Original Research Article

Efficacy of Aloe vera gel and Water-leaf Extracts for Removal of Egg Adhesiveness During Artificial Propagation of African Catfish *(Clarias gariepinus*, *Burchel 1822)*

ABSTRACT:

The paper aims to study the best immersion period and concentration of the plant extracts under study (water-leaf and aloe vera gel) that can efficiently remove egg adhesiveness of *Clarias gariepinus*. Data generated were subjected to Multivariate Analysis of Variance Test, Tukey multiple range tests was used as a follow up procedure. Third order Polynomial regression analysis was then used to determine the best concentration and immersion period that neutralizes adhesiveness in eggs of *C. gariepinus*. The experiment was carried out at The Teaching and Research Fish Farm, Federal University of Technology, Akure, between October 2018 to December 2018. One male and one female*C. gariepinus* brood stock weighing 1.0kg and 1.3kg, respectively were used for the experiment. Three different concentrations (1, 3, and 5)% of the plant extracts were used. 2g of urea diluted in 4g NaCl/L of water was used as reference de-adhesion agent, while water without urea solution nor plant extracts was used as control. The fish eggs were rinsed with the solutions at different durations of 1, 3 and 5 minutes. Each concentration and rinsing time was recorded in duplicates. Results of the study showed that there were no significant differences (p=0.05) in the non-adhesive egg hatching of eggs immersed in Aloe vera gel and Urea solution. Number of non-adhesive egg and hatching increased in waterleaf extract which was significantly different *(P=0.05)* from eggs immersed in Aloe vera gel and water but not significantly different from urea solution. Therefore this study shows that waterleaf extract at 1% concentration and 1minute immersion period can efficiently remove egg adhesiveness and increase hatching in *C. gariepinus.*

Keywords: Aloe vera, Clarias gariepinus, egg adhesiveness, non-adhesiveness, waterleaf.

1.0. Introduction

Aquaculture has evolved as the fastest growing sector of agriculture in the world (1). It is perceived as a means of protein security, poverty alleviation, economic and community development for the populace in many developing countries (2). African catfish, *Clarias gariepinus* is considered as one of the most economically important culturable freshwater fish species that dominated local fish production in developing countries like Nigeria (3). The speciesis known for its favourable food conversion, resistance to diseases, excellent food meat quality (4) possibility for high stocking density under culture conditions and can tolerate wide ranges of environmental conditions (5). *C. gariepinus* is easily induced for breeding activity in the hatchery, and possess high feed efficiency and utilization (6). However, egg adhesiveness of *C. gariepinus* is one of the problems affecting hatching and cause high larval mortality which discourage Nigeria fish farmers (7). This problem is probably due to the demersal nature of catfishes eggs which becomes sticky after encountering water thereby adhering themselves to substrata (8). This problem can be solved either by rinsing the eggs of *C. gariepinus*

with certain solutions or coating the eggs with certain powders. For instance, (9) used urea solution to removed egg stickiness of *Clarias gariepinus* for 1 minute. Pineapple juice solution had effectively reduces stickiness of *Heterobranchus bidosalis* eggs for about 3minutes (10), tannic acids have also been used as rinsing agent in himri barbel, *Barbus luteus* (11) and enzymes (α-Chymotrypsin and Alcalase) in the common carp (12). Aloe Vera and waterleaf (*Aloe barbadensis* and *Talinum triangulare)* are readily available plants that contain many antioxidants, polysaccharides, minerals, proteins, enzymes vitamins (13; 14).To date, no study has been done to remove adhesiveness of the African catfish eggs using these plants. Therefore, this study focused on the optimum concentration and immersion period of Aloe vera gel and water leaf extract that efficiently removed adhesiveness of *C. gariepinus* eggs.

2.0 Materials and Methods

2.0.1 Study Zone

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

2.0.2 Fish Holding Facility

Apparently healthy male and female *C. gariepinus* weighing 1.0kg and 1.3kg, respectively were procured from a reputable fish farm in Akure prior to the commencement of the experiment. Brood stocks were kept in separate holding tanks (40 x 30 x 35cm^3)containing aerated water where they were acclimatized and fed with commercial diet for five days. The brooders were starved for 24hours before the commencement of the breeding operation.

