De-adhesion of Common Carp Fish Eggs (*Cyprinus carpio* L.) Using Strawberry Natural Juice Solution During Artificial Reproduction Process

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Abstract:

One of the most important problems during artificial reproduction of Common carp is the adhesion of its egg. Disposal of adhesive material on eggs increasing its hatching rate activity. In this research three different concentrations of Strawberry Juice were used (10, 15 and 20% respectively) to treat the eggs of Common carp fish in order to remove the adhesive materials comparing with the control (salt, urea and Tannin solutions). The results showed that using Strawberry Juice after eggs fertilization with concentration of 20 and15 % have the possibility of removing the adhesive substances with short time of about 5 and 7 minutes respectively with significant effect ($p<0.05$) as compared with the control treatment which it takes about75 minutes to remove this substance. While the concentration of10 %the adhesive materials were not removed. Additionally there was a surpassing effect ($p<0.05$) at the rat of eggs fertilization and hatching in the both concentrations (15%; 20 %) which was higher than that treated with Tannin and fertilizing solution, and the results also showed that eggs at the two treatments (T2 and T3) with Strawberry Juice , have a shorter period of incubation which was(66 and 64 hours)as compared with the control (71 hours to hatches at 22Cº).

Keywords: De-adhesion Eggs, Strawberry Juice, fertilizing solution, Cyprinus carpio

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Introduction

In most Common carps the external layer of eggs is not viscous before touching water, and it became sticky when it touch water. This viscosity makes eggs sticks with each other or with other surfaces in the natural water to prevent their drift or eaten by predators (Riehl and Patzner, 1998). The eggs were not viscous inside the ovaries but it become like that quickly when it touch water within 30seconds (Mansour,*et al.*,2009). Adhesion of eggs causes problems during fertilization and prevents Oxygen from reaching the embryos within the adjoining eggs and the risk of developing fungal diseases (Siddique, *et al*., 2014). This viscosity is caused by the presence of glycoproteins and this substance can be disintegrated by enzymes or substances make it deposit such as sodium chloride with urea and then Tannin, or disable the adhesion mechanism through the formation of glue compounds such as milk, clay and starch. For this purpose many different traditional materials were used such as urea solution, food salt and Tannin solution(Horvath,1985).Pomegranate appeals and white alum also been used (Nife,2011). Grazina *et al.* (2018) referred that Tannic acid, milk and salt solutions were used to remove the sticky substances from Common carp egg, Tibor, *et al.*, (2011) used milk and starch ,while (Linhart, *et al*., 2008 ; Sunil, 2012) used Alkaline enzyme DX PLM4715 and NA2EDTAenzyme of disodium ethylene diaminetetraacetic acid salt on *Pike perch* fish egg(Uros,*et al*.,2017).While clay was used for *Huso huso* fish by

(Hosseini and Khara ,2015) , and Pineapple extract juice for Mud fish egg, (Ekokotu and Nwachi, 2014) and also for Cat fish *Clarias gariepinu*s eggs, and Common carp fish *Cyprinus carpio* (AL-humairi,2016).The dependence on artificial reproduction in fish farms is to support this expansion in fish farming for the purpose of producing genetically modified fortified varieties(Al-Jebory,2011 ; Al-Jebory,2012).As well as increasing fertilization and hatching rateto increase the number of larvae produced during one season of breeding and the interplay between them(AL-Gezali,2010).Common carp fish considered as one of the most important cultivated fish in fresh water and there is several ways are used to artificially reproduce them ((Brzuska, 2006).One of the most important things in artificial reproduction of Common carp is the removal of adhesion of eggs (Grazina*,et al.*,2018). Adhesion of eggs considered a serious problem in the process of artificial reproduction, which reduces the rate of fertilization and hatching. It also requires a lot of effort, at skill because of the nature of used material (AL-humairi,2016).The viscous material can be removed from Common carp eggs by using of different methods but in most cases it can depend on (Woynarovich,1962),in this method the eggs is washed by a fertilization solution which is composed of 4 gm. food salt plus 3gm urea /L water, after that it is treated for a short period with Tannin solution, the first part of this process doesn't have any role in the growth of the embryo, but Tannicacid solution may affect the embryonic growth and even it may lead it to die depending on the concentration of the solution used and time of treatment (Kujawa*, et al*., 2010), so it is necessary to look for an alternative ways safe and efficient such as strawberry fruit,(*Fragaria* × *ananassa Duch*) which belongs to the family Rosacea which is a famous at wide spread fruits, It's name was derived from the Latencies word *Fragrans* , the English word is *Strawberry* while in Iraq and Syria it is called *Toot alardh* . In Egypt it is called *Farawlla*, In Turky called *Chillaik*(AL-Saadi, 2000). Fruit consist of many Important nutrients such as vitamins, minerals, folic acid and fibers which is a rich source of Phytochemical compounds presents as Polyphenols known as antioxidant and anti – inflammatory such as Anthocyanin Elago tanin (USDA,2006),by using it as extracts or solution .The purpose of this study is the attempt of using a new methods such as Strawberry extract solution to remove the adhesion of the eggs(in a short time) and enhancing the rate of eggs fertilization and hatching .

