Journal of Agriculture and Environment
Vol. 19 No. 2, 2023: 143-152

Effect of different exposure time of papain on adhesion and some reproductive indices of *Clarias gariepinus* (Burchell, 1822) eggs under semi-arid environment of Sokoto, north-western, Nigeria

M.Y. Abubakar[1], L.A. Argungu[1], B.A. Shinkafi[2], I.S. Jega[3], G. Abdullahi[1] and H.F. Maradun[1]

[1]Department of Fisheries and Aquaculture, Faculty of Agriculture, Usmanu Danfodio University, Sokoto
[2]Department of Bilogical Sciences, Federal Univeristy, Gusau, Zamfara State
[3]Department of Forestry and Fisheries, Faculty of Agriculture, Kebbi State University of Science and Technology, Aliero

ABSTRACT

Egg adhesiveness can cause major problems affecting the hatchability of *Clarias gariepinus*. However, appropriate media for de-adhesion in Catfish eggs is not yet well established. In this study, the effect of the anti-adhesion properties of Papain enzyme (extracted from pawpaw fruit peel) on Clarias eggs was investigated in terms of non-stickiness of eggs, fertilization rates hatchability and fry survival. Milt and eggs obtained from African Catfish broodstock of the same lineage were used for induced breeding following standard procedure. 4g of eggs were subjected to four (4) different exposure times of Papain – 30, 60, 120 and 300 seconds – and compared against a control. Treatment efficacy was assessed by comparing the percentage of non-sticky eggs, fertilization, hatching and survival rate in the treatments and control. Exposure time of 30 seconds was found to be most effective in preventing adhesiveness in the eggs. Also, eggs subjected to 30, 120 and 300 seconds of papain exposure had the best hatchability (50.58±1.84**,** 53.91±4.53**,** 59.21±2.97). Survival rate was similar among the treatments and control, except for exposure time of 300 (81.66±7.15) seconds with significantly lower survival rate. For effective hatchability and survival of *Clarias gariepinus* eggs, exposure to papain at 120 seconds is recommended.

Keywords: *Clarias gariepinus*; exposure time; enzyme papain; de-adhesion

INTRODUCTION

The African catfish, *Clarias gariepinus* is considered as one of the most economically important freshwater fish in Nigeria and is a source of high-quality protein in developing countries. This species is known for its resistance to diseases, high growth rate, resistance to handling stress, ability to tolerate a wide range of environmental stressors and high stocking densities under culture conditions (Rasowo *et al.,* 2007; Wachirachaikarn *et al.,* 2009).

ISSN: 1595-465X (Print) 2695-236X (Online) https://dx.doi.org/10.4314/jagrenv.v19i2.15

According to Ojutiku (2008), the scarcity of fingerlings of this widely acceptable species constitutes a major constraint to the rapid development of fish farming in Nigeria. A previous study by Atanda (2007) indicated that the total seed production and supply in Nigeria from all sources amount to 55 million fingerlings while the immediate need of the market is 500 million per annum. This shortfall is traceable to low hatching and survival rates which could be linked to the adhesiveness of eggs (Muchlisin *et al.,* 2010).

Under natural condition, egg adhesiveness is a reproductive strategy of most teleost for protecting the eggs from drifting with currents. 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. According to Abigail *et al.,* (2010), adhesiveness of eggs can cause high larval mortality. This is because the adhesiveness covers the micropyle and hinders the sperms from fertilizing the eggs (Prinsloo *et al.,* 1987), as such the chances of sperm getting in contact with the eggs are reduced and hence the chances of the eggs getting fertilized are compromised.

Interestingly, several researches have attempted the use of various materials to control adhesiveness in eggs of different fish species such as milk for common carp (El-Gamal *et al.,* 2008), tannic acid for pike perch (Demska *et al.,* 2005), kaolin for shishamo smelt (Mizuno *et al.,* 2004), mud solution in Japanese dace (Nakamura, 1966) and urea solution in carp (Rothbard, 1978). However, only 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 exposure time for papain enzyme required to eliminate the stickiness of the African catfish eggs for improve fertilization and hatching rates.

