IMPACT OF GARLIC AQUEOUS EXTRACT ON THE FERTILIZATION, HATCHING RATES, AND EARLY GROWTH OF *Clarias gariepinus* L. (Burchell 1822)

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ABSTRACT
The efficacy of garlic aqueous extract on the fertilization, hatching rates, and early growth of *Clarias gariepinus* l. (burchell 1822)*) were investigated. The aim of the study was to minimize the use of synthetic chemicals by* evaluating the potential of an aqueous extract of garlic in the prevention of the pathogenic fungus *Saprolegnia* species on *Clarias gariepinus* eggs*. Fresh* Allium sativum *was procured from Maiduguri Monday Market, peeled, washed, and ground to obtain* the *garlic juice. The effects of garlic on the fertilization and hatching rate were obtained by fertilization of eggs after induced breeding. Fertilized eggs were submerged into different concentrations of the garlic extract for 5 minutes. Effects of garlic on early growth were obtained by stocking 50 fry each into different treatments for a period of 42 days feeding with commercial feed (3 times per day). The experiment was carried out using a completely randomized experimental design with six (6) treatments and five (5) replicates. The data obtained were subjected to one-way analysis of variance, and the differences between the means were separated using Duncans Multiple Range test. The results showed fertilization and hatching rates of 90.42% and 63.88% in T6 respectively. Therefore, based on the results obtained it is recommended that (2.5ml/l) of garlic extract was effective in saprolegnia on egg, and improved fertilization and hatching rate of Clarias gariepinus. The result obtained on growth performance showed the highest specific growth rate of (1.42%/day) was recorded in T6 and the highest survival rate 95.10% was also recorded in T6.*

Keywords: aqueous extract, pathogenic, saprolegnia, fertilization, stocking

INTRODUCTION

African catfish *Clarias gariepinus* is a freshwater fish species of African origin and is one of the world's most cultured fish species. This fish species is valued for its fast growth rate, diseases resistance, and high stocking density (Emiroglu *et al*., 2018). Charo and Oirere (2000) equally submitted that the major constraint to the intensification and expansion of fish culture in Nigeria lies in the inadequate supply of quality fingerlings and juveniles for stocking cages, ponds and pens. Atanda (2006) stressed that fish farmers in most parts of the country (especially the Northern part) are perpetually in need of hatchery produced fish seed for their farm which is mostly not available Catfish larva production impediment is traceable to low hatching and survival rates (Muchlisin *et al*., 2010) which could be linked to the adhesiveness of eggs.

Disease in fish management plays a critical role in lower fertilization and hatching rate, fungi infection has been identified as a major cause of mortalities and economic losses in cultured fish (Ashour *et al.*, 2017). Saprolegniasis is one of the diseases responsible for the mass mortality of fish eggs, larval and post-larval stage of freshwater fish species (El-Deen *et al*., 2018). Among fungal infections, Saprolegniasis is one of the most aggressive and problematic diseases for several wild and farmed species of fish in different life stages, especially eggs, while the available drugs are neither safe for the fish nor for human consumption of the treated fish, Products such as malachite green, formalin, hydrogen peroxide, potassium permanganate, and iodine are commonly used to combat harmful microorganisms in fish egg incubators (Fuangsawat *et al.*, 2011). In fish farms, these chemicals present a risk of contaminating aquatic environments and of having cumulative and carcinogenic effects, as well as being unreliable, leading to the search for potential substitutes (Reverter *et al.*, 2014).

Natural products can be an alternative to the above chemicals. Some plants have active compounds with antimicrobial, immunostimulating, and nutritional properties and are being used in aquaculture (Pereira *et al*., 2016). Plant-derived products are a promising source of bioactive molecules, while being readily available, less costly, and biocompatible (Bulfon *et al.*, 2015). Among these medicinal plants, garlic *Allium sativum* L. can help in the control of pathogens, especially bacteria and fungi, because it has antiviral, antifungal, and antibacterial properties (Santhosha *et al.*, 2013), and increase the welfare of fish (Corzo-Martinez, 2007). Garlic is a bulb plant, containing sulfide compounds such as allicin and ajoene, in addition to bioflavonoids (Lee and Gao, 2012). The objective of the current study was to evaluate the effects of garlic (*Allium sativum*) aqeous extract on the rates of egg fertilization and hatching, of African catfish *Clarias gariepinus.*

