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Effect of stickiness elimination agents at different rinsing time on Common Carp (*Cyprinus carpio* L.) eggs

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Abstract

Common carp (*Cyprinus carpio*) is one of the commercially important and commonly cultured fishes. The common carp are multiple breeders that produce adhesive eggs. The aim of this study is to reduce egg stickiness and increase the fertilization and hatching rate.The study was conducted from October to November 2021 at Ma Fatema fish hatchery, Chanchra, Jashore Sadar Upazila, Bangladesh. In this study, pineapple juice, milk powder and urea+salt with three different rinsing times in each were used to observe the impact on adhesiveness, fertilization and hatching rate of *C. carpio* eggs.In the case of pineapple juice solution, 3 minutes rinsing treatment showed the highest percentage of free eggs (90.00±4.54%), fertilization rate (85.00±7.00%) and hatching rate (87.00±7.54%) with significant difference ($P<0.05$) from 5 minutes and control group. Among three different rinsing times, 20 minutes of treatment of milk powder solution exposed the maximum percentage of free eggs (84.00±7.93%), fertilization rate (80.00±5.56%) and hatching rate (85.00±6.00%). Twenty (20) minutes of treatment was significantly different ($P<0.05$) from 15 minutes treatment for the percentage of free eggs and with 25 minutes treatment for the fertilization and hatching rate. The highest percentage of free eggs (94.00±2.00%), fertilization rate (90.00±3.60%) and hatching rate (82.00±2.64%) was observed at 5 minutes rinsing treatment of urea+salt solution and it was significantly different ($P<0.05$) from 1-minute rinsing treatment for the percentage of free eggs and fertilization rateand for the hatching rate of 10-minute rinsing treatment.

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Introduction

Bangladesh's fisheries and aquatic resources are commercially, environmentally, culturally, and aesthetically vital. It contributes 3.5 percent of the national GDP and 26 percent of agricultural GDP, 1.5 percent of total export profits, 60 percent of animal protein supply by taking 62.6 g/capita/day and employs more than 12% of the populace (DoF, 2020). This proportion comprises women who contribute directly and indirectly to food security, poverty alleviation, and the enhancement of fisherman's livelihoods (DoF, 2020). Total fisheries output in Bangladesh, based on culture and capture fisheries, come from closed and open inland water bodies (84.69 percent) and marine water (15.31 percent). Small-Scale Fisheries (SSF), often known as artisanal fisheries, account for around 1.5 million fishermen in marine fishing. More than 10 million coastal fishermen rely on these fisheries for a living, either directly or indirectly (DoF, 2018).

Carps are the most important species in Bangladesh's aquaculture industry, and their production is entirely dependent on timely and appropriate supplies of quality seeds. *Cyprinus carpio* output is the world's second greatest farmed fish production, primarily in Asia (Saikia and Das 2009). Many recreational fisheries, notably in Europe, such as the United Kingdom, the Czech Republic, and Germany, value common carp (Wedekind *et al*., 2001; Arlinghaus and Mehner 2003).

Common carp can reach sexual maturity as early as the end of its first year in some parts of its natural habitat, although it usually takes three to four years. Male carp reach maturity before female carp (Pinto *et al.*, 2005). In general, common carp breeds in natural bodies of water. The industrial community and farm level are critical for the effective spread of aquaculture and the economic status of farmers. Artificial fish breeding techniques and low-cost hatchery designs have been effectively implemented in Bangladesh since 1975.Egg adhesiveness has been observed in other fish species, primarily freshwater teleosts, where various chemical or enzymatic treatments and modes of application have been successfully used to either prevent eggs from becoming adhesive after artificial fertilization or, in a few studies, to remove the existing adhesive gum layer in naturally spawned eggs. The time and treatments employed to remove egg adhesiveness, however, varied between species. When applied at various time intervals immediately following artificial fertilization of manually stripped gametes, substances like urea and salt solutions, powdered milk, and clay have traditionally been used to inhibit egg adherence or to cover the egg surface (Kowtal*et al.,* 1986; Rottmann*et al.,* 1991; Ringle *et al.,* 1992; Linhart *et al.,* 2000; El-Gamal and El-Greisy, 2008).