MATERIALS AND METHODS

Experimental Site

The research was carried out in the Fish hatchery of the Department of Fisheries and Aquaculture, Faculty of Agriculture, Usmanu Danfodiyo University, Sokoto state. The annual average temperature is 28.3ºC (82.9ºF) with maximum daytime temperature for most of the year at 40ºC (104.0ºF). The warmest months are between March and June when daytime temperature can exceed 45ºC, the rainy season is from June to October while the other times of the year are characterized by Harmattan (Mamman *et al.,* 2014).

Papain Enzyme and Extraction

The enzyme used in this research work was Papain, extracted from pawpaw (*Carica papaya*) latex. The Unripe Pawpaw fruit was sourced from a backyard farm in Arkilla, Sokoto.

Other materials used include blender, test tube, freeze-drying machine, refrigerator, centrifuge machine, conical flask, funnel, bowls, knife, and polythene bag. NaOH, $(NH_4)_2SO_4$ and Distilled H_2O were used as reagents. The procedure for extraction was carried out as follows:

1. Unripe pawpaw was peeled inside polythene bag and latex and immersed inside a 5-liters bowl containing ice to prevent the enzyme from denaturing.

2. The peeled pawpaw was grinded using an electric grinder and NaOH was added to prevent foaming and to raise the pH to 9.0. Few drops of ethanol were also added for precipitation and the extract was sieved into a conical flask using a colander.
3. 200g of $(NH_4)_2SO_4$ was added into the extract and the extract was precipitated using a centrifuge ran at 500 rpm.
4. The precipitate was then collected inside a beaker ethanol and water was added to remove the ammonium sulphate.
5. The extract was stored in a refrigerator and was observed to be solid after 24hours and the solid extract was then freeze dried for another 24 hours using a freeze dryer at a pressure of 000009pa.
6. After the extract got dried, it was preserved at room temperature. When ready for use, it was re-activated by adding water.

Plate 1: Unripe pawpaw (*Carica papaya*)

Plate 2: Peeled Papaya fruit

Experimental Design

The experiment consisted of five (5) treatments based on the exposure time of the just fertilized eggs to papain enzyme solution. Each treatment was replicated three (3) times in a Completely Randomized Design (CRD) using 70 litres plastic bowls as incubation/spawning tanks. The bowls were labelled as T_1, T_2, T_3, T_4, and T_5., replicated as R_1, R_2 and R_3 respectively.

Experimental Procedure

The bowls were filled with water almost to $^2/3$ level, constantly aerated with aerator pumps and temperature was maintained at a range of 27.4 - 30.8^{o}C. Prepared spawning mats were washed and placed inside each bowl where the eggs were to be placed. For each treatment, 4g of fertilized eggs were rinsed and retained in 2% of papain solution according to treatment. T1 (control) had no exposure to papain, while T2, T3, T4 and T5 had 30, 60,120 and 300 seconds exposure times respectively before incubation.

Incubation of Eggs

The eggs were incubated using a plastic bowl of 70 litres capacity, and spawning mat was laid inside the spawning tank. Before starting the experiment, the net and the bowl were disinfected with salt and filled with clean and well aerated water. The fertilized eggs and treatments were poured and incubated in separate tanks.

Data Collection

Percentage Non-stickiness of Eggs: Egg adhesiveness was assessed by calculating the percentage of whole eggs. The number of whole (non-clumped) eggs and aggregated eggs in each container were counted after 1 hour of incubation. The percentage of non-stickiness was estimated as follows:

$$\text{Non stickiness of eggs} = \frac{Number\ of\ non{-}sticky\ eggs}{Initial\ number\ of\ eggs} \text{x}100$$ Kereem *et al.* (2017)

Percentage Fertilization rate (Fr): Fertilization was evaluated after 6 hours of incubation by counting the transparent embryos relative to the white colour unfertilized or dead eggs. The percentage fertilization was then calculated as:

$$\text{Percentage Fertilization rate} = \frac{Number\ of\ fertilized\ eggs}{Initial\ number\ of\ eggs} \text{x}100$$ (Hogendoorn, 1979)

