

Efficacy of Bitter Leaf (*Vernonia Amygdalina*) Extract for Removal of Egg Adhesiveness during Artificial Propagation of African Catfish (*Clarias Gariepinus*, Burchell 1822)

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ABSTRACT

This study evaluated the optimal immersion period and concentration of bitter leaf (*Vernonia amygdalina*) extract for effectively removing egg adhesiveness in African catfish (*C. gariepinus*). Two males (1.3 kg) and two females (1.4 kg) were selected as broodstock for induced breeding. Three concentrations of bitter leaf extract (0.5%, 1.0%, and 1.5%) were tested at immersion durations of 30, 60, and 90 seconds. Tannic acid (0.75 g/L) served as the reference de-adhesion agent, while water alone was used as the control. Each treatment was conducted in triplicate. Data were analysed using one-way ANOVA, followed by Tukey's multiple range test, while third-order polynomial regression was applied to identify the most effective concentration and immersion period. The findings showed no significant differences ($p > 0.05$) in non-adhesive egg percentage and hatchability between eggs treated with bitter leaf extract and those treated with tannic acid. However, the combination of 0.5% bitter leaf extract with a 30-second immersion period exhibited the highest de-adhesion efficiency, resulting in 97.40% fertilisation, 95.07% non-adhesive eggs, and 90.09% hatchability. The study concludes that bitter leaf extract is an effective, eco-friendly, and low-cost alternative to synthetic agents such as tannic acid for removing egg adhesiveness in *C. gariepinus*. Its use at 0.5% concentration with a short immersion period (30 seconds) is recommended for hatchery operations to enhance seed production efficiency.

Keywords: Bitter leaf, *Clarias gariepinus*, Egg stickiness, Non-adhesiveness, Hatching.

INTRODUCTION

The artificial propagation of African catfish (*Clarias gariepinus*) is essential in aquaculture but faces challenges due to the adhesive nature of their eggs (Kwirikiza *et al.*, 2025). When released into the water, these eggs tend to clump together, reducing fertilisation and hatching rates and increasing larval mortality (Ojebuola *et al.* 2024). This adhesiveness poses a significant obstacle in hatcheries, necessitating effective solutions to ensure higher survival and productivity rates.

Over the years, several physical and chemical methods have been employed to mitigate the adhesiveness of *C. gariepinus* eggs. Solutions such as urea, mud, milk, kaolin, and tannins have been used, sometimes requiring species-specific applications (Kareem *et al.*, 2016). While tannin solutions are effective for some species like pikeperch (Żarski *et al.*, 2015), they can be costly and may not be readily available in rural aquaculture settings. Enzymatic treatments, such as Alcalase, show promise but also face issues of cost and availability (Kristan *et al.*, 2017; Ljubobratović *et al.*, 2018). Aloe Vera gel and water leaf extracts have been used with immersion periods of five and one minute, respectively, for African catfish (Fawehinmi *et al.*, 2019). Ojebuola *et al.* (2024) also reported that 1% concentration of okra leaf extract with a one-minute immersion period yielded the best results for *Clarias gariepinus*. However, these methods often require precise control over treatment times and conditions, which can be challenging to maintain consistently.

Given these limitations, there is a need for more accessible, cost-effective, and reliable methods to address egg adhesiveness in *Clarias gariepinus*. One promising solution is the use of bitter leaf (*Vernonia amygdalina*), an

abundant and inexpensive plant native to tropical Africa, commonly found in Nigeria, Cameroon, and Ghana. This bushy plant, which can grow up to three meters tall, is characterised by its dark green leaves with a bitter taste. The leaves contain beneficial compounds, including antioxidants, polysaccharides, minerals, proteins, enzymes, and vitamins (Nwachukwu *et al.*, 2014).

According to Ogwu and Ikhajagbe (2023), bitter leaf is traditionally used in many African cultures for its medicinal properties. It is used to treat ailments such as malaria, diabetes, gastrointestinal disorders, and to improve general health. Its antimicrobial and anti-inflammatory properties are well-documented, making it a valuable resource in both traditional medicine and modern pharmacology.

Despite its widespread use in other applications, limited research exists on the use of bitter leaf extract for removing egg adhesiveness in *Clarias gariepinus*. Usunobun and Ngozi (2016) found that saponins, tannins, alkaloids, flavonoids, triterpenoids, steroids and cardiac glycosides were high in *V. amygdalina*. Given its bioactive components and accessibility, it stands as a potential solution that could be both effective and economically viable for fish farmers, particularly in rural areas. This study aims to explore the efficacy of bitter leaf extract in removing egg adhesiveness and to determine the optimal concentrations and immersion periods needed to enhance fertilisation and hatching rates.

By investigating the use of bitter leaf extract, this research seeks to provide an alternative that could improve the efficiency of African catfish hatcheries. The goal is to develop a method that addresses the issue of egg adhesiveness while aligning with the practical and economic constraints faced by many aquaculture operations. This could ultimately lead to higher productivity and sustainability in the cultivation of *Clarias gariepinus*.

MATERIALS AND METHODS

Study Area and brood fish

The experiment was conducted at the Teaching and Research Fish Farm of the Federal University of Technology, Akure, located in Obaekere, Akure. Two healthy male and two female *C. gariepinus*, weighing approximately 1.3 kg and 1.4 kg, respectively, were procured from a reputable fish farm in Akure. The fish were placed in separate holding tanks ($40 \times 30 \times 35$ cm³) supplied with aeration and acclimated for five days. During this period, they were fed with a local commercial diet, which was withdrawn 24 hours before artificial induction of ovulation.

Collection and Identification of Plant Material

Fresh bitter leaf plants were collected within the Teaching and Research Fish Farm, Department of Fisheries and Aquaculture, The Federal University of Technology, Akure. It was identified as *Vernonia amygdalina* at the Herbarium of the Department of Crop, Soil and Pest Management, The Federal University of Technology, Akure.

Preparation of Bitter Leaf Extract

Fresh, healthy bitter leaves were selected, identified by their dark green colour, elliptical shape, and bitter taste due to compounds such as sesquiterpene lactones and tannins, the latter being an active agent in egg de-adhesion (Żarski *et al.*, 2015). About 500 g of leaves were harvested, inspected to ensure they were free from pests and diseases, and thoroughly washed under running water until the rinse water was clear.