2.1 EXPERIMENT 1

2.1.1 Collection and Identification of Aloe Vera Plant

A. vera leaves were cut with a sharp, clean knife at Alaba hostel around The Federal University of Technology, Akure. The leaves were identified as *Aloe barbadensis* by a Botanist in the Department of Crop, Soil and Pest Management, The Federal University of Technology, Akure.

2.1.2 Processing of Plants to Form Aqueous Extract

Aloe vera leaves were thoroughly washed with clean water, serrated edges of the plants were cut and the green barks stripped off with the use of a sharp knife. The bitter yellow latex was carefully skimmed out with a knife into a clean transparent nylon and put inside warm water. The gel gradually melted to form colourless liquid as the water temperature decreased.

2.1.3 Preparation of Aqueous Solution of Aloe Vera

Three different concentrations of aqueous solutions of Aloe vera were prepared at different concentrations:

1% = 1.0ml of aqueous plant extracts in 99ml of water.

3% = 3.0ml of aqueous plant extracts in 97ml of water.

5% = 5.0ml of aqueous plant extracts in 95ml of water.

2.2 EXPERIMENT 2

2.2.1 Collection and Identificationof Water-Leaf Extract

A sample of fresh waterleaf plants were collected within the Teaching and Research Fish Farm, Federal University of Technology, Akure and was identified as *Talinum triangulare* at the Herbarium of the Department of Crop, Soil and Pest Management, Federal University of Technology, Akure.

2.2.2 Preparation of Water Leaf Extract

Waterleaf leaves were thoroughly under a running tap. Leaves were plucked without the stem and extract squeezed out using an electrical blender, filtered using a muslin bag. The greenish extract gotten was stored in a clean, dry air tight transparent plastic container, labelled and refrigerated at 4^{o}C.

The greenish extract gotten was prepared into percentages as follows:

1% = 1.0ml of waterleaf extract in 99ml of water.

3% = 3.0ml of waterleaf extract gel in 97ml of water.

5% = 5.0ml of waterleaf extract in 95ml of water.

2.3 Preparation of Urea Solution (Reference de-adhesion agent)

Urea/NaCl solution that served as reference de-adhesion agent was prepared by diluting 2 g of urea in 4g NaCl in one litre of water.

Water without any of the extracts served as the control.

2.3.1 Preparation of Spawning Bowls

Fifty six spawning bowls of 4 litres capacity each, labelled according to the inclusion levels of the treatments and immersion periods. The bowls were filled with 100 ml of water (control), 99 ml of water (1 %), 97 ml of water (3 %) and 95 ml of water (5 %), respectively.

2.4 Milt and Egg Collections

Female brooder was injected with ovaprim at angle 45^{o} with the needle pointing towards the gonad region. The injected brooder was kept inside separate plastic tanks containing water and tightly covered with perforated lid. After a latency period of 12 hours, slight pressure was applied on the abdominal cavity to express the eggs inside a clean bowl. The male testes was removed by abdominal dissection and cleaned with a towel and milt was gently squeezed out and collected in a beaker

2.4.1 Fertilization and Immersion

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

2.4.2 Experimental Design

Each treatment replicate received 1g of eggs, (1g of eggs measured 650eggs using Metler balance, Model: Toledo PB 8001).

2.4.2.1 EXPERIMENT 1:

The fertilized eggs were placed in three treatment concentration of Aloe vera gel extract (1%, 3% and 5%), urea solution (reference de-adhesion agent) and water (control). There were two replicates to each of the inclusion levels. The exposure time was (1, 3 and 5 minutes) respectively to determine the optimum concentration and immersion period of Aloe vera extract. After the speculated exposure period, the concentrated water were decanted, eggs were replaced with clean aerated water and left to incubate in the spawning bowls.

2.4.2.2 EXPERIMENT 2:

The eggs were placed in the three treatment concentration of waterleaf (1%, 3% and 5%), urea solution (reference de-adhesion agent) and water (control). There were two replicates to each of the inclusion levels. The exposure time was (1, 3 and 5 minutes) to determine the optimum concentration and immersion period of

waterleaf extract. After the speculated exposure period, the concentrated water was decanted and eggs were replaced with clean aerated water.

