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PRELIMINARY STUDY ON THE EFFECT OF WATERMELON JUICE AND MILK SOLUTION ON DE-ADHESION, DEVELOPMENTAL STAGES AND HATCHING RATE OF EGGS AND SURVIVAL OF FRY OF *CLARIAS GARIEPINUS*

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ABSTRACT
Sticky layer of *Clarias gariepinus* eggs cause the clumping of eggs immediately after fertilization, this create low oxygen, and increase pathogenic infection, thereby reducing fertilization and hatching rate hence reduces in mass production of *C. gariepinus* seed. Although various substances are use in the removal of the sticky layer, no information is available about the use of watermelon. Therefore, the present study, aim in exploring the potential of using watermelon in removing the sticky layer of *C. gariepinus* to enhance production. Fertilized eggs were treated with watermelon juice (T1) for comparison with 14 g L^{-1} milk solution (T2 as de-adhesion reference) and water as control (T_0 as adhesion reference). The treatments were agitated with aeration for 30 minutes, rinsed with water, and poured on respective netted hatching trough, with water recirculation for incubation at 27 ^{0}C for 24 h period. Except for T_0, no clumping of eggs was observed in the remaining groups. Slight variation in attaining a developmental stage among eggs was observed in eggs treated with watermelon. Hatching and survival rate in T2 are higher than T1 and T_0 ($P<0.05$). However, watermelon indicated the potential, as it removed the sticky layer while maintaining hatching and survival rate as in the control (T_0) ($P>0.05$), more should be explored before adoption of watermelon for de-adhesion and incubation of *C. gariepinus* eggs.

Keywords: Watermelon Juice, Milk solution, Sticky layer of egg, water recirculation

INTRODUCTION

The egg of *C. gariepinus* have sticky layer, which compose of glycoprotein. This layer becomes activated once the eggs made contact with water after fertilization (FAO, 1987). In the wild, the presence of sticky layer is adaptive strategy that enhance survival of embryo by allowing the eggs to adhere to the substrate, enhance exposure to oxygen, and avoid predation or being carried away by the water current (Riehl and Patzner, 1998). However, such layer pose challenge for mass hatching in hatchery, as the eggs need to be incubated at high density with constant flow through of water, otherwise the eggs clumps to create low oxygen, and increase fungal infection, thereby enhancing the mortality (Ljubobratović et al., 2017). According FAO (1987) report, removal of sticky layer allows the spread of eggs to form several layers in hatching trays or jars.

The choice of materials for the chemical or enzymatic removal of sticky layer defends on the ability to breakdown the peptide bond or dissolve glycoprotein in the layer (Pooley et al., 2019; Ringle et al., 1992). For example, the selection of pineapple juice was because of the abundance of proteolytic enzymes which can digest protein (Adogbeji and Francis, 2015; Thai and Ngo, 2004), while tannin solution for it phenolic compound content that bind and precipitate glycoprotein (Maulidiyah et al., 2019). Therefore, substances that can inhibit the activities of glycoprotein could have the potential of removing sticky layer of fish egg.

Different part of watermelon (i.e. pulp, rind and seed) contain tannin, saponin, alkaloid, phenol, and flavonoids in varying quantities (Johnson et al., 2014). Polyphenols (e.g. tannin and flavonoids) bind and precipitate or shrink proteins (Ashok & Upadhyaya, 2012). Hence, in view of the chemical constituents, the present study was preliminarily designed to explore the potential of watermelon juice in removing the sticky layer of *C. gariepinus* eggs.

MATERIALS AND METHODS

Study site and source of experimental seed

The study was carried out in the Aquarium unit of Biological Sciences Department, Bayero University, Kano. Matured male and female brooder weighing 1.9 kg and 2.5 kg respectively were used to obtain the seeds artificially through induce breading followed by incubation of fertilized eggs (de Graaf and Janssen 1996).

Preparation of treatments and Experimental design

Concentrated watermelon juice was obtained by blending, squeezing and filtering the reddish portion (Pulp) of the fruit. Milk solution was prepared by dissolving 14 g of powdered milk into a liter of distilled water (Muchlisin et al., 2014). 120 g of fertilized eggs were treated with watermelon juice (T1) for comparison with 14 g L^{-1} milk solution (T2 as de-adhesion reference) and water as control (T_0 as adhesion reference). The eggs in the treatments were agitated with aeration for 30 minutes then rinsed with hatchery water thrice. 10 g from each treatment is poured on respective netted hatching troughs, with water recirculation for incubation at 27 ^{0}C for 24 h. The developmental stages of embryo were observed from each treatment at 2 hours intervals using a light microscope at ×40, up to the time when movement of the tail to either side of the chorion was observed and hatching rates were measured following the incubation of the eggs. The identification of the stages were done following (Sule and Adikwu, 2004). Picture of the important stages were taken using a phone mounted to the microscope.

After the eggs were hatched, 3 days old fry were placed into 3 groups (each 200 fry) in duplicate against control, maintaining the order in which the treatments were used. The survival rate of fry was measure after two weeks of satiation feeding with artemia.

