

Effect of *Azanza garckeana* (Goron Tula) Pulp Powder on the Reproductive Indices of *Clarias gariepinus* Broodstock and Fry Survival

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Abstract

The rising cost and limited practicality of synthetic spawning agents in aquaculture have increased interest in affordable nutritional approaches, particularly functional feed additives. This study investigated the influence of *Azanza garckeana* pulp powder on the reproductive performance and fry survival of *Clarias gariepinus*. A total of 120 gravid broodfish were allocated to five dietary treatments (0, 10, 15, 20, and 25 g/kg; T1–T5), each replicated three times. Fifteen ponds were stocked with eight broodfish (four males and four females) per replicate. Brood stocks were fed the experimental diets twice daily for 56 days, with breeding initiated in week five. Each week, one male and one female from each treatment were sampled to evaluate gonadosomatic index, fecundity, fertilization rate, hatchability, and fry survival. Male broodfish were also examined for semen characteristics, including motility, volume, viscosity, colour, morphology, and total sperm count. Responses varied across treatments and sampling periods, reflecting both dose- and time-dependent effects. Overall, hatchability increased with higher *A. garckeana* inclusion, and fry survival reached 100% in T4 and T5 during the first two weeks. These findings indicate that *A. garckeana* pulp powder enhances key reproductive parameters in *C. gariepinus* and represents a promising natural alternative to synthetic fertility stimulants, supporting improved brood stock performance and sustainable seed production.

Keywords: *Azanza garckeana*, *Clarias gariepinus*, broodstock, reproductive indices, Fry survivability.

Introduction

Aquaculture now provides nearly half of the world's seafood and continues to expand rapidly (FAO 2024). However, intensified production has made maintaining reproductive performance in cultured fish increasingly challenging (Abdul Kari 2025). The African catfish (*Clarias gariepinus*), a major aquaculture species accounting for approximately 75% of global catfish production (Naylor et al. 2021), plays a critical role in food security across developing countries (Ouma & Barasa, 2022). Its popularity stems from rapid growth, hardiness, tolerance to low oxygen conditions, acceptance of pelleted feeds, and the ability to achieve large body sizes (Mehrim et al. 2022). Despite its importance, production is constrained by

limited seed availability. African catfish rarely spawn naturally in captivity, and reliance on wild fry collection is seasonal, unreliable, and inefficient. Hormonal induction using pituitary extracts, hormone analogues, or human chorionic gonadotropin has been widely employed to stimulate ovulation and spawning, but these synthetic hormones are often costly and impractical for widespread use (Shokr 2020). Thus, improving reproductive performance requires enhanced feed additives and effective reproductive management (FAO, 2015).

In recent years, medicinal plants have emerged as natural, low-side-effect alternatives to synthetic reproductive stimulants (Oyedemi et al. 2018). Herbal feed supplements contain bioactive compounds, including alkaloids, flavonoids, phenols, and terpenoids, which can promote sexual maturation and reproductive processes in fish (Sepehrfar et al. 2023; Barad et al. 2024). Several plants, such as *Desmodium adscendens* (Oke et al. 2019) and *Garcinia kola* (Olaniyi et al. 2020), have been tested as fertility enhancers in African catfish. Similarly, pomegranate peel extract improves gonadal development and fecundity in *Oreochromis niloticus* (EA Badrey et al. 2021), and *Vitex agnus-castus* enhances reproductive behavior and egg production in zebrafish (*Danio rerio*) (Enayat Gholampour et al. 2020).

Azanza garckeana (Malvaceae), locally known as Goron Tula, is native to several African regions and cultivated exclusively in Tula village, Gombe State, Nigeria (Iyojo et al. 2022). Traditionally, its leaves, roots, and fruits have been used to treat infertility, respiratory disorders, and liver ailments (Chanda et al. 2020; Ibrahim et al. 2023). The plant's bioactive phytochemicals flavonoids, tannins, and alkaloids possess antioxidant, anti-inflammatory, and antimicrobial properties, suggesting its potential as a natural reproductive enhancer in aquaculture species (Ibrahim et al. 2023).

Previous studies have reported that *A. garckeana* improves egg quality in *Clarias gariepinus* broodstock (Oniya et al. 2015) and positively affects hematological and biochemical parameters, as well as reproductive toxicity, in New Zealand rabbits (Iyojo et al. 2022). Additionally, *A. garckeana* has been shown to reverse fluoxetine-induced sexual dysfunction in female rats and enhance antioxidant activity while mitigating toxicological effects (Nurudeen et al. 2024). While these studies focus primarily on female reproductive indices, research on the effects of *A. garckeana* on male reproduction is limited. Therefore, this study was undertaken to evaluate the effects of dietary *A. garckeana* pulp powder as a reproductive enhancer for both male and female African catfish broodstock.

Statement of Problem

Fish farming in Nigeria faces major challenges related to brood stock and seed quality. Broodfish are often expensive, and using early-maturing fish for breeding is discouraged because their eggs are typically not fully developed. Since healthy brood stock and high-quality seed are essential for successful aquaculture, the difficulty of obtaining viable gametes that can produce robust, well-nourished larvae poses a significant constraint. Additionally, several studies have reported declining reproductive performance in cultured fish, particularly reduced male fertility, further complicating hatchery productivity.

Aim of the study

The aim of this research is to examine the effect of *Azanza garckeana* (Goron Tula) Pulp Powder on the Reproductive Indices of *Clarias gariepinus* Brood stock and Fry Survival

Specific objectives of the study are to:

1. determine egg diameter and milt quality of experimental broodfish fed varying levels of *Azanza garckeana*.
2. determine the gonadosomatic index (GSI) of broodfish fed varying levels of *Azanza garckeana* dieta.
3. determine the hatchability and fry survivability of broodfish fed varying levels of *Azanza garckeana*.