2.4.2 Non-adhesive, Incubating Period, Hatching and Percentage Survival Examination

Percentage non-adhesive egg, incubation period, hatching and survival were assessed to determine the efficacy and efficiency of Aloe vera gel and waterleaf on egg adhesiveness. The parameters assessed were computed according to the methods described by Adebayo (2006).

Non-adhesive eggs (%) = number of non − adhesive eggs/initial number of eggs × 100

Hatchability (%) = number of eggs hatched/Total number of eggs counted × 100

Survival (%) = number of hatched larvae/Total number of hatchling × 100

2.5 Data analysis

All data generated were subjected to multivariate Analysis of Variance Test. Also, Tukey's multiple range tests 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.

3.0 RESULTS

3.1 EXPERIMENT 1: Effects of Aloe vera gel on *Clarias gariepinus* eggs

3.1.1Non-Adhesive Eggs of *C. gariepinus* exposed to varying concentrations and immersion periods of Aloe vera gel.

Result of eggs of *C. gariepinus* exposed to varying concentrations and immersion periods of Aloe vera gel is shown in table 3.1. Eggs of *C. gariepinus* in varying concentrations and immersion periods of Aloe vera gel showed no significant effect on the non-adhesive eggs (P=0.05). Eggs of *C. gariepinus* immersed in water was significantly different *(P=0.05)* from the non-adhesive eggs of *C. gariepinus* exposed to Urea solution and Aloe vera gel. However, Urea solution and Aloe vera were not significantly different (p=0.05) from one another.

3.1.2 Incubation Period of *C. gariepinus* exposed to varying concentrations and immersion periods of Aloe vera gel.

Incubation period of *C. gariepinus* was not affected by the varying concentrations and immersion periods of Aloe vera gel. Hatching was fast in eggs exposed to Aloe vera gel compared to the reference de-adhesion agent. However, eggs of *C. gariepinus* in control and urea solution are not significantly different (p>0.05) from one another statistically.

3.1.3 Hatching Rate Examination of *C. gariepinus* exposed to varying concentrations and immersion periods of Aloe vera gel

Percentage hatchability and survival showed that hatching rate increased with increasing concentration and immersion period of Aloe vera gel. This result was not significantly different (p>0.05) from percentage hatchability of urea solution. However, percentage of hatched larvae in the control group were significantly different from percentage hatchability of urea solution and aloe vera gel. Eggs immersed in 1 % concentration of Aloe vera gel were not hatched out probably due to high stickiness.

3.1.4 Percentage survival of *C. gariepinus* exposed to varying concentrations and immersion periods of Aloe vera gel

The percentage larvae that survived showed that survival increased with an increase in immersion period of Aloe vera gel. There was significant difference *(P=0.05)* at 5% concentration with immersion period of 3 minutes and 5 minutes of Aloe vera gel when compared with the control group. There was no significant difference ($p>0.05$) at 5% concentration level of Aloe vera with immersion period of 3 minutes and 5 minutes immersion periods of Aloe vera when compared with Urea at concentration levels of 5% and 3% with immersion period of 3 minutes and 5 minutes of Aloe vera.

Table 3.1: Percentage of non-adhesive eggs, hatchability, survival and incubation period of Aloe vera gel, Urea solution and water

