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Effect of Some Commonly Used Anti-Fungus Treatment Chemicals on the Hatching Rate of Eggs, Survival and Growth Performance of Fry of the African Catfish (*Clarias Gariepinus*)

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Abstract: *Hatchability and survivability rates are an essential part of the African catfish rearing, seeing that it is the most farms fish in Nigeria and even the African continent. Adequate analysis of the various disinfectant chemicals used by fish farmers was carried out to determine the chemical with the most favourable hatchability rate in percentage and the chemical with the most favourable survivability rate. Four bowls of thirty Clarias eggs each were treated with iodine (100mg/l), hydrogen peroxide (250mg/l), malachite green (5mg/l), and formalin (250mg/l) in bath treatments for 10 minutes, 15 minutes, 10 minutes, and 15 minutes respectively for each treatment type excluding the control bowl (untreated). Percentage hatchability was evaluated by dividing the number of hatched eggs by the total number of eggs and multiplying by 100, and percentage survivability was evaluated by dividing the number of surviving fries by the number of hatched fries. Formalin accounted for 70% hatchability, with iodine recording the least 30%; meanwhile, hydrogen peroxide accounted for 83.33% survivability, with iodine recording the least survivability of 0%. This study further suggests using formalin in disinfecting catfish egg diseases, while it promotes hydrogen peroxide usage in disinfecting newly hatched fry and enhancing their survivability.*

1. Introduction

Fish are aquatic vertebrates that have gills throughout life and limbs, if any, in the shape of fins (Nelson, 1994). The popular concept of a fish as an animal with fins and scales, and lives in water, is not strictly correct. All fishes have a backbone or a notochord, and all breath using gills. Some animals that are not fish also breathe using gills. However, these animals have fully formed limbs lacking in fishes (Helfman *et al.,* 1997). If we allow room for these and other exceptions, a fish can be defined as a poikilothermic, aquatic chordate with appendages (when present) developed as fins, whose chief respiratory organ are gills and whose body is usually covered with scales (Berra, 2001).

The African catfish, *Clarias gariepinus* is a sizeable eel-like fish, usually of dark grey or black colouration on the back, fading to a white belly. In Africa, this catfish has been recorded to be second in size only to the Vundu of the Zambian waters. Morphologically, the *Clarias garipienus* is a scaleless fish with smooth skin and soft ray fin, dorsal-ventrally flattened shrunken head, an elongated body (Yinka *et al*., 2005; Idodo-Umeh, 2003). Its dorsal fin has about 61-80 rays and an anal fin of 50 -65 rays. Its head is between rectangular and pointed dorsal outlines with a broad snout. It is often described as a depressed and long-body-shaped fish. The eyes are positioned in the flat, depressed head and are relatively small in size. The depth of its body is 6-8 times its standard length. Its teeth are vomerine, granular, delicate, and are arranged in rows. The common species of *Clarias* possess characteristically elongated four pairs of barbells like the cat whiskers, which are 20-50% as long as the head when the fish is longer than 30cm and 50-80% of the head when the length is smaller in size. *Clarias gariepinus* has several gill rakers, long, slender, and closely fitted with about 24 -110 in numbers that increase as the fish grows. The fish has short dorsal fins that extend as far as the caudal fin. Some of them show-band of pigmentation on both sides and irregular black spots (Idodo-Umeh, 2003). *C. gariepinus* has pseudo-lungs, long bodies, and a high capacity to produce mucous as adaptations for living in stagnant environments or out of the water (Doneelly, 1973). In its natural range, it is omnivorous, feeding on plant material, plankton, arthropods, mollusks, fish, reptiles, and amphibians (Yalcin *et al*., 2001a). Its reproduction is seasonal, with gonadal maturation associated with periods of flooding. The maturation process is influenced by changes in water temperature and photoperiod,

but the increase of water level is the principal factor for their reproduction (Van der Waal, 1974; De Graaf *et al*., 1975; Yalcin *et al*., 2001b).

