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ORIGINAL RESEARCH ARTICLE

Synergistic Effects of Hormonal Inducers and *Terminalia catappa* Extract on the Breeding Efficiency of *Pangasianodon hypophthalmus* (Sauvage, 1878)

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ABSTRACT

The improvement in global aquaculture has motivated the varying levels of improvement in fish seed and grow-out production methods. Successful breeding practices are vital to meeting the increasing demand for high-quality fingerlings, with hormonal agents playing a pivotal role in stimulating ovulation and spermiation in culturable fish species. The study assessed the impact on the reproductive productivity of the striped catfish (*Pangasianodon hypophthalmus)* of three common hormonal inducing agents in Nigeria: pituitary gland extract, Ovatide, and Ovaprim. *An* extract from Indian almond leaves (*Terminalia catappa*), *a natural* supplement, was utilized for a successful breeding outcome. The pituitary gland was extracted using standardized procedures, and the synthetic hormones were sourced from reputable retailers. Early-stage fry survival and hatchability rates were used to evaluate successful breeding. Results reveal significant variations amongst the treatments; the most successful in promoting egg production per broodstock was Ovatide, weighing 144.00±0.22g, followed by Ovaprim, 142.00±0.22g and pituitary gland extract, 122.60±0.22g, respectively. Conversely, pituitary gland extract showed a higher hatchability rate of 82.67±0.51%, therefore proving its effectiveness in guaranteeing successful spawning. *T. catappa* increased hatchability by 18% ($p<0.05$) across all hormonal treatments, with larvae achieving a survival rate of over 71% for all agents. The first demonstration on synergistic action of hormonal therapies and natural additives has shown promising results in the breeding of *P hypophthalmus*. The optimal use of *T. catappa* leaf extract under varying environmental conditions to further enhance breeding efficiency is recommended.

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INTRODUCTION

The growth in global aquaculture production has enjoyed various forms of improvement, ranging from intensification of fish culture, development of improved strains, efficient fish seed production, and introduction of new culturable fish species, among others (Omitoyin, 2018; Ojewole *et al*., 2024).

Optimizing breeding protocols, particularly in commercially significant species such as striped catfish (*Pangasianodon hypophthalmus*), an exotic species in Nigeria, will be an addendum to culturable freshwater fish species in Nigeria. The striped catfish, *P. hypophthalmus*, is indigenous to the Mekong River basin and is known for its rapid growth rate, adaptability to varying environmental conditions, and contributing to the country's aquaculture productivity and the world aquaculture industry (De Silva and Phuong, 2011; Chowdhury et al., 2020; Irabor et al., 2022). These features prime striped catfish as a good aquaculture candidate for Nigerian fish farms in order to improve Nigeria's domestic fish production. According to Dasuki et al. (2013), the country's aquaculture production is dominated by the small-scale fish farm; hence, improvement and sustainability can be attained via the production and availability of good seeds and the introduction of new culturable species.

Production of *Pangasianodon hypophthalmus* depends on the quality of fish seed that is available and is related to the number and quality of overall seeds produced. It is of note that *Pangasianodon hypophthalmus* hardly reproduce in captivity, therefore limiting the breeder with the option of hormonal inducement. Hormonal agents stimulate ovulation and spermiation, synchronizing breeding and improving overall reproductive efficiency (Zohar & Mylonas, 2001). Despite their widespread use, the effectiveness of various hormonal agents in inducing spawning and optimizing hatchability varies depending on the species, dosage, and environmental conditions (Ashraf et al., 2024).

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Commonly used hormonal agents according to Amirah et al. (2022) for induced fish spawning include synthetic analogues of gonadotropin-releasing hormone (GnRH), such as Ovaprim, human chorionic gonadotropin (HCG), Ovaplant, Ovatide and pituitary extracts. According to Woo *et al.* (2021) and Benini *et al.* (2022), each of these hormonal agents operates via different physiological pathways, and they influence gamete maturation, ovulation, and fertilization success. Chaturvedi et al. (2015) noted that different studies recorded the variations in response of different catfish species following the administration of hormones. Ashraf et al. (2024) reported that using a 0.7ml/Kg body weight of Ovaprim recorded a 65% fertilization rate in *P. hypophthalmus* with a lowered latency period, while Okomoda et al. (2017) recorded an 86 – 89% fertilization rate with a 0.5ml Kg^{-1} for pure and reciprocal fertilization of Clarias and Pangasius. Dasuki (2021) reported a 98% success rate on Anabas strains using 0.5ml Kg^{-1} weight with Ovatide.