Rinsing Agents	Concentration (%)	Immersion time(mins)	Non-adhesive eggs (%)	Incubation period (mins)	Hatching (%)	Survival (%)
Water (Control)			19.00±1.73^{b}	1515±5.00^{a}	17.93±3.15^{b}	16.00±2.00^{b}
Aloe vera	1	1	72.16±3.39^{a}	0.00±0.00	0.00±0.00	0.00±0.00
		3	68.23±4.69^{a}	0.00±0.00	0.00±0.00	0.00±0.00
		5	**66.93±5.70^{a}**	0.00±0.00	0.00±0.00	0.00±0.00
Urea (Reference de-adhesion agent)	1	1	75.70±3.39^{a}	1524±6.00^{a}	22.85±3.16^{b}	**10.91±1.53^{b}**
		3	70.16±7.85^{a}	1511±0.50^{a}	29.00±0.08^{b}	15.52±0.89^{b}
		5	**58.00±1.53^{a}**	1509±4.00^{a}	**22.00±1.69^{b}**	20.29±1.96^{b}
Aloe vera	3	1	81.31±5.00^{a}	1454±4.00^{a}	12.97±1.80^{b}	0.00±0.00
		3	77.54±5.39^{a}	1488±8.00^{a}	11.77±0.38^{b}	0.00±0.00
		5	78.00±1.23^{a}	1509±1.00^{a}	13.08±0.16^{b}	0.00±0.00
Urea (Reference de-adhesion agent)	3	1	81.47±1.15^{a}	1524±9.00^{a}	23.08±0.62^{b}	15.17±1.07^{b}
		3	77.16±8.39^{a}	1525±10.0^{a}	47.85±0.46^{a}	41.58±4.60^{a}
		5	73.34±5.34^{a}	1563±2.50^{a}	43.31±0.39^{a}	41.66±1.66^{a}
Aloe vera	5	1	79.92±4.84^{a}	1511±1.00^{a}	**11.23±0.92^{b}**	**5.53±1.53^{b}**
		3	**83.00±1.15^{a}**	1509±1.00^{a}	41.47±1.93^{a}	29.91±5.72^{a}
		5	73.00±0.85^{a}	1575±3.00^{a}	49.31±2.55^{a}	**39.78±0.88^{a}**
Urea (Reference de-adhesion agent)	5	1	79.24±3.85^{a}	1521±12.50^{a}	27.54±0.78^{b}	23.46±1.43^{b}
		3	81.54±2.77^{a}	1530±0.00^{a}	32.08±4.23^{ab}	27.39±2.50^{ab}
		5	**84.85±4.70^{a}**	1521±12.50^{a}	**50.23±5.77^{a}**	**49.25±3.83^{a}**

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

3.2 EXPERIMENT 2: Effects of Waterleaf extract on *Clarias gariepinus* eggs

3.2.1 Non Adhesive Eggs of *C. gariepinus* exposed to varying concentrations and immersion periods of waterleaf extract

Result of eggs of *C. gariepinus* exposed to varying concentrations and immersion periods of waterleaf extract is shown in table 3.2. Detachment of eggs reduces in waterleaf and urea solution with increasing concentration. Percentage non-adhesive eggs exposed to waterleaf and urea solution were not significantly different (p=0.05) from one another. However, urea and waterleaf were not significantly different *(P=0.05)* from one another. Percentage non-adhesive eggs exposed to waterleaf and urea solutions were not significantly different (p=0.05) from one another.

3.2.2 Incubation period of *C. gariepinus* exposed to varying concentrations and immersion periods of waterleaf extract

Incubation periods of eggs in urea solution and water were not significantly different (p>0.05) from each other but waterleaf was significantly different from both urea solution and water (control) *(P=0.05)*.Percentage hatching decreases with increasing concentrations of waterleaf extract. Hatching was delayed in eggs exposed to waterleaf extract and were significantly different *(P=0.05)* from both the reference de-adhesive agent and the control.

3.2.3 Hatching Rate of *C. gariepinus* exposed to varying concentrations and immersion periods of waterleaf extract

Percentage hatching decreased with increasing concentrations of waterleaf extract. Percentage hatched larvae in waterleaf extract at 1 % concentration and 1 minute immersion period was significantly different *(P=0.05)* from the control and other rinsing agent at varying concentrations and immersion periods.

3.2.4 Survival of *C. gariepinus* exposed to varying concentrations and immersion periods of waterleaf extract

There was no significant difference (p>0.05) at the concentration levels and immersion periods of larvae that survived in waterleaf extract but there was significant differences across the immersion periods *(P=0.05)* in the larvae that survived in urea solution.

Table 3.2: Percentages of non-adhesive eggs, hatchability, survival and incubation period of waterleaf extract, Urea solution and water.