The cleaned leaves were cut into pieces of about 2–3 cm to increase surface area and facilitate extraction. They were then manually crushed and squeezed in a clean basin, releasing their liquid extract. The mixture of crushed leaves and liquid was filtered through a 1 mm mesh hand net to separate the aqueous extract from leaf residues. Filtration was repeated until sufficient extract was obtained. The resulting greenish extract was collected in a clean basin.

The extract was transferred into a dry, airtight plastic container and kept at room temperature ($\approx 25^{\circ}\text{C}$) in a shaded, dry place. It was allowed to stand for 30 minutes so that fine particles could settle, after which the

clear supernatant was carefully decanted for use. The extract was freshly prepared and applied on the same day as egg stripping to maintain quality and effectiveness.

The greenish extract was prepared into percentages as follows:

0.5% = 0.5ml of Bitter leaf extract in 99.5ml of water.

1% = 1.0ml of Bitter leaf extract in 99ml of water.

1.5% = 1.5ml of Bitter leaf extract in 98.5ml of water.

Preparation of Tannic Acid Solution (Reference de-adhesion agent)

Tannic acid solution that served as a reference de-adhesion agent was prepared by diluting 0.75g of tannic acid into one litre of water according to Żarski *et al.* (2015).

Water without any of the extracts served as the control.

Preparation of Spawning Bowls

Fifty-seven spawning bowls of 4 litres capacity were used for the experiment. The bowls were thoroughly washed and dried. The bowls were labelled according to the inclusion levels of the treatments, tannic acid solution (0.5%, 1% and 1.5%) and control as well as the immersion periods (30 seconds, 60 seconds and 90 seconds). The bowls were filled with 100ml of water (control), 99.5ml of water (0.5%), 99ml of water (1%) and 98.5ml of water (1.5%) respectively.

Milt and Egg Collection

The female brooders were injected with hormone (Ovaprim Syndel Laboratories Ltd., Nanaimo, BC Canada V9S 4M9) at an angle 45° with the needle pointing towards the gonad region. The injected brooder was kept inside separate plastic tanks (24 x 12 x 12 cm³) containing water and tightly covered with a perforated lid. After a latency period of 12 hours, slight pressure was applied to the abdominal cavity to express the eggs into a clean bowl. The male testes were removed by abdominal dissection and cleaned with a towel; the milt was gently squeezed out and collected in a beaker.

Fertilisation and immersion

Wet fertilisation was used in the experiment. Milt collected was then mixed with a small quantity of saline solution. 1g of the striped eggs was carefully weighed on nylon, and each measured egg was fertilised with the prepared milt (0.01ml of milt to 1g of eggs (FAO 1996). The eggs were randomly rinsed inside the spawning bowls and subjected to the treatments.

Experimental Design

Each treatment triplicate received 1g of eggs (1g of eggs contained 700 eggs using a Metler balance, Model: Toledo PB 8001).

The fertilised eggs were placed in three treatment concentrations of Bitter leaf extract, tannic acid solution (reference de-adhesion agent) at (0.5, 1 and 1.5) %, and water (control). There were three replicates for each of the inclusion levels. The exposure time was 30, 60 and 90 seconds, respectively, to determine the optimum concentration and immersion period of bitter leaf extract. After the speculated exposure period, the concentrated water was decanted, and then clean water was replaced to incubate the eggs in the spawning bowls.

Evaluation of non-adhesive eggs, hatchability, survival and deformity indices

To determine the efficacy and efficiency of bitter leaf extract in removing egg adhesiveness, the parameters

assessed were computed according to the method described by Adebayo (2006).

Non-adhesive eggs (%) = number of non – adhesive egg/initial number of eggs $\times$ 100

Hatchability (%) = (Number of eggs hatched)/(Total number of eggs incubated) $\times$ 100

Survival (%) = number of hatchling at 72h/Total number of hatchling at 0h $\times$ 100

Hatching index: For each treatment, a hatching index expressed as the percent viable embryos at 72 h post-hatch/initial number of eggs was calculated. This index represented the percentage (%) of the hatched larvae obtained from the initial number of eggs.

Deformity (%) = (Number of deformed larvae)/(Total number of larvae 72 h post-hatch) $\times$ 100

Larvae Size: The mean total larval length at 72 h post-hatch was calculated using ImageJ 1.34 software (Rasband 1997–2011) as described by Ben Khemis *et al.* (2014).

Water Quality Parameters

Water quality parameters such as temperature, pH and dissolved oxygen concentration were monitored twice throughout the study period using a mercury-in-glass thermometer (YSI-DO 550, U.S.A), a pH meter (Hanna H198106 model) and a dissolved oxygen meter (JPP-607 model) as described by APHA (1987).

Statistical analysis

All percentage data at different concentrations and immersion periods were subjected to an Analysis of Variance test. Also, Tukey's Honestly Significant Difference test was used as a follow-up procedure. Polynomial regression analysis was then used to determine the best concentration and immersion period of bitter leaf extract treatment that effectively removed egg adhesiveness. All analysis was performed at a 0.05 significance level.

RESULTS

Effects of bitter leaf extract on *Clarias gariepinus* eggs

Adhesiveness of eggs of *C. gariepinus* exposed to varying concentrations and immersion periods of Bitter leaf extract

The results of egg adhesiveness in *C. gariepinus* exposed to different concentrations and immersion periods of bitter leaf extract are presented in Table 1. The highest percentage of non-adhesive eggs (95.07%) was recorded at 0.5% concentration with a 30-second immersion period, while the lowest (73.41%) occurred at 1.5% concentration with a 90-second immersion period. Similarly, eggs treated with tannic acid solution showed non-adhesive values ranging from 92.52% at 0.5% concentration with a 60-second immersion period to 80.34% at 1.5% concentration with a 90-second immersion. In contrast, the control group recorded only 25.62% non-adhesive eggs. Statistical analysis revealed no significant difference ($p > 0.05$) between bitter leaf extract and tannic acid treatments, but both differed significantly from the control. Generally, egg detachment decreased in both bitter leaf and tannic acid solutions as concentration and immersion periods increased. Figure 1 presents the highest percentage of non-adhesive *C. gariepinus* eggs treated with 0.5% bitter leaf extract at different immersion times, compared with tannic acid as the reference agent.