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

$$\text{Percentage Hatchability} = \frac{Number\ of\ hatchlings}{Total\ number\ of\ fertilized\ eggs} \text{x}100$$ (Ayinla and Nwadukwe, 1990)

Percentage Survival rate: The survival rate of larva was monitored for 3 days for each treatment. The percentage survival rate was estimated as follows:

Percentage Survival rate = $\frac{Final\ number\ of\ hatchlings}{Initial\ number\ of\ hatchlings}$x100 (Ayinla and Nwadukwe, 1990)

Data Analysis

Data collected on fertilization, hatchability, survival and growth were subjected to analysis of variance (ANOVA), and means were separated using New Duncan's Multiple Range Test (DMRT) (Gomez & Gomez, 1984). The analysis was carried out using the SPSS V: 20.0 package for Windows.

RESULTS

Effect of Papain on Non- sticky Eggs

The result obtained on percentage non-stickiness of eggs at different exposure time as presented in table 4.1 shows that eggs exposed to papain at 30 secs were significantly higher ($p<0.05$) in percentage of non-sticky eggs with a mean value of (32.34±6.41 %) than eggs exposed to papain at 120 secs. However, there was no significant difference when compared to the control and exposure time of 60 secs and 300 secs.

Effect of Papain on Fertilization:

The effect of papain on the percentage fertilization as presented in Table 4.1 below showed that the control had the highest percentage fertilization rate with a mean value of 49.75±6.63 % and was significantly higher than the other treatments. There was no significant difference ($p>0.05$) among eggs exposed to papain for 30 secs, 60 secs, 120 secs and 300 secs.

Effect of Papain on Hatchability:

Table 1 shows that there was no significant difference in hatchability between the eggs exposed to 30, 120 and 300 seconds of papain. These were however significantly higher than the control with mean hatchability percentage of 37.71±5.72 %.

Effect of Papain on Survival:

The result obtained on percentage survival of eggs at different exposure time as presented in Table 1 shows that there was no significant difference ($P>0.05$) among eggs exposed to papain for 30 secs, 60 secs, 120 secs and control. An exposure time of 300 secs produced larva with the least survival with mean value of 81.66±7.15 %.

Table 1: Egg reproductive indices

	Exposure time				
Parameters	TRT1	TRT2	TRT3	TRT4	TRT5
% of Non-sticky eggs	25.70±0.26^{ab}	32.34±6.41^{a}	27.15±0.76^{ab}	21.08±2.04^{b}	25.43±1.97^{ab}
% Fertilization rate	49.75±6.63^{a}	37.45±0.57^{b}	37.86±0.86^{b}	34.38±3.20^{b}	39.04±1.34^{b}
% Hatchability	37.71±5.72^{b}	50.58±1.84^{a}	47.67±1.17^{ab}	53.91±4.53^{a}	59.21±2.97^{a}
% Survival rate	98.95±0.58^{a}	96.13±2.44^{a}	98.95±0.45^{a}	98.57±0.16^{a}	81.66±7.15^{b}

TRT1 = Control; TRT2 = 30 seconds; TRT3 = 60 seconds; TRT4 = 120 seconds; TRT5 = 300 seconds

Water Temperature

The result obtained for water temperature during this experiment as presented in Table 2 below; indicated that the highest water temperature recorded was in the evening with mean value (29.65°±0.14) while the least was recorded in the morning (28.12±0.16). However, the minimum temperature recorded was 27.40 while maximum was 30.80.

pH

The results obtained for pH was presented in Table 2, revealed that the highest pH was recorded in the afternoon (7.69±0.04) while the least was in the morning (7.62±0.04). The minimum pH recorded was 7.19 while the maximum pH recorded was 7.90.

Electrical Conductivity

The result obtained for conductivity as presented in Table 2, showed that the highest conductivity observed was (489.15±2.20) in the morning while the least was (484.15±2.69) in the afternoon. The minimum electrical conductivity recorded was 463 in the afternoon while the maximum recorded was 500.

