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CLIMATE CHANGE PARTNERSHIP ACTIONS FOR SUSTAINABLE FUTURE AND RESTORING LIFE ON EARTH

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PREFACE

There is a growing concern on the adverse impacts of climate on biodiversity. This phenomenon is greatly manifested in form of shifting weather patterns threatening global food security, health and species existence. Humanity is at the receiving end of the consequences of climate change hence there is a need to step up actions on all fronts- overtime, everywhere all at once.

This calls for collaboration, partnership and networking to strengthening synergy among relevant stakeholders in a bid to tackling climate change menace. This forms the basis for the theme of this year world Environmental conservation conference: **CLIMATE CHANGE PARTNERSHIP ACTIONS FOR SUSTAINABLE FUTURE AND RESTORING LIFE ON EARTH.** The theme is conceived with a view to create an interface for information sharing and offer opportunities for participants to refine their commitments and pledges in the quest to achieving Sustainability in the face of climate change.

This year World Environmental Conservation Conference is memorable in the sense that it received overwhelming funding from the host - West African Science Service on Climate Change and Adapted Land use). WASCAL is posed to provide information and knowledge at the local, national and regional level to cope with the adverse impacts of climate change. Thus, this conference will offer opportunities for participants to learn from good practices demonstrated and showcase by WASCAL during the course of the conference. It will also strengthen staff-student exchange and provide prospect for Doctorate Research Doctoral Research in West Africa Climate System Programme (DRP WACS) – WASCAL among others.

Special appreciation goes to the management of The Federal University of Technology, Akure the host institution, National Park Service and African Regional Center for Space Science and Technology Education-English (ARCSSTE-E) that co-host this conference. We equally acknowledge other private, individual and corporate organizations that have contributed towards the success recorded in this event.

All the submitted articles were subjected to strict double blind peer-review process by the reviewers that are experts in the area of the particular submitted manuscript. The accepted manuscripts are published in WECC 2023 proceedings and also available for download on the organization website (www.necorng.org).

The accepted manuscripts fall within the underlisted subthemes:

- Climate change adaptation strategies in Agriculture, Forestry and Other Land Use (AFOLU)
- Climate smart city and architectural landscape design
- Retrofitting and decarbonization in tourism and hospitality industry
- Indigenous knowledge and local innovation in climate change adaptation
- Climate risk management, health, safety and hygiene
- Carbon credit-offset marketing/circular economy
- ICT development in environmental conservation (image processing and acquisition, computer vision, graphics, speed, interface technology, HMD devices, GIS: Body Tracking, AI and IOT, VRT, IVE).

We commend our keynote speaker Prof. Douda Kone Director Capacity Building Department, WASCAL Headquarter, Ghana and other guest speakers Prof. Babatunde Rabiu, Director General, Chief Executive Office, African Regional Centre for Space Science and Technology Education-English (ARCSSTE-E) and Dr. Goni I. M., Conservator General National Park Service.

It is hoped that researchers, students and policy makers will find the papers in this book very useful. Even though all the papers were reviewed and edited, the content and option expressed remain essentially that of the authors and not necessarily that of Netlink Environmental Conservation Organization.

Dr. Oladeji S. O.

President Netlink Environmental Conservation Organization

Convener World Environmental Conservation Conference

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ABSTRACT

The study assessed the best immersion period and concentrations of the Cape gooseberry root Physalis peruviana that can efficiently remove egg adhesiveness of Clarias gariepinus. One male and one female C. gariepinus brood stock weighing 1.0 kg and 1.3 kg, respectively were used for the experiment. Three different concentrations (0.5%, 1%, and 2%) of Tannin extract from Cape gooseberry root were used. Tannic acid (0.75g) diluted in 1 litre of water was used as reference de-adhesion agent, while water was used as control. The fish eggs were rinsed with the solutions at different durations of 0.5, 1 and 2minutes. Each concentration and rinsing time was recorded in triplicates. Data generated were subjected to Univariate Analysis of Variance Test, and third order Polynomial regression analysis was then used to determine the best concentration and immersion period that neutralizes adhesiveness in eggs of C. gariepinus. Results of the study showed that there were no significant differences ($p \leq 0.05$) in the non-adhesive eggs and hatching of eggs immersed in Tannin extract from Cape gooseberry rootand Tannic acid solution but significantly different from the control. Therefore this study showed that Tannin extract from Cape gooseberry root at 1% concentration and 1minute immersion period is more efficient for egg adhesion and increase hatching in C. gariepinus.

