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Effect of chemical disinfectant (Izal) on hatching of eggs of African catfish (*Clarias gariepinus*), survival and growth performance of fry.

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Abstract
Eggs of matured *Clarias gariepinus* size ranging from 550-800 g total body weight (TBW) were treated with 0.5 ml and 1 ml izal concentrations for 60 seconds to determine its efficacy and effect on hatching, survival and growth performance of fry. Each treatment was replicated three times. The research was conducted at the Toxicology Unit Fish Farm, Federal University of Technology, (FUT), Bosso Campus, Minna. Mean fecundity did not differed significantly (P>0.05); 113943.1^{a}, 109281.9^{a} and 116081.9^{a} for 0.5 ml, 1 ml and control among the treatments. Percentage fertilization differed significantly (P<0.05) between 1 ml, and 0.5 ml and control (64.85^{a}, 61.64^{b} and 64.13^{ab}) respectively and (37.94^{a} and 46.43^{b}) 1 ml and control for hatching. The bred hatchlings were maintained for 8 weeks and total percentage mortality was 12, 16 and 24 for 0.5 ml, 1 ml and control respectively. Mean Total Body Weight (TBW) gain differed significantly (P<0.05) between 1 ml (3.08^{a}) and 0.5 ml (2.47^{b}). Treatment two (1 ml izal solution) gave better result in terms of fertilization, hatching and growth performance. This shows that eggs treated with izal at 1 ml concentration for 60 seconds is appropriate to disinfect eggs. The treatment is hence recommended for disinfecting *Clarias gariepinus* eggs before incubation.

Keywords: Disinfectant, Induced spawning, hatching, *Clarias gariepinus* egg.

1. Introduction
Aquaculture practice in Nigeria, Africa and indeed globally is on the increase, this has made hatchery propagation of culturable fish species important in order to meet the increasing demand for fingerlings. Fungal infections on eggs causes disease problem which resulted into egg mortality, reduces hatching of fertilized eggs and survival of larvae [6]. The external surface of fish eggs is easily colonized by bacteria, such as *Flavobacterium* sp, *Pseudomonas* sp, *Aeromonas* sp. and *Vibrio* sp. [7, 8]. Eggs are externally disinfected at the green and/or eyed stage to minimize the possibility of infection by bacteria, fungi or parasites. Formalin is a generic term which describes a solution of 37% formaldehyde gas dissolved in water [1]. Solutions of formalin for use on fish should contain 10 to 15% methanol which inhibits formation of par formaldehyde, a highly toxic compound. Formalin has long been used as traditional treatment for fish ecto parasites but izal seems to be innovative in this regard. It is extremely effective against most protozoan as well as some monogenetic trematodes through bath, flush or flowing treatment methods [6]. Formalin is also one of the most commonly used chemical treatments for fungal control in fish hatcheries and effective in the control of fungus on eggs without adverse effect on hatchability and post-hatch survival as reported by Pedersen *et al*. (2008) [11]. Egg disinfection is an important and routine bio-security practice among hatchery operators. Egg disinfection helps to prevent the transfer of external pathogens from brood stock to larvae and thus helps reduce the mortality associated with these pathogens. The use of izal to treat fish eggs (*Clarias gariepinus*) before incubation has not been a common practice in Nigeria by fish breeders and hatchery operators. African catfish (*Clarias gariepinus*) is specie with high economic value in Nigeria. It is widely cultured owning to its hardiness, fast growth and highly priced food fish. The efficacy of various disinfection methods has been studied using many different species of fish eggs [5, 10, 12]. Izal unlike formalin has not been widely used for treating fungal infection on fish eggs in intensive aquaculture operations to improve the hatchability and survival of larvae because there is

problem of appropriate concentration of chemical and period of time the treated eggs are to be in contact with the chemical before incubation in order to reduce potential toxic effect on fish. The objective of this study therefore was to determine the efficacy of izal solution (0.5 ml and 1 ml) diluted at 99.5 ml and 99 ml respectively on treated eggs for 60 seconds post-fertilization.

