EFFICACY OF FORMALIN AND HYDROGEN PEROXIDE ON FUNGAL - INFECTED AND UNINFECTED TILAPIA, *Oreochromis mossambicus,* EGGS

A Research Paper Presented
to the Faculty of
Philippine Science High School - Western Visayas

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in Science Research II

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ABSTRACT

Formalin and hydrogen peroxide were tested for their efficacy to prevent the spread of fungal infection (Saprolegniasis) on *Oreochromis mossambicus* eggs. 50 eggs were assigned to each of the 14 treatment trays. The trays were then divided into two groups: the positive, which was infected with *Saprolegnia*, and the negative, which was uninfected. Positive and negative controls were not given chemical treatments.

One way Analysis of Variance proved that formalin and hydrogen peroxide have significant effect ($p<0.05$) on preventing Saprolegniasis on the fish eggs.

0.5 mL/L of water concentration of hydrogen peroxide had the highest efficacy on both positive and negative groups; 1.0 and 1.5 mL/L of water concentrations of formalin were not tolerated by *Oreochromis mossambicus* eggs resulting to 100% mortality on the final treatment.

T102

APPROVAL SHEET

This research paper entitled "Efficacy of Formalin and Hydrogen Peroxide on Fungal-infected and Uninfected Tilapia, *Oreochromis mossambicus,* Eggs" submitted by Jenny Aurielle B. Babon, Sheila Mae T. Nolido and Filame Joy D. Uyaco in partial fulfillment of the requirements in Science Research II, has been examined and is recommended for acceptance and approval.

_____ _____

Date Prof. Josette T. Biyo
Science Research II Consultant

This research paper is accepted and approved in partial fulfillment of the requirements in Science Research II.

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Date Prof. Rebecca V. Yandog
PSHSWV Director

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CHAPTER I
INTRODUCTION

A. BACKGROUND AND RATIONALE OF THE STUDY

There are many waterborne fungal infections such as Saprolegniasis that affect the fish and fish eggs. These infections may spread rapidly causing fish mortality and will affect many fish cultivators who would want to meet the growing demands for fish products.

Many ways of preventing fungal infection in fish eggs have been used by many fish cultivators. One of these is malachite green. But in 1991, the United States Food and Drug Administration (FDA) terminated its use because of its teratogenic effects (Schreier, Rach and Howe, 1995). Other methods of preventing fungal infection of fish eggs like removing the infected eggs and eliminating the fungal spores using copper sulfate or quicklime (Natividad, 1984) may prove to be impractical for it will take much time to sort out the infected fish eggs.

There is a growing need of fungicides for aquaculture use. Formalin and hydrogen peroxide are two most promising candidate fungicides.

Historically, hydrogen peroxide has been used to treat fish for ectoparasites in freshwater since the 1930s (Schreier, et. al., 1995). It is also used for the control of sea lice in USA, Canada, Scotland, Ireland, Norway, and Chile (Schreier, et. al., 1995). Hydrogen peroxide is also being used as a disinfectant, as treatment for drinking water, as an antimicrobial agent in food processing and as bleaching agent of cellulose and textiles. It also has potential for the control of bacterial gill rot disease based on its bacteriostat property.

Formalin has been used as an effective fungicide in the USA, but is only approved for use on the eggs of salmonids and esocids (Schreier, et. al., 1995). Also, the use of formalin has increased causing more awareness about user's safety and the chemical's impact on the environment.

This study tested the efficacy of formalin and hydrogen peroxide on fungal-infected and uninfected tilapia, *Oreochromis mossambicus*, eggs. It

is hoped that the result of the study will provide other alternative fungicides that will be used in preventing outbreaks of fungal infection.