Keywords: *Egg De-adhesion, African Catfish, Tannin extract, Cape gooseberry, Artificial propagation.*

INTRODUCTION

Aquaculture is one of the fastest growing global food production systems with developing countries contributing significantly to its growth (Haruna, 2003). It is an efficient means of providing income, job opportunities and food rich in protein. FDF (2015) estimated that Nigeria has a potential of producing 1.3 million metric tons of fish from aquaculture but current contributions to fish production stood at 278,706 metric tons.

The culture of clariid catfish dominated local fish production in Nigeria and has grown rapidly since 1985 till now because it grows fast and feeds on a large variety of food, tolerate a wide range of water quality conditions, relatively easy to reproduce in captivity and can be raised in high density resulting in high yield (Okechi, 2004). Ojutiku (2008) however, noted that the scarcity of fingerlings of this widely acceptable species is a major constraint to the rapid development of fish farming in Nigeria. Atanda (2006) stressed that fish farmers in most part of the country (especially the Northern part) are constantly in need of hatchery-produced fish seed for their farms which is usually not readily available. Documented evidences indicated that the total seed production and supply in Nigeria from all sources amount to 55 million fingerlings while the immediate market need stands at 500 million per annum (Atanda, 2007).

The relatively low production of Catfish larva is traceable to low hatching and survival rates (Muchlisin*et al.*, 2010) which could be linked to the adhesiveness of eggs. The clumping together of egg when they are released into the water leads to low fertilization and hatching rates (El-Gamal and El-Greisy, 2008). Adhesiveness of eggs can also cause high larval mortality leading to low survival rate and low production (Abigail *et al.*, 2010). In African catfish**,** eggs are covered with a layer of mucus that makes them stick together. In their natural environment, egg adhesiveness is a reproductive strategy for most teleost to protect the eggs from water drifting (Kareem *et al.*, 2017). In the wild, African catfish spawn at night in shallow water with temperatures above 22ºC and the eggs stick to the leaves and stems of vegetation (Little *et al.*, 1994). Adhesiveness covers the microphiles and hinders the sperms from fertilizing the eggs thus reducing chances of sperm getting in contact with the eggs (Prinsloo *et al.*, 1987). When the eggs come into contact with water during the incubation period, it clumps together and this reduces the chance of the eggs to hatch.

One of the ways to solve this problem is to rinse the eggs in certain solutions such as urea, mud, milk, kaolin etc. However, these solutions are sometimes species specific. Hence, there is a need for a significant study to find the best solution to eliminate the stickiness of the African catfish eggs so that fertilization, hatching and survival rates could be increased (Asraf *et al.*, 2013).Therefore, this study is focused on the optimum concentration and immersion period of tannin extract from Cape gooseberry root (*Physalis peruviana*) that efficiently remove adhesiveness of *C. gariepinus* eggs, increasehatching and survival of the larva produced. The results obtained from this study would help to improve hatchability, thereby increasing the supply of fish seed for production.

MATERIALS AND METHODS

Location of the Site for the Experiment

The experiment was carried out at The Teaching and Research Fish Farm of the Federal University of Technology, Akure, Ondo State.

Procurement of Experimental Fish

Apparently healthy male and female *C. gariepinus* with weight 1.0kg and 1.3kg respectively were procured from a reputable fish farm in Akure prior to the commencement of the experiment. Criteria for selection of brood stocks such as distended abdomen and free ooze of egg when slightly pressed in female brooder and reddish colour of the top of genital papillae in male brooder were considered.