The African catfish, *Clarias gariepinus*, is widely distributed in Africa. Its main habitats are calm lakes, rivers, and swamps that flood regularly (De Graaf and Janssen, 1996; Winemiller and Keso-Winemiller, 1996)

Aquaculture in Nigeria is the beginning of catfish culture, and the hope of fish supply in Nigeria hangs on the development and culture of catfish. The growth of aquaculture in Nigeria now is mainly being boosted by a steady rise in catfish culture. Inadequate availability of seed for stocking and feed used to be a significant problem. Tremendous progress is now being made. Today's total value is eight hundred US Dollars from the value of fingerlings, feed, and farmed fish. Since the culture of *Clarias gariepinus* through hypophysation was initiated in Western Nigeria in 1973, the procedure has been widely practiced throughout Nigeria, thus increasing farm-raised catfishes from the 80s to date (Adewumi, Olaleye; 2010). *Clarias gariepinus* is commonly referred to as the African Catfish, Sharptooth Catfish, Common Catfish, Mudfish, Barbel, Sharptoothed Catfish and North African Catfish. The farming of *Clarias gariepinus* extended outside natural habitats and was introduced to Argentina and other parts of the world (Freyhorf, 2013). Although spawning occurs naturally in the wild, artificial propagation of *Clarias gariepinus* is now carried out in hatcheries with hormonal induction. Farmers have found the homoplastic pituitary gland suspension cheaper, practical and more highly reliable than the imported synthetic hormonal analogues. The Clarias gariepinus broodstock weight used for artificial breeding ranges between 0.3kg to 2kg (Olaleye, 2005).

Despite the breakthrough with the use of the hormone in induced spawning, fry survival is still beset with a number of biotic and abiotic factors. The biotic factors include cannibalism, heavy predation by frogs/aquatic insects, and abiotic factors, including water temperature, dissolved oxygen, and ammonia levels.

2. Methodology

Location: The study was conducted in one station in the Animal Unit of the Department of Animal and Environmental Biology, in the Faculty of Life Sciences, University of Benin, Edo State, Nigeria.

Materials: The experiment was carried out with a total of five plastic circular tanks. Other materials used for the experiment include A pack of saline water; a razorblade; a syringe; a 10ml bottle of the hormone, Ova Prim; an electrical weighing balance; water heater; five separate meshes of loosened sack; five small bowls; two hand towels; iodine, malachite green, formalin, hydrogen peroxide; artemia, and siphon.

Experimental Procedure: The physicochemical analysis was carried out for the water supply to each tank before the broodstock was introduced. The experiment had four treatments with water as control; T1: Water in this tank was not treated (control); T2: Water in this tank was treated daily with formalin; T3: Water in this tank was treated daily with iodine; T4: Water in this tank was treated daily with hydrogen peroxide; and T5: Water in this tank was treated daily with malachite green.

Parameters Measured During the Experiment: The following parameters were measured for the experiment: Water temperature using a thermometer, pH using a pH meter, conductivity using a Conductivity meter, Total Dissolved Solids (TDS) using a TDS meter, and Dissolved Oxygen (DO) using Wrinkler's method of DO determination. The broodstock's live weight(g) and the number of the eggs separated into each tank using an electronic weighing balance and a siphon, respectively.

Analysis of Chemical Parameters

Dissolved Oxygen (DO): The water sample was collected from the water supply to the five experimental tanks into a 250ml reagent glass bottle. The dissolved oxygen was then fixed by adding 1.5ml of Wrinkler's solutions A and B to produce a characteristic brown precipitate. Essentially, the brown precipitate is trapped oxygen; this occurs to a degree proportional to the amount of dissolved oxygen present in the sample. In the presence of 2ml of concentrated sulphuric acid, the brown precipitate dissolved to produce a golden yellow colour. Then two drops of the starch indicator were added to 200ml of the sample in a conical flask. The sample turned blue-black and was titrated with dilute sodium thiosulphate, and an endpoint was deduced when the solution was clear and colourless.