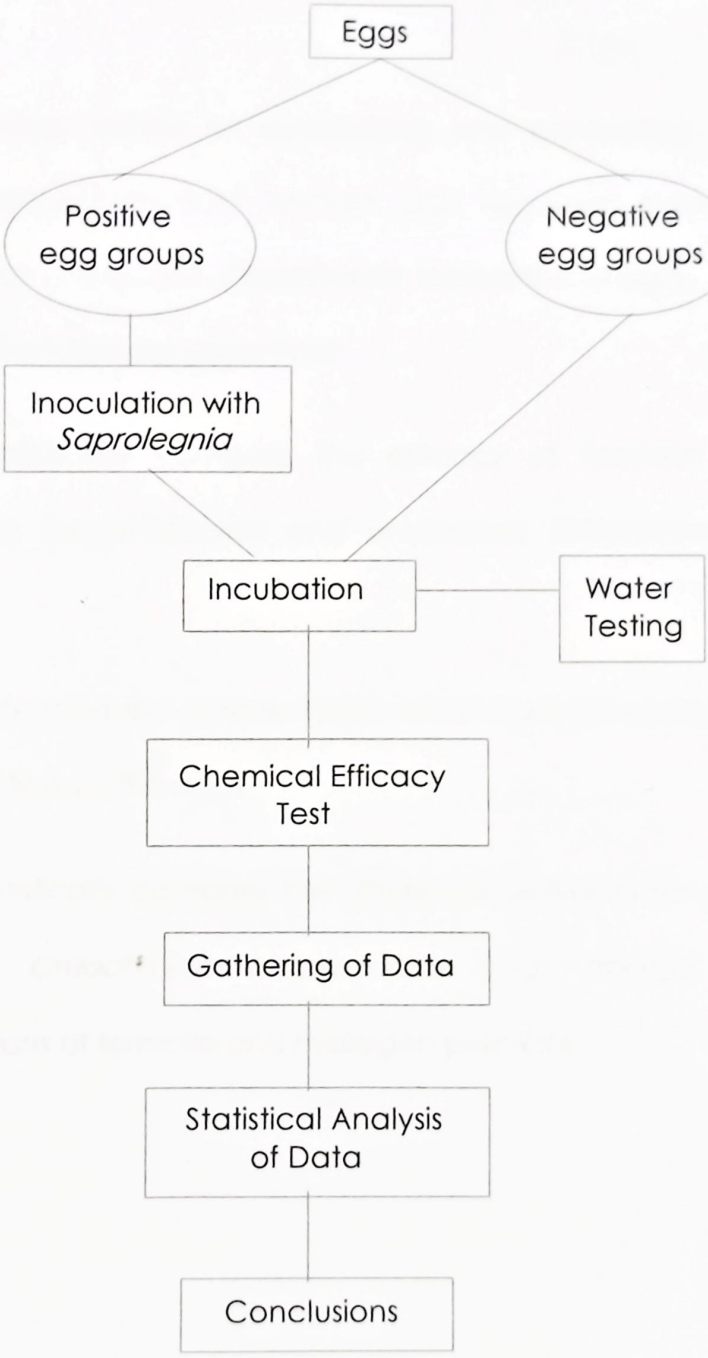

FIGURE 1.0 Flowchart of the methodology

B. OBJECTIVES OF THE STUDY

This study aimed at determining and comparing the efficacy of different concentrations of formalin and hydrogen peroxide on fungal-infected and uninfected *Oreochromis mossambicus* eggs. Specifically the study had the following objectives:

1. Statistically compare the efficacy of formalin and hydrogen peroxide on fungal-infected and uninfected *Oreochromis mossambicus* eggs.

2. Determine the concentration which best prevented the spread of fungal infection on the eggs.

3. Statistically compare the difference between fungal-infected and uninfected *Oreochromis mossambicus* eggs treated with different concentrations of formalin and hydrogen peroxide.

C. HYPOTHESIS OF THE STUDY

It is hypothesized that different concentrations of formalin and hydrogen peroxide will have no effect on fungal-infected and uninfected *Oreochromis mossambicus* eggs.

D. SIGNIFICANCE OF THE STUDY

Outbreaks of waterborne fungal infections (Saprolegniasis) on fish and fish eggs continue to cause problems among cultured fish (Schreier, et. al., 1995). Crowded conditions of the fish could promote fungal infections. Infection of eggs would lessen the hatching rate of fishes. To meet the demands of the growing market of the fish product, there is a need for finding new ways to prevent infection. The use of fungicides has been proven to be more effective than the rest.

This study will prevent the spread of infection on other fish eggs, by using the most effective fungicide. Uninfected eggs will be safe from infection and fish cultivators can expect an increase in the fish production.

E. SCOPE AND LIMITATIONS OF THE STUDY

The study focused only on *Oreochromis mossambicus* eggs in freshwater. A temperature of 27° Celsius was measured for tap water. It only focused on fungal infection on fish eggs caused by *Saprolegnia.* Two treatments were made: the positive treatment (infected) and the negative treatment (uninfected). There were also two control treatments: one for the positive group and the other for the negative group. Each treatment were given different amounts of formalin and hydrogen peroxide according to the methodology. Other chemicals were not used in the study. Water flow rate was not considered.

F. DEFINITION OF TERMS

Concentration

In this study, it refers to the amount of formalin and hydrogen peroxide present in the water where the eggs are being incubated.

Efficacy

It is sometimes called intrinsic activity and it describes the ability of the drug-receptor complex to produce a physiological response. Together, the affinity and efficacy of a drug determine its potency. (Encyclopedia Britannica, 1993).

Formalin

A solution containing 40 percent formaldehyde and 60 percent water or water and methyl and methyl alcohol. It is employed as a disinfectant, insecticide, fungicide, and deodorant. (Encyclopedia Britannica, 1993).

Hydrogen Peroxide

A best-known and widely used peroxy compound. It is a colorless liquid usually produced as an aqueous solution of various strength. It is used

as a disinfectant, substitute for chlorine in the treatment of water, and bleaching agent. (Encyclopedia Britannica, 1993).

Malachite Green

Also known as aniline green, benzaldehyde green, or china green. Malachite green is effective against fungi and gram positive bacteria. In the fish-breeding industry, it has been used to control *Saprolegnia*, a water mold that kills the eggs and young fry. It is prepared from benzaldehyde and dymethylaniline. (Encyclopedia Britannica, 1993).

Oreochromis mossambicus

It is one of the species of Tilapia under the family Cichlidae. It has dark color and bright orange margin on the dorsal and caudal fins. It is also known as native Tilapia.

Saprolegnia

It is of Order Saprolegniales under class Oomycetes. Represented by about 30 species, majority of them occur regularly as saprophytes on various types of substrata found in water. They are also known as water molds.

Saprolegnia grow saprophytically on dead bodies of many insects, tadpoles, fishes as well as on their eggs and causes diseases. They also love to grow on aquatic substrata rich in proteins.