Conditioning of Broodstocks

Selected brood stocks were kept in separate plastic holding tanks ($40x30x35cm^3$)containing aerated water (Oxyguard, London) and were acclimated and fed with commercial diet for five days prior to the commencement of the experiment. The brooders were then starved for 24 hours before the commencement of the breeding operation to ensure that their intestines are empty thereby preventing faeces from mixing neither with the eggs when stripping nor with the milt during abdominal dissection.

Collection of Cape Gooseberry (*Physalis peruviana*)

Fresh Cape gooseberry plants were collected within the Teaching and Research Fish Farm of the Federal University of Technology, Akure. It was identified as *Physalis peruviana* at the Herbarium of the Department of Crop, Soil and Pest Management, The Federal University of Technology, Akure using the taxonomic key of Odugbemi (2006).

Extraction of Tannin Extract from Cape Gooseberry

Air-dried root samples (from bulk collections) were pulverised and mixed with 70% acetone (200ml). The samples were filtered using a Whatmann filter paper No. 1, 18.5cm disc and the residual material rinsed in additional solvent. Two portions each of 50 ml acetone were used for additional rinsing. The sample extracts were then dried in a vacuum oven with model number LCBN53CF at 60^0C until a solid material is obtained according to Elgailani and Ishak (2016).

Test for Tannins

Two (2) grams of the extract was boiled with 5ml of 45% ethanol for five (5) minutes. The mixture was cooled and filtered. The filtrate was subjected to ferric chloride test which involves diluting 1ml of the filtrate in distilled water and adding two (2) drops of ferric chloride. A transient greenish to black color indicates the presence of tannins (Ukoha *et al.,* 2011).

Preparation of Tannin from Cape Gooseberry

The tannin extract was prepared into different concentrationsas follows:
0.5% = 0.5g of tannin extract in 99.5 ml of water.
1% = 1.0g of tannin extract gel in 99 ml of water.
2% = 2.0g of tannin extract in 98 ml of water.

Preparation of Tannic Acid Solution (De-Adhesion Agent)

Tannic acid solution that served as the reference de-adhesion agent was prepared by diluting 0.75g of tannic acid into one litres of water according to Żarski *et al.*,(2015).Water without any of the extracts served as the control.

Preparation of Spawning Bowls

Fifty three (53) spawning bowls of four (4) litres capacity were procured at Oja Oba, Akure for the experiment. The bowls were thoroughly washed and dried. The bowls were labelled according to the inclusion levels of the treatments (0.5%, 1% and 2%), control and tannic acid as well as the immersion periods (30seconds, 1minute and 2minutes). The bowls were filled with 100ml of water (control), 98ml of water with 2% tannin extract, 99ml of water 1%tannin extract and 99.5ml of water tannin extract0.5% respectively.

Administration of Hormone (Ovulin)

Female brooder (*Clarias gariepinus*) was removed carefully from the holding tank and placed on a slab where it was injected with synthetic hormone (ovulin) at a dosage of 0.5 ml per kg of fish. Injection was given intramuscular above the lateral line at angle 45^o with the needle pointing towards the gonad region according to Adebayo (2006). The needle was retracted after injection and the injected area was rubbed gently with a finger in order to ensure even distribution of ovulin throughout the muscle and also prevent backflow of the ovulin. The injection was done at 21.00 hour GMT and the injected brooder was kept inside separate plastic tank containing water and tightly covered with perforated lid to prevent it from jumping out.

Stripping of Eggs

After a latency period of twelve (12) hours, the female broodstock was carried gently; the body was cleaned with towel to avoid the eggs coming in contact with water which could make the micropyle close up. The brooder was tightly held with towel, slight pressure was applied on the abdominal cavity to strip the eggs inside a clean dried bowl.

Procurement of Milt

Testes was removed by abdominal dissection and cleaned with a tissue paper after which a small incision was made on the lobes of the sperm sac. Milt was gently squeezed out and collected in a clean beaker.

Fertilization and Immersion

Wet fertilization was used in the experiment. Milt collected was mixed with saline solution. 1g of the striped eggs was carefully weighed on nylon and each measured eggs was fertilized with the prepared milt. The eggs was randomly rinsed inside the spawning bowls and subjected to the different treatments.

