Original Research Article

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

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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.

Materials and Methods

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. Selected broodstocks were kept in separate holding tanks (40 x 30 x 35cm^3) containing aerated water on the farm where they were acclimatized and fed with commercial diet for five days prior to exposure period. The brooders were starved for 24hours before the commencement of the breeding exercise.

Plants collection and identification

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

Processing of Plants to Form Aqueous Extract

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

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

Rinse solution preparation

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

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

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

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

Preparation of Urea Solution (Reference de-adhesion agent)

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

Water without any of the extracts served as the control.

Preparation of Spawning Bowls

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

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

Sperm 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 to prevent it from jumping out. After latency period of 12 hours, the female was squeezed abdominally to collect the eggs inside a clean bowl. Then the testes of the male was removed by abdominal dissection and cleaned with a towel and milt was gently squeezed out and collected in a beaker. Milt collected was then mixed with small quantity of saline solution.

Rinsing Procedure

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

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 method described by Adebayo (2006).

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

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

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

Data analysis

All percentage data at different concentrations and immersion periods were subjected to multivariate Analysis of Variance test. Also, Tukey 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.

Results

Result of eggs of *C. gariepinus* exposed to varying concentrations and immersion periods of Aloe vera gel is shown in table 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. 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 statistically, eggs of *C. gariepinus* in control and urea solution are not significantly different ($p>0.05$) from one another

Percentage hatchability and survival showed that hatching rate and hatched larvae increases 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 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 did not hatch out due to high stickiness.

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]**

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

Result of eggs of *C. gariepinus* exposed to varying concentrations and immersion periods of waterleaf extract is shown in table 2. Percentage non-adhesive eggs exposed to waterleaf and urea solution were not significantly different ($p>0.05$) from one another. Detachment of eggs reduces in waterleaf and urea solution with increasing concentration. However, detachment of eggs in waterleaf and urea solution were significantly different ($p<0.05$) 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. Incubation across the different concentration levels and immersion periods were similar and there was no significant difference ($p<0.05$). 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. 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 agents at varying concentrations and immersion periods.

Hatched larvae that survived in waterleaf with concentration of 1% were not significantly different from hatched larvae that survived in urea solution with concentrations of 3% and 5% with immersion periods of 3minutes and 5minutes, respectively. 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 difference across the immersion periods ($p<0.05$) in the larvae that survived in urea solution.

172 **Table 2:** Percentages of non-adhesive eggs, hatchability, survival and incubation period of waterleaf extract, Urea solution
173 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}

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

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

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178 Table 3: The main effect of varying concentration against immersion periods of aloe vera, waterleaf extract and urea solution on the
179 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}

180 The mean values in the same column with different superscript is significantly different (P<0.05).
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The optimum concentration that can efficiently remove egg adhesiveness and increase hatching in *C. gariepinus* using waterleaf extract was observed at concentration of 1.6% using 3rd order polynomial regression as shown in figure 1.

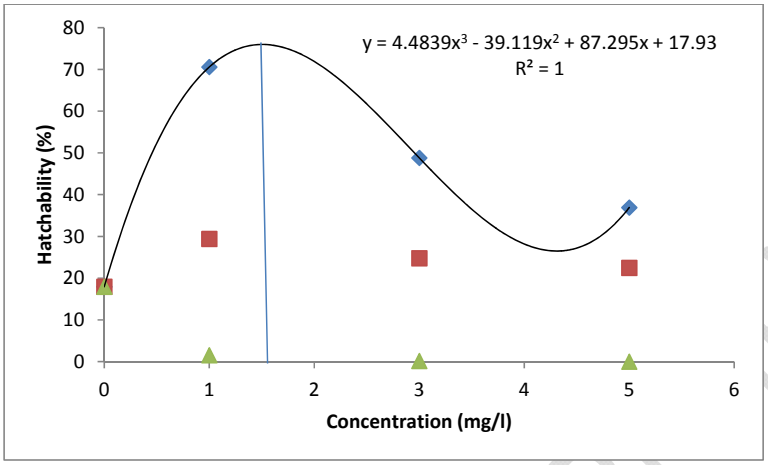

Figure 1: The Optimum Concentration of Waterleaf Extract used as de-adhesive agent during artificial propagation of *C. gariepinus*

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 from urea solution. 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. 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. 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. Survived hatched larvae in the three rinsing agents were not significantly different from one another but survived larvae in the control group were significantly different from all the rinsing agents used in the experiment.

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

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

Discussion

This finding revealed that eggs detachment increases 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. (15) Used Alkaline protease at its highest concentration and immersion period to remove egg adhesiveness and increase hatching in Walleye (*Sander vitreus).* Urea was not significantly different from Aloe vera gel, enhanced hatching rate, this result is similar to the result obtained by (16) (17) during the use of urea to reduce stickiness of carp eggs. The present study indicates that the optimal time needed to rinse the African catfish eggs was 5minutes using Aloe vera and urea solution which does not conform to the finding of (9) in that the optimal time needed to rinse African cat fish eggs was one minute. 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. Hence, egg stickiness was not effectively reduced by Aloe vera gel and urea solution.

Incubation period is directly affected by temperature and exposure period (18). Temperature of eggs immersed in Aloe vera and urea solution ranged from 27.4^0c and 28.8^0 which falls within the temperature range stated by (19), in that the best temperature for *C. gariepinus* hatching is between the range of 23.89^o C -29.44^o C.

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

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

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

Waterleaf at 1% concentration and immersion period of 1minute gave the highest hatchability and with a prolong exposure period of 5minutes, hatchability decreases. Hatchability was successful in groups exposed to waterleaf due to the presence of tannin and tannic acid in waterleaf. A similar recommendation was proposed by (24) that the application of tannin solution with immersion period of 1min in pikeperch eggs gave the highest hatching rate of 95%. Asraf *et al.,* 2013 used urea solution to removed egg stickiness of *Clarias gariepinus* for 1minute. Thai *et al.,*2004 observed hatching rate of 70.2% of hatching in common carp when treated with salt/urea/tannin for 1minute. Thus, the decrease in hatching rate may be due to antinutritional factors present in *waterleaf* as reported by (25) and Horvath *et al.,* 2002 indicated that tannin 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, the best immersion period needed to rinse African catfish eggs when using waterleaf extract is 1 minute and this result conform to the finding of (9).

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

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

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

Conclusion and recommendation

This present study 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.

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