They appear as fringes of colorless hyphae. These hyphae first appear as tiny tufts of cotton fibers. (Textbook of Thallophytes, 1992).

CHAPTER II
REVIEW OF RELATED LITERATURE

A. ANTIFUNGAL AGENTS FOR TREATING INFECTED FISH AND FISH EGGS

Antifungal agents, according Schreier, Rach and Howe (1995), are essential for the maintenance of healthy stocks of fish and fish eggs in intensive aquaculture operations. Many of these agents have already been tested for their efficacy in the prevention of fungal infection in fish and fish eggs.

A.1 Formalin

Formalin is an inexpensive and popular chemoprophylactic and chemotherapeutic agent (Diseases of Fishes, Book 6). Formalin is restricted to use with eggs of salmonids and esocids (Marking, Rach and Schreier, 1994). User safety and the effect of effluents on the environment are also concerns related to formalin treatments. When using formalin (Diseases of Fishes, Book 6), contamination with paraformaldehyde must be avoided and special care must be taken to ensure through mixing.

In the USA, formalin is the only fungicide approved for fish culture (Schreier et. al., 1995). Formalin at a concentration of 1,000 ppm appeared to be an effective alternative to the standard hatchery practice of treating eggs with formalin at a concentration of 1667 ppm (Waterstat and Marking, 1995). Also Schreier, et. al., (1995) recorded 1000 ppm to 1500 ppm as the more effective concentrations. In a study conducted by Armin-Walser and Phelps (1993) on controlling *Saprolegnia* infections on channel catfish, *Ictalarus punctatus*, eggs with formalin showed that the latter administered twice daily as a flush at concentrations of 100, 200, or 400 mg/L increased percent hatch of the eggs compared to non-treated ones. Formalin at 400 mg/L was most effective in controlling *Saprolegnia* sp. infections and the resultant percent hatch was 93.7%. Marking, Rach and Schreier (1994) evaluated different antifungal agents for fish culture. Formalin effectively prevented fungal infections on eggs at concentrations as low as 250 ppm. A 1000 ppm treatment of formalin not only prevented infection but also decreased existing infection and hatching rates at exposure of 15, 30 and 60 min.

A.2 Hydrogen Peroxide

Preliminary studies (Dawson, Rach and Schreier, 1994) at the National Fisheries Research Center, La Crosse, USA indicate that hydrogen peroxide is effective for controlling of *Saprolegnia* sp. fungus on incubating eggs of rainbow trout. It is also effective against a wide variety of other organisms such as bacteria, yeasts, viruses, and spores and has been proposed as a treatment for sea lice on salmon. Dawson et. al. (1994) also noted that hydrogen peroxide and its primary decomposition products, oxygen and water, are not systemic poisons and are considered environmentally compatible.

Hydrogen peroxide was recently classified by the US Food and Drug Administration as a low regulatory compound when used and it is the antifungal agent of choice for further development (Marking et. al. 1994).

Marking et. al. (1994) tested hydrogen peroxide to control fungus on infected eggs. Concentrations of 500 and 1,000 ppm were most effective and also increased hatching rates of treated eggs. Schreier et. al. (1995) also got 500 and 1,000 ppm concentrations as effective in treating

uninfected and infected rainbow trout eggs. Hydrogen peroxide at these concentrations appear to be effective alternatives to the standard 1,667 ppm formalin concentration treatment (Waterstat et. al., 1995).

A.3 Other Agents

An evaluation of antifungal agents for fish culture against species of *Saprolegnia* infecting eggs of rainbow trout (*Oncorhynchus mykiss*) was conducted by Marking et. al. (1994). Fourteen compounds were ineffective for control of rainbow trout eggs or were toxic to the eggs. The seven compounds that effectively controlled fungus on infected eggs and provided a reasonable margin of safety were Abbott A-73336, amorolfine, formalin, glutaraldehyde, H_2O_2, melaleuca and sodium chloride (salt).

Salt solution at a concentration of 30,000 ppm controlled fungal infection on fall chinook salmon eggs (Waterstat, 1995) but the large quantity of salt required to treat eggs over the 35-day incubation period, coupled with an apparent increase in egg mortality, rendered salt impractical for current hatchery operations.

Guerrero and Paycana (1982) conducted a study on the prophylactic treatment of tilapia and crucian carp infected with the ectoparasite Trichodina. Results showed that $KMnO_4$ at 0.1 mg/50 L of water was the most effective treatment for the parasite. Copper sulfate ($CuSO_4$) and formalin were effective after two to four hours. Sodium chloride was the least effective at 12.5 ml/50 L of water.

Zinc-free malachite green is probably the most popular agent for controlling Saprolegniasis. The popularity of this chemical stems from the fact that it is an inexpensive and effective fungicide and, in general allows a wide margin of error between therapeutic and toxic dosages (Diseases of Fishes, Book 6). But O.P. Sharma (1992) wrote that the treatment of rainbow trout eggs with malachite green can cause genetic defects.

B. FUNGI THAT INFECT FISH AND FISH EGGS

Many fishes, mollusks and crustaceans are infected by fungi (Sharma, 1992). About 250 species of molds, yeasts and other fungi that live in the sea have been identified (Lerman, 1986). Various investigations have identified

morphological and physiological differences among water mold isolates (Noga, 1993).