Experimental Design

Experimental design was Completely Randomized Design (CRD). Each treatment replicate received 1g of eggs (1g of eggs was measured using Metler balance, Model: Toledo PB 8001) using the reference value of 1g of egg = 700 eggs according to Viveen *et al.*,(1985).

The fertilized eggs were placed in three (3) treatment concentrations of Tannin extract from Cape gooseberry (0.5%, 1% and 2%), tannic acid solution (reference de-adhesion agent) and water (control). There were two(2) replicates to each of the inclusion levels. The exposure time was 30seconds, 1minute and 2minutes, respectively to determine the optimum concentration and immersion period of tannin extract from Cape gooseberry. After the speculated exposure period, the concentrated water was decanted, and replaced with clean aerated water to incubate the eggs in the spawning bowls.

Hatching Rates Determination

Incubation was monitored and observed after 24-36 hours depending on temperature. Numbers of larvae produced was then counted.

Hatching Index

Hatching index was calculated by multiplying 72-h embryo survival and hatching rates (Żarski *et al*., 2015).

Determination of Egg Adhesiveness Percentage

After the experimental trial, the percentage of completely free (non-adhesive) eggs in each experimental bowls was counted. The percentage of non-adhesive is estimated as:

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

Determination of Percentage Fertility and Hatchability

Fertility was evaluated after 30 minutes of incubation and the number of unfertilized eggs was counted, the fertilized egg (%) as well as the percentage hatchability was computed according to the method described by Adebayo (2006).

$$\text{Fertility (\%)} = \frac{Number\ of\ fertile\ eggs}{Total\ number\ of\ eggs} \times 100$$

$$\text{Hatchability (\%)} = \frac{Number\ of\ eggs\ hatched}{Total\ number\ of\ eggs}\ 100$$

Incubating Period

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

Deformity

The deformed larvae were counted in each treatment and their percentage was determined as:

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

Survival

After hatching and determination of hatching rate, the unhatched eggs was siphoned out of the spawning bowls in order to ensure the survival of the hatched ones and the water was partly changed to improve dissolved oxygen level of the water. The larvae were observed daily after hatching to determine the survival and percentage survival. The percentage survival was computed according to the method described by Adebayo (2006).

$$\text{Survival (\%)} = \frac{Number\ of\ larvae\ alive\ up\ to\ larvae\ stage}{Total\ number\ of\ hatchings} \times 100$$

Larval Rearing

Larval fed on their yolk sac for three (3) days and on the fourth day, the fry was fed with shell free artemia for two (2) weeks after which they were fed with commercial feed (0.5mm) twice daily for another two (2) weeks. Survival was monitored and recorded.

Larvae Size at Hatching (mm)

The total length of the larvae was calculated using Image J 1.34 software (Rasband 1997–2011) as described by Ben Khemis *et al* (2014).

Water Quality Parameters

Water quality parameters including temperature, pH and dissolved oxygen concentrations were monitored twice throughout the study period using mercury-in-glass thermometer (YSI-DO 550,U.S.A), pH meter (Hanna H198106 model) and dissolved oxygen meter (JPP-607 model) as described by APHA (1987). The water parameter kit to be used for the experiment was inserted into each bowls and readings were recorded.

Statistical Analysis

All percentage data at different concentrations and immersion periods were subjected to Univariate Analysis of Variance test. Also, Tukey Honestly significant different test was used as a follow up procedure. Polynomial regression analysis was then used to determine the best concentration and immersion period that effectively removed egg adhesiveness during artificial propagation at 0.05 significance level.

RESULT AND DISCUSSION

Percentage fertility of eggs of *C. gariepinus* exposed to varying concentrations and immersion periods of Tannin extract from Cape gooseberry root

The present study revealed that fertility of eggs immersed in tannin extract from Cape gooseberry root ranged from 54.67% at 2% concentration (2minutes) to 99.23% at 1% concentration (1minute) as shown in the Table below. The eggs immersed in tannic acid solution had fertility which varied from 94.58% at 2% (2minutes) to 98.04% at 0.5% concentration with the immersion period of 2 minutes. In control, fertility of 92.80% was recorded. Hence, there was no significant difference ($p>0.05$) in fertility between concentrations and immersion periods of eggs immersed in tannin extract from Cape gooseberry root, tannic acid solution and in control except for the eggs rinsed in 2% concentration and immersion period of 1 and 2minutes.The fertility reduces as concentration and immersion period increases for eggs immersed in tannin extract Cape gooseberry root.