The eggs of common carp is often sticky, which increases the adhesion properties among the different eggs, reducing the rate of fertilization and hatching. One of the essential objectives during commercial seed production of Common carp in hatchery systems by artificial reproduction is the reduction of egg adhesiveness. Traditional treatments for reducing egg stickiness include treating with a solution of sodium chloride and carbamide (40 g of urea and 30 g of salt diluted in 10 L clean water), followed by a 2 tannin solution (5 g tannin in 10 L of clean water, Rottmann *et al.,* 1991). Tannic acid use has the downside of causing egg envelope encrustation, which has a negative influence on hatching (Chebanov and Galich, 2013). This procedure, however, needs great work and skill, and it takes at least one hour. Pineapple is widely available in many tropical countries. Pineapple juice is high in proteolytic enzymes, particularly bromalinases, which may degrade protein (Michael, 2001). Proteolytic enzymes have been employed successfully in hatcheries to eliminate egg stickiness (Linhart *et al.,* 2002).

This research describes a simple and effective strategy for reducing egg stickiness in common carp and enhancing fertilization and hatching rates under certain hatchery settings. The study was carried out to discover the optimal way to decrease the adhesiveness of common carp (*C. carpio*) eggs, as well as the fertilization and hatching rates at different

stickiness reduction agents with different rinsing times.

Materials and methods

Study location and duration of time

Selection of the study location is the basic consideration for any research work. The experiment was conducted in Mafatema fish hatchery,Chanchra, Jessore,from Octoberto November 2021.

Selection of brood fish

The species used in this experiment was the common carp - *Cyprinus carpio* Linnaeus, 1758. It is vital to select appropriate brood fish for effective induced breeding (Penman and McAndrew, 1998., Islam and Chowdhury, 1976). The weight and age of the brood fishes were taken into consideration. Brood fish selection was mostly centered on physical qualities such as size, colour, and development rate, with little consideration given to the long-term genetic influence that such selection had on populations.

Sex determination

Males and females who were healthy and mature were carefully chosen. The mature producers were chosen based on the following criteriashown in Table 1.

Conditioning

Mature males and females from the brood raising ponds were chosen and promptly transported to the hatchery, where they were housed in a rectangular tank for around 24 hours. They were treated to water showers to stimulate the breeding state. During the conditioning phase, no feed was given.

Collection and preparation of pituitary glands (PG)

As an inducing agent, locally accessible dry carp pituitary glands (PG) were obtained from the market and stored in airtight vials. At first, the pituitary glands were carefully extracted from the container with a pair of tweezers and dehydrated and then using the filter paper for 2-3 minutes and then measured by an experimental digital balance (College B204-S, Switzerland) (College B204-S, Switzerland). Using the following calculation, the amount to be weighed out was computed based on the total body mass of all the fishes.

$$\text{Weight of PG(mg)} = \frac{\text{Wt} \times \text{Pt}}{1000} \ldots\ldots\ldots\ldots (1)$$

Where Wt indicates the total body weight (g) of all the fish to be injected and Pt is the rate of PG injection in mg/kg body weight under a specific therapy. The weighted PG was placed in a tissue homogenizer and vigorously crushed. The crushed PG was then dissolved in distilled water and centrifuged using a hand centrifuge for precipitation. The freshly made supernatant solution of hormone was then slowly injected into a 1 ml hypodermic syringe.

Administration of pituitary gland hormone

Following the production of the PG solution, it was administered into brood fish. The fish was carefully captured with a net and preserved in a sponge. They were draped in soft linen before being injected with PG around the base of the pectoral fin. The volume of PG mixture with each fish was previously established based on the body mass of the broods given in Table 2.

Preparations of treatment solutions

Pineapple juice was prepared by squeezing peeled fresh fruit. The solution was made up of 1% (10 ml juice to 1 L clean water. Milk powder solution was made up of 1% (10 g milk powder to 1L clean water.Urea+salt solution was made up at 0.4% (4 g Urea + 4salt to 1L clean water).