Table 2: Water quality parameters

Parameters	Morning	Afternoon	Evening
Water temperature (°C)	28.12±0.16	29.25±0.17	29.65±0.14
Minimum	27.40°C	28.50°C	28.60°C
Maximum	29.20°C	30.50°C	30.80°C
pH	7.62±0.04	7.69±0.04	7.67±0.06
Minimum	7.20	7.41	7.19
Maximum	7.89	7.87	7.90
Electrical Conductivity (µs/cm)	489.85±2.20	484.15±2.69	485.08±2.02
Minimum	476	463	476
Maximum	500	492	498

DISCUSSION

In this experiment, the percentage non-stickiness of eggs shows that the reduction of adhesiveness in *Clarias gariepinus* eggs can be achieved using papain. The highest percentage (32%) was obtained in treatment 2 exposed to papain for 30 secs and was

significantly different (P<0.05) from control, and longer exposure times. This is in line with Kareem *et al.,* (2017) who reported that every rinsing agent has a specific exposure time when they are most effective. The result obtained is also in line with other research that reported significant differences in adhesion between control and the use of rinsing agents. Fadholi and Sanoesi (2018), recorded more than 90% non-sticky eggs for 60 secs exposure time, leading to 99.17% fertilization rate using papain on Iridescent Sharks eggs (*Pangasianodon hypophthalmus*). Also, Feledi *et al.* (2011) in Poland, recorded 89% non-sticky eggs for 15 mins exposure time using Siberian Sturgeon.

The percentage fertilization was highest in control (49%), while treatment 4 had the lowest percentage fertilization (34%) which was significantly difference (P<0.05). The fertilized eggs usually develop normally if the incubation conditions (temperature, cleanliness, dissolved oxygen) are adequate (FAO, 2011). These factors were taken care of during the experimental period. The percentage fertilization obtained from this experiment is within the range of Kareem *et al.,* (2017), who reported his percentage fertilization ranged as 34.17% to 97.86% in 6 g of urea solution at 5 minutes exposure time and in 2 g urea with 10 minutes exposure time, respectively. Also, Asraf *et al.* (2013) recorded 38 % fertilization rate on *Clarias gariepinus* using a solution of 2 g urea + 4 g NaCl/L water for 1 min exposure time. The success of a breeding program is determined by various factors. The quality of eggs and sperm are two of the important factors in a breeding program. It is very likely that the quality of eggs and sperms in this study was sub-optimal and therefore resulted in lower fertilization and hatching rates.

Proteolytic enzymes have been successfully applied to common carp, tench and African catfish eggs, leading to a hatching rate of over 80% (Linhart *et al.,* 2000, 2004; Zarski *et al.,* 2015). From the study, treatment 5 had the highest hatchability percentage (59 %) and was not significant (P>0.05) among the treatments while treatment 1 (control) in this experiment had the lowest percentage hatchability (37 %) which was significantly different (P<0.05) among other treatments. Żarski *et al.* (2015) were the first to report high hatching rates after using solely tannic acid to remove adhesiveness in pikeperch eggs. This result correlated with the result obtained by Kareem *et al.,* (2017), who recorded highest percentage hatching rate of 62.50% from 10 g Powdered milk at 25 minutes exposure time. The control (0 g PM) gave the lowest hatching of 31.81% at 25 minutes rinsing time. Al-Hazzaa and Hussein (2003) recorded 58% hatching using tannic acid+2 g of NaCl on himri eggs, Asraf *et al.,* (2013) reported 25% hatching using 2 g urea+4 g NaCl/L of solution on *Clarias gariepinus* eggs and Fadholi and Sanoesi recorded the best percentage hatching (80.31%) on Iridescent shark using papain from Carica papaya leaf solution. There are several factors that influence the production and quality of seed quality of brood stock (Marteinsdottir and Steinarsson, 1998; Al-Hazzaa and Hussein, 2003), by incubation condition and other physiological influences (Schreck *et al.,* 2001; Al-Hazzaa and Hussein, 2003).

