Effects of Tannic Acid on Stickiness Removal and Time of Hatching of African Catfish, *Clarias gariepinus* Eggs

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ABSTRACT

The effects of different tannic acid concentrations and treatment duration to eliminate the stickiness of African Catfish, *Clarias gariepinus*, eggs was carried out. Four different concentrations of tannic acid (0.3, 0.50, 0.80 and 1.00 g/l) and a control sample were used. After fertilization, fish eggs were treated in these concentrations at different durations (10, 30, and 60 seconds) to reduce stickiness before incubation. Each concentration and treatment duration was conducted in replicates. Stickiness elimination increased from 43.50±0.00 to 69.25±0.25 and time of hatching of eggs increased from 23.15 to 26.40 hours as the concentration and duration of treatment increased. It was therefore concluded that, the higher the concentration of tannic acid, the faster the rate of stickiness removed with longer duration of hatching.

(Keywords: *Clarias gariepinus*, concentrations, hatchability, stickiness elimination, photophilic eggs, Tannic acid)

INTRODUCTION

Certain fish species including the African catfish (*Clarias gariepinus*) have so-called photophilic eggs that have sticky protein material on the surface of the fertilized swollen egg that facilitates attachment to vegetation in nature (5, 6). However, during artificial breeding, the stickiness covers the micropyles and hinders the sperms from fertilizing the eggs (6). This reduced the chances of sperm getting in contact with the eggs and hence the chances of the eggs to be fertilized are reduced. Moreover, when the eggs come into contact with water during the incubation period, they become clumped and this reduces the chance of the eggs hatch because trapped eggs cannot obtain sufficient oxygen and the developing embryo dies (16).

Tannic acid, also known as acidum tannicum or tannin is a highly tanning astringent, polyphenolic plant compound. It is a synonym for hydrolysable tannins, which are widely distributed in nature (e.g. *Qnercusin fectoria*, *Caesalpini aspinosaor* the genus Rhus) (24). During artificial breeding in hatcheries, technicians often add tannin to eggs after fertilization for the irreversible termination of stickiness. However, if the concentration of tannin and the duration of treatment are not appropriately defined the quality of eggs is impaired (6).

Different methods of eliminating eggs stickiness have been developed, but most related research has been conducted on cyprinids (7, 12), European catfish (15, 14), percids such as walleye (21), carp and wel catfish (6), Beluga (23), and pikeperch (11; 25). In the present study, the effect of tannic acid treatment in respect to stickiness removal and time of first hatched of African catfish (*Clarias gariepinus*) eggs were investigated.

MATERIALS AND METHODS

The study was carried out at the Hatchery Unit of the Department of Fisheries and Aquaculture, University of Agriculture Makurdi, Benue State-Nigeria. One female and one male broodstock were procured from the University of Agriculture Makurdi fish farm to ensure uniformity. These were identified and selected based on their external sexual characteristics (1; 19).

Artificial breeding was carried out prior to hormone injection. The hormone (ovaprim^{R}) was administered at 9:00pm (21 hours) following standard protocols with modifications (18). Both

sexes were injected intramuscularly above the lateral line towards the dorsal section and pointed towards the ventral side. The female and male *Clarias gariepinus* were given a single dose of 0.5 and 0.25 ml/kg of hormone (ovaprim^{R}), respectively (13). After the latency period of 11 hours at a temperature of 27^{o}C, eggs and milt were collected from the female and male brood stock, respectively.

Fertilization of eggs was carried out using the "wet" method of fertilization (18). In replicates, fertilized eggs were incubated at a temperature range of 27±0.5 - 29±0.5. Four (4) different concentrations of tannic acid (0.3, 0.5, 0.8, and 1g/l) and a control were used with three treatment durations (10, 30, and 60 sec) to reduce the stickiness. Each treatment combination was applied to three portions of eggs. Each egg portion was treated with different concentration–time combinations (making a total of 12 treatment groups) and a control sample.

After tannic acid application, a total number of 975 eggs required for stocking 75 eggs per treatment were estimated following (10), that, the number of eggs per 1 gram varies from 600 – 900 eggs, with a mean number of 750 eggs (600+900/2) for accurate calculations.

Hence, 750 eggs = 1 gram of eggs
975 eggs = x gram of eggs

=(975 eggs ×1gram of egg)/(750 eggs)
X gram of eggs = 1.3 x 1 gram of eggs

= 1.3 gram of eggs

0.1 gram of egg (75 eggs) was weighed using a sensitive weighing balance. Treated eggs were placed in bowls (plate 3) and incubated in 2 liters under a drip-system, using de-chlorinated municipal water. The eggs were spread in single layers on the suspended nylon mesh net for incubation. Hatching occurred 23.15±0.00 to 26.55±0.25 hours. Egg portions were closely monitored and the time of hatching in each bowl was recorded.