2. Materials and Methods

Ten (10) samples, (5 males and 5 females) gravid *Clarias gariepinus* brood stock size ranging from 550-800 g total body weight (TBW) were procured from Tunga Mallam along Paiko road, Minna, Niger State. They were acclimatized for 2 weeks in holding indoor concrete tanks of 725.76 L water holding capacity of indoor hatchery Federal University of Technology, (F.U.T.), Bosso Campus Minna. They were maintained under optimum temperature and fed with 40% crude protein commercial diet. The brood stocks were examined for gonad development according to the method of Blythe *et al.* (1994) [3] and reported by Yisa *et al*. (2010) [13]. Males were examined for rigid and reddish infusion of the genital orifice and for females, genital orifice for reddish infusion, distension of the belly and release of eggs when gentle pressure was applied on the abdomen. The selected samples were properly maintained separately before being used for breeding. Matured female brood fish were treated with a single dose of hormone (Ovaprim) according to the method of Goudie *et al*. (1992) [4] and hand stripped for eggs after a minimum latency period of twelve hours at water temperature of between 25-29 ^{0}C. Izal solution was prepared by diluting 0.5 ml with 99.5 ml and 1 ml izal into 99 ml distilled water respectively. Twelve and half grams (12.5 g) of total eggs stripped were used to fertilize milt. Milt was obtained by sacrificing the male and testis removed, cleaned with cotton wool to remove all the stained blood and then kept in a clean Petri dish and thereafter macerated to squeeze out milt. The milt and eggs were mixed together gently with a plastic spoon for 2-3 minutes. The fertilized eggs were divided into three equal portions and was used for the three treatments (T_1 (control), T_2 (0.5 ml) and T_3) (1 ml). Treatment two and three (T_2 and T_3) were treated with 20 ml diluted izal concentration for 60 seconds. Each treatment was replicated three times. Small quantity of saline solution was then pour onto the eggs to avoid sticking together. The fertilized eggs were rinsed with distilled water and taken to the incubator for incubation. Incubator made of net hapa with kakabarns, placed inside glass aquarium tanks filled with clean water was used for the purpose. Fertilized eggs were spread in a monolayer on the kakabarns in the incubator. Aeration was maintained by flow through system. The hapa was constructed from a coated nylon net with 1.5 mm mesh size. When hatching was completed the hapa with un- hatched eggs and shells was lifted out of the incubation tank and washed. 450 fries for each treatment at stocking rate of 150 fries per glass aquaria tank was reared for 8 weeks. After yolk absorption, the hatchlings were fed with decapsulated artemia. Water quality parameters including temperature, Dissolved Oxygen, pH and conductivity were monitored and maintained at optimum level. The weights of the hatchlings were determined using sensitive electronic balance (P.E. mx Rady).

Fecundity, percentage fertilization and hatchability were determined according to method described by (Oyelese, 2006) [9] using the formulae:

$$\text{Fecundity} = \frac{\text{Total weight of stripped eggs}}{\text{Weight of eggs in sub-sample}} \times \text{Total No. of eggs in sub-sample} \quad (1)$$

$$\text{Percentage Fertilization} = \frac{\text{No. of fertilized eggs}}{\text{No. of eggs stripped}} \times 100 \quad (2)$$

$$\text{Percentage Hatchability} = \frac{\text{No. of fry}}{\text{No. of fertilized eggs}} \times 100 \quad (3)$$

Percentage mortality and survival rates were determined with the following formula:

$$\text{Percentage Survival} = \frac{\text{Cumulative Survival}}{\text{Total number stocked}} \times 100 \quad (4)$$

(After Bargenal, 1978) as adopted by Yisa *et al*. (2010).

$$\text{Percentage Mortality} = \frac{\text{Cumulative Mortality}}{\text{Total number stocked}} \times 100 \quad (5)$$

One way Analysis of Variance (ANOVA) was used as statistical tool for the analysis. Data obtained were pooled from the replicate and mean values was calculated per treatment. Also Duncan Multiple range Test was used for mean separation. All differences in mean values of parameters were determined at P = 0.05 level of significance.

3. Results

The result of fecundity, percentage fertilization and hatchability from the brood stocks *Clarias gariepinus* are presented in Table 1. It showed that fecundity did not differed significantly (P>0.05) 113943.1^a, 109281.9^a and 116081.9^a for 0.5 ml, 1 ml and control among the treatments. Results also showed that percentage fertilization differed significantly (P<0.05) among the treatments. Table 2, 3 and 4 shows the cumulative mortality and survival rates that was recorded for 8 weeks of rearing. Cumulative mortality was 12, 16 and 24 respectively for 05 ml, 1 ml and control. Table 4 indicated highest mortality in the control. Mean Total Body Weight (TBW) gain differed significantly (P<0.05) between 1 ml (3.08^a) and 0.5 ml (2.47^b) as indicated in Table 5).

