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Research Article

Effect of Different Fertilization and Egg De-adhesion Methods on Hatching and Survival of *Clarias gariepinus* (Burchell 1822) Fry

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Abstract: Egg adhesiveness has been a major problem affecting the hatchability of *Clarias gariepinus*. The removal of egg stickiness will improve aquaculture production through enhanced hatching rates. However, appropriate media for removal of adhesiveness in Catfish eggs is not yet well established. This study therefore, determine the optimum concentration and rinsing time of powdered milk (PM), Urea (UR) and Tannic acid (TA) for removal of adhesiveness of African catfish eggs and their effect on fertility and hatching success. Milt and eggs obtained from African Catfish broodstock of the same lineage were used for induced breeding following standard procedure. Four hundred fertilized eggs each were rinsed in the three different media at varying concentrations and exposure time using dry and wet fertilization methods. Treatment efficacy was assessed by comparing the percentage of non-stick eggs, fertilization, hatching and survival rate in the treatment groups and the control. Data were subjected to multivariate general linear model and Duncan multiple range tests at $\alpha_{0.05}$. Under the wet fertilization method eggs exposed to 10 g PM at 20 minutes gave the highest number of free eggs (87.50%) while 6g UR at 5 minutes recorded the lowest (27.09%). In dry method, 14 g PM at 20 minutes recorded the highest number of free eggs (90.00%) whereas, the lowest (47.50%) was recorded in 6g urea at one minute rinsing time. The highest number of hatchlings for PM, UR and TA were 53.26%, 33.24%, and 13.13%, respectively. The survival rate was high in all treatments except in 1.5 g TA. Eggs treated with PM solution performed best in adhesiveness removal and recorded highest percentage fertilization and hatching rate. However, more studies should be conducted to establish the appropriate media for other culture fish species.

Keywords: Powdered milk; Urea; Tannic acid; Stickiness; Catfish

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Introduction

Aquaculture is one of the fastest growing food production systems in the world with developing countries contributing significantly to its growth (Haruna, 2003). It is acknowledge as the efficient means of providing food rich in protein, income and employment opportunities for the populace. FDF (2005) estimated potential of Nigeria in aquaculture as 1.3 million metric tonnes but its current contributions stood at 278,706 metric tonnes (FDF, 2015). With stagnating yields from many capture fisheries and increasing demand for fish and fishery products, there is high expectation for aquaculture to increase its contribution to the world's aquatic food production. It is also hoped that aquaculture will continue to strengthen its role in contributing to food security and poverty alleviation in many developing countries (FAO, 2010). 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) noted that the scarcity of fingerlings of this widely acceptable species constitute a major constraint to the rapid development of fish farming in Nigeria. Charo and Orirere (2000) equally submitted that the major constraint to intensification and expansion of fish culture in Nigeria lies in inadequate supply of quality fingerlings and juveniles for stocking of ponds cages and pens. Atanda (2006) stressed that fish farmers in most part of the country (especially the Northern part) are perpetually in need of hatchery produced fish seed for their farm which is mostly not available. Documented evidences indicated that the total seed production and supply in Nigeria from all sources amount to 55 million fingerling while the immediate needs of the market is 500 million per annum (Atanda, 2007). Catfish larva production impediment is traceable to low hatching and survival rates (Muchlisin et al., 2010) which could be linked to the adhesiveness of eggs. The clump together of egg when they are released into the water results in low fertilization and hatching rates (El-Gamal and El-Greisy, 2008). In addition, adhesiveness of eggs can cause high larval mortality (Abigail et al., 2010). In African catfish, eggs are covered with a layer of mucus that gives them adhesiveness. Under natural condition, egg adhesiveness is a reproductive strategy of most teleost for protecting the eggs from water drifting. In the wild, African catfish spawning takes place at night in shallow water with temperatures above 22ºC and the eggs stick to the leaves and stems of vegetation (Little et al., 1994). However, in artificial spawning, the adhesiveness of the eggs could reduce fertilization and hatching rates. This is because the adhesiveness covers the microphiles and hinders the sperms from fertilizing the eggs (Prinsloo et al., 1987); the chances of sperm getting in contact with the eggs are reduced and hence the chances of the eggs getting fertilized are compromised. Also, when the eggs come into contact with water during the incubation period, it becomes clumped and this reduces the chance of the eggs to hatch. However, various techniques have been adopted to control reproduction in cultured fish species with the aim of achieving high fertilization of eggs and hence production of large number of fingerlings (Delinc et al., 1987). Materials in use to remove egg adhesiveness of fishes, includes: milk for common carp, *Cyprinus carpio* (El-Gamal et al., 2008), tannic acid for pikerpich, *Sander lucioperca* (Demska et al., 2005), kaolin for shishamo smelt, *Spirinchus lanceolatus* (Mizuno et al., 2004), mud solution in Japanese dace, *Tribolodon hakonensis* (Nakamura, 1966) and urea solution in carp (Rothbard, 1978). To date, few studies have been done on how best to remove adhesiveness of the African catfish eggs in Nigeria despite dominating local fish production. Therefore, this study is carried out to find the best solution required to eliminate the stickiness of the African catfish eggs for improve fertilization and hatching rates. Herein, powdered milk, urea solution and tannic acid at different concentrations were evaluated.