Egg stickiness was determined by counting the number of completely free (non-stickiness) eggs and aggregated eggs in each group after 15 minutes of incubation as described by (17). The rate (percentage) of non-stickiness (P) was calculated as:

P=(No of non-stickiness eggs)/(Total No. of eggs incubated per group)×100

De-chlorinated municipal water was used for the experiment. Some physic-chemical parameters (temperature, pH, dissolved oxygen, biochemical oxygen demand, and total alkalinity) of the water used for the treatments were determined following (2).

One way analysis of variance (ANOVA) was used to analyze data using Minitab 17th edition. Significant Difference (F-LSD) was used to separate means at $p<0.05$ (9).

RESULTS

Table 1 shows the mean water temperature and dissolved oxygen in all the experimental bowls as 27.3^{o}C (atmospheric air temperature of 29.2^{o}C) and 3.7mg/l, respectively. Other water quality parameters such as Biochemical Oxygen Demand (BOD), hydrogen ion concentration (pH), and total alkalinity were also recorded as 1.9 mg/l, 7.3 , and 28.9 mg/l, respectively during the experiment. This falls within the recommended range reported by Bhatnagar and Singh, 2010 in studying fishpond culture in related to water quality parameters.

The result in Table 2 shows that there were significant ($p<0.05$) effects of tannic acid on the removal of egg stickiness of the African catfish, *Clarias gariepinus*, eggs. The number of aggregated (clumps) eggs and non-stickiness (free) eggs decreased from 31.50±0.00 to 6.15±0.15c and increased from 43.50±0.00 to 69.25±0.25a, respectively with increased concentrations of tannic acid and increased duration of egg treatment.

The clumping effects as from the control sample (0.00g/l for 0.00sec.) of tannic acid gave the highest (31.50±0.00) number of aggregated eggs and lowest (6.15±0.15C) as the concentration of tannic acid increased to 1.0 g/l for 60 sec.

1.00g/l for 60 seconds and lowest (43.50±0.00) as compared to the control sample.

The time of hatching in Table 2 also ranged from 23.15±0.00 to 26.55±0.25 hours after incubation with the control sample having the shortest (23.15±0.00) time of hatching.

Table1: Physico-Chemical Parameters of the Tested Water used in Hatching of *Clarias gariepinus* at Different Tannic Acid Concentrations.

Rinsing Agents	Rinsing Time	Temp	pH	DO	BOD	Alk 26.29±0.08
0.0g Tannic Acid (control)	0	27.15±0.30	7.88±0.13	3.80±0.27	1.94±0.25	
		-	-	-		
0.3g Tannic Acid	10	27.13±0.31	7.91±0.12	3.75±0.25	1.93±0.25	26.28±0.10
	30	27.12±0.30	7.93±0.13	3.71±0.24	1.93±0.25	26.27±0.11
	60	27.10±0.31	7.93±0.13	3.73±0.25	1.93±0.25	26.27±0.11
P-Value		*0.99^{ns}*	*0.98^{ns}*	*0.99^{ns}*	*0.99^{ns}*	*0.99^{ns}*
0.5g Tannic Acid	10	27.15±0.30	7.95±0.13	3.72±0.24	1.93±0.25	26.28±0.10
	30	27.15±0.30	7.93±0.13	3.71±0.24	1.93±0.24	24.26±0.10
	60	27.10±0.32	7.94±0.13	3.70±0.24	1.93±0.25	26.27±0.10
P-Value		*0.99^{ns}*	*0.99^{ns}*	*0.99^{ns}*	*0.99^{ns}*	*0.99^{ns}*
0.8g Tannic Acid	10	27.23±0.31	7.93±0.13	3.70±0.24	1.92±0.24	26.28±0.10
	30	27.23±0.31	7.94±0.13	3.70±0.24	1.93±0.24	26.27±0.10
	60	27.14±0.32	7.95±0.13	3.71±0.24	1.92±0.24	26.26±0.10
P-Value		*0.97^{ns}*	*0.99^{ns}*	*0.99^{ns}*	*0.99^{ns}*	*0.99^{ns}*
1g Tannic Acid	10	27.15±0.31	7.95±0.13	3.71±0.24	1.93±0.25	26.27±0.10
	30	27.16±0.31	7.94±0.13	3.72±0.24	1.91±0.24	26.28±0.10
	60	27.20±0.32	7.94±0.13	3.72±0.24	1.81±0.24	26.27±0.10
P-Value		*0.99^{ns}*	*0.99^{ns}*	*0.99^{ns}*	*0.99^{ns}*	*0.99^{ns}*

Means on the same column with different superscript are statistically significant ($p<0.05$); ns = not significant

Keys: Temp. (Temperature), pH (Hydrogen ion concentration), DO (Dissolved oxygen), BOD (Biochemical Oxygen Demand), and Alk (Alkalinity)

Table 2: Effects of Tannic Acid Concentration on Egg Stickiness and Time of First Hatched of *Clarias gariepinus.*