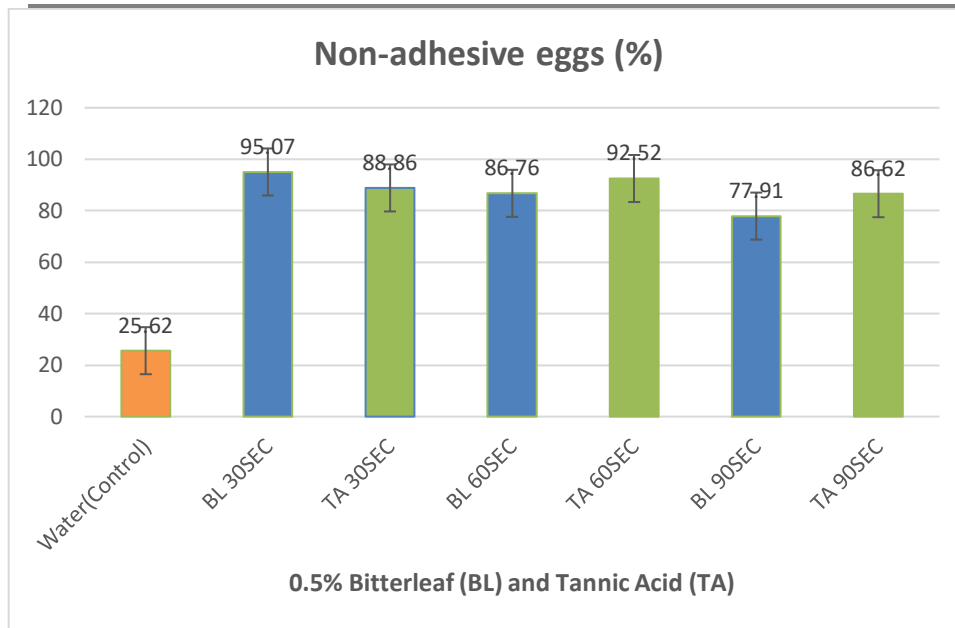


Figure 1: Egg adhesiveness of *C. gariepinus* treated with 0.5% bitter leaf extract at varying immersion times.

Incubation period of *C. gariepinus* exposed to varying concentrations and immersion periods of Bitter leaf extract

The incubation period of eggs treated with bitter leaf extract ranged from 23 hours 50 minutes at 1% concentration (30 seconds) to 24 hours 50 minutes at 1.5% concentration (90 seconds), as shown in Table 1. For eggs treated with tannic acid solution, incubation time varied from 23 hours 53 minutes at 0.5% concentration (30 seconds) to 24 hours 65 minutes at 1.5% concentration (90 seconds). In contrast, eggs incubated in water (control) recorded the longest incubation period of 25 hours 73 minutes. Statistical analysis showed no significant difference ($p > 0.05$) between incubation periods of eggs immersed in bitter leaf extract and tannic acid solution, though both were shorter than the control.

Percentage hatchability of *C. gariepinus* exposed to varying concentrations and immersion periods of Bitter leaf extract

The percentage hatchability decreased with increasing concentrations of bitter leaf extract, as presented in Table 1. The highest hatchability (90.09%) was obtained in eggs treated with 0.5% bitter leaf extract for 30 seconds, while eggs exposed to 0.5% tannic acid solution for 30 seconds recorded a hatchability of 85.07%. Hatchability values obtained from treatments with 0.5% bitter leaf extract and tannic acid solution were high and not significantly different ($p > 0.05$). However, both were significantly higher ($p < 0.05$) than the control group, which recorded the lowest hatchability of 43.24%. Figure 2 presents the highest percentage hatchability of *C. gariepinus* treated with 0.5% bitter leaf extract at different immersion times, compared with tannic acid as the reference agent.

Percentage hatching index of *C. gariepinus* exposed to varying concentrations and immersion periods of Bitter leaf extract

The percentage hatching index decreased with increasing concentrations of bitter leaf extract, corresponding with the observed hatchability trends as presented in Table 1. The lowest hatching index (9.55%) was recorded in the control group, while the highest value (72.60%) occurred in eggs exposed to 0.5% bitter leaf extract for 30 seconds. In comparison, eggs treated with 0.5% tannic acid solution for 30 seconds achieved a hatching index of 57.14%. Notably, the hatching index observed at the lowest concentration of bitter leaf extract (0.5% with 30 seconds immersion) was significantly higher ($p < 0.05$) than that of the control and other treatments across varying concentrations and immersion periods.

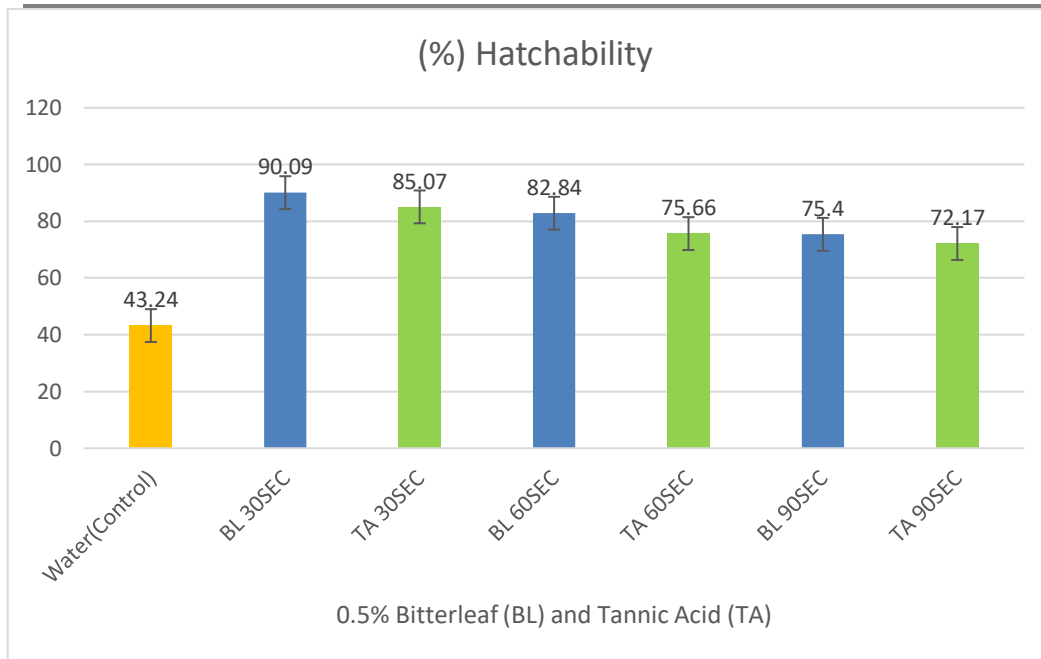


Figure 2: Percentage Hatchability of *C. gariepinus* treated with 0.5% bitter leaf extract at varying immersion times.