There was no significant difference (P>0.05) among the treatments and the control, treatment 3 and control had the highest percentage survival rate (98%). However, there was significant difference (P<0.05) with treatment 5 (81%). This result was in line with Neitali *et al.* (2013), who reported a higher survival rate (more than 80%) when trypsin was used for de-adhesion of Persian sturgeon eggs compared to the use of clay suspension (less than 40%). In pike perch, use of tannic acid resulted in more hatching and survival rates of alevins (Zarski *et al.,* 2015). According to Istvan *et al.* (2017), he recorded 36.7%, 89%, and 93 % for tannic acid, Enzyme and Milk respectively after 3 days of incubation as percentage survival rate. Kareem *et al*. (2017), reported highest percentage survival rate (94-100%) on *Clarias*

gariepinus using papain enzyme for 30 secs, 1 min, 2 mins, 5 mins and 10 mins as exposure time.

CONCLUSION

This study shows that papain enzyme has significant effect on removing stickiness associated with *Clarias gariepinus* eggs. Treatment 2 (papain solution exposed for 30 seconds) removed adhesiveness in African catfish eggs better than all the other treatments. However, the control proof to be the best when fertilization is considered. Treatment 2,4 and 5 (30,120 and 300 seconds) yielded the better hatchability rate. There was high survival rate in all the treatments except for the treatment 5. The results therefore confirm that catfish eggs rinsed in papain enzyme solution can reduce the clumping of eggs and also enhance fertilization and hatching rate.

Papain extract is therefore recommended to be used to remove adhesion on *Clarias gariepinus* on exposure time for 120 seconds eggs and improve hatchability and survival.

REFERENCES

Abigail, B.W., Ward, D., Duclos, K., Peters, M. and Berlinsky, D.L (2010). Surface disinfection and removal of adhesiveness from rainbow smelt eggs. *North American Journal of Aquaculture*, 72: 158-163.

Al-Hazzaa, R. and Hussein, A. (2003). Stickiness elimination of himri barbel (*Barbus lutes*, Heckel) eggs. *Turkish Journal of Fisheries and Aquatic Sciences*, 3: 47-50.

Asraf, A., Muchlisin, Z.A. and Siti-Azizah, M.N. (2013). Removal of eggs Adhesiveness of African catfish (*Clarias gariepinus*) at different concentrations of urea solution. *Aceh International Journal of Science and Technology*, 2(3): 94-97.

Atanda, A.N. (2007). Freshwater fish seed resources in Nigeria. In: M.G. Bondad-Reantaso. *Assessment of Freshwater Fish Seed Resources for Sustainable Aquaculture*. FAO: 361-380.

Ayinla, O.A. and F.O. Nwadukwe (1990). Effect of season on controlled propagation of the African Catfish, *Clarias gariepinus* (Burchel 1822). *Nigerian Institute for Oceanography and Marine Research Technical Paper* No. 62, 15p.

Demska-Zakęś, K., Zakęś, Z. and Roszuk, J. (2005). The use of tannic acid to remove adhesiveness from pikeperch, *Sander lucioperca*, eggs. *Aquaculture Research*, 36(14): 1458-1464.

El-Gamal, A.H.E. and El-Greisy, Z.A. (2008). Effect of removal of egg adhesiveness on hatchability and effect of different levels of salinity on survival and larval development in common carp, *Cyprinus carpio*. *J of App Sci Res*, 4: 1935-1945.

FAO (2014). The State of World Fisheries and Aquaculture. Opportunities and Challenges. Food and Agricultural Organisation of the United Nations, Rome. 22.

Fadholi, M.R., and Sanoesi, E. (2018). The effect of immersion length in Papaya leaf solution (*Carica papaya* L.) toward fertilization and hatching of Iridescent Sharks (*Pangasianodon hypophthalmus* S.). *RJOAS:* 2(74).

FAO (2011). Regional Compendium on Aquaculture and Inland Fisheries Legislation (Asian Region). Rome: FAO Development Law Service.

Feledi, T., Kucska, B., & Rónyai, A. (2011). Effect of different fertilization and egg de-adhesion methods on the artificial propagation of Siberian sturgeon. *Archives of Polish Fisheries*. 19(2): 119-122.

Gomez, K.A. and A.A. Gomez (1984). *Statistical procedure for Agricultural Research*. 2nd Edition. New York: John Wily and sons, 680pp.

Horgendoorn, H. (1979). Controlled propagation of the African catfish *Clarias lazera* (C. and V.). *Aquaculture*, 17:323-333.