MATERIALS AND METHODS

The study was conducted in the teaching and research fish farm of the Department of Fisheries, University of Maiduguri, between latitude 11° 48' 16" North and longitude 13° 12' 12" East. The mean monthly temperature is (40.2°C) before the onset of the rain in June and the lowest (31.3°C) during the peak of the rainy period in August. The average annual rainfall is about 550 mm[3]

Collection and preparation of garlic extract

Six hundred grams (600g) of garlic bulbs were purchased from the local market in Maiduguri. The garlic were peeled and washed with tap water. The peeled garlic were weighed using a digital electronic scale (Gallenkomp, England). It was then ground using a wooden pestle and mortar to obtain the garlic juice. The garlic was filtered with muslin cloth as described by Suleria *et al.* (2013).

Experimental fish

To obtain the fry for the experiment, one-year-old gravid female and male fish of *C. gariepinus* (1000-1500g) were procured from a reputable fish farm for induced breeding. The gravid fish were being transported in 25L capacity horizontally cut plastic container half filled with water to the fish hatchery complex of the Department of Fisheries, University of Maiduguri. The gravid fish were acclimatized for 15 days in a polyethylene-lined earthen pond (7x5x1.2M). During the acclimatization, the fish were fed commercial feed with 35% crude protein before the commencement of the inducement.

The breeding was done using one female and one male. The female brood fish was induced with hormone (ovaprim) at 0.5 ml/kg of fish and kept in a separate tank ($2m^2$) before the latency period. At the end of the latency period, milt from the male was obtained using the method described by Diyaware *et al*. (2010). The milt sacs were washed to remove blood and fat using physiological saline solution and placed in a 100-ml beaker containing 0.9% saline solution. The milt was squeezed into a 10 ml beaker container with 1-2ml of physiological solution (0.9%) saline. Then eggs from the female were stripped into a clean receptacle. The milt in the beaker was used to fertilize the stripped eggs.

Experimental design

A total of 1g of fertilized eggs was placed on a nylon net (2cm) as an egg substrate. The fertilized eggs on the nylon egg substrates were placed in six (6) different concentrations of garlic aqueous extract: T1 (0 control), T2 (0.5 ml), T3 (1.0 ml), T4 (1.5 ml), T5 (2.0 ml), and T6 (2.5 ml) ml per liter of water for five (5) minutes as treatments (T1-T6). The treatment was replicated five times in a complete Randomized Design (CRD) manner. The aqueous garlic-treated eggs were incubated in a two (2)-liter capacity trough (10cm diameter x 5cm depth) filled with one (1) liter of water using a flow-through system.

Determination of fertilized and hatched rates

After eight (8) hours post-fertilization, the number of fertilized and dead eggs were counted photo-metrically using a digital camera. A snapshot of each treatment was transferred into a computer for a clearer and larger view of the eggs for counting. Translucent eggs containing embryonic eyes were considered fertilized eggs. While white or opaque eggs were considered unfertilized or dead eggs. The hatching rate was estimated photo-metrically after 24 hours post-hatching.

The fertilization and hatching rate were determined using the following formulae:

$$\text{Fertilization (\%)} = \frac{Number\ of\ fertilized\ eggs}{Number\ of\ eggs} x100$$

$$\text{Hatching rate (\%)} = \frac{Number\ of\ hatched\ eggs}{Number\ of\ fertilized\ eggs} x100$$

Determining the growth performance

A total of fifty (50) fry, each with recorded weight and standard length were stocked in a 40 cm diameter by 20 cm depth incubation trough containing five (5) liters of water for early growth. The fry was fed with a 40% crude protein commercial diet 3 times daily at the rate of 3% body weight. Dead fish were picked up immediately from the trough to avoid deterioration of water. At the end of the rearing period (42 days), the final weight (g), final length (cm), feed applied and mortality for each treatment was recorded. The following growth parameters were estimated for each treatment using the following formulae:

(i) Weight gain (WG) = final weight (FW) – initial weight (IW)

(ii) Daily weight gain (DWG) = $\frac{W_2 - W_1}{T}$

Where: W_2 = final weight, W_1 = initial weight, T = rearing period in days.