Deformed larvae of *C. gariepinus* exposed to varying concentrations and immersion periods of Bitter leaf extract

No deformity of larvae was observed in this experiment. The survived larvae were very active and responsive to feeding.

Percentage survival of *C. gariepinus* exposed to varying concentrations and immersion periods of Bitter leaf extract

The survival larvae percentage showed that survival decreased as concentration and immersion period increased, as shown in Table 1. 80.90% was the highest, and this was observed in 0.5% concentration with a 30-second immersion period, and the lowest (51.30%) was in 1.5% concentration (90 seconds) of bitter leaf extract. Also, 67.41% was the highest and this was observed in 0.5% concentration with 30 seconds immersion period and lowest (54.89%) in 1.5% concentration (90 seconds) of tannic acid solution while survival of hatched larvae from the control group was 22.11% which was the least when compared with survived larvae exposed to varying concentrations and immersion periods of bitter leaf extract and tannic acid solution.

However, there was a significant difference ($p < 0.05$) between the highest larvae survival (80.90%) recorded in 0.5% concentration with a 30-second immersion period of bitter leaf extract and other rinsing agents at varying concentrations and immersion periods, including the control.

Larvae size of *C. gariepinus* exposed to varying concentrations and immersion periods of Bitter leaf extract

The result of larvae size of *C. gariepinus* exposed to varying concentrations and immersion periods of bitter leaf extract is shown in Table 1. The larvae size of *C. gariepinus* obtained for eggs immersed in bitter leaf extract ranged from 0.32mm to 0.38mm in 1.5% (90 seconds) and 0.5% (30 seconds), respectively. Also, larvae size of *C. gariepinus* obtained for eggs immersed in tannic acid solution varied between 0.29mm to 0.35mm in 1.5% (90 seconds) and 0.5% (30 seconds), respectively while 0.23mm was recorded in the control, which is the smallest size compared to other larvae sizes of those immersed in varying concentrations and immersion periods. Hence, there was no significant difference ($P > 0.05$) in larvae size between concentrations and immersion periods of eggs immersed in bitter leaf extract and tannic acid solution but there was significant different ($P < 0.05$) when compared with the control.

Table 1: Percentages of egg adhesiveness, fertility, hatchability, survival and incubation period of bitter leaf extract, tannic acid solution and water.

Rinsing Agents	Concentration (%)	Immersion time(sec)	Non-adhesive eggs (%)	Incubation period (mins)	Hatching (%)	Hatching Index (%)	Survival (%)	Larvae size (mm)
Water (Control)			25.62±0.10 ^b	1544±2.90 ^b	43.24±0.17 ^b	9.56±0.04 ^d	22.11±0.09 ^c	0.23±0.01 ^b
Bitter leaf	0.5	30	95.07±0.36 ^a	1411±2.65 ^a	90.09±0.34 ^a	72.60±0.28 ^a	80.90±0.31 ^a	0.38±0.01 ^a
		60	86.76±0.33 ^a	1415±2.66 ^a	82.84±0.31 ^a	59.65±0.23 ^b	68.14±0.26 ^b	0.37±0.01 ^a
		90	77.91±0.30 ^a	1416±2.66 ^a	75.40±0.29 ^a	49.28±0.19 ^b	57.89±0.22 ^b	0.36±0.01 ^a
Tannic Acid	0.5	30	88.86±0.34 ^a	1412±2.65 ^a	85.07±0.33 ^a	57.14±0.22 ^b	67.41±0.26 ^b	0.35±0.01 ^a
		60	92.52±0.35 ^a	1413±2.65 ^a	75.66±0.29 ^a	56.63±0.22 ^b	67.12±0.26 ^b	0.36±0.01 ^a
		90	86.62±0.33 ^a	1421±2.67 ^a	72.17±0.27 ^a	41.90±0.16 ^c	56.15±0.20 ^b	0.34±0.01 ^a
Bitter leaf	1	30	89.60±0.34 ^a	1410±2.65 ^a	82.77±0.31 ^a	63.44±0.24 ^{ab}	76.93±0.29 ^a	0.36±0.01 ^a
		60	81.42±0.31 ^a	1413±2.66 ^a	71.80±0.27 ^a	51.61±0.20 ^b	63.31±0.24 ^b	0.35±0.01 ^a
		90	73.73±0.28 ^a	1421±2.67 ^a	69.43±0.26 ^a	40.80±0.16 ^c	51.53±0.20 ^b	0.33±0.01 ^a
Tannic Acid	1	30	86.35±0.33 ^a	1413±2.66 ^a	81.44±0.31 ^a	51.65±0.20 ^b	65.28±0.25 ^b	0.33±0.01 ^a
		60	86.57±0.33 ^a	1415±2.66 ^a	72.25±0.27 ^a	49.25±0.19 ^{bc}	62.37±0.24 ^b	0.32±0.02 ^a
		90	82.86±0.31 ^a	1416±2.66 ^a	71.77±0.27 ^a	44.57±0.17 ^c	62.33±0.24 ^b	0.31±0.02 ^a
Bitter leaf	1.5	30	85.23±0.32 ^a	1419±2.67 ^a	78.20±0.30 ^a	54.69±0.21 ^b	70.18±0.27 ^a	0.35±0.01 ^a
		60	78.03±0.30 ^a	1421±2.67 ^a	64.50±0.25 ^b	44.36±0.17 ^c	59.74±0.23 ^b	0.34±0.01 ^a
		90	73.41±0.28 ^a	1470±2.77 ^a	60.78±0.23 ^b	36.20±0.14 ^c	51.30±0.20 ^b	0.32±0.01 ^a
Tannic Acid	1.5	30	82.18±0.31 ^a	1421±2.67 ^a	71.77±0.27 ^a	38.99±0.14 ^c	56.96±0.22 ^b	0.33±0.01 ^a
		60	80.53±0.31 ^a	1454±2.74 ^a	72.04±0.00 ^a	38.21±0.15 ^c	56.64±0.22 ^b	0.31±0.02 ^a
		90	80.34±0.31 ^a	1479±2.78 ^a	69.77±0.00 ^a	31.61±0.12 ^c	54.89±0.21 ^b	0.29±0.01 ^{ab}

The mean values in the same column with different superscript were significantly different (P<0.05)

Water quality parameters of varying concentrations and immersion periods of Bitter leaf extract

The water quality parameters of eggs immersed in bitter leaf extract are presented in Table 2. The lowest temperature (27.02°C) was recorded at 0.5% concentration with a 30-second immersion period, while the highest temperature (27.18°C) occurred at 1% and 1.5% concentrations with a 90-second immersion period. The lowest pH (7.05) was observed at 0.5% concentration with a 30-second immersion period, whereas the highest pH (7.18) was recorded at 1% concentration with a 90-second immersion period. Dissolved oxygen ranged from a minimum of 5.50 mg/L at 1.5% concentration with a 90-second immersion period to a maximum of 6.05 mg/L at 0.5% concentration with a 60-second immersion period. All results obtained were not significantly different (P > 0.05) from those recorded in the tannic acid solution. Similarly, the water quality parameters of eggs immersed in bitter leaf extract, tannic acid solution, and the control showed no

significant differences ($P > 0.05$). Overall, the monitored parameters remained within suitable ranges to support fish growth across all treatments.