Literature Review

In captive fish, the quality of the diet administered can affect gonadal development and fertility rate if there is a deficiency of essential nutrients (Sharma, and Gangwar, 2001). Likewise, the nutrients supplied in the diet influence maturation, egg viability, and larval survival Awotimehin et al (2025)

Azanza garckeana has been observed to exert significant antioxidants, antimicrobial, analgesic, anti-inflammatory, anti-arthritis, and wound healing effect in experimental studies. Furthermore, methanol extract has been avowed to be most potent. Though, the exact mechanism of activities were not identified, its beneficial effects could possibly be correlated with the presence of important bioactive metabolites particularly phenols, alkaloids and flavonoids identified by Yusuf et al. (2020).

Studies have shown that treatments with antioxidants improve steroidogenesis through enhancement of the Leydig cell and endocrine function and, consequently, increasing circulating concentrations of testosterone, which stimulates spermatogenic functions (Meli et al. 2020). Adienbo et al. (2013) reported increased sperm motility and concentration of hydro-methanolic extract of Ethiopian pepper (*Xylopi aethiopica*) in rabbits.

Azanza garckeana is also a potential source of fertility compounds, possessing both spermatogenic and aphrodisiac activities, which may be utilized in traditional or alternative health-care systems (Edward et al. 2021).

Materials and Methods

Experimental Site

This study was carried out at the Research and Teaching Farm, Department of Fisheries and Aquaculture, Faculty of Agriculture, Federal University of Lafia, Nasarawa State. The facility is located at the Gandu Lafia campus in the Southern Guinea Savannah Zone of Nigeria, at latitude 08°28'7.78"N and longitude 08°33'31.11"E. The area experiences an average minimum temperature of 23°C and a maximum of 36.9°C, with a mean monthly

relative humidity of 74%. The mean annual rainfall is approximately 823 mm, and the mean monthly temperature is 35.06°C (NIMET, 2024).

Experimental Fish

The experimental broodfish were sourced from NUBWA Fish Farm, located at 6 Hymbula Crescent, off Old Karu Road, near NUBWA Shopping Complex, Mararaba, Nasarawa State. The fish were transported to the Research and Teaching Farm of the Department of Fisheries and Aquaculture, Federal University of Lafia, using plastic troughs measuring 50 cm in diameter and 30 cm in depth. Upon arrival, the broodstock were acclimatized for one week before the start of the experiment.

Experimental Diet

The experimental diets were formulated to contain 40% crude protein using FeedWin software. Ingredients including fish meal, soybean, wheat bran, vitamin premix, vitamin C, lysine, methionine, and cassava starch (binder) were obtained from the Lafia market, while soybean meal was sourced from a village near Abuja. All ingredients were weighed using a Sartor top-loading scale, with micro-ingredients measured on a sensitive scale. Each ingredient was ground separately with a hammer mill, dried, thoroughly mixed, and then pelleted. The diet formulation is presented in the table below.

Table 1: Gross composition of the formulated diet

Composition AGM in g/kg					
Ingredients	AG1	AG2	AG3	AG4	AG5
Maize	8	8	8	8	8
Rice bran	8	8	8	8	8
GNC	32	32	32	32	32
Fish meal	14	14	14	14	14
SBC	32	32	32	32	32
Casava	4.5	4.5	4.5	4.5	4.5
Bone meal	0.25	0.25	0.25	0.25	0.25
Vitamin premix	0.25	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25	0.25
fish oil	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25
Azanza (Goron Tula)	0	10g/kg	15g/kg	20g/kg	25g/kg
Total	100	100	100	100	100

AG1= 0% Azanza garckeana. AG2=10% Azanza garckeana. AG3=15% Azanza garckeana. AG4=20% Azanza garckeana. AG5=25% Azanza garckeana.

Collection, Processing, and Preparation of *A. garckeana* Pulp Meal

Azanza garckeana fruits were purchased from the Lafia market in Nasarawa State, Nigeria. The samples were authenticated using a plant identification manual (Hunt & Handoo) by experts in the Department of Forestry and Wildlife, Federal University of Lafia. The seeds were removed, and the pulp was sun-dried, pounded using a mortar and pestle, and sieved through a 0.8 mm mesh. The resulting pulp powder was packaged in black polythene bags and stored at room temperature until use.

Experimental Design

Five dietary treatments were prepared by incorporating *Azanza garckeana* pulp meal (AGPM) at 0, 10, 15, 20, and 25 g/kg feed, designated as T₁ (control), T₂, T₃, T₄, and T₅, respectively. A total of 120 gravid *Clarias gariepinus* brood stock were used and randomly allotted into five treatment groups, each replicated three times. Fifteen experimental ponds were stocked, with each pond containing eight broodfish (four males and four females).

Experimental Procedure

The AGPM-based diets were assigned to brood stock in concrete ponds (3 × 2 × 2 m). Fish were fed their respective diets *ad libitum* twice daily (morning and afternoon) for 56 days. The brood stock was weighed weekly to monitor biomass and growth performance, while water quality parameters were also assessed weekly. Breeding activities began in week five. At the end of each week, one male and one female broodfish were randomly selected from each treatment for reproductive evaluation, including fecundity, fertilization rate, hatchability, and larval survival. Male broodfish were taken to the laboratory for semen analysis, where semen viscosity, volume, colour, motility, morphology, and total sperm count were measured. Fertilization was induced using Ovatide at 0.5 mL/kg. After a 10-hour latency period, females were stripped and the eggs mixed with milt to initiate fertilization. Male broodfish were sacrificed and the testes removed; each testis was halved, and spermatozoa were gently squeezed onto the stripped eggs. A saline solution was added after 1 minute of gentle stirring to activate the sperm. Approximately 700 eggs from each treatment were fertilized and placed in duplicate spawning trays with well-aerated water. Fertilized eggs were counted 7 hours post-fertilization, while hatchlings were recorded 24–28 hours post-fertilization depending on water temperature. One hundred fry from each treatment were then transferred into transparent bowls for survival assessment, recorded 3–4 days post-hatching after yolk sac absorption. The gonadosomatic index (GSI), percentage fertilization, hatchability, and larval survival were calculated as follows:

$GSI = \text{Weight of the gonads} / \text{Weight of the fish} \times 100$

$\text{Fecundity} = \text{Number eggs per gram} \times \text{total ovary weight}$

$\text{Relative fecundity} = \text{total egg number} / \text{total body weight}$

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

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

Survival rate (%) = Number of fries survive at the end of yolk stage/Number of fry stocked × 100.