Rinsing Agents	Concentration (%)	Time (minute)	Non-adhesive eggs (%)	Incubation period	Hatching (%)	Survival (%)
Water (Control)			19.00±1.73^{c}	1515±5.00^{a}	17.93±3.15^{c}	16.00±2.00^{b}
Waterleaf	1	1	93.77±4.08^{a}	1673±2.50^{b}	70.59±1.23^{a}	48.09±6.53^{a}
		3	88.16±3.70^{a}	1603±2.50^{b}	48.81±0.95^{b}	40.00±7.54^{a}
		5	78.16±5.85^{a}	1432±2.00^{b}	36.92±1.23^{b}	32.95±0.37^{a}
Urea (Reference de-adhesion agent)	1	1	75.70±3.39^{a}	1524±6.00^{a}	22.85±3.16^{c}	10.91±1.53^{b}
		3	70.16±7.85^{a}	1511±0.50^{a}	29.00±0.08^{bc}	15.52±0.89^{b}
		5	58.00±1.53^{b}	1509±4.00^{a}	22.00±1.69^{c}	20.29±1.96^{b}
Waterleaf	3	1	83.24±0.16^{a}	1605±2.50^{b}	29.39±0.77^{bc}	16.90±1.78^{b}
		3	58.85±1.93^{b}	1613±2.50^{b}	24.77±0.92^{c}	17.33±1.55^{b}
		5	60.70±1.31^{ab}	1678±2.50^{b}	22.46±2.00^{c}	16.70±0.23^{b}
Urea (Reference de-adhesion agent)	3	1	81.47±1.15^{a}	1524±9.00^{a}	23.08±0.62^{c}	15.17±1.07^{b}
		3	77.16±8.39^{a}	1525±10.0^{a}	47.85±0.46^{b}	41.58±4.60^{a}
		5	73.34±5.34^{a}	1563±2.50^{a}	43.31±0.39^{b}	41.66±1.66^{a}
Waterleaf	5	1	63.10±2.69^{ab}	1462±2.50^{b}	1.54±0.31^{d}	3.08±1.07^{c}
		3	61.47±0.54^{ab}	1474±0.00^{b}	0.16±0.16^{d}	0.08±0.08^{c}
		5	63.93±5.31^{ab}	0.00±0.00	0.00±0.00	0.00±0.00
Urea (Reference de-adhesion agent)	5	1	79.24±3.85^{a}	1521±12.50^{a}	27.54±0.78^{c}	23.46±1.43^{b}
		3	81.54±2.77^{a}	1530±0.00^{a}	32.08±4.23^{b}	27.39±2.50^{ab}
		5	84.85±4.70^{a}	1521±12.50^{a}	50.23±5.77^{b}	49.25±6.64^{a}

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

The optimum concentration that can efficiently remove egg adhesiveness in *C. gariepinus* using waterleaf extract was observed at concentration of 1.6 % using 3rd order polynomic regression as shown in Figure 1.

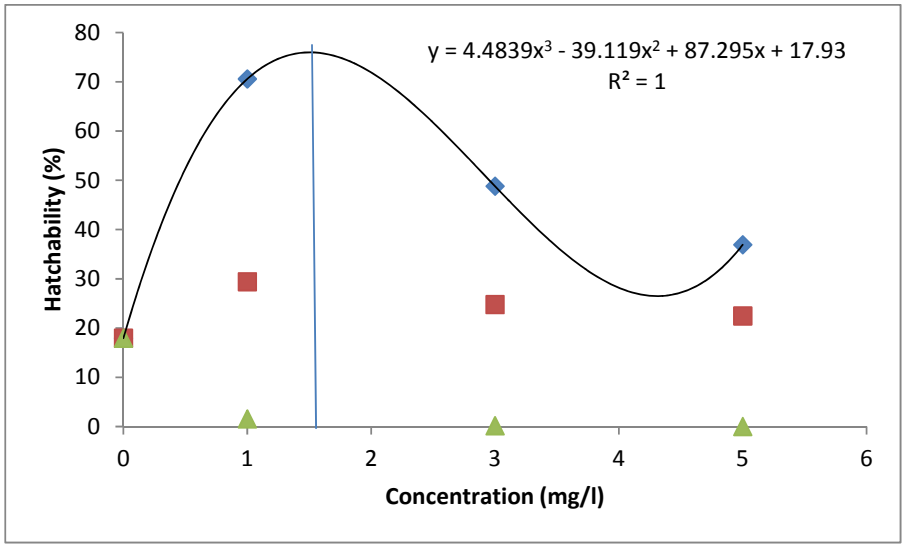

Fig. 1: The Optimum Concentration of Waterleaf Extract used as de-adhesive agent during artificial propagation of *C. gariepinus*