Determination of fertilization

After 6 hours of the final dosage, eggs and sperm were recovered from ovulated females and males by gently stripping the abdomen. The eggs were initially gathered in a plastic dish. Milts were then spread over the same jar. Following the given rinsing time of each treatment solution, the eggs and sperms were mixed together for 1 minute and then pineapple juice solution, milk powder solution; and urea + salt solution were utilized individually to eliminate the stickiness of the eggs to prolong the fertilizing capacity of milt. Each treatment study employed 400

eggs, with three duplicates and one control condition maintained. A small volume of treatment solution was poured separately over the eggs and stirred continuously with a feather, then observed under different exposure times. All the treated eggs were rinsed with clean tap water prior to the transfer of the eggs to the hatchery funnels. The number of completely free eggs and aggregated eggs in each funnel were counted (Table 3).

Estimation of free eggs

The proportion of free eggs was calculated by dividing the proportion of clumping eggs by the number of clumping eggs. After 15 minutes of incubation, the number of entirely free (non-stickiness) eggs and clustered eggs in each jar were counted. The following is an estimate of the proportion of free eggs:

$$\text{Free eggs (\%)} = \frac{\text{No. of free eggs} \times 100}{\text{Initial no. of eggs}} \quad \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Estimation of fertilization rate

The appearance of "eye mark" and enlargement of the fertilized eggs distinguished them from the unfertilized eggs. Unfertilized eggs were white and opaque, but fertilized eggs were clear. The fertilization rate was calculated using the following formulae.

$$\text{Fertilization rate (\%)} = \frac{\text{No. of fertilized eggs} \times 100}{\text{Total no. of eggs}} \quad \ldots\ldots\ldots\ldots (3)$$

Estimation of hatching rate

For hatching, the fertilized eggs were put in funnel-style incubators. A constant flow of water was maintained through the incubators' inlets. The hatchery's thermostatic heater was adjusted at 260 degrees Celsius, ensuring that the eggs hatched. After 482 hours after fertilization, the eggs began to hatch. After 962 hours after hatching, the yolk sac was absorbed. When hatching was finished, the larvae were gathered in a pot (dish), counted visually with a magnifying lens, and documented. The hatching rate was then calculated using the procedure below.

$$\text{Hatching rate (\%)} = \frac{\text{No. of hatchlings} \times 100}{\text{Total number of fertilized eggs}} \quad \ldots\ldots\ldots\ldots\ldots (4)$$

Statistical analysis

The experiment's findings were examined. All types of data were analyzed qualitatively and quantitatively. MS Excel was also utilized to show the tables and graphs derived from various sources of data. Using SPSS 16.00 software, the influence of broodstock management on fertility, fertilization rate, and hatching rate of common carp (*C. carpio*) was studied using analysis of variance (one way) and the Tukey-Kramer test for differences between means.

Results and discussion

Effect of different rinsing times of pineapple juice solution on the percentage of free eggs, fertilization and hatching rate of common carp C. carpio

The eggs of common carp, *Cyprinuscarpio*, are characterized as those of some other teleostfishes by having egg adhesiveness which may lead tothe delay of fertilization and embryonic development. Many methods have been used to separate the carp eggs from each other. *C. carpio* eggs were rinsed by three types of desticking agents with three different rinsing times in each. In the case of pineapple juice solution, 3 minutes rinsing treatment showed the highest percentage of free eggs (90.00±4.54%), fertilization rate (85.00±7.00%) and hatching rate (87.00±7.54%) (Fig. 1, 2 and 3).

Table 1. Criteria followed to select mature breeders.

Male	Female
(a) Small in size	(a) Relatively large in size
(b) Abdomen normal; not bulky like female	(b) Abdomen bulging, elastic and soft
(c) Pectoral fins were rough	(c) Pectoral fins were slimy
(d) Slightly protruding reddish vent seemed best criteria for male	(d)Small amount of eggs from the ovary were used to observe

The hatching rate at 3 minutes of treatment was significantly different ($P<0.05$) from 5 minutes and control groups. The present results are in agreement with THAI and NGO (2004). On the other hand, the percentage of free eggs, fertilization and hatching rate was decreased with the increased rinsing time of pineapple juice solution. It may happen for the presence of ascorbic acid in Pineapple fruit.

The ascorbic acid reacts with water and lowers the pH value of water. Low pH has a negative impact on the fertilization and hatching rate of fish eggs.

Table 2. Doses of PG for female and male broods.