Formulae and Analysis of the Data

Formula

The following formulae were used:

$$\text{Hatching Rate\%} = \frac{\text{no of eggs incubated}}{\text{Total number of eggs}} \times 100$$

$$\text{Survival rate \%} = \frac{\text{no of fry at the end of experiment}}{\text{innitial number of fry}} \times 100$$

In the formula, total number of eggs and total number of incubated eggs in each group were determined according to the method of de Graaf et al., (1995).

Statistical Analysis of the Data

One-way analysis of variance was used to compare the differences in the effects of the de-adhesion treatments on the hatching and survival rate of egg of *C. gariepinus* using Sigma Stat package V. 3.5.

RESULT AND DISCUSSION

Egg de-adhesion efficiency

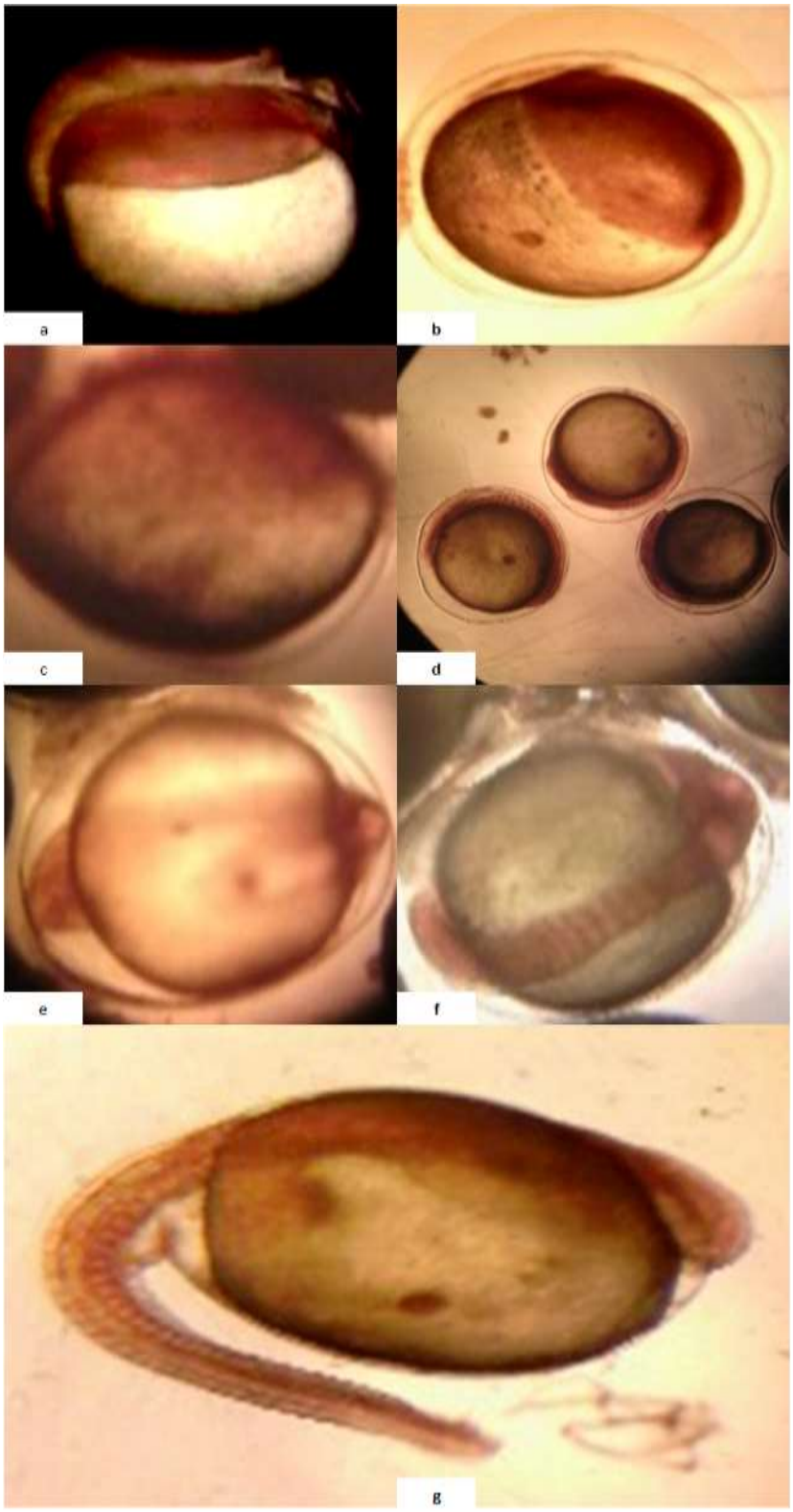
Except for control, all the treatments were observed to have removed the adhesiveness or sticky layer of *C. gariepinus* eggs to certain degrees. Base on physical observation, the segregation of eggs treated with milk solution is slightly higher than the watermelon treatment.

Embryonic developmental stages

The embryonic developmental stages of all treatments shared similar stages at the same time interval of microscopy with slight difference of attaining a particular stage in eggs treated with watermelon.