Data Analysis

Analysis of variance (ANOVA) at a 95% confidence level was used to assess differences among treatment means for egg size, milt quality, fecundity, GSI, hatchability and survival rate. Data were expressed as mean ± standard deviation and analyzed using one-way ANOVA in SPSS (version 23). Fisher's Least Significant Difference (LSD) test determined significant pairwise differences, and mean separation was further verified using Duncan's Multiple Range Test.

Results

Effect of Dried *Azanza garckeana* Pulp Powder on the egg size of *Clarias gariepinus* Brood stock

Table 2 presents the weekly effects of dried *A. garckeana* pulp powder on the egg size of *Clarias gariepinus* brood stock. In the first week, no significant differences ($p > 0.05$) were observed among all treatment groups. By week two, egg size in treatments T2–T5 did not differ significantly ($p > 0.05$), but all were significantly larger ($p < 0.05$) than the control (T1), which recorded the lowest value (1.00 ± 0.00). In week three, T5 produced the largest eggs (1.19 ± 0.00), significantly higher than T1–T4, which did not differ from one another ($p > 0.05$). In week four, T3 recorded the highest egg size (1.17 ± 0.02), while the smallest eggs (1.03 ± 0.05) were again observed in the control.

Table 2: Weekly Effect of Dried *Azanza garckeana* Pulp Powder on the egg size of *Clarias gariepinus* Brood stock

Weeks	Treatments					p value
	T1	T2	T3	T4	T5	
1	1.03 ± 0.05^a	1.15 ± 0.05^a	1.06 ± 0.05^a	1.15 ± 0.05^a	1.20 ± 0.26^a	0.514
2	1.00 ± 0.00^a	1.07 ± 0.06^b	1.11 ± 0.07^b	1.11 ± 0.08^b	1.18 ± 0.02^b	0.049
3	1.06 ± 0.07^a	1.11 ± 0.02^a	1.09 ± 0.03^a	1.09 ± 0.01^a	1.19 ± 0.00^b	0.021
4	1.03 ± 0.05^a	1.08 ± 0.02^{ab}	1.17 ± 0.02^c	1.14 ± 0.05^{bc}	1.06 ± 0.05^{ab}	0.018

Values are mean±SD (n=3). ^{ab} Rows with different superscript are significantly different ($p < 0.05$).

Weekly Effect of Dried *Azanza garckeana* Pulp Powder on the Milt Quality of *Clarias gariepinus* Broodstock

Table 3 summarizes the weekly effects of *Azanza garckeana* pulp powder on milt quality in *Clarias gariepinus*. In week 1, T2 produced the highest milt weight and length, while T5 yielded the greatest milt volume and motility; T3 showed the highest normal morphology. T2 also recorded the highest TMC. In week 2, T2 again had the highest milt weight, whereas

T₃ had the longest milt length. Milt motility peaked in T₅, and TMC was highest in T₅ and T₃. By week 3, all milt parameters differed significantly among treatments. T₄ produced the highest milt weight, length, volume, and morphology, while T₅ exhibited the greatest motility. T₃ and T₄ recorded the highest TMC values. In week 4, significant differences persisted across all parameters. T₄ had the highest milt weight, and T₁ produced the longest milt length. Motility was greatest in T₁–T₃, whereas T₅ achieved the highest TMC. Overall, *A. garckeana* enhanced milt quality in a dose- and time-dependent manner, with T₄ and T₅ showing the strongest improvements

Table 3: Weekly Effect of Dried *Azanza garckeana* Pulp Powder on the Milt Quality of Male *Clarias gariepinus* Broodstock

Week	Parameters	Treatments					P value
		T ₁	T ₂	T ₃	T ₄	T ₅	
1	Weight (g)	3.50±0.05 ^d	5.17±0.02 ^e	2.81±0.02 ^b	3.13±0.02 ^c	2.43±0.02 ^a	0.000
	Length (cm)	4.20±0.10 ^c	4.80±0.30 ^d	3.30±0.20 ^a	3.90±0.20 ^{bc}	3.50±0.30 ^{ab}	0.000
	Volume (ml)	1.10±0.10 ^{ab}	1.20±0.10 ^{abc}	1.00±0.10 ^a	1.30±0.20 ^{bc}	1.40±0.20 ^c	0.053
	Motility (%)	56.66±2.88 ^a	65.00±5.00 ^{abc}	70.00±5.00 ^{bc}	60.00±10.00 ^{ab}	75.00±5.00 ^c	0.025
	Normal morphology (%)	82.00±4.00 ^{ab}	78.00±4.00 ^a	87.00±2.00 ^b	80.00±5.00 ^{ab}	81.00±2.00 ^{ab}	0.100
	TMC (x10 ⁶ cells/ml)	27.00±3.00 ^a	35.00±3.00 ^c	31.00±2.00 ^b	24.00±1.00 ^a	33.00±1.00 ^{bc}	0.000
2	Weight (g)	4.70±0.20 ^{bc}	4.98±0.21 ^c	4.48±0.20 ^b	2.36±0.03 ^a	2.50±0.20 ^a	0.000
	Length (cm)	4.40±0.20 ^b	5.00±0.20 ^{bc}	5.40±0.30 ^c	5.00±0.30 ^{bc}	3.00±0.50 ^a	0.000
	Volume (ml)	1.00±0.10 ^a	1.00±0.20 ^a	1.20±0.20 ^a	1.30±0.20 ^a	1.10±0.10 ^a	0.201
	Motility (%)	55.00±10.00 ^a	60.00±5.00 ^a	55.00±5.00 ^a	56.00±5.00 ^a	80.00±5.00 ^b	0.004
	Normal morphology (%)	81.00±2.00 ^a	80.00±3.00 ^a	85.00±4.00 ^a	83.00±3.00 ^a	80.00±5.00 ^a	0.400
	TSC (x10 ⁶ cells/ml)	30.00±1.00 ^{bc}	24.00±2.00 ^a	33.00±2.00 ^c	26.00±4.00 ^{ab}	34.00±3.00 ^c	0.003
3	Weight (g)	4.50±0.10 ^c	3.07±0.02 ^a	4.68±0.10 ^c	4.98±0.21 ^d	3.68±0.02 ^b	0.000
	Length (cm)	3.90±0.20 ^{ab}	3.50±0.40 ^a	4.30±0.10 ^b	5.30±0.20 ^c	4.90±0.30 ^c	0.000
	Volume (ml)	1.00±0.00 ^a	1.00±0.20 ^a	1.10±0.10 ^a	1.40±0.20 ^b	1.20±0.10 ^{ab}	0.030
	Motility (%)	50.00±2.00 ^a	65.00±5.00 ^b	65.00±5.00 ^b	75.00±4.00 ^c	80.00±2.00 ^c	0.000
	Normal morphology (%)	80.00±2.00 ^a	82.00±1.00 ^a	80.00±5.00 ^a	89.00±5.00 ^b	85.00±2.00 ^{ab}	0.041
	TSC (x10 ⁶ cells/ml)	28.00±3.00 ^{ab}	34.00±4.00 ^c	36.00±1.00 ^c	27.00±2.00 ^a	32.00±1.00 ^{bc}	0.005
	Weight (g)	5.85±0.05 ^d	2.90±0.10 ^a	3.99±0.07 ^c	6.37±0.16 ^e	3.73±0.03 ^b	0.000
	Length (cm)	5.15±0.05 ^c	3.10±0.10 ^a	4.30±0.30 ^b	4.50±0.30 ^b	4.30±0.20 ^b	0.000

