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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Research Article

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Abstract

The best immersion period and concentration of bitter leaf plant extract that can efficiently remove egg adhesiveness of Clarias gariepinus was assessed. One male and a female C. gariepinus broodstock weighing 1.3kg and 1.4kg respectively were used for the breeding. Three different concentrations comprising (0.5, 1 and 1.5) % of bitter leaf extract were tested. Tannic acid of 0.75g diluted into one liter of water was used as reference de-adhesion agent while water without solution or extract was used as control. The fish eggs were rinsed with the solutions at different durations of 30, 60, and 90 seconds. Each concentration and rinsing time were recorded in triplicates. Data generated were subjected to Analysis of Variance test, Tukey multiple range tests was used as a follow up procedure. Third order polynomial regression analysis was used to determine the best concentration and immersion period that neutralizes adhesiveness in eggs of C. gariepinus. The result of the study showed that there were no significant differences (p = 0.05) in the non-adhesive egg, percentage hatchability of eggs immersed in bitter leaf plant extract and tannic acid solution. The use of bitter leaf extract at the lowest concentration of 0.5% and 30 seconds immersion period showed great de-adhesion efficiency which gave highest percentage fertility, non-adhesive eggs and percentage hatchability of 97.40%, 95.07% and 90.09% respectively.

Introduction

Aquaculture has evolved as the quickest growing sector of agriculture in the world (FAO, 2018). World production including aquatic plants in 2016 was 110.2 million tonnes, with the first-sale worth calculable at USD 243.5 billion (FAO, 2018). Development of fish seeds production has been known as a rational means of augmenting the dwindling fish provision from the capture fisheries (Dada and Fagbenro, 2008). The African catfish Clarias gariepinus is acknowledged for its favourable food conversion, resistance to diseases, low technology farming system, wonderful food meat quality (Fagbenro, et al., 2003). It additionally has high feed potency and utilization and may be simply used for breeding (Adebayo and Olaneewaju, 2000; Rasowo et al., 2007; Wachirachaikarn et al., 2009). Adebayo (2006) reported that fish culture these days is hardly possible while not the substitute or semi artificial mass propagation of fish seeds of cultured fish species.

However, there are some issues in production of African catfish larvae. One among the many issues is low hatching and survival rates (Muchlisin et al., 2010). This drawback might be because of the eggs, the eggs clumped together when they are discharged into the water, and therefore leading to low fertilization and hatching rates (El-Gamal and El-Greisy, 2008). Additionally, when eggs clumped together, it causes high larval mortality (Abigail et al., 2010). One among the ways to deal with this problem is to rinse the eggs with certain solutions. For instance, tannin was used in pikeperch (Sander lucioperca L.) at 0.75g/L best result was obtained in group of eggs submerged in one and two minutes (86.5% and 80.5% of larvae were obtained respectively) (Zarski et al., 2015), Alcalase enzyme treatment for elimination of adhesiveness in pikeperch, (Sander lucioperca L.) was done (Kristan et al., 2017), Alcalase enzyme solution was used for total elimination of stickiness in pikeperch (Sander lucioperca) (Ljubobratović et al., 2018), Aloe Vera gel and Water leaf extracts has been used with immersion period of five minutes and one minutes respectively for African catfish Clarias gariepinus (Fawehinmi et al., 2019).

Bitter leaf (Vernonia amygdalina) is a readily available plant with very little or no cost and contain several antioxidants, polysaccharides, minerals, proteins, enzymes, vitamins (Nwachukwu et al., 2014). There is dearth of data using this plant on the removal of adhesiveness of African catfish eggs. There is a need for a study to find the best solution to eliminate the stickiness of the African catfish eggs and increase the fertilization and hatching rates. Therefore, this study focused on the efficacy of bitter leaf extract on optimum concentrations and immersion period that efficiently remove egg adhesiveness in Clarias gariepinus.