Materials and Methods

The experiment was conducted in 72 plastic aquaria of sizes 0.39 m×0.27 m×0.26 m at the Wet Laboratory, Department of Aquaculture and Fisheries management, University of Ibadan from May to July, 2015. A male and a female broodstock of African catfish weighing 700-1200 grams were obtained from a commercial farm in Ibadan and acclimatized in separated tanks for 24 hours. The female brooder was artificially induced following Olanrewaju et al. (2009). Twelve hours after injection, the milt was collected from the male broodstock by sacrificing the male broodstock. This is necessary as catfish milt cannot be released by abdominal pressing (Muchlisin et al., 2014). Gentle pressure was applied on the abdomen of the female brooder and the ovulated eggs that were ooze out easily from the genital opening was collected in a clean dry plastic bowl (Nwachi et al., 2014). For all the treatments, both wet and dry methods of fertilization were used. For the dry method, the milt and eggs were mixed with plastic spoon and two drops of saline water added to activate the milt. For wet method, Milt was mixed with the saline solution in a flat bowl, poured onto the eggs and stirred gently with a plastic spoon to fertilize the eggs (Lawson and Ishola, 2003). For both methods, fertilized eggs were left for five minutes to allow the eggs make sufficient contact with the sperm.

Three different concentrations of rinsing agents (powdered milk, Urea and Tannic acid) were prepared by dissolving appropriate quantity of solute in a liter of water and adding 1 gram and 4 grams sodium chloride respectively. Tannic acid solution was also prepared in the same way but without sodium chloride. 400 eggs were randomly counted and rinsed thoroughly in each concentration of urea, tannic acid and powdered milk and observed under different exposure time **(Table 1)**. Each of the mixture was rinsed with water prior to transfer of eggs into the 20 L plastic container at room temperature. Flow-through water exchange method was used during incubation. The fertilized eggs from each of the treatments were spread on 30 cm×20 cm strips of mosquito netting. Incubation was monitored for 24 h with gentle water flow through.

Egg adhesiveness was assessed by rating the percentage of clumping eggs. The number of completely free (non-stickiness) eggs and aggregated eggs in each container were counted after 15 minutes of incubation. The percentage of non-stickiness is estimated as follows:

Table 1: Concentrations and rinsing time of different rising agents for removing egg adhesiveness. Demska-Zakes et al. (2005), Muchlisin et al. (2010), Amirul et al. (2013).

Rinsing agents	Rinsing time (minutes)
Powdered milk Treatments (PM)	
0 g+1 g Nacl/L of water (Control)	
10 g powdered milk+1 g Nacl/L of water	15.0, 20.0, 25.0
12 g+1 g Nacl/L of water	
14 g +1 g Nacl/L of water	
Urea Treatments (UR)	
0 g+4 g NaCl/L water(Control)	
2 g urea+4 g NaCl/L water	
4 g urea+4 g NaCl/L water	1.0, 5.0, 10.0
6 g urea+4 g NaCl/L water	
Tannic Acid Treatments (TA)	
0 g Tannic acid/L of water	
0.5 g Tannic acid/L of water	
1g Tannic acid/L of water	0.5, 2.0, 5.0
1.5 g Tannic acid/L of water	

$$\text{Non stickiness eggs }(\%) = \frac{\text{Number of non}-\text{stick eggs }(\text{R1})}{\text{Initial number of eggs }(\text{R2})} \times 100$$

Fertilization was evaluated after 30 minutes of incubation by counting the transparent embryos in relative to the white colour unfertilized or dead eggs. The percentage fertilization was calculated thus:

$$\text{Percentage of fertilization rate }(\text{Fr}) = \frac{\text{Number of fertilized eggs}(\text{F2})}{\text{Initial number if incubated eggs}} \times 100$$

