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

ABSTRACT

This study evaluated the most effective immersion duration and concentration of okra leaf extract for removing the adhesive properties of *Clarias gariepinus*eggs. For the breeding experiment,a male and a female *C. gariepinus* broodstock, weighing 1.3 kg and 1.4 kg respectively, were used. The okra leaf extract was tested at three different concentrations: 1%, 3%, and 5%. Tannic acid at a concentration of 0.75 g per liter of water served as the reference de-adhesion agent, while plain water (without tannic acid or plant extract) was used as the control. The fish eggs were rinsed with the solutions for one, three, and five minutes. Each concentration and rinsing time were recorded in triplicate. The generated data was subjected to an Analysis of Variance Test, followed by a third order polynomial regression analysis to determine the best concentration and immersion period for removing *C. gariepinus* egg adhesiveness.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 found that okra leaf extract at 1% concentration with a one-minute immersion period can effectively 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). Global aquaculture production reached a record high of 122.6 million tonnes in 2020, worth 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).

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The most commonly Nigeria farmed fish species 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). *Clarias gariepinus* also demonstrates high feed utilization efficiency and can be easily bred in hatcheries.(Linhart et al., 2004;Adebayo and Olanrewaju, 2000; Wachirachaikarn et al., 2009). According to Adebayo (2006) andBrzuska et al., (2003), modern fish farming heavily relies on the artificial or semi-artificial mass propagation of fish seeds[39]. 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, the artificial production of African catfish larvae faces significant challenges, especially regarding the low hatching and survival rates of the 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.

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2. MATERIALS AND METHODS

2.1 Study Zone and Holding Facility

The experiment took place at the Teaching and Research Fish Farm of The Federal University of Technology, Akure, situated in Obakekere, Akure. Healthy male and female *Clarias gariepinus*, weighing 1.3 kg and 1.4 kg respectively, were obtained from a reputable fish farm in Akure prior to the start of the experiment.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, properly labeled, and used 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, employed as the reference de-adhesion agent, was formulated by diluting 0.75 g of tannic acid in one liter of water, following the procedure outlined by Żarski et al. (2015). Water without tannic acid or extract was utilized as the control.

2.5 Preparation of Spawning Bowls

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Fifty-seven spawning bowls, each with a capacity of 4 liters, were obtained for the experiment. They were meticulously cleaned, dried, and subsequently labeled based on the treatment inclusion levels, including tannic acid (1%, 3%, and 5%), as well as the control, along with the immersion periods (1 minute, 3 minutes, and 5 minutes). The bowls were then filled with specific volumes of water: 100ml for the control, 99ml for the 1% treatment, 97ml for the 3% treatment, and 95ml for the 5% treatment, 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 testiswas removed by abdominal dissection, cleaned with a towel. Then,the milt was gently squeezed out into a beaker.

2.7 Experimental design

In the experiment, wet fertilization was employed. Milt collected into a clean beaker was mixed with a small quantity of saline solution just before fertilizing the eggs. The striped eggs were carefully divided into 1 g aliquots, each containing approximately 700 eggs. Each aliquot was then fertilized using the saline solution-activated milt. Subsequently, the eggs were randomly rinsed inside the spawning bowls, which contained either 100ml of 1%, 3%, and 5% okra leaf extract, tannic acid solution, or plain water, in triplicates for 1, 3, and 5 minutes, respectively, to determine the optimal concentration of okra leaf extract and immersion period. Upon completion of the predetermined exposure time, the liquid content of all bowls was decanted and replaced with aerated clean water for the incubation of eggs, which took place in the same bowls.

2.8 Evaluation of fertility, non-adhesive eggs, Hatchability, survival and deformity indices

To assess the effectiveness and efficiency of bitter leaf extract in removing egg adhesiveness, key parameters such as percent fertility, the ratio of non-adhesive eggs, hatching rate, and survival rate were calculated following the methodology outlined 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

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Hatching index

According to Żarski et al. (2015), a hatching index (HI) was determined based on the 72-hour embryo survival rate of incubation and the hatching rate. The formula used to calculate HI is as follows:

HI = (S1 * S2) / 100,

Where:

S1 represents the 72-hour embryo survival rate (%),

S2 represents the hatching rate (%).

This index reflects the percentage of hatched larvae obtained from the initial number of eggs.

Deformity (%) = (Number of deformed larvae)/(Total number of larvae) × 100

Furthermore, the total length of the larvae was measured using ImageJ 1.34 software (Rasband, 1997–2011), following the methodology described by Ben Khemis et al. (2014).

2.9 Water quality parameters

Water quality parameters, including temperature, pH, and dissolved oxygen concentration, were monitored twice during the study period. Temperature was measured using a mercury-in-glass thermometer (YSI-DO 550, U.S.A), pH was measured using a pH meter (Hanna H198106 model), and dissolved oxygen concentration was measured using a dissolved oxygen meter (JPP-607 model). The monitoring procedure followed the guidelines outlined by APHA (1987).