The optimum concentration of bitter leaf extract used as a de-adhesive agent during artificial propagation of *C. gariepinus*

At the end of the experimental trial, the optimum concentration that can efficiently remove egg adhesiveness in *C. gariepinus* using bitter leaf extract was observed at a concentration of 0.65% using 3rd order polynomial regression, as shown in Figure 3

Table 2: Physico-chemical parameters of test solutions of varying concentrations and immersion periods of Bitter leaf

Rinsing agents	Concentration (%)	Immersion time(mins)	Temperature	pH	DO
Water (Control)			27.00±0.99 ^a	7.17±0.04 ^a	6.05±1.20 ^a
Bitter leaf	0.5	30	27.02±1.01 ^a	7.05±0.02 ^a	5.70±0.71 ^a
		60	27.08±0.89 ^a	7.08±0.03 ^a	6.05±1.13 ^a
		90	27.09±1.08 ^a	7.14±0.02 ^a	5.82±0.54 ^a
Tannic Acid (Reference de-adhesion agent)	0.5	30	27.04±1.00 ^a	7.08±0.04 ^a	5.65±0.64 ^a
		60	27.10±0.96 ^a	7.16±0.04 ^a	5.75±0.78 ^a
		90	27.12±0.95 ^a	7.18±0.03 ^a	5.70±0.72 ^a
Bitter leaf	1	30	27.12±0.04 ^a	7.12±0.04 ^a	5.61±0.57 ^a
		60	27.17±0.01 ^a	7.17±0.01 ^a	5.66±0.50 ^a
		90	27.18±0.04 ^a	7.18±0.04 ^a	5.75±0.77 ^a
Tannic Acid (Reference de-adhesion agent)	1	30	27.09±1.09 ^a	7.11±0.04 ^a	5.65±0.78 ^a
		60	27.17±0.90 ^a	7.18±0.06 ^a	5.60±0.57 ^a
		90	27.10±0.94 ^a	7.19±0.04 ^a	5.65±0.79 ^a
Bitter leaf	1.5	30	27.17±1.05 ^a	7.10±0.06 ^a	5.75±0.64 ^a
		60	27.10±0.94 ^a	7.12±0.04 ^a	5.85±0.92 ^a
		90	27.18±0.95 ^a	7.13±0.03 ^a	5.50±0.71 ^a
Tannic Acid (Reference de-adhesion agent)	1.5	30	27.06±0.94 ^a	7.14±0.04 ^a	5.65±0.50 ^a
		60	27.11±1.00 ^a	7.18±0.05 ^a	5.95±1.20 ^a
		90	27.13±0.80 ^a	7.21±0.01 ^a	6.00±1.27 ^a

The mean values in the same column were not significantly different ($P > 0.05$).

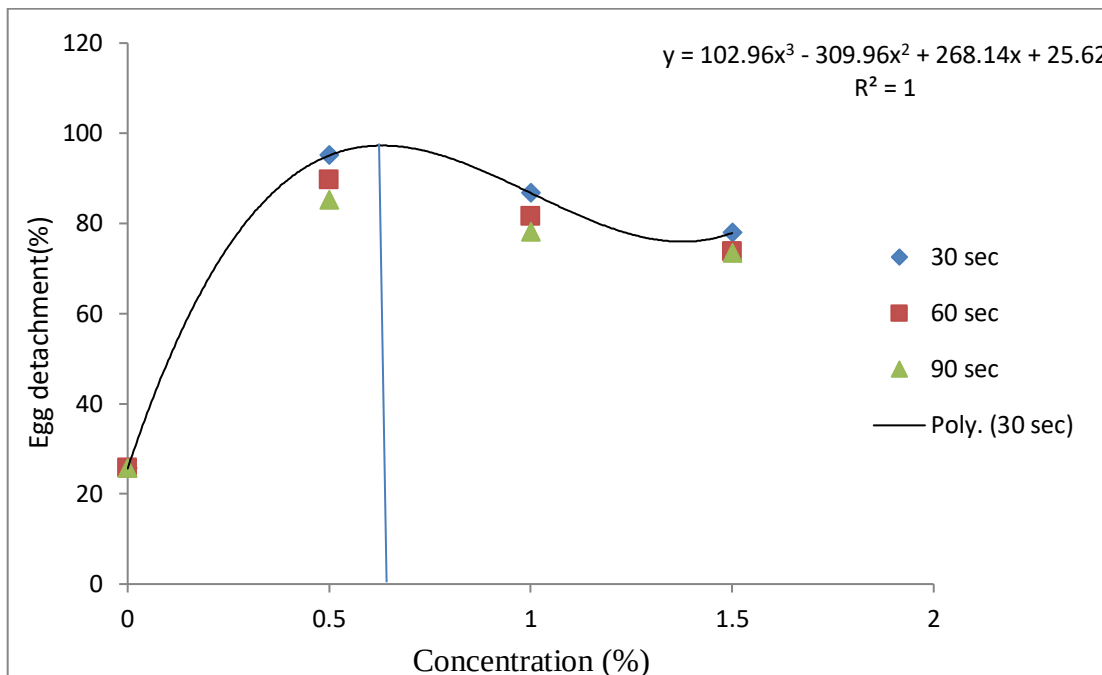


Fig. 3: The optimum concentration of bitter leaf extract used as a de-adhesive agent during artificial propagation of *C. gariepinus*

DISCUSSION

Effects of bitter leaf extract on *Clarias gariepinus* eggs.

