

Research Article
Open Access

Evaluation of Bromelain as Hormonal Diluent and Egg De-Adhesion Agent During Artificial Propagation of African Catfish, *Clarias gariepinus*

Adebayo OT¹, Ojebuola TO^{1*}, Gbadamosi OK¹, TM Oladipupo¹ and Olanipekun OO²

¹Department of Fisheries and Aquaculture Technology, School of Agriculture and Agricultural Technology, The Federal University of Technology, Akure, Ondo State, Nigeria

²Chemistry Department, The Federal University of Technology, Akure, Ondo State, Nigeria

ABSTRACT

This study evaluated bromelain, a proteolytic enzyme from *Ananas comosus*, as a hormonal diluent and egg de-adhesion agent in the artificial propagation of *Clarias gariepinus*, aiming to identify the concentration that enhances reproductive performance, improves de-adhesion efficiency, and maximises hatchery outcomes compared to undiluted ovaprim. The experiment was conducted at the Teaching and Research Fish Farm, Federal University of Technology Akure, Nigeria, using a completely randomized design with five treatments (0%, 25%, 50%, 75% and 100% bromelain concentration) in triplicate. Apparently healthy female and male broodstock with average weight of 1 kg were induced, stripped manually, and monitored for reproductive parameters. Egg samples were incubated in flow-through systems, and data on fecundity, relative fecundity, fertilization rate, de-adhesion percentage, incubation duration, hatchability, and larval survival were analyzed using ANOVA, Tukey HSD, and polynomial regression at 0.05 significance level. Results showed no significant differences in broodstock weight, egg weight, or fecundity across treatments ($p > 0.05$), confirming that bromelain does not impair ovulation or egg production. However, significant improvements were observed in de-adhesion performance, hatchability, incubation period, and larval survival. The 50% bromelain concentration consistently produced the most favourable outcomes, yielding the highest percentage of non-adhesive eggs (96.86%), the highest fertilization rate (94.33%), the shortest incubation duration, the highest hatchability (86.03%), and the greatest larval survival (76.07%), all significantly higher than the control. Polynomial regression further identified an optimal bromelain concentration of approximately 52% for both de-adhesion and hatchability. The findings demonstrate that bromelain is a safe, efficient, and biologically compatible natural enzyme that enhances reproductive success in *C. gariepinus*, offering an eco-friendly alternative to traditional synthetic or chemical agents. The study concludes that bromelain within the range of 50–52% concentration, can significantly improve hatchery productivity and contribute to sustainable aquaculture through low-cost, biodegradable, and waste-valorised enzyme utilisation.

*Corresponding author

Ojebuola TO, Department of Fisheries and Aquaculture Technology, School of Agriculture and Agricultural Technology, The Federal University of Technology, Akure, Ondo State, Nigeria.

Received: December 04, 2025; **Accepted:** December 08, 2025; **Published:** December 30, 2025

Keywords: Bromelain, *Ananas Comosus*, *Clarias Gariepinus*, Hormonal Diluent, Egg De-Adhesion

Introduction

Aquaculture has become a critical sector in meeting the growing global demand for fish protein, particularly as wild fish stocks decline due to overfishing and environmental degradation. Global aquaculture output reached a new peak in 2022, with approximately 130.9 million tonnes, an increase of 8.1 million tonnes compared to 122.8 million tonnes recorded in 2020 [1]. In Nigeria, fish culture has gained prominence due to abundant water resources, heightened awareness of aquaculture practices, and the economic potential of fish production [2]. Despite these advantages, local production continues to fall short of demand, as Nigeria is among the largest fish-consuming countries in Africa and globally [3]. Consequently, effective artificial propagation of key aquaculture species, particularly *Clarias gariepinus* (African catfish), is critical for sustaining the industry.

Clarias gariepinus is highly valued for its rapid growth, adaptability to diverse environments, and high-quality flesh [4]. However, artificial propagation of this species is often constrained by challenges such as suboptimal milt quality, low egg fertilisation rates, and high larval mortality, commonly linked to egg adhesiveness and difficulties in gamete handling [5]. Hormonal induction is widely employed to overcome reproductive asynchrony, with agents such as luteinising hormone-releasing hormone analogs (LHRHa) and human chorionic gonadotropin (hCG) commonly used to stimulate gamete maturation and spawning [6, 7]. While effective, these synthetic hormones present challenges including high cost, variable efficacy, and potential adverse effects on fish health and human consumers due to endocrine disruption [8, 9].

According to Sharma and Vimal, bromelain facilitates the breakdown of follicular walls, enhancing gamete release during spawning, while also demonstrating anti-inflammatory and immunomodulatory effects that support fish health under the

stress of artificial propagation. In addition to its role in hormonal induction, bromelain reduces egg adhesiveness, which improves egg handling, fertilisation rates, and larval survival [10, 11]. Several studies have further highlighted bromelain's utility in aquaculture; Khazaeel reported enhanced milt motility and fertilisation rates in bromelain-treated sperm, attributed to reduced viscosity and improved sperm-egg interaction [12]. Similarly, Daramola observed that bromelain-containing extenders improved milt motility, acrosome integrity, and membrane stability, while reducing sperm abnormalities [13]. These findings demonstrate that bromelain can enhance both gamete quality and fertilisation efficiency, positioning it as a sustainable and cost-effective alternative to conventional synthetic hormones.

