

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

Abstract

The present study has been focused on the optimum concentration and immersion period of Aloe vera gel and water leaf extract that efficiently removed adhesiveness of *C. gariepinus* eggs. revealed that 1% (10mls) of waterleaf extract with 1 minute immersion period gave the lowest eggs sticky rate, highest fertilization, hatchability and survival of *C. gariepinus*. In view of this, elimination of stickiness of *C. gariepinus* eggs using waterleaf extract with 1% concentration level at 1 minute immersion period is therefore recommended to fish hatcheries operators/ fish breeders because of the effective, quick, simple technology and at affordable price than other methods. Urea solution which served as the reference de-adhesion agent was not different from waterleaf in term of hatching and survival however, it is more expensive. Waterleaf extract is therefore recommended for its efficacy, efficiency, cost effectiveness, availability, handling and processing.

Keywords: *Clarias gariepinus*, Artificial Propagation, Egg Adhesiveness, Aloe vera gel.

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 species is 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 coat the eggs with certain powders. For instance, (9) used urea solution to removed egg stickiness of *Clarias gariepinus* for 1minute. 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

45 available plants that contain many antioxidants, polysaccharides, minerals, proteins, enzymes
46 vitamins (13; 14). To date, no study has been done to remove adhesiveness of the African
47 catfish eggs using these plants. Therefore, this study focused on the optimum concentration
48 and immersion period of Aloe vera gel and water leaf extract that efficiently removed
49 adhesiveness of *C. gariepinus* eggs.

50 **Materials and Methods**

51 **Fish Holding Facility**

52 Apparently healthy male and female *C. gariepinus* weighing 1.0kg and 1.3kg, respectively
53 were procured from a reputable fish farm in Akure prior to the commencement of the
54 experiment. Selected broodstocks were kept in separate holding tanks (40 x 30 x 35cm³)
55 containing aerated water on the farm where they were acclimatized and fed with commercial
56 diet for five days prior to exposure period. The brooders were starved for 24hours before the
57 commencement of the breeding exercise.

58 **Plants collection and identification**

59 Aloe vera plant were collected at Alaba hostel around The Federal University of Technology,
60 Akure while fresh waterleaf plants were collected within the Teaching and Research Fish
61 Farm, FUTA and were identified as *Aloe barbadensis* and *Talinum triangulare*, respectively
62 by a Botanist in the Department of Crop, Soil and Pest Management, FUTA before
63 transported to the experimental site.

64 **Processing of Plants to Form Aqueous Extract**

65 Aloe vera leaves were thoroughly washed with clean water, the serrated edges of the plants
66 were cut and the green barks were stripped off with the use of a sharp knife. The bitter yellow
67 latex was carefully skimmed out with the knife into a clean transparent nylon and put inside
68 boiled water (45°C). The gel gradually melted to form colourless liquid as the water
69 temperature decreases.

70 Waterleaf leaves were washed free from dust, soil, organic and inorganic matters under a
71 running tap. The leaf was plucked without the stem and the extract was squeezed out using
72 an electrical blender and a muslin bag. The greenish extract gotten was stored in a dry, clean
73 air tight transparent plastic container, labelled and refrigerated at 4°C

74 **Rinse solution preparation**

75 Three different concentrations of rinse solution were used in this experiment namely. The
76 aqueous solution of Aloe vera and waterleaf was prepared into the percentages
77 (concentrations) as follows:

78 1% = 1.0ml of aqueous aloe vera gel in 99ml of water.

79 3% = 3.0ml of aqueous aloe vera gel in 97ml of water.

80 5% = 5.0ml of aqueous aloe vera gel in 95ml of water.

81 **Preparation of Urea Solution (Reference de-adhesion agent)**

82 Urea/Nacl solution that served as reference de-adhesion agent was prepared by diluting 2g of
83 urea in 4g Nacl into one litres of water.

84 Water without any of the extracts served as the control.

85 **Preparation of Spawning Bowls**

86 Fifty six spawning bowls of 4litres capacity labelled according to the inclusion levels of the
87 treatments (1%, 3% and 5%), control and urea/Nacl as well as the immersion periods

88 (1mins, 3mins and 5mins). The bowls were filled with 100mls of water (control), 99mls of
89 water (1%), 97mls of water (3%) and 95mls of water (5%) respectively.

90 **Sperm and Egg Collections**

91 Female brooder was injected with ovaprim at angle 45° with the needle pointing towards the
92 gonad region. The injected brooder was kept inside separate plastic tanks containing water
93 and tightly covered with perforated lid to prevent it from jumping out. After latency period
94 of 12 hours, the female was squeezed abdominally to collect the eggs inside a clean bowl.
95 Then the testes of the male was removed by abdominal dissection and cleaned with a towel
96 and milt was gently squeezed out and collected in a beaker. Milt collected was then mixed
97 with small quantity of saline solution.