		Treatments					
4	Volume (ml)	1.00±0.10 ^a	1.00±0.00 ^a	1.10±0.10 ^a	1.10±0.10 ^a	1.20±0.20 ^a	0.274
	Motility (%)	55.00±5.00 ^b	55.00±2.00 ^b	50.00±2.00 ^b	35.00±4.00 ^a	40.00±2.00 ^a	0.000
	Normal morphology (%)	84.00±3.00 ^{ab}	88.00±3.00 ^b	80.00±2.00 ^a	78.00±6.00 ^a	83.00±2.00 ^{ab}	0.046
	TSC (x10 ⁶ cells/ml)	41.00±1.00 ^c	30.00±2.00 ^b	32.00±2.00 ^b	20.00±2.00 ^a	48.00±4.00 ^d	0.000

Values are mean±SD (n=3). ^{ab} Rows with different superscript are significantly different ($p < 0.05$).

Effect of Dried *Azanza garckeana* Pulp Powder on *Clarias gariepinus* Broodstock on Fecundity

The weekly effects of dried *Azanza garckeana* pulp powder on the fecundity of *Clarias gariepinus* brood stock are shown in Table 4. Ovary weight and fecundity differed significantly among treatments throughout weeks 1–4 ($p < 0.05$). In week 1, T₂ produced the highest ovary weight (118.31 ± 15.47 g) and fecundity ($76,903.66 \pm 10,060.04$), while T₁, T₃, T₄, and T₅ did not differ significantly. A similar trend was observed in week 2, with T₂ again exhibiting the highest ovary weight (113.61 ± 8.70 g) and fecundity ($73,848.66 \pm 5,660.38$), whereas T₁, T₃, and T₄ remained statistically similar. In week 3, T₂ continued to show the greatest reproductive output, recording the highest ovary weight (129.35 ± 17.46 g) and fecundity ($84,077.50 \pm 11,351.71$). No significant differences were observed among T₁, T₃, and T₄. By week 4, T₂ again produced the highest ovary weight (130.17 ± 19.62 g) and fecundity ($84,610.50 \pm 12,755.55$), whereas T₅ showed the lowest values for both parameters (34.17 ± 3.23 g and $22,214.83 \pm 2,102.02$, respectively). Overall, *A. garckeana* supplementation, particularly in T₂, consistently enhanced fecundity and ovary development across the experimental period.

Table 4: Weekly effect of Dried *Azanza garckeana* Pulp Powder on the Fecundity of *Clarias gariepinus* Broodstock

		Treatments					
Week	Parameters	T ₁	T ₂	T ₃	T ₄	T ₅	<i>p</i> value
1	Ovary weight (g)	82.23±4.34 ^a	118.31±15.47 ^b	83.96±5.70 ^a	98.99±2.03 ^a	81.02±13.89 ^a	0.004
	Fecundity	53453.83±2823.43 ^a	76903.66±10060.04 ^b	54578.33±3707.37 ^a	64345.66±1323.34 ^a	52663.00±9029.06 ^a	0.004
2	Ovary weight (g)	85.20±5.66 ^b	113.61±8.70 ^c	89.79±9.22 ^b	96.76±1.31 ^b	67.70±8.86 ^a	0.000
	Fecundity	55380.00±3679.87 ^b	73848.66±5660.38 ^c	58367.83±5993.62 ^b	62898.33±857.21 ^b	44005.00±5762.27 ^a	0.000
3	Ovary weight (g)	85.86±7.73 ^b	129.35±17.46 ^c	80.88±1.46 ^b	78.25±2.84 ^b	57.39±1.82 ^a	0.000
	Fecundity	55813.33±5026.23 ^b	84077.50±11351.71 ^c	52572.00±954.57 ^b	50866.83±1851.00 ^b	37307.83±1181.14 ^a	0.000

		Treatments					
4	Ovary weight (g)	86.31±4.99 ^c	130.17±19.62 ^e	61.53±6.76 ^b	107.19±12.98 ^d	34.17±3.23 ^a	0.000
	Fecundity	56103.66±3245.35 ^c	84610.50±12755.55 ^e	39996.66±4399.88 ^b	69675.66±8437.99 ^d	22214.83±2102.02 ^a	0.000

Values are mean±SD (n=3). ^{ab} Rows with different superscript are significantly different ($p < 0.05$).