The use of bromelain derived from pineapple core, a commonly discarded waste product, also aligns with sustainable aquaculture practices by valorising agro-industrial by-products and reducing reliance on synthetic chemicals [11]. Given the combined challenges of poor milt quality, egg adhesiveness, and high larval mortality in *Clarias gariepinus*, evaluating bromelain as a hormonal diluent and egg de-adhesion agent presents a practical and environmentally friendly approach. The present study therefore seeks to assess the efficacy of bromelain from pineapple waste (core) in enhancing gamete quality, facilitating spawning, and improving fertilisation success, with the ultimate goal of promoting sustainable and efficient artificial propagation of African catfish in Nigerian aquaculture.

Materials and Methods

Site of the Experiment

The experiment was conducted in the experimental section of the Teaching and Research Fish Farm, Federal University of Technology, Akure, located at Obakekere, Akure.

Procurement and Identification of Pineapple (*Ananas Comosus*)

Fresh Smooth Cayenne cultivar pineapples (*A. comosus*) were procured from Oja Oba Market, Akure, Ondo State. Identification and authentication were carried out at the Herbarium, Department of Crop, Soil, and Pest Management, FUTA.

Extraction of Bromelain

Bromelain extraction was conducted in the Department of Chemistry, Federal University of Technology Akure (FUTA), using deionised water under cold laboratory conditions to preserve enzyme stability. Fresh pineapple fruits were washed thoroughly under running tap water to remove dust, sand, and other extraneous contaminants. The fruits were peeled and the cores were separated from the edible pulp. The pineapple cores were weighed to obtain approximately 500 g of fresh material for extraction. The cores were manually chopped into smaller pieces and homogenised using a laboratory blender for two minutes with the addition of chilled deionised water at a ratio of 1:1 (w/v), maintaining the temperature at 4°C. The homogenised slurry was placed on ice and allowed to stand for five minutes to facilitate maceration. The mixture was then filtered twice through sterile muslin cloth with an effective pore size of 0.22 µm to remove coarse fibre and larger particulates. The first filtration removed solid debris, while the second ensured finer clarification of the crude extract. The filtrate was immediately transferred into pre-cooled centrifuge tubes to prevent enzymatic denaturation.

Centrifugation was performed at 7,000 rpm for 40 minutes at 4°C using a high-speed refrigerated centrifuge to remove insoluble fractions and residual impurities. Following centrifugation, the

clear supernatant containing the crude bromelain extract was carefully decanted into sterile amber glass bottles to minimise light-induced degradation. The pH 7.0 was maintained, as bromelain exhibits optimal stability at neutral pH. All extraction steps were carried out on ice or at low temperature to preserve enzymatic activity, in accordance with the adapted procedure of Lakshminarasimaiah [14]. The final crude extract was stored at 4°C until required for subsequent experimental analyses.

Procurement and Selection of Broodstock

Twenty-two (22) apparently healthy *Clarias gariepinus* broodstocks, comprising fifteen females and seven males with an average weight of approximately 1 kg each, were sourced from a reputable fish farm in Akure. Broodstock selection followed standard physical criteria: females were identified by their distended abdomens and the free release of eggs upon gentle abdominal pressure, while males were confirmed by the reddish colouration at the tip of the genital papilla.

Upon arrival, the broodstock were acclimatised in separate concrete tanks (2.44 × 2.44 × 1.52 m) to reduce stress and minimise intersexual aggression. They were fed a commercial diet containing 40% crude protein for five days prior to the experiment to enhance reproductive readiness.

Feeding was withheld for 24 hours before breeding to ensure empty digestive tracts, thereby reducing the risk of faecal contamination during egg and milt collection. Maintaining clean, uncontaminated gonadal materials was essential for achieving successful fertilisation and optimal larval development.

Experimental Design

A total of fifteen (15) trials were conducted, consisting of five treatments in triplicate. The treatments were based on varying inclusion levels of undiluted generic Ovaprim and bromelain, a natural enzyme extracted from pineapple. The treatments were designated as follows:

Treatment A: 0% Ovaprim (control group)

Treatment B: 25% Bromelain

Treatment C: 50% Bromelain

Treatment D: 75% Bromelain

Treatment E: 100% Bromelain

Hormone Injection

Female brooder (*Clarias gariepinus*) was removed carefully from the holding tank and placed on a slab where it was injected with synthetic hormone (Ovaprim, Syndel Laboratories Ltd., Nanaimo, BC, Canada V9S 4M9) at a dosage of 0.5ml per kg of fish. Injection was given intramuscular above the lateral line at an angle 45° with the needle pointing towards the gonad region to enhance the effective delivery of the solution. The injections contained varying concentrations of bromelain: 0%, 25%, 50%, 75%, and 100%. This procedure ensured accurate dosing and consistent administration across treatment groups.

After injection, each female brooder was transferred to a separate holding trough to prevent injuries from aggressive interactions. This precaution reduced stress and minimised physical harm, which could negatively affect spawning efficiency and broodstock health.