98 **Rinsing Procedure**

99 1g of the striped eggs was carefully weighed and each measured eggs was fertilized with the
100 prepared milt. The eggs were randomly rinsed inside the spawning bowls containing varying
101 concentration of aloe vera and water leaf extract, and left for 1, 3, 5 minutes, respectively in
102 the experimental bowls (in duplicates). After completion of each duration period, the
103 concentrated water were decanted, eggs were replaced with clean aerated water and left to
104 incubate in the spawning bowls.

105 **Non-adhesive, Incubating Period, Hatching and Percentage Survival Examination**

106 Percentage non-adhesive egg, incubation period, hatching and survival were assessed to
107 determine the efficacy and efficiency of Aloe vera gel and waterleaf on egg adhesiveness.
108 The parameters assessed were computed according to the method described by Adebayo
109 (2006).

110 **Non- adhesive eggs (%)** = number of non – adhesive egg/initial number of eggs × 100

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

112 **Survival (%)** = number of hatchling/Total number of hatchling × 100

113 **Data analysis**

114 All percentage data at different concentrations and immersion periods were subjected to
115 multivariate Analysis of Variance test. Also, Tukey multiple range tests was used as a follow
116 up procedure. Polynomial regression analysis was then used to determine the best
117 concentration and immersion period that effectively removed egg adhesiveness during
118 artificial propagation at 0.05 significance level.

119 **Results**

120 Result of eggs of *C. gariepinus* exposed to varying concentrations and immersion periods of
121 Aloe vera gel is shown in table 1. Eggs of *C. gariepinus* in varying concentrations and
122 immersion periods of Aloe vera gel showed no significant effect on the non-adhesive eggs
123 ($P>0.05$). Eggs of *C. gariepinus* immersed in water was significantly different ($P<0.05$) from
124 the non-adhesive eggs of *C. gariepinus* exposed to Urea solution and Aloe vera gel.
125 However, Urea solution and Aloe vera were not significantly different ($p>0.05$) from one
126 another. Incubation period of *C. gariepinus* was not affected by the varying concentrations
127 and immersion periods of Aloe vera gel. Hatching was fast in eggs exposed to Aloe vera gel
128 compared to the reference de-adhesion agent. However statistically, eggs of *C. gariepinus* in
129 control and urea solution are not significantly different ($p>0.05$) from one another

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

UNDER PEER REVIEW

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138 **Table 1:** Percentages 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 |

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

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142 Result of eggs of *C. gariepinus* exposed to varying concentrations and immersion
143 periods of waterleaf extract is shown in table 2. Percentage non-adhesive eggs exposed
144 to waterleaf and urea solution were not significantly different ($p>0.05$) from one another.
145 Detachment of eggs reduces in waterleaf and urea solution with increasing
146 concentration. However, detachment of eggs in waterleaf and urea solution were
147 significantly different ($p<0.05$) Hatching was delayed in eggs exposed to waterleaf
148 extract and were significantly different ($p<0.05$) from both the reference de-adhesive
149 agent and the control. Incubation across the different concentration levels and immersion
150 periods were similar and there was no significant difference ($p<0.05$). Incubation periods
151 of eggs in urea solution and water were not significantly different ($p>0.05$) from each
152 other but waterleaf was significantly different from both urea solution and water
153 (control) ($p<0.05$). Percentage hatching decreases with increasing concentrations of
154 waterleaf extract. Percentage hatched larvae in waterleaf extract at 1 % concentration
155 and 1 minute immersion period was significantly different ($p<0.05$) from the control and
156 other rinsing agents at varying concentrations and immersion periods.
157 Hatched larvae that survived in waterleaf with concentration of 1% were not
158 significantly different from hatched larvae that survived in urea solution with
159 concentrations of 3% and 5% with immersion periods of 3minutes and 5minutes,
160 respectively. There was no significant difference ($p>0.05$) at the concentration levels and
161 immersion periods of larvae that survived in waterleaf extract but there was significant
162 difference across the immersion periods ($p<0.05$) in the larvae that survived in urea
163 solution.

164 **Table 2:** Percentages of non-adhesive eggs, hatchability, survival and incubation period of waterleaf extract, Urea solution
 165 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 |

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

167 The concentration and immersion period that performed best in all the rising agents were compared in order to determine the best extract,
 168 concentration and immersion period that reduced egg stickiness of *C. gariepinus* as shown in table 3.

