EFFECT OF PIRIMIPHOS-METHYL ON ESTERASE ACTIVITIES IN *OREOCHROMIS NILOTICUS* IN MOROGORO, TANZANIA

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A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN PUBLIC HEALTH AND FOOD SAFETY OF SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA

2014

ABSTRACT

This study was conducted to investigate the effect of Actellic Super (pirimiphos-methyl) in *Oreochromis niloticus (Nile tilapia)* in Mvomero District, Morogoro, Tanzania. Field surveys using questionnaire interviews were used to collect sociological data from households that own fish ponds. The response of Acetylcholinesterase (AChE) and Pseudocholinesterase (BuChE/PChE) activities in *O. niloticus* as a result of Organophosphates (OPs) and carbaryl water borne exposure was determined in plasma and brain samples of fish using Ellman's method and 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) chromophore. . The *in vivo* dose-effect relationships were assessed using pirimiphos-methyl at 0.04, 0.08 and 0.16 µM. The majority, 56% (n=25) of respondents with the age above 51 years practiced fish farming. Males 64.0% (n=25) were more than females. Sixty eight percent of all respondents (n=25) had primary education. *Oreochromis niloticus* was the most kept fish species because of its good and lucrative market. Moreover, 24 percent (n=25) of respondents used pirimiphos-methyl. Concentrations that inhibited 50% (IC_{50}) of AChE activities in brain in *in vitro* exposures were 0.004, 0.005 and 1.307 mM and in BuChE exposure were 0.743, 0.007 and 0.031 mM for carbaryl, pirimiphos-methyl, and profenofos respectively. Also, concentrations that inhibited 50% (IC_{50}) of AChE and BuChE activities in plasma in *in vitro* exposure were 1.801, 0.031 and 0.630 mM for carbaryl, profenofos and pirimiphos-methyl and 0.045, 0.153 and 0.091 mM for carbaryl, pirimiphos-methyl and profenofos respectively. Following *in-vivo* exposure of fish to pirimiphos-methyl at concentrations of 0.16µM a significant inhibition of AChE (53%), PChE (90%) activities in plasma and AChE (50%), PChE (75%) in brain was observed respectively. The investigation from this work revealed that inhibition of AChE and BuChE activity in *O. niloticus* is a useful biomarker for assessing aquatic environmental pollutants by anticholinesterases.

DECLARATION

I, Boniface Erick Kilanga do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work and has neither been submitted nor being concurrently submitted for a degree award in any other University.

Signed:…… Date:……

Boniface Erick Kilanga

(MSc. Public Health and Food Safety candidate)

The above declaration is confirmed by

Signed:……... Date…….....

Prof. R.H. Mdegela

(Supervisor)

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ACKNOWLEDGEMENTS

I am grateful to Almighty God, the most Merciful, the Creator of Heaven and the Universe, for giving me the gift of life, strength and mostly, the chance to study. I am also grateful to my wife Dativa Ignas Lyimo, who was tolerant and inspirational throughout the course of my study. Also, I am grateful to my father Erick D. Kilanga and my mother Foebe Paul for their help and for their good upbringing. .

I am deeply indebted to my supervisor, Prof. Mdegela, R.H. for his tireless supervision, constructive advice throughout the study and for the sponsorship he provided to carry out the experimental studies and analysis of samples in the Ecotoxicology Laboratory. In fact, I appreciate much, and I feel honored to work with him. I am very grateful to my Lecturers at SUA, Prof. Mlangwa, J.E.D ., Prof. Mellau, L.S.B., Prof. Kazwala, R.R., Prof. Kimera, S.I., Prof. Madundo, M.A., Dr. Karimuribo, E.D., Dr. Ngowi, H.A., Dr. Nonga, H.E., Dr. Kasanga, C.J., and Dr. Kayunze, K. for their lectures during coursework, which built a foundation to this work and successful completion of my studies. Also I am so grateful for the assistance I got from laboratory staff in the Faculty of Veterinary Medicine at SUA, Mr. Ramadhani, A. and Mr. Jingu. P; they were very helpful and available whenever I needed their assistance.

Similarly, I value and acknowledge the moral and material support that I got from my fellow MSc. Public Health and Food Safety and other MSc. Degree programs students of 2010/2011 and 2011/2012intakes particularly Mr. Nyambega, M., Dr. Muse, A., Dr. Komba, L., Mr. Mwalukasa, M, and Dr. Barnabas, E. Despite the fact that it is difficult to mention all, I articulate my appreciation to all individuals who assisted me in one way or another to carry out this study. May God bless you all.

DEDICATION

I would like to dedicate this work to the Almighty God for giving me good health, and ability to study from the beginning up to this level of accomplishing thesis. Also the work is dedicated to my beloved parents: Mr. Erick Damson Kilanga and Mrs. Foebe Kilanga for building a strong background and their good upbringing that have enabled me to achieve this success.

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ABBREVIATIONS AND SYMBOLS

%:	percent
µg:	microgram
AChE:	Acetylcholinesterase
BL:	Blank
CNS:	Central Nervous System
FAO:	Food and Agriculture Organization
GAP:	Good Agricultural Practices
HD:	High dose
HuBChE:	Human Serum BChE
LD:	Low dose
MD:	Medium dose
mg:	milligram
Op:	Organophosphates
PChE:	Pseudocholinesterase
RBC:	Red blood cells
USA:	United States of America
WHO:	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

The fisheries industry is among the important economic sub sectors of the economy in Tanzania. The sector provides employment, income, foreign earnings and revenue to the country. In 2009 the fisheries sector contributed 1.3% to GDP, and the per capita fish consumption was 8.0 kilogram and about 30% of animal protein consumption (Ministry of Livestock Development and Fisheries, 2010). In the country, private subsistence fish ponds dominate freshwater fish culture systems and many new ponds continue to be constructed each year. This is because fish ponds provide many important and practical benefits such as erosion control, fire control, livestock watering, irrigation, swimming, picnicking, wildlife enhancement, animal protein and income to households (Quarainic, 2007).

Contamination of running water directly or from run-off during spraying operations of pesticides can occur to the fish ponds during their routinely employment in integrated farming practices to protect crops and animals from insects, weeds and diseases. The use of pesticides at different stages of crop production, starting from seed processing to storage of agricultural produce may cause risks to aquatic environment (Singh *et al.*, 2010). Nearly three million cases of pesticides poisoning occur annually (*ibid*). Basically, contamination by pesticides is an important public health problem, mainly in developing countries. It is estimated that only 0.1% of the applied pesticides reach the target pests, while the rest spreads throughout the environment (Hart and Pimentel, 2002).

Additionally, pesticides can be grouped into; organochlorines, organophosphates, carbamate, synthetic pyrethroids and miscellenius compounds which are chemical families. Organophosphates are the most widely used worldwide (Chambers *et al*., 2002). The most commonly used organophosphates in Morogoro Tanzania include pirimiphosphosmethyl, diazinon, chlorfenvinphos, diamethoate, fenitrothion and profenofos (Mdegela *et al*., 2010). Among the different classes of pesticides, organophosphates are the most frequently used, because of their high insecticidal property, low mammalian toxicity and less persistence and rapid environmental biodegrability. The majority of pesticides in particular organophosphates like pirimiphos-methyl, carbamates, and some synthetic pyrethroids that are currently in use, produce toxicity by inhibiting the cholinesterase enzymes in the nervous system which is responsible for hydrolysis of the neurotransmitter, acetylcholine, into choline and acetic acid (Walker, 2001).

1.2 Problem Statement and Justification

In Morogoro region, fishes are consumed by large population as a major source of protein due to their easy availability. Fish farmers in this area use mostly maize bran to feed fish in their ponds. The maize used are preserved mostly by Actellic super (Pirimiphos-methyl at 16g/kg and permethrin at 3g/kg) pesticides. In addition, farmers alongside ponds use pesticides including fungicides, insecticides, rodenticides in the group of carbamates, organophosphate, organochlorides, and miscellaneous compounds to control pests attack in vegetables. Also, fish farmers use vegetables likely to be contaminated by pesticides to feed fish in their ponds. Environmental pesticide residues are also drained into the ponds during the rainy

season. Apparently, there is no any scientific data regarding contamination of ponds by pirimiphos-methyl in the fish pond farms in Mvomero District. For this case, the absence of such information bolded the need of present study, i.e. to study the effect of pirimiphos-methyl used to store maize for human consumption and fish bran. Equally, the study went further to assess the effect of profenofos used to preserve vegetables in Mvomero District. The results obtained from this study are expected to fill the gaps in knowledge regarding food safety and aquatic environmental contamination resulting from the use of pirimiphos-methyl in storing of maize. Similarly, the results have generated data for working out the safety management strategies in using these pesticides and reducing their public health risks.

1.3 Objectives

1.3.1 Overall Objective

The overall objective of the study was to investigate the effect of pirimiphos-methyl, profenofos and carbaryl in fish kept in ponds in Mvomero District in a way of devising mitigation measures related to risks associated with pesticide exposures to fish.

1.3.2 Specific Objectives

The specific objectives of the study were:

1. To assess the knowledge, attitude and practices related to contamination of fish ponds with pesticides particularly with an emphasis on pirimiphos-methyl and permethrin.
2. To determine the *in vitro* IC_{50} and activities of AChE and PChE in plasma and

brain of *O. niloticus* exposed to pirimiphos-methyl, profenofos and carbaryl.

3. To assess the *in vivo* dose-effect relationships using different concentrations of Pirimiphos-methyl in fish under experimental conditions.

1. 4 Research Hypothesis

H_0: There is no significant difference in people's knowledge and attitude related to contamination of fish ponds with pesticides (pirimiphos-methyl).

H_a: There is significant difference in people's knowledge and attitude related to contamination of fish ponds with pesticides (pirimiphos-methyl).

H_0: There is no significant effect of pirimiphos-methyl, profenofos and carbaryl on the IC_{50} and activities of AChE and PChE in plasma and brain of *O. niloticus.*

H_a: There is significant effect of pirimiphos-methyl, profenofos and carbaryl on the activity of AChE and PChE in plasma and brain of *O. niloticus*.

H_0: There are no significant effects of pirimiphos-methyl, profenofos and carbaryl on fish behaviour.

H_a: There is a significant effect of pirimiphos-methyl, profenofos and carbaryl on fish behaviour.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Fish Farming

Fish farming refers to the process of raising fish in ponds, tanks, net enclosures, cages, or raceways (Murnyak, 2010). It is one of the economic activities in many parts of the world, and it is considered a good source of protein. It has great potential to produce high quality protein in relatively shorter periods and in small areas. Fish farming is one of resource that poor farmers throughout the world can provide protein that is often lacking in the family diet and too expensive to purchase (Murnyak, 2010). In addition, fish farming can generate high interest and excitement. Generally, fish farming is a historical activity where people have raised fish for thousands of years. In some areas, the farmers are experienced and the techniques are quite developed, though in others they are just starting.

In the past 30 years, there has been a dramatic increase in fish farm production especially in Asian countries (Fitzsimmons and Naim, 2010). This increase is partly because of the depletion of the natural fish stock and harvests from freshwater and ocean fisheries. Next, is because of the increased promotion of fish farming as a means for local communities to improve their nutrition and economic opportunities. Although the supply of fish from the wild is decreasing, the demand continues to increase. Fish farming is trying to meet the deficiency in worldwide fish supply.

Tilapia is a commonly raised fish throughout the world, second only to Carp. According to Fitzsimmons and Naim,(2010) in 2009 more than 3 million metric tons of tilapia was raised. Tilapia thrives in warm tropical areas. It is a good fish for

resource for poor farmers to grow because tilapia are easy to raise, fast growing and tasty, able to eat many types of foods low on the food chain, highly disease resistant, able to reproduce easily, hardy and can tolerate poor water quality conditions (*ibid*). In Tanzania, Nile tilapia culture is a promising aquaculture enterprise. There are over 100 different species of tilapia, each with unique characteristics, behavior, and suitability to fish farming (*ibid*). A few of the most commonly farmed are the Nile tilapia (*O.niloticus*), blue tilapia (*O. aureus*), Mozambique tilapia *(O. mossambicus*), red belly tilapia (*Tilapia zillii*), and the red breasted tilapia (*T. rendalli*) (Balarin, 1979).

2.2 Pesticides in Fish Farming

Usually the goal of raising fish is to grow the fish as fast and economically as possible to a harvestable size. Therefore, farmers use various options/factors to manipulate and influence growth the rate of fish. These include manipulation of pond environment, type and density of fish, food, fertilizer, water quality, and growth period (Murnyak, 2010). With these manipulations, farmers use food material that contains pesticides such as pirimiphos-methyl in maize grains and profenofos in vegetables. Pirimiphos-methyl is a broad-spectrum organophosphorus insecticide and acaricide, with both contact and fumigant action. In plants, it penetrates leaf tissue and exhibits translaminar action, but is of short persistence. When applied to stored agricultural commodities (such as grain and nuts) it provides longer-lasting pest control. It is also effective on controlling various mites on vegetables and fruits (FAO, 2004). On the other hand, Profenofos 40% EC is the cholinesterase inhibitor insecticide.

Again, pirimiphos-methyl and other organophosphate pesticides, carbamates, and some synthetic pyrethroids that are at present in use produce toxicity by inhibiting the cholinesterase enzymes in the nervous system. Cholinesterase enzymes are responsible for hydrolysis of the neurotransmitter, acetylcholine, into choline and acetic acid (Walker, 2001). As well, the enzyme controls the flow of ionic currents at the synapses and neuromuscular junctions. Alongside with that, organophosphates are more frequently used, because of their high insecticidal property, low mammalian toxicity, less persistence and rapid biodegrability in the environment. Many OPs and carbamates degrade rapidly in the environment .As a result, levels often fall below detectable thresholds within hours.

Nevertheless, esterase inhibition following OPs and carbamate exposure can persist for days or weeks (Fulton and Key, 2001). Aquatic organisms demonstrate a broad range of AChE inhibitory responses to OPs and carbamate pesticides. Such responses may vary, depending on the type of pesticide, exposure time, dose and route, water quality and species of fish (Coppage and Mathews, 1974; Sancho *et al.*, 1998). The primary and most known target for the action of organophosphorus and carbamate compounds is a family of enzymes (Cholinesterases; ChEs) formed by acetylcholinesterase and butyrylcholinesterase. Cholinesterases are enzymes that hydrolyze the acetylcholine released at central and peripheral sites. There are two types of cholinesterase: AChE, or true cholinesterase, and PChE. On one hand, cholinesterases are synthesized in hematopoiesis, occurring in the brain, endplate of skeletal muscle, erythrocyte membrane, and its main function is to regulate neuronal communication by hydrolyzing the ubiquitous neurotransmitter acetylcholine in

synaptic cleft (Quinn, 1987; Silman and Sussman, 2005). On the other hand, Acetylcholinesterase is found in large amounts in red cells and neurons.

Pseudocholinesterase, also known as butrylcholinesterase (BuChE) or plasma cholinesterase is primarily synthesized in liver and is present in plasma, smooth muscle, pancreas, adipocytes, skin, brain and heart (Çokugras, 2003). Organophosphate and carbamate pesticides can inhibit both enzymes. Although its physiological function is not well defined, BuChE is pointed out as one of the main detoxifying enzymes able to hydrolyze or scavenge a broad range of xenobiotic compounds like cocaine, heroine, anaesthetics, and pesticides (Soreq and Zakut, 1990; Çokugras, 2003; Nicolet *et al*., 2003). For instance, some studies hypothesize that one of the functions of BChE is to protect AChE against anticholinesterasic agents (Whitaker, 1980; Whitaker, 1986). The difference between the two type of cholinesterase is substrates, that acetylcholinesterase hydrolyses acetylcholine more quickly and pseudocholinesterase hydrolyses butyrylcholine more quickly (Wang and Tang, 2005).

2.3 Development of Toxicity

Organophosphates such as pirimiphos-methyl and carbamate pesticides inhibit both AChE and PChE activities. The role of AChE is to hydrolyse the neurotransmitter acetylcholine into choline and acetate. Butyrylcholinesterase (also known as pseudocholinesterase, plasma cholinesterase BCHE, or BuChE) is a non-specific cholinesterase enzyme that hydrolyses many different choline esters. In humans, it is found primarily in the liver and is encoded by the BCHE. It is very similar to the

neuronal acetylcholinesterase, which is also known as RBC or erythrocyte cholinesterase, The term "serum cholinesterase" is generally used in reference to a clinical test that reflects levels of both of these enzymes in the blood. Butyrylcholine is a synthetic compound and does not occur in the body naturally. It is used as a tool to distinguish between acetyl- and butyrylcholinesterase. BuChE can be lowered and inhibited by these pesticides . Acetyl cholinesterase can be inactivated as the pesticide binds to the active site of the enzyme and inhibit its function. The inhibition of these enzymes cause accumulation of acetylcholine (Ach) in the synapse resulting in increased stimulation of the post synaptic neuron and cholinergic overstimulation (Follansbee and Durkin, 2004). Generally, serum cholinesterase is used in reference to a clinical test that reflects levels of these enzymes in the blood. Pseudocholinesterase is lowered by organophosphate pesticide, the depression of plasma enzyme basically persists several days to few weeks, but RBC enzyme may not reach its minimum for several days and usually remains depressed for longer time, sometimes 1-3 months until new enzyme replaces the inactivated one (Reigart, 2009).

2.4 Signs and Symptoms of Poisoning with Organophosphorus Compounds in Fish and Human beings

Clinical signs of toxicosis observed in the behavior of fish exposed to pirirniphos-methy1 before the eventual death includes lack of balance, erratic swimming and restlessness. Other clinical signs according to Sandahl *et al*. (2005) included reduced spontaneous swimming rate, low swimming rate during feeding, latency to first strike, and total food strikes. Murthy *et al*. (2013) reported clinical signs in exposed

fish that included increased stress, reduced swimming ability, which in turn can reduce the ability to feed and the interrupt schooling behavior. On the other hand, symptoms in human beings include excessive salivation, sweating, rhinorrhea and tearing, muscle twitching, weakness, lack of coordination, headache, dizziness, nausea, vomiting, abdominal cramps, diarrhea, respiratory depression, tightness in chest, wheezing, productive cough, and fluid in lungs, pin-point pupils, sometimes with blurred or dark vision. In addition, there are severe cases which include seizures, incontinence, respiratory depression and loss of consciousness. Pseudocholinesterase deficiency results in delayed metabolism of only a few compounds of clinical significance including succinylcholine, mivacurium, procaine and cocaine (Maiorana *et al*., 2003). The higher levels of these compounds especially succinylcholine molecules reaching receptors in the neuromuscular junction causing duration of paralytic effect to continue for as long as eight hours.

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The accumulation of acetylcholine in the synapse has been grouped into four groups. At sufficient dosage, there is loss of enzyme function allowing the accumulation of Ach peripherally at cholinergic neuro-effector junctions (muscarinic effects), skeletal nerve-muscle junctions and autonomic ganglia (nicotinic effects). At cholinergic nerve junctions with smooth muscle and gland cells high concentration of Ach causes muscle contractions respectively. At skeletal muscle junction, high concentration of Ach causes excitatory (muscle twitching), but may also weaken or paralyses the cell by depolarizing the end-plate. In the CNS, high Ach concentration causes sensory and behavioral disturbances in coordination depressed motor function and respiratory depression. The increased pulmonary secretions coupled with respiratory failure are the usual causes of death.

In mammalian, inhibition of these enzymes produces a variety of systemic effects including:salivation, urination, lacrimation, convulsions, increased bronchial secretions, respiratory depression and even death (Follansbee and Durkin, 2004). The mechanism of action of organophosphate and carbamate differ in such a way that the action of carbamate is shorter in duration and milder in intensity than that of organophosphate.

2.5 Management of Organophosphate Poisoning in Animals and Human beings

The major diagnostic tools and measures of exposure to organophosphate insecticides is the determination of cholinesterase activity in various tissues, most often red blood cells and plasma. Inhibition of RBC, AChE is generally regarded as a more clinically significant index of organophosphate exposure, compared with inhibition of plasma PChE, as erythrocyte AchE (Follansbee and Durkin, 2004). Detection of intact organophosphates in the blood is usually not possible except during or soon after absorption of a substantial amount. Generally, organophosphates are hydrolyzed in the blood for few minutes or hours, unless the quantity absorbed is or the hydrolyzing liver enzymes are inhibited. Diagnosis is usually done careful.

The current antidote such as the tropine sulfate which is combined with an oxime is used to combat the effects of the acute organophosphates poisoning. Also diazepam is sometimes administered in combination with the atropine and oximes (Jokanovic and Kosanovic, 2010). These drugs work to counteract the effects of excess acetylcholine and reactivate AChE. Therefore, atropine can be used as an antidote in conjunction with pralidoxime or other pyridinium oximes such as trimedoxime or obidoxime (Rahimi *et al.*, 2006).

Bioscavenger Enzymes are being developed as a pretreatment to sequester highly toxic organophosphates before they can reach their physiological target and prevent the toxic effects from occurring. The significant advances with cholinesterase (ChEs), specifically human serum BChE (HuBChE) have been made. HuBChE can offer a broad range of protection for nerve agents including soman, sarin and tabun. HuBChE also poses a very long retention time in the human circulation system,because it is from a human source. Consequently, it will not produce any antagonistic immunological responses. To prevent and control OP poisoning, care should be taken to prevent drainage of organophosphates especially by adjoining pastures in pond's field streams or other premises outside the treated area.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The field work was conducted in Langali and Mkindo villages located in Mgeta and Hembeti Wards in Mvomero District respectively. The district is situated between 6° 20' South and 37° 25' East. The study was carried in 11 fresh water fish farms in Langali and 14 in Mkindo villages. Some of the fish ponds were near the homes and others away from homes. The pond sizes ranged from 8 to 15m^2 and 20 to 35m^2 . This study was conducted for one year from September 2011 to September,2012

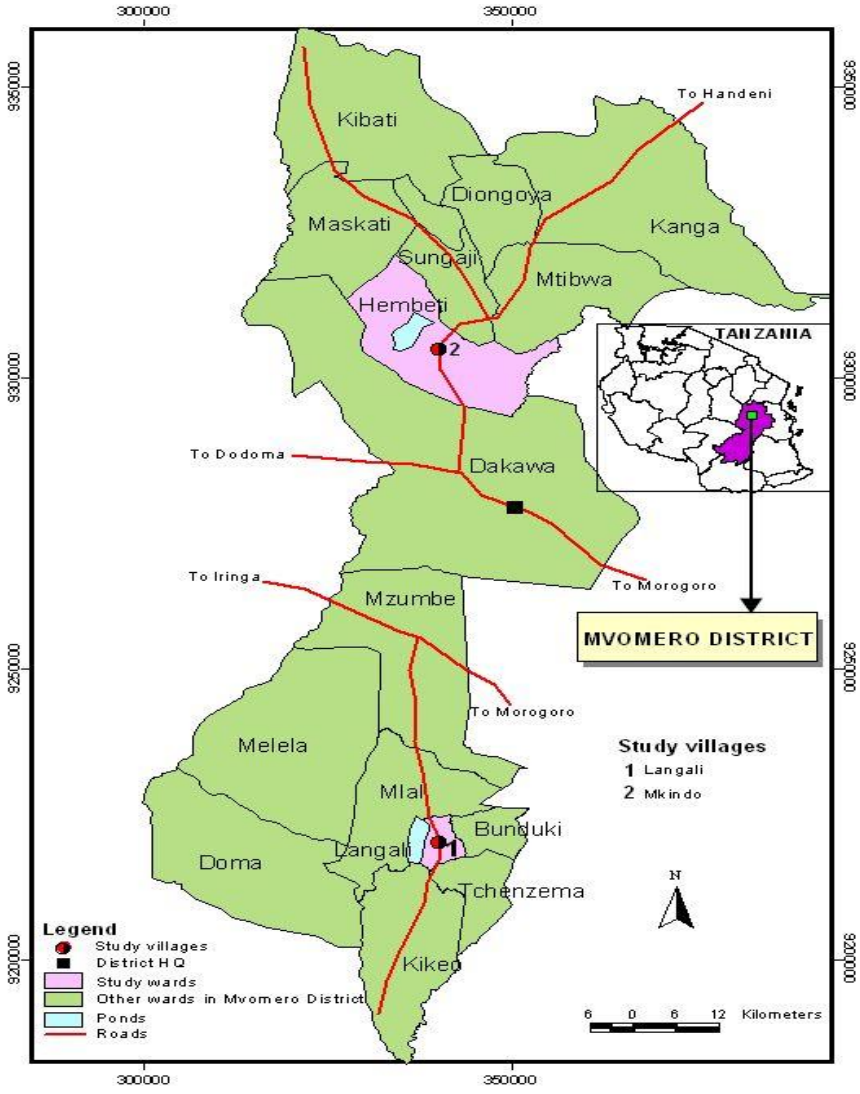

Figure 2: Map of Mvomero district showing Langali (1) village in Mgeta ward and Hembeti (2) ward where Mkindo village is found. Samples were collected in the two villages.

Source: SUA-GIS Department.

3.2 Study Population

This study targeted small-scale fish farmers in Mvomero District in Langali and Mkindo villages. Due to limited number of farmers, the study population was selected purposively.

3.3 Study Design

Cross-sectional and experimental study designs were employed in this study. The cross-sectional study design allows data to be collected at one point in time without repetition from the target population. Experimental study design allows manipulation of contributing variables.

3.4 Sampling and Sample Size Determination

A purposive sampling technique was employed in this study. Due to limited time, lack of resources and availability of fish farmers, the sample size used was 25 (100%) in the two fish farming villages. Eleven and fourteen respondents were selected from Langali and Mkindo villages respectively. The sample size was obtained as determined by Kirk and Sterne, (2003) formula. Absolute sampling error of 10% and confidence interval of 90% was used to obtain the sample size of 27 fish.

$$n = \frac{[u\sqrt{\pi(1-\pi)} + \nu\sqrt{\pi_{null}(1-\pi_{null})}]^2}{(\pi - \pi_{null})^2}$$

Where:

n = the required minimum sample size, π = the proportion of interest (0.8), π_{null} = the null hypothesis proportion (0.2), u = the one sided percentage point of the normal

distribution corresponding to 100% (With 90% power, u = 1.28), ν = percentage of the normal distribution corresponding to the required (two sided) significance level (with 5% level of significance, ν = 1.96). The sample size of experimental fish ranged from 4 to 6 per group. The limiting factors for sample size were availability, size and weight of fish, time required for exposure and termination of the experiment and duration of analysis of the samples. With such small sample sizes, it has been difficult to demonstrate statistical differences in some parameters because of low detective power attributed to sample sizes.

3.5 Data Collection and Management

3.5.1 Questionnaire Surveys

Questionnaire surveys were carried out through face-to-face interviews with one respondent from each of the selected households. The household heads were the targeted respondent (father or mother) and in case of absence, another permanently resident-adults (> 18 years) in the selected household took part in the interview. The questionnaire was designed in English and then translated into Kiswahili, a national language, which is understood by the majority of the respondents in the study area**.** The questionnaire had closed and open ended questions seeking to capture information regarding the demographic information that included fish farmer's location, age, gender, occupation and level of education. Fish farmer's knowledge on methods for preservation of maize and other cereals, preparation methods of maize bran, contamination of water with pesticides and the type of pesticides used during storage of maize was also assessed . The use of vegetables to feed fish and if vegetables were contaminated with pesticides were investigated. The questionnaire collected information on the area of the cropped land and fish farming so that to

understand if pesticides were used on the crops. Likewise, it collected information on the availability of water bodies, sources of agrochemicals, and on the use of agrochemical application devices. Furthermore, information on water bodies and on application devices, especially where they purchased and how they were collected and stored was collected. The other questions involved the person's responsible for application of pesticides and their knowledge on human and animal health hazards associated with the pesticide usage. The assessment of respondents' knowledge and attitude of using pesticides, direct observation of the chemicals was done on the day of farm visits that included observation of expirer dates, containers and presence of instructions for application and handling. Again, the information was also collected in respect to how the farmers fed fish and whether there were risks of pond contamination with pesticides.

3.5.2 Experimental Study

3.5.2.1 Materials and Reagents

Data for estrase activity were collected through *in vitro* and *in vivo* studies. *In vitro* $IC_{50,}$ measurement of AChE and BuChE activity were made in plasma and brain of tilapia fish respectively. In this experiment, pesticides used, included commercial preparation of pirimiphos-methyl (O-(2-diethylamino-6-methylpyrimidin-4-yl) O,O-dimethyl phosphorothioate), profenofos (O-4-bromo-2-chlorophenyl O-ethyl S-propylphosphorothioate), and carbaryl (1-napthyl N-methylcarbamate). All these pesticides (organophosphate and carbamates) were used as insecticides in crops. The stock solutions of pesticides were prepared by dissolving them in acetone. The chemicals used included acetylcholine iodide, Butylrylcholine, DTNB, sodium

bicarbonate and sodium phosphate (monobasic and dibasic). A Erba-Mannheim total protein determination kit was used to determine the protein concentrations of plasma and brain homogenates. All the chemicals and solvents used in total protein determination were obtained from Transasia Bio-medicals LTD, and were of analytical grade. Laboratory work was undertaken at Eco-toxicology laboratory in the department of Veterinary Medicine and public health at Sokoine University of Agriculture in Morogoro.

3.5.2.2 Management and Treatment of Experimental Fish

After being caught, fish were starved to avoid faeces which may contaminate water and cause consumption of dissolved oxygen during transportation. The fish were transported in 60 liter plastic tanks containing water with ice cube to maintaining temperature required for fish survivor to SUA, where they were acclimatized in five different glass tanks, with a capacity of 180 liters for five days without food while observing the behaviours like, opercula movement, erratic swimming, searching for food, chasing each other, swimming equilibrium and lethargy. In the glass tanks, the water was aerated with air pumps and photoperiod was 12 hours light and 12 hours dark cycle.

The dose-effect relationships in *O. niloticus* after water borne exposure to pirimiphos-methyl pesticides were determined. During challenge experiments a total of 27 fish were used and were divided in groups of 5 that were kept in separate glass tanks each with a capacity of 180 liters. The first group contained four fish and were not feed nor treated with pesticides, the second group contained five fish and fed

with maize bran as feed without being treated with pesticides, the third group containing six fish was fed with maize bran and treated with low dose of pirimiphos-methyl, the fourth and fifth groups contained six fish and were given maize bran with medium and high doses of pirimiphos-methyl respectively. The fish with size of (mean + SD; 13.54 + 1.629cm) long and with weight of (36.57 + 9.92g) were used. They were exposed to 0.04, 0.08 and 0.16µM concentration of pirimiphos-methyl in the range finding test. Exposure doses were at concentrations of environmental relevance and were estimated from the IC_{50} results. Waterborne exposure was for 24 hours with 12 hours natural light and 12 hours natural darkness.

3.5.2.3 Collection, Preparation and Storage of Samples

During sampling the fishes were restrained manually; blood was collected from the heart using a 2cc, 23G needle syringe and about 1ml of blood was collected and immediately transferred into EDTA vacutainer tubes. The total length, standard length and weight were recorded. The collected blood was centrifuged (Hettich) for 15 min, 3000XG, at 4ºC and the plasma was separated and frozen at -21ºC, until the analysis for AChE and BuChE activities. After the collection of blood, the fish was sacrificed by decapitation and pithing, and the head was immediately frozen at -21ºC overnight. After overnight storage of fish heads, the whole brain were removed, weighed and immediately homogenized manually, using a Potter-Elvehjem homogenizer, in ice-cold 0.1M phosphate buffer pH 8.0 (1:5 w:v). The aliquots of homogenates were stored at -21ºC until analysis.

Plate 1: Photograph of *O.niloticus* used in the study.

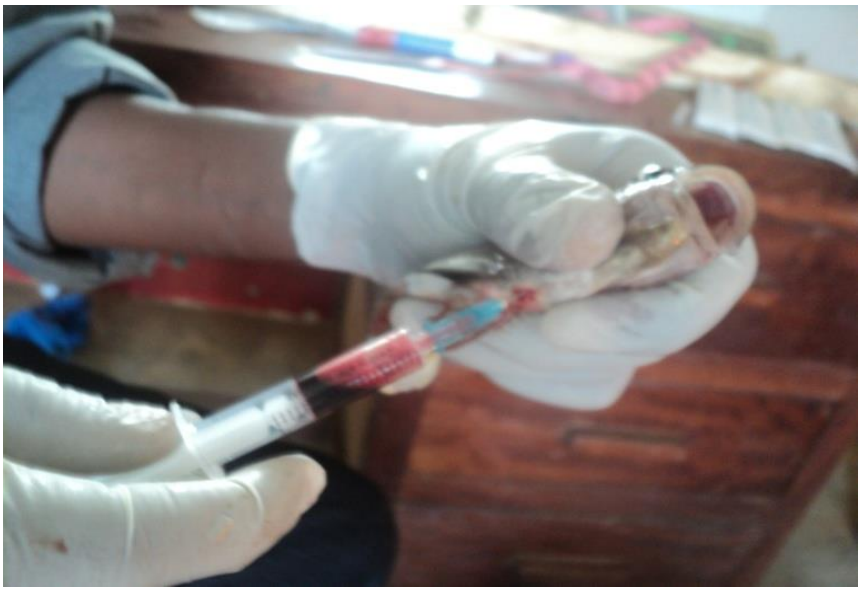

Plate 2: Photograph of blood being taken from *O. niloticus.*

3.5.2.4 Protein Analysis of Plasma and Brain Homogenates

The protein concentration for plasma and brain homogenates was determined using a total protein concentration determination kit (Erba Mannheim) manufactured by Transasia Bio-Medicals LTD. Three ml of phosphate buffer were added into the cuvette followed by a chromogenic agent DTNB sample and then pesticides. The measurement of enzyme activity was initiated by the addition of substrates. The absorbance of the DTNB from the reaction was recorded at 412 nm for three minutes at the intervals of 5 seconds at room temperature using a spectrophotometer. Spontaneous substrate hydrolysis was assessed using a blank without substrate. The kinetic was calculated in the linear range using Lambert's Beer law.

3.5.2.5 *In-vitro* Determination of IC_{50}

The IC_{50} for pirimiphos-methyl, profenofos and cabaryl pesticides were determined in plasma and brain samples from *O. niloticus*. Measurements of AChE and BuChE activity was done using a spectrophotometric methods described by Ellman *et al.* (1961). The stock solutions of all pesticides were prepared at a concentration of 1M in acetone and were diluted by 0.1M phosphate buffer, pH 8.0, to the desired experimental concentrations. Homogenates of brain samples (25 µL) from individual fish were incubated in duplicate with 3000 µL Na-phosphate buffer (0.1 M, pH 8.0) containing the pesticides at different concentrations(de la Torre *et al.*, 2002). After 10 minutes of incubation at controlled room temperature (20˚C), 25 µL of 8mM DTNB chromophore and 25 µL of 45 mM acetylcholine iodide and 25µL pseudocholine substrate were added. The contents were mixed and changes in absorbance were read continuously at 412 nm for 3 minutes, at intervals of 10 seconds using spectrophotometer (Cole Parma 1100 series, UNICO, Dayton New

Jersey, USA). Each activity measurement was at least in duplicate. The AChE activity was calculated using Beer Lambert's Law, with molar extinction coefficient of DTNB of 13,600 M^{-1} cm^{-1}. The IC_{50} for the pesticides tested were calculated by plotting a regression line through the linear part of the graph, and the concentration of pesticide that inhibited 50% of the activity was calculated from the graph/equation (Rickwood and Galloway, 2004). The percentage of AChE inhibition was derived by expressing the activity in the tissues exposed to different pesticide concentrations as a percentage activity in unexposed tissues.

3.5.2.6 Measurement of AChE and BuChE Activity in Plasma and Brain

Measurements of AChE and PChE activity were performed using a spectrophotometric method described by Ellman *et al.* (1961) as described above. To measure the activity, 20 µL of plasma sample was added to the cuvette containing 3000 µL of 0.1 M phosphate buffer (pH 8.0), 25 µL of 8 mM DTNB chromophore and 25 µL 45 mM acetylcholine iodide and pseudocholine substrate, at controlled room temperature (20º C). For brain homogenates, the sample volumes were 25 µL and the buffer volume was 3000 µL, as for the analysis of plasma. The contents in the cuvette were mixed and the absorbance was read continuously for 3 minutes at the intervals of 10 seconds as described above.

3. 6 Statistical Data Analysis

Data collected were summarized, coded and verified before the analysis. SPSS version 16.0 and Microsoft Excel 2010 Office analytical tool pack computer software were employed in data analysis. Descriptive statistical package of different factors

were used to obtain proportions and their 95% confidence intervals (CIs) where necessary. The gradients of unexposed and exposed plasma and brain homogenates were also calculated. Percentages inhibitions against concentrations were plotted. The figures with extrapolated 50% enzyme activity against concentrations (IC_{50}) were made.

CHAPTER FOUR

4.0 RESULTS

4.1 Socio-demographic Characteristics of Respondents

The table below shows demographic information of the respondents in Langali and Mkindo Villages.

Table 2: Socio-demographic characteristics of respondents in Langali and Mkindo villages. The values are in percentages

Characteristics	Categories	Langali (n=11)	Mkindo (n=14)	Total (n=25)	p-value
Age	21-30 years	0.0	21.4	12.0	0.263
	31-40 years	27.3	7.1	16.0	
	41-50 years	18.2	14.3	16.0	
	Above 51years	54.5	57.1	56.0	
Sex	Female	45.5	28.6	36.0	0.383
	Male	54.5	71.4	64.0	
Marital status	Single	9.1	0.0	4.0	0.250
	Married	90.9	100.0	96.0	
Education level	No formal education	0.0	7.1	4.0	0.216
	Adult education	0.0	28.6	16.0	
	Primary school	81.8	57.1	68.0	
	Secondary education	9.1	7.1	8.0	
	Tertiary education	9.1	0.0	4.0	
Family size	1-3 people	0.0	21.4	12.0	0.185
	4-6 people	27.3	7.1	16.0	
	7-9 people	45.5	28.6	36.0	
	10 and above people	27.3	42.9	36.0	

4.1.1 Age of Respondents

The study found that the majority 56% (n=25) of respondents were of the age above 51 years and were practicing fish farming more than any other age group. In both villages, fishing was dominated by old people being 54.5% (n=11) and 57.1% (n=14) for Lingali and Mkindo respectively. However, there was no significant difference on the age distribution between the two villagers ($p>0.05$).

4.1.2 Sex of Respondents

The study showed that the majority 64.0% (n=25) of the respondents who practiced fish farming were males. Furthermore, it was found that in Langali village there were more females 45.5% (n=11) practicing fish farming than Mkindo 28.6% (n=14). However, there was no significant difference on the sex distribution between the two villagers ($p>0.05$).

4.1.3 Marital Status of the Respondents

The marital status was characterized into; single and married. The majority of the respondents 96.0% (n=25) were married. The distribution of marital status confirms that fish farming activities in the study area attract mostly adults, whose main activity for their wellbeing was farming.

4.1.4 Education Level of the Respondents

The results show that the majority 68.0% (n=25) of the respondents had attended primary education. Moreover, on individual villages more than a half of the respondents who attained primary education, were practicing fish farming more than any other level of education. The results revealed that level of education of the

respondents did not differ between the villages ($p>0.05$).

4.1.5 Family Size of Respondents

The results show that majority of respondent from Langali 45.5 % (n=11) had family size between seven and nine people while 42.9% (n=6) from Mkindo had household size more than nine people. It is because high household size results from extended family. Therefore, large number of household would be able to provide the labour that might be required to perform different activities..

4.2 Type of Fish Kept

The study sought to ascertain the type of fish kept on the study area. It was found that the majority of the respondents both in Langali and Mkindo villages kept *O. nioticus* (Figure 2), but also few of them kept catfish (*Clarius garipinas*). During the interviews, most participants said one reason for keeping Nile tilapia was availability of market. It was found that the market for tilapia is high due to the demand from the consumers, but also tilapia is among the species which show some resistance towards the varying environmental conditions.

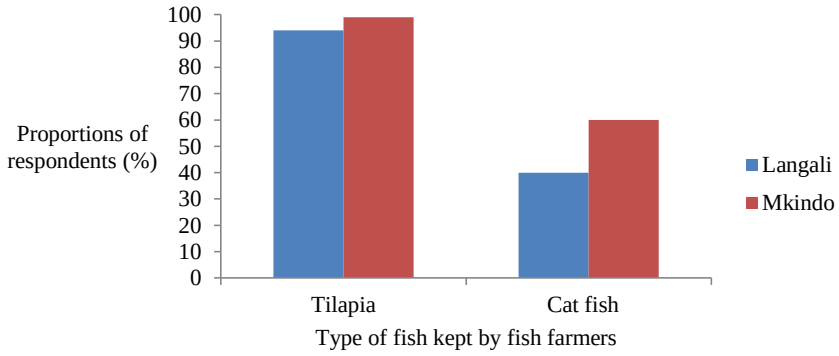

Figure 3: Proportions of types of fish kept by farmers in Langali and Mkindo study area. Tilapia fish kept more than catfish in both villages.

4.3 The Fish Ponds

Respondents were asked to state where they cultivated the fish. It was found that majority of them keep on their own constructed ponds (Figure 3). Very few respondents said they conduct their fishing activities on the nearby natural constructed ponds.

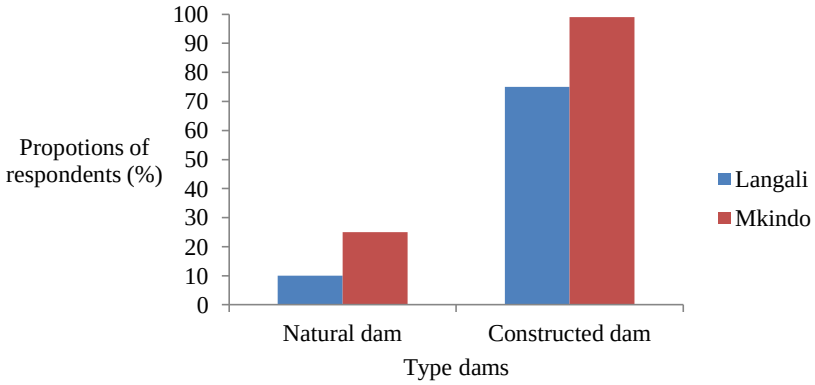

Figure 4: Proportions of types of ponds (dam) used for fishing at Langali (Mgeta) and Mkindo (Hembeti) villages.

4.4 Fish Farming Practices

The results show that the majority of respondents 88.0% (n=25) used maize bran and vegetables to feed fish. The remaining few of the respondents used maize bran and food remnants. Inclusion of vegetables on fish feeds was based on their availability in the study area. The common storage facilities for feeds were fiber and plastics bags. The majority of the respondents used fibers bags (Table 2).

Table 3: Fish farming practices

Parameter	**Categories**	**Percentages of fish farmers**		
		Langali (n=11)	**Mkindo (n=14)**	**Overall (n=25)**
Feeds given to fish	Maize bran/ food remnants	18.2	7.1	12.0
	Maize bran/ vegetables	81.8	92.9	88.0
Storage facility for fish feeds	Fibers bags	90.9	81.8	76.0
	Plastic baskets	45.5	54.5	44.0

The results further show that 56% and 44% were the fish farmers at Mkindo (n= 14) and Langali (n=11). The types of pesticide to store maize are shown in Table 3. It was found that the majority of the respondents 24% (n=25) used pirimiphos-methyl while very few 4 % (n=25) used famazeb. In Langali, all respondents used profenofos as the pesticide for storing maize and as a pest control to vegetable growing.

Table 4: Type of pesticides used in storing of maize and vegetables

Parameter	Categories	Percent of fish farmers		
		Langali (n=11)	Mkindo (n=14)	Overall (n=25)
Use of pesticides for storage of maize and vegetables.		12.0	44.0	56.0
Types of pesticides used to store maize	Pirimiphos-methly	0.0	24.0	24.0
	Profenofos	4.0	4.0	8.0
	Profenofos/ Pirimiphos-methly	4.0	16.0	20.0
	Profenofos/ Famazeb	4.0	0.0	4.0

Comment [H2]: Use chemical names

4.5 Attitude towards Sources of Contamination of Fish Ponds

From the results, 92% agreed that maize bran contaminated with pesticides, kills fish where by 48% were from Mkindo and 44% from Langali.as shown in Table 4**.**

Table 5: Attitude towards sources of contamination to fish ponds

Parameter	Categories	Percent of fish farmers		
		Langali (n=11)	Mkindo (n=14)	Overall (n=25)
Effects of feeding pesticides contaminated maize bran to fish	Kills fish	28.0	36.0	64.0
	Unknown	16.0	20.0	36.0
Fish kills from	Maize bran contaminated with pesticides	44.0	48.0	92.0
	Vegetables sprayed with pesticides	32.0	44.0	76.0
	Irrigation water contaminated with pesticides dripping to fish ponds	40.0	40.0	80.0

4.6 Effect of Pirimiphos-methyl, Profenofos and Cabaryl in Plasma and Brain Homogenates of *O. niloticus*

Comment [u3]: Is it brain or bran?

The *in-vitro* residual AChE and PChE activities of pirimiphos-methyl, profenofos and cabaryl in plasma and brain homogenates of *O. niloticus* are as shown in Figures 4, