UNIVERSITI MALAYSIA KELANTAN

EFFECT OF WOOD VINEGAR ON SURVIVABILITY OF NILE TILAPIA, *Oreochromis niloticus*.

By

MOHAMAD FIKREE BIN JAMALUDDIN

A report submitted in fulfilment of the requirements for the degree of Bachelor of Applied Science (Animal Husbandry Science) with Honours

Faculty of Agro Based Industry
UNIVERSITI MALAYSIA KELANTAN

2018

DECLARATION

I hereby declare that the work embodied in this report is the result of the original research and has not been submitted for a higher degree to any universities or institutions.

____

Student

Name: Mohamad Fikree Bin Jamaluddin

Date:

I certify that the report of this final year project entitled "Effect of wood vinegar on survivability of Nile Tilapia, *Oreochromis niloticus*" by Mohamad Fikree Bin Jamaluddin matric number F14A0138 has been examined and all the correction recommended by examiners have been done for the degree of Applied Science (Animal Husbandry Science) with honours, Faculty of Agro-Based Industry, Universiti Malaysia Kelantan.

Approved by:

____

Supervisor

Name: Dr. Suniza Anis binti Mohamad Sukri

Date:

ACKNOWLEDGEMENT

First of all, praised to Allah the almighty one who gave me an opportunity to still breathing till this days and gave me chance to complete my final year project research and thesis. Without the help of Allah, there's no way I could complete my final year project neither experiment nor thesis writing.

This gratitude also for my ex-supervisor Dr. Tan Sze Huey which just quit from becoming lecturer of Universiti Malaysia Kelantan few months ago. I would like to give thousands of thanks to her because guiding me and giving me ideas to complete my research. I hope she's having a wonderful life afterwards.

Not forget to mention, my new supervisor for this project, Dr. Suniza Anis Binti Mohamad Sukri, for giving me chances to supervise my FYP research and gave me guidance and advice in order to complete this research. Thousands of thanks also to her for giving correcting my thesis writing so far.

Other than that, I would gave token of appreciate to the lab assistant En.Shamsul because providing me full equipment for my experiments for free.

I would also love to thanks my friends and families which keep supporting me during this thesis writing and final year project experiments. Thanks to my friends because helping me to understand the guideline of thesis writing and etc.

Effect of wood vinegar on survivability of Nile Tilapia, *Oreochromis niloticus*

ABSTRACT

Wood vinegar is a pyroligneous acid which is the byproduct that was created from the charcoal production. It was used in the agriculture and livestock industry for the reason to enhance the production of their product. The use of this wood vinegar in aquaculture species remains elusive. A research was conducted in order to determine the survivability of Nile tilapia, *Oreochromis niloticus* by adding the wood vinegar in fish culturing water with two different concentrations which is 1:1500 and 1:1000. 1:1500 concentrations were labelled as Treatment 1, concentration of 1:1000 was labelled as Treatment 2 and control fish was labelled as Treatment 3. The tilapia fish were observed for every 4 hours until 72 hours. The mortality of the fish was recorded during the observation period. The fish have shown unfavourable towards the concentration of wood vinegar 1:1000 which is considered as concentrated. Mortality of the fish in the concentration of wood vinegar 1:1000 reach over 32 % while there is no mortality recorded for the concentration of wood vinegar 1:1500 and the control fish. The findings of this research provide additional information about the use of wood vinegar in aquaculture which will be helpful for the future.

Keywords: Wood vinegar, concentration, survivabililty, fish culturing water, *Oreochromis niloticus*

Kesan cuka kayu ke atas kemandirian ikan tilapia nil, *Oreochromis niloticus*

ABSTRAK

Cuka kayu merupakan acid pyroligneous dimana ia adalah produk sampingan yang dicipta dari pembuatan arang. Ia digunakan di dalam industri asas tani dan ternakan bagi meningkatkan lagi penghasilan produk mereka. Kegunaan cuka kayu dalam industri akuakultur masih lagi tidak diketahui. Satu kajian telah dijalankan bagi mengetahui kemadirian ikan tilapia Nil, *Oreochromis niloticus* dengan meletakkan cuka kayu kedalam air kultur ikan dengan dua kepekatan yang berbeza dimana ia adalah 1:1500 dan 1:1000. Kepekatan 1:1500 dilabel sebagai Rawatan 1, kepekatan 1:1000 dilabel sebagai Rawatan 2 dan ikan kawalan dilabel sebagai Rawatan 3. Ikan tilapia akan diperhati setiap empat jam sehingga tujuh puluh dua jam. Kematian ikan telah dicatatkan sepanjang tempoh pemerhatian. Ikan tilapia telah menunjukkan ketidakselesaan dalam kepekatan cuka kayu 1:1000 dimana ia dianggap sebagai pekat. Kematian ikan tilapia dalam kepekatan 1:1000 mencecah 32% manakala tiada kematian dicatatkan bagi ikan tilapia di dalam kepekatan 1:1500 dan ikan kawalan. Penemuan di dalam kajian ini memberi informasi tambahan terhadap kegunaan cuka kayu dalam industri asas tani di mana ia dapat membantu pada masa akan datang.