(iii) Specific growth rate (SGR) = $\frac{Log\ W_2 - Log W_1}{T} \times 100$

Where: $LogW_2$ = logarithm of the final weight of fish, $LogW_1$ = logarithm of the initial weight of fish, T = rearing periods in days.

(iv) Survival (%) = $\frac{N_o - N_e}{N_o} \times 100$

Where No = Initial total number of fingerlings, Ne = Total number of mortalities at the end of feeding trial of *Clarias gariepinus*

Monitoring of water quality parameters

Dissolved Oxygen (DO) (mg/l) and water temperature (^{o}C) were recorded daily using a digital DO/temperature analyzer (Model: JPB-608 DO). Also, pH was recorded daily using a digital pen pH meter (Model: ATC pH).

Statistical analyses

Data obtained from the study were subjected to one-way Analysis of variance (ANOVA), to test for significant differences in treatment means. Differences between the means were separated using Duncan's Multiple Range Teat (DMRT) at 95% level of confidence with aid of statistic 8.0.

RESULTS

The efficacy of garlic (*Allium sativum*) aqueous solution on the fertilization rate and hatching rate is presented in Table 4.1, while the percentage of dead eggs is presented in Figure 2. The result revealed that the highest fertilization rate was recorded in T6 with a value of 90.42%, followed by (88.44%) in T5 of *A. sativum* aqueous extract. However, the lowest fertilization rate (72.70%) was recorded in T1. However, there are significant differences ($p<0.05$) in all the treatments.

The highest hatching rate (63.88%) was observed in T6, followed by 58.64%, which was observed in T5 (A. *sativum* aqueous extract). The lowest hatching rate (40.84%) was recorded in T1 of *A. sativum* aqueous extract. However, the results showed a significant difference between the treatment groups with increased hatched eggs as the concentration of *A. sativum* aqueous extract increased.

Effect of *Allium sativum* aqueous extract on the early growth of *Clarias gariepinus*