Materials and methods

Male and female Common carp fishes of 3-4 kg weight were used in this research. These fishes were put in small Tanks inside the hatchery building. The Male and Female fishes were injected with Pituitary gland extracts (Carp Pituitary Gland, CPG) to stimulate the fishes according to (Horvath ,*et al.*, 1985; Mengxi Cao, *et al*., 2014) methods. The Collected eggs from the female were mixed with the collected sperms from more than one male fish in the dried methods because it's the best way (Jerome and Lionel, 2002). The eggs were divided into four treatments after it was mixed with sperms and left for 5 minutes where the fertilization completed, each treatment composed of two replicates, each one (hatching glass) with about (100 gm / egg/replicate).Removal of adhesion substance depend the following stages:

T1: using Strawberry fruit juice solution with concentration of 10 %(10 ml completed to 100 ml fertilized solution).

T2: using Strawberry fruit juice solution with concentration of 15 %(15 ml completed to 100 ml fertilized solution).

T3: using Strawberry fruit juice solution with concentration of 20 %(20 ml completed to 100 ml fertilized solution).

The treated eggs with different concentrations of Strawberry juice mixed softly with bird feather and washed with fresh water twice then transferred to the hooligans (Zug Jars).

T4:(control): Eggs were treated with fertilized solution was added (4 gm. food salt +3gm urea / L. water) started with 20 % of egg volume then increased gradually with mixing according to (Woynarovich and Horvath,1980).

The treatment continued with fertilized solution by gentle mixed with feather for 60-75 minutes to complete the swelling of eggs and adhesive materials was removed (Brania, *et al.,*1998), after that the eggs washed with Tannin solution 0.5 gm/L. to remove the residues of adhesion materials and hardened of egg shell for 20 seconds (Bakos,1984).

The fruit of Strawberry was bought from local market, the leaves were removed, washed with water, then it cut into small pieces and squashed by using a plastic clasp (10 micron).

After the treatment, about 100 gm of eggs were transferred into labeled Zug Jars of 7 L. A clip is placed at the top of the hatching Jar to prevent the eggs of larvae from leaving after hatching (the clip was cleaned every four hours to prevent clogged holes with shells of unfertilized egg or after hatching). The temperature of water was 22 c◦ and it should be stable during the experiment since the source of water is a ground water according to(AL-humairi,2011), and the pH was 7.5 , O2= 6 mg/L. and it's within ideal limits (Al-Abbadi, 2010).Water flow was adjusted (0.6 L/min.) and then increased gradually until it is reach 2 L/minutes near the end of hatching . Eggs twice daily with Malachite green solution ,5 ppm (Horvath, *et al.*,1985). Observing incubation of eggs until hatching stage, the following calculations were performed:

Estimation of fertilization rate:

The fertilized eggs were differentiated from the unfertilized ones after 6-8 hour of stripping by the presence of "eye spot" and the swelling of the fertilized egg. The unfertilized eggs were white and opaque while the fertilized eggs were transparent. The rate of fertilization was estimated by using the following formula (Bagenal and Braumm, 1971; RALC, 1981):

Fertilization rate (%) =**Number of fertilized eggs in sample/Total number of eggs in sample× 100**

Estimation of hatching rate :

The rate of hatching was calculated after larvae collection from each Jar by siphon method after closing the water supply and placed in a cube cage (15×10×10 cm) dimensions, number was calculated by volume method after taking a volume sample and calculating the hatching ratio according to the following equation:

Hatching rate (%) =Number of hatchlings in sample/Total number of fertilized eggs in sample× 100.

(Shigang, 1989 ; Alameen,2001).