Kata kunci: Cuka kayu, kepekatan, kemandirian, air kultur ikan, *Oreochromis niloticus*

TABLE OF CONTENT

LIST OF FIGURES

No. **Page**

LIST OF TABLES

LIST OF ABBREVIATION AND SYMBOLS

FAO	Food and Agriculture Organization
m	Meter
cm	Centimetres
℃	Degree Celcius
g	Gram
%	Percentage
P/E	Protein and Energy
kCal	Kilo Calorie
NR	Natural Rubber
HPA	Hypothalamic-Pituitary Axis
LF	Lactofferin
CFU	Colony Forming Unit
LC	Lethal Concentration
ppt	Parts per thousand
p.p.m	Parts per million
ml	millilitres

CHAPTER 1

INTRODUCTION

1.1 Research background

Tilapia fish (*Oreochromis niloticus*) is recognized as one of the important aquaculture species that contribute substantially to the commercial industry and was also known as number three after catfish and carp (Trewavas, 1983). However, the invasion of pathogenic bacteria likes *Streptococcus* and *Edwardsiella* bacteria has been a threat for the farmers and also wiped out the populations of the tilapia fish in tilapia fish farming. Since the incidents, there are many different approaches were taken in order to evade the problems. In order to overcome this problem, many researcher need to find a way to produce healthier and stronger fish. One of the ways to investigate on how to produce healthier tilapia fish is by using the wood vinegar in the fish culturing water. It is because the use of wood vinegar towards the aquaculture activity still remains elusive. Testing the effect of wood vinegar on the stress level of tilapia fish (*O. niloticus*) can help for a further research in order to discover a way to prevent the diseases as the uses of wood vinegar were proves that it enhance the production of livestock (Nikhom, 2010).

Wood vinegar is also known as pyrolignous acid, which is a dark liquid produced by the destructive distillation (heating with a high temperature) of wood and other plants materials (Webster, 1913: de Guzman, 2009). The principal components of pyroligneous acid are acetic acid, acetone, and methanol. Wood vinegar is one of the natural extracts from the woods which were used in agricultural activity and were used in animal fodders.

The study on the uses of wood vinegar was established to the agriculture and livestock production. The study found that the wood vinegar can increase the production of livestock and agriculture. The uses of wood vinegar in the crop also can increase the crop resistance to bad condition. For enhanced plant production, the solution can be showered over plant shoots. Wood vinegar, which the function similar to hormones, were assimilated into twigs, trunks, or leaves, bringing about more grounded plants and leaves that are greener and more impervious to vermin and illnesses also, the wood vinegar can also be used as a disinfectant against the pathogenic diseases (de Guzman, 2009). This study may give us vision on the study of the effect of the wood vinegars usage in the fish culturing water for the further research of the effect of using the wood vinegars for aquaculture activity.

The study about the effects of wood vinegars on the stress level of the fish can helps in further study about the functions and application of the wood vinegar on the fish which may help in the aquaculture industry as for the tilapia fish, the mycotic (fungal) infection become significance if they are under constant stress (Macnive, 2001).

1.2 PROBLEM STATEMENT

Wood vinegars have been used in agricultural and livestock production. However, very few has reported on the uses and benefits of the wood vinegar in aquaculture activity. Until now, there are not many researches that have been conducted to test the effectiveness of wood vinegar in the aquaculture activity. Thus, this test result will be one of the most important information or discoveries for the aquaculture industry to determine the compatibility of using the wood vinegar solution into the fish culturing water. Although the effectiveness of the wood vinegar was confirmed in agricultural and livestock, the effectiveness of using the wood vinegar on the aquaculture species has not been reported. Hence, the experimental test will be conducted to tilapia fish in order to observe the effect of the uses of wood vinegar on the stress level of the tilapia fish *Oreochromis niloticus*.

1.3 OBJECTIVES

1. To determine the compatibility of the wood vinegar on the tilapia fish by observing their behavioural and stress level.
2. To determine the survivability of tilapia fish stress challenged with wood vinegar.

1.4 FIELD OF STUDY

This research focuses on the uses of the wood vinegar in the fish culturing water and the effect of using different concentration of wood vinegar which is 1:1500 (Treatment 1) and 1:1000 (Treatment 2) on the aquaculture species survivability. The results were then compared to the fish tanks that not been added with wood vinegar which labelled as Treatment 3 (control).

1.5 SIGNIFICANCE OF THE STUDY

This project specifically focused on this tilapia fish (*Oreochromis niloticus*.)The tilapia fish growth has several barriers for example the infection of pathogenic bacteria which can cause fatality of the fish. So far, there are not many research have been done in order to study the effect of the wood vinegar on the fishes even though the use of wood vinegar shows a positive results on agricultural and livestock industry. The aim of this project is evaluate the effectiveness of wood vinegar on the fish tanks water and to deduce the effect of the uses of wood vinegar on the stress level and mortality of the tilapia fish. The outcome of this project will suggest further study about the effect of using the wood vinegar on the growth or immunity level of the fish.

Thus, the study on the effect of uses of wood vinegar stress challenged with fish water may contribute to the improvement of many responses of the tilapia fish (*O. niloticus*).

1.6 LIMITATION OF THE STUDY:

This experiment used the fingerlings for study which may be hard to rear. The fingerlings may injure during the transportation that it may die if not handled carefully. The risk of the fish to die is high due to the sensitivity of the fingerlings towards the environment. Insufficient oxygen may cause the fish to suffocates and die. Other than that, the lack of information on the way to apply or use the wood vinegar is one of the limitations that were faced during this study.

1.7 HYPOTHESIS

H_0 Fish that was cultured in fish culturing water that contain high concentration of wood vinegar may survive.

H_1 Fish that was cultured in fish culturing water that contain high concentration of wood vinegar may die

CHAPTER 2

LITERATURE REVIEW

2.1 Tilapia fish (*Oreochromis niloticus)*

Nile Tilapia (*Oreochromis niloticus*) is a species of tilapia native fish from the Africa from Egypt South to East and the Central Africa, and West Gambia (Froese *et al.,* 2015). Tilapia is the members of cichlidae family which are the group of warm water fish and also have become one of the most important warm water aquaculture fish group in the world (FAO, 2003). Tilapia fish, (*O. niloticus*) usually inhabit in a wide variety of freshwater natural surroundings like waterways, lakes, sewage trenches and water system channels (Bailey, 1994). Nile tilapia (*O. niloticus)* usually feed on phytoplankton, or benthic algae.