Hydrogen ion concentration (pH): The pH of the water sample collected was determined in the laboratory using the Hanna pH meter (Hi-1922 model). The meter was first calibrated using buffer 7.0 and rinsed severally with distilled water.
Conductivity/ Total Dissolved Solids: A conductivity meter (WTW Series Cond 730) was used to measure the conductivity of water samples. The meter was first calibrated with 0.01M potassium chloride solution (KCL). 100ml of the sample was measured into a beaker and the conductivity/tds was determined by placing the meter probe into the sample. The probe was held in the beaker for a few minutes until the digital display reading stabilized.

Experimental Methods

The method of spawning used in this experiment was the induced spawning method. In induced spawning, the female is injected with a hormone to prepare her eggs for a higher fertilization percentage. For this experiment, Ova Prim, the hormone used, although there are other cheaper hormones available in Nigeria such as Suprefact and Motilium. The Ova Prim was injected at 0.5ml/kilogramme of fish body weight, and the fish weighed 1.8 kilogramme. The needle was inserted at 2-3cm at the angle of 30-45 degrees in the dorsal muscle while retracting the syringe after injecting the hormone. After this, a finger was used to rub the injection area to move the hormone throughout the muscle. This injection was done at 10 am on August 5th. Following the injection was a 10-hour wait for the eggs to mature and be ready for spawning. Since inducing was carried out by 10 am, the 10-hour wait for spawning fell at 8 pm that same day. Ten hours later, the male's milt was collected by sacrificing the fish and dissecting the testis with a razor blade. The two testes were removed without squeezing them and put on a plastic plate. The blade was used to cut each testis into smaller pieces before there were lightly squeezed to let the spermatozoa out to be collected in a recipient. The testis pieces were rinsed with saline water to ensure the complete collection of spermatozoa.

If the female has responded well to the earlier injection, the mature eggs will efficiently run out from her genital opening. The female was held gently with a wet towel and then stripped. Her eggs were collected into a bowl until there were no more eggs, after which saline water was added to the eggs to prevent them from sticking together. The siphon was then used to pick thirty (30) eggs each into each one of the five (5) small bowls. Then the spermatozoa were poured over the eggs and mixed thoroughly with a plastic spoon by stirring. Some water was added to the mixture before the mixture was stirred for another half minute. The eggs were then transferred to another set of five (5) small bowls with nets suspended in them. Eggs hatch in about 24 hours at 28-30 degrees in Celsius. After stripping, females can be returned to earthen ponds, where they can be ready to spawn again in three months with good feeding. The different waters in four of the five bowls were treated with each of the four (4) chemicals, and the last bowl of water was left untreated as a control. Before the eggs were spread on the nets, the waters were treated.

For the bowl treated with formalin, the one (1) litre volume of water was treated with 250mg/l. After the eggs were spread on the net in the bowl, the treatment was left for fifteen (15) minutes, after which the water in the bowl was begun to be siphoned out as the water was being replaced into the bowl to clear the formalin treatment.

For the bowl treated with malachite green, the one (1) litre volume of water was treated with 5mg/l. After the eggs were spread on the net in the bowl, the treatment was left for ten (10) minutes, after which the water in the bowl was begun to be siphoned out as the water was being replaced into the bowl to clear the malachite green treatment.

For the bowl treated with iodine, the one (1) litre volume of water was treated with 100mg/l. After the eggs were spread on the net in the bowl, the treatment was left for ten (10) minutes, after which the water in the bowl was begun to be siphoned out as the water was being replaced into the bowl to clear the iodine treatment.