Relative Fecundity of *Clarias gariepinus* Broodstock Fed Dried *Azanza garckeana* Pulp Powder

The weekly effects of dried *Azanza garckeana* pulp powder on the relative fecundity of *Clarias gariepinus* broodstock are shown in Table 5. Fish weight did not differ significantly among treatments throughout weeks 1–4 ($p > 0.05$). However, both total egg number and relative fecundity varied significantly across treatments each week ($p < 0.05$). In weeks 1–4, T₂ consistently recorded the highest total egg production and relative fecundity, with values of 76,903.66 ± 10,060.04 eggs and 104.64 ± 23.17 in week 1; 73,848.66 ± 5,660.38 eggs and 92.99 ± 12.17 in week 2; 84,077.50 ± 11,351.71 eggs and 99.73 ± 18.84 in week 3; and 84,610.50 ± 12,755.55 eggs and 96.44 ± 19.82 in week 4. Across these weeks, T₁, T₃, and T₄ generally showed no significant differences from one another. T₅ consistently performed poorest, recording the lowest total egg number and relative fecundity in week 4 (22,214.83 ± 2,102.02 eggs; 23.27 ± 2.83). Overall, *A. garckeana* supplementation particularly at the T₂ level substantially improved reproductive output, while T₅ produced the weakest response.

Table 5: Weekly Effect of Dried *Azanza garckeana* Pulp Powder on the Relative Fecundity of *Clarias gariepinus* Broodstock

		Treatments					
Week	Parameters	T ₁	T ₂	T ₃	T ₄	T ₅	<i>p</i> value
1	Fish weight (g)	826.66±115.47 ^a	746.66±80.82 ^a	800.00±0.00 ^a	845.00±39.68 ^a	820.00±34.64 ^a	0.479
	Total egg no.	53453.83±2823.43 ^a	76903.66±10060.04 ^b	54578.33±3707.37 ^a	64345.66±1323.34 ^a	52663.00±9029.06 ^a	0.004
	Relative fecundity	65.62±10.74 ^a	104.64±23.17 ^b	68.22±4.63 ^a	76.31±5.26 ^a	64.52±13.04 ^a	0.020
2	Fish weight (g)	856.66±125.03 ^a	800.00±87.17 ^a	878.33±46.45 ^a	861.66±46.45 ^a	848.33±44.81 ^a	0.776
	Total egg no.	55380.00±3679.87 ^b	73848.66±5660.38 ^c	58367.83±5993.62 ^b	62898.33±857.21 ^b	44005.00±5762.27 ^a	0.000
	Relative fecundity	65.84±12.68 ^{ab}	92.99±12.17 ^c	66.33±3.46 ^{ab}	73.10±3.18 ^b	52.00±7.87 ^a	0.003
	Fish weight (g)	893.33±120.96 ^a	850.00±78.10 ^a	975.00±122.57 ^a	913.33±90.18 ^a	898.33±63.31 ^a	0.646

		Treatments					
3	Total egg no.	55813.33±5026.23 ^b	84077.50±11351.71 ^c	52572.00±954.57 ^b	50866.83±1851.00 ^b	37307.83±1181.14 ^a	0.000
	Relative fecundity	63.63±13.35 ^b	99.73±18.84 ^c	54.54±7.58 ^{ab}	55.97±4.51 ^{ab}	41.60±1.67 ^a	0.001
4	Fish weight (g)	940.00±144.22 ^a	884.33±66.90 ^a	1026.66±155.34 ^a	993.66±110.18 ^a	956.66±40.41 ^a	0.612
	Total egg no.	56103.66±3245.35 ^c	84610.50±12755.55 ^e	39996.66±4399.88 ^b	69675.66±8437.99 ^d	22214.83±2102.02 ^a	0.000
	Relative fecundity	60.72±11.00 ^b	96.44±19.82 ^c	39.11±1.76 ^a	70.70±11.19 ^b	23.27±2.83 ^a	0.000

Values are mean±SD (n=3). ^{ab} Rows with different superscript are significantly different ($p < 0.05$).

Gonado-Somatic Index of Male *Clarias gariepinus* Broodstock Fed Dried *Azanza garckeana* Pulp Powder The weekly effects of dried *Azanza garckeana* pulp powder on the gonado-somatic index (GSI) of male *Clarias gariepinus* broodstock are shown in Table 5. Fish weight did not differ significantly among treatments throughout the four weeks ($p > 0.05$). In contrast, gonad weight and GSI varied significantly across treatments in all weeks except week 3, where GSI showed no significant differences ($p > 0.05$). In weeks 1 and 2, T2 consistently produced the highest gonad weight (0.66 ± 0.01 g and 0.60 ± 0.01 g, respectively) and GSI values (0.08 ± 0.00 and 0.07 ± 0.00), outperforming all other treatments ($p < 0.05$). In week 3, gonad weight was highest in T1 and T4 (0.51 ± 0.00 g), although GSI did not differ significantly. By week 4, T1 and T4 again recorded the highest gonad weight and GSI, while T2 showed the lowest values. Overall, *A. garckeana* supplementation influenced male reproductive development, with T2 enhancing gonadal growth in the early weeks, whereas T1 and T4 showed stronger responses by week 4.

Table 6: Weekly Effect of Dried *Azanza garckeana* Pulp Powder on the Gonado-Somatic Index of Male *Clarias gariepinus* Brood stock

		Treatments					
Week	Parameters	T1	T2	T3	T4	T5	<i>p</i> value
1	Fish weight (g)	800.00±20.00 ^a	800.00±40.00 ^a	860.00±103.92 ^a	766.66±61.10 ^a	780.00±87.17 ^a	0.556
	Gonad weight (g)	0.45±0.02 ^c	0.66±0.01 ^d	0.35±0.00 ^a	0.39±0.00 ^b	0.37±0.02 ^{ab}	0.000
	GSI (%)	0.05±0.00 ^b	0.08±0.00 ^c	0.04±0.00 ^a	0.05±0.00 ^b	0.04±0.00 ^{ab}	0.000
2	Fish weight (g)	833.33±14.33 ^a	850.00±43.58 ^a	918.33±79.10 ^a	856.66±92.91 ^a	791.66±80.36 ^a	0.304
	Gonad weight (g)	0.54±0.02 ^d	0.60±0.01 ^e	0.51±0.01 ^c	0.25±0.00 ^a	0.29±0.00 ^b	0.000

		Treatments					
	GSI (%)	0.06±0.00 ^c	0.07±0.00 ^d	0.05±0.00 ^c	0.03±0.00 ^a	0.04±0.00 ^b	0.000
3	Fish weight (g)	908.33±45.36 ^a	881.00±61.26 ^a	977.66±123.51 ^a	909.00±95.35 ^a	811.00±62.95 ^a	0.252
	Gonad weight (g)	0.51±0.00 ^b	0.41±0.05 ^a	0.45±0.02 ^a	0.51±0.00 ^b	0.43±0.00 ^a	0.005
	GSI (%)	0.05±0.00 ^a	0.04±0.00 ^a	0.04±0.00 ^a	0.05±0.00 ^a	0.05±0.00 ^a	0.186
4	Fish weight (g)	951.66±49.33 ^a	920.00±72.11 ^a	1023.33±166.23 ^a	955.66±48.64 ^a	831.66±53.92 ^a	0.207
	Gonad weight (g)	0.63±0.02 ^c	0.31±0.01 ^a	0.42±0.02 ^b	0.64±0.00 ^c	0.42±0.01 ^b	0.000
	GSI (%)	0.06±0.00 ^c	0.03±0.00 ^a	0.04±0.00 ^{ab}	0.06±0.00 ^c	0.05±0.00 ^b	0.000

Values are mean±SD (n=3). ^{ab} Rows with different superscript are significantly different ($p < 0.05$). GSI = Gonado-Somatic Index

Effect of Dried *Azanza garckeana* Pulp Powder on the Hatchability and Survival Rates of *Clarias gariepinus* Fry

The weekly effects of dried *Azanza garckeana* pulp powder on the hatchability and survival of *Clarias gariepinus* fry are shown in Table 6. The number of hatched eggs, hatchability percentage, and fry survival rate all differed significantly among treatments across the four weeks ($p < 0.05$). In week 1, T2 produced the highest number of hatched eggs ($47,796.33 \pm 5,494.90$), whereas hatchability was highest in T5, T4, and T3. Survival rate reached 100% in T4 and T5 but was lowest in T1. In week 2, T4 recorded the highest number of hatched eggs, while T5 achieved the highest hatchability. Survival again remained highest in T4 and T5. By week 3, T2 had the greatest number of hatched eggs, while T5 and T4 showed the highest hatchability. Survival followed the same pattern as earlier weeks, favoring the supplemented treatments. In week 4, T2 and T4 produced the most hatched eggs, and T5 recorded the highest hatchability. Survival rates for T2, T3, T4, and T5 did not differ significantly. Overall, *A. garckeana* supplementation influenced reproductive performance, with T2 enhancing egg hatching in multiple weeks and T5 consistently improving hatchability and survival.

Table 7: Weekly Effect of Dried *Azanza garckeana* Pulp Powder on the Hatchability and Survival Rate of *Clarias gariepinus* Fry

Week	Parameters	Treatments					P value
		T1	T2	T3	T4	T5	
1	No. of fertile eggs	37932.66±1798.84 ^a	63764.66±7628.54 ^c	46727.66±2581.41 ^{ab}	55761.00±791.40 ^{bc}	46996.66±9734.45 ^{ab}	0.002
	No. of hatched eggs	26918.66±1092.26 ^a	47796.33±5494.90 ^c	37699.00±1064.42 ^b	46020.33±2084.28 ^{ab}	39610.66±8056.18 ^{ab}	0.002

		Treatments					
	Hatchability rate (%)	70.98±1.99 ^a	74.99±2.00 ^a	80.76±2.24 ^b	82.53±3.55 ^b	84.33±1.52 ^b	0.000
	Survival rate (%)	80.66±2.30 ^a	89.66±0.57 ^b	93.33±1.53 ^c	100.00±0.00 ^d	100.00±0.00 ^d	0.000
2	No. of fertile eggs	42847.33±3508.71 ^{ab}	60117.66±5673.12 ^c	47257.33±4672.53 ^b	50535.33±802.66 ^b	36639.00±4423.01 ^a	0.001
	No. of hatched eggs	26954.66±1523.81 ^a	39699.33±4085.45 ^d	32899.66±2798.16 ^{bc}	37067.33±1362.66 ^{cd}	28183.00±3035.71 ^{ab}	0.001
	Hatchability rate (%)	63.00±1.99 ^a	66.00±0.99 ^b	69.69±1.53 ^c	73.33±1.52 ^d	77.00±1.00 ^e	0.000
	Survival rate (%)	84.66±2.51 ^a	89.33±2.51 ^b	97.00±2.64 ^c	100.00±0.00 ^c	100.00±0.00 ^c	0.000
3	No. of fertile eggs	36140.33±4280.67 ^{ab}	57006.66±5929.07 ^c	39244.33±105.76 ^b	39331.33±1411.36 ^b	31087.66±71.29 ^a	0.000
	No. of hatched eggs	19328.00±1419.37 ^a	34794.00±3966.94 ^c	23415.33±2010.57 ^{ab}	26627.33±1512.14 ^b	22589.66±430.35 ^{ab}	0.000
	Hatchability rate (%)	53.66±2.51 ^a	61.00±1.00 ^b	59.66±5.13 ^b	67.66±1.52 ^c	72.66±1.52 ^c	0.000
	Survival rate (%)	94.66±1.52 ^a	96.00±1.00 ^a	97.00±3.00 ^a	97.00±1.00 ^a	98.00±2.00 ^a	0.310
4	No. of fertile eggs	40586.00±2665.48 ^b	62802.66±11735.38 ^c	30912.33±2070.65 ^b	55282.00±7329.18 ^c	18817.00±1958.13 ^a	0.000
	No. of hatched eggs	19736.33±1423.20 ^a	34417.00±8211.90 ^b	19057.00±782.33 ^a	32159.33±5687.53 ^b	13088.66±1929.02 ^a	0.001
	Hatchability rate (%)	48.69±3.34 ^a	54.45±2.86 ^{ab}	61.83±4.89 ^b	58.03±4.21 ^b	69.38±4.45 ^c	0.001
	Survival rate (%)	91.66±2.51 ^a	96.00±1.00 ^b	96.33±1.52 ^b	97.33±1.15 ^b	98.00±1.00 ^b	0.004