Hatching rates were observed after 24 hours of incubation, by counting the number of larvae produced. The hatchability percentage was estimated as follows:

$$\text{Hatchability }(\text{Fr}) = \frac{\text{Number of Hatchlings }(\text{H3})}{\text{Total number of fertilized eggs }(\text{F2})} \times 100$$

The survival rate of larva was monitored for seven days for each treatment. The percentage survival rate was estimated as follows:

$$\text{Percentage Survival rate }(\text{Sr}) = \frac{\text{Final number of hatchlings }(\text{H4})}{\text{Initial number of hatchlings }(\text{H3})} \times 100$$

Water quality parameters were monitored during the experiment. Temperature, pH and Dissolved oxygen was determined daily using mercury-in-glass thermometer, pH meter (Hanna, model H198107) and dissolved oxygen meter (Labtech, model AVI-660), respectively. The raw data were tested by multivariate general linear model using SPSS (version 17) to examine the main effect of milk, Urea and tannic acid concentrations and interactions at different times on free, fertilized and hatching and survival rates. Duncan's multiple range tests were used to determine differences among treatments.

Results

The Percentages of Non-stick eggs, fertilization, hatching and survival rate based on rinsing agents and interaction effects between rinsing agents and rinsing time is shown in **Table 2**. The interaction between the rinsing concentration and rinsing time for wet method of fertilization revealed that 10 g and 14 g PM gave the highest quantity of non-stick eggs (90.00% each) at 15 and 25 minutes, this was significantly different from other treatments ($p<0.05$). The results obtained in the control ranges from 43.75% at 25 minutes rinsing time to 61.25% at 20 minutes rinsing time. The fertilization rate was higher in 10 g PM at 15 minutes (95.75%) and this was significantly different from other treatment ($p<0.05$). The highest hatching of 62.50% was obtained from 10 g PM at 25 minutes exposure time. The control (0 g PM) gave the lowest hatching of 31.81% at 25 minutes rinsing time. The result from PM treatment gave high survival rate of between 91.67% and 100%. PM treatment using the dry method revealed that, 14g PM had the highest de-sticking ability (90.00%) at 20 minutes exposure time while the lowest was the control (31.67%) at 15 minutes rinsing time. The fertilization rate was higher in 12 g PM (97.50%) at 20 minutes; this was significantly different ($p<0.05$) from other treatment except 14 g PM at 25 minutes exposure time (92.09%). The lowest fertilization was recorded in the control with 15 minutes rinsing time. The result of hatchability indicated that, 10g PM at 20 minutes rinsing time gave the highest (72.66%), this was significantly different from other treatment ($p<0.05$). The result obtained implied high survival rate of between 94.00% and 100.00%.

Table 3 showed the effects of UR treatments on the Stickiness removal, fertilization and hatching rates ($P<0.05$). For wet method of fertilization, the non-sticky rate varied from 38.75% to 69.20% in control (0 g UR) at 10 minutes rinsing time and in 6 g UR also at 10 minutes rinsing time, respectively. The fertilization rate ranged from 34.17% to 97.86% in 6 g UR at 5 minutes rinse time and in 2 g UR with 10 minutes rinsing time, respectively. In addition, the hatchability result ranges from 16.15% to 40.24% in 4 g UR at 10 minutes rinsing time and in 2 g UR at 10 minutes rinsing time. Survival rate ranges from 81.25% to 100.00% in 6 g urea at 10 and 5 minutes exposure time respectively. Among the treatments, the concentration of 6 g urea at 10 minutes rinsing time gave the best results for removing stickiness of eggs (69.20%), this was significantly different from other treatments ($p<0.05$). On the other hand, the 2 g UR at 10 minutes rinse time recorded the highest fertilization (97.86%), which was significantly different ($p<0.05$) from other treatments. In addition, the same concentration of UR gave the highest numbers of hatchlings (40.24%), but this was lower than the control at 1 minute rinsing time (47.75%). However, these results were not significantly different from other treatments ($p>0.05$). The results obtained from survival rates indicated high percentage survival rates in all the treatments. On the effect of rinsing agent on stickiness removal under dry method of fertilization, 4 g urea gave the highest percentage of free eggs, (60.42%) at 5 minutes rinsing time. However, this was not significantly different from other treatments. The same concentration of 4 g urea at 5 minutes rinsing time had the highest fertilization (91.84%), and hatching rate (51.59%). The result of fertilization indicated a significant different among treatments, while for hatchability, there was no significant difference among

Table 2: Percentages of Non-stick eggs, fertilization and hatching and survival rate based on interaction effects between rinsing agent (powdered milk) and rinsing time.