For the bowl treated with hydrogen peroxide, the one (1) litre volume of water was treated with 250mg/l. After the eggs were spread on the net in the bowl, the treatment was left for fifteen (15) minutes, after which the water in the bowl was begun to be siphoned out as water was being replaced into the bowl to clear the hydrogen peroxide treatment. The treatments were repeated every 24 hours, after nine days (), the hatchability and survivability rates were calculated as follows:

The hatchability rate was calculated for each of the four chemicals alongside the control (water).

For formalin: $\frac{\text{No of hatched eggs}}{\text{Total no of eggs}} \times 100$;

Hence, $\frac{21}{30} \times 100 = 70\%$.

For hydrogen peroxide: $\frac{\text{No of hatched eggs}}{\text{Total no of eggs}} \times 100$

Hence, $\frac{18}{30} \times 100 = 60\%$.

For water: $\frac{\text{No of hatched eggs}}{\text{Total no of eggs}} \times 100$

Hence, $\frac{19}{30} \times 100 = 63.33\%$.

For malachite green, $\frac{\text{No of hatched eggs}}{\text{Total no of eggs}} \times 100$

Hence, $\frac{12}{30} \times 100 = 40\%$.

For iodine, $\frac{\text{No of hatched eggs}}{\text{Total no of eggs}} \times 100$

Hence, $\frac{9}{30} \times 100 = 30\%$

The survivability was also calculated for each of the four chemicals, alongside water (water)

For formalin: $\frac{No\ of\ remaining\ eggs}{No\ of\ hatched\ eggs} \times 100$

Hence, $\frac{16}{21} \times 100 = 76.2\%$

For hydrogen peroxide, $\frac{No\ of\ remaining\ eggs}{No\ of\ hatched\ eggs} \times 100$

Hence, $\frac{15}{18} \times 100 = 83.33\%$

For water, $\frac{No\ of\ remaining\ eggs}{No\ of\ hatched\ eggs} \times 100$

Hence, $\frac{12}{19} \times 100 = 63.16\%$

For malachite green, $\frac{No\ of\ remaining\ eggs}{No\ of\ hatched\ eggs} \times 100$

Hence, $\frac{2}{12} \times 100 = 16.67\%$

For iodine, $\frac{No\ of\ remaining\ eggs}{No\ of\ hatched\ eggs} \times 100$

Hence, $\frac{0}{9} \times 100 = 0\%$

3. Results

Physicochemical parameters: The water used for the experiment (temperature, pH, conductivity, Total dissolved solids (TDS), and dissolved oxygen) of the various treatments is shown in Tables 1. The pH value of the water ranged between 7.40 and 8.00. The dissolved oxygen (DO) in the water ranged between 4.00 – 5.90. Furthermore, the Total Dissolved Solids in the water was 34.132.

Number of Eggs Hatched and Embryo Survival for Each Treatment: Table two below shows the results for the experiment on catfish eggs, showing the various hatchability rates (day 1) and survivability rates (day 2 – day 9) of the catfish eggs with effects of formalin, hydrogen peroxide, iodine, malachite green and water (as control). Figure 1 graphically shows the survivability rate values plotted against the remaining 8 days after the first day of hatching under chemical observation. The various colour keys indicate the various chemicals used for the control of the catfish eggs. The dark blue colour indicates the survivability rate of the catfish eggs controlled with water. The maroon colour indicates the survivability rate of catfish eggs treated with formalin.

The lemon bars represented on the graph indicate the survivability rate of *Clarias gariepinus* eggs treated with iodine. The purple bars on the graph indicate the survivability rate of catfish eggs treated with malachite green. The light blue coloured bar on the graph indicates the survivability rate of the catfish eggs that were treated with hydrogen peroxide. Figure 2 graphically shows the values of the hatchability rate of the catfish eggs plotted against the different chemicals and the control (water), for the first 24 hours after fertilization. Tables 3 and 4 show the length and weight relationships, respectively, of the *Clarias* hatchlings treated with each of the chemicals and the control (water). The length relationship was measured in centimetres, while the weight was measured in (mg).