They also practice mouth brooding which they hold off the newly hatched fry in their mouth to give protection for them (Trewavas, 1983: Yonas, 2006). After the male prepared nest, the young fry or the egg were carried in the mother's mouth for 12 days. They also demonstrate parental care if they face danger or threat. However, carrying the young fry will reduce the reproductive fitness of the mother (Mendoza *et al.,* 2005).

2.1.1 Water Quality Parameters

The operations involved in water quality assessment are many and complex. During 1950s, in the beginning of modern era water quality checking, activity were rarely concentrated or focused on specific issues. However, the water quality evaluation process has now developed into an arrangement of refined observing activities including the utilization of water science, particulate material and aquatic biota. Numerous manuals on water quality observing methods as of now exist (Boyd, 1979).

The principle explanation behind the appraisal of quality of the aquatic environment has been, generally, needed to verify whether the observed water quality is reasonable for intended users. Monitoring which is used to get important data ought to mirror the information needs of the different users involved (Boyd, 1979). Single-target checking which might be set up to address one issue zone as it were. This includes a straight forward set of variable, for example, pH, alkalinity and a few cation for acid rain; supplements and chlorophyll colors for eutrophication; different nitrogenous compound for nitrate contamination; or sodium, calcium, chloride and a couple of different components for water system. Multi-target checking which may cover different water uses and give information to more than one evaluation program, for example, drinking water supply, industrial manufacturing, fisheries or aquatic life, thereby involving a large set of variables (Boyd, 1979).

Hypothetically, expanded carbon: nitrogen ration, C:N proportion through carbon addition improves change of the toxic inorganic nitrogen species to microbial biomass accessible as food for culture animals. The ideal C:N proportion in an aquaculture framework can be kept up by including distinctive locally accessible cheap carbon sources and reduction of protein content in the feed (Boyd, 1979).

2.1.2 Effects salinity to *Oreochromis niloticus*

Nile tilapia (*Oreochromis niloticus*) is one of the most important freshwater fish in aquaculture, although it grows fast, it is less salt tolerance compared to *Oreochromis aureus, Oreochromis mossambicus* and tilapia zilli (Likongwe, 1996*).* Even though *O. mossambicus* and tilapia zilli are high salt tolerant, they are not the most popular tilapia culture compared to the nile tilapia *O. niloticus* (Stecko, 1996). It is found that the *O. niloticus* can only survive briefly under the salt water (Stauffer, 1996). Researchers indicate that the hybrid of *O. mossambicus* and *O. niloticus* have higher salinity tolerance compared to the *O. niloticus* alone, and suggested that this type of hybridization may develop a progeny that can be transferred in salt water at very small sizes and thus reducing the fresh water requirement (Carline, 1996). Early exposure through spawning and incubation to elevated salinities can enhance the salinity tolerance level of *O. niloticus* fry effectively (Likongwe *et al.,* 1996).

A research have been conduct to observe the spawning of *O. niloticus* in the salinity of water ranging from freshwater to the full seawater which the salinity for seawater is 32 g 1^{-1} however, the egg that were spawned in the seawater shows poor quality and hatching success (Likongwe, 1996). The egg and fry production per unit are greater in the yearling females in the salinity ranging 5 to 15 g 1^{-1} compared to the older females in the freshwater (Stauffer, 1996). Tilapia fish can tolerate the high temperature however, it has been reported that the salinity can modify the effects of temperature on the growth of the tilapia (Stauffer, 1996). Salinity may affect the temperature preference of the fish, and the temperature may also influence the salinity selection (Carline *et al.,* 1996).

2.1.3 Effect of temperature on Nile Tilapia

In Tunisia, tilapia fish was first introduced in the year of 1999. These events occur in order to develop enormous geothermal water resources in the country. The tropical and subtropical of Tunisia was clearly reflected in their thermal preferendum; in which this fish growth does not develop well with the temperature below 16℃ and can't survive for more than a few days in the temperature of below 10^{0}C (Azaza, 2008).

Tilapia fish are remarkably highly tolerance in the high temperature which it can tolerate the temperature up to 40℃ (Azaza, 2008). Temperature is one of the factors that affecting the growth, food intake, and the food conversion of the fish (Krai, 2008). Fish water will move to the deeper water when the surface water temperature decrease or increased beyond their preferred range in the natural aquatic system (Dhrai,2008).

For the Nile Tilapia studies, most of the studies used the fish at a late stage of development when factors other than growth can affect permanently on the growth (Azaza, 2008). It may be possible to maintain or control the optimum temperature requirement of the species under captivity. The temperature plays an important role for optimization of the growth development of younger stages, especially at the early stages (Azaza *et al.,* 2008).

In addition, this research also found information about the effect of the temperature on the sex determination of the fish during the early stage of development. Ambient temperature during the period of sex differentiation of fry are strongly influences the sex ratio in nile tilapia (*Oreochromis niloticus*) (Baroiller, 1995). High temperature results in high favour of males while low temperature results in high favour of females tilapia (Krai *et al.,* 2008).

2.1.4 Protein and Energy Requirement of Tilapia Fish (*Oreochromis niloticus*).

Nile Tilapia fry (0.020g-0.028g) that reared at four salinities and fed on fish meal diet base required 28%-30% daily protein in order to achieve maximum growth (El-Sayed, 1992). For the fingerling tilapia (1-1.29g) fed on protein-based diet requires 40% dietary protein in order to get the maximum growth of the fish (Teshima *et al.,* 1992). The problem faced by the researcher in order to determine the dietary protein of tilapia is that the use of substance that called "isocaloric diets" (El-Sayed *et al.,* 1992).

However, response of the tilapia fry for different protein and energy (P/E) ratios was not fully understood. The protein and energy ratios in fish diets play an important role in order to determine their dietary protein and energy requirements (El-Sayed & Teshima, 1992). More protein used for the energy, the more ammonia will be produced and thus, the more energy will be used as heat (Teshima, 1992).

