter leaf) extract on egg hatchability, as well as the growth performance and survival rate of *Clarias gariepinus* larvae fed three different experimental diets: *Artemia*, zooplankton, and black soldier fly larva meal. The physicochemical parameters of the culture water, hatchability and survival percentages, proximate composition of the feed, and various growth indices were analyzed.

Water Quality Parameters (Table 1)

The temperature in the experimental tanks ranged from 22.43 ± 0.60°C to 23.86 ± 0.39°C. A significant difference was found only in the temperature values, with Treatment 3 (zooplankton) being significantly lower ($p < 0.05$). Dissolved oxygen levels were sub-optimal, ranging from 0.43 ± 0.10 to 0.96 ± 0.36 mg/L, which is below the WHO-recommended 5 mg/L for aquaculture (W.H.O., 1987). This could be due to poor water circulation and borehole water limitations, as also observed by James et al. (2017). The pH values (7.62–7.81) and conductivity (1472.36–1508.48 µS/cm) were within acceptable ranges, although conductivity exceeded the ideal threshold of 1000 µS/cm (Ross, 2000).

Table 1: Physico-chemical parameters of water used for culture

Water parameter	T1 Blackfly larvae meal	T2 *Artermia* feed	T3 Zooplankton
Temperature (ºC)	**23.3^{b}±0.41**	**23.86^{b}±0.39**	**22.43^{a}±0.60**
Dissolved Oxygen (mg/l)	**0.43^{a}±0.10**	**0.96^{a}±0.36**	**0.78^{a}±0.27**
pH	**7.81^{a}±0.03**	**7.73^{a}±0.03**	**7.77^{a}±0.03**
Conductivity	**1472.36^{a}±28.34**	**1506.3^{a}±27.71**	**1508.46^{a}±20.84**

Mean values with different superscript letters are significantly different (P < 0.05).

Hatchability and Bitter leaf Extract (Table 2)

The application of bitter leaf extract at different concentrations failed to support successful hatching. Fertilized eggs in all treatments containing the extract developed until the 18th hour and subsequently died. Conversely, the control group (no bitter leaf) achieved 60.96 ± 0.65% hatchability and 33.33 ± 0.77% survival. This suggests that bitter leaf extract, despite its known antimicrobial properties, may be toxic to developing *C. gariepinus* embryos. This contradicts previous reports of its beneficial use in aquaculture, such as its successful use in juvenile rearing as reported by Okukpe *et al*. (2018).

Table 2: Hatchability and Survival percentage of Fish larvae production using Bitter leaf extract as a disinfectant.

Parameters	**Hatchability (%)**	**Survival (%)**
Water (control)	60.96±0.65	33.33±0.77
Treatment 1(bitter leaf extract 5g/L)	0.00±0.00	0.00±0.00
Treatment 2 (bitter leaf extract 10g/L)	0.00±0.00	0.00±0.00
Treatment 3 (bitter leaf extract 20g/L)	0.00±0.00	0.00±0.00

Proximate Composition of Feed

Black soldier fly larva meal had the highest moisture (8.0%), crude fiber (21.9%), and fat/oil (10.43%) content, indicating its high energy potential. Artemia feed had the highest protein (29.89%) and ash content (11.6%), while zooplankton had the highest carbohydrate content (56.08%). Variations in feed composition influenced the growth and survival of larvae receiving the different dietary treatments.