This agreed with the findings of Asraf *et al.,* (2013), who recorded that one minute rinsing time gave the highest fertilization and lower clumping rate when immersed in urea. Fertilization of 96.17% was recorded for eggs immersed in tannic acid at 1% concentration with the immersion period of 1minute which was not significantly different from that of tannin extract from Cape gooseberry root.

The result obtained from the percentage fertility showed that fertilization decreases with increasing concentration and immersion period of tannin extract from Cape gooseberry rootand tannic acid solution. This study corroborates the findings of Asraf *et al.*, (2013) who reported that fertilization increased at lowest concentration of urea and decreased when concentration of urea was increased.

Adhesiveness of eggs of *C. gariepinus* exposed to varying concentrations and immersion periods of Tannin extract from Cape gooseberry root

The non- adhesive eggs of *C. gariepinus* obtained after exposure to tannin extract from Cape gooseberry root ranged from 52.37% at 2% concentration (30 seconds) to 94.67% in 1% concentration (1 minute)as shown in the Tablebelow. The eggs of *C. gariepinus* in the varying concentrations and immersion periods of tannin extract Cape gooseberry root showed no significant effect on the adhesiveness of eggs ($p>0.05$). The eggs immersed in tannic acid solution had non-adhesive eggs which varied from 80.34% at 2% (2 minutes) to 92.52% at 0.5% with the immersion period of 1minute. The eggs of *C. gariepinus* immersed in water which served as control had 25.62% non-adhesive eggs which was significantly different ($p<0.05$) from the non-adhesive eggs of *C. gariepinus* exposed to tannic acid solution and tannin extract Cape gooseberry root.

The result for the percentage non-adhesive eggs showed thatdetachment was successful in lower concentration and immersion period of tannin extract from Cape gooseberry root and tannic acid solution with 94.67% and 92.52%, respectively with no significant difference.

This study compared favorably with the studies of Żarski *et al.,* (2015), who reported best result in groups of eggs submerged in tannic acid solution for 1 and 2 minutes (86.5% and 80.5%), respectively. It is also in line with the findings of Fawehinmi *et al.,* (2019), who reported that waterleaf extract which contains tannic acid was effective at concentration of 1% with the immersion period of 1minute to give the highest fertilization and lowest clumping rate. It as well agrees with the studies of Asraf*et al.* (2013) who reported that the optimal time needed to rinse African cat fish eggs was one (1) minute with urea.

Hence, tannin extract from Cape gooseberry root can be used at a concentration 1% with low immersion period of 1minute to effectively reduced *Clarias gariepinus* egg stickiness. At higher immersion period, de-adhesion occurs, but the hatching rate is affected. This agrees with the research of Kujawa*et al.*, (2009) who reported that increasing the concentration and immersion period resulted in a high mortality of embryos during hatching because high concentration of tannin could harden egg capsule.

Incubation period of *C. gariepinus* exposed to varying concentrations and immersion periods of tannin extract from Cape gooseberry root

Incubation period of eggs immersed in tannin extract from Cape gooseberry root ranged between 25hours 11minutes at 0.5% concentration (2minutes) to 23hours 27minutes at 0.5% concentration (30seconds) while incubation period in eggs immersed in tannic acid solution varied from 23hours 32minutes at 0.5% concentration with immersion period of 30 seconds to 24hours 39minutes at 2% concentration (2minutes). Hence, there was no significant difference ($p>0.05$) between concentrations and immersion periods of eggs immersed in tannin extract Cape gooseberry root, tannic acid solution including the control.