Tannic Acid Concentration (g/L)	Duration(sec)	Free Eggs (%)	Aggregate Eggs (%)	Time of Hatching (Hrs)
0.0g Tannic Acid (control)	0	43.50±0.00	31.50±0.00	23.15±0.00
		-	-	-
0.3g Tannic Acid	10	49.55±0.05^{b}	25.40±0.10^{a}	23.80±0.50
	30	49.40±0.10^{b}	22.55±0.05^{b}	23.40±0.10
	60	52.40±0.10^{a}	22.40±0.10^{b}	23.50±0.50
P-Value		*<0.01*	*<0.01*	*0.79^{ns}*
0.5g Tannic Acid	10	54.35±0.15^{b}	20.40±0.10^{a}	24.36±0.13^{b}
	30	55.15±0.15^{ab}	20.50±0.50^{a}	24.44±0.06^{b}
	60	56.50±0.50^{a}	18.95±0.05^{b}	25.27±0.12^{a}
P-Value		*0.03*	*0.04*	*0.01*
0.8g Tannic Acid	10	56.15±0.15^{a}	19.10±0.10^{a}	25.55±0.05^{b}
	30	57.40±0.10^{b}	17.55±0.05^{b}	26.07±0.02^{a}
	60	29.65±0.15^{c}	16.50±0.50^{b}	26.26±0.13^{a}
P-Value		*0.55^{ns}*	*0.01*	*0.01*
1g Tannic Acid	10	62.41±0.11^{c}	12.40±0.10^{a}	26.26±0.04
	30	63.45±0.05^{b}	11.40±0.10^{b}	26.39±0.14
	60	69.25±0.25^{a}	6.15±0.15^{c}	26.55±0.25
P-Value		*<0.01*	*<0.01*	*0.64^{ns}*

Means on the same column with different superscript are statistically significant ($p<0.05$); ns = not significant

The longest (26.55±0.25 hours) time, of hatching was recorded at a tannic acid concentration and duration of treatment of 1.00g/l for 60 seconds, respectively.

DISCUSSION

The mean physico-chemical parameters of the water used was recorded in Table 1. In light of the present study the non-significant change of the water quality parameter of the various experimental bowls indicate that tannic acid did not adversely affect the water quality as the values observed were all within the recommended range reported by (4) in studying fishpond culture in related to water quality parameters.

The results in Table 2 on egg stickiness elimination using tannic acid shows that, the number of completely free (non-stickiness) eggs was 69.25±0.25[a] (92.3%) at a tannic acid concentration of 1.00g/l for 60sec as compared to the control 43.50±0.00 (58.0%) for 0.00 seconds. This implies that, the number of free (non-stickiness) eggs increased as the concentration and duration of tannic acid treatments increased and this respectively decreased the number of aggregated (clumps) eggs from 31.50±0.00 (42.0%) to 6.15 (8.2%). This is in line with (22) and (8) in their study on carp using urea solution. They reported that, urea effectively removed stickiness of carp eggs and enhanced fertilization and hatching rates of carp (25) reported the effectiveness of tannic acid application in egg stickiness elimination of pike perch, *Sander lucioperca L.* at varied moments of the egg swelling process increases with time. In their study, the best result was obtained in groups of eggs submerged for 1 and 2 minutes (86.5% and 80.5%) of larvae were obtained, respectively, 30 min following gametes activation. In contrast, (3) in their study on removal of egg stickiness of African catfish, *Clarias gariepinus* using urea solution at different concentrations where the sticky nature of catfish eggs were not effectively reduced by urea solution, instead lower clumping of eggs were observed in control. Thus, they came to a conclusion that, the difference observed in their study might be due to the unique stickiness apparatus on *Clarias gariepinus* eggs as earlier reported by (20).

Table 2 also shows the time of hatching of the African catfish, *Clarias gariepinus* eggs using tannic acid. The time of hatching using tannic acid to eliminate egg stickiness range from 23.15±0.00 hrs. to 26.55±0.25 hrs. with the shortest (26.55±0.25 hrs.) time of hatching recorded in the control sample and the longest (26.55±0.25 hrs.) time of hatching recorded at a concentration of 1.00g/l for 60 seconds. It was therefore observed that increased in the concentration of tannic acid with longer treatment durations increased the time of hatching. This was also noted by (6) in their study on common carp (*Cyprinus carpio*) and Wel catfish (*Silurus glanis)* that high concentrations of tannic acid harden wall of fish egg and consequently making the developing embryo hatched with difficulty.

CONCLUSION AND RECOMMENDATIONS

There were significant ($p<0.05$) effects of tannic acid on the removal of egg stickiness of the African catfish, *Clarias gariepinus* eggs as the concentration and duration of egg treatment increases.

The time of egg hatched increased as the concentration and duration of tannic acid treatment to eggs increased with the control sample having the shortest (23.15±0.00 hrs.) as against the 1.00g/l (26.55±0.25 hrs.) for 60 sec., respectively. Stickiness removal with tannic acid is a promising method of eliminating *Clarias gariepinus* eggs.

All African catfish culturist have to bear in mind that, even thus tannic acid effectively removed egg stickiness also delays the time of egg hatching. Full scale study on methodology of tannic acid treatment for direct removal of egg stickiness during artificial propagation of African catfish in a commercial setting as well as the effect of tannic acid on longer egg hatching time, needs to be optimized and should be of interest for further studies.

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