Adhesiveness of eggs of *C. gariepinus* exposed to varying concentrations and immersion periods of bitter leaf extract

Clarias gariepinus eggs immersed in bitter leaf extract, which contains tannins as the main active compound, achieved 95.07% egg detachment. The highest effectiveness was observed at the lowest concentration (0.5%) with the shortest immersion period (30 seconds). Tannins are polyphenolic compounds known to precipitate proteins and disrupt mucopolysaccharides in the egg's adhesive layer, thereby reducing stickiness and enabling easier separation (Salisu et al., 2021). This biochemical action likely explains the rapid and efficient de-adhesion observed in the present study.

The result compares favourably with Fawehinmi et al. (2019), who reported 93.77% detachment using waterleaf extract containing tannic acid at 1% concentration with a 1-minute immersion. It also aligns with Asraf et al. (2013), who identified one minute as the optimal rinsing period for African catfish eggs using urea, and with Źarski et al. (2015), who reported high detachment rates (86.5% and 80.5%) in eggs treated with tannic acid for 1–2 minutes. However, these results contrast with Demska-Zakes et al. (2005), who found that low tannic acid concentrations combined with short immersion periods were ineffective, possibly due to insufficient exposure to initiate protein precipitation.

However, the findings suggest that bitter leaf extract is an effective natural de-adhesion agent, offering optimal fertilisation and minimal egg clumping at just 0.5% concentration with a 30-second immersion. Prolonged exposure or higher concentrations may reduce efficiency, likely due to excessive protein precipitation leading to damage or over-hardening of the chorion.

Incubation period of *C. gariepinus* exposed to varying concentrations and immersion periods of bitter leaf extract

The incubation periods of eggs exposed to bitter leaf extract did not differ significantly across treatments.

According to SRAC (2006), incubation duration is strongly influenced by temperature and exposure period. In this study, the first hatching was observed at 23 hours 50 minutes in eggs treated with 1% bitter leaf extract for 30 seconds. This finding aligns with Adebayo and Olayinka (2009), who reported the first hatching at 24.5 hours under the lowest formalin concentration, noting that longer exposure of *C. gariepinus* eggs to formalin increased hatching time. Similarly, Ayoola *et al.* (2012) reported an incubation range of 21–26 hours, which corroborates the present results.

Furthermore, incubation time in this study appeared unaffected by the relatively short immersion period. The recorded water temperature (27.00–27.18°C) falls within the optimal range of 23.89–29.44°C reported by Adebayo (2006) for *C. gariepinus* hatching, confirming that suitable thermal conditions supported normal embryonic development.

Percentage hatchability of *C. gariepinus* exposed to varying concentrations and immersion periods of bitter leaf extract

The eggs immersed in 0.5% bitter leaf extract for 30 seconds achieved the highest hatchability of 90.09%. This is consistent with Źarski *et al.* (2015), who reported peak hatching rates of 95% in pikeperch eggs treated with tannin solution for 1 minute. Similar results were reported by Thai and Ngo (2004), who achieved 86.3% hatchability using pineapple juice, while salt/urea/tannin at 1% concentration produced 70.2%. Fawehinmi *et al.* (2019) also documented about 70% hatchability in eggs immersed in waterleaf extract for 1 minute.

The superior performance of bitter leaf extract can be linked to its tannin content, which effectively removes the adhesive mucopolysaccharide coating while minimising prolonged chemical stress on the eggs. By breaking down the sticky layer quickly at a mild concentration, tannins ensure better oxygen circulation and nutrient absorption during incubation, which are critical for embryo development. This explains why hatchability peaked at the lowest concentration with the shortest immersion time.

These findings reinforce the importance of optimising both concentration and exposure time. They agree with Źarski *et al.* (2015), who stressed that the shortest possible immersion at the lowest effective concentration maximises hatching outcomes when using tannic acid. Likewise, Asraf *et al.* (2013) reported that a 1-minute rinsing period yielded high fertilisation and hatchability with minimal egg clumping, supporting the effectiveness of mild but timely treatment.

Percentage hatching index of *C. gariepinus* exposed to varying concentrations and immersion periods of bitter leaf extract

A significantly higher hatching index ($P < 0.05$) of 72.60% was obtained in the group treated with 0.5% bitter leaf extract for 30 seconds. Similarly, Zarski *et al.* (2015) reported the highest hatching index in groups exposed to 1–2 minutes of immersion in tannic acid. The hatching index represents the percentage of larvae hatched relative to the initial number of eggs incubated, thereby providing a reliable measure of the actual production efficiency of *C. gariepinus* larvae from the total eggs used.

Deformed larvae of *C. gariepinus* exposed to varying concentrations and immersion periods of bitter leaf extract

No deformities were observed in the larvae throughout the experiment. The surviving larvae were highly active and exhibited strong feeding responses. This finding is consistent with the report of Zarski *et al.* (2015), who noted that immersion period and duration did not significantly affect deformity rates in hatched larvae treated with tannic acid.

Percentage survival of *C. gariepinus* exposed to varying concentrations and immersion periods of bitter leaf extract

The highest survival rate (80.90%) was observed in eggs exposed to the lowest concentration of bitter leaf extract (0.5%) with a 30-second immersion period, while survival declined progressively with increasing extract concentrations. This outcome aligns with the findings of Akpoilih and Adebayo (2010), who reported

reduced survival rates with higher concentrations of formalin. Similarly, Ljubobratović *et al.* (2018) documented embryo survival rates of 82.5% and 87.7% in Alcalase-treated eggs and those treated with milk and kaolin, respectively, which corroborates the present study.

Larvae size of *C. gariepinus* exposed to varying concentrations and immersion periods of bitter leaf extract

The eggs immersed in 0.5% bitter leaf extract for 30 seconds produced the highest larval size (0.38 mm), whereas higher concentrations resulted in reduced larval size. This finding is consistent with Hakim *et al.* (2008), who reported increased larval growth in common carp at lower salinity levels. Conversely, Demska-Zakęś *et al.* (2005) highlighted that prolonged immersion of eggs in rinsing agents such as tannic acid could decrease egg size or even cause disruption due to osmotic pressure.

Water quality parameters of varying concentrations and immersion periods of bitter leaf extract

Water quality parameters play a crucial role in the growth, development, and survival of different stages of fish. Thus, determining the optimal range of these variables is essential for successful aquaculture practices (Marimuthu *et al.*, 2019). In this study, the recorded water temperature ranged between 27.18°C and 27.23°C. This finding aligns with Adebayo (2006), who reported an optimal hatching temperature range of 23–29°C for *Clarias gariepinus*. Similarly, Viveen *et al.* (1986) and Amaechi and Solomon (2015) suggested suitable temperature ranges of 20–30°C and 26–27°C, respectively, for *C. gariepinus* larvae, which are consistent with the present observations.