Sex	First dose	Second dose	Time interval (h)
Female	2mg/kg body wt.	4mg/kg body wt.	6
Male	-	2mg/kg body wt.	6

Table 3. Treatment trials for removing stickiness.

Treatment solution	Treatment time (Minute)
Pineapple juice solution (1%)	1
	3
	5
Milk powder solution (1%)	15
	20
	25
Urea+salt solution (0.4%)	1
	5
	10
Control	-

According to Gao *et al.* (2011), fertilization and hatching rates were decreased in lower pH.Kim *et al.* (2020) also found that low pH negatively affects the fertilization and hatching rate. Marimuthu *et al.*, 2019). Pineapple fruits have a high concentration of ascorbic acid (Vitamin C) in their composition.

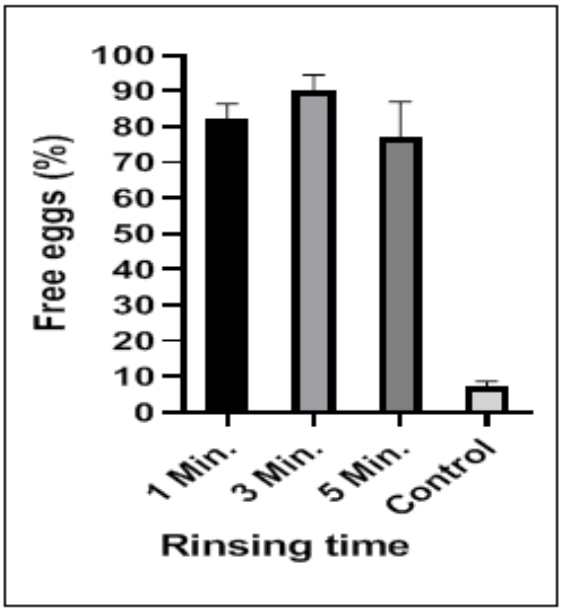

Fig. 1. Percentage of free eggs at different rinsing times of pineapple juice solution.

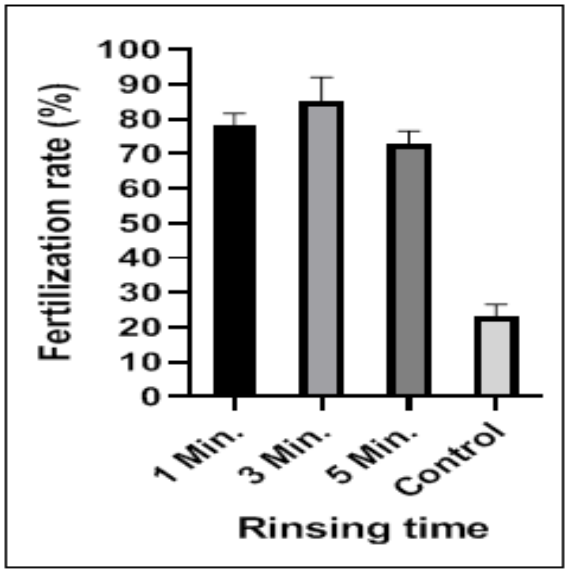

Fig. 2. Percentage of fertilization at different rinsing times of pineapple juice solution.

The juice is extracted and mixed with water; the solution (juice and water) is added to the egg, which reacts with the eggshell, thereby reducing the blood vessel and the hardness (thickness) of the eggshell. This helps to improve hatchability (Adogbeji and Nwachi, 2014).

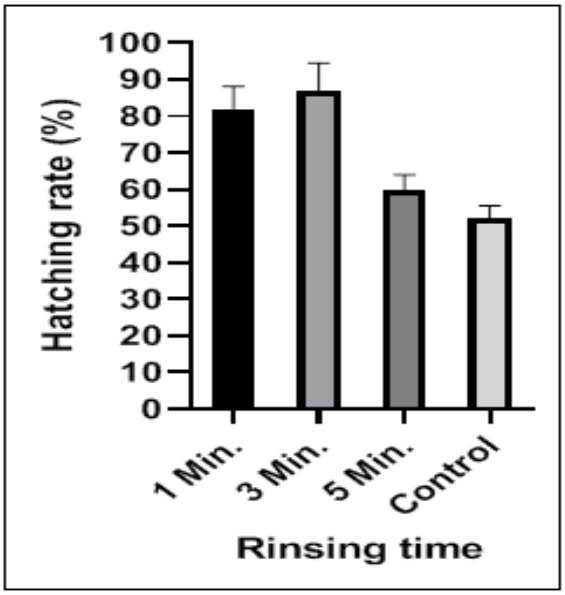

Fig. 3. Percentage of hatching at different rinsing times of pineapple juice solution.