The best known and most widely distributed mycotic infections of fishes are those caused by the freshwater Oomycetes (Diseases of Fishes, Book 6). The class Oomycetes is divided into four orders: Lagenidiales, Peronosporales, Leptomitales and Saprolegniales. Species included in ten presently recognized oomycete genera have been reported to be either naturally occurring or artificially induced parasites of fishes. Oomycetes that infect fishes produce an easily recognized cottony mycelium on the surface of the affected animal.

Water molds grow rapidly in dead tissue (Noga, 1993) yet do not easily cause disease in healthy, unstressed hosts. They are usually relegated to the skin and superficial muscle. Trauma, nutritional deficiencies, endocrine changes and stressful water quality have been implicated. There may be major differences in how different fish species respond to such stress.

Gill rot disease is caused by the fungus *Saprolegnia.* The fungus is not considered as an obligatory parasite, but as a secondary invader of fish

following an injury or general weakening (Natividad, 1984). *Saprolegnia* infections frequently occur in incubated eggs and newborn fry (Lio-Po, 1996). Saprolegniales like *Saprolegnia* and *Achlya* are the common fungal parasites of fishes (Sharma, 1992). The fishes of domestic aquaria are commonly infected by *Saprolegnia ferax* and *S. parasitica.* El Sharouny and Badran (1995) recovered seventeen fungal species belonging to six genera from the four organs of Tilapia fish and most common were *Saprolegnia ferax, S. diclina, Achlya dubia, A. americana, A. racemosa* and *A. flagellata, Dictyuchus sterile, Pythium undulatum* and *Aphanomyces sp..* Severe infection followed by death *of* all fish was incited by *S. parasitica* and *S. ferax* through experiment I.

Kitancharoen and Hatal (1996) conducted an experimental infection of *Saprolegnia* spp. In rainbow trout eggs. Four strain of *Saprolegnia* were tested for their pathogenecity to rainbow trout eggs. Three experimental groups were prepared: Group I containing 20 living eggs, Group II containing 20 dead eggs and Group III with 10 living and 10 dead eggs. Infection and mortality rates were investigated for one week. No fungal hyphae were observed on eggs of Group I, whereas most eggs in Group II

were entangled with fungal hyphae on the second day; Group III showed a considerable degree of infection.

CHAPTER III
METHODOLOGY

A. MATERIALS AND EQUIPMENT

A.1 Sterilization of glass wares

QTY	UNIT	EQUIPMENT/MATERIALS
1		autoclave
all		glass wares

A.2 Growing the disease agent

QTY	UNIT	EQUIPMENT/MATERIALS
6		petri dishes
1		light microscope

A.3 Assigning of egg groups

QTY	UNIT	EQUIPMENT/MATERIALS
28		aluminum trays
5		aerators
3	100 mL	graduated cylinders
6	250 mL	beakers
2		spoons
		plastic tubing
		air stones

A.4 Water testing

QTY	UNIT	EQUIPMENT/MATERIALS
1		thermometer
1		pH meter

A.5 Positive egg group inoculation

QTY	UNIT	EQUIPMENT/MATERIALS
3		inoculating needles
1		alcohol lamp burner
2		petri dishes

A.6 Chemical efficacy tests

QTY	UNIT	EQUIPMENT/MATERIALS
	100 mL	Formalin
	100 mL	Hydrogen Peroxide
2	10 mL	pipettes
2		pipettors

B. METHODS

B.1 Test Organism

Oreochromis mossambicus eggs were used as test organism. The researchers chose *Orechromis mossambicus* eggs among other cultured fish because they hatch easily and they are an important food source.

Because *Oreochromis mossambicus* is a mouth brooder, the eggs were taken directly from the mouth of the mother fish. The eggs were transferred five times before they were placed in their respective treatment trays. The eggs were acquired from Iloilo State College of Fisheries in Barotac Nuevo, Iloilo.

B.2 Disease Agent

A species of *Saprolegnia* was used to infect the positive egg groups. *Saprolegnia* was grown from dead insects placed in sterile petri dishes with pond water as noted by Sharma (1992). The insect used was the common fruit fly and the pond water was taken from Bitoon fishpond.

B.3 Test Chemicals

Formalin and hydrogen peroxide (commercial grade) for the efficacy tests were taken from the Philippine Science High School-Western Visayas laboratory.

B.4 Assigning of Egg Groups

Aliquots of 50 eggs were randomly placed in aluminum trays. The eggs were counted manually. The trays were then assigned to two treatment groups: the positive and the negative group. Each treatment group composed of seven aluminum trays. The first treatment trays of both groups were assigned as control and the other treatment trays were assigned different concentrations of formalin and hydrogen peroxide. Tap water was used for egg incubation and was subjected to pH and temperature testing.

B.5 Production of Infection

This particular method was patterned from Schreier, Rach and Howe, 1995.

To produce infections, the positive egg group was inoculated with *saprolegnia* by placing 5 randomly selected eggs in a sterile petri dish with pond water and suspending the inoculating needle containing the fungi in the petri dish. The uninfected egg group was not inoculated with fungus and were allowed to incubate until all the treatment trays of the positive egg group were infected. Infection was determined by the visible fungal growth on the eggs.