Stripping and Fertilisation

Injected female brooders were removed from their troughs after a 12-hour latency period and stripped into dry bowls. From

each sample, 1 g of eggs was collected into pre-labelled bowls for proper identification. The milt from the male brooders was obtained after dissection, with the testes lacerated gently using a clean razor blade to release the milt.

A fertilization ratio of 0.01 ml of milt to 1 g of eggs, as recommended by FAO (1996), was applied. The milt and eggs were thoroughly mixed to ensure effective fertilization for each treatment.

Evaluation of Reproductive Performance

Fecundity

The fecundity of the female fish was determined for each treatment. Each fish was weighed before hormone injection, and reweighed after stripping to determine the approximate weight of the eggs. One gram of eggs was counted and multiplied by the total egg mass to estimate fecundity. Both fecundity and relative fecundity were calculated following the method described by Adebayo and Fawole [15].

$$(\text{Fecundity} = \text{Weight of eggs}) / (\text{No. of eggs in 1g of egg mass})$$

$$\text{Relative Fecundity} = (\text{Total no of egg}) / (\text{weight of fish}) \times 100$$

Determination of Percentage Fertility

Fertility was evaluated after 20 minutes of incubation. Eggs that appeared translucent and showed visible embryonic eyes at the time of polar cap formation were classified as fertilized, while opaque eggs that turned whitish were recorded as unfertilized. The percentage of fertilized eggs and percentage hatchability were calculated using the procedure described by Adebayo [16].

$$\% \text{ Fertility} = (\text{Number of fertilized eggs}) / (\text{Total number of eggs counted}) \times 100$$

Determination of Egg Adhesiveness Percentage

The percentage of non-adhesive (completely free) eggs in each experimental bowl was determined. The proportion of non-adhesive eggs was then calculated accordingly.

$$\text{Non-adhesive eggs (\%)} = (\text{Number of non-adhesive egg}) / (\text{Initial number of eggs}) \times 100$$

Incubating Period

The incubation period for each solution was determined by calculating the time difference between fertilization and hatching.

Hatching Rates Determination

Incubation was monitored between 23 and 36 hours. The number of hatched larvae was counted to determine hatching rates.

$$\% \text{ Hatchability} = (\text{Number of egg hatched}) / (\text{Total number of eggs counted}) \times 100$$

Deformity

All deformed larvae in each treatment were counted, and their percentage was calculated.

$$\% \text{ Deformity} = (\text{Number of deformed larvae}) / (\text{Total number of larvae}) \times 100$$

Survival

After hatching and assessing hatching rates, unhatched eggs were siphoned out of the spawning bowls to improve larval survival. Partial water changes were carried out to enhance dissolved oxygen levels. The larvae were observed daily, and survival percentages

were calculated following the method described by Adebayo [16].

$$\% \text{ Survival} = (\text{Number of hatchling}) / (\text{Total number of hatchling}) \times 100$$

Statistical Analysis

All percentage data obtained across the different concentrations and immersion periods were analysed using a one-way Analysis of Variance (ANOVA) to determine significant differences among treatments. Tukey's Honestly Significant Difference (HSD) test was applied as the post-hoc procedure to compare treatment means. In addition, polynomial regression analysis was conducted to identify the concentration that provided optimal performance both as a hormonal diluent and as an effective egg de-adhesion agent during artificial propagation. All statistical tests were performed at the 0.05 significance level.

Results

The weight of female broodstock used across treatments did not differ significantly ($p > 0.05$), indicating effective uniformity in fish selection and conditioning before hormonal induction. Mean weights ranged from 1012.02 g in the 50% bromelain treatment to 1066.34 g in the 75% group, while the control averaged 1023.43 g. Although minor numerical variations were observed, the absence of significant differences confirms that bromelain inclusion did not influence baseline broodstock characteristics.

Egg weight also varied numerically but remained statistically similar across treatments ($p > 0.05$). The highest egg weight occurred at 25% bromelain (222.54 g), followed by 50% (190.51 g), whereas the control produced 171.02 g. The 75% treatment recorded 181.45 g, while no spawning occurred at 100%. These results indicate that bromelain did not adversely affect ovulation or egg release, and broodstock responded consistently to hormonal induction except at the highest concentration.

Fecundity showed no significant differences among treatments ($p > 0.05$), demonstrating that bromelain did not suppress egg production. The highest fecundity was obtained at 25% (155,778 eggs), followed by 50% (133,359 eggs), both numerically higher than the control (119,714 eggs). Although fecundity decreased slightly at 75% (127,012.67 eggs), all bromelain treatments maintained comparable reproductive output. The upward trend in bromelain groups suggests that enzyme-assisted hormonal dilution may enhance hormone diffusion and ovarian response without disrupting pre-spawning development.

Relative fecundity followed the same general pattern and also showed no significant differences ($p > 0.05$). The 25% bromelain group recorded the highest value (14,790.22 eggs/kg), followed by 50% (13,229.26 eggs/kg) and the control (11,705.56 eggs/kg). The 75% group produced 11,877.47 eggs/kg. These trends suggest that bromelain may support more efficient reproductive output at moderate concentrations by improving hormonal utilisation and ovarian hydration.