Materials And Methods

Study Area and brood fish

The experiment was carried out in the experimental section at the Teaching and Research Fish Farm of The Federal University of Technology, Akure; located at Obakekere, Akure. Apparently healthy male and female C. gariepinus weighing 1.3kg and 1.4kg respectively were obtained from a reputable fish farm in Akure and were stocked into separate holding tanks (40 x 30 x 35) cm³ containing aerated for five days. During this five-day acclimatization period, they were fed with local commercial diet until 24 hours before the artificial induction 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

Bitter leaves plucked without the stem were thoroughly washed under a running tap water, extracts was squeezed out manually by hand and then filtered using a hand net (mesh size 1mm). The greenish aqueous extract was stored in a dry, clean air tight transparent plastic container and labelled prior to use. It was done before stripping to ensure the quality of the extract.
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 reference de-adhesion agent was prepared by diluting 0.75g of tannic acid into one litres 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**

Female brooder was injected with hormone (Ovaprim Syndel laboratories Ltd., Nanaimo, BC Canada V9S 4M9) at 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 perforated lid. After a latency period of 12 hours, slight pressure was applied on the abdominal cavity to express the eggs inside a clean bowl. The male testes was removed by abdominal dissection and cleaned with a towel, the milt was gently squeezed out and collected in a beaker.

**Fertilization And Immersion**

Wet fertilization was used in the experiment. Milt collected was then mixed with small quantity of saline solution. 1g of the striped eggs was carefully weighed on nylon and each measured eggs was fertilized 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 under the treatments.

**Experimental Design**

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

The fertilized eggs were placed in three treatment concentration 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 to 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 were decanted, then clean water was replaced to incubate the eggs in the spawning bowls.

**Evaluation Of Fertility, Non-adhesive Eggs, Survival And Deformity Indices**

To determine the efficacy and efficiency of bitter leaf extract in removing egg adhesiveness, percent fertility, ratio of non-adhesive eggs, hatching and survival were computed according to the method described by Adebayo (2006).

\[
\text{Percent fertility} = \frac{\text{Number of fertilized eggs}}{\text{Total number of eggs counted}} \times 100
\]

\[
\text{Ratio of Non-adhesive eggs } (\%) = \frac{\text{numberofnon-adhesiveeggs}}{\text{initial numberofeggs}} \times 100
\]

\[
\text{Hatchability } (\%) = \frac{\text{Number of egg hatched}}{\text{Total number of eggs incubated}} \times 100
\]

\[
\text{Survival (} \%) = \frac{\text{numberofhatchlingat72h}}{\text{Total numberofhatchlingat0h}} \times 100
\]

**Hatching Index**

According to Żarski et al., (2015), based on 72-h embryo survival of incubation and based on the hatching rate, a hatching index (HI) was determined. HI was calculated based on the below formula:
HI = (S1 S2) 100-1,
Where S1 – survival 72-h embryo survival (%),
S2 – hatching rate (%).

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) × 100

Additionally, the total length of the larvae 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 mercury-in-glass thermometer (YSI-DO 550, U.S.A), pH meter (Hanna H198106 model) and 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 Analysis of Variance test. Also, Tukey Honestly significant different 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 0.05 significance level.

**Results**

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

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

The result of the eggs of *C. gariepinus* exposed to varying concentrations and immersion periods of bitter leaf are shown in Table 1. The fertility of eggs immersed in bitter leaf extract ranged from 94.76% at 1.5% concentration (90 seconds) to 98.13% at 0.5% concentration (60 seconds). The eggs immersed in tannic acid solution had fertility which varied from 94.58% at 1.5% (90 seconds) to 98.04% at 0.5% concentration with the immersion period of 90 seconds. Also, 92.80% fertility was recorded in control. Percentage fertility was high across different concentrations and immersion periods, hence, there were no significant difference (P>0.05) in fertility between concentrations and immersion periods of eggs immersed in bitter leaf solution, tannic acid solution with control inclusive.