Table 1: Concentrations of the physicochemical parameters of the test media

PARAMETERS	Water Mean±S. E (Min-Max)	Formalin Mean±S. E (Min-Max)	Iodin Mean±S. E (Min-Max)	Malachite Green Mean±S. E (Min-Max)	Hydrogen Peroxide Mean±S. E (Min-Max)
PH	7.65±0.061 (7.40 – 8.00)	7.39±0.038 (7.20 – 7.60)	7.55±0.057 (7.4 – 7.8)	7.16±0.0529 (7.0-7.40)	7.37±0.0539 (7.2 – 7.6)
Electric conductivity (EC) µScm-1	64.4±3.565 (50.00 – 80.0)	138.13±3.95 (120 – 160)	239.83±7.36 (225-289)	326. 0 ±6.164 (300 - 350)	740±10.645 (700 – 780)
Dissolved Oxygen (DO) mg/L	4.52±0.192 (5.90 – 4.0)	4.16±0.0202 (3.0 – 4.8)	3.97±0.109 (3.50 – 4.5)	3.22±0.143 (2.7 – 3.7)	0.95±0.288 (0.0 – 2.1)
Temperature ^{0}C	26.73±0.13 (26.0 – 26.8)	26.75 ± 0.144 (26.0 – 26.9)	26.7±0.151 (26.0 – 26.8)	26.6±0.173 (26.0 – 26.9)	26.8±0.144 (26.0 – 26.9)
Total dissolved solids (TDS)	34.13±1.88 (26.3 - 39.75)	73.2±5.552 (63.6 – 84.8)	134.695±3.901 (119.2 -153.17)	172.78±3.267 (159 – 185.5)	392.2±5.641 (371 – 413.4)

Table 2: Hatchability and survivability rates of the catfish eggs with effects of formalin, hydrogen peroxide, iodine, malachite green, and water.

Treatments / Hours Post-Hatch	Water	Formalin	Iodine	Malachite green	Hydrogen peroxide
24	19	21	9	12	18
48	17	21	7	11	18
72	17	19	7	11	18
96	16	19	7	8	18
120	16	19	5	8	17
144	13	19	5	6	16
168	12	17	3	5	16
192	12	17	3	5	16
216	12	16	0	2	15

Table 3: The length relationship between the fry in all four treatments and the control

Day	formalin	iodine	malachite green	Hydrogen peroxide	water
1	0.4cm	0.4cm	0.3cm	0.4cm	0.4cm
3	0.6cm	0.5cm	0.4cm	0.5cm	0.5cm
7	0.8cm	0.8cm	0.6cm	0.8cm	0.9cm

Table 4: The weight relationship between the fry in all four treatments and the control

Day	formalin	iodine	malachite green	hydrogen peroxide	water
1	0.09	0.08	0.07	0.09	0.08
3	0.14	0.14	0.09	0.14	0.12
7	0.16	0.17	0.12	0.17	0.15

