Original Research Article

Efficacy of Okra Leaf (*Abelmoschus esculentus L.*) Extract for Removal of Egg Adhesiveness during Artificial Propagation of African Catfish (*Clarias gariepinus*, Burchel 1822).

ABSTRACT

The best immersion period and concentration of okra leaf plant extract that can efficiently remove egg adhesiveness of *Clarias gariepinus*were assessed in this study. One male and a female *C. gariepinus* broodstock weighing 1.3 kg and 1.4 kg respectively were used for the breeding.Three different concentrations comprising 1%, 3%, and 5% of okra leaf extract were used. Tannic acid of 0.75 g in one litre of water was used as a reference de-adhesion agent, while water without no tannic acid or plant extract was used as a control. The fish eggs were rinsed with the solutions at different durations of 1, 3, and 5 minutes. Each concentration and rinsing time was recorded in triplicates. Data generated were subjected to an Analysis of Variance Test, third order Polynomial regression analysis was then used to determine the best concentration and immersion period that removed egg adhesiveness of *C. gariepinus*. The results of the study showed that the use of okra leaf extract at 1% lowest concentration and 1 minute immersion period was not significantly different ($p>0.05$) from the use of tannic acid solution. Therefore, this study showed that okra leaf extract at 1% concentration with 1minute immersion period can efficiently remove egg adhesiveness and increase hatching in *C. gariepinus.*

Keywords: *Okra leaf, Clarias gariepinus, Egg adhesiveness, Non-adhesiveness, Hatching*

1. INTRODUCTION

Aquaculture, or aqua farming, involves raising aquatic species such as fish, crustaceans, mollusks, and aquatic plants. In 2016, aquaculture production exceeded that of capture fisheries, accounting for about 53% of global fish output (Boyd et al., 2022). In 2020, global aquaculture production had reached a record 122.6 million tonnes, valued at USD 281.5 billion (FAO, 2022). In Nigeria, fish demand greatly surpasses local production, as the country is the largest fish consumer in Africa and ranks among the top fish consumers globally (Nwuba et al., 2022).

The most commonly farmed fish species in Nigeria and other parts of Africa belong to the catfish family, Clariidae. Among these, the African catfish, *Clarias gariepinus*, and Heterobranchus species are the most extensively cultured (Oluwalola et al., 2019). *Clarias gariepinus* is an important food source for both low-income and wealthy families worldwide (Langi et al., 2024). This species is known for its efficient food conversion ratio, disease resistance, suitability for low-technology farming systems, and high-quality meat (Fagbenro et al., 2003). It also has high feed utilization efficiency and can be easily bred in hatcheries (Adebayo and Olanrewaju, 2000; Rasowo et al., 2007; Wachirachaikarn et al., 2009). According to Adebayo (2006), modern fish farming heavily relies on the artificial or semi-artificial mass propagation of fish seeds. Ojutiku (2008) identified the scarcity of broodstock for this popular species as a major barrier to the rapid development of fish farming in Nigeria. Additionally, Atanda (2006) noted that fish farmers across much of the country, particularly in the northern regions, consistently need hatchery-produced fish seeds for their operations. Thus, the growth and success of fish farming in Nigeria depend on the production of large quantities of high-quality fry for stocking in ponds, cages, and pens (Charo and Orirere, 2000).

However, there are challenges in the artificial production of African catfish larvae, particularly concerning the low hatching and survival rates of their eggs (Muchlisin et al., 2010). The eggs tend to become adhesive and clump together when released into the water, resulting in reduced fertilization and hatching rates (El-Gamal and El-Greisy, 2008). This adhesiveness also contributes to high larval mortality (Abigail et al., 2010). To address this issue, rinsing the eggs with various solutions, such as urea, mud, milk, or kaolin, has been proposed. These solutions can be species-specific and may affect the eggs differently based on their composition (Muchlisin et al., 2014). Therefore, extensive research is needed to identify the most effective solution for reducing the stickiness of African catfish eggs, thereby improving fertilization and hatching rates.