4. Discussion

The significant difference in percentage fertilization was attributed to egg viability and milt quality as observed by Yisa (2012) [14] that the viability of egg and milt quality is a determining factor for higher fertilization. Similar trend was observed in hatching. Pedersen *et al*. (2008) [11] reported that formalin is one of the most commonly used chemical treatments for fungal control in fish hatcheries and effective in the control of fungus on eggs without adverse effect on hatchability and post-hatch survival, izal in similar way has proven to be effective as shown in this study. The low mortality recorded in 0.5 ml and 1ml izal concentrations was indicative of the fact that izal like other disinfectants had effectively reduced fungi infection on eggs and larvae of *Clarias gariepinus*. This result corroborated the report of Akpoilih and Adebayo (2010) [1] where they recorded percentage survival to be high (85.53 ± 9.56^a) post egg disinfection. This was also attributed to egg and milt quality and viability resulted in vigour hatchlings which increase chances of high survival as observed by (Yisa (2012) [14]. Mean Total Body Weight (TBW) was highest in 1ml. Fungi infection on the fry was reduced hence free from disease problem, this facilitate their growth rate.

5. Conclusion

Eggs of *Clarias gariepinus* treated with 1 ml diluted izal concentration for 60 seconds in terms of fertilization, hatching, survival and growth performance was most effective and therefore recommended.

Table 1: Mean Fecundity, % Fertilization and % Hatching of *Clarias gariepinus.*

Parameters	0.5 ml	1 ml	Control	±S.E.
Fecundity	113943.1[a]	109281.9[a]	116081.9[a]	6089.94
% Fertilization	61.64[b]	64.85[a]	64.13[ab]	1.422
% Hatching	53.80[a]	37.94[a]	46.43[b]	1.453

Values carrying different superscript on the same row differed significantly from each other ($p<0.05$).

Table 2: Mean cumulative Mortality and Survival Rates and Percentages for *Clarias gariepinus* Fry Eggs Treated at 0.5 ml Izal and Reared in Plastic Bowls for 8 Weeks.

Initial Stock Per Plastic Bowl 150				
Period (Weeks)	**Mortality**	**% Cumulative Mortality**	**Survival**	**% Cumulative Survival**
1	1	0.66	149	96.35
2	7	4.66	143	95.33
3	10	6.66	140	93.33
4	11	7.33	139	92.66
5	12	8.00	138	92.00
6	12	8.00	138	92.00
7	12	8.00	138	92.00
8	12	8.00	138	92.00
Mean		6.41		93.58

Table 3: Mean cumulative Mortality and Survival Rates and Percentages for *Clarias gariepinus* Fry Treated at 1 ml Izal and Reared in Plastic Bowls for 8 Weeks.

Initial Stock Per Plastic Bowl 150				
Period (Weeks)	**Mortality**	**% Cumulative Mortality**	**Survival**	**% Cumulative Survival**
1	5	3.33	145	96.66
2	10	6.66	140	93.33
3	13	8.66	137	91.33
4	14	9.33	136	90.66
5	16	10.66	134	89.33
6	16	10.66	134	89.33
7	16	10.66	134	89.33
8	16	10.66	134	89.33
Mean		8.83		91.16

Table 4: Mean cumulative Mortality and Survival Rates and Percentages for *Clarias gariepinus* Fry Control and Reared in Plastic Bowls for 8 Weeks.

Initial Stock Per Plastic Bowl 150				
Period (Weeks)	**Mortality**	**% Cumulative Mortality**	**Survival**	**% Cumulative Survival**
1	11	7.33	139	92.66
2	19	12.66	131	87.33
3	21	14.00	129	86.00
4	22	14.66	128	85.33
5	23	15.33	127	84.66
6	24	16.00	126	84.00
7	24	16.00	126	84.00
8	24	16.00	126	84.00
Mean		13.99		85.99

Table 5: Body Weight Gain of *Clarias gariepinus* Fry Treated Eggs with Izal at 0.5 ml, 1 ml and Control Reared in Plastic Bowls for 8 Weeks.

Parameter	Weeks	T1 0.5 ml	T2 1 ml TBW (g)	Control
	1	0.15 [a]	0.18 [a]	0.14 [a]
	2	0.29 [a]	0.28 [a]	0.24 [c]
	3	0.36 [a]	0.35 [a]	0.30 [a]
	4	0.20 [a]	0.26 [a]	0.36 [a]
	5	0.25 [c]	0.38 [b]	0.53 [a]
	6	1.38 [a]	2.41 [a]	2.65 [a]
	7	1.25 [b]	1.62 [a]	1.53 [a]
	8	2.47 [b]	3.08 [a]	3.22 [a]
	Mean	0.79	1.07	1.12

Values with different superscript on the same row differed significantly from each other ($p<0.05$).

Key: TBW= Total Body Weight.

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