This agrees with the studies of Adebayo and Olayinka (2009) who reported that the first hatching after fertilization was 24.5hrs in lowest formalin treatment concentration, he stated that the more the exposure period of *C.gariepinus* eggs to Formalin, the higher the hatching time. Incubation period is directly affected by temperature and exposure period (SRAC, 2006).

Percentage hatchabilityof *C. gariepinus* exposed to varying concentrations and immersion periods of tannin extract from Cape gooseberry root

The lowest percentage egg hatchability (35.93%) was recorded at 2% concentration of tannin extract from Cape gooseberry root at immersion period of 2minutes while the highest hatching rate (87.37%) was recorded in the group exposed to 1% of tannin extract from Cape gooseberry root. 43.24% hatchability was recorded for the control. The egg hatchability of tannic acid (Reference de-adhesion agent) ranged from 71.77% with concentration of 1% and 2% with immersion period 1minute and 30seconds respectively to 85.07% at 0.5% concentration with 30seconds immersion period.

The percentage hatching decreases with increasing concentrations of tannin extract from Cape gooseberry root, this agrees with the findings of Riehl and Appelbaum (1991) who reported that the hatching rates decreases as urea concentration increased. Thai and Ngo (2004) reported the highest hatching rate of 86.3% in pineapple juice and the hatching rate of 70.2% in salt/urea/tannin with 1% concentration. Fawehinmi *et al.,* (2019) also reported that eggs immersed in waterleaf extract at immersion period of 1 minute gave the highest hatchability of about 70%.

Strongly tanned egg membrane hindered the ability of the embryos to hatch. Although they were fully ready to hatch, they were unable to break the egg membrane and therefore died of exhaustion. Sometimes the egg membrane was broken in a few places and the embryo was partly outside, and would be unable to free itself and died (Kujawa *et al.,* 2009).Zarski *et al.,* 2015 reported that application of longer immersions or higher tannin concentrations resulted in "inactivation" of this structure at an early egg swelling stage (about 5 min following fertilization) thus leading to hardening of an egg envelope and prevented larvae hatching (Demska-Zakes *et al.,* 2005; Kujawa *et al.,* 2010). Thus, the best immersion period needed to rinse African catfish eggs when using tannin extract from Cape gooseberry root is 1 minute and this result agrees with the finding of Zarski *et al.,* 2015 who concluded that it is crucial to apply the shortest possible immersion in a tannic acid at the lowest possible concentration. Also, Asraf *et al.,* (2013) stated that the optimal time needed to rinse the African catfish eggs was one (1) minute because fertilization and hatching rates were high and clumping rate was lowest when the eggs were rinsed for one (1) minute.

Percentage hatching index of *C. gariepinus* exposed to varying concentrations and immersion periods of tannin extract from Cape gooseberry root

The percentage hatching index decreases with increasing concentrations of tannin extract from Cape gooseberry root in relation with the hatchability as shown in the table below. The lowest percentage hatching index (9.55%) was recorded in the control while the highest hatching index (86.67%) was recorded in the group exposed to 1% of tannin extract from Cape gooseberry root with 1minute immersion period while percentage hatching index (57.14%) was recorded in group exposed to 0.5% of tannic acid solution with 30seconds immersion period. However, hatching index recorded in 1% concentrations with 1minute immersion periods of tannin extract from Cape gooseberry root and tannic acid solution were not significantly different ($p>0.05$) but were significantly different ($p<0.05$) from that of the control and other rinsing agents at varying concentrations and immersion periods.

This corroborate the findings of Zarski *et al.,* (2015) who recorded highest HI (P <0.05) in groups subjected to a 1 and a 2 minute immersions in tannic acid. This index represented the percentage (%) of the hatched larvae obtained from the initial number of eggs. It provided data at real production of *C. gariepinus* larvae form total number of eggs which were initially used for incubation (Zarski*et al.,* 2015).

Deformed larvae of *C. gariepinus* exposed to varying concentrations and immersion periods of tannin extract from Cape gooseberry root

No deformity of larvae was observed in this experiment. The survived larvae were very active and responsive to feeding. This result compared favourably with the findings of Zarski *et al.,* (2015) who recorded that both the periods and the immersion duration did not have an effect (p> 0.05) on the deformity rate in the hatched larvae when immersed in tannic acid.