3.3 EXPERIMENT 3: Comparative Appraisal of The Effects of Aloe Vera Gel, Waterleaf Extract and Urea Solution on *C. gariepinus* Eggs

The concentration and immersion period that performed best in all the rinsing agents were compared in order to determine the best extract, concentration and immersion period that reduced egg stickiness of *C. gariepinus* as shown in Table 3.3.

Table 3.3: The main effect of varying concentration against immersion periods of aloe vera, waterleaf extract and urea solution on the removal of *C. gariepinus* egg adhesiveness.

Rinsing Agents	Concentration (%)	Time (minute)	Non-adhesive eggs (%)	Incubation period (minutes)	Hatching (%)	Survival (%)
Water			19.00±3.00^{c}	1515±5.00^{a}	17.93±3.15^{c}	16.00±2.00^{b}
Urea (Reference de-adhesion agent)	5	5	84.85±4.70^{ab}	1521±12.50^{a}	50.23±5.77^{ab}	49.25±3.83^{a}
Aloe vera gel	5	5	73.00 ± 0.85^{b}	1515±3.00^{a}	49.31±2.55^{b}	39.78±0.88^{a}
Waterleaf extract	1	1	93.77±4.08^{a}	1673±2.50^{b}	70.59±1.23^{a}	48.09±6.53^{a}

The mean values in the same column with different superscript is significantly different *(P=0.05)*.

3.3.1 Non adhesive eggs of *Clarias gariepinus*

The highest number of non-adhesive eggs was observed in waterleaf extract at 1.6% concentration which was significantly different *(P=0.05)* from aloe vera gel and the control but not significantly different (p>0.05) from urea solution as shown in Table 3.3 Percentage non- adhesive eggs in urea solution was not significantly different from eggs immersed in aloe vera gel but significantly different from eggs immersed in the control group.

3.3.2 Incubation period of *Clarias gariepinus*

The de-adhesive agents used in the experiment had no significant effect on the incubation period except waterleaf in which hatching was delayed.Incubation period in water leaf was significantly different from the other two rinsing agents and control, however, urea solution was not significantly different from the control and aloe vera gel.

3.3.3 Hatching percentage of *Clarias gariepinus*

Highest hatched larvae was recorded in group exposed to waterleaf which was not significantly different from group exposed to urea solution but significantly different from the control group and aloe vera gel.

3.3.4 Survival Rateof *Clarias gariepinus* Eggs

Survived hatched larvae in the three rinsing agents were not significantly different from one another but survived larvae in the control group was significantly different *(P=0.05)* from all the rinsing agents used in the experiment.

4.0 Discussion

4.1 EXPERIMENT 1: Effects of Aloe vera gel on *Clarias gariepinus* eggs.

4.1.1 Non-Adhesive Eggs of *C. gariepinus* exposed to varying concentrations and immersion periods of Aloe vera gel.

This finding revealed that eggs detachment increased as the concentration and immersion period increased, at 5% concentration with immersion period of 5minutes, Aloe vera gel effectively reduced *C. gariepinus* egg stickiness. This reduction could be attributed to the presence of alkaline protease which is one of the active ingredients in Aloe vera gel. Zakes *et al.,*(2006)used Alkaline protease at its highest concentration and immersion period to remove egg adhesiveness and increase hatching in Walleye (*Sander vitreus).* It was also observed that urea was not significantly different from Aloe vera gel. It effectively reduced clumping of eggs in *C. gariepinus* at the highest concentration and immersion period with enhanced fertilization and hatching rate. This result is similar to that obtained by Rothburd (1978) and El-Gamal and El-Greisy(2008) during the use of urea to reduce stickiness of carp eggs. Aloe vera gel concentration of 5% at 3minutes immersion period gave the best