Effect of different rinsing times of milk powder solution on the percentage of free eggs, fertilization and hatching rate of common carp C. carpio

The powdered milk proved to be the most appropriate media for the removal of egg adhesiveness due to the inherent ability of milk to reduce the rate of egg aggregation by coating it with milk particles.

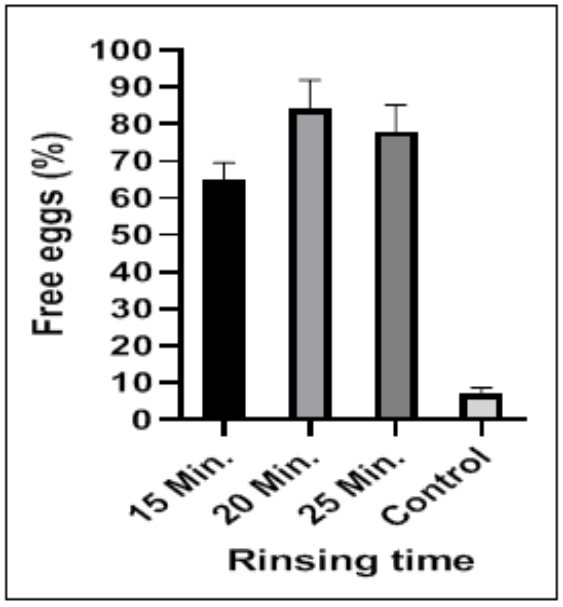

Fig. 4. Percentage of free eggs at different rinsing times of milk powder solution.

The coating prevents the eggs from sticking to each other. The use of powdered milk as organic dissolvent to remove egg stickiness has been reported by Soin (1977). Among three different rinsing times, 20 minutes of treatment of milk powder solution exposed the maximum percentage of free eggs (84.00±7.93%), fertilization rate (80.00±5.56%) and hatching rate (85.00±6.00%) (Fig. 4, 5 and 6). Twenty minutes treatment was significantly different ($P<0.05$) from 15 minutes treatment for the percentage of free eggs.

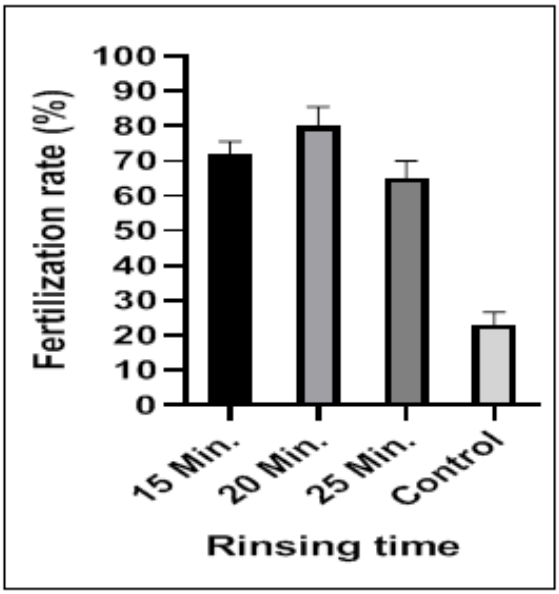

Fig. 5. Percentage of fertilization at different rinsing times of milk powder solution.