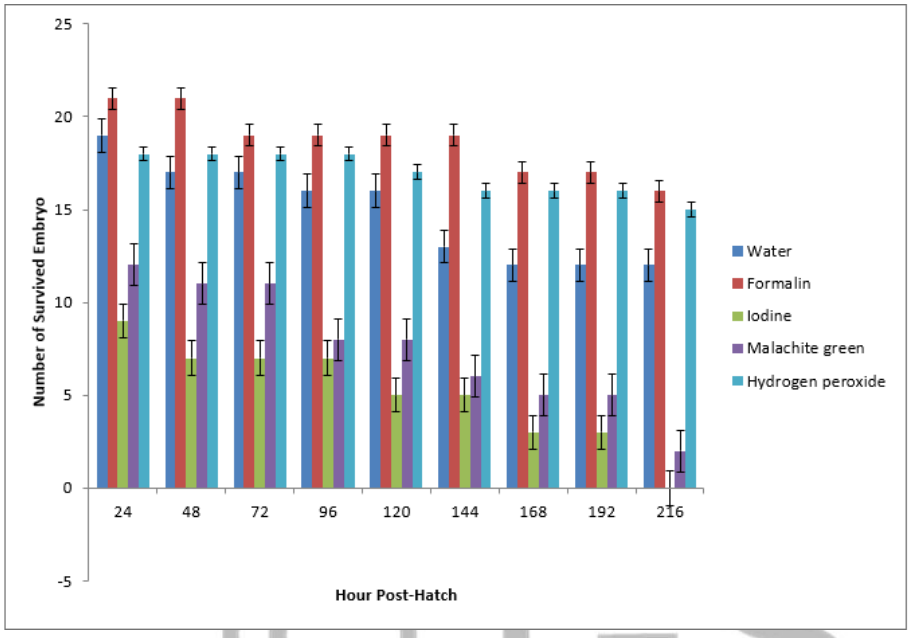

Figure 1: Graph showing the number of survived eggs for each of the chemicals and the control (water).

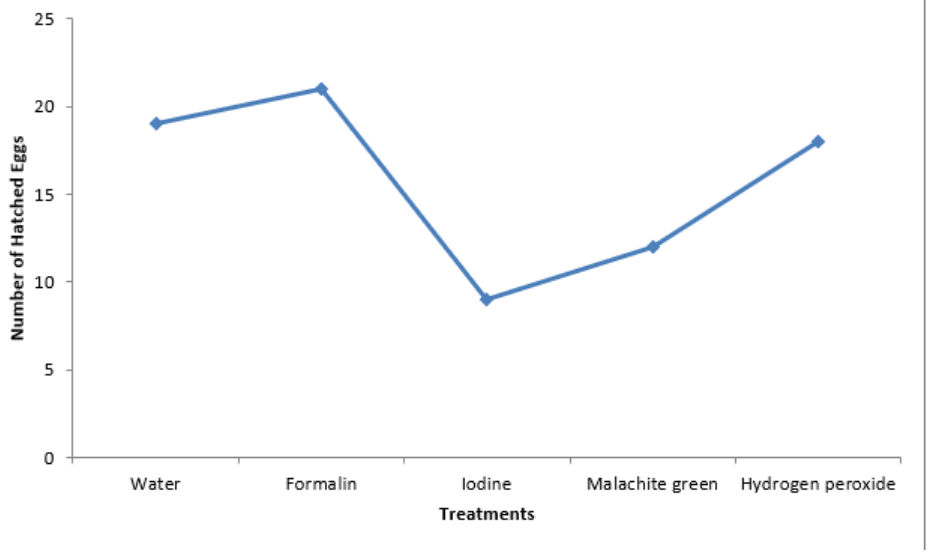

Figure 2: Line graph showing the number of hatched eggs for each of the four chemicals and the control (water).

4. Discussion

The result of the range finding test showed that the hatchability rate was between 30%-70% Typically, under poor conditions, fish will succumb to bacterial infections, fungus or parasites.

Formalin is widely used as a disinfectant for fish eggs in most fish farms as one of the registered fungicides. Formalin is a solution of 37-40% formaldehyde dissolved in water (Van Waters and Rogers, 1988; Schnick, 1973). The effect of formalin on the hatching rate of eggs of the African catfish and the subsequent survival of the larvae have been investigated using range finding and definitive tests. 0mg/l (control), 250mg/l, 500mg/l, 750mg/l and 1000mg/l of formalin were used to define the threshold limit of tolerance of eggs to formalin. There were significant differences in the hatching rates and survival of early larvae between the control and all four formalin concentrations. The result of the range finding test showed that hatching rate was between 65% and 69% with mean hatching rate of 65.3±3.51% in the control, which was significantly different from 250mg/l concentration of formalin-treated eggs which ranged between 3%-4% with a mean hatching rate of 3.67±0.58%. No hatching was noted in the concentration of 500mg/l, 750mg/l and 1000mg/l of formalin. This showed that formalin has an effect on the hatching rate of the African catfish, decreasing it with increasing concentration from 50mg/l-250mg/l. although survival rate followed the same trend as hatching rate, decreasing with increasing concentration, there was however no significant difference between control and 250mg/l concentration of formalin.