Fertilization percentage increased across bromelain-treated groups, although differences were not statistically significant ($p > 0.05$). The control recorded 87.44%, while fertilization improved to 89.81% at 25% bromelain and peaked at 94.33% at 50%, before declining slightly to 88.16% at 75%. The improved rates highlight bromelain's potential role in enhancing gamete interaction and creating a more favourable fertilization environment, especially at mid-range concentrations.

Egg de-adhesion showed significant differences among treatments ($p < 0.05$), confirming bromelain's effectiveness as a de-adhesion agent. The control produced 53.60% non-adhesive eggs, reflecting natural stickiness characteristic of *C. gariepinus*. Bromelain markedly increased de-adhesion, with 25% yielding 88.73% and 50% achieving the highest value at 96.86%. The 75% treatment also performed well (86.61%), though slightly lower than the optimal mid-range concentration. These results demonstrate strong enzymatic activity by bromelain in breaking down adhesive mucopolysaccharides, with 50% identified as the most efficient concentration.

Incubation period decreased progressively with higher bromelain levels. The control recorded the longest duration (1458.00 min), while the shortest occurred at 75% (1412.60 min). The 50% treatment (1433.60 min) and 25% (1453.20 min) showed reductions relative to the control. Significant differences between the control and higher bromelain concentrations indicate that improved egg surface permeability and enhanced aeration following de-adhesion supported faster embryonic development.

Hatching onset occurred earlier in bromelain-treated groups. The control began hatching at 24.30 h, while 75% bromelain initiated hatching at 23.54 h, followed by 50% at 23.89 h and 25% at 24.22 h. Earlier hatching aligns with reduced incubation time and improved egg aeration, indicating that bromelain creates more favourable conditions for embryo development at concentrations of 50–75%.

Despite variations in onset time, all treatments recorded the same hatching end time (32.00 h). This consistency suggests that bromelain accelerates early development without disrupting overall hatching synchrony, ensuring normal and uniform embryonic progression across treatments.

Hatchability differed significantly among treatments ($p < 0.05$). The control showed the lowest value (46.72%), while hatchability increased markedly at 25% bromelain (69.40%) and peaked at 50% (86.03%). A slight reduction at 75% (66.71%) suggests that very high concentrations may marginally affect membrane stability. The strong performance at 50% reflects the direct advantage of efficient de-adhesion and improved oxygen availability during incubation.

Survival rates followed the same trend as hatchability, with significant differences across treatments ($p < 0.05$). The control recorded the lowest survival (40.04%), while survival increased to 58.41% at 25% and reached the highest value at 50% (76.07%). The 75% treatment produced moderate survival (54.86%), higher than the control but lower than the optimal concentration. The improvement in survival among bromelain-treated groups demonstrates the importance of effective de-adhesion in reducing stress, enhancing aeration, and promoting larval robustness during and after hatching.

Table 1: Effect of Bromelain as Hormonal Diluent and Egg De-Adhesion Agent During Artificial Propagation of *Clarias gariepinus*

Bromelain	Weight of fish (kg)	Weight of eggs	Fecundity	Relative Fecundity	% Fertilized	Non-adhesive eggs (%)	Hatching time (Start)	Hatching Period (End)	Incubation period (mins)	% Hatchability	% Survival
Control	1023.43±4.00 ^a	171.02±17.12 ^a	119714.00±11985.60 ^a	11705.56±1210.20 ^a	87.44±3.97 ^a	53.60±2.62 ^a	24.30±0.02 ^a	32.00±0.00 ^a	1458.00±1.04 ^a	46.72±2.23 ^c	40.04±1.94 ^c
Bromelain (25%)	1041.71±70.60 ^a	222.54±34.94 ^a	155778.00±24461.13 ^a	14790.22±1613.90 ^a	89.81±0.97 ^a	88.73±1.38 ^{ab}	24.22±0.05 ^a	32.00±0.00 ^a	1453.20±3.08 ^a	69.40±2.34 ^b	58.41±3.06 ^b
Bromelain (50%)	1012.02±62.24 ^a	190.51±18.28 ^a	133359±12799.43 ^a	13229.26±1337.70 ^a	94.33±1.90 ^a	96.86±1.01 ^a	23.89±0.19 ^{ab}	32.00±0.00 ^a	1433.60±11.25 ^{ab}	86.03±0.79 ^a	76.07±0.74 ^a
Bromelain (75%)	1066.34±58.73 ^a	181.45±14.92 ^a	127012.67±10444.96 ^a	11877.47±358.80 ^a	88.16±4.56 ^a	86.61±3.03 ^b	23.54±0.23 ^b	32.00±0.00 ^a	1412.60±1.74 ^b	66.71±3.07 ^b	54.86±3.89 ^b
Bromelain (100%)	1017.07±16.38 ^a	0.00	-	-	-	-	-	-	-	-	-

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

The Optimum Concentration of Bromelain Used as a Hormonal Diluent and De-Adhesion Agent During Artificial Propagation of *C. gariepinus*

At the end of the experimental trial, the extract that gave the highest fertilisation, non-adhesive eggs, highest hatchability and survival rates was subjected to statistics in order to ascertain the best concentration.