As a result, less protein will be retained in the fish body. When the amounts of energy intake are excessive at the moderate protein intake, it will lead to the fat deposition. (El-Sayed, 1992). Thus, the best fish growth obtained is achieved from the intake of the diet that contains 45% and 400kCal /100g protein and energy, respectively.

Increasing the dietary protein and energy beyond this level will not affect or improve anything on the growth performance of the fish and feed utilization efficiency (El-Sayed, 1992).

2.2 Wood vinegar

Wood vinegar was also known as the pyroligneous acid or wood acid (George, 2010). The colour of the wood vinegar is dark brown which is manufactured by the process of destructive distillation of woods and the other plant. Pyroligneous acid was first investigated by German chemist, Johann Rudolph Gaulber (George, 2010). Other than that, this acid was also eaten as a substitute for vinegar. Wood vinegars also function as the remedy for treating wounds, ulcers and the other ailments. Salt can be made by neutralizing the wood vinegar acid with lye that was made from the ashes of the burnt wood (Johann Rudolph Gaulber, 1651: George, 2010). Pyroligneous acid was used for ages as the sterilizing agent, deodorizer, fertilizer and antimicrobial (George, 2010).

A number of potential agricultural applications was also reported which the wood vinegar was blended with water with the ratio of 1:50 (1 liter wood vinegars to 50 litres water) to 1:800 (De Guzman, 2009). The production of wood vinegar in number of farm have been reported for several years, which it includes spraying the wood vinegar solution to control insect pests (Nikhom, 2010). Mae Jo University also reported that the use of wood vinegar in the soil help to increase the population of the microbe that can enhance the growth of the plant root growth (Nikhom, 2010).

Wood vinegar is safe for the organism in the food chain, that including the pollinating insects. However, according to Thailand Department of Agriculture, the substance will be slightly toxic to fish and very toxic to the plant if the wood vinegar is applied excessively also, the wood vinegar with excessive tar also can be harmful to the plant (Nikhom, 2010).

2.2.1 Wood Vinegars as Antifungal Agents.

Natural rubber (NR) latex from the *Hevea brasiliensis* tree usually was coagulated by the acid in for preparation of sheets production. (Niamsa, 2009). The wood vinegar contain of many chemical component with acetic acid as the main substance (Baimark, 2009). The remaining of the wood vinegar is called the tar-extracted wood vinegar. Use of the wood vinegar as the coagulant in NR sheets for the preparation process have been reported (Baimark, 2009). The high moisture content of the NR is the main cause of the fungi growth. It may affect the quality of the NR production so that the antifungal agents must be added during the production of the NR (Baimark, 2009).

The antifungal efficiency of coagulants was compared from a fungi growth area on the pre-drying natural rubber (NR) sheet surface (Niamsa, 2009). The research found that the percentage of the fungi growth areas of pre-drying NR sheets which were prepared with the use of formic acid and acetic acids were 100% (Niamsa, 2009).The use of wood vinegars had higher efficiency for antifungal action compared to the formic acid and acetic acids (Baimark *et al.,* 2009). The phenolic components which were found in the wood vinegar might as well exhibit some antifungal property. Wood vinegars contains the phenolic compounds meanwhile acetic and formic acids does not contain phenolic compounds.(Baimark & Niamsa, 2009)

2.2.2 Antifungal and antitermitic activities of wood vinegar from *Vitexpubescens*

The biodegradation of the wood which were caused by the fungi and termites are renowned as one of the most problematic matters. (Meyer *et al.,* 2005). Chemical control has been a successful method of preventing fungal and termite attack for a long time (Preston, 2000). The controlling of parasites and termites by utilizing the manufactured pesticides are considered to have negative impact on human wellbeing and the nature because of their residual impacts (Preston *et al.,* 2000).

Wood vinegar, which is likewise called as pyroligneous acid or fluid smoke, is an item by preparing of high temperature carbonization of wood with absent of oxygen (Verma *et al.,* 2009). Wood vinegars acquired from a wide range of many sources of wood are perceived as sheltered regular inhibitors with different applications, in which they have different bioactivities such as antifungal, termiticidal, and creepy crawly repulsing (insect repelling) activity (Sharma, 2009).

During the previous study, there is a report about the effect of the mixed wood vinegar from acacia mangium wood (*Vitex pubescens*) fighting against the *Aspergillus flavus* (Imamura *et.al* 2004). *Vitex pubescens* is an animal type that attacks the meadows overwhelmed by *Imperata cylindrical* in West Kalimantan, Indonesia. The results show that the use of wood vinegar from *V. pubescens* can function as antifungal and anti-termite agents.

This outcome can be a decent preparatory sign for future application of wood vinegar that were produced by using *V. pubescens* to wood security, in spite of the fact that in the present work just two growths and two termites were utilized as test living beings (Kartal *et al.,* 2004).

2.3 Definition of Stress

Stress was defined as the state of decreasing in the terms of fitness, or any other external agents which challenge the homeostatic power of any of the organism or threatened its survival. Impact of the aquaculture related to the stressors can lead to the fish disease (Bruce, 2002).

Animal which were kept in the artificial habitats confront a wide range of potentially provocative environmental challenge which can lead to a physical or mental disruption which is called "stress". "Stress" was used in so many different contexts that have been argued by some to be essentially useless term (Bruce, 2002).

Generally, stress was defined as the experience of having intrinsic or extrinsic demands that exceed the individual's resources of responding to those demands (Bruce *et al.*, 2002). Tendency of the system to maintain the steady state was referred or known as hemeostasis and for some other authors, it was known as something that challenges the homeostasis (Bruce *et.al,* 2002).

A "stressor may be an actual physical challenge to homeostasis for example the sudden exposure to a sudden change of the environmental such as temperature, physical restraint or combat. Other than that, it can also be a threat stress cause such as direct stare from more dominant individuals, or approaching of human-with-handling-gloves (Bruce, 2002).