Statistical analysis:

Results of the methods were statistically analyses the obtained data were presented as mean ± standard deviation and subjected to the analysis of variance (ANOVA)followed by the least significant differences (LSD) Test at 0.05 level using IBM SPSS statistics 22. All statistical tests were conducted based on Al-Haiti (2004).

Results and discussion

Results of the statistical analysis shown in Table (1) indicated that the egg adhesion rate was very high at level of ($p< 0.05$) in T1(100%) as compared with (T2,T3,T4) which means that at T1 treatment, the adhesive materials were not removed which is in agreement with(Sunil, 2012 ; Tibor, 2011; Ekokotu and Nwachi , 2014) , and that means if the adhesive materials were not removed the eggs will completely lumped and aggregate in the hatching Jar and that led to reduce the hatching rates. While the time of removing of the adhesive materials after fertilization in T3 treatment was 5 minutes which was out standing up on other treatments, it was also noticed that T2 was better than control treatment and the time was 7 minutes because of the highly increasing rate very low (zero) compared with other treatments, because the of the arrival of dissolved oxygen to the fertilized eggs leading to death of the embryos (Al-Abbadi, 2010; FAO, 2012; Nife,2011; Chattopadhyay, 2014). The results also showed a significant improvement ($p<0.05$)in fertility ratios for T3

which reached 94% on all other treatments, while T2was superior up on T1 and T4 and this results of fertilizing ratio falls within normal numbers of this indicator and came close to what was mentioned by (Horvath, *et al.*,1985;Nife,2005;AL-humairi,2011; Monirul, *et al*., 2016), who mentioned that the fertilization ratio range from 75 - 95 % in Common carp fish ,while the hatching ratio was very low as it is seen in Table 1in T1compared with other treatments because of eggs conglomeration. The hatching ratio of T2,T3 and T4 was 85,87 and 80 % respectively, although there was significant differences between T2,T3 and control but the hatching ratio was within the normal limits as mentioned by (Salih and Abdul Karim,2013). Yeasmin(2015) mentioned that the time required for eggs to hatch after fertilization depends upon the temperature around it. In recent study it was noticed that time required for eggs to be hatched reached of high significant at ($p< 0.05$) in control (T4) which was 71minutes compared with other treatments treated with Strawberry Juice solution which was64 and 66minutes forT3 and T2respectively. At level of ($p<0.05$) and it is similar with the results of (AL-humairi,2016) when he used Pineapple Juice solution , while the time used to incubate and hatch the eggs for T4 was approximately the same with (Horvath & Seagrave, 1992;Brania, *et al.,*1998; Al-Mukhtar, *et al.,*2010 and Al-Abbadi, 2015).The reason why the eggs hatch early in this experiment because of egg shell was not solidify and Strawberry solution composed of natural nutrients such as Anthocyanin , Elago tanine compared with T4control treated with Tannin which make egg shelled become harder. The Results of this study indicated the concentration of Strawberry solution in a T2 and T3 was more positive with good results as compared with T1and Control especially at the time when adhesive material was removed and the time required to fertilize and incubate the eggs , so it is recommended in this study to use the Strawberry Juice solution in concentration of 15 and 20 % because of it's importance to remove the viscosity of adhesive material from fishes egg shelled in a short time and also reduces the time required for egg hatching.

Table 1: Effect of different concentrations of strawberry juice solution on the studied traits and their comparison with control treatment.

	Concentrations %				
Traits studied	**T1** **10%**	**T2** **15 %**	**T3** **20 %**	**T4** **(Salt+ urea ,Tannin)**	**Significance**
Adhesion rate%	**100±0** **b**	**0±0** **a**	**0±0** **a**	**0±0** **a**	*
Time of removal (mint)	**0** **d**	**± 71.24** **b**	**5 ± 1.43** **a**	**751.53±** **c**	*
Fertility rate%	**0± 0** **d**	**90 ± 1.34** **b**	**±942.13** **a**	**77 ±1.31** **c**	*
Hatching rate%	**0± 0** **c**	**85 ±2.13** **a**	**87 ±1.43** **a**	**80 ±1.41** **b**	*
Hatching period (hours)	**0** **c**	**66±0.73** **a**	**64 ±0.71** **a**	**±711.21** **b**	*

Different letters in the horizontal lines refers to significant differences ($P< 0.05$).

CONCLUSION

The use of a natural strawberry juice solution in a concentrations(15 ,20)% led to the removal of the adhesive material while reducing the effort and time of hatching eggs compared with conventional method (salt + urea and Tannin acid).

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