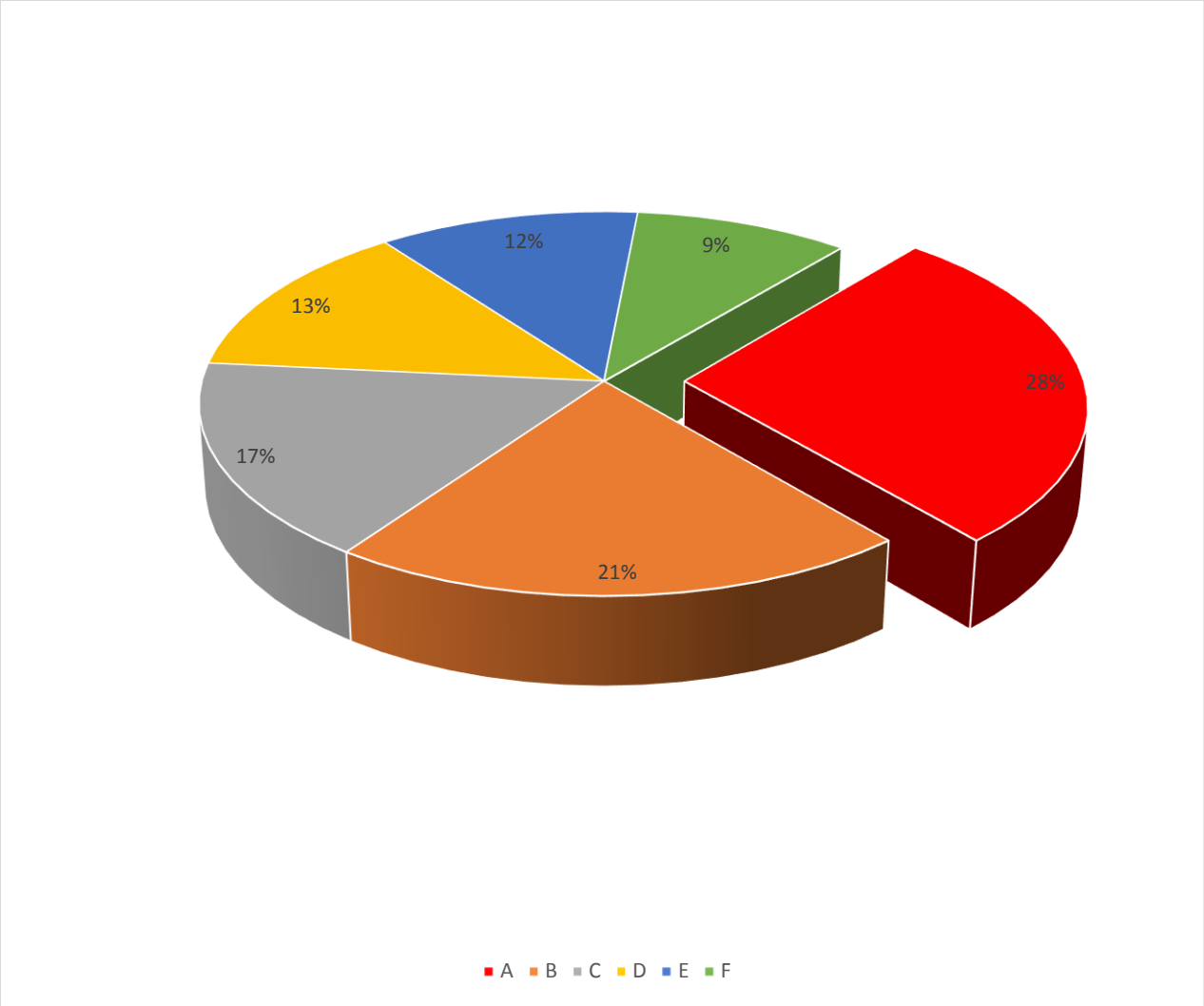
The effect of *Allium sativum* on the early growth of *Clarias gariepinus* is presented in Table 4.2. The initial weight values ranged from 0.34-0.60g from T1 to T6. The result indicates that he highest daily weight gain (0.090g) was observed in T6 of *A. sativum* aqueous extract, followed by (0.089g), which was recorded in T5 of *A. sativum* aqueous extract, while the lowest daily weight gain (0.044g) was observed in T1 of *A. sativum* aqueous extract. However, there was significant differences ($p<0.05$) between T1 and the remaining treatments of *A. sativum* aqueous extract.

Table 1: Efficacy of Garlic Aqueous Extract on the Fertilization and Hatching rates of *Clarias gariepinus*

	CONCENTRATIONS						
PARAMETERS	T1 (0.0ml^{-1})	T2 (0.5ml^{-1})	T3 (1.0ml^{-1})	T4 (1.5ml^{-1})	T5 (2.0ml^{-1})	T6 (2.5ml^{-1})	SEM
TNOE (g)	355.80^{a}	353.80^{a}	354.60^{a}	355.80^{a}	353.80^{a}	355.20^{a}	1.2463
NODE%	97.00^{a}	73.40^{b}	58.40^{c}	46.40^{d}	40.80^{e}	33.60^{f}	1.7682
FR%	72.70^{f}	79.20^{e}	83.48^{d}	86.32^{c}	88.44^{b}	90.42^{a}	0.6332
HR%	40.84^{f}	44.13^{e}	48.76^{d}	53.70^{c}	58.64^{b}	63.88^{a}	0.3897

*Means with different superscripts across each row, differ significantly ($p\leq0.05$).

Key: SEM= Standard Error of Mean, TNOE= Total Number of Eggs, NODE=Number of Dead Eggs, FR%=Fertilization ate, HR= Hatching Rate.



KEYS: A=T1(0ml), B=T2(0.5ml), C=T3(1.0ml), D=T4(1.5ml), E=T5(2.0ml), F=T6(2.5ml).