detachment of 83%, it was however not significantly different from 5% with immersion period of 5minutes which gave percentage detachment of about 85%. The optimal time needed to rinse the African catfish eggs according to findings from this research is 5 minutes using Aloe vera and urea solution. This does not conform the findings of Asraf *et al.* (2013). He reported that the optimal time needed to rinse African cat fish eggs was one minute. Effective reduction of eggs stickiness at the highest concentration may also be due to the presence of enough Amylase at higher concentration in Aloe vera gel which helps to digest mucopolysaccharides by extracting the mucus and breaking the bonds (Mooi *et al.,* 1990; Abraham *et al.,* 1993) because at the lowest concentration of 1%, egg detachment was very poor. The result gotten from the percentage non-adhesive eggs showed the adhesive elimination was not successful for all the groups of eggs in Aloe vera gel, at concentration of 1%, *C. gariepinus* eggs stick together. Aloe vera is mostly used in the hatcheries for the control of fungi on fish eggs thus its ineffectiveness in the control of clumpiness may be due to microphyl, a unique adhesion apparatus present on C. *gariepinus* eggs as reported by Riehl and Appelbaum (1991).

4.1.2 Incubation Period of *C. gariepinus* exposed to varying concentrations and immersion periods of Aloe vera gel.

Incubation periods were not significantly different from one another in eggs exposed to Aloe vera gel. Incubation period is directly affected by temperature which is determined by the prevalent weather condition during the research period and exposure period (SRAC, 2006). Temperature of eggs immersed in Aloe vera and urea solution ranged from 27.4^0c and 28.8^0c. It was reported by (Adebayo, 2006) that the best temperature for *C. gariepinus* hatching is between the range of 23.89 ^{o} C -29.44^o C. This finding falls within the temperature range stated by Adebayo, (2006). Also, incubation period was not affected due to the relatively short exposure period ranging f 1 minute to 5minutes.

4.1.3 Hatching of *C. gariepinus* exposed to varying concentrations and immersion periods of Aloe vera gel.

In most popularly cultured species such as *C. gariepinus,* hatching rate is an important criterion for evaluating the efficacy of artificial reproduction as reported by Rottmann *et al.,* (1991). The result gotten from the percentage hatchability showed that hatching rate increases with increasing concentration and immersion period of Aloe vera gel; same thing was recorded for urea solution. However, this is in contrast with the findings of Riehl and Appelbaum (1991) who reported that the hatching rates decreased as urea concentration increases. Hatching rate observed in urea and Aloe vera at highest concentration and immersion period could be due to the high rate of eggs detachment which allowed the eggs more space and reduced the incidence of suffocation.

4.1.4 Survival of *C. gariepinus* exposed to varying concentrations and immersion periods of Aloe vera gel.

The percentage survival increased with an increase in immersion period. This result was not in agreement with the result of Akpoilih and Adebayo (2010) who reported that survival decreases with an increase in concentration level in formalin. It should be noted that formalin is a petrochemical and poisonous for fish at high concentrations while Aloe vera extract is extracted from plantwhich is harmless to *Clarias gariepinus*

4.2 EXPERIMENT 2: Effects of waterleaf extract on *Clarias gariepinus* eggs

4.2.1 Non-Adhesive Eggs of *C. gariepinus* exposed to varying concentrations and immersion periods of waterleaf extract

Waterleaf extract contains tannic acid which was effective at the lowest concentration of 1% (10ml) with shortest immersion period of 1minute to give the highest fertilization and lowest clumping rate, this was in contrast to Demska-Zakes *et al.,* (2005) who reported that the application of low concentration of tannic acid solution for a short exposure period is not effective to reduced egg stickiness. Suggestively, the marked contrast might be due to difference in prevailing environmental conditions at the time of carrying out both experiments.

4.2.2 Incubation Period of *C. gariepinus* exposed to varying concentrations and immersion periods of waterleaf extract

Incubation periods of eggs exposed to waterleaf extract were significantly different from other treatments. Incubation period of eggs immersed into waterleaf extract were delayed. This delay may be due to the presence of nitrate concentrations present in waterleaf. Ariole and Okpokwasili (2013) reported that high nitrate concentrations delay hatching in fish eggs.