Rinsing agent	Rinsing Time (mins)	Fertilization Methods	Non Stickiness (%)	Fertilization (%)	Hatchability (%)	Survival rate (%)
0 g PM/L of water	15.0	Wet	50.00 ± 0.00$_{abc}$	76.25 ± 5.30$_{bcd}$	34.29 ± 12.45$_{ab}$	94.60 ± 4.49$_{abc}$
		Dry	43.34 ± 2.35$_{ab}$	49.17 ± 12.96$_{a}$	51.67 ± 20.82$_{abcd}$	100.00 ± 0.00$_{c}$
	20.0	Wet	61.25 ± 1.77$_{bcde}$	73.75 ± 1.77$_{bcd}$	34.80 ± 8.84$_{ab}$	96.78 ± 1.73$_{abc}$
		Dry	43.75 ± 8.84$_{ab}$	70.00 ± 21.21$_{abcd}$	41.71 ± 17.31$_{abc}$	98.89 ± 1.56$_{bc}$
	25.0	Wet	43.75 ± 8.84$_{ab}$	62.50 ± 15.38$_{ab}$	31.81 ± 6.52$_{a}$	100.00 ± 0.00$_{c}$
		Dry	31.67 ± 2.35$_{a}$	75.67 ± 23.81$_{bcd}$	34.78 ± 17.66$_{ab}$	98.00 ± 2.82$_{bc}$
10 g PM/L of water	15.0	Wet	90.00 ± 7.07$_{e}$	95.75 ± 4.59$_{e}$	50.25 ± 0.35$_{abcd}$	99.50 ± 0.71$_{bc}$
		Dry	86.25 ± 5.30$_{ef}$	93.13 ± 0.88$_{cd}$	62.50 ± 17.68$_{abcd}$	94.17 ± 5.89$_{ab}$
	20.0	Wet	87.50 ± 3.50$_{ef}$	70.00 ± 7.07$_{abc}$	50.70 ± 14.57$_{abcd}$	100.00 ± 0.00$_{c}$
		Dry	83.75 ± 1.77$_{ef}$	92.50 ± 0.00$_{cd}$	72.66 ± 24.98$_{d}$	97.11 ± 1.06$_{bc}$
	25.0	Wet	83.67 ± 5.42$_{ef}$	78.75 ± 5.30$_{bcd}$	62.50 ± 17.68$_{cd}$	96.89 ± 15.55$_{abc}$
		Dry	73.75 ± 1.77$_{cdef}$	78.75 ± 1.77$_{bcd}$	47.50 ± 3.54$_{abcd}$	98.00 ± 0.00$_{bc}$
12 g PM/L of water	15.0	Wet	75.00 ± 21.21$_{cdef}$	68.75 ± 5.30$_{abc}$	37.00 ± 2.12$_{abc}$	100.00 ± 0.00$_{c}$
		Dry	77.50 ± 10.61$_{def}$	85.00 ± 14.14$_{bcd}$	51.68 ± 15.81$_{abcd}$	99.55 ± 0.0.64$_{bc}$
	20.0	Wet	81.25 ± 22.90$_{de}$	78.75 ± 8.77$_{bcd}$	53.35 ± 18.89$_{abcd}$	91.67 ± 2.35$_{a}$
		Dry	88.75 ± 8.83$_{e}$	97.50 ± 4.33$_{e}$	57.82 ± 11.05$_{abcd}$	98.52 ± 0.74$_{bc}$
	25.0	Wet	71.67 ± 11.79$_{cdef}$	86.26 ± 10.4$_{bcd}$	61.95 ± 21.14$_{abcd}$	99.50 ± 0.71$_{bc}$
		Dry	52.50 ± 3.54$_{abcd}$	81.25 ± 15.91$_{de}$	40.23 ± 14.83$_{abcd}$	98.75 ± 2.17$_{bc}$
14 g PM/L of water	15.0	Wet	75.00 ± 21.21$_{cdef}$	91.25 ± 1.77$_{cd}$	44.70 ± 0.42$_{abcd}$	97.50 ± 3.54$_{bc}$
		Dry	80.00 ± 3.54$_{ef}$	91.25 ± 1.77$_{cd}$	48.34 ± 25.93$_{abcd}$	96.21 ± 4.07$_{abc}$
	20.0	Wet	72.50 ± 28.28$_{cdef}$	86.25 ± 15.90$_{def}$	41.00 ± 15.56$_{abc}$	98.34 ± 2.35$_{bc}$
		Dry	90.00 ± 3.54$_{e}$	95.00 ± 0.00$_{def}$	56.61 ± 28.72$_{abcd}$	97.84 ± 1.65$_{bc}$
	25.0	Wet	90.00 ± 3.54$_{e}$	81.25 ± 15.91$_{de}$	65.70 ± 8.06$_{bcd}$	99.50 ± 0.71$_{bc}$
		Dry	82.92 ± 0.59$_{ef}$	92.09 ± 0.59$_{e}$	60.05 ± 16.09$_{abcd}$	94.00 ± 2.82$_{ab}$

Table 3: Effect of Urea as anti-sticking agent on fertilization, hatchability of Clarias gariepinus eggs and survival of the hatchlings.