Water pH is another critical parameter influencing fish physiology, particularly in maintaining homeostasis (Marimuthu *et al.*, 2019). In the current study, pH values ranged from 7.05 to 7.21. These values are within the recommended range of 6.7–7.5 suggested by Marimuthu *et al.* (2019) for optimal hatching and larval survival of African catfish. Furthermore, Santhosh and Singh (2007) reported a broader suitable pH range of 6.7–9.5 for fish breeding, which also corresponds with the present findings.

Dissolved oxygen (DO) is equally vital for fish development, as it influences both embryonic and larval survival. The present study recorded DO levels between 5.50 mg/L and 6.05 mg/L. These values fall within the range of 4.5–8.0 mg/L reported by Bhatnagar and Sangwan (2009) as suitable for fish breeding, thereby confirming the adequacy of the water quality conditions during the experiment.

CONCLUSION AND RECOMMENDATION

This study established that 0.5% bitter leaf (*Vernonia amygdalina*) extract with a 30-second immersion period produced the best results in reducing egg adhesiveness, improving hatchability, and enhancing larval survival of *Clarias gariepinus*. While tannic acid solution at 0.5% concentration with a 60-second immersion period showed similar effectiveness, its higher cost makes it less feasible for routine hatchery application.

The findings highlight bitter leaf extract as an effective, affordable, and easily prepared natural alternative for egg de-adhesion. Its wide availability and low cost make it particularly suitable for small- and medium-scale hatchery operators who often face resource constraints. Adoption at a larger scale could help reduce dependence on synthetic chemicals, lower production costs, and improve seed availability for farmers.

From a sustainability perspective, the use of bitter leaf extract offers additional advantages. As a biodegradable, plant-based material, it presents minimal risk of harmful residues entering aquatic systems, supporting environmentally friendly aquaculture practices. Its preparation requires no specialised equipment, which increases its practicality and promotes ease of adoption across different production systems, including rural hatcheries.

It is therefore recommended that hatchery operators adopt bitter leaf extract at a 0.5% concentration with a 30-second immersion period for egg de-adhesion. Further research should, however, explore large-scale applications, long-term environmental effects, and possible integration with other hatchery management

practices. By investigating these dimensions more deeply, bitter leaf extract has the potential to contribute not only to improved hatchery efficiency but also to the broader goal of sustainable aquaculture development.

Statements And Declarations

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Competing Interest

Authors have declared that no competing interests exist. The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

Author Contributions

This work was carried out in collaboration among all authors. First Author designed the study, managed the literature searches and wrote the first draft of the manuscript. The second Author wrote the protocol. The third Author performed the statistical analysis, managed the analyses of the study, wrote the review and edited. All authors read and approved the final manuscript.

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REFERENCES

1. Abigail, B. W., Ward, D., Duclos, K., Peters, M. and Berlinsky, D.L., (2010). Surface disinfection and removal of adhesiveness from rainbow smelt eggs. *North American Journal of Aquaculture*, 72: 158-163.
2. Adebayo, O.T. (2006). Reproductive performance of African Clariid Catfish *Clarias gariepinus* broodstocks on varying maternal stress. *Journal of Fisheries International*, 1 (1-2): 17-20.
3. Adebayo, O.T. and Olarewaju, J.O. (2000). Reproductive performance of African Catfish, *Heterobranchus bidorsalis* under different feeding regimes. *Proceedings of the 6th International Symposium on Reproductive Physiology of Fish*, 4-9
4. Adebayo, O.T. and Olayinka, S.O. (2009). Efficacy of formalin in the removal of adhesiveness from *Clarias gariepinus* eggs during artificial propagation. In: 24th Annual Conference of the Fisheries Society of Nigeria (FISON), Akure, Nigeria, pp. 143-147.
5. Akpoilih B.U. and Adebayo, O.T. (2010). Effect of Formalin on the Hatching Rate of Eggs and Survival of Larvae of the African Catfish *Clarias gariepinus*. *Journal of Applied Science Environment*, 14 (4): 31-34
6. Al Hazaa R. and Hussein A. (2003) Stickiness elimination of Himri Barbel (*Barbus luteus*, Heckel) eggs. *Turkish Journal of Fisheries and Aquatic Sciences* 3: 47–50.
7. Amaechi, C. and Solomon, J.R. (2015). Calculation of the physiochemical parameters of catfish (*Clarias gariepinus*) fed locally formulated feeds (earthworm). *International Journal of bioassays*, 4(16):3941-3947.
8. APHA, (1987). *Standard Method for the Examination Water and Wastewater*. 17th ed. Washington D.C. Pp.1268.
9. Asraf, A., Muchlisin Z.A. and Siti-Azizah. M.N. (2013). Removal of eggs adhesiveness of African catfish *Clarias gariepinus* at different concentrations of urea solution. *Aceh International Journal of Science and Technology*, 2(3): 94-97.