Fig.1: Percentage of dead eggs recorded.

Table 2: Effect of aqueous extract on early growth of *Clarias gariepinus*

	CONCENTRATION						
EARLY GROWTH PARAMETER(g)	0.0 (ml^{-1})	0.5 (ml^{-1})	1.0 (ml^{-1})	1.5 (ml^{-1})	2.0 (ml^{-1})	2.5 (ml^{-1})	SEM
IW	0.34^{d}	0.44^{c}	0.50^{bc}	0.56^{ab}	0.60^{a}	0.60^{abc}	0.0477
DWG	0.044^{b}	0.081^{a}	0.086^{a}	0.092^{a}	0.089^{a}	0.090^{a}	0.0110
FW	2.12^{b}	3.88^{a}	4.14^{a}	4.46^{a}	4.40^{a}	4.36^{a}	0.5230
SGR	0.058^{b}	1.276^{ab}	1.354^{a}	1.414^{a}	1.380^{a}	1.420^{a}	0.2116
SR%	52.68^{e}	83.84^{d}	86.14^{c}	94.08^{b}	94.50^{b}	95.10^{a}	0.3266

*Means with different superscripts across each row, differ significantly ($p \leq 0.05$).

Key: (IW)= Initial weight, (DWG)= Daily weight gain, (FW)= Final weight (SGR)= Specific growth rate, (SR)= Survival rate.

Water quality parameters

Figure 3 presents the water quality parameters recorded during the experimental period. The water temperature ranged from 24.28 to 27.24ºC throughout the research period. The pH values obtained were alkaline throughout the study; they ranged from 7.0 to 7.5 while the dissolved oxygen (DO) concentration was measured from 7.4 to 9.7 mg/l across the treatments