Rinsing agent	Rinsing Time (mins)	Fertilization Methods	Non Stickiness (%)	Fertilization (%)	Hatchability (%)	Survival rate (%)
0 g UR/L of water	1.0	Wet	41.84 ± 0.23$_{ab}$	73.83 ± 15.80$_{ab}$	47.75 ± 3.18$_{a}$	98.18 ± 0.25$_{a}$
		Dry	51.67 ± 9.42$_{ab}$	52.50 ± 3.54$_{ab}$	40.77 ± 6.39$_{a}$	96.66 ± 4.72$_{a}$
	5.0	Wet	42.30 ± 10.32$_{ab}$	62.50 ± 17.68b$_{cde}$	34.63 ± 1.24$_{a}$	99.17 ± 1.18$_{a}$
		Dry	47.91 ± 2.95$_{ab}$	55.67 ± 3.77$_{abc}$	34.15 ± 4.09$_{a}$	93.65 ± 7.98$_{a}$
	10.0	Wet	38.75 ± 5.30$_{ab}$	53.75 ± 5.30$_{ab}$	39.25 ± 4.24$_{a}$	98.46 ± 0.52$_{a}$
		Dry	40.00 ± 14.14$_{ab}$	52.09 ± 7.66$_{ab}$	33.50 ± 4.95$_{a}$	100.00 ± 0.00$_{a}$
2 g UR/L of water	1.0	Wet	42.50 ± 19.70$_{ab}$	94.14 ± 0.19$_{de}$	39.03 ± 29.66$_{a}$	97.50 ± 3.54$_{a}$
		Dry	45.50 ± 7.07$_{ab}$	67.71 ± 16.21$_{abcd}$	45.45 ± 0.00$_{a}$	98.33 ± 0.00$_{a}$
	5.0	Wet	53.64 ± 15.05$_{ab}$	69.70 ± 33.76$_{abcd}$	31.11 ± 33.79$_{a}$	99.09 ± 1.29
		Dry	60.42 ± 17.09$_{ab}$	79.59 ± 0.59$_{abcd}$	29.54 ± 14.79$_{a}$	97.75 ± 0.35$_{a}$
	10.0	Wet	48.81 ± 21.88$_{ab}$	97.86 ± 0.00$_{e}$	40.24 ± 49.17$_{a}$	99.34 ± 0.94$_{a}$
		Dry	50.42 ± 6.48$_{ab}$	72.50 ± 31.82$_{bcde}$	36.19 ± 40.75$_{a}$	92.09 ± 9.02$_{a}$
4 g UR/L of water	1.0	Wet	50.42 ± 24.12$_{ab}$	64.05 ± 11.95$_{abcd}$	26.79 ± 32.81$_{a}$	100.00 ± 0.00$_{a}$
		Dry	54.33 ± 22.15$_{ab}$	84.25 ± 13.79$_{bcde}$	51.59 ± 2.26$_{a}$	97.34 ± 3.77$_{a}$
	5.0	Wet	61.46 ± 27.99$_{ab}$	69.58 ± 20.04$_{abcd}$	33.77 ± 44.17$_{a}$	96.93 ± 4.35$_{a}$
		Dry	48.50 ± 12.02$_{ab}$	91.84 ± 9.67$_{cde}$	35.07 ± 35.26$_{a}$	95.83 ± 3.54$_{a}$
	10.0	Wet	53.13 ± 4.42$_{ab}$	56.40 ± 2.26$_{abc}$	16.15 ± 12.52$_{a}$	100.00 ± 0.00$_{a}$
		Dry	49.45 ± 14.92$_{ab}$	81.52 ± 25.13$_{bcde}$	21.79 ± 25.75$_{a}$	98.75 ± 1.77$_{a}$
6 g UR/L of water	1.0	Wet	37.50 ± 24.75$_{ab}$	58.25 ± 6.01$_{abcd}$	32.20 ± 17.25$_{a}$	99.17 ± 1.18$_{a}$
		Dry	47.50 ± 8.89$_{ab}$	62.78 ± 4.59$_{abcde}$	20.70 ± 25.37$_{a}$	87.55 ± 7.32$_{a}$
	5.0	Wet	27.09 ± 2.94$_{a}$	34.17 ± 12.96$_{a}$	27.50 ± 10.61$_{a}$	100.00 ± 0.00$_{a}$
		Dry	49.84 ± 11.55$_{ab}$	76.88 ± 23.86$_{bcde}$	32.72 ± 38.59$_{a}$	89.96 ± 11.84$_{a}$
	10.0	Wet	69.20 ± 18.31$_{b}$	68.93 ± 1.66$_{abcde}$	18.65 ± 16.05$_{a}$	81.25 ± 26.52$_{a}$
		Dry	50.62 ± 7.95$_{ab}$	60.00 ± 14.14$_{abcd}$	23.70 ± 23.05$_{a}$	91.99 ± 7.78$_{a}$