2.10 Statistical analysis

All percentage data were subjected to an ANOVA test and Tukey HSD procedure to assess significant differences among treatment groups. Polynomial regression analysis was employed to identify the optimal concentration and immersion period of okra leaf extract treatment for effectively removing egg adhesiveness. All statistical analyses were conducted at a significance level of 0.05.

3. RESULTS AND DISCUSSION

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

Table 1 shows the results of *C. gariepinus* eggs exposed to various concentrations and immersion periods in okra leaf extract. The fertility of eggs immersed in okra leaf extract varied between 89.50% at a 5% concentration for 5 minutes and 97.34% at a 1% concentration for 1 minute. Similarly, the fertility of eggs immersed in tannic acid solution ranged from 88.50% at 5% concentration for 5 minutes to 96.17% at 1% concentration for one minute of immersion.In the control group, a fertility rate of 92.80% was

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recorded. Therefore, there was no significant difference (P > 0.05) in fertility observed among the various concentrations and immersion periods of eggs immersed in okra leaf solution, tannic acid solution, and the control group. However, it's worth noting that fertility tended to decrease as both concentration and immersion period increased.

This is consistent with the findings of Asraf et al. (2013), who discovered that a one-minute rinsing time resulted in the highest fertilization rate and lowest clumping rate when using urea.Similarly, a fertilization rate of 96.17% was documented for eggs immersed in tannic acid at a 1% concentration with a one-minute immersion period, which did not differ significantly from the outcome observed 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 study is consistent with the findings of Asraf et al. (2013), who found that fertilization rates increased at low urea concentrations and decreased as urea concentrations 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 adhesiveness of *C. gariepinus* eggs was not significantly affected by varying concentrations and immersion periods in okra leaf extract (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 the 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. The adhesiveness of eggs increased with increasing concentration and immersion period.

The present findings indicate that egg detachment decreases as both the 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%.

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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.

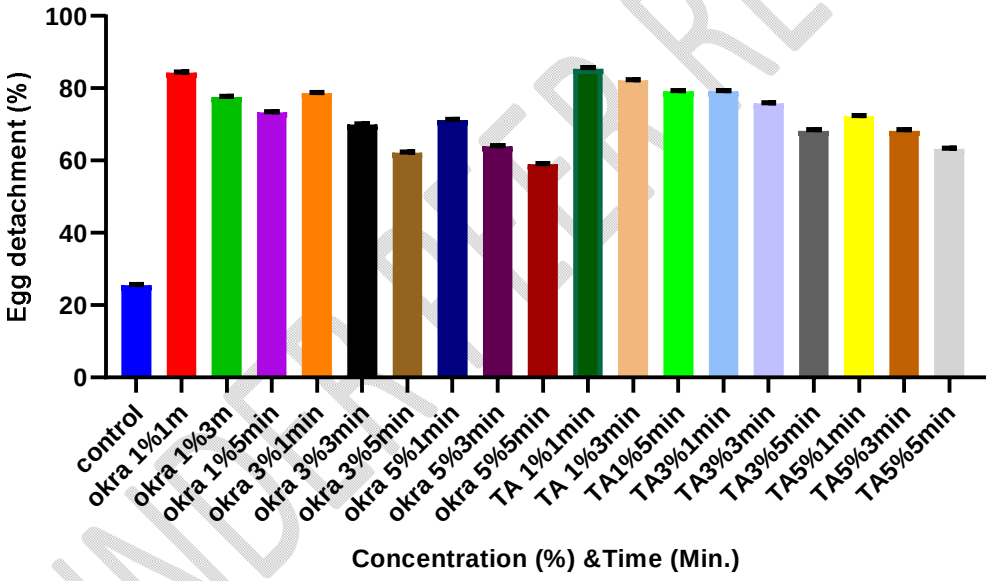

Figure 1 Effect of varying concentration of okra leaf extracts and tannic acid solution at different exposure periods on removal of adhesiveness of *Clarias gariepinus*egg.

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 time of eggs immersed in tannic acid solution ranged from 24 hours 3

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minutes at 1%, 3%, and 5% concentrations with a one-minute immersion period to 25 hours 17 minutes at the 5% concentration (1 minute).The eggs incubated in water for 25 hours and 73 minutes. As a result, no significant difference ($P > 0.05$) was found between the concentrations and immersion times of eggs in okra leaf solution, tannic acid solution, or the control group.