Okra, a tall dicotyledonous plant related to cotton, cocoa, and Hibiscus, can be either annual or perennial and grows to about 2 meters in height. A study by Caluête et al. (2015) revealed that okra leaves contain phytochemicals such as lectin, tannin, and total phenolic compounds, along with calcium, magnesium, and potassium. Tannin, in particular, has been identified as effective in reducing the adhesiveness of *Clarias gariepinus* eggs (Fawehinmi et al., 2019). Therefore, the study was conducted to evaluate the effectiveness of okra leaf extract at various concentrations and immersion periods in removing the adhesiveness of *Clarias gariepinus* eggs.

2. MATERIALS AND METHODS

2.1 Study Zone and Holding Facility

The experiment was conducted at the Teaching and Research Fish Farm of The Federal University of Technology, Akure, located in Obakekere, Akure. Healthy male and female *Clarias gariepinus*, weighing 1.3 kg and 1.4 kg respectively, were sourced from a reputable fish farm in Akure before the experiment began. The selected broodstock were housed in separate holding tanks (40 x 30 x 35 cm^3) with aerated water at the research farm, where they were acclimatized and fed a commercial diet for five days prior to the exposure period. The brooders were then starved for 24 hours before the breeding exercise commenced.

2.2 Plants collection and identification

Fresh okra leaves were collected from within the Teaching and Research Fish Farm at The Federal University of Technology, Akure. These leaves were identified as *Abelmoschus esculentus L.* at the Herbarium of the Department of Crop, Soil, and Pest Management, The Federal University of Technology, Akure.

2.3 Preparation of Okra Leaf Extract

Okra leaves, plucked without stems, were thoroughly washed under running tap water. The leaves were then hand-squeezed to obtain the extract, which was subsequently filtered through a hand net with a 1 mm mesh size. The greenish extract was collected and stored in a dry, clean, airtight transparent plastic container and labelled for use on the same day. To ensure quality, both leaf collection and extract preparation were conducted early in the morning, just before the strip spawning of the brood fish. Different concentrations of the extract were prepared as follows:

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

3% = 3.0ml of Okra leaf extract in 97ml of water.

5% = 5.0ml of Okra leaf extract in 95ml of water.

2.4 Preparation of Tannic acid Solution (Reference de-adhesion agent)

A tannic acid solution, which served as the reference de-adhesion agent, was prepared by diluting 0.75 g of tannic acid in one liter of water, following the method described by Żarski et al. (2015). Water without tannic acid or extract was used as the control.

2.5 Preparation of Spawning Bowls

Fifty-seven spawning bowls of 4 litre capacity used for the experiment were procured, thoroughly washed and dried first. Then, they were labelledaccording to the inclusion levels of the treatments, tannic acid (1%, 3% and 5%), and control as well as the immersion periods (1 minute, 3minutes and 5minutes). The bowls were filled with 100ml of water (control), 99ml of water (1%), 97ml of water (3%) and 95ml of water (5%) respectively.

2.6 Milt and Egg Collection

For artificial induction of ovulation, the female brooder was injected with ovaprim at angle 45° with the needle pointing towards the gonad region. The injected female was kept inside separate plastic tanks, tightly covered with a perforated lid. After a latency period of 12 hours, slight pressure was applied to abdomen and the eggs inside a clean bowl. The male ~~testes~~ testiswas removed by abdominal dissection, cleaned with a towel. Then, milt was gently squeezed out into a beaker.

2.7 Experimental design

Wet fertilization was used in the experiment. Milt collected into a clean beaker was mixed with small quantity of saline solution just before the fertilization of eggs. The striped eggs were~~as~~ carefully divided into 1 g aliquots containing 700 eggs. Each aliquot was fertilized with the saline solution activated milt. Then, the eggs were randomly rinsed inside the spawning bowls containing 100ml 1, 3, and 5% okra leaf extract, tannic acid solution or just water in triplicates for 1, 3, and 5 minutes to determine the optimum okra leaf extract concentration and immersion period. At the end of the predetermined exposure time, the liquid content of all bowls was decanted and replaced with aerated clean water. Incubation of eggs was performed in the same bowls.

2.8 Evaluation of fertility, non-adhesive eggs, Hatchability, 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).

Percent fertility = (Number of fertilized eggs)/(Total number of eggs counted) × 100

Ratio of Non- adhesive eggs (%) = number of non – adhesive egg/initial number of eggs × 100

Hatchability (%) = (Number of eggs hatched)/(Total number of eggs incubated) × 100

Survival (%) = number of hatchling at 72h/Total number of hatchling at 0h × 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).