4.2.3 Hatching and Survival of *C. gariepinus* exposed to varying concentrations and immersion periods of waterleaf extract

Eggs immersed in waterleaf extract at immersion period of 1minute gave the highest hatchability of about 70%. Application of tannin solution with immersion period of 1min in pikeperch eggs gave the highest hatching rate of 95% (Zarski *et al.,* 2015), Thai *et al.* (2004) observed hatching rate of about 70% in common carp when treated with salt/urea/tannin for 1minute, Colesante and Youmans (1983) observed 65% hatching of walleyes *(Sander vitreus)* eggs when treated with tannic acid for 4mins.These results are similar to this present study, at 1 minute immersion period, *C. gariepinus* gave the highesthatching rate and with a prolong exposure period of 5minutes, hatchability decreases. Decrease in hatching rate may be due to presence of polyphenols (tannin) in waterleaf. Krise *et al.,* (1986) and Horvath *et*

al., 2002 indicated that tannin is an antinutritional factor present in waterleaf which has a detrimental effect during incubation and can be toxic to eggs if not well used or at a contact exceeding a few seconds.

Thus, according to table 3.2 , the best period for immersing African catfish eggs when using waterleaf extract is 1 minute and this result conform to the finding of Asraf *et al.,*(2013) who urea solution to remove eggs adhesiveness of African catfish Clarias gariepinus at different concentrations.

4.3 EXPERIMENT 3: Comparative Appraisal of The Effects of Aloe Vera Gel, Waterleaf Extract and Urea Solution on *Clarias gariepinus* Eggs

4.3.1 Non adhesive eggs of *Clarias gariepinus*

The results obtained from the comparative appraisal of all the de-adhesive agents under study showed that waterleaf extract was more efficient in the detachment of *C. gariepinus* eggs. It was significantly more efficient in effect than Aloe vera gel and the control group (water) but was not significantly different from urea solution (reference de-adhesion agent). Thai *et al.,(2004)*observed hatching rate of about 70% in common carp when treated with salt/urea/tannin for 1minute. This means that waterleaf and urea solutions can effectively remove *C. gariepinus* eggs adhesiveness.

4.3.2 Incubation Period

All the treatments except waterleaf have no effect on the incubation period of *C. gariepinus*. Waterleaf incubation period was delayed because of the presence of polyphenols notably tannin, nitrites and nitrate. This was also reported by Horvath *et al.,* (2002)that the polyphenols to be toxic to eggs at prolong exposure period.

4.3.3 Hatching and survival percentage

Hatching rate of about 70% was obtained in waterleaf extract at 5% concentration and 50% in urea solution at 5% concentration. Thai *et al.,*(2004) reported highest hatching rate of about 86% in Pineapple juice and hatching rate of about 70% in salt/urea/tannin with 1% concentration. His findings are in conformity with the use of waterleaf and urea solutions. Eliminating stickiness using urea and waterleaf does not affect the survival rates of *C. gariepinus*(Thai *et al.,*2004).

5.0 Conclusion and recommendation

The present study revealed that 1% (10mls) of waterleaf extract with 1minute immersion period gave the lowest sticky rate, highest fertilization, hatchability and survival of *C. gariepinus*larvae. However, 5% (50mls) concentration of 2g urea + 4grams Nacl/L solution with 5minutes immersion period is also optimum for detachment of *C. gariepinus* eggs without affecting the incubation period, hatchability and percentage survival. Aloe vera gel at

concentration level of 5% and 5minutes immersion period was not significantly different from urea solution but with a poorer detachment of eggs and hatchability.

In view of this, elimination of stickiness of *C. gariepinus* eggs using waterleaf extract with 1% concentration level at 1minute immersion period is therefore recommended to fish hatcheries operations because of the effective, quick, simple technology involved. It requires less time and affordable than the other two anti-adhesive agents. Although, urea which served as the reference de-adhesion agent is not significantly different from waterleaf in term of hatching and survival but it is more expensive. Waterleaf extract is therefore recommended due to its efficacy, efficiency, cost effectiveness, availability, handling and ease of processing.

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