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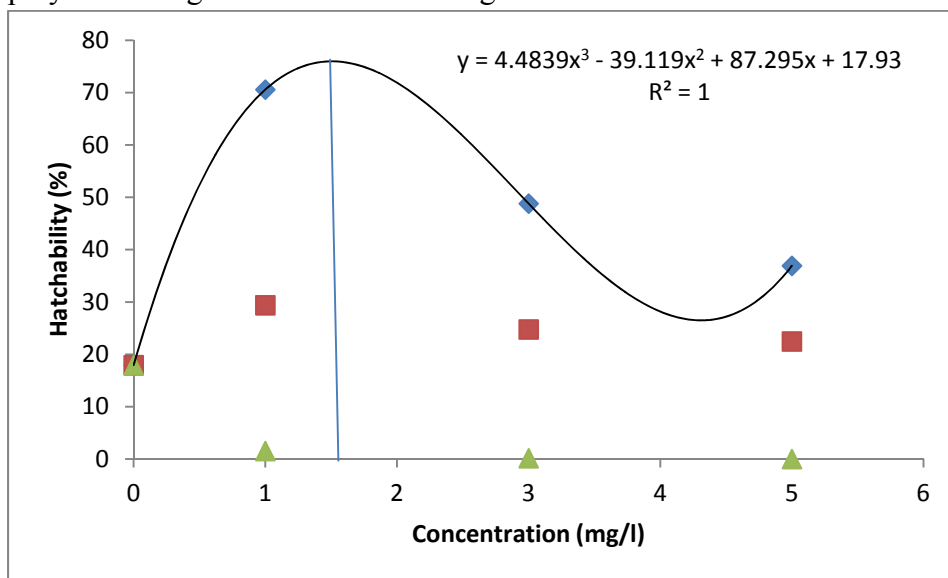
170 Table 3: The main effect of varying concentration against immersion periods of aloe vera, waterleaf extract and urea solution on the
 171 removal of *C. gariepinus* egg adhesiveness.

| 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).

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175 The optimum concentration that can efficiently remove egg adhesiveness and increase hatching
176 in *C. gariepinus* using waterleaf extract was observed at concentration of 1.6% using 3rd order
177 polynomial regression as shown in figure 1.



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

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184 The highest number of non-adhesive eggs was observed in waterleaf extract at 1.6%
185 concentration which was significantly different ($p < 0.05$) from aloe vera gel and the control but
186 not significantly different from urea solution. Percentage non-adhesive eggs in urea solution
187 was not significantly different from eggs immersed in aloe vera gel but significantly different
188 from eggs immersed in the control. Incubation period in water leaf was significantly different
189 from the other two rinsing agents and control, however, urea solution was not significantly
190 different from the control and aloe vera gel. Highest hatched larvae was recorded in group
191 exposed to waterleaf which was not significantly different from group exposed to urea solution
192 but significantly different from the control group and aloe vera gel. Survived hatched larvae in
193 the three rinsing agents were not significantly different from one another but survived larvae in
194 the control group were significantly different from all the rinsing agents used in the experiment.

195 Waterleaf extract efficiently removed egg adhesiveness of *C. gariepinus* at concentration of 1%
196 and within short period of 1 minute, It gave the best egg detachment although statistically, it was
197 not significantly different ($p > 0.05$) from urea. Aloe vera gel and urea were not significantly
198 different ($p > 0.05$) from each another but waterleaf extract is significantly different ($p < 0.05$)
199 from Aloe vera. The control gave the least egg detachment and was significantly different from
200 all the treatments ($p < 0.05$). Hatchability was highest in waterleaf extract followed by urea but
201 they were not significantly different from one another ($p < 0.05$). Aloe vera gel and urea solution
202 were not significantly different from each other but waterleaf was significantly different from
203 Aloe vera and control

204 The control had the least number of larvae that survived at the end of the experiment and it was
205 significantly different from other treatments. Highest survival rate was recorded in urea solution
206 followed by waterleaf and Aloe vera but they were not significantly different ($p>0.05$) from one
207 another. The de-adhesive agents used in the experiment had no significant effect on the
208 incubation period except waterleaf in which hatching was delayed.

209 Discussion

210 This finding revealed that eggs detachment increases as the concentration and immersion period
211 increased, at 5% concentration with immersion period of 5minutes, Aloe vera gel effectively
212 reduced *C. gariepinus* egg stickiness. This reduction could be attributed to the presence of
213 alkaline protease which is one of the active ingredients in Aloe vera gel. (15) Used Alkaline
214 protease at its highest concentration and immersion period to remove egg adhesiveness and
215 increase hatching in Walleye (*Sander vitreus*). Urea was not significantly different from Aloe
216 vera gel, enhanced hatching rate, this result is similar to the result obtained by (16) (17) during
217 the use of urea to reduce stickiness of carp eggs. The present study indicates that the optimal
218 time needed to rinse the African catfish eggs was 5minutes using Aloe vera and urea solution
219 which does not conform to the finding of (9) in that the optimal time needed to rinse African cat
220 fish eggs was one minute. The result gotten from the percentage non-adhesive eggs showed the
221 adhesive elimination was not successful for all the groups of eggs in Aloe vera gel, at
222 concentration of 1%, *C. gariepinus* eggs stick together. Hence, egg stickiness was not
223 effectively reduced by Aloe vera gel and urea solution.