the treatments. In addition, high survival rates were recorded in all the treatments.

On the effect of TA treatment on stickiness removal, fertilization, hatching and survival rate, it could be deduced that TA had significant effect on stickiness removal. For wet method of fertilization, treatment with 1.5 g TA at 0.5 minute exposure time recorded the highest numbers of free eggs (96.17%) while for dry method; 0.5 g at 5 minutes exposure time recorded the highest number of free eggs (85.84%). These were significantly different from other treatments. The fertilization rate showed that, wet method with 1.5 g TA at 2 minutes exposure time gave the highest fertilization rate (97.19%), which was significantly different ($p<0.05$) from other treatments **(Table 4)**.

The results obtained for stickiness removal using dry method showed that, 0.5 g at 5 minutes rinsing time achieved the highest (96.33%) result, which was not significantly different for the same concentration at 2 minutes exposure time ($p<0.05$). As regard the effect of TA on hatching rate, there were no significant difference among the treatments ($p>0.05$), except for the control. Generally, low hatching rate was recorded in the treatments with tannic acid. The highest number of hatchlings obtained was at 0.5 minute exposure time for wet (41.50%) and dry (51.25%) methods respectively. Treatment with 1.5 g TA recorded the lowest survival rates.

Discussion

The powdered milk proved to be the most appropriate media for the removal of egg adhesiveness when compared with tannic acid and urea. This may be due to the inherent ability of milk to reduce the rate of egg aggregation by coating it with the milk particles. The coating prevents the eggs from sticking to each other. This in-turn increased fertilization rate as the sperm can have a direct contact with the eggs without the obstacle of the mucus layer on the egg surface. The hatching rate also increased in the treatment with powdered milk solution when compared with the control. The high survival rate observed under this treatment indicated that, at this optimum concentration the rinsing agent concentration do not have any negative effect on the survival of hatchlings. Mohammed et al. (2015) reported that introduction of rinsing agent makes the eggs discrete and non-sticky after treatment which invariably allowed easy spread of eggs in the incubator and hence facilitate easy separation of hatchlings from egg cases. Kudo (1982) submitted that the cortical envelope of fish egg is rich in protein and acid phosphates which are responsible for stickiness of eggs when in contact with water. Elimination of stickiness is therefore, critical to reduce adhesive effect, increase embryonic development, hatching and easy management of the incubator. The use of powdered milk solution for the removal of stickiness of *Clarias gariepinus* eggs showed that the optimum concentration and rinsing time under wet method of fertilization

Table 4: Percentage of non-stickiness, fertilization, hatchability and survival of Clarias gariepinus eggs exposed to varying rinsing time and concentrations of Tannic acid solution.