Table 1 The developmental stages of *C. gariepinus* eggs in different treatments

Parameters	T_0	T_1	T_2
Initial (after 30 mins)	Formation of animal and vegetal pole	Formation of animal and vegetal pole	Formation of animal and vegetal pole
2hr:30 mins	32-cells stage	32-cells stage	32-cells stage
4hr:30 mins	Blastula stage	Blastula stage	Morula stage
6hr:30 mins	Gastrula stage	Gastrula stage	Gastrula stage
8hr:30 mins	Gastrula stage	Gastrula stage	Gastrula stage
10hr:30mins	Gastrula stage fully achieved	Gastrula stage	Gastrula stage
22hr :30mins	First wriggling movement of embryo	First wriggling movement of embryo	First wriggling movement of embryo



Formation of animal vegetal pole **b**. 32 cells stage **c**. Morula Stage **d**. Blastula Stage **e.** Gastrula stage **f.** First wriggling movement **g.** Violent movement of the tail

Figure 1 Embryonic developmental stages of *C. gariepinus* egg

Hatching rate

The effect of different level of treatments differ among the treatments (P<0.05). In the recirculation hatching trough system, the hatching rate in T1 (81.00%) differ significantly with T1 (63.85%) and the T_0 (65.55%) (*P<0.05*).

Table 2 Egg hatching rate and Fry survival rate of *C. gariepinus*

Parameters	T_0	T_1	T_2	LSD
Hatching Rate (%)	65.55 ± 1.48^b	63.85 ± 2.05^b	81.00 ± 1.98^a	8.345
Survival Rate (%)	32.6 ± 1.13^b	23.06 ± 4.67^b	56.2 ± 8.63^a	18.149

*The same superscript on same row indicate no significant differences
*LSD: Least significant difference at which the mean differs

DISCUSSION

The study reveals that watermelon juice removed the sticky layer of eggs to certain degree compares to control. The removal is the possible action of certain phytochemicals content of watermelon that inhibits the activities of glycoprotein responsible for the egg's adhesion. Watermelon (i.e. pulp, rind and seed) contain tannin, saponin, alkaloid, phenol, and flavonoids in varying quantities (Johnson et al., 2014). Polyphenols (e.g. tannin and flavonoids) bind and precipitate or shrink proteins (Ashok and Upadhyaya, 2012). Studies show that saponin (Kiersnowska et al., 2011; Li et al., 2014), alkaloid (Lei et al., 2013; Tropes et al., 1989), flavonoids (Bansal et al., 2009; Pietro et al., 2002), tannin (Kitagawa et al., 2007) and polyphenol (Kitagawa, 2006), inhibit the activities of p-glycoproteins of different cells. Unlike phytochemicals of watermelon that needs to react with protein for action, milk reduces the aggregation of eggs by directly coating the eggs with milk particles, which prevent the eggs from sticking to each other. Hence, the observed high de-adhesion in milk treatment beyond the treatment with watermelon.

The hatching rate of eggs treated with milk solution increases when compare with watermelon juice and control. Although the sticky layer of the egg was removed, the concentration of phytochemical constituent of watermelon juice may be the course of delay in attaining certain of stages embryonic development of the eggs which in turn affect the hatching rate, thereby causing the statistically similar degree of hatching and fry survival rate between watermelon and control. This could be attributed to the possible absorptions of ions (iron, calcium, magnesium) by some phytochemicals that create ionic imbalances during embryonic development. Tannin leads to deficiency of calcium, magnesium, iron (Ashok and Upadhyaya, 2012; Ljubobratović et al., 2017), and flavonoid affect the expression and activity of proteins involved the systemic regulation of iron metabolism and iron absorption (Lesjak, 2019). Meanwhile fresh watermelon is characterized by high concentration of flavonoid (mg/100 g) of around 58.10 ± 0.33 (Pulp); 40.16 ± 0.01 (Seed); 8.71 ± 0.01 (Rind) (Johnson et al., 2014). Alafiatayo et., al (2019), observed high mortality of embryo and larvae deformity of zebra fish at high concentration (125.0 ug/mL) of *C. longa* flavonoid extract. Furthermore, 1 to 5 µg/ml saponin affect the hatching time by 18 h, and exposure to 5 µg/ml saponin exhibited 100% embryonic mortality of zebra fish - *Danio rerio*.

CONCLUSION

While the milk solution shows high hatching, and survival rate because the action on egg is restricted to the surface of the egg. Ljubobratović et al., (2017) suggested that, the usage of milk as de-adhesion substance appears to be impractical. Watermelon indicated the potential, as it removed the sticky layer while maintaining hatching and survival rate as in the control (T_0), but, more should be explored before adoption of watermelon for de-adhesion and incubation of *C. gariepinus* eggs. Like milk, the use of watermelon pulp as de-adhesion substance may be impracticable as they may add the cost of fish seed production or compete with human. Similarly, the concentration of phytochemicals in watermelon juice is higher. However, the rind of watermelon will serve the purpose of de-adhesion since it constitutes the same phytochemicals in lower concentration compare with pulp portion.

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