224 Incubation period is directly affected by temperature and exposure period (18). Temperature of
225 eggs immersed in Aloe vera and urea solution ranged from 27.4⁰c and 28.8⁰ which falls within
226 the temperature range stated by (19), in that the best temperature for *C. gariepinus* hatching is
227 between the range of 23.89⁰ C -29.44⁰ C.

228 In most cultured species such as *C. gariepinus*, hatching rate is an important criterion to
229 evaluate the efficacy of artificial reproduction (20). Result from the current study showed that
230 hatching rate increases with increasing concentration and immersion period of Aloe vera gel
231 and urea solution. However, this is in contrast with the findings of(8) who reported that the
232 hatching rates decreased as urea concentration increases. The percentage survival increased with
233 an increase in immersion period. This result was not in agreement with the result of (21) who
234 reported that survival decreases with an increase in concentration level in formalin. The high
235 concentration and immersion period of Aloe vera that support survival depict that this plant is
236 not harmful to *C. gariepinus*.

237 Waterleaf extract was effective in reducing egg stickiness at the lowest concentration of 1%
238 (10ml) with shortest immersion period of 1minute. The reduction in the stickiness may be due
239 to the presence of tannic acid in waterleaf which is a good agent that reduces stickiness.
240 Although the percentage non adhesive eggs contradict (22) who reported that the application of
241 low concentration of tannic acid solution for a short exposure period is not effective to reduced
242 egg stickiness.

243 Incubation period of eggs immersed in waterleaf extract were delayed. This delay may be due to
244 the presence of nitrate concentrations present in waterleaf. (23) reported that high nitrate
245 concentrations delay hatching in fish eggs.

246 Waterleaf at 1% concentration and immersion period of 1minute gave the highest hatchability
247 and with a prolong exposure period of 5minutes, hatchability decreases. Hatchability was
248 successful in groups exposed to waterleaf due to the presence of tannin and tannic acid in
249 waterleaf. A similar recommendation was proposed by (24) that the application of tannin
250 solution with immersion period of 1min in pikeperch eggs gave the highest hatching rate of
251 95%. Asraf *et al.*, 2013 used urea solution to removed egg stickiness of *Clarias gariepinus* for
252 1minute. Thai *et al.*,2004 observed hatching rate of 70.2% of hatching in common carp when
253 treated with salt/urea/tannin for 1minute. Thus, the decrease in hatching rate may be due to
254 antinutritional factors present in *waterleaf* as reported by (25) and Horvath *et al.*, 2002 indicated
255 that tannin has a detrimental effect during incubation and can be toxic to eggs if not well used or
256 at a contact exceeding a few seconds. Thus, the best immersion period needed to rinse African
257 catfish eggs when using waterleaf extract is 1 minute and this result conform to the finding of
258 (9).

259 The result obtained from the comparative appraisal of all the de-adhesive agents showed that
260 waterleaf extract was more efficient in the detachment of *C. gariepinus* eggs. The detachment
261 might be due to the present of tannic acid in water leaf, in that(22) reported the application of
262 tannic acid in the elimination of pikeperch, *Sander lucioperca*, eggs.

263 Waterleaf incubation period of eggs was delayed in waterleaf because of the presence of tannin,
264 nitrites and nitrate which was reported by (26) to be very toxic to eggs at prolong of time 20
265 seconds.

266 Hatching rate of 70.59% was obtained in waterleaf extract and 50.23% in urea solution at
267 concentration of 1%. (27) reported highest hatching of 70.2% in salt/urea/tannin with 1%
268 concentration which was in conformity with the use of waterleaf and urea solution. Eliminating
269 stickiness of eggs using urea and waterleaf does not affect the survival rates of *C. gariepinus*

270 **Conclusion and recommendation**

271 This present study revealed that 1% (10mls) of waterleaf extract with 1 minute immersion
272 period gave the lowest eggs sticky rate, highest fertilization, hatchability and survival of *C.*
273 *gariepinus*. In view of this, elimination of stickiness of *C. gariepinus* eggs using waterleaf
274 extract with 1% concentration level at 1 minute immersion period is therefore recommended to
275 fish hatcheries operators/ fish breeders because of the effective, quick, simple technology and at
276 affordable price than other methods. Urea solution which served as the reference de-adhesion
277 agent was not different from waterleaf in term of hatching and survival however, it is more
278 expensive. Waterleaf extract is therefore recommended for its efficacy, efficiency, cost
279 effectiveness, availability, handling and processing.

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