Rinsing agent	Rinsing Time (mins)	Fertilization Methods	Non Stickiness (%)	Fertilization (%)	Hatchability (%)	Survival rate (%)
0 g TA/L of water	0.5	Wet	$41.32 \pm 3.44_{ab}$	$50.75 \pm 1.77_{a}$	$41.50 \pm 5.66_{b}$	$100.00 \pm 0.00_{c}$
		Dry	$40.63 \pm 4.42_{ab}$	$58.50 \pm 1.41_{abc}$	$51.25 \pm 2.47_{c}$	$98.90 \pm 1.56_{c}$
	2.0	Wet	$29.86 \pm 10.80_{a}$	$57.75 \pm 0.35_{abc}$	$38.00 \pm 6.01_{b}$	$93.26 \pm 1.99_{bc}$
		Dry	$42.08 \pm 1.77_{abc}$	$56.25 \pm 1.77_{abc}$	$49.50 \pm 0.71_{c}$	$99.21 \pm 1.12_{c}$
	5.0	Wet	$48.13 \pm 0.88_{abcd}$	$53.88 \pm 1.59_{ab}$	$41.00 \pm 5.66_{b}$	$96.19 \pm 2.26_{bc}$
		Dry	$43.75 \pm 8.83_{abc}$	$59.95 \pm 1.70_{abc}$	$49.38 \pm 4.41_{c}$	$97.30 \pm 3.82_{c}$
0.5 g TA/L of water	0.5	Wet	$49.17 \pm 1.81_{abcd}$	$52.71 \pm 2.06_{abc}$	$4.13 \pm 1.24_{a}$	$87.50 \pm 17.68_{bc}$
		Dry	$65.10 \pm 13.49_{abcdef}$	$96.22 \pm 0.30_{cd}$	$2.25 \pm 0.35_{a}$	$100.00 \pm 0.00_{c}$
	2.0	Wet	$53.13 \pm 4.42_{abcd}$	$53.13 \pm 8.83_{a}$	$1.25 \pm 0.0.00_{a}$	$66.67 \pm 23.57_{bc}$
		Dry	$81.72 \pm 19.57_{cdef}$	$81.04 \pm 23.68_{abc}$	$3.38 \pm 0.53_{a}$	$83.07 \pm 23.19_{bc}$
	5.0	Wet	$71.78 \pm 35.21_{bcdef}$	$65.97 \pm 40.26_{abc}$	$1.00 \pm 0.00_{a}$	$50.00 \pm 70.71_{abc}$
		Dry	$85.84 \pm 15.32_{def}$	$96.33 \pm 2.67_{cd}$	$1.13 \pm 0.66_{a}$	$100.00 \pm 0.00_{abc}$
1 g TA/L of water	0.5	Wet	$91.67 \pm 7.07_{ef}$	$71.34 \pm 34.88_{abc}$	$3.13 \pm 0.18_{a}$	$70.84 \pm 5.89_{bc}$
		Dry	$77.60 \pm 21.46_{bcdef}$	$83.42 \pm 17.79_{abc}$	$2.88 \pm 0.53_{a}$	$99.60 \pm 0.37_{bc}$
	2.0	Wet	$77.91 \pm 21.80_{bcdef}$	$73.94 \pm 31.80_{abc}$	$0.88 \pm 0.18_{a}$	$75.00 \pm 35.36_{bc}$
		Dry	$80.90 \pm 16.80_{bcdef}$	$85.27 \pm 16.29_{abc}$	$1.13 \pm 0.17_{a}$	$99.60 \pm 0.57_{c}$
	5.0	Wet	$82.27 \pm 19.11_{cdef}$	$82.61 \pm 15.17_{abc}$	$0.50 \pm 0.00_{a}$	$50.00 \pm 70.71_{abc}$
		Dry	$80.89 \pm 15.40_{bcdef}$	$81.03 \pm 11.17_{abc}$	$0.50 \pm 0.00_{a}$	$100.00 \pm 0.00_{abc}$
1.5 g TA/L of water	0.5	Wet	$96.17 \pm 0.86_{e}$	$93.05 \pm 2.75_{abc}$	$0.82 \pm 0.26_{a}$	$50.00 \pm 70.71_{abc}$
		Dry	$69.38 \pm 25.63_{abcdef}$	$74.65 \pm 24.25_{abc}$	$0.50 \pm 0.00_{a}$	$66.67 \pm 57.74_{abc}$
	2.0	Wet	$87.07 \pm 12.01_{def}$	$97.19 \pm 2.31_{c}$	$0.50 \pm 0.50_{a}$	$25.00 \pm 43.30_{abc}$
		Dry	$80.63 \pm 16.79_{bcdef}$	$85.59 \pm 12.31_{abc}$	$1.50 \pm 0.00_{a}$	$49.00 \pm 69.30_{abc}$
	5.0	Wet	$85.39 \pm 14.69_{def}$	$82.61 \pm 15.17_{abc}$	$1.25 \pm 1.06_{a}$	$50.00 \pm 70.71_{a}$
		Dry	$68.24 \pm 24.28_{abcdef}$	$67.93 \pm 24.44_{abc}$	$0.50 \pm 0.50_{a}$	$25.00 \pm 43.30_{ab}$

was 10 g powdered milk at 20 minutes rinsing time while the best concentration of rinsing agent and rinsing time recorded for dry method of fertilization was 14 g powdered milk at 20 minutes rinsing time. This was in accordance with the findings of Muchlisin et al. (2014) who reported optimum concentration of 14 g powdered milk/L solution at 25 minutes rinsing time for African catfish eggs under dry fertilization method. It could however, be seen from the result of this experiment that, the concentration of 14 g powdered milk recorded the highest fertilization rate for both dry and wet method of fertilization, but at different rinsing time of 15 minutes (wet) and 20 minutes (dry) respectively. This agreed with the findings of Muchlisin et al. (2014), who recorded higher fertilization rate in 12 g powdered milk. Generally, there was high survival in both the treatment with powdered milk solution and the control.