Chronic or long-term stress occur in prolonged elevation of the glucocorticoids (GCC's) levels that in effect becoming the self-sustaining, as the prolonged level of the GCC that circulates will damaged the area of the brain that is responsible for terminating the stress response mechanism (Jiang *et al.,* 2008). Physiologically, the chronic stress may indicated by the suppressed of the reproductive cycling (Kumar, 2008). Blunted activation by the hypothalamic pituitary-adrenal axis (HPA) response to the acute stress, suppressed immune, response, reduced in the growth hormone levels and the inhibited or retarded growth rate, and reduced body weight (Weng *et al.,* 2008).

From the behavioral view, chronic stress may be detected by the reduced of the reproductive behavior, increased in abnormal behavior, reduced exploratory behavior and increased behavioral inhibition, increased vigilance behavior and increased hiding, reduced behavioral complexity, increased aggression, increased fearfulness and frequency startle and increasing freezing behavioral and decreased latency to freeze. All of these behavioral and physiological sign is the measures that were used to define the chronic stress (Biology, 2017).

2.3.1 Environmental Stress Challenge

It is prior to the introduction of the concept of the environmental enrichment, most artificial environment usually were constructed simple and unresponsive to the animal behavior. These environmental do not allow the animal to interact with their surrounding in way which developed the sensory and cognitive behavior, or allow that display of the species-typical behavior (Morgan *et al.,* 2007). Occurring at the same time, the view of animal welfare have substantially have changed the nature of the captured environment. Scientist has developed strategies in order to improve the condition of captivities that including the way to increase the animal's behavioral opportunities.

The researchers have investigated the effect of the modification of the structure, complexity, and interactivity of the traditional and artificial environment on the behavior and health captive of the animal (Morgan, 2007). The contemporary methods for improving the artificial environmental were included, but no limited to, enhancing the biotic parameters, such as the increasing in the natural sound and improving the substrate complexity (Tromborg, 2007).

Finally, some of the instance, interactive or automated technologies have employed the increase in opportunities for animal to engage in the complex problem solving while challenged by the variation in the physical properties of their environment (Shepherdson *et al.*, 1998). Other than that, the intelligence idea of the zoological gardens attempt to preserved the species-typical behavior was introduced (Tromborg, 2007). Many of these environmental enrichment strategies were undoubtedly improving the conditions of captivities (Morgan *et al.,* 2007).

2.3.2 Fish immune and stress challenge of *Oreochromis niloticus* with bovine lactoferrins.

Number of chemical agents, polysaccharides, plant nutrient, and some nutrients was included in the fish diets as immune stimulants in order to increase the resistance to infectious disease by enhancing the immune system (Welker, 2007). This diet was promoted in the intensive aquaculture farming as a means of overcoming the immune suppressive effect of stress, even though few have published their report to support the claims (Welker, 2007). Lactoferrin (LF) is a bibolate iron-binding glycoprotein functioned as an antimicrobial component of the milk and other exocrine secretions in mammals and was also released by the activated neutrophils during inflammatory process (Lim, 2007).

Lactoferrins also functioned to increase the natural killer cytotoxicity which stimulates the production cytokines from the macrophages (Lim, 2007). Other than that, Lactoferrins also function to increase the production of macrophages, granulocytes, and neutrophils (Klesius *et al.,* 2007).

For the stress challenge method, ten fish was transferred from each aquarium into new aquaria. The fish was adjusted to twenty, then, all of the fish were injected by IP injection method with 100 µl of *Streptococcus iniae* culture containing 1.6 x 10^8 CFU/ml. The fish was returned to respective aquaria after injection. Each fish in the aquaria will be fed twice daily. The fish mortality was recorded twice a day for 14 days (Welker, 2007).

2.3.3 Use of Oxidative Stress Response as Biomarkers in Nile Tilapia.

Rivers, streams and lakes are the major source of drinking water. Pollution of these water hugely affect the environmental today. Selection of appropriate biological effects marker for the research of contaminant-effect/low-dose response relationship is usually became a controversial issue whenever the info about the mechanism of action the contaminant is not sufficient (Almeida, 2002).

However, even very low exposure to the pollutants may connect with various biological effects such as the shift in the activity of the some enzymes (Almeida *et al.,* 2002) and also change in metabolic fuels (Marques, 2002). The biochemical marker was frequently used for diagnosing and detecting sub-lethal effect in fish (Diniz, 2002).

Cadmium is widely used in the modern industry (Almeida *et al.,* 2000). It shown exerts adverse effect to the organism (Diniz *et al.,* 2002). Some of the toxics effect of cadmium in fish has been connected to each other (Ribas *et al.,* 2002). However, some of the species can maintain their growth rate under the exposure of the cadmium for a very long period due to the great variation response and resistance of metabolic development (Faine *et al.,* 2002).

2.3.4 Stress Challenge Methodology for Nile Tilapia (*Oreochromis niloticus*).

Fish seed quality was defined as the grade of excellence regarding with the survival, growth, health, productivity and marketability (Macniven, 2001). Poor quality of seed was identified as one of the obstacle for the development of freshwater fish culture in the Asia (Little *et.al,* 2001). Stress was identified as the effect that impacts the physiological systems (Macniven *et al.,*2001). These effects include the retarded growth, limited reproduction and immunity problem which cause the major issue on achieving the good seed quality. Acute stress challenge was widely used for the crustacean aquaculture as measurement of good quality (Macniven *et.al,* 2001).

There was a research conducted in order to evaluate a stress challenge on nile tilapia (*Oreochromis niloticus*). Formalin and iodize salt was used as the treatment. LC_{50}

values were estimated to be used in the test for the marketable-sized fry to saline and formalin solution. 24 ppt and 500 ppm for saline and formalin respectively for the concentration test were used as reference for the toxicants in acute, static bioassays for the challenge of the fry reared under different condition s during the nursery period (Little *et al.,* 2001).