Percentage survival of *C. gariepinus* exposed to varying concentrations and immersion periods of tannin extract from Cape gooseberry root

The survived larvae percentage showed that survival decreased with an increase in concentration and immersion period as shown in the table below. 99.80% was the highest and this was observed in 0.5% with one(1) minute immersion period and lowest (51.60%) in 2% with two (2)minutes immersion period of tannin extract from Cape gooseberry root. Also, 67.41% was the highest and this was observed in 0.5% with 30seconds immersion period and lowest (54.89%) in of 2% with two (2) minute immersion period of tannic acid solution while survival of hatched larvae from the control group was 22.10% which was the least when compared with survived larvae exposed to tannin extract Cape gooseberry root and tannic acid solution.

This result was in agreement with the result of Akpoilih and Adebayo (2010) who reported that the survival decreased with an increase in the concentration level in formalin. This result was similar with the findings of Ljubobratović *et al.,* (2018) who observed 82.5% and 87.7% embryo survival in Alcalase-treated eggs and eggs treated with milk and kaolin, respectively.

Larvae size of *C. gariepinus* exposed to varying concentrations and immersion periods of tannin extract from Cape gooseberry root

The larvae size of *C. gariepinus* obtained for eggs immersed in tannin extract from Cape gooseberry root ranged from 3.03mm to 4.20mm at 1% (2 minutes) and 2% (1minute), respectively. Also, larvae size of *C. gariepinus* obtained for eggs immersed in tannic acid solution varied between 3.30mm to 4.43mm at 0.5% (30seconds) and 2% (30seconds) respectively while 3.43mm was recorded for that of control. Hence, there was no significant difference (p>0.05) in larvae size between concentrations and immersion periods of eggs immersed in tannin extract Cape gooseberry root and tannic acid solution but there was significant different (p<0.05) when compared with the control. Demska-Zakęś *et al.,* (2005) opined that when eggs are immersed too long in the rinsing agent (tannic acid), the egg size decreases or the eggs may even rupture due to the osmotic pressure.

Percentages of Egg adhesiveness, Fertility, Hatchability, Survival and Incubation period of Tannin extract from Capegooseberry root, tannic acid solution and water