B.6 Chemical Efficacy Tests

The treatment chemicals were administered to the treatment tray through a pipette. Treatments were administered every other day until eggs began to hatch (approximately five days) as patterned by Schreier et.al. (1995).

Mortality rates and number of eggs hatched were assessed before the initial treatment and after the final treatment. Percent mortality were determined by counting the number of dead eggs; those whose appearance was white. Fry hatch was assessed after all viable eggs had hatched. The percentage hatch was corrected by subtracting the initial mortality from 50 eggs according to the following formula by Schreier et.al. (1995).

$$\text{Percent hatch} = \frac{\text{number hatched}}{\text{50 eggs - initial morts}} \times 100$$

B.7 Treatments

N.B. (per 500 mL of water)

POSITIVE GROUP

Control^{+} - infected, untreated

Treatment A_1^{+} - 0.250 mL Formalin

A_2^{+} - 0.500 mL Formalin

A_3^{+} - 0.750 mL Formalin

B_1^{+} - 0.075 mL Hydrogen Peroxide

B_2^{+} - 0.125 mL Hydrogen Peroxide

B_3^{+} - 0.250 mL hydrogen peroxide

NEGATIVE GROUP

Control^{-} - uninfected, untreated

Treatment A_1^{-} - 0.250 mL Formalin

A_2^{-} - 0.500 mL Formalin

A_3^{-} - 0.750 mL Formalin

B_1^{-} - 0.075 mL Hydrogen Peroxide

B_2^{-} - 0.125 mL Hydrogen Peroxide

B_3^{-} - 0.250 mL Hydrogen Peroxide

B.8 Statistical Analysis

Analysis of Variance was used as statistical tool to determine significant differences between treatments. This was used as basis for the researchers' conclusions.

CHAPTER IV
RESULTS AND DISCUSSIONS

Saprolegnia was first cultured September 3, 1997 to verify O.P. Sharma's method. After four days, cottony growth was observed under the microscope.

The negative group was conducted last January 25 to January 28, 1998. The *Saprolegnia* for the positive group was cultured January 4, 1998. The first set-up for the positive group was conducted last January 8 to January 13, 1998. The second set-up was done last January 25 to January 28, 1998. The treatment groups were not done simultaneously because of the minimal supply of eggs.

Water temperature averaged to 27° Celsius and pH was seen at an average of 6.89.

Table 1.0 Mortality rates before initial treatment and after final treatment

TREATMENT	INITIAL MORTALITY		FINAL MORTALITY	
POSITIVE	R_1	R_2	R_1	R_2
Control	1	20	11	30
0.250 mL Formalin	3	19	50	28
0.500 mL Formalin	1	17	50	50
0.750 mL Formalin	1	19	50	50
0.075 mL Hydrogen Peroxide	1	25	17	33
0.125 mL Hydrogen Peroxide	1	23	6	30
0.250 mL Hydrogen Peroxide	1	25	4	29
NEGATIVE				
Control	4	22	10	27
0.250 mL Formalin	13	20	23	30
0.500 mL Formalin	17	18	50	50
0.750 mL Formalin	15	19	50	50
0.075 mL Hydrogen Peroxide	12	13	26	26
0.125 mL Hydrogen Peroxide	16	13	25	25
0.250 mL Hydrogen Peroxide	12	20	20	27

Table I shows the mortality rates of each treatment tray before initial treatment and after final treatment. The eggs of the formalin groups A_2 and A_3 for the negative egg groups in both replicates and the A_2 and A_3 of the first set-up of the positive egg group died after the final treatment. The eggs of the first replicate of the positive A_1 also died. A probable reason for this must be the concentration of formalin in water. This showed that *Oreochromis mossambicus* eggs did not tolerate concentrations of 1.0 and 1.5 mL/L of water for formalin.

Table 2.0 Percent hatch for each treatment

TREATMENT	PERCENT HATCH		MEAN
	R_1	R_2	
POSITIVE Control	79.59	66.67	73.13
0.250 mL Formalin	0	70.97	35.49
0.500 mL Formalin	0	0	0
0.750 mL Formalin	0	0	0
0.075 mL Hydrogen Peroxide	67.35	68	67.68
0.125 mL Hydrogen Peroxide	89.8	74.07	81.94
0.250 mL Hydrogen Peroxide	93.88	84	88.94
NEGATIVE Control			
0.250 mL Formalin	86.96	82.14	84.55
0.500 mL Formalin	72.97	66.67	69.82
0.750 mL Formalin	0	0	0
0.075 mL Hydrogen Peroxide	0	0	0
0.125 mL Hydrogen Peroxide	63.16	64.86	64.01
0.250 mL Hydrogen Peroxide	73.53	67.57	70.55
	78.95	76.67	77.81

Table 2 shows the percent hatch of each treatment for both replicates. Mean percent hatch were taken by adding percent hatches of both replicates and dividing the sum by two. The mean percent hatch for each treatment were the values considered for One-way Analysis of Variance.