The use of powdered milk as organic dissolvent to remove egg stickiness has been reported by Soin (1976). However, Linhart et al. (2003) reported the use of enzymes. According to Palikoval and Krejci (2011), a combination of whole milk and talc result in 80%-90% anti stickiness and hatching rate while whole milk only gave 70% results. This confirmed that urea has positive effect on removal of egg adhesiveness. From this experiment, 4 g urea at 10 minutes rinsing time was the optimum concentration and rinsing time for removing the stickiness of eggs in African catfish under wet method of fertilization while 2 g urea at 5 minutes rinsing recorded the highest numbers of free eggs in dry method. El-Gamal et al. (2008) examined the removal of egg adhesiveness in Common carp (*Cyprinus carpio)* using a mixture of 3-5 gm of urea and 4 gm of NaCl/L of water. The authors however, obtained the best result in 4 gm urea/L of water. According to Woynarovich and Woynarovich (1962) and Rothbard (1978), the function of urea in adhesiveness removal is to remove glutinosity of egg. However at higher concentrations, the urea may cause the basal membrane of the egg to be overly and disturb the chorion development resulting in lower fertilization and hatching rates as corroborated by Amirul et al. 2013. Overall, the number of free eggs, fertilization and hatching rates recorded in this study were higher than that of Amirul et al*.* 2013. The post hock test showed that tannic acid concentration had significant effect in removing the adhesiveness of African catfish eggs, but the rinsing time and interaction between rinsing agent and rinsing time does not indicate any significant effects. This contradict the report of Demska et al. (2005) which submitted that, both the concentration of tannic acid and exposure time significantly impacted the degree of stickiness removal and embryo survival. Ozdal et al. (2013) adduced variability in result when using tannic acid to different in water quality parameters which plays a significant role in the reaction between phenol groups and proteins. The result revealed that concentration of tannic acid of between 1 g to 1.5 g at 2-5 minutes exposure time was the best in removing the stickiness of African catfish eggs for both method of fertilization. This is similar to the result of Demska-Zakes et al. (2005) who reported that the adhesiveness of pike perch eggs disappeared completely at 5 minutes exposure to tannic acid solution of 0.5-1.5 g L^{-1}. The authors further submitted that, the tannic acid solution of ≤0.5 g L^{-1} applied at 0.5-2 minutes was not effective; the eggs clumped and it was impossible to separate them even with intensive mixing while better results were obtained using higher tannic acid concentrations and/or by lengthening exposure time. Treatment with 1.5 g produced the highest fertilization rate under wet method of fertilization while 5 g was recorded as best for dry. For hatching, the result indicated that Tannic acid has a negative effect on African Catfish eggs as the highest hatching recorded for wet and dry method were 2.13% and 2.25% respectively, notwithstanding its significant effect on removing egg stickiness. Similar observation was reported by Demska-Zakęś et al. (2005) when tannic acid was used on the pikeperch, *Sander lucio perca*eggs. At high concentration, tannic caused the chorion to harden, thus decreasing the chorionase activity. Also, Amirul et al. (2013) reported that higher concentrations of urea may cause the basal membrane of the egg to be overly abraded and disturb the chorion development resulting in lower fertilization and hatching rates. From this study, 0.5 g tannic acid gave the highest hatching rate. This implies that concentration of 0.5 g tannic and below will be the appropriate concentration for better hatching rate. This is supported by Demska-Zakes et al. (2005) who opined that high tannic acid concentration had a negative impact on the pikeperch larvae hatching.

Conclusion

This study shows that powdered milk, urea and tannic acid solution have significant effect on removing stickiness associated with *Clarias gariepinus* eggs. 10 g powdered milk at 20 minutes rinsing time remove adhesiveness in African catfish eggs better than all the other treatments. However, 14 g powdered milk at 15 minutes rinsing time proof to be the best concentration when fertilization and hatching rate are considered. The dry method of fertilization gave the best fertilization and hatching rate for the three media when compare with the wet method. There was high survival rate in all the treatments except for the treatment with 1.5 g tannic acid. The results therefore confirm that catfish eggs rinsed in powdered milk can reduce the clumping of eggs and also enhance fertilization and hatching rate.

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