For this reason, the practical aspect of this project was carried out with 250mg/l measure of formalin (for optimum results) for a 15 minutes bath period in 10 litres of water. As observed from the tabular result, formalin had the most numbers of hatched eggs in the experiment, alongside the most number for survived larvae. With a 70% survivability rate success, formalin was the most successful disinfectant used for this experiment. It is also the most widely approved fungicide for catfish eggs around the world (although, under controlled use) as it has little or no effects on catfish eggs.

Aquatic fungi are ubiquitous in natural water supplies of fish hatcheries, often causing serious disease problems. Malachite green is effective in control of fungus on fish and fish eggs, but due to suspected teratogenicity, that is potential carcinogenicity (Meyer and Jorgenson, 1983; Fitzpatrick *et al*, 1995) and /or mutagenic properties (Marking *et al*, 1994), its use was limited to the treatment of non-food fish (that is egg or adult salmon held for spawning).

During this practical, it was observed that the treatment of malachite green on the catfish eggs yielded negative effects. The malachite green was administered at 5-10mg/l for 10-15 minutes, for eggs; and 0.05-0.1mg/l for 60 minutes, for hatchlings. The chemical yielded a 40% hatchability rate with a 16.67% survivability rate which is extremely low and very not advisable for fish farms. It was also noticed that the fish in the tank treated with malachite green had some body defects, as they got lighter in colour and sluggish every day.

Iodine is also an aquaculture drug of low regulatory priority. Although it has been recorded to improve hatching rate by 10%, in this practical it was recorded to have the least value for hatchability and survivability rates. Iodine is not an approved treatment for catfish eggs but it is used by most fish farmers anyway. The dosage recorded to have had the most positive effects is 100ppm for 10 minutes, daily for 9 days. The experiment showed a 30% hatchability rate for eggs treated with iodine, ten percent less than the hatchability rate of malachite green. The fishes in the bowls treated with iodine showed the least percentage of hatchability and survivability rates.

Hydrogen peroxide is also an aquaculture drug of low regulatory priority. It is expected that hydrogen peroxide will eventually be approved as a new animal drug and that the label will include the treatment of catfish eggs. The dosage for hydrogen peroxide as a fungi disinfectant for catfish eggs is 250mg/l for 15 minutes, although at cooler temperatures (26 degrees Celsius), hydrogen peroxide is observed to be less toxic and higher concentrations are more effective. Daily application of hydrogen peroxide is as effective as treatments with formalin. In this experiment, the bowl of catfish eggs treated with hydrogen peroxide recorded a hatchability rate of 18% and a survivability rate after 9 days of 83.33%, closest to the most successful treatment (formalin).

Water which was used as control for the experiment was untreated with no chemicals, despite this, it was the third most successful bowl of catfish eggs from day 1- day 9. Although it is unadvisable to go through with catfish farming without using any chemical disinfectants for the eggs or hatchlings, from the experiment of the project carried out, it was observed that the bowl of catfish eggs with untreated water was actually more successful in hatchability and survivability than malachite green and iodine, respectively. No modern catfish farms operate without chemical disinfection of eggs and hatchlings, for the fear of possible increase in the mortality rate of both the eggs and hatchlings, but from this experiment, it is proven that untreated water is more favourable for farmers

than treatments with malachite green and iodine although affected by certain factors like water quality and temperature.

5. Conclusion

Although many factors can cause poor hatch rates and survivability rates, knowing the optimal conditions for handling and hatching catfish eggs and following good hatchery practices will reduce problems of disease and poor survival. The availability and cheap prices of certain chemical disinfectants does not guarantee the fact that they will yield positive effects on catfish eggs and hatchlings.

6. References

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