Values are mean±SD (n=3). ^{ab} Rows with different superscript are significantly different (*p* < 0.0)

Discussion

Egg Quality of *Clarias gariepinus* Fed Dried *Azanza garckeana* Pulp Powder

Fish egg quality is highly dependent on broodstock nutrition, which directly impacts egg size, yolk composition, embryo viability, and early larval survival. Recent studies emphasize that providing a balanced diet containing adequate proteins, essential amino acids, fatty acids, and vitamin is crucial for maximizing reproductive performance in broodstock (Hernandez-de-Dios et al., 2022; Engdaw and Geremew, 2024). The results indicate that dietary inclusion of dried *Azanza garckeana* pulp powder had a positive effect on the egg

size of *Clarias gariepinus* broodstock, with higher concentrations generally enhancing egg development over the experimental period. While no significant differences were observed in the first week, suggesting that short-term exposure to AGPP may not immediately affect oocyte development. This is consistent with previous findings that herbal feed additives often require sustained feeding periods to elicit measurable effects on reproductive traits (Abdul Kari, 2025).

Subsequent weeks showed that certain supplementation levels significantly improved egg size compared to the control, suggesting a cumulative or delayed effect of the phytochemicals on ovarian development. These findings are consistent with recent studies demonstrating that bioactive compounds in medicinal plants, such as flavonoids, tannins, and alkaloids, can enhance reproductive performance in fish by stimulating vitellogenesis and yolk deposition (Engdaw and Geremew, 2024; Barad et al., 2024; Sepehrfar et al., 2023;). Improved egg size is particularly important because larger eggs generally contain greater yolk reserves, which can enhance embryo development, larval survival, and early growth performance (FAO, 2024).

Furthermore, the variation in egg size response across different supplementation levels aligns with previous reports suggesting that optimal dosing of plant-based feed additives is critical to achieve maximum reproductive benefits, as excessively high or low inclusion levels may not produce significant improvements (Oke et al., 2019; Olaniyi et al., 2020).

Milt Quality of *Clarias gariepinus* Fed Dried *Azanza garckeana* Pulp Powder

Milt quality is a key determinant of successful larval production in African catfish during artificial propagation (Kowalski & Cejko, 2019). It reflects the ability of sperm to fertilize eggs and is influenced by factors such as seminal fluid composition, milt volume, sperm density, and motility (Ogidan et al., 2018). Nutrition plays a critical role in male gamete quality, with well-balanced broodstock diets significantly enhancing semen characteristics and overall reproductive performance (Engdaw & Geremew, 2024; Izquierdo et al., 2001). Adequate dietary protein, lipids, and essential nutrients are particularly important to support the high energy demands of gonadal development and spermatogenesis in cultured species (Izquierdo et al., 2001). In this study, supplementation with dried *A. garckeana* pulp powder enhanced milt quality in male *Clarias gariepinus* including increases in milt volume, sperm motility, morphology, and total sperm count. These findings resonate with recent evidence that brood stock diets enriched in lipids and fatty acids can enhance sperm quality and functionality across various fish species (Figuerola-Villalobos et al., 2025). Specifically, the fatty acid composition of the diet is known to impact sperm membrane structure, energy production, and motility, because spermatozoa rely heavily on mitochondrial fatty acid oxidation for ATP generation required for motility (Köprücü et al., 2015).

Moreover, sperm motility, a key predictor of fertilization success improved in treatments with higher inclusion of *A. garckeana* powder. This aligns with results from other studies

showing that diets supplemented with polyunsaturated fatty acids (PUFAs) significantly increase sperm motility and overall seminal quality (Ciji et al., 2025). The improved sperm morphology observed in this study may be attributable to antioxidative protection afforded by phytochemicals present in the pulp, which could protect sperm cell membranes and DNA from oxidative damage a mechanism recognized in fish reproductive studies (Sepehrfar et al., 2023).

Total sperm count was significantly higher in the supplemented groups, indicating that dietary inclusion of *A. garckeana* pulp may enhance spermatogenesis and milt production by providing nutritional and biochemical support (Mir & Bhat, 2021). Similar improvements in milt quality have been reported in male *Clarias gariepinus* fed *Kigelia africana* seed powder (Adeparusi et al., 2010) and bitter kola seed meal (Diyaware et al., 2012), in striped catfish (*Pangasianodon hypophthalmus*) receiving varied diets (Pamungkas et al., 2023), and in rainbow trout (*Oncorhynchus mykiss*) supplemented with dietary n-3 fatty acids (Kayal et al., 2020).

Fecundity of *Clarias gariepinus* Broodstock Fed Dried *Azanza garckeana* Pulp Powder

The results in Table 4 indicate that dietary supplementation with dried *Azanza garckeana* pulp powder had a significant positive effect on fecundity and ovary development in *Clarias gariepinus* brood stock. Female fish fed the 10 g/kg *A. garckeana* diet (T2) consistently showed higher ovary weights and total egg production compared to other treatments and the control across the four-week period. This suggests that the bioactive compounds in the pulp, likely including bioflavonoids, xanthones, and essential micronutrients, may enhance gonadal development and stimulate oocyte production, thereby improving reproductive output (Barde et al., 2023).

Enhanced fecundity in response to plant-based dietary supplementation has been documented in several fish species. For instance, supplementation with medicinal plants such as *Garcinia kola* and *Kigelia africana* has been shown to increase ovary weight and fecundity in *Clarias gariepinus* by promoting steroidogenesis and vitellogenesis (Dada & Ajilore, 2009; Adeparusi et al., 2010; Dada, 2012). Similarly, Oniya et al. (2015) reported improved reproductive performance in African catfish fed *A. garckeana* pulp. These studies collectively support the role of phytochemicals as reproductive enhancers, possibly through antioxidative protection of gonadal tissues and modulation of estrogen synthesis, which is critical for oocyte maturation (Izquierdo et al., 2001; Sepehrfar et al., 2023).