Indian almond *Terminalia catappa*, Linn. (Combretaceae), is a tropical tree with ethnomedicinal properties and one of the commonly used plants by aquarists and fish farmers worldwide to improve the health status of cultured fish (Wang et al., 2024). The leaf has been affirmed to contain some therapeutic compounds which have anti-bacterial and anti-fungal properties (Ahmed et al., 2005; Mahardika et al., 2024); some natural chemicals that can reduce the pH of water (Caruso et al., 2013) and decrease water acidity (Ikhwanuddin et al, 2014). Among the Indonesian farmers, the red leaves of the India almond are soaked directly in the fish-rearing water, and sometimes, the dried powder is infused in the fish feed to reduce infection and mortality during fish culture.

Several authors have incorporated the extract into feeds like Orisasona et al. (2025) for *Heterobranchus longifilis,* Waris et al. (2018) for Betta species, Priyanto et al. (2016) for *Oreochromis niloticus* or as immunotherapy against diseases Chansue (2007) Goldfish; Lusiastuti *et al.* (2017) Tilapia and as immunostimulant in breeding protocols Dasuki (2021) *Anabas testudineus*, Mahardika et al. (2024) in hybrid groupers (*Epinephelus fuscoguttatus* x *E. lanceolatus*) attesting to its effectiveness as an anti-parasitic, anti-bacteria and anti-fungi qualities and thereby promoting growth. The use of *T. catappa* extract on striped catfish is to assess its potentiality to reduce the apparent mortalities reported during the early stages of live, to optimize the induction protocol and improve water quality. Further, the role of *T. catappa* leaf extract on the hatchability and survival of striped catfish productivity using different commonly used hormonal agents will be assessed, as there is a dearth of information on studies of *T. catappa* interaction with hormones in pangasius culture.

The variations in the production outcome of different hormones on hatchability for a breeding protocol cannot be overemphasized for a sustainable aquaculture practice. Thus, this study compared the efficiency of three common hormonal agents in Nigeria: pituitary gland extract, Ovatide, and Ovaprim on the egg production, hatchability, and survival rates of *Pangasianodon hypophthalmus,* exploring the role of *Terminalia catappa* extract on early fry survival.

MATERIALS AND METHODS

Description of the Study Area

The study was conducted in the Department of Fisheries and Aquaculture Laboratory, Faculty of Renewable Natural Resources, Federal University Dutsin-Ma, located in Dutsin-Ma Local Government Area, Katsina State. The region is located at latitude 12°27'14.11" N and longitude 7°29'50.03" E, within the Sudan Savannah ecological zone. Distinct wet and dry seasons are experienced in this semi-arid area.

Procurement and Management of Broodstock.

A total of twenty (20) *Pangasianodon hypophthalmus* broodstocks were procured from a reputable source consisting of fifteen (15) females (average weight of 1.65kg) and five (5) males (average weight of 1.20kg) stock. They were carefully acclimatized for a period of two (2) weeks in a 2m by 3m concrete tank. Selection of gravid fish was achieved by checking the sexual readiness and availability of eggs in the broodstocks ahead of use for the study according to the methods of Wardani et al. (2021) and Nwachi et al. (2024).

Collection and Management of Hormones

Pituitary glands were obtained from male African catfish (*Clarias gariepinus*), with an average weight of 1.20kg, which were procured from a reputable fish farm. The pituitary glands were extracted using the method described by Usman et al. (2021) and stored in vials containing acetone for future use. Ovatide and Ovaprim were sourced from a trusted fish agrovet shop in Kaduna, Kaduna State, Nigeria.