2.9 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), pH meter (Hanna H198106 model), and dissolved oxygen meter (JPP-607 model) as described by APHA (1987).

2.10 Statistical analysis

All percentage data was subjected to ANOVA test and Tukey HSD procedure. Polynomial regression analysis was used to determine the best concentration and immersion period of okra leaf extract treatment that effectively removes egg adhesiveness. All analysis was performed at a 0.05 significance level.

3. RESULTS AND DISCUSSION

3.1 Effects of different concentrations of okra leaf extract on the fertility of *C. gariepinus* eggs

The results of the eggs of *C. gariepinus* exposed to varying concentrations and immersion periods of okra leaf extract are presented in Table 1. The fertility of eggs immersed in okra leaf extract ranged from 89.50% at 5% concentration (5 minutes) to 97.34% at 1% concentration (1 minute). Similarly, the fertility of eggs immersed in tannic acid solution varied from 88.50% at 5% concentration (5 minutes) to 96.17% at 1% concentration with a one-minute immersion period. In the control group, a fertility rate of 92.80% was recorded. Therefore, there was no significant difference ($P > 0.05$) in fertility among the

concentrations and immersion periods of eggs immersed in okra leaf solution, tannic acid solution, and the control group, although fertility decreased as concentration and immersion period increased.

This aligns with the findings of Asraf et al. (2013), who observed that a one-minute rinsing time resulted in the highest fertilization and lowest clumping rate when using urea. Similarly, a fertilization rate of 96.17% was recorded for eggs immersed in tannic acid at a 1% concentration with a one-minute immersion period, which was not significantly different from the result with okra leaf extract.

The study also showed that fertilization rates decreased with increasing concentrations and immersion periods of both okra leaf extract and tannic acid solution. This finding is consistent with Asraf et al. (2013), who reported that fertilization rates increased at the lowest concentration of urea and decreased as the concentration of urea was increased.

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

The non-adhesive eggs of *C. gariepinus* obtained after exposure to okra leaf extract ranged from 59.11% at 5% concentration (5 minutes) to 84.42% at 1% concentration (1 minute), as shown in Table 1. Similarly, the eggs of *C. gariepinus* in varying concentrations and immersion periods of okra leaf extract showed no significant effect on the adhesiveness of eggs ($P > 0.05$). In comparison, the eggs immersed in tannic acid solution had non-adhesive eggs ranging from 63.28% at 5% concentration (5 minutes) to 85.54% at 1% concentration with a one-minute immersion period. Conversely, the eggs of *C. gariepinus* immersed in water, serving as the control, had 25.62% non-adhesive eggs, which was significantly different ($P < 0.05$) from the non-adhesive eggs of *C. gariepinus* exposed to tannic acid solution and okra leaf extract.

The percentage of non-adhesive eggs indicates that adhesive elimination was successful at lower concentrations and immersion periods of okra leaf extract and tannic acid solution, with 84.42% and 85.54%, respectively, showing no significant difference. Adhesiveness of eggs increased with increasing concentration and immersion period.

The present findings indicate that egg detachment decreases as both concentration and immersion period increase. As shown in Figure 2, at the lowest concentration of 1% with an immersion period of one minute, okra leaf extract effectively reduced the stickiness of *Clarias gariepinus* eggs by 84.42%. This reduction in stickiness is attributed to the presence of tannin, an active ingredient in okra leaf extract. Similarly, an 85.54% reduction was recorded with a tannic acid solution at the same 1%

concentration and one-minute immersion period, which was not significantly different from the result with okra leaf extract.

These results are consistent with the findings of Żarski et al. (2015), who reported optimal results with eggs submerged in tannic acid solution for one and two minutes (86.5% and 80.5%, respectively). Additionally, the findings align with Fawehinmi et al. (2019), who found that waterleaf extract containing tannic acid was most effective at the lowest concentration of 1% and a one-minute immersion period, resulting in the highest fertilization and lowest clumping rates. Similarly, Asraf et al. (2013) reported that the optimal time for rinsing African catfish eggs was one minute using urea.

Therefore, okra leaf extract can be used at a 1% concentration with a one-minute immersion period to effectively reduce the stickiness of *Clarias gariepinus* eggs.