Challenge test for the replicates was conducted for three consecutive days (MacNiven, 2001). The data was present as the $^{+}_{-}$ standard deviation (SD). Two hours-LC_{50} was calculated from eye fitted, probit line which have been drawn on a graph which show the percentage of mortality against concentration (Macniven, 2001).

The acute stress challenges are carried out minimum period of 48 hours with a preferred period of 96 hours (Little, 2001). The tolerance of Nile Tilapia towards the salinity can be increase by early exposure in order to increase the salinity at spawning or hatching (Macniven *et al.,* 2001).

2.3.5 Acute Stress Challenge in Fish

The Nile tilapia, *Oreochromis niloticus*, which originates from the upper Nile in Uganda, is generally restricted to a fresh water distribution (Breves *et al.,* 2010). Nile tilapia can routinely acclimate to saline water although some lab strains can endure full-strength (Hasegawa *et al.,* 2010).

The survival condition and activities that was used in aquaculture practices may cause stress either acute or chronic where it can reduce the involvement of their welfare, which the mainly relevant factors for the reduction of the farmed fish can be list as following: genetic, environmental factors, stocking density during growth period, malnutrition, starvation, cataracts, deformities, transport, handling, selection, and overcrowding (Yoshioka *et al.,* 2010).

Crowding stress may be one of the important factors of the fish in which the rearing density also could affect the physiology of the fish (Fox *et al.,* 2010).

Intermediate level of the density has the tendencies to increase the growth efficiency of the fish also to decrease the disease development (Takei, 2010). In order to reduce the metabolic rate of the fish, stress response, oxygen consumption, and production of the discarded product, fish starving was practiced. Starving can also be a natural behavior of the fish according to its water temperature, age, species and the season (Davis *et al.,* 2010).

CHAPTER 3

MATERIALS AND METHOD

3.1 Tilapia fish, *Oreochromis niloticus.*

The tilapia fish (*Oreochromis niloticus*) were reared in the big tank with aeration for three days for acclimatization of the fish. After three days, the tilapia fish were transferred in nine aquarium tanks with stocking density of 30 fingerlings per tanks. The fish were being observed for a several days before wood vinegar dilution were added in the tanks. Wood vinegar with concentration of 1:1000 was recorded as Treatment 1 while wood vinegar with concentration of 1:1500 was recorded as Treatment 2. The tank with normal culture water was recorded as Treatment 3 (control).

3.2 Preparation of tanks

Nine (9) tanks were used for this experiment. The tanks were prepared with 25 L of water for each of the tanks. Before adding up the water, the tanks were cleaned to prevent the contamination of water.

3.3 Preparation of wood vinegar

The wood vinegar was diluted in the aquarium water tanks with the ratio of 1: 1500 and ratio of 1: 1000. The aquarium water in the tanks volume is 25 litres thus, the use of wood vinegar for 25 litres of water were 25ml of wood vinegar for 1:1000 ratios and 16.67ml of wood vinegar for 1:1500ml respectively. Table 3.1 below showed the concentration of wood vinegar for the stress challenge.

Treatment Group	Ratio	Formula
T1	1:1500	25ml wood vinegar + 25L culture water
T2	1:1000	16.67ml wood vinegar + 25L culture water
T3	0	25L culture water

Table 3.1 Formula of wood vinegar

3.4 Stress Challenge Method

3.4.1 Observing the fish period

The tilapia fish in each of the treatment was observed for every four (4) hours until seventy-two (72) hours or three (3) days. The acute stress challenges must be carried out for the minimum period of 48 hours with a preferred period of 96 hours (EIFAC, 1983).

3.4.2 Removing the dead fish from the tank

The dead fish were removed immediately after the dead were recorded. This must be done in order to prevent the contamination of the water from the dead fish. Other than that, removing the dead fish must be done in order to prevent the infection of pathogen from the dead fish which may possibly die from the disease (Howells *et al.*, 2015).

3.4.3 Observing the fish behavioural

The fish behaviour were observed and recorded for each four hours observation been made. The fish may suffocate due to the addition of the wood vinegar as it reduces the pH of the water. The wood vinegar is slightly toxic to the fish if wood vinegar were applied excessively (Nikhom, 2010).

3.5 Feeding the fish

During this experimental period, each of the tilapia fish were fed using commercial feed 5% from their weight per day (El-Sayed *et al.,* 1992).

3.6 Water parameter

Water quality parameter such as temperature, salinity and turbidity were checked and recorded before and after addition of the wood vinegar. Tilapia fish is a warm water fish, so the ideal temperature for the water is between the ranges of 23.89℃-32.22℃. (Boyd *et al.,*1979).

3.7 Statistical analysis

The survivability of the fish stress challenged with wood vinegar were determined by using One-way ANOVA and followed by using the post-Duncan multiple range test at $p<0.05$.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Fish behavioural and stress level

Treatment Group	Fish behavioural after addition of wood vinegar
Treatment 1	Fish swimming uncomfortably, normal anxiety
Treatment 2	Fish gasping for air and swimming uncomfortably
Treatment 3	Normal behaviour

Table 4.1 Behaviour of the fish after addition of wood vinegar

The concentrations of wood vinegar used for the treatment are 1:1500 and 1:1000. The different concentration of the wood vinegar was used in order to determine suitability of the wood vinegar in aquaculture species. From the result, the fish shows different reaction towards the concentration applied. From the observation for every four hours, the fish with higher concentration which is Treatment 2 with the concentration of wood vinegar 1:1000 showed an extreme anxiety as they gasped for the air and swimming uncomfortably while the other wood vinegar concentration which is 1:1500 that labelled as Treatment 1 showed normal anxiety level because they just swimming uncomfortably just in the early duration of the observation period. The result showed that use of high concentration of wood vinegar does not suitable for the fish as it caused high mortality level of the fish if this situation continues. It is because that the wood vinegar was toxic to the fish if applied excessively (Thailand Department of Agriculture, 2010).