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

Result of egg adhesiveness of *C. gariepinus* exposed to varying concentrations and immersion periods of bitter leaf extract is shown in Table 1. The percentage of non-adhesive eggs (95.07%) was highest at 0.5% concentration with 30 seconds immersion period and lowest (73.41%) in 1.5% concentration with 90 seconds immersion period. The eggs of *C. gariepinus* exposed to varying concentrations and immersion periods of tannic acid solution had non-adhesive eggs which varied from 80.34% at 1.5% (90 seconds) and 92.52% at 0.5% with the immersion period of 60 seconds. The eggs immersed in the control group had 25.62% non-adhesive eggs. The percentage non-adhesive eggs exposed to bitter leaf and tannic acid solution were not significantly different (p>0.05) from one another compared to that of the control. Detachment of eggs reduced in bitter leaf and tannic acid solutions with increasing concentration and immersion periods.

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

Incubation period of eggs immersed in bitter leaf extract varied between 23hours 50minutes at 1% concentration (30 seconds) to 24hours 50minutes at 1.5% concentration (90 seconds) as shown in Table 1, incubation period of eggs immersed in tannic acid solution varied from 23hours 53minutes at 0.5% concentrations with immersion period of 30 seconds to 24hours 65minutes at 1.5% concentration (90 seconds) while incubation period of the eggs immersed in water was 25hours 73minutes. Hence, the incubation period of the eggs immersed in bitter leaf extract and tannic acid solution were not significantly different (p>0.05) from one another compared to that of the control.

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

The percentage hatchability decreases with increasing concentrations of bitter leaf extract as shown in Table 1. The highest percentage hatchability (90.09%) was recorded in the group exposed to 0.5% concentration of bitter leaf extract with 30 seconds immersion period, percentage egg hatchability of (85.07%) was recorded in group exposed to 0.5% concentration of tannic acid solution with 30 seconds immersion period. Percentage hatchability recorded in 0.5% concentration of bitter leaf extract and tannic acid solution were high and not significantly different (p>0.05) but were significantly different (p<0.05) from that of the control with (43.24%).

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

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The percentage hatching index decreases with increasing concentrations of bitter leaf extract in relation with the hatchability as shown in Table 1. The lowest percentage hatching index (9.55%) was recorded in the control while the highest hatching index (72.60%) was recorded in the group exposed to 0.5% concentration of bitter leaf extract with 30 seconds immersion period while hatching index (57.14%) was recorded in group exposed to 0.5% of tannic acid solution with 30 seconds immersion period. However, hatching index recorded in lower concentration 0.5% with 30 seconds immersion periods of bitter leaf extract was significantly different ($p<0.05$) from the control and other rinsing agents at varying concentrations and immersion periods.

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 survived larvae percentage showed that survival decreased as concentration and immersion period increases as shown in Table 1. 80.90% was the highest and this was observed in 0.5% concentration with 30 seconds immersion period and lowest (51.30%) 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 significant difference ($p<0.05$) between highest larvae survival (80.90%) recorded in 0.5% concentration with 30 seconds 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 control which is the least size compared to other larvae size 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.
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 result of water quality parameters of eggs immersed in bitter leaf extract are shown in Table 2. The lowest temperature of 27.02°C was recorded at 0.5% concentration with immersion period of 30 seconds while the highest temperature of 27.18°C was recorded at 1% concentration with 90 seconds immersion period. Lowest pH of 7.05 was recorded at 0.5% concentration with immersion period of 30 seconds while the highest pH of 7.18 was recorded at 1% concentration with immersion period of 90 seconds. All results observed were not significantly different from those observed in tannic acid solution. The water quality parameters of the eggs immersed in bitter leaf extracts, tannic acid solution with the control were not significantly different (P > 0.05). The water quality parameters monitored in the course of this study were adequate for fish growth in each treatment.

**The optimum concentration of bitter leaf extract used as 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 concentration of 0.65% using 3rd order polynomial regression as shown in Figure 1.

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

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

**Discussion**
50 minutes at 1% concentration (30 seconds) The study corroborates with findings of Adebayo and Olayinka (period is directly affected by temperature and exposure period. Incubation period of eggs immersed in bitter leaf extract has first hatching at 23 hours. The incubation periods were not significantly different from one another in the eggs exposed to bitter leaf extract. According to SRAC, (2006), incubation rate as detachment of eggs reduces with increasing concentration. Hence, Bitter leaf extract can be used for egg de-adhesion in shortest immersion period of 30 seconds to give the highest fertilization and lowest clumping rate as detachment of eggs reduces with increasing concentration.