10. Ayoola, S.O, Kuton, M.P and Chukwu, S.C. (2012). Comparative study of piscine and non-piscine pituitary extract and ovulin for inducing spawning in catfish (*Clarias gariepinus*). African Journal of Food, Aquaculture, Nutrition and Development, 12 (6): pp 6809-6822.
11. Ben Khemis I. B, Hamza N, Messaoud N. B, Rached S. B and M’Hetli M (2014) Comparative study of pikeperch *Sander lucioperca* (Percidae; Linnaeus, 1758) eggs and larvae from wild females or from captive females fed chopped marine fish. Fish Physiology Biochemistry 40:375–384.
12. Bhatnagar A. and Sangwan, P. (2009). Impact of Mass Bathing on Water Quality, International Journal of Environment Research, 3 (2):247-252.
13. Dada, A.A and Fagbenro, O.A (2008). Catfish fingerlings production in Nigeria. Proceeding 4th Annual Conference School Agric. Agric. Technology. The Federal University of Technology, Akure pp. 107-110.
14. Demska-Zakes K., Zakes, Z. and Roszuk, J. (2005). The use of tannic acid to remove adhesiveness from pikeperch, *Sander lucioperca*, eggs. Aquaculture Research, 36: 1458–1464.
15. El-Gamal, A.H.E. and El-Greisy, Z.A. (2008). Effect of removal of egg adhesiveness on hatchability and effect of different levels of salinity on survival and larval development in common carp, *Cyprinus carpio*. Journal of Applied Sciences Research, 4(12): 1935-1945.
16. Fagbenro, O.A., Adeparusi, E.O. and Fapounda, O.O. (2003). Feedstuffs and dietary substitution for the farmed fish in Nigeria. In; National Workshop on fish Feed Development and Feeding Practices in Aquaculture Organized by FISON in collaboration with NIFFR and FAO Special Programme on Food Security (SPFS), New Bussa, 15th-19th Sept. Pp 60-72.
17. FAO, (1996). Artificial Reproduction and Pond Rearing of the African Catfish *Clarias gariepinus* in Sub-Saharan Africa- A Handbook. Rome: Paper 362
18. FAO, (2018). The state of world fisheries and aquaculture. Meeting the Sustainable Development Goals. Rome. Licence: CC BY-NC-SA 3.0 IGO
19. Fawehinmi S. J., O. T. Adebayo and O. K. Gbadamosi (2019). Efficacy of Aloe vera Gel and Water-leaf Extracts for Removal of Egg Adhesiveness during Artificial Propagation of African Catfish (*Clarias gariepinus*, Burchell 1822), Asian Journal of Fisheries and Aquatic Research 3(3): 1-13.
20. Hakim A.E., El-Gamal and Zeinab A. El-Greisy (2008). Effect of Removal of Egg Adhesiveness on Hatchability and Effect of Different Levels of Salinity on Survival and Larval Development in Common Carp, *Cyprinus Carpio*. Journal of Applied Sciences Research, 4(12): 1935-1945
21. Křišťan J, Blecha and M, Policar T (2017) Alcalase treatment for elimination of stickiness in pikeperch (*Sander lucioperca* L.) eggs under controlled conditions. Aquaculture Resources 47:3998–4003.
22. Kwikiriza, G., Tebitendwa, S.M., Rwezawula, P., Mwanja, W.W., Abaho, I. and Meimberg, H., 2025. Enhancing African Catfish (*Clarias gariepinus*) Aquaculture in Uganda: Insights into Hatchery Propagation, Population Suitability, and Broodstock Management. Fishes, 10(6), p.290.
23. Ljubobratović U, Péter G, Horváth Z, Demény F and Rónyai A (2018) HANDBOOK – State-of-the-art in zander (*Sander lucioperca*) artificial reproduction at NAIK HAKI institute. In: Poleksić V, Marković Z (eds) Proceedings of the VIIIth International conference “Water and Fish”. University of Belgrade - Faculty of Agriculture, Belgrade-Zemun, 500–517
24. Marimuthu K., H. Palaniandy and Z. A. Muchlisin (2019) Effect of different water pH on hatching and survival rates of African catfish *Clarias gariepinus* (Pisces: Clariidae) Journal of Animal Science 4(2): 80-88
25. Marteinsdottir, G. and Steinarsson, A. (1998). Maternal influence on the size and viability of Iceland cod *Gadus morhua* eggs and larvae. Journal of Fish Biology, 52: 1241-1258.
26. Muchlisin, Z.A., Nadiya, N., Nadiyah, W.N., Musman, M. and Siti-Azizah, M.N. (2010) Preliminary study on the natural extenders for artificial breeding of African catfish *Clarias gariepinus* (Burchell, 1822). AACL Bioflux 3: 119-124
27. Nwachukwu, E. C, Nulit, R., and Rusea, Go. (2014). Nutritional and biochemical properties of Malaysian okra variety. Advancement in Medicinal Plant Research, 2(1): 16-19.
28. Ojebuola, T. O., Adebayo, O. T., and Gbadamosi, O. K. (2024). Efficacy of Okra Leaf [*Abelmoschus esculentus* L.] Extract for Removal of Egg Adhesiveness During Artificial Propagation of African Catfish [*Clarias gariepinus*, Burchell 1822]. Asian Journal of Fisheries and Aquatic Research, 26(7), 12–24. <https://doi.org/10.9734/ajfar/2024/v26i7780>

29. Oyelese, O.A. (2006). Water temperature a determinant of fertilization and hatchability rate in artificially induced breeding of *Clarias gariepinus* (Teleostei: Clariid). Resource Journal of Biological Science, 1(1-4): 87.
30. Rasband WS (1997–2011) ImageJ. U.S. National Institutes of Health, Bethesda, Maryland, USA
31. Rasowo, J., Okoth, O.E. and Ngugi, C.C. (2007). Effects of formaldehyde, sodium chloride, potassium permanganate and hydrogen peroxide on hatch rate of African catfish *Clarias gariepinus* eggs. Aquaculture, 269: 271–277.
32. Salisu, A., Umar, B. I., Usman, A. U., Muhammad, M. N., Yakubu, M. S., & Yusuf, F. M. (2021). Preliminary Study on the Effect of Watermelon Juice and Milk Solution on De-Adhesion, Developmental Stages and Hatching Rate of Eggs and Survival of Fry of *Clarias gariepinus*. FUDMA Journal of Sciences, 5(1), 314-318.
33. Santhosh, B. and Singh, N.P. (2007). Guidelines for water quality management for fish culture in Tripura, ICAR Research Complex for NEH Region, Tripura Center, Publication no.29.
34. Southern Regional Agriculture Center and the Texas Aquaculture Extension Service (2006).
35. Thai, B.T and Ngo, T.G., (2004). Use of pineapple juice for elimination of egg stickiness of common carp (*Cyprinus carpio*). Asian Fisheries Science, 17: 159-162.
36. Usunobun, U. and Ngozi, O. (2016). Phytochemical analysis and proximate composition of *Vernonia amygdalina*. International Journal of Scientific World, 4(1): 11.
37. Viveen, C. (1986). Practical manual for culture of the African catfish *Clarias gariepinus*, The Netherlands Ministry of Development Co-operation, Selection for Research and Technology. p93.
38. Wachiracaikarn A., Rungsin, W., Srisapoome, P. and Na-Nakorn, U. (2009). Crossing of African Catfish (*Clarias gariepinus*) strains based on strain selection using genetic diversity data. Aquaculture, 290: 53-60.
39. Źarski D, Krejszeff S, Kucharzyk D, Palińska K, Targońska K, Kupren K, Fontaine P, Kestemont P (2015) The application of tannic acid to the elimination of egg stickiness at varied moments of the egg swelling process in pikeperch, *Sander lucioperca* (L.). Aquaculture Resource 46:324–334.