The optimum concentration that serves best as a hormonal diluent and efficiently removes egg adhesiveness in *C. gariepinus* using bromelain was observed at a concentration of 52% in both de-adhesion and hatchability using 3rd order polynomial regression as shown in Figures 1 and 2 below.

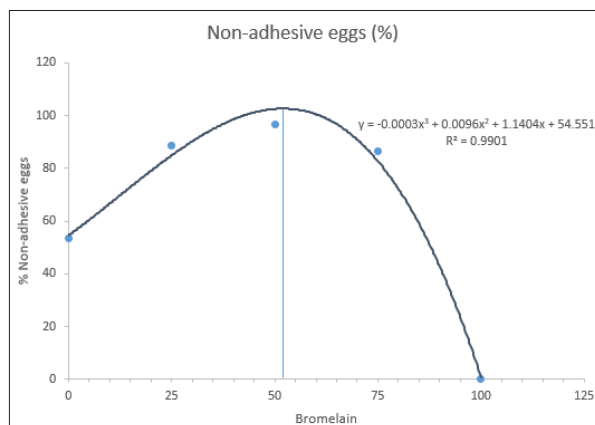


Figure 1: The Optimum Concentration of Bromelain Used as a De-Adhesion Agent During Artificial Propagation of *C. gariepinus*

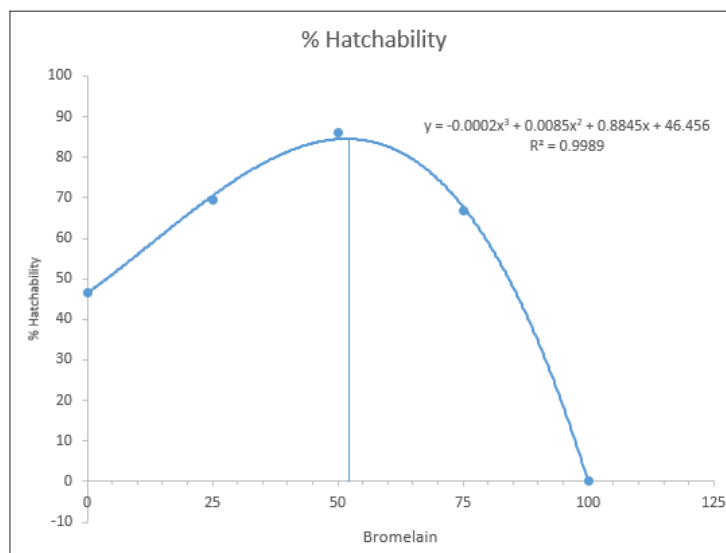


Figure 2: The Optimum Concentration of Bromelain Used Hormonal Diluent and its effect on Percentage Hatchability During Artificial Propagation of *C. gariepinus*

Discussions

The lack of significant variation in broodstock weight across treatments confirms that maternal size was effectively standardised and therefore could not have influenced subsequent reproductive responses. This aligns with observations by Amoah and Egwenomhe, who emphasised that *Clarias gariepinus* body weight strongly predicts egg output and should be harmonised across treatments to avoid confounding [17, 18]. Since broodstock mass directly affects fecundity, egg size, and overall reproductive potential, ensuring uniformity supports the reliability of treatment effects recorded in this study. Similar to the approach adopted by Olumuji and Mustapha and Assan, broodstock matching ensured that the reproductive outcomes observed were attributable to bromelain-based dilutions rather than differences in maternal physiological condition [19, 20]. This practice aligns with established hatchery standards, reinforcing the internal validity of the experiment [21].

Egg wet weight revealed numerical differences but no statistical variation among treatments, indicating that bromelain did not disrupt oocyte hydration or alter yolk water content during ovulation. The relatively stable egg mass across dilutions up to 75% suggests that the enzyme did not cause premature membrane weakening or excessive fluid loss during stripping. These results are consistent with earlier work by Olubiyi and Olumuji and Mustapha, who reported that suitable diluents maintain egg integrity when exposure conditions are well controlled [19]. Tiamiyu similarly demonstrated that certain diluents preserve egg weight until critical dilution thresholds are exceeded [22]. The present findings therefore confirm that bromelain, at moderate concentrations, is compatible with the structural requirements of *C. gariepinus* eggs at spawning.

Absolute fecundity did not differ significantly but was numerically higher in bromelain treatments, with 25% producing the greatest values. This pattern indicates that bromelain neither impaired hormone efficacy nor hindered oocyte release. Two mechanisms may explain the improved egg recovery: enhanced hormone diffusion due to bromelain's proteolytic activity, and reduced egg clumping during stripping, which minimises mechanical loss. This interpretation aligns with reports by Olumuji and Mustapha and Assan, who observed that certain diluents can improve handling

efficiency and egg retrieval [19, 20]. Furthermore, the stable fecundity across treatments supports the observations of Tiamiyu, who noted that excessive dilution reduces fecundity, whereas optimal diluent composition preserves normal ovarian response. Bromelain, within the tested range, did not show such detrimental effects [22].