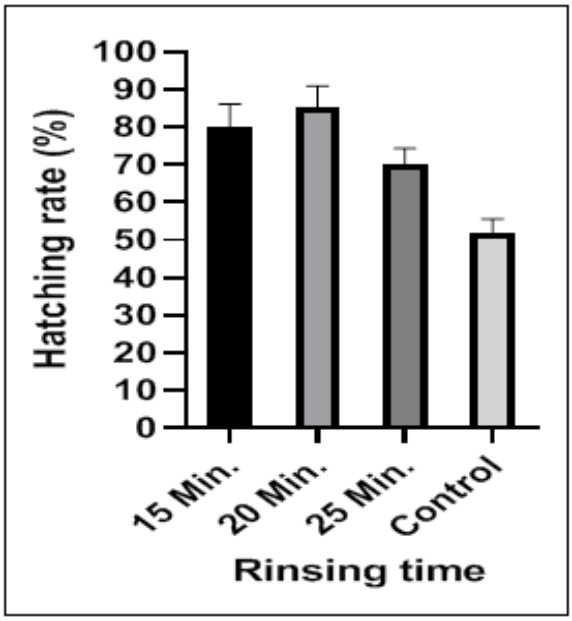

Fig. 6. Percentage of hatching at different rinsing times of milk powder solution.

This 20 minutes rinsing treatment also showed a significant difference ($P<0.05$) with 25 minutes

treatment of milk powder solution for the fertilization and hatching rate.

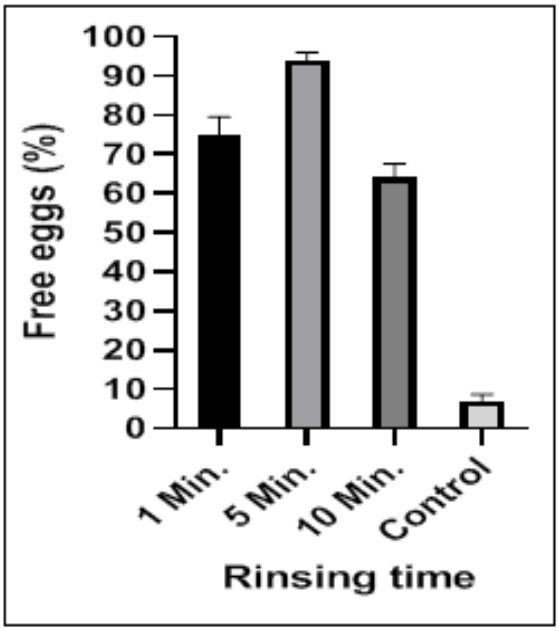

Fig. 7. Percentage of free eggs at different rinsing times of urea+salt solution.

According to Kareem *et al.* (2017), 10 g milk powder treatment at 20 minutes gave the highest number of free eggs, fertilization and hatching rate in the case of *Clariasgariepinus*. Due to the high price of milk powder in Bangladesh perspective, the application of milk powder in a large amount of egg treatment is not cost-effective.

Effect of different rinsing times of urea+salt solution on the percentage of free eggs, fertilization and hatching rate of common carp C. carpio

The highest percentage of free eggs (94.00±2.00%) fertilization rate (90.00±3.60%) and hatching rate (82.00±2.64%) was observed at 5 minutes rinsing treatment of urea+salt solution and it was significantly different ($P<0.05$) from 1-minute rinsing treatment for the percentage of free eggs and fertilization rate (Fig. 7, 8 and 9). In the case of hatching rate,5 minutes rinsing treatment of urea+salt solution also exposed the significant difference ($P<0.05$) with 10-minute rinsing treatment.On the effect of rinsing agent on stickiness removal under the dry method of fertilization, 4 g urea gave the highest percentage of free eggs (60.42%) at 5 minutes rinsing time.

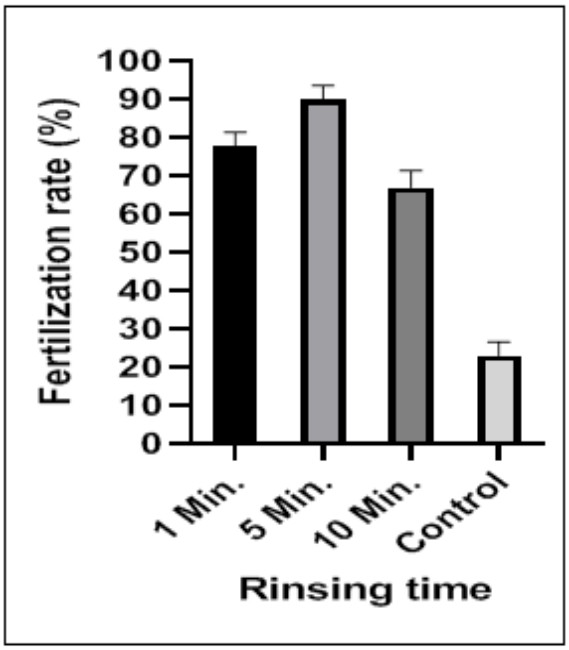

Fig. 8. Percentage of hatching at different rinsing times of urea+salt solution.