Table 3: Proximate formulation of experimental feed (%)

Parameters	**Zooplankton**	***Artemia***	**Black fly larvae**
Moisture content	**6.0**	**6.73**	**8.0**
Ash content	**8.1**	**11.6**	**10.87**
Crude fiber content	**1.28**	**4.48**	**21.9**
Fat/oil content	**10.43**	**9.9**	**17.2**
Crude protein content	**18.28**	**29.89**	**21.7**
Carbohydrate content	**56.08**	**46.92**	**20.33**

Growth and Survival Performance

The specific growth rate (SGR) was highest in fries fed with zooplankton (138.93 ± 69.34), although no significant differences were observed across treatments. Artemia-fed fries recorded the highest weight gain (19650 ± 7150), followed by blackfly (10533.33 ± 6382.88) and zooplankton (1825.33 ± 594.94). However, zooplankton-fed fries had the highest survival rate (3.17 ± 2.42), which might be linked to the significantly lower culture temperature in Treatment 3 and a condition factor value (0.52 ± 0.22) closer to the ideal of 1. Conversely, Artemia-fed larvae had the lowest survival (1.06 ± 0.53) and the highest mortality rate (6.23 ± 2.10), despite achieving the highest thermal growth coefficient (0.19 ± 0.03).

Growth parameter	**T1 Blackfly larvae meal**	**T2 *Artemia* feed**	**T3 Zooplankton**
Specific growth rate (%/day)	**39.32[a]±10.34**	**52.1[a]±3.84**	**138.93[a]±69.34**
Condition factor	**18.95[a]±12.84**	**33.74[a]±3.02**	**0.52[a]±0.22**
Percentage weight gain (%)	**10533.33[a]±6382.88**	**19650[a]±7150**	**1825.33[a]±594.943**
Percentage survival (%)	**1.59[a]±0.92**	**1.06[a]±0.53**	**3.17[a]±2.42**
Thermal growth coefficient	**0.10[a]±0.04**	**0.19[a]±0.03**	**0.06[a]±0.02**
(%) Mortality	**6.20 ± 2.45**	**6.23 ± 2.10**	**6.10 ± 1.99**

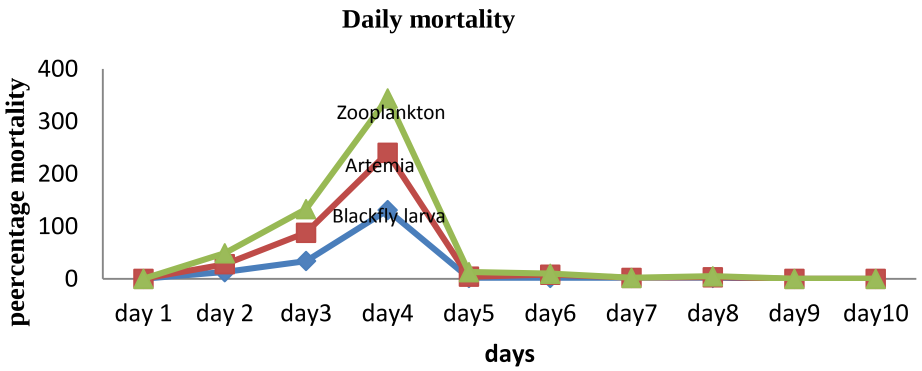


Figure 1: Daily mortality

These findings show an apparent trade-off between growth rate and survival. Although Artemia has a high protein content that likely contributes to better weight gain, it may simultaneously lead to stress or physiological imbalances resulting in higher mortality. Similar observations were made by Abubakar *et al*. (2013), who noted the performance boost Artemia provides, but also acknowledged challenges related to survival. In contrast, zooplankton appears to provide a more balanced nutritional profile conducive to steady growth and better survival, as supported by findings from Adande *et al*. (2017).

Conclusion and Recommendation

The research concludes that while bitter leaf extract is ineffective for egg disinfection and hatchability enhancement, it could still be explored in post-hatch larval rearing. Among the feed types tested, zooplankton offered the best results in terms of specific growth and survival, while Artemia promoted greater weight gain but was associated with higher mortality. Future studies could explore combining feeds to balance growth and survival while optimizing water quality management to mitigate low dissolved oxygen levels. Therefore, the use of *V. amygdalina* as a disinfectant for *Clarias gariepinus* eggs is not recommended, as it proved ineffective in promoting hatchability and may be toxic to embryos.

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