Relative fecundity followed the same trend as absolute fecundity, with slightly elevated values in the 25% and 50% treatments, implying improved reproductive efficiency after normalising for broodstock mass. Because relative fecundity accounts for maternal size, these findings suggest that bromelain may enhance hormone utilisation or facilitate more complete egg release per unit body weight. This is consistent with the conclusions of Assan, who demonstrated that appropriately diluted ovaprim maintains relative fecundity comparable to undiluted hormone. In contrast, Tiamiyu reported declines under extreme dilution, highlighting the importance of maintaining optimal diluent potency, conditions clearly met by bromelain at the concentrations used in this study [20, 22].

Fertilisation percentage increased in bromelain-treated eggs, peaking at 50%, which exceeded the control and other treatments. This finding suggests that bromelain enhances fertilisation by reducing adhesiveness and improving egg dispersion, thereby increasing micropyle accessibility and uniform sperm-egg contact. The concentration-dependent response, improvement at moderate levels and slight decline at 75%, corresponds with the concept of an optimal enzyme window. Excessive enzymatic activity may begin to alter chorionic microstructure or create osmotic stress that reduces fertilisation efficiency. The trend observed mirrors reports by Olumuji and Mustapha and Assan, where moderate dilutions supported higher fertilisation, whereas extreme dilutions were detrimental [19, 20]. Tiamiyu also documented sharp declines beyond critical dilution limits [22]. The fertilisation pattern observed in this study, therefore, fits well within established reproductive responses to modified diluent composition.

Egg de-adhesion exhibited the most pronounced and statistically significant treatment effect, with bromelain at 50% producing nearly complete de-adhesion. This confirms strong proteolytic activity against the adhesive mucopolysaccharide matrix

that typifies *Clarias* eggs. The concentration–response curve, characterised by optimal performance at 50% and a slight decline at 75%, suggests that excessive enzymatic activity may begin to compromise chorion stability. These observations are consistent with findings by Zarski, Fawehinmi, who reported high de-adhesion with enzyme- or tannin-based agents but warned that elevated concentrations can impair hatching by altering the egg envelope [23]. Egwenomhe also demonstrated that pineapple-based extracts effectively reduced adhesiveness in *C. gariepinus*. The present findings further align with Tiamiyu, who noted that while some diluents reduce adhesiveness, their efficiency declines rapidly at extreme dilutions [22]. Bromelain, however, demonstrated superior activity within an optimal range.

Incubation period shortened progressively with increasing bromelain concentration, with the 75% treatment exhibiting the fastest development. This can be attributed to improved oxygen exchange and reduced micro-anoxia following de-adhesion, allowing embryos to develop under more favourable hydrodynamic conditions. The reduction in incubation time, coupled with increased hatchability at 50%, suggests physiologically enhanced, not stress-induced embryogenesis. These observations agree with the findings of Ojebuola and Adebayo and Olayinka, who noted that removal of egg adhesiveness improves water circulation and accelerates developmental processes. Similarly, Olumuji and Mustapha reported enhanced incubation efficiency in eggs treated with suitable diluents [24, 5].

Hatching onset occurred earlier in bromelain treatments, particularly at 75% and 50%, tracking the reductions in incubation duration. Earlier onset likely reflects improved embryo readiness under better aeration conditions and reduced egg massing. Tiamiyu reported similar shifts in hatching timing depending on diluent potency [22]. Despite early onset, the hatching end time remained consistent across treatments, indicating that bromelain accelerates early-stage development without disrupting clutch synchrony.

Hatchability improved markedly in bromelain treatments, with the highest rate recorded at 50%. The close association between non-adhesive egg percentage and hatchability strongly suggests that bromelain's de-adhesion effect contributes directly to improved hatch success by enhancing oxygen availability and reducing clumping-related infections. The reduced hatchability at 75% supports the presence of an optimal enzymatic range beyond which chorion properties may be affected. These results correspond with the patterns reported by Olubiyi et al. (2005), who observed favourable hatchability at mid-level dilutions of ovaprim. Egwenomhe also demonstrated enhanced hatchability using pineapple-derived extracts. Similarly, Olumuji and Mustapha and Assan reported increased hatchability with appropriate dilutions and warned against extreme concentrations [19, 20]. The present findings provide further evidence that bromelain-based diluents, when optimised, match or exceed the performance of traditional diluents.

Larval survival followed the same trend as hatchability, peaking at 50% and remaining lowest in the control. Enhanced survival in bromelain-treated groups likely resulted from reduced fungal load, improved aeration, and minimal mechanical abrasion owing to lower egg adhesion. The correspondence between hatchability and survival indicates that bromelain does not induce latent embryonic damage but supports healthier early life stages. Similar conclusions have been reported by Thai and Ngo and Ekokotu and Nwachi, who linked de-adhesion to improved larval robustness

[25, 26]. The findings also agree with Assan, whose recommended diluent protocols similarly enhanced survival outcomes. The high survival recorded in this study therefore confirms that bromelain, particularly at 50%, is a viable and effective option for improving larval performance in artificial propagation [27-30].