Hormones and Pituitary gland Application

An inducement protocol was carried out by injecting hormones intramuscularly into the dorsal musculature of the female pangas fish. Each extracted pituitary gland was crushed and dissolved in 12% saline solution at 2mL/kg (Abdulkareem, 2023). The synthetic hormones, Ovatide and Ovaprim were administered according to methods of Okomoda et al. (2017), as slightly modified at 0.5 ml/kg full dose with 0.2mL/Kg as preliminary injection and 0.3mL/Kg for stimulatory injection after 8 hours while, the male fish received an injection of 0.3mL/Kg at first simultaneous injection of the female pangas. A selection ratio of two (2) females to one (1) male, in duplicates, was grouped for each individual treatment during the experiment. The injected gravid fish (9 females and three males) were kept in separate plastic culture tanks.

Preparation of Indian Almond (Terminalia catappa) Leaf Extract

Fresh leaves of Indian almond were sourced from the Take-off Campus of Federal University Dutsin-Ma. The leaves were collected, sorted of foreign materials, and washed under running tap water to eliminate contaminants and dried at room temperature. The dried leaves were crushed to facilitate the release of the bioactive compounds. The infusion from the Indian almond leaf was obtained using the methods of Dasuki (2021) as slightly modified; 100g of the powdered leaves were boiled in 2 litres of dechlorinated water for 15 minutes (5000mg/100ml), and left to cool at room temperature. The resulting solution was filtered through a fine sieve to remove leaf residues.

Egg Collection and Fertilization of Pangasianodon hypophthalmus

Eight (8) hours after hormonal induction, matured eggs were collected from each of the induced female broodstock using the stripping method. Care was taken to avoid contamination and ensure that the eggs remained undamaged. Also, milt was obtained from the male broodstock, and the milt was diluted with a saline solution to maintain sperm viability before being spread evenly over the stripped eggs. The eggs and the milt were mixed using the wet fertilization method, ensuring thorough contact. The fertilized eggs were gently rinsed with *Terminalia catappa* infusion to remove excess sperm and debris before being transferred to aerated incubation systems under optimal water quality conditions (Temperature, Dissolved Oxygen, Total Dissolved Solids and pH). 500ml of the leaf infusion was added to each tank (164 × 48 × 38 cm), which was filled halfway to promote successful hatching in the experimental units. A flow-through water exchange was utilized in the hatching chamber to continuously optimize the water quality once eggs were hatched throughout the breeding period.

Data Collection

Data such as the number of eggs per kilogram of fish, the hatchability and survival rates were collected using standard methods. The total number of eggs per fish was calculated by estimating the number of eggs in 1g multiplied by the total weight of stripped eggs per fish. The number of eggs per kilogram of fish was calculated by dividing the total number of eggs obtained from a fish by the total weight of the fish. The hatchability rate (%) was estimated as the number of hatched larvae recorded and expressed as a percentage of the fertilized eggs, while the survival rate (%) was estimated as the number of surviving larvae after 48 hours. The effectiveness of the Indian almond leaf extract was determined by comparing the hatchability rates with and without the leaf extract. The experiment was conducted thrice, and data were obtained for each of the three trials.

Statistical Analysis

Data obtained were analyzed using descriptive and inferential statistics. Percentages, mean, and standard error were used to describe the number of eggs, hatchability and survival rates. One-way ANOVA was used to compare the effects of different hormonal agents on the quantity of eggs produced, hatchability, and survival rates. Means were separated using Tukey's HSD test at a 5% significance level using SPSS Statistics 23 (Statistical Program for Social Science) Software.