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 difference among treatments, while for hatchability, there was no significant difference among the treatments.

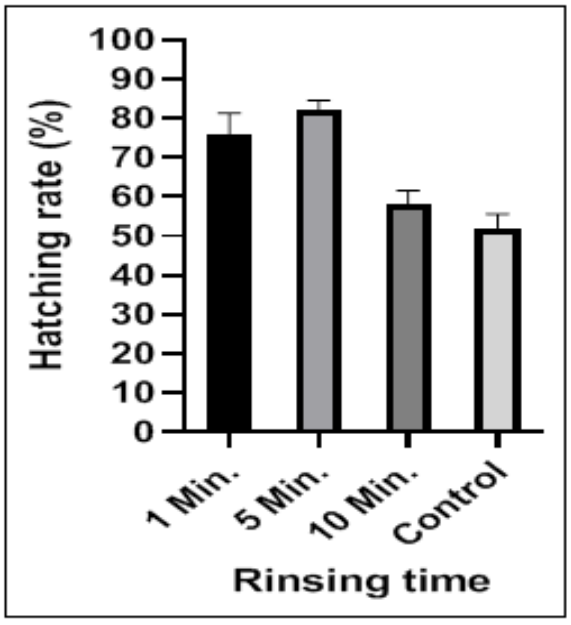

Fig. 9. Percentage of hatching at different rinsing times of urea+salt solution.

In addition, high survival rates were recorded in all the treatments (Kareem *et al.*, 2017).The best results

were obtained after rinsing 5 minutes of fertilized eggs with a mixture of 4 g urea and 4 g NaCl/L of water and the percentage of completely free eggs was 98% (Abdel-Hakim *et al.*, 2008).

Conclusion

The current study indicated that a 1 percent pineapple juice solution rinsed for 3 minutes is extremely efficient at reducing egg stickiness (90.00±4.53 percent) and increasing fertilization (85.00±7.00 percent) and hatching rate (87.00±7.54 percent). It works quite rapidly. However, the Pineapple fruit is not accessible all year. In the case of milk powder solution 1 percent dilution with 20 minutes rinse duration, the proportion of free eggs, fertilization rate, and hatching rate are 84.00±7.93 percent, 80.00±5.56 percent, and 85.00±6.00 percent, respectively. Among all treatments, 0.4 percent urea+salt solution rinsed for 5 minutes produced the greatest percentages of free eggs, fertilization, and hatching (94.00±2.00 percent, 90.00±3.60 percent, and 82.00±2.64 percent, respectively).

References

Aaronson DS, Horvath CM. 2002. A road map for those who don't know JAK-STAT. Science **296(5573),** 1653-1655.

Abdel-Hakim E, **Gamal E**, **Zeinab A, Greisy E.** 2008. Effect of removal of eggs adhesiveness on hatchability and effect of different levels of salinity on survival and larval development in common carp *Cyprinus carpio*. Journal of Applied Sciences **4(12),** 1935-1945.

Adogbeji EP, **Nwachi OF.** 2014. Use of pineapple juice in the elimination of egg stickiness in mudfish (*Heterobranchus bidorsalis*).

Arlinghaus R, **Mehner T.** 2003. Socio-economic characterization of specialized common carp (*Cyprinus carpio* L.) anglers in Germany, and implications for inland fisheries management and eutrophication control. Fisheries Research **61(1-3),** 19-33.

Chebanov MS, Galich EV. 2013. Sturgeon hatchery manual. FAO Fisheries and Aquaculture Technical Paper **558,** 1-17.

Department of Fisheries, Do F. 2020. National fish week compendium (In Bengali): Department of Fisheries, Ministry of Fisheries and Livestock, Bangladesh, 21–32.