4.2 pH of the fish culturing water

Treatment group	pH level
Treatment 1	5.5
Treatment 2	4.5
Treatment 3	6.8

Table 4.2 pH level of fish culturing water

During the observation period, no mortality was recorded for the Treatment 1 which 1:1500 concentration of wood vinegar were added in the fish culturing water. The average pH recorded for the fish culturing water after addition of wood vinegar was 5.5. There was no mortality recorded for this treatment. The pH of this treatment dropped after the addition of wood vinegar as the wood vinegar was an acidic type.

The fish mortality level was higher in the concentration of wood vinegar of 1:1000 (Treatment 2). The high concentration of wood vinegar changes the pH into acidic form (de Guzman, 2009) which was not suitable for the fresh water fish such as tilapia. This intact with the research about effect of the pH on tilapia fish growth in which the results from the research found that pH 7 was the ideal pH for the growth of fish and the acidic pH will considered as lethal for the fish (Yonas *et al.,* 2006). The pH recorded for Treatment 2 was 4.5. From the data obtained, more than 37% mortality recorded for the tilapia fish that were cultured in the fish culturing water that contained 1:1000 (Treatment 2) concentration of wood vinegar. The results obtained from this experiment showed that the higher concentration level of the wood vinegar will cause lethal effect to the fish which is in line with the expected outcome of this experiment.

For the control fish (Treatment 3), no mortalities recorded on the three replicates tank. The pH value for the culturing water for Treatment 3 is 6.8. pH value was different with Treatment 1 and Treatment 2 because no wood vinegar were added in this Treatment. There was no mortality recorded for Treatment 3.

4.3 Survivability of the fish

Time	0	4	8	12	16	20	24	28	32	36	40	44	48	52	56	60	64	68	72
Treatment 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Treatment 2	0	0	0	0	3	3	0	0	2	0	0	1	1	1	1	1	0	0	0
Treatment 3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 4.3 Mortality of Nile Tilapia, *Oreochromis niloticus*.

Previous study focused on the use of bamboo vinegar as alternatives for antibiotics for the growth performance of the fattening pigs. The research found that use of bamboo vinegar increase the average daily gain (ADG) and the feed efficiency the same as using antibiotics. Bamboo vinegar has increase the growth performance and the physical condition of the pig compared to the pigs that used antibiotics (Chu, 2012).

This study focused more on the effect of the wood vinegar on the survivability of the Nile Tilapia, *Oreochromis niloticus.* The average mortality for Treatment 2 is 37.78%. However, in the Treatment 1 and Treatment 3, all the fish in the tanks survive. It shows us that the use of wood vinegar may be applicable for aquaculture species but the concentration of wood vinegar used may varies depends on the species pH tolerance. (Nikhom, 2010) stated that, the excessive use of wood vinegar was highly toxic to the plant and slightly toxic to the fish. This may be the cause of the high mortality recorded which is over 37% in Treatment 2. The amount of wood vinegar that applied in the Treatment 2 may excessive so that it were toxic to the fish which were then cause mortality to occur.

The Treatment 1 with concentration of wood vinegar 1:1500 does not have mortality even though the addition of wood vinegar reduces the pH level of the fish culturing water. This indicates that the concentration of wood vinegar 1:1500 may not be excessive for the fish culturing water. From result obtained, the results were significance after analysed using the One-way ANOVA followed by post-hoc Duncan.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

The result obtained from this research show important information about the application of wood vinegar in fresh water fish. This may help further research about the effect of application of wood vinegar in the fish culturing water. Other than that, this research also proved that, wood vinegar can be apply to aquaculture. However, suitability of wood vinegar depends on the species pH tolerance because the wood vinegar will decrease the pH of water after application ranging from 3-5.

As a conclusion from this experiment, the concentration of wood vinegar of 1:1500 is better than 1:1000 as there is no mortality recorded from that concentration during 72 hours of observation. The results from this research were also significance. Treatment 2 with the concentration of wood vinegar 1:1000 shows extreme anxiety level as they gasping for the air while the Treatment 1 with concentration of wood vinegar 1:1500 just show normal anxiety level as they show uneasy traits just in the early periods of observation. From the result obtained from this research, Treatment 1 and Treatment 3 are better compared to Treatment 2. Thus, the H_0 is rejected and vice versa.

5.2 RECOMMENDATION

The research about the stress challenge of tilapia fish (*Oreochromis niloticus*) by application of the wood vinegar showed good results. This research was important information about the use of wood vinegar for aquaculture. The effect of wood vinegar on aquaculture species can be study further. The example of research that related to the use of wood vinegar in aquaculture is the effect of wood vinegar on the growth performances, and also the effect of spraying the wood vinegar on the commercial pallets. If further research conducted, the effect and benefits of the use of wood vinegar in aquaculture will be revealed and thus may help in the development of aquaculture industry in Malaysia.

REFERENCES

Abdel-Fattah, M., El-Sayed, & Shin-Ichi Teshima. (1992). Protein and energy requirement of Nile Tilapia, (*Oreochromis niloticus*). 103, 55-63.

Adams, S. M. (ed.) 1990. Biological indicators of stress in fish. American Fisheries Society Symposium Series 8, Bethesda, Maryland.

Almeida, J. A., Diniz, Y. S., Marques, S. F. G., Faine, L. A., & Ribas, B. O. (2002). The use of the oxidative stress responses as biomarkers in Nile tilapia (*Oreochromis niloticus*) exposed to in vivo cadmium contamination, *27*, 673–679.

Azaza, M. S. Ã., Dhraı, M. N., & Kraı, M. M. (2008). Effects of water temperature on growth and sex ratio of juvenile Nile Tilapia *Oreochromis niloticus* (Linnaeus) reared in geothermal waters in southern Tunisia, *33*, 98–105.