The observed increases in ovary weight and fecundity in T2 compared to the control and other treatment groups align with the principle that brood stock nutrition directly influences gametogenesis. Adequate levels of dietary proteins, lipids, vitamins, and antioxidants provide the energetic and biochemical resources necessary for gonadal development and egg production (Engdaw & Geremew, 2024). The variability observed among other treatment groups may reflect differences in the bioactive concentration or digestibility of *A. garckeana* at varying inclusion levels, as well as possible interactions with other dietary components (Frías-Moreno et al., 2021).

Gonado-Somatic Index of Male *Clarias gariepinus* Broodstock fed dried *Azanza garckeana* Pulp Powder

Male body mass remained statistically unchanged across treatments, indicating that the observed differences in gonad weight and GSI were attributable to direct effects on reproductive tissues rather than somatic growth. Dietary inclusion of *Azanza garckeana* pulp powder elicited clear dose- and time-dependent modifications in gonadal development, with certain inclusion levels enhancing early testicular growth and others exerting more pronounced effects during later stages of the feeding period. These patterns are consistent with recent evidence demonstrating that targeted broodstock nutrition can modulate gonadal maturation independently of overall body mass (Torsabo et al., 2024). Two mechanistic pathways likely underpin these responses. First, the nutritional composition of the pulp particularly its lipid fraction and essential nutrients may support spermatogenesis and steroidogenic activity, thereby contributing to increased gonad mass and elevated GSI. Previous studies have shown that brood stock diets enriched with balanced fatty acid profiles and adequate macronutrients directly enhance testicular development, sperm production, and reproductive indices in finfish (Sándor et al., 2024). Second, the phytochemical constituents of *A. garckeana*, including flavonoids, tannins, and xanthenes, may provide antioxidant and endocrine-modulating effects that promote testicular function and protect germ cells from oxidative stress. Plant-derived bioactive compounds are increasingly recognized for their capacity to improve semen quality, stimulate gonadal growth, and mitigate oxidative damage in reproductive tissues (Sepehrfar et al., 2023). Experimental work on *A. garckeana* and related botanical supplements further supports their ability to enhance male reproductive organ integrity under both normal and stress-induced conditions.

The temporal variability in GSI observed across sampling weeks is characteristic of short-term nutritional trials. Gonadal tissues often exhibit delayed or cyclical responses due to endocrine rhythms, nutrient assimilation dynamics, and the sequential progression of spermatogenesis. Similar fluctuations have been reported in broodstock fed phytochemical or functional dietary additives, with peak responses frequently emerging only after sustained dietary exposure (Eissa et al., 2024).

Effect of Dried *Azanza garckeana* Pulp Powder on the Hatchability and Survival Rate of *Clarias gariepinus* fry

Male reproductive performance is central to hatchery success in *Clarias gariepinus*, and the present results show that dried *Azanza garckeana* pulp powder can markedly enhance hatchability and fry survival. The superior performance of supplemented groups supports the role of *A. garckeana* as a functional broodstock additive that improves both gamete quality and progeny viability, consistent with recent studies highlighting broodstock nutrition as a critical driver of reproductive efficiency in aquaculture (Pandey, 2024; Engdaw & Geremew, 2024). Improved hatchability observed in treated groups may be linked to

better yolk quality and higher energetic reserves, as adequate brood stock intake of lipids and essential fatty acids is known to support embryogenesis and early fry fitness (Izquierdo et al., 2001). Additionally, the phytochemicals in plant-derived supplements particularly antioxidants and polyphenols can protect reproductive cells from oxidative damage, enhance membrane stability, and promote overall brood stock health, thereby improving larval survival (Rashidian et al., 2023). Similar benefits of herbal additives for reproductive performance have been documented in other fish species (Ashry et al., 2024). The treatment-dependent variation observed here further indicates that reproductive responses to *A. garckeana* are dose-sensitive, reflecting a common pattern in functional feed trials where optimal supplementation promotes maximal benefits while excessive or insufficient levels reduce effectiveness (Engdaw & Geremew, 2024).

The hatchability rates observed in this study were consistently high across all weeks, with values of 80.66–100.00% in week one, 84.66–100.00% in week two, 94.66–98.00% in week three, and 91.66–98.00% in week four. The control group consistently showed the lowest hatchability, whereas the 40 mg/kg (T5) diet produced the highest rates throughout the trial. Overall, these results indicate a substantial improvement in reproductive performance compared with earlier studies. Diyaware et al. (2012) reported much lower hatchability (42.78–58.55%) in *Clarias gariepinus* brood stock fed *Garcinia kola*. Similarly, Dada (2012) found hatchability rates of 48.99–70.63% when brood stocks were fed *Garcinia kola* seed meal. Diyaware et al. (2010) observed a hatching range of 63–88.27% in *C. anguillaris* fertilized with milt obtained through male ablation. Dada and Ogunduyile (2011), also documented hatchability values between 61–91.37% in *C. gariepinus* brood stock treated with varying doses of *Mucuna pruriens* seed meal. Compared with these previous findings, the current study demonstrates that *Azanza garckeana* pulp supplementation supports significantly higher hatchability, emphasizing its potential as a superior natural broodstock dietary additive.

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Conclusion and Recommendation

The findings indicate that *A. garckeana* pulp powder enhances both male and female *C. gariepinus* brood stocks gonadal development and increases GSI through complementary nutritional and phytochemical mechanisms. The observed improvement in egg fertilization success, hatchability rates, and early fry survival associated with *Azanza garckeana* pulp powder supplementation supports the idea that brood stock diet optimization including functional plant-based additives can substantially enhance seed production in *Clarias gariepinus*. By improving yolk quality, supporting embryonic development, and protecting reproductive cells, such supplementation could offer a sustainable, low-cost strategy for improving hatchery output. Given the promising results, further studies should focus on defining optimal inclusion levels, assessing long-term effects on progeny performance, and characterizing the biochemical composition of eggs and larvae to fully understand the mechanisms behind the enhancement and support the development of effective, natural reproductive enhancers for sustainable catfish aquaculture.

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