The duration of incubation is directly impacted by temperature and exposure duration (SRAC, 2006). This observation is consistent with the findings of Adebayo and Olayinka (2009), who reported the first hatching occurring at 24.5 hours in the lowest formalin treatment concentration. They noted that prolonged exposure of *C. gariepinus* eggs to formalin resulted in extended 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

Table 1 shows that as the concentration of okra leaf extract increased, the percentage of hatching decreased. The control group had the lowest percentage of egg hatchability (43.24%), while the group that was exposed to 1% okra leaf extract for one minute had the highest hatching rate (81.54%). Similarly, the group that was immersed in a 1% tannic acid solution for one minute had a 79.54% egg hatchability rate. The hatchability percentage in 1% concentrations of okra leaf extract and tannic acid solution was high and not significantly different ($p > 0.05$), but significantly different ($p < 0.05$) from the control group.

The decrease in percentage hatching with increasing concentrations of okra leaf extract is consistent with the findings of Riehl and Appelbaum (1991), who discovered decreased hatching rates with increasing urea concentrations. Thai and Ngo (2004) reported the highest hatching rate of 86.3% with pineapple juice and 70.2% with a 1% salt/urea/tannin solution. Similarly, Fawehinmi et al. (2019) found that eggs immersed in waterleaf extract for one minute had the highest hatchability, around 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

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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).

Therefore, 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 time in tannic acid at the lowest feasible concentration. Additionally, Asraf et al. (2013) reported that the optimal rinsing time for African catfish eggs was 1 minute, resulting in high fertilization and hatching rates, along with the lowest clumping rate, when 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

Table 1 shows that as the concentration of okra leaf extract increases, the percentage hatching index decreases, which correlates with hatchability. The control group had the lowest hatching index (9.55%), while the group that was exposed to 1% okra leaf extract for one minute had the highest hatching index (57.23%). Similarly, the group that was immersed in 1% tannic acid solution for one minute had a hatching index of 55.05%.However, the hatching index recorded in 1% concentrations with one-minute immersion periods of both 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 times.

This finding is consistent with Zarski et al. (2015), who found the highest hatching index in groups exposed to one- and two-minute immersions in tannic acid. The hatching index calculates the percentage of hatched larvae from the total number of eggs used for incubation, providing information on the actual production of *C. gariepinus* larvae (Zarski et al., 2015).

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

During this experiment, no abnormalities were detected in the larvae, and those that survived exhibited vigorous activity levels and responsiveness to feeding. This finding is consistent with the findings of Zarski et al. (2015), who discovered that neither the duration nor the immersion period had a significant effect ($P > 0.05$) on the incidence of deformities in hatched larvae when exposed to tannic acid.

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

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The percentage of surviving larvae decreased with increasing concentration and immersion period, as shown in Table 1. The highest survival rate (71.44%) was observed in the group exposed to 1% okra leaf extract for a one-minute immersion period, while the lowest survival rate (40.32%) occurred in the group exposed to 5% okra leaf extract for a five-minute immersion period. Similarly, the group exposed to 1% tannic acid solution for a one-minute immersion period had the highest survival rate (69.47%), whereas the group exposed to 5% tannic acid solution for a five-minute immersion period had the lowest survival rate (46.89%). In contrast, the survival rate of newly hatched larvae in the control group was 22.11%, the lowest of those exposed to okra leaf extract and tannic acid solution.However, there was no significant difference ($p > 0.05$) in the survival rates of larvae exposed to a 1% concentration of okra leaf extract and a one-minute immersion period in tannic acid solution. Nonetheless, significant differences were found when compared to the control group andother treatments with different concentrations and immersion times.

This finding is consistent with Akpoilih and Adebayo's (2010) findings, which showed a decrease in survival rates as formalin concentrations increased. Ljubobratović et al. (2018) found 82.5% and 87.7% embryo survival rates in eggs treated with Alcalase, milk, and kaolin, respectively.

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

Table 1 shows the results of *C. gariepinus*larvae size after exposure to various concentrations and immersion periods of okra leaf extract. Eggs immersed in okra leaf extract produced larvae ranging in size from 0.29 mm to 0.37 mm, with the largest size observed at a 1% concentration for a one-minute immersion period and the smallest size at a 5% concentration for a five-minute immersion period. Similarly, the size of *C. gariepinus* larvae obtained from eggs immersed in tannic acid solution ranged from 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.

As a result, there was no significant difference ($P > 0.05$) in larvae size between the various concentrations and immersion times of eggs in okra leaf extract and tannic acid solution. The comparison of these groups to the control group revealed a significant difference ($P < 0.05$).

This is consistent with the findings of Hakim et al. (2008), who observed increased length (mm) in the growth of common carp at the lowest concentration with varying salinity levels. Additionally, Demska-

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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.

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Table 1: Egg adhesiveness, fertility, hatchability, survival, and incubation period percentages for okra leaf extract, tannic acid solution, and water.