Rinsing Agents	Concentration (%)	Immersion time (secs)	% Fertility	Non-adhesive eggs (%)	Incubation period (mins)	Hatchability (%)	Hatching Index (%)	Survival (%)	Larvae size (mm)
Water (Control)			92.80±0.35^{a}	25.62±0.10^{c}	1543±3.00^{a}	43.24±0.17^{c}	9.55±0.04^{d}	22.10±0.10^{c}	3.43±0.51^{a}
Cape gooseberry Root Tannin extract	0.5	30	98.80±0.20^{a}	67.87±3.02^{a}	1407±65.86^{a}	64.13±6.13^{b}	60.17±4.08^{b}	94.07±6.33^{a}	4.03±0.76^{a}
		60	98.40±0.43^{a}	77.53±6.44^{a}	1427±58.29^{a}	72.97±7.54^{a}	72.83±7.41^{a}	99.80±0.20^{a}	3.60±0.27^{a}
		120	93.27±0.35^{a}	57.53±6.44^{a}	1432±27.93^{a}	72.30±0.85^{a}	71.87±0.81^{a}	99.40±0.72^{a}	4.13±0.55^{a}
Tannic Acid	0.5	30	95.96±0.36^{a}	88.86±0.34^{a}	1412±2.65^{a}	85.07±0.33^{a}	57.14±0.22^{b}	67.41±0.26^{b}	3.30±1.01^{a}
		60	94.97±0.36^{a}	92.52±0.35^{a}	1413±2.65^{a}	75.66±0.29^{a}	56.63±0.22^{b}	67.12±0.26^{b}	4.07±0.75^{a}
		120	98.04±0.37^{a}	86.62±0.33^{a}	1421±2.67^{a}	72.17±0.27^{a}	41.90±0.16^{c}	56.15±0.20^{b}	3.80±0.66^{a}
Cape gooseberry Root Tannin extract	1	30	98.87±0.15^{a}	86.23±0.76^{a}	1469±7.64^{a}	85.37±0.95^{a}	85.03±0.83^{a}	99.57±0.12^{a}	3.50±0.36^{a}
		60	99.23±0.20^{a}	94.67±1.82^{a}	1468±9.50^{a}	87.37±2.39^{a}	86.67±2.88^{a}	99.20±0.66^{a}	3.90±0.82^{a}
		120	86.40±0.70^{a}	77.73±4.73^{a}	1470±8.51^{a}	84.30±1.40^{a}	79.43±4.77^{a}	94.20±4.24^{a}	3.03±0.35^{a}
Tannic Acid	1	30	96.80±0.37^{a}	86.35±0.33^{a}	1413±2.66^{a}	81.44±0.31^{a}	51.65±0.20^{b}	65.28±0.25^{b}	4.03±1.01^{a}
		60	95.06±0.36^{a}	86.57±0.33^{a}	1415±2.66^{a}	72.25±0.27^{a}	49.25±0.19^{c}	62.37±0.24^{b}	3.53±1.27^{a}
		120	96.91±0.37^{a}	82.86±0.31^{a}	1416±2.66^{a}	71.77±0.27^{a}	44.57±0.17^{c}	62.33±0.24^{b}	3.83±1.10^{a}
Cape gooseberry Root Tannin extract	2	30	77.77±4.87^{a}	52.37±0.46^{a}	1490±18.58^{a}	54.67±5.01^{b}	58.13±0.72^{b}	60.20±6.17^{a}	3.77±0.57^{a}
		60	60.13±1.42^{b}	63.17±0.38^{a}	1497±15.53^{a}	45.00±2.10^{c}	31.20±0.70^{c}	69.40±3.38^{b}	4.20±0.85^{a}
		120	54.67±1.29^{b}	67.27±0.29^{a}	1511±30.62^{a}	35.93±2.87^{c}	18.50±2.60^{d}	51.60±7.34^{b}	3.43±0.51^{a}
Tannic Acid	2	30	94.97±0.36^{a}	82.18±0.31^{a}	1421±2.67^{a}	71.77±0.27^{a}	38.99±0.14^{c}	56.96±0.22^{b}	4.43±0.21^{a}
		60	95.61±0.36^{a}	80.53±0.31^{a}	1454±2.74^{a}	72.04±0.00^{a}	38.21±0.15^{c}	56.64±0.22^{b}	4.07±0.74^{a}
		120	94.58±0.36^{a}	80.34±0.31^{a}	1479±2.78^{a}	69.77±0.00^{b}	31.61±0.12^{c}	54.89±0.21^{b}	4.10±0.69^{a}

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

CONCLUSION AND RECOMMENDATION

This present study revealed that 1% of tannin extract from Cape gooseberry root with 1minute immersion period gave the highest fertilization, lowest sticky rate, highest hatchability and survival of *C. gariepinus.* However, tannic acid solution at 0.5% and 1% concentration with 1minute immersion period was also optimum for detachment of *C. gariepinus* eggs without affecting the fertilization, incubation period, hatchability and percentage survival rate. The use of tannin extract from Cape gooseberry root at lowest concentration and immersion period was not significantly different from the use of tannic acid solution. Therefore, the use of 1% of tannin extract from Cape gooseberry root with 1minute immersion period is recommended to fish hatcheries operators/ fish breeders in Nigeria because of its effectiveness, quick and simple technology.

Although, tannic acid which served as the reference de-adhesion agent was not significantly different from result gotten from tannin extract from Cape gooseberry root but it is more expensive. Hence, tannin extract from Cape gooseberry root is recommended for its efficacy, efficiency, cost effectiveness, availability, handling and processing. However, further study can be conducted to test the efficacy of tannin extract from other plant materials and compare it tannin extract from Cape gooseberry root. Also, this test should be carried out on more fish species apart from *Clarias gariepinus*. This will help us to develop a natural insemination protocol for fish egg de-adhesion which then lead to increase in fish productivity.

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