### Table 1: Effects of bitter leaf extract on Clarias gariepinus eggs.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Immersion time(mins)</th>
<th>Temperature</th>
<th>pH</th>
<th>DO</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>30</td>
<td>27.00±0.99(^a)</td>
<td>7.17±0.04(^a)</td>
<td>6.05±1.20(^a)</td>
</tr>
<tr>
<td>90</td>
<td>60</td>
<td>27.02±1.01(^a)</td>
<td>7.05±0.02(^a)</td>
<td>5.70±0.71(^a)</td>
</tr>
<tr>
<td>60</td>
<td>90</td>
<td>27.08±0.89(^a)</td>
<td>7.08±0.03(^a)</td>
<td>6.05±1.13(^a)</td>
</tr>
<tr>
<td>90</td>
<td>60</td>
<td>27.09±1.08(^a)</td>
<td>7.14±0.02(^a)</td>
<td>5.82±0.54(^a)</td>
</tr>
</tbody>
</table>

The quality of eggs and sperm are two of the important factors in a breeding program as there are several factors that influence the production and quality of seed quality of broodstock (Marteinsdottir and Steinarsson, 1998; Al-Hazzaa and Hussein, 2003), the quality of eggs and sperms in this study was optimal and therefore resulted in high fertilization.

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

*C. gariepinus* eggs immersed in bitter leaf extract which contains tannin as an active ingredient gave (95.07%) egg detachment, this was effective at the lowest concentration of 0.5% with the shortest immersion period of 30 seconds which compared favorably with the study of Fawehinmi et al.; (2019), who reported that waterleaf extract which contains tannin as an active ingredient gave 93.77% which was effective at the lowest concentration of 1% with the shortest immersion period of 1 minute. It was similar as well with the studies of Asraf et al. (2013) who reported that the optimal time needed to rinse African cat fish eggs was one minute with urea. Žarski et al., (2015) also reported best result in groups of eggs submerged in tannic acid solution for 1 and 2 min (86.5% and 80.5%) respectively. This is in contrast to Demska-Zakes et al., (2005) who reported that the application of low concentration of tannic acid solution for a short exposure period is not effective to reduced egg stickiness.

Hence, Bitter leaf extract can be used for egg de-adhesion in shortest immersion period of 30 seconds to give the highest fertilization and lowest clumping rate as detachment of eggs reduces with increasing concentration.

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

The incubation periods were not significantly different from one another in the eggs exposed to bitter leaf extract. According to SRAC, (2006), incubation period is directly affected by temperature and exposure period. Incubation period of eggs immersed in bitter leaf extract has first hatching at 23 hours 50 minutes at 1% concentration (30 seconds) The study corroborates with findings of Adebayo and Olayinka (2009) who reported that the first hatching
after fertilization was 24.5hrs in lowest formalin treatment concentration, he stated that the more the exposure period of *C. gariepinus* eggs to Formalin, the higher the hatching time. Similarly, a research conducted by Ayoola et al., (2012) reported incubation period of 21–26 hours.

However, incubation period was not affected due to the relatively exposure time. 27.00°C to 27.18°C recorded in this study falls within the temperature range reported by Adebayo (2006) who stated that the best temperature for *C. gariepinus* hatching is ranged between of 23.89 °C -29.44°C.

**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 at immersion period of 30 seconds gave the highest hatchability of 90.09%. This result is similar to the findings of Zarski *et al.* (2015) who recorded highest hatching rate of 95% in application of tannin solution with immersion period of 1 min for pikeperch eggs. Thai and Ngo (2004) reported the highest hatching rate of 86.3% in pineapple juice and the hatching rate of 70.2% in salt/urea/tannin with 1% concentration. Fawehinmi et al. (2019) also reported that eggs immersed in waterleaf extract at immersion period of 1 minute gave the highest hatchability of about 70%.