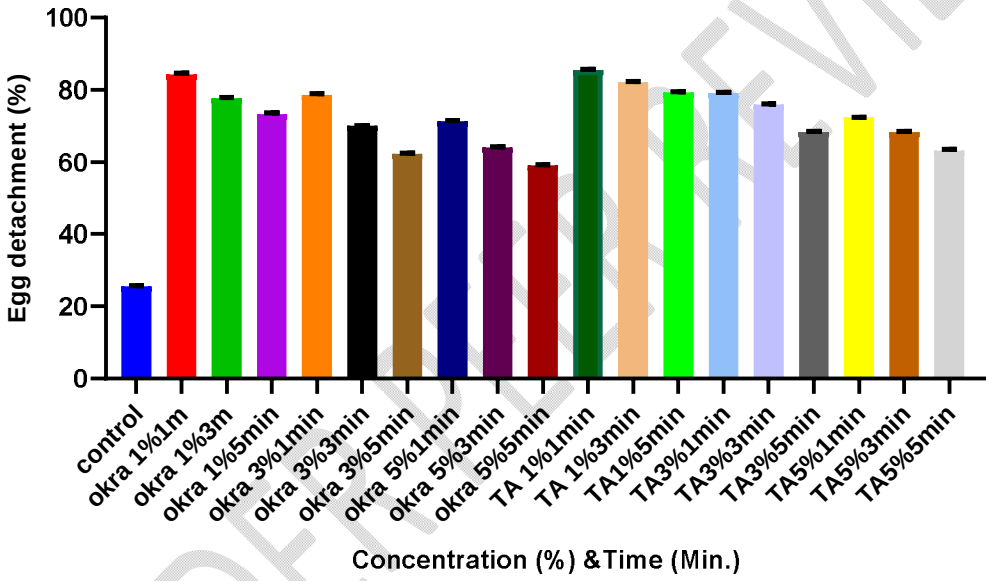

Figure 1 Effect of varying concentration of okra leaf extracts and tannic acid solution at different exposure periods on removal of adhesiveness of *Clarias gariepinus*egg.

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

The incubation period of eggs immersed in okra leaf extract ranged between 23 hours 70 minutes at 1% concentration (1 minute) to 25 hours 18 minutes at 5% concentration (5 minutes), as shown in Table 1. Conversely, the incubation period of eggs immersed in tannic acid solution varied from 24 hours 3 minutes at 1%, 3%, and 5% concentrations with a one-minute immersion period to 25 hours 17 minutes at 5% concentration (1 minute). The incubation period of the eggs immersed in water was 25 hours 73

minutes. Therefore, there was no significant difference (P > 0.05) observed between concentrations and immersion periods of eggs immersed in okra leaf solution, tannic acid solution, including the control group.

The incubation period is directly influenced by temperature and exposure duration (SRAC, 2006). This finding aligns with Adebayo and Olayinka (2009), who reported the first hatching at 24.5 hours in the lowest formalin treatment concentration, noting that longer exposure of *C. gariepinus* eggs to formalin resulted in longer hatching times.

However, the incubation period was not significantly affected by the relatively short exposure times ranging from one to five minutes. This observation is consistent with Fawehinmi et al. (2019), who found that varying concentrations and immersion periods of Aloe vera gel did not significantly affect the incubation period for *C. gariepinus*.

3.4 Percentage hatchabilityof *C. gariepinus*exposed to varying concentrations and immersion periods of Okra leaf extract

The percentage of hatching decreased with increasing concentrations of okra leaf extract, as shown in Table 1. The lowest percentage of egg hatchability (43.24%) was recorded in the control group, while the highest hatching rate (81.54%) was observed in the group exposed to 1% okra leaf extract with a one-minute immersion period. Similarly, a percentage egg hatchability of 79.54% was recorded in the group exposed to 1% tannic acid solution with a one-minute immersion period. The percentage hatchability recorded in 1% concentrations of okra leaf extract and tannic acid solution was high and not significantly different (p > 0.05), but it was significantly different (p < 0.05) from that of the control group.

The percentage hatching decreased with increasing concentrations of okra leaf extract, aligning with the findings of Riehl and Appelbaum (1991), who reported decreased hatching rates with increasing urea concentration. Thai and Ngo (2004) reported the highest hatching rate of 86.3% using pineapple juice and 70.2% using a salt/urea/tannin solution at 1% concentration. Similarly, Fawehinmi et al. (2019) noted that eggs immersed in waterleaf extract for 1 minute achieved the highest hatchability, approximately 70%.