Kareem, O.K., E.K. Ajani, M.A. Akintunde, A.N. Olanrewaju and O.B. Oduntan (2017). Effect of different fertilization and egg de-adhesion methods on hatching and survival of *Clarias gariepinus* (Burchell 1822) fry. *Journal of Fisheries Sciences*, 11(1): 021-027.

Linhart, O., Gela, D., Flajshans, M., Duda, P., Rodina, M., Novak, V. (2000). Alcalase enzyme treatment for elimination of egg stickiness in tench, *Tinca tinca* L. Aquaculture. 191:303–308.

Linhart, O., Gela, D., Rodina, M., Kocour, M. (2004). Optimization of artificial propagation in European catfish, *Silurus glanis* L. *Aquaculture*. 235: 619–632.

Mamman, T., Ipinjolu, J.K., and Bilyaminu,G.D.(2014). Effect of drying methods on the nutritive value of some aquatic macrophytes in River Rima, North Western Nigeria. *Agriculture and Food Sciences Research*,1(1):11-14.

Marteinsdottir, G. and Steinarsson, A. (1998). Maternal influence on the size and viability of Iceland cod *Gadus morhua* eggs and larvae. *Journal of Fish Biology*. 52: 1241-1258.

Mizuno, S., Sasaki, Y., Omoto N. and Imada, K. (2004). Elimination of adhesiveness in the eggs of shishamo smelt Spirinchus lanceolatus using kaolin treatment to achieve high hatching rate in an environment with a high iron concentration. *Aquaculture*, 242: 713-726.

Muchlisin, Z.A., Nadiya, N., Nadiah, W.N., Musman, M. and Siti-Azizah, M.N. (2010). Preliminary study on the natural extenders for artificial breeding of African catfish *Clarias gariepinus* (Burchell, 1822). *AACL Bioflux*. 3: 119-124.

Nakamura, K. (1966). Method to eliminate egg adhesion in Japanese dace. *The Aquaculture* 14: 37-40.

Neitali, B.K., Amiri, B. M, Kalbassi M. R, Nouri A. (2013). A Comparative Study on the Effect of Trypsin Treatment and Clay Method on Jelly Coat Removal of the Persian *Sturgeon* (*Acipenser persicus*). *Journal of Fishery (in Persian)*, 67 (1): 109-122.

Ojutiku, R.O. (2008). Comparative Survival and Growth Rate of *Clarias gariepinus* and Heteroclarias Hatchlings Fed Live and Frozen Daphnia. *Pakistan Journal of Nutrition*, 7: 527-529.

Prinsloo, J.F., Schoonbee, H.J. (1987). Growth of Chinese grass carp Ctenopharyngdon idella fed on cabbage wastes and kikyu grass. *Water. S.A*. (13) 125-132.

Rasowo, J., Okoth, O.E. and Ngugi, C.C. (2007). Effects of formaldehyde, sodium chloride, potassium permanganate and hydrogen peroxide on hatch rate of African catfish *Clarias gariepinus* eggs. *Aquaculture*, 269: 271–277.

Rothburd, S. (1978). Notes on the induced spawning of the common carp, *Cyprinus carpio* a summary of work done at the fish breeding station at Gan Shmuel Israel. *Fish Farmer*, 22: 21-27.

Schreck, C.B., Contreras-Sanchez, W. and Fitzpatrick, M.S. (2001). Effects of stress on fish reproduction, gamete quality, and progeny. *Aquaculture*, 197: 3–24.

Wachirachaikarn A., Rungsin, W., Srisapoome, P. and Na-Nakorn, U. (2009). Crossing of African catfish (*Clarias gariepinus*) strains based on strain selection using genetic diversity data. *Aquaculture*, 290: 53-60.

Zarski, D., Krejszeff, S., Kucharczyk, D., Paliska-zarska, K., Targonska, K., Kupren, Fontaine, P. and Kestemont, P. (2015). The application of tannic acid to the elimination of egg stickiness at varied moments of the egg swelling process in pikeperch, *Sander lucioperca* (L.). *Aquaculture Research*, 46: 324–334.