Bailey, R.G. (1994). Guide to the fishes of the River Nile in the Republic of the Sudan. J. Nat. Hist. 28:937-970

Baimark, Y., & Niamsa, N. (2009). Study on wood vinegars for use as coagulating and antifungal agents on the production of natural rubber sheets. Biomass and Bioenergy, *33*(6–7), 994–998. https://doi.org/10.1016/j.biombioe.2009.04.001

Baroiller, J. F., Chourrout, D., Fostier, A., & Jalabert, B. (1995). Temperature and sex chromosomes govern sex ratios of the mouth brooding cichlid Fish *Oreochromis niloticus*, *223*.

Biology, C. (2017). Stress in fishes : A diversity of responses with particular reference to changes in, *525*, 517–525.

Boyd, C.E. (1979). Water quality in warm water fish ponds. Agriculture Experiment Station, Auburn, Alabama. 358 pp.

Breves, J. P., Hasegawa, S., Yoshioka, M., Fox, B. K., Davis, L. K., Lerner, D. T., &Grau, E. G. (2010). General and comparative endocrinology acute salinity challenges in Mozambique and Nile Tilapia : Differential responses of plasma prolactin , growth hormone and branchial expression of ion transporters. General and comparative endocrinology, *167*(1), 135–142.

Bruce, Department of Biology and Missouri River institute & University of South Dakota (2002). Stress in fishes: A diversity of responses with particular reference to changes in circulating corticosteroids, 42:517-525.

Gyo Mun Chu, Cheol Kyu Jung, Hoi Yun Kim, Ji Hee Ha, Jong Hyun Kim, Min Seob Jung, Shin Ja Lee, Yuno Song, Rashid Ismael Hag Ibrahim, Jae Hyon Cho, Sung Sill Lee & Young Min Song (2012). Effects of bamboo charcoal and bamboo vinegar as alternatives on growth performance, immune responses and fecal microflora population in fattening pigs. 144, 240-246.

de Guzman, C.B. (2009). “Exploring the beneficial uses of wood vinegar”. Retrieved on September 2010 from Republic of Philippines Department of Agriculture, Bureau Agriculture Research website: http://www.bar.gov.ph/news/woodvinegar.asp

FAO, 2003. Etat de l’Aquaculture dans le monde. Circulaire sur les peˆches no. 886, re´ v. 2, 114pp.

Fessehaye, Yonas, El-Bialy, Zizy, Rezk, Mahmoud, A., Croojimans, Richard, Bovenhuis, Henk, Komen & Hans (2006). “Mating systems and male reproductive success in Nile Tilapia (*Oreochromis niloticus*) in breeding hapas: A microsatellite analysis”. Aquaculture, 256 (1-4): 148-158.

Froese, Rainer, Pauly & Daniel (2015). “*Oreochromis niloticus*”. Retrieve on November 2015 from fish base website: http://fishbase.org/manual/english/contents.htm

George A. & Burdock. (2010), "Pyroligneous acid", Fenaroli's Handbook of Flavor Ingredients (6th ed.), Taylor & Francis, pp. 1774–1775.

Jiang, I., Kumar, V. B., Lee, D., & Weng, C. (2008). Fish & shellfish immunology acute osmotic stress affects Tilapia (*Oreochromis mossambicus*) innate immune responses. *Fish and shellfish immunology*, *25*(6), 841–846.

Kartal, S.N., Imamura, Y., Tsuchiya, F., & Ohsato, K. Evaluation of fungicidal and termiticidal activitiesof hydrolysates from biomass slurry fuel production from wood. Bioresource technology 2004;95:41-7

Likongwe, J. S., Stecko, T. D., Stauffer, J. R., & Carline, R. F. (1996). Combined effects of water temperature and salinity on growth and feed utilization of juvenile Nile, *146*, 37–46.

Macniven, A. M., & Little, D. C. (2001). Development and evaluation of a stress challenge testing methodology for assessment of Nile tilapia (*Oreochromis niloticus , Linn*) fry quality.

Meyer, J.R. (2005). Isoptera.Department of Entomology, NC State University.

Morgan, K. N., & Tromborg, C. T. (2007). Sources of stress in captivity §, 102, 262–302.

Nakayama, F.S., Vinyard, S.H., Chow, P., Bajwa, D.S., Youngquist, J.A., Muehl, J.H.et al. (2001). Guayule as a wood preservative. Industrial Crops and Products;14:105-11.

Nikhom, Laemsak, Faculty of Forestry Kasetsart University. (2010). "Wood vinegar". Retrieved on September 2010 from author stream website: http://www.authorstream.com/Presentation/Cannes-50452-Wood-vinegar Background-Product-Carbonization-Cont-Recover-Pyroligneous-Liquor-Collector Procedure-Im-as-Education-ppt-powerpoint/.

Preston, A.F. (2000). Wood preservation: trends of today that will influence the industry tomorrow. Forest Production Journal, 50:13-19.

Trewavas, E. (1983). Tilapiine fishes of the genera *Sarotherodon, Oreochromis* and *Danakilia* British Mus. Nat. Hist., London, UK.583 p.

Verma, M., Sharma, S., Prasad, R. (2009). Biological alternatives for termite control: a review. International Biodeterior Biodegrad, 63:1-14.

Welker, T. L., Lim, C., Yildirim-aksoy, M., & Klesius, P. H. (2007). Growth , immune function , and disease and stress resistance of juvenile Nile tilapia (*Oreochromis niloticus*) fed graded levels of bovine lactoferrin, *262*, 156–162.

APPENDIX

Table A.1: One-way ANOVA

mortality

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	13.520	2	6.760	8.129	.000
Within Groups	139.719	168	.832		
Total	153.240	170			

Table A.2: Post Hoc Analysis using Duncan Multiple test for Total Phenolic Content

Duncan[a]

concentration	N	Subset for alpha = 0.05	
		1	2
1.00	57	.000	
3.00	57	.000	
2.00	57		.596
Sig.		1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 57.000.