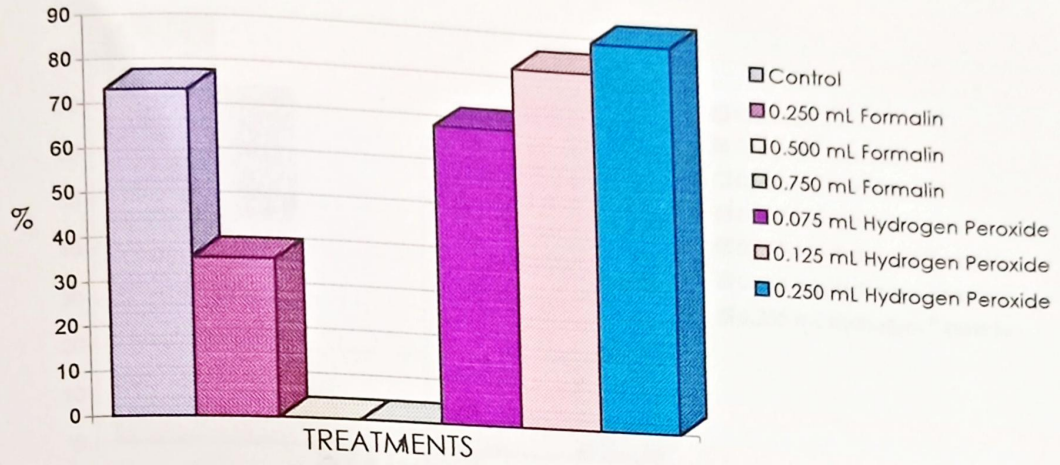

FIGURE 2.0 Mean percent hatch of positive egg group

Figure 2 illustrates the mean percent hatch of the treatments belonging to the positive egg group. For the hydrogen treatments, there is an increasing trend in the mean percent hatch.

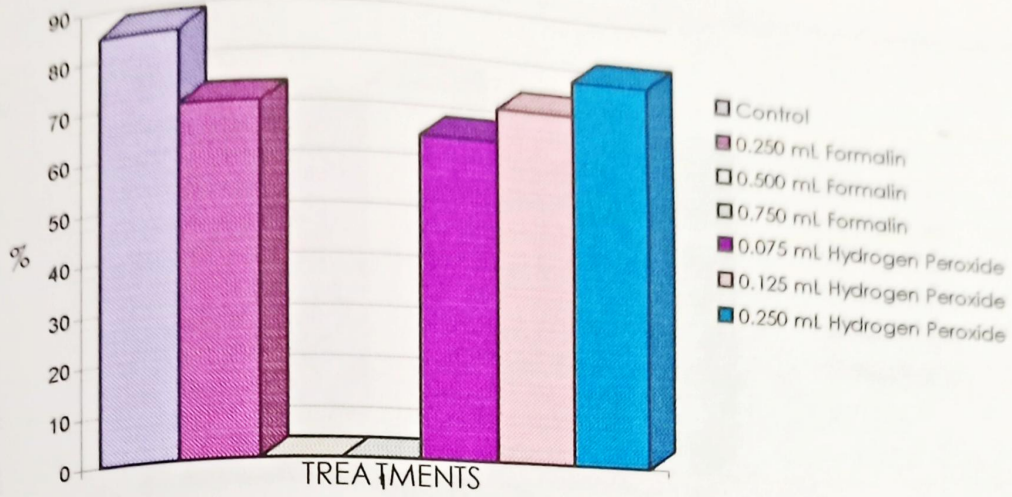

FIGURE 3.0 Mean percent hatch of negative egg group

Figure 3 illustrates mean percent hatch of treatments of the negative group. The hydrogen treatments of the negative group also exhibits the same trend as that in the positive egg group.

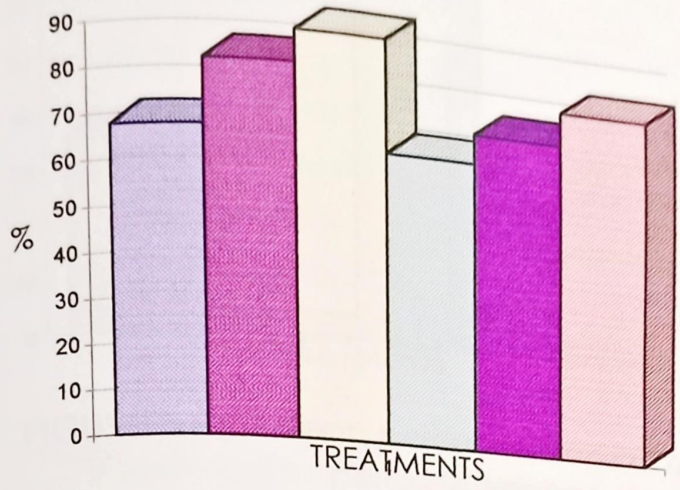

FIGURE 4.0 Mean percent hatch of formalin treatments

Figure 4 illustrates mean percent hatch of formalin treatments. The figure clearly shows that only a concentration of 0.5 mL/L of water hydrogen peroxide was tolerated by the *Oreochromis mossambicus* eggs.