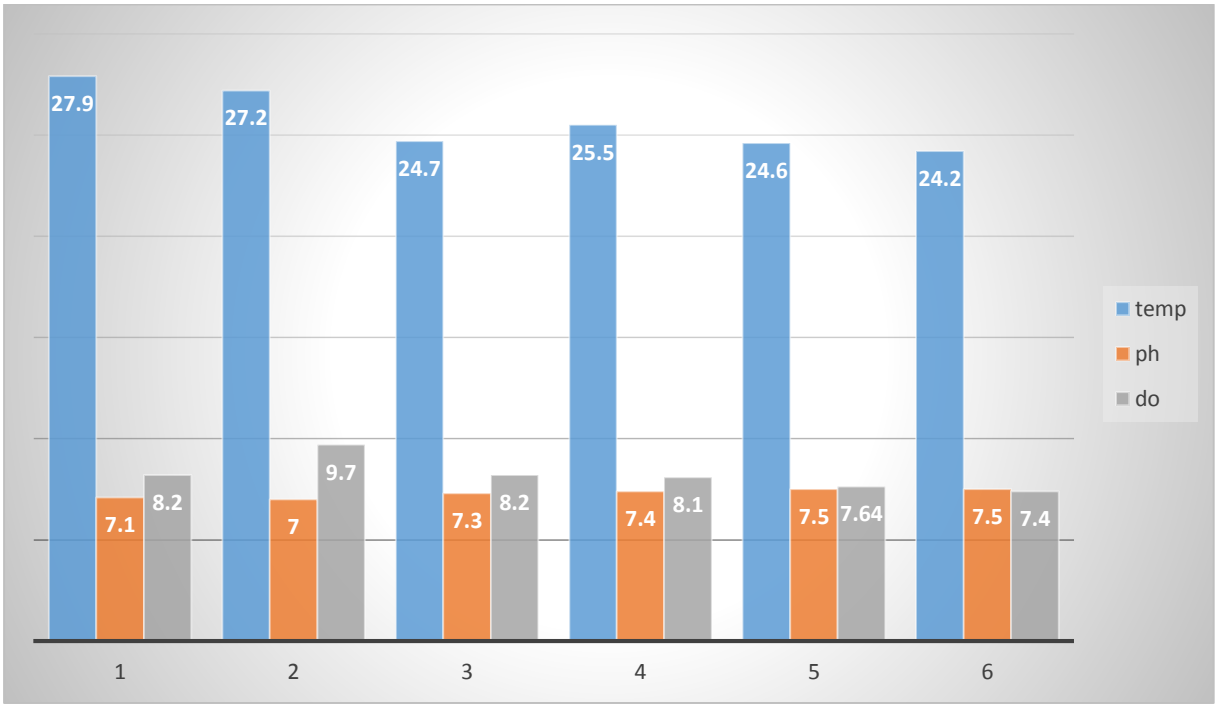


Fig. 2**: Mean** water quality showing parameters recorded in each treatment during the research period.

DISCUSSION

Higher fertilization and hatching rate success increased the production yield and economy. Sulfur compounds present in garlic act as antimicrobials that give protection to the egg from fungal attack. A higher fertilization rate was recorded in this study, which is in line with the report of Marengoni *et al.* (2017), who reported that garlic essential oil at higher concentrations increased egg fertilization with average of 74.86%. Also, Mousavi *et al.* (2009), reported a 70% hatching rate with a higher concentration of blended essential oils obtained from different medicinal plants (*Thymus vulgaris, Salvia officinalis, Eucalyptus globulus,* and *Mentha*

piperita) in rainbow trout (*Oncorhynchus mykiss*) egg incubation. *Matricaria chamomilla* extract on *Oncorhynchus mykiss* eggs (hatching rate 75.90%) and garlic skin aqueous methanolic extract Amiri and Meshkini (2019). *Cutcherry kaempferia* at higher concentrations improved the hatching rate by up to 66.5% (Junianto, 2017).

Fish continue to grow throughout their life, but due to fungal infection, the growth rate tends to be slow; in this study, the growth rate increased as the concentration of garlic increased. The improvement in growth parameters recorded may be due to the bioactive components of garlic, which include allin and allicin. Allicin is the most abundant compound, representing almost 70% of all present thiosulfinates (Mehrim, 2014). Physiological properties of allicin include an improvement in the performance of intestinal flora, thus improving digestion and consequently enhancing the utilization of energy and leading to an increase in the speed of bodily development (Kaur, 2020). The growth promotion effect of garlic can also be associated with its flavour, which increases food intake, improves digestion, and increases the availability of nutrients, leading to higher growth rates. A similar result was reported by Effendi *et al*. (2022) who reported a specific growth rate of 2.9% recorded using guava leaves at a dose of 1.5 g per 100 g of commercial feed for 2 months. Also, Megbowon *et al.* (2013) recorded a 1.97 specific growth rate at a dose of 30 g of garlic diet fed to tilapia for 12 weeks.

A similar result was obtained by Paulin *et al. (2021)* who reported fish fed 1% garlic had the best growth performance in terms of final weight (33.01 ± 2.99g). Also, Effendi *et al.* (2022) reported 69.67g of weight gain recorded using guava leaves at a dose of 1.5 g per 100 g of commercial feed for 2 months.

The higher survival rate is associated with the allicin content of the extract which enhanced immune response resulting from improved defense mechanism. A similar result was obtained by Sahu *et al*. (2007), who reported controlling *A. hydrophila* infection in *Labeo rohita* fingerlings, and they noted that the 0.1 and 0.5% added groups showed the highest level of survival (85%) compared to the control group (57%). Also, Aly and Mohamed (2010) also found that *O. niloticus* fed a 3% garlic-supplemented feed showed a significantly increased survival rate (85%) after a challenge with *A. hydrophila.* Also, Megbowon *et al*. (2013) recorded a 95.4% survival rate using 30g of garlic incorporated in tilapia feed fed for 12 weeks.

The water quality parameters recorded during this study were within the recommendations of Viveen *et al*. (1985) for the earing of African catfish fish.

CONCLUSION

Garlic extract at concentration of 2.5ml/l was able to improved fertilization and hatching rate of *Clarias gariepinus*. It means that garlic extract at concentration of 2.5m/l can be use in hatchery in the improvement of fertilization and hatching rate of *Clarias gariepinus*.

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