Thus, the best immersion period needed to rinse African catfish eggs when using bitter leaf extract is 30 seconds which is as well similar to Zarski *et al.*, (2015) who opined it is crucial to apply the shortest possible immersion in a tannic acid at the lowest possible concentration. Also Asraf et al., (2013) stated that the optimal time needed to rinse the African catfish eggs was one minute because fertilization and hatching rates were high and clumping rate was lowest when the eggs were rinsed for 1 minute.

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

Highest survival rate (80.90%) was recorded in the group exposed to lowest concentration 0.5% of bitter leaf extract with 30 seconds immersion period. Zarski *et al.*, (2015) recorded highest hatching index in groups subjected to 1 and 2 minutes immersion in tannic acid. This index represented the percentage (%) of the hatched larvae obtained from the initial number of eggs. It provided data at real production of *C. gariepinus* larvae from total number of eggs which were initially used for incubation Zarski *et al.*, (2015).

**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. This corroborate with findings of Zarski *et al.*, (2015) who recorded that both the periods and the immersion duration did not have an effect on the deformity rate in the hatched larvae when immersed in tannic acid.

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

The eggs immersed in bitter leaf extract at 0.5% concentration of bitter leaf extract with 30 seconds immersion period obtained highest larvae size (0.38mm) while at highest concentration, the larvae size reduced. Hakim et al., (2008) observed an increased length (mm) in growth of common carp at lowest concentration in different levels of salinity which is in line with this study. However, Demska-Zakęs *et al.*, (2005) opined that when eggs are immersed too long in the rinsing agent (tannic acid), the egg size decreases or the eggs may even disrupt due to the osmotic pressure.

**Water Quality Parameters Of Varying Concentrations And Immersion Periods Of Bitter Leaf Extract**

Water quality parameters influence the growth and survival of different developmental stages of fishes and hence determining the optimal water quality variables is greatly important for any aquaculture farming (Marimuthu et al., 2019). The temperature observed during this study ranged between 27.18°C – 27.23°C, this agrees with work of Adebayo (2006) who reported that the best temperature for *C. gariepinus* hatching is between the ranges of 23°C-29°C. Similarly, Viveen *et al.*, (1986), Amaechi and Solomon (2015) reported a suitable temperature range of 20°C- 30°C and 26-27°C respectively for *C. gariepinus* larvae which was in line with the present study.

Water pH is considered the key factors and plays an important role in the maintenance of the homeostasis in fishes (Marimuthu et al., 2019). The result of Marimuthu et al., (2019) who recommended a water pH level of 6.7–7.5 for ideal hatching and the greatest larval survival of African catfish is in agreement with this present study in which pH ranged between 7.05–7.21. Santhosh and Singh (2007) which reported that suitable pH range for fish breeding ranged between of 6.7–9.5 which was in line with this present study.

Bhatnagar and Sangwan (2009) who reported that dissolved oxygen in the range of 4.5-8.0mg/l was suitable for fish breeding corroborate with this study which falls within the range between 5.50mg/l- 6.05mg/l.

**Conclusion And Recommendation**
This present study revealed that 0.5% of bitter leaf extract with 30 seconds immersion period gave the highest fertilization, lowest sticky rate, highest hatchability and survival of *C. gariepinus*. However, tannic acid solution at 0.5% concentration with 60 seconds immersion period was also optimum for detachment of *C. gariepinus* eggs without affecting the fertilization, incubation period, hatchability and percentage survival rate. The use of bitter leaf extracts at lowest concentration and immersion period was not significantly different from the use of tannic acid solution.

Therefore, the use of 0.5% of bitter leaf extract with 30 seconds immersion period is recommended to fish hatcheries operators because of its effective, quick and simple technology. The plant source is cheap, affordable and requires less time. Although, tannic acid which served as the reference de-adhesion agent was not significantly different from result gotten from bitter leaf extract but it is more expensive. Hence, Bitter leaf extract is recommended for its efficacy, efficiency, cost effectiveness, availability, handling and uncomplicated processing.

**Declarations**

**DECLARATION OF 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.”

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**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. Second Author wrote the protocol. Third Author performed the statistical analysis, managed the analyses of the study, writing review and editing. All authors read and approved the final manuscript.

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**References**


**Figures**
Figure 1

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