RESULTS AND DISCUSSION

The number of eggs per kg of the broodstock is presented in Table 1. The table shows that the pituitary gland extract led to an average egg production of 122.60g per broodstock, with a low standard error of 0.345, indicating consistent performance across trials. However, this treatment produced the lowest egg yield among the hormonal agents tested. The broodstock treated with Ovatide showed the highest efficacy in terms of egg production, producing an average of 144.00g of eggs per broodstock with minimal variability (SE = 0.224). This indicates both superior effectiveness and high consistency. The significant improvement in egg yield compared to the pituitary gland extract highlights Ovatide's stronger potential for enhancing reproductive performance in *Pangasianodon hypophthalmus*. Ovaprim-treated broodstock produced an average of 142.30 g of eggs, slightly less than Ovatide, with a higher standard error of 1.435. Nonetheless, Ovaprim's performance is still markedly better than that of the pituitary gland extract and far superior to the control group. The control group, which received no hormonal treatment, produced no eggs; this finding underscores the importance of hypophysation agents in artificial breeding programs, as natural spawning without induction appears to be ineffective in captive conditions. These findings align with existing studies that highlight the superior performance of synthetic hormonal inducers like Ovatide and Ovaprim over natural extracts in boosting reproductive output (Das et al., 2020; Sahoo et al., 2005).

Table 2 provides information on the hatchability rates of *Pangasianodon hypophthalmus* eggs across the three trials, illustrating the effectiveness of these agents in improving reproductive outcomes. The result demonstrates that the pituitary gland extract was the most effective hatching agent, yielding the highest average hatchability rate of 82.67%, compared to 61.33% hatchability rates recorded in Ovatide and Ovaprim treatments. The high performance displayed by pituitary gland extract, combined with a low standard error of 0.51, suggests a consistent and reliable impact on egg hatching across the three trials. The consistent performance indicates the pituitary gland extract's strong potential for use in artificial breeding programs. Although Ovatide and Ovaprim treatments resulted in significantly lower hatchability rates (61.33%), subtle differences in variability were observed, with Ovatide displaying a slightly higher standard error (0.63) compared to Ovaprim (0.54). This difference suggests that Ovaprim may provide more predictable outcomes, even though its average hatchability is no better than Ovatide. These findings corroborate earlier research showing that natural pituitary extract, being rich in gonadotropins, is highly effective in mimicking natural spawning conditions (Kumar et al., 2021). However, the relatively lower performance of Ovatide and Ovaprim suggests that their effectiveness may depend on specific environmental conditions or dosages, as reported by Olaniyi et al. (2022).

The survival rates of the hatchlings after 48 hours are presented in Table 3. Larvae from the broodstock treated with pituitary gland extract had lower survival rates ranging between 70% and 73% across the three trials, with an average of 71.67%. However, the minimal variability (SE= 0.14) demonstrates pituitary's consistent effectiveness in supporting larval survival. Larvae from Ovatide-treated broodstock showed a slightly higher average survival rate of 72%, with survival percentages ranging from 71 – 73%, with a slightly higher standard error (0.24) than that of the pituitary gland extract. Also, Ovaprim-treated broodstock produced larvae with an average survival rate of 72%, with the standard error being 0.20, which indicates slightly less consistency compared to the pituitary gland extract but more stability than Ovatide. However, there were no statistically significant differences ($p > 0.05$) in the survival rates among the three hormonal agents, indicating that all of them had similar performance in enhancing early-stage larval viability. Furthermore, each agent achieved survival rates above 70%, demonstrating their reliability in promoting successful hatching and subsequent larval development. The control group resulted in no surviving larvae, emphasizing the indispensable role of hormonal induction in the artificial breeding of *Pangasianodon hypophthalmus*.

Table 1: Weight of stripped eggs per female *Pangasianodon hypophthalmus* broodstock

Agent	First Trial (g)	Second Trial (g)	Third Trial (g)	Average (g)	Standard Error
Pituitary gland	120.00	123.00	125.00	122.60^{b}	0.35
Ovatide	145.00	146.00	141.00	144.00^{a}	0.22
Ovaprim	142.00	142.00	143.00	142.30^{a}	1.44
Control	0.00	0.00	0.00	0.00	0.00

Means followed by different superscripts in the same column are significantly different (P<0.05).

The absence of egg production in the control group reiterates the necessity of hormonal intervention for artificial breeding of this species.