Conclusion and Recommendations

This study evaluated bromelain extracted from pineapple core waste as a hormonal diluent and egg de-adhesion agent in the artificial propagation of *Clarias gariepinus*. The findings show that bromelain effectively improved key reproductive outcomes, including fertilisation, egg de-adhesion, hatchability, and larval survival. Concentrations between 25% and 50% performed best, with 50% producing the most consistent and favourable results. The regression analysis further identified an optimal functional concentration of about 52%, confirming that moderate bromelain levels provide strong enzymatic activity without damaging egg structure.

The study demonstrates that bromelain is a reliable, eco-friendly alternative to synthetic de-adhesion agents and can support efficient hatchery operations. Because pineapple core waste is readily available and easy to process, bromelain offers a low-cost option that can enhance productivity and promote sustainable waste utilisation in aquaculture.

It is recommended that hatcheries adopt bromelain at concentrations of 52% for routine propagation of *Clarias gariepinus*. This study contributes to the growing evidence supporting the use of natural enzymes in hatchery management and offers practical guidance for hatchery managers, fish breeders, and aquaculture researchers in Nigeria and across other African aquaculture systems.

Acknowledgements

The authors gratefully acknowledge the support of the Tertiary Education Trust Fund (TETFUND), Nigeria, for funding this research under the Institutional-Based Research Grant with Reference Number: TETF/DR&D/CE/UNI/AKURE/IBR/2024. We also extend our sincere appreciation to the Federal University of Technology, Akure (FUTA), for providing the enabling environment and institutional support required for the successful execution of this study.

Competing Interests

The authors declared that they have no competing interests

Author's Contributions

- Adebayo OT conceived the study, provided expertise in fish breeding and biotechnology, supervised the research, and coordinated all project components.
- Ojebuola, TO conducted the experimental work, assisted in fish breeding and physiological assessments, collected and analysed data, and contributed to manuscript preparation.
- Gbadamosi OK carried out reproductive physiology analyses, provided expertise in biometrics and waste management, contributed to experimental design, data analysis, and manuscript writing. Oladipupo TM supported aquaculture and nutritional analyses, managed laboratory setup and water quality, and contributed to data interpretation and writing.
- Olanipekun OO performed phytochemical identification and analysis of bromelain and lignan, also contributed to laboratory work.