These findings are consistent with the present study, where the highest hatching rate was observed at the lowest concentration (1%) with a 1-minute immersion period. Prolonged exposure and higher concentrations, such as 5% for 5 minutes, resulted in decreased hatchability. This decline in hatching rate may be attributed to tannin, an antinutritional factor present in okra leaf. Zarski et al. (2015) found that longer immersions or higher tannin concentrations led to the "inactivation" of the egg envelope at an

early egg swelling stage, which caused hardening of the egg envelope and prevented larvae hatching (Demska-Zakez_s et al., 2005; Kujawa et al., 2010).

Thus, the optimal immersion period for rinsing African catfish eggs using okra leaf extract is 1 minute. This conclusion is supported by Zarski et al. (2015), who emphasized the importance of using the shortest possible immersion in tannic acid at the lowest possible concentration. Asraf et al. (2013) also reported that the optimal time for rinsing African catfish eggs was 1 minute, as it resulted in high fertilization and hatching rates and the lowest clumping rate when the eggs were rinsed for this duration.

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

The percentage hatching index decreases with increasing concentrations of okra leaf extract, correlating with hatchability, as shown in Table 1. The lowest percentage hatching index (9.55%) was recorded in the control group, while the highest hatching index (57.23%) was observed in the group exposed to 1% okra leaf extract with a one-minute immersion period. Similarly, a hatching index of 55.05% was recorded in the group exposed to 1% tannic acid solution with a one-minute immersion period. However, the hatching index recorded in 1% concentrations with one-minute immersion periods of okra leaf extract and tannic acid solution was not significantly different ($p > 0.05$) but was significantly different ($p < 0.05$) from that of the control group and other rinsing agents at varying concentrations and immersion periods.

This finding corroboratewith Zarski et al. (2015), who reported the highest hatching index in groups subjected to one- and two-minute immersions in tannic acid. The hatching index represents the percentage of hatched larvae from the initial number of eggs, providing data on the actual production of *C. gariepinus* larvae from the total number of eggs initially used for incubation (Zarski et al., 2015).

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

No deformities were observed in the larvae during this experiment, and the surviving larvae displayed high activity levels and responsiveness to feeding. This outcome corroborates with the findings of Zarski et al. (2015), who reported that both the duration and immersion period did not have a significant effect ($P > 0.05$) on the deformity rate in hatched larvae when immersed in tannic acid.

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

The percentage of surviving larvae indicated a decrease with an increase in concentration and immersion period, as illustrated in Table 1. The highest survival rate (71.44%) was observed in the group exposed to 1% okra leaf extract with a one-minute immersion period, while the lowest survival rate (40.32%) was recorded in the group exposed to 5% okra leaf extract with a five-minute immersion period. Similarly, the highest survival rate (69.47%) was observed in the group exposed to 1% tannic acid solution with a one-minute immersion period, while the lowest survival rate (46.89%) was recorded in the group exposed to 5% tannic acid solution with a five-minute immersion period. In contrast, the survival rate of hatched larvae from the control group was 22.11%, the lowest among those exposed to okra leaf extract and tannic acid solution.However, there was no significant difference ($p > 0.05$) between the survival rates of larvae exposed to 1% concentration with a one-minute immersion period in okra leaf extract and tannic acid solution. Nevertheless, significant differences were observed compared to the control group and other rinsing agents at varying concentrations and immersion periods.

This finding aligns with the results of Akpoilih and Adebayo (2010), who reported a decrease in survival with increasing concentration levels of formalin. Similarly, this result is consistent with the findings of Ljubobratović et al. (2018), who observed 82.5% and 87.7% embryo survival in eggs treated with Alcalase and eggs treated with milk and kaolin, respectively.