Table 2: Hatchability rates of *P. hypophthalmus* broodstocks treated with different hormones

Treatment	First Trial	Second Trial	Third Trial	Average Hatchability (%)	Standard Error
Pituitary gland	80.00%	83.00%	85.00%	82.67^{b}	0.51
Ovatide	62.00%	61.00%	61.00%	61.33^{a}	0.63
Ovaprim	64.00%	60.00%	60.00%	61.33^{a}	0.54

Means followed by different superscripts in the same column are significantly different (P<0.05).

Table 3: Survival rate of *Pangasianodon hypophthalmus* larvae after 48 hours post-hatching

Treatment	1st Trial (%)	2nd Trial (%)	3rd Trial (%)	Average Survival Rate (%)	Standard error
Pituitary gland	70.000	73.00	72.00	71.67^{a}	0.14
Ovatide	72.00	71.00	73.00	72.00^{a}	0.24
Ovaprim	73.00	72.00	71.00	72.00^{a}	0.20
Control	Nil	Nil	Nil	Nil	Nil

Note: Means followed by different superscripts in the same column are significantly different ($p < 0.05$).

Table 4: Effect of *Terminalia catappa* leaf extract on hatchability rate of *Pangasianodon hypophthalmus*

Treatment	Average hatchability rate with leaf extract (%)	Average hatchability rate without leaf extract (%)	Standard Error (%)
Pituitary gland	83.00	65.00	1.43
Ovatide	82.00	64.00	1.42
Ovaprim	83.00	64.00	1.41
Control	0.00	0.00	0.00

The slight differences in variability among treatments align with the findings of Ali et al. (2020), which suggest that while hormonal agents ensure high survival rates, external factors such as water quality and larval handling might influence variability. Table 4 shows the influence of Indian almond leaf (*Terminalia catappa*) extract on the hatchability rate of *Pangasianodon hypophthalmus* eggs when combined with the three hypophysation agents: pituitary gland extract, Ovatide, and Ovaprim. Marked improvement in hatchability rates was observed in all of the treatments when they were treated with Indian almond leaf extracts; a rise from 64 – 65% hatchability rates to 82 – 83% was recorded among the treatments. This rise in hatchability (about 18% increase) highlights the synergistic effect of *Terminalia catappa* on the efficiency of the pituitary gland, Ovatide and Ovaprim in promoting hatchability. These findings are consistent with previous findings of Adedokun et al. (2021), Rath et al. (2022) and Orisasona et al. (2025), which reported the antimicrobial, antifungal, and water-conditioning properties of *Terminalia catappa*, as well as its capacity to create a conducive environment for egg development and larvae survival.

The consistent improvement in hatchability rates suggests that the bioactive compounds in the leaf extract are effective across different hormonal treatments. Previous works have reported the potentiality of plant extracts in improving and promoting survival in fish, as well as their affordability and safety considerations both environmentally and for human consumption (Abdel-Tawwab *et al.*, 2018; Omitoyin et al., 2019; Ajani et al., 2020; Orisasona et al., 2025). This result emphasizes the versatility of *Terminalia catappa* as a natural enhancer in aquaculture systems.

Implications for Aquaculture Practices

Synthetic agents like Ovatide and Ovaprim are more effective for egg production compared to the natural extracts (Pituitary gland), making them suitable for high-yield operations. The pituitary gland extract, while less effective for egg production, remains superior for achieving higher hatchability rates. It is also of economic value as pituitary glands from sacrificed male *Clarias gariepinus* can be stored for future use. This study shows that *Terminalia catappa* leaf extract significantly enhances hatchability across all hypophysation agents. A natural solution to improve reproductive outcomes in *P. hypophthalmus* larvae. The improved survival (18%) suggests an enhanced nonspecific immune system, and a low variability across trials for all treatments suggests that these methods can be reliably applied, provided environmental parameters are optimized.

FUTURE RESEARCH DIRECTIONS

- **Optimization of *Terminalia catappa* Use:** Further studies should explore optimal dosages and application methods to maximize its benefits.
- **Environmental Interactions:** Investigate how water quality parameters interact with hormonal and natural additives to affect reproductive performance.
- **Broader Species Applicability:** Assess the effectiveness of these treatments across other economically important aquaculture species.

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