References

1. FAO (2024) FishStat: Global production by production source 1950–2022. In FishStatJ <https://www.fao.org/fishery/en/statistics/software/fishstatj>
2. Ogunji, J, Wuertz S (2023) Aquaculture Development in Nigeria: The Second Biggest Aquaculture Producer in Africa. *Water* 15: 4224.
3. Nwuba LA, Ude EF, Ogbonnaya HF (2022) Current trends in fisheries and aquaculture. *International Journal of Agriculture, Food and Biodiversity* 1: 64-69.
4. Kari ZA, Kabir MA, Razab MK AA, Munir MB, Lim PT, et al. (2020) A replacement of plant protein sources as an alternative of fish meal ingredient for African catfish, *Clarias gariepinus*: A review. *Journal of Tropical Resources and Sustainable Science (JTRSS)* 8: 47-59.
5. Adebayo OT, Olayinka SO (2009) Efficacy of formalin in the removal of adhesiveness from *Clarias gariepinus* eggs during artificial propagation. In: 24th Annual Conference of the Fisheries Society of Nigeria (FISON), Akure, Nigeria 24: 143-147.
6. Garcia LM, Genotiva AMI, Cortes JR (2023) Homoplastic Hypophysation of African Catfish (*Clarias gariepinus*, Burchell 1822) Using Catfish Pituitary Gland with Coconut Water as Extender Agent. *Asian Journal of Fisheries and Aquatic Research* 25: 123-129.
7. Zamri AS, Zulperi Z, Esa Y, Syukri F (2022) Hormone Application for Artificial Breeding Towards Sustainable Aquaculture—A Review. *Pertanika Journal of Tropical Agricultural Science* 45.
8. Arthur RI, Skerritt DJ, Schuhbauer A, Ebrahim N, Friend RM, et al. (2022) Small-scale fisheries and local food systems: Transformations, threats and opportunities. *Fish and Fisheries* 23: 109-124.
9. Khatun P, Saha P, Islam MZ, Islam A, Islam MA, et al. (2024) The reality of the use of growth hormones in fish (*Rui* (*Labeo rohita*), *Catla* (*Catla catla*), and *Monosex Tilapia* (*Oreochromis niloticus*) production. *Current Research in Food Science* 8: 100709.
10. Habtamu T, Abelneh Y (2024) The Use of Pine Apple Juice in the Elimination of Egg Stickiness in Catfish (*Clarias gariepinus*), Reared in a Hatchery, Sebeta, Ethiopia. *Aquaculture and Fisheries Studies* 6: 1-6.
11. Marinus E, Yusuf A, Ihenyen E, Sadiq HO, Nwabuma EI, et al. (2022) The Effects of Pineapple and Orange Juice as De-Adhesion Agents on *Clarias gariepinus* (African catfish) Eggs. *Dutse Journal of Pure and Applied Sciences (DUJOPAS)* 8: 70-76.
12. Khazaeel K, Rad OR, Jamshidian J, Tabandeh MR, Mohammadi G, et al. (2022) Effect of bromelain on sperm quality, testicular oxidative stress and expression of oestrogen receptors in BISPENOL-A treated male mice. *Andrologia* 54: e14584.
13. Daramola JO, Adekunle EO, Onagbesan OM, Oke OE., Ladokun AO, et al. (2018) Protective effects of fruit-juices on sperm viability of West African Dwarf goat bucks during cryopreservation. *Animal Reproduction (AR)* 13: 7-13.
14. Lakshminarasimaiah N, RajaRajeshwari B, Vibhuti BG (2014) Extraction of bromelain from pineapple waste. *International Journal of Scientific & Engineering Research* 5: 763.
15. Adebayo O, Fawole FJ (2012) Growth and reproductive performance of African giant catfish, *Heterobranchus longifilis* Valenciennes 1840 broodstock on ascorbic acid supplementation. *Indian Journal of Fish* 59: 135-140.
16. Adebayo OT (2006) Reproductive performance of African Clariid Catfish *Clarias gariepinus* broodstocks on varying maternal stress. *Journal of Fisheries International* 1: 17-20.
17. Amoah K, Adu Asiamah P, Dong XH, Ampofo Yeboah A, Abarike ED (2020) A comparative study on the hatchability and survival rate of African catfish, *Clarias gariepinus* (Burchell, 1822), induced with catfish's pituitary gland hormone from farmed and wild sources. *Aquaculture International* 28: 2221-2234.
18. Egwenomhe M, Otutu H, (2023) Female Broodstock Size and Maternal Effects on Progeny of *Clarias gariepinus* (BURCHELL 1822). *African Journal of Health, Safety and Environment* 4: 55-65.
19. Olumuji OK, Mustapha MK (2012) Induced breeding of African mud catfish, *Clarias gariepinus* (Burchell 1822), using different doses of normal saline diluted Ovaprim. *Journal of Aquaculture Research and Development* 3: 1-4.
20. Assan D, Anane K, Abarike ED, Alhassan EH, Ampofo Yeboah A (2022) Evaluation of induced breeding of catfish (*Clarias gariepinus*), using different doses of normal saline diluted ovaprim. *Journal of Applied Aquaculture* 34: 456-468.
21. Lucas JS, Southgate PC, Tucker CS (2019) Aquaculture: Farming aquatic animals and plants. John Wiley & Sons [https://www.wiley.com/en-
jp/e%3A+Farming+Aquatic+Animals+and+Plants%2C+3rd+Edition-p-9781119230861](https://www.wiley.com/en-
jp/e%3A+Farming+Aquatic+Animals+and+Plants%2C+3rd+Edition-p-9781119230861).
22. Tihamiyu LO, Okomoda VT, Oyeniyi ME, Aperegh J (2015) Spawning performance of *Clarias gariepinus* administered serially diluted doses of ovaprim. *Banat's Journal of Biotechnology* 11: 30-35.
23. Fawehinmi SJ, OT Adebayo, OK Gbadamosi (2019) Efficacy of Aloe vera Gel and Water-leaf Extracts for Removal of Egg Adhesiveness during Artificial Propagation of African Catfish (*Clarias gariepinus*, Burchell 1822), *Asian Journal of Fisheries and Aquatic Research* 3: 1-13.
24. Ojebuola TO, Adebayo OT, Gbadamosi OK (2024) Efficacy of Okra Leaf [*Abelmoschus esculentus* L.] Extract for Removal of Egg Adhesiveness During Artificial Propagation of African Catfish. *Asian Journal of Fisheries and Aquatic Research* 26: 12-24.
25. Thai BT, Ngo TG, (2004) Use of pineapple juice for elimination of egg stickiness of common carp (*Cyprinus carpio*). *Asian Fisheries Science* 17: 159-162.
26. Ekokotu PA, Nwachi OF (2014) Use of pineapple juice in the elimination of egg stickiness in Mudfish (*Heterobranchus bidorsalis*). *Global Journal of Bio-science and Technology* 3: 161-163.
27. Esa YB, Dadile AM, Syukri F, Christianus A, Diyaware MY (2023) Evaluation of fecundity, fertilisation, hatching, and gonadosomatic index of exotic *Clarias gariepinus* (Burchell, 1822) and native *Clarias macromystax* (Gunther, 1864) under semi-arid conditions of Nigeria. *Animals* 13: 1723.
28. Źarski D, Krejszef S, Kucharzyk D, Palińska K, Targońska K, et al. (2015). The application of tannic acid to the elimination of egg stickiness at varied moments of the egg swelling process in pikeperch, *Sander lucioperca* (L.). *Aquaculture Resource* 46: 324-334.
29. Sharma G, Vimal A (2023) Bromelain: an enzyme expanding its horizon from food to pharmaceutical industry. *Current Pharmaceutical Biotechnology* 24: 1715-1726.
30. Egwenomhe M, Yusuf A, Ihenyen E, Sadiq HO, Nwabuma EI (2022) The effects of pineapple and orange juice as e-adhesion agents on *Clarias gariepinus* (African catfish) eggs. *Dutse Journal of Pure and Applied Sciences* 8: 70-76.

Copyright: ©2025 Adebayo OT1, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.