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

The results of larvae size of *C. gariepinus* exposed to varying concentrations and immersion periods of okra leaf extract are presented in Table 1. The larvae size of *C. gariepinus* ranged from 0.29mm to 0.37mm for eggs immersed in okra leaf extract, with the largest size observed at 1% concentration for a one-minute immersion period and the smallest size at 5% concentration for a five-minute immersion period. Similarly, the larvae size of *C. gariepinus* obtained for eggs immersed in tannic acid solution varied between 0.26mm to 0.35mm, with the largest size observed at 1% concentration for a one-minute immersion period and the smallest size at 5% concentration for a five-minute immersion period. In contrast, the larvae size of the control group was 0.23mm, the smallest size compared to other larvae sizes of those immersed in varying concentrations and immersion periods of okra leaf extract and tannic acid solution.

Hence, there was no significant difference ($P > 0.05$) in larvae size between concentrations and immersion periods of eggs immersed in okra leaf extract and tannic acid solution, but there was a significant difference ($P < 0.05$) when compared with the control group.

This is in line with the observations of Hakim et al. (2008), who noted increased length (mm) in the growth of common carp at the lowest concentration with different levels of salinity. Additionally, Demska-Zakęś et al. (2005) suggested that prolonged immersion of eggs in a rinsing agent such as tannic acid could lead to a decrease in egg size or even disruption of the eggs due to osmotic pressure.

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

Rinsing Agents	Concentration (%)	Immersion time(min)	% Fertility	Non-adhesive eggs (%)	Incubation period (mins)	Hatching (%)	Hatching Index (%)	Survival (%)	Larvae size (mm)
Water(Control)			92.80±0.35^{a}	25.62±0.10^{c}	1544±2.90^{a}	43.24±0.17^{b}	9.55±0.04^{d}	22.11±0.09^{c}	0.23±0.01^{b}
Okra leaf	1	1	97.34±0.37^{a}	84.41±0.32^{ab}	1422±2.68^{a}	81.54±0.31^{a}	58.25±0.22^{a}	71.44±0.27^{a}	0.37±0.01^{a}
		3	96.81±0.37^{a}	77.74±0.30^{b}	1456±2.74^{a}	80.85±0.31^{a}	42.78±0.16^{b}	54.09±0.21^{b}	0.36±0.01^{a}
		5	94.37±0.36^{a}	73.36±0.28^{b}	1472±2.77^{a}	73.35±0.28^{a}	31.24±0.12^{b}	45.76±0.18^{b}	0.34±0.01^{a}
Tannic Acid	1	1	96.17±0.36^{a}	85.54±0.32^{ab}	1458±2.74^{a}	79.54±0.30^{a}	55.05±0.21^{a}	69.47±0.26^{a}	0.35±0.01^{a}
		3	95.28±0.36^{a}	82.18±0.31^{b}	1422±2.68^{a}	78.99±0.29^{a}	45.13±0.17^{b}	57.35±0.22^{b}	0.34±0.01^{a}
		5	89.43±0.34^{a}	79.25±0.30^{ab}	1477±2.78^{a}	73.79±0.28^{a}	41.60±0.16^{b}	56.58±0.22^{b}	0.33±0.01^{a}
Okra leaf	3	1	97.18±0.37^{a}	78.73±0.30^{b}	1461±2.75^{a}	59.47±0.23^{b}	37.16±0.14^{b}	62.72±0.24^{ab}	0.35±0.01^{a}
		3	95.38±0.36^{a}	70.15±0.30^{b}	1468±2.76^{a}	57.38±0.22^{b}	28.88±0.11^{c}	50.52±0.19^{b}	0.33±0.03^{a}
		5	93.10±0.35^{a}	62.32±0.24^{b}	1477±2.78^{a}	48.36±0.18^{b}	19.42±0.08^{c}	42.31±0.15^{b}	0.32±0.01^{a}
Tannic Acid	3	1	94.45±0.36^{a}	79.17±0.30^{ab}	1458±2.74^{a}	74.05±0.28^{a}	45.49±0.17^{b}	61.66±0.23^{ab}	0.34±0.01^{a}
		3	94.60±0.36^{a}	75.99±0.29^{ab}	1464±2.75^{a}	70.80±0.27^{a}	37.15±0.14^{b}	52.68±0.20^{b}	0.32±0.02^{a}
		5	87.37±0.33^{a}	68.35±0.26^{ab}	1480±2.78^{a}	68.22±0.26^{a}	31.86±0.12^{c}	47.89±0.18^{b}	0.31±0.01^{a}
Okra leaf	5	1	96.07±0.36^{a}	71.33±0.27^{b}	1467±2.76^{a}	57.51±0.22^{b}	27.87±0.11^{c}	48.66±0.19^{b}	0.34±0.01^{a}
		3	96.05±0.36^{a}	64.05±0.24^{b}	1475±2.78^{a}	46.10±0.18^{b}	19.54±0.08^{c}	44.50±0.17^{b}	0.32±0.01^{a}
		5	89.58±0.34^{a}	59.11±0.23^{b}	1511±2.84^{a}	45.28±0.17^{b}	17.78±0.07^{c}	40.32±0.16^{b}	0.29±0.01^{ab}
Tannic Acid	5	1	94.15±0.36^{a}	72.33±0.28^{ab}	1458±2.74^{a}	69.39±0.26^{a}	41.94±0.16^{b}	60.66±0.23^{ab}	0.33±0.00^{a}
		3	90.87±0.34^{a}	68.35±0.26^{b}	1460±2.75^{a}	63.88±0.24^{b}	31.61±0.12^{c}	49.67±0.19^{b}	0.27±0.01^{a}
		5	88.50±0.34^{a}	63.28±0.24^{ab}	1510±2.84^{a}	60.80±0.23^{b}	28.40±0.11^{c}	46.89±0.18^{b}	0.26±0.02^{ab}