Department of Fisheries, **Do F**, 2018. Yearbook of Fisheries Statistics of Bangladesh 2016–17. Fisheries Resources Survey System (FRSS), Department of Fisheries. Bangladesh, 129.

El-Gamal AHE, **El-Greisy ZA.** 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*. Journal of Applied Sciences Research **4(12),** 1935-1945.

Gao Y, **Kim SG, Lee JY.** 2011. Effects of pH on fertilization and the hatching rates of far eastern catfish *Silurus asotus*. Fisheries and aquatic sciences **14(4),** 417-420.

Islam MZ, **Chowdhury AQ.** 1976. Induced spawning of major carps for commercial production of fry for fish seed in Bangladesh. Bangladesh Journal of Zoology **4(2),** 51-61.

Kareem OK, **Ajani EK, Akintunde MA, Olanrewaju AN, Oduntan OB.** 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),** 21.

Kim KH, **Moon HN**, **Noh YH, Yeo IK.** 2020. Influence of Osmolality and Acidity on Fertilized Eggs and Larvae of Olive Flounder (*Paralichthys olivaceus*). Development & reproduction **24(1),** 19.

Kowtal GV,**Clark WH, Cherr GN.** 1986. Elimination of adhesiveness in eggs from the white

sturgeon, *Acipensertrans montanus*: chemical treatment of fertilized eggs. Aquaculture **55(2),** 139-143.

Linhart O, **Stech L, Svarc J, Rodina M, Audebert JP, Grecu J, Blliard R.** 2002. Present state of the culture of the European catfish (*Silurus glanis* L.) in Czech Republicand France. Aquatic Living Resources **15,** 109-112.

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

Linhart O, Štětch L, Švarc J, Rodina M, Audebert JP, Grecu J, Billard R. 2002. The culture of the European catfish, *Silurus glanis*, in the Czech Republic and in France. Aquatic Living Resources **15(2),** 139-144.

Marimuthu K, **Palaniandya H, Muchlisin ZA.** 2019. Effect of different water pH on hatching and survival rates of African catfish *Clarias gariepinus* (Pisces: Clariidae). Aceh Journal of Animal Science **4(2),** 80-88.

Michael TM. 2001. What are proteolytic Enzyme. Natural Factor Nutritional Products Ltd.

Penman DJ, **McAndrew BW.** 1998. Practical aspects of selection and fitness of stocked fish. FAO-FisheriesTechnical-Paper No. **374,** 223-233.

Pinto L, Chandrasena N, Pera J, Hawkins P, Eccles D, Sim R. 2005. Managing invasive carp (*Cyprinus carpio* L.) for habitat enhancement at Botany Wetlands, Australia. Aquatic Conservation: Marine and Freshwater Ecosystems **15(5),** 447-462.

Ringle JP, **Nickum JG, Moore A.** 1992. Chemical separation of channel catfish egg masses. Progressive Fish-Culturist **54,** 73–80.

Rottmann RW, **Shireman JV**, **Chapman FA**. 1991. Techniques for taking and fertilizing the spawn of fish. Stoneville, Mississippi: Southern Regional Aquaculture Center.

Rottmann WH, **Peter GF, Oeller PW, Keller JA, Shen NF, Nagy BP, Theologis A.** 1991. 1-Aminocyclopropane-1-carboxylate synthase in tomato is encoded by a multigene family whose transcription is induced during fruit and floral senescence. Journal of molecular biology **222(4),** 937-961.

Saikia SK, Das DN. 2009. Feeding ecology of common carp (*Cyprinus carpio* L.) in a rice–fish culture system of the *Apatani plateau* (Arunachal Pradesh, India). Aquatic Ecology **43(2),** 559-568.

Soin SG. 1977. Some features of the development of carp *Cyprinus carpio* under hatchery conditions. Ichthyology **17(5),** 759-768.

Thai BT, Ngo TG. 2004. Use of pineapple juice for elimination of egg stickiness of common carp (*Cyprinus carpio* L.). Asian Fisheries Science **17(1/2),** 159-162.

Wedekind H, **Hilge V**, **Steffens W.** 2001. Present status, and social and economic significance of inland fisheries in Germany. Fisheries Management and Ecology **8(4-5),** 405-414.