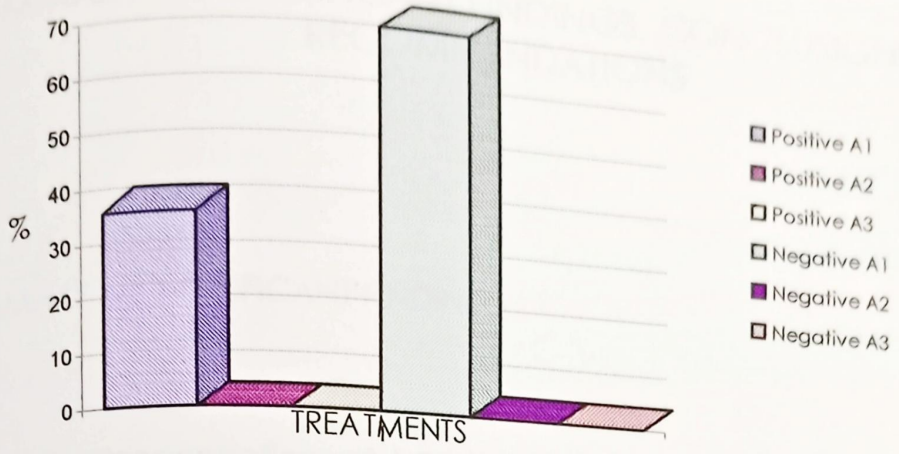

FIGURE 5.0 Mean percent hatch of hydrogen peroxide treatments

Figure 5 illustrates mean percent hatch for hydrogen peroxide treatments. The figure displays the increasing percent hatch pattern in both of hydrogen peroxide's positive and negative egg groups.

One-way Analysis of Variance (ANOVA) showed that formalin and hydrogen peroxide have a significant effect on the spread of fungal infection on *Oreochromis mossambicus* eggs. Statistical computations can be seen in Appendix C of this research paper.

CHAPTER V
SUMMARY OF SIGNIFICANT FINDINGS, CONCLUSIONS AND RECOMMENDATIONS

A. SUMMARY OF SIGNIFICANT FINDINGS

1. Formalin concentrations of 1.0 to 1.50 mL/L of water proved to be toxic to *Oreochromis mossambicus* eggs.

2. For the positive egg group, treatment B_3+ (Hydrogen Peroxide at 0.5 mL/L of water) best prevented the spread of fungal infections on the eggs as exhibited by its high mean percent hatch compared to the positive control.

3. For the negative egg group, all the other treatments had a slightly lower percent hatch than the negative control.

4. There was a direct relationship between the percent hatch and the concentration of hydrogen peroxide in water: the higher the

concentration, the higher the percent hatch. This was exhibited by both egg groups.

5. One-way ANOVA proved that formalin and hydrogen peroxide had significant effects on fungal-infected and uninfected *Oreochromis mossambicus* eggs.

B. CONCLUSIONS

1. Hydrogen peroxide was found to be a more effective fungicide compared to formalin.

2. Hydrogen peroxide in the negative group did not effectively prevent the spread of fungal infection on *Oreochromis mossambicus* eggs.

3. Formalin concentrations of 1.0 and 1.5 mL/L of water were found to have toxic effects on *Oreochromis mossambicus* eggs.

C. RECOMMENDATIONS

The researchers recommend that the amount of formalin administered to *Oreochromis mossambicus* eggs be lowered. The eggs do not qualify for the standard 1,000 - 1,667 ppm concentrations of formalin used in hatcheries.

The researchers also suggest that water quality be considered in future studies to avoid variations in stress conditions of the eggs. It is also recommended that formalin and hydrogen peroxide be tested in large-scale ponds to verify their effectiveness in vitro. It is also recommended that further studies on fish mycology.

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APPENDICES

APPENDIX A. Systematic Position of *Saprolegnia*

Division - Eumycota

Subdivision - Mastigomycotina

Class - Oomycetes

Order - Saprolegniales

Family - Saprolegniaceae

Genus - *Saprolegnia*

APPENDIX B. Bactericide Effects of Formaldehyde and Hydrogen Peroxide

Chemical Agent	Fungi	Spores
formaldehyde (vaporized)	positive	positive
hydrogen peroxide	positive	positive

APPENDIX C. One-way ANOVA results

C.1 Descriptive Statistics

TREATMENT	N	Mean	Standard Deviation	Minimum	Maximum
Control$^+$	2	73.13	9.1358	66.67	79.59
A_1^+	2	35.485	50.1834	0	70.97
A_2^+	2	0	0	0	0
A_3^+	2	0	0	0	0
B_1^+	2	67.675	0.4596	67.35	68
B_2^+	2	81.935	11.1228	74.07	89.8
B_3^+	2	88.94	6.9862	84	93.88
Control$^-$	2	84.55	3.4083	82.14	86.96
A_1^-	2	69.82	4.4548	66.67	72.97
A_2^-	2	0	0	0	0
A_3^-	2	0	0	0	0
B_1^-	2	64.01	1.2021	63.16	64.86
B_2^-	2	70.55	4.2144	67.57	73.53
B_3^-	2	77.81	1.6122	76.67	78.95

C.2 ANOVA results for Control$^+$ and A_1^+

SOURCE	Sum of Squares	Degrees Freedom	Mean Square	F Ratio	Prob.
between	1417.146	1	1417.146	1.089	0.4062
within	2601.834	2	1300.917		
total	4018.98	3			

C.3 ANOVA results for Control^{+} and $A_2{}^{+}$

SOURCE	Sum of Squares	Degrees Freedom	Mean Square	F Ratio	Prob.
between	5347.997	1	5347.997	128.152	7.713E-03
within	83.463	2	41.732		
total	5431.46	3			