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

Table 2: Physico-chemical parameters of test solutions of varying concentrations and immersion periods of Okra 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]
Okra leaf	1	1	26.90±0.98[a]	7.15±0.05[a]	5.75±0.78[a]
		3	26.95±0.91[a]	7.16±0.06[a]	5.80±0.85[a]
		5	27.10±1.13[a]	7.18±0.02[a]	5.60±0.71[a]
Tannic Acid (Reference de-adhesion agent)	1	1	27.00±0.99[a]	7.17±0.05[a]	5.90±0.85[a]
		3	27.07±1.04[a]	7.19±0.02[a]	5.65±0.6[a]
		5	27.13±0.90[a]	7.23±0.06[a]	5.70±0.9[a]
Okra leaf	3	1	27.07±1.09[a]	7.14±0.05[a]	5.55±0.64[a]
		3	27.13±1.03[a]	7.21±0.02[a]	5.60±0.85[a]
		5	27.11±2.29[a]	7.22±0.06[a]	5.55±0.78[a]
Tannic Acid (Reference de-adhesion agent)	3	1	27.03±1.03[a]	7.21±0.01[a]	5.95±0.92[a]
		3	27.05±0.94[a]	7.22±0.02[a]	5.70±0.85[a]
		5	27.10±1.00[a]	7.23±0.02[a]	5.55±0.64[a]
Okra leaf	5	1	27.05±1.06[a]	7.19±0.01[a]	5.70±0.71[a]
		3	27.19±0.87[a]	7.21±0.01[a]	5.60±0.71[a]
		5	27.00±0.99[a]	7.24±0.02[a]	5.45±0.92[a]
Tannic Acid	5	1	27.16±1.03[a]	7.22±0.03[a]	5.80±1.13[a]

(Reference de-adhesion agent)	3	27.16±0.98[a]	7.23±0.01[a]	5.60±0.71[a]
	5	27.23±2.37[a]	7.24±0.01[a]	5.50±0.99[a]

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

3.9 Water quality parameters of varying concentrations and immersion periods of Okra leaf extract

The physico-chemical parameters of water are known to significantly influence fish breeding, particularly in terms of temperature, dissolved oxygen, and pH (Akombo et al., 2018).

During this study, the observed temperature ranged between 26.90°C to 27.23°C, which is consistent with the findings of Adebayo (2006), who reported that the optimal temperature for *C. gariepinus* hatching falls within the range of 23°C to 29°C. Similarly, Viveen et al. (1986) identified a suitable temperature range of 20°C to 30°C for *C. gariepinus* larvae, corroborating the temperature range observed in the present study.

Water pH is a critical factor influencing water quality and plays a vital role in maintaining homeostasis in fishes (Parra and Baldisserotto, 2007). The pH observed in this study ranged from 7.16 to 7.24, which aligns with the findings of Amaechi and Solomon (2015), who reported that a pH range of 7.10 to 8.70 favored the fertilization and hatchability of *Clarias gariepinus*.

Dissolved oxygen is another important criterion affecting the distribution of many fish species. In this study, dissolved oxygen levels ranged between 5.50 mg/L to 6.05 mg/L, falling within the range reported by Bhatnagar and Sangwan (2009), who identified a dissolved oxygen range of 4.5 mg/L to 8.0 mg/L as suitable for fish breeding.

3.10 Polynomial regression showing effects of Okra leaf extract and Tannic acid solution on *C. gariepinus* eggs

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

At the end of the experimental trial, the extract that gave the highest fertilization, non-adhesive eggs, highest hatchability and survival rates were subjected to statistics in order to ascertain the best extract among the rising agents, best concentration and immersion period.

The optimum concentration that can efficiently remove egg adhesiveness in *C. gariepinus*using okra leaf extract was observed at concentration of 1.7%

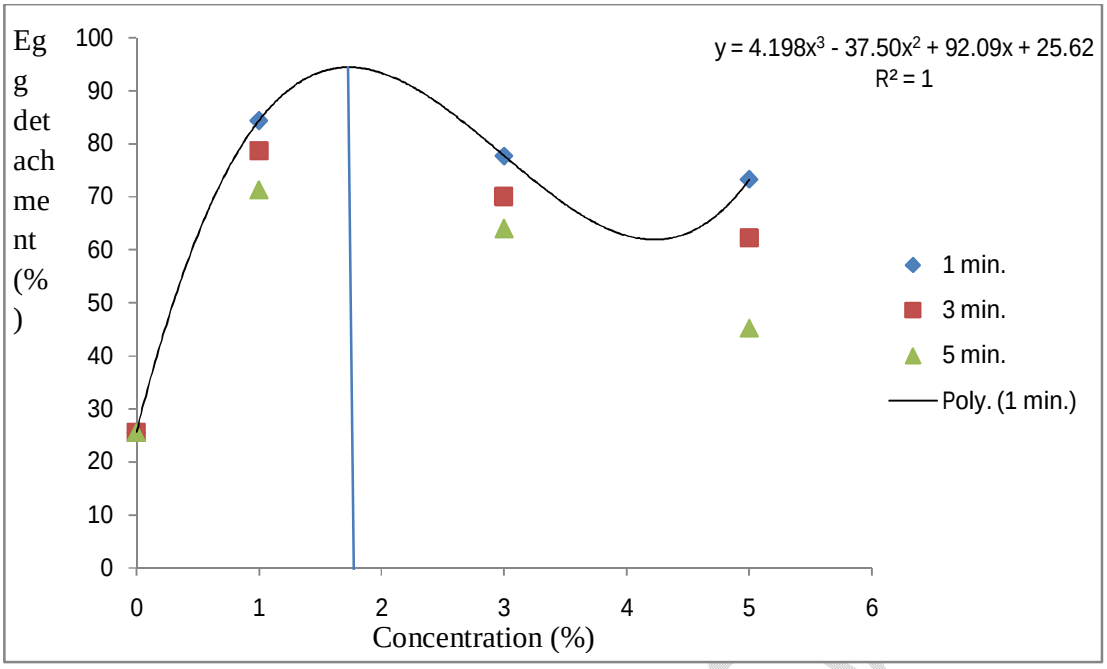

Figure. 2: The optimum concentration of okra leaf extract used as de-adhesive agent during artificial propagation of *C. gariepinus*

4. CONCLUSIONS

This study found that a 1% concentration of okra leaf extract with a 1-minute immersion period resulted in the highest fertilization rates, lowest stickiness, and highest hatchability and survival rates for *Clarias gariepinus*. Similarly, a 1% tannic acid solution with a 1-minute immersion period was also effective for detaching *C. gariepinus* eggs without negatively impacting fertilization, incubation, hatchability, or survival rates. The performance of the okra leaf extract at the lowest concentration and immersion period was comparable to that of the tannic acid solution.

Therefore, the use of a 1% okra leaf extract with a 1-minute immersion period is recommended for fish hatchery operators due to its quick, effective, and simple application. The plant source is inexpensive, readily available, and requires minimal preparation time. Although tannic acid, the reference de-adhesion agent, produced similar results, it is more costly. Therefore, okra leaf extract is recommended for its efficacy, efficiency, availability, cost-effectiveness, ease of handling, and simple processing.

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