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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)

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Table 2: Physicochemical parameters of test solutions with different concentrations and immersion times 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 (Reference de-adhesion agent)	5	1	27.16±1.03[a]	7.22±0.03[a]	5.80±1.13[a]
		3	27.16±0.98[a]	7.23±0.01[a]	5.60±0.71[a]

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	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).

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

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 temperature ranged from 26.90°C to 27.23°C, aligning with Adebayo's (2006) report the optimal temperature for *C. gariepinus* hatching is 23°C to 29°C.Likewise, Viveen et al. (1986) found a suitable temperature range of 20°C to 30°C for *C. gariepinus* larvae,which corroborates the findings of this study. Water pH, a crucial factor influencing water quality, plays an essential role in maintaining homeostasis in fish (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 factor influencing the distribution of many fish species. In this study, dissolved oxygen levels ranged from 5.50 mg/L to 6.05 mg/L, which falls within the range reported by Bhatnagar and Sangwan (2009). They 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 ideal concentration of okra leaf extract utilized as a de-adhesive agent in the artificial propagation of *C. gariepinus.*

At the end of the experiment, the extract with the highest fertilization, non-adhesive eggs, hatchability, and survival rates was statistically analyzed to determine the most effective extract among the rinsing agents, optimal concentration, and immersion time.

The optimal concentration of okra leaf extract for effectively reducing egg adhesiveness in *C. gariepinus* was found to be 1.7%.

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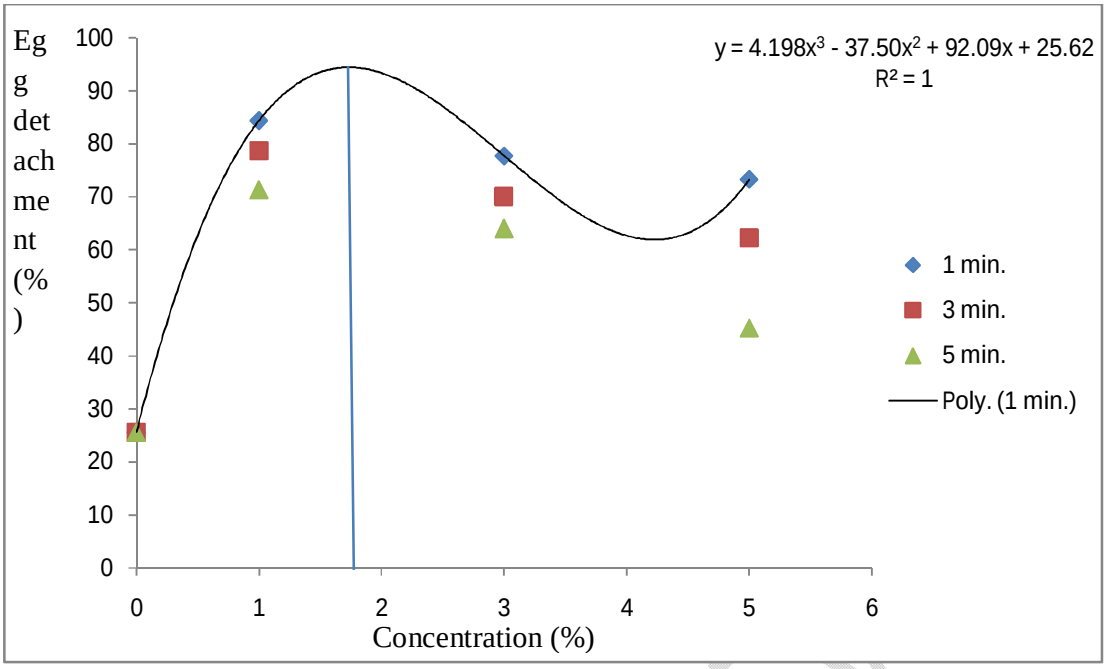

Figure. 2: The ideal concentration of okra leaf extract utilized as a de-adhesive agent in the artificial propagation of *C. gariepinus*.

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4. CONCLUSIONS

This study revealed that a 1% concentration of okra leaf extract with a 1-minute immersion period yielded the best fertilization rates, low egg stickiness, and high hatchability and survival rates for *Clarias gariepinus*.Similarly, a 1-minute immersion in a 1% tannic acid solution was effective in detaching *C. gariepinus* eggs while leaving fertilization, incubation, hatchability, and survival rates unchanged. At the lowest concentration and immersion period, okra leaf extract performed similarly to the tannic acid solution.

Hence, it is recommended for fish hatchery operators to utilize a 1% okra leaf extract with a 1-minute immersion period due to its rapid, efficient, and straightforward application. The plant source is cost-effective, widely accessible, and requires minimal preparation time. While tannic acid, the reference de-adhesion agent, yielded comparable outcomes. Because of the higher price, an alternative is preferred. Okra leaf extract is popular because of its efficacy, efficiency, availability, cost-effectiveness, ease of use, and simple processing.

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1.

2.

3.

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