C.4 ANOVA results for Control^{+} and $A_3{}^{+}$

SOURCE	Sum of Squares	Degrees Freedom	Mean Square	F Ratio	Prob.
between	5347.997	1	5347.997	128.152	7.713E-03
within	83.463	2	41.732		
total	5431.46	3			

C.5 ANOVA results for Control^{+} and $B_1{}^{+}$

SOURCE	Sum of Squares	Degrees Freedom	Mean Square	F Ratio	Prob.
between	29.757	1	29.757	0.771	0.4878
within	83.674	2	41.837		
total	113.431	3			

C.6 ANOVA results for Control^{+} and $B_2{}^{+}$

SOURCE	Sum of Squares	Degrees Freedom	Mean Square	F Ratio	Prob.
between	77.528	1	77.528	0.748	0.4782
within	207.18	2	103.59		
total	284.708	3			

C.7 ANOVA results for Control^{+} and $B_3{}^{+}$

SOURCE	Sum of Squares	Degrees Freedom	Mean Square	F Ratio	Prob.
between	249.956	1	249.956	3.779	0.1913
within	132.27	2	66.135		
total	382.226				

C.8 ANOVA results for Control^{-} and $A_1{}^{-}$

SOURCE	Sum of Squares	Degrees Freedom	Mean Square	F Ratio	Prob.
between	216.973	1	216.973	13.793	0.0655
within	31.461	2	15.731		
total	248.434	3			

C.9 ANOVA results for Control$^-$ and A_2^-

SOURCE	Sum of Squares	Degrees Freedom	Mean Square	F Ratio	Prob.
between	7148.703	1	7148.703	1230.816	8.115E-04
within	11.616	2	5.808		
total	7160.319	3			

C.10 ANOVA results for Control$^-$ and A_3^-

SOURCE	Sum of Squares	Degrees Freedom	Mean Square	F Ratio	Prob.
between	7148.703	1	7148.703	1230.816	8.115E-04
within	11.616	2	5.808		
total	7160.319	3			

C.11 ANOVA results for Control$^-$ and B_1^-

SOURCE	Sum of Squares	Degrees Freedom	Mean Square	F Ratio	Prob.
between	421.892	1	421.892	64.602	0.0151
within	13.061	2	6.531		
total	434.953	3			

PLATES

PLATE 1.0 *Egg acquisition.* The eggs were taken from the mouth of the mother fish.

PLATE 2.0

Assigning of egg groups. Fifty eggs were placed in individual aluminum trays and were designated corresponding treatments.

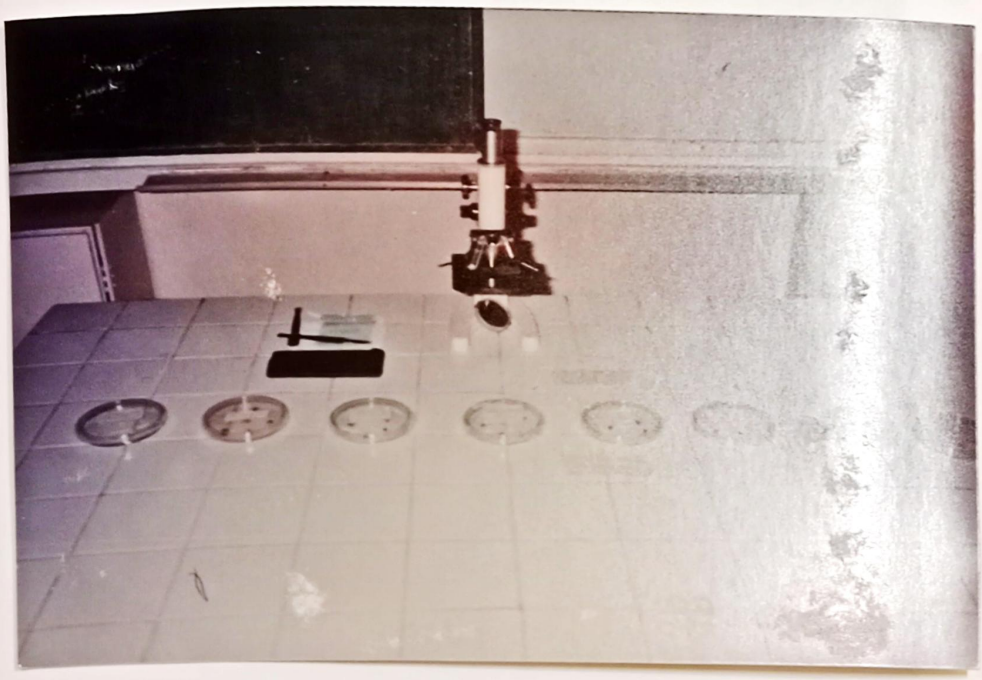

PLATE 3.0 *Growing the disease agent.* Saprolegnia was grown from sterile petri dishes with dead flies placed in pond water.

PLATE 4.0

Promotion of infection. 10% of the eggs were inoculated with Saprolegnia to promote infection on the positive egg group.

PLATE 5.0 *Counting of fish mortality.* Initial fish mortality were counted before the initial treatment.

PLATE 6.0 *Chemical efficacy tests.* Test chemicals were administered to each treatment tray through a pipette.

PLATE 7.0

Post-treatment procedures. Final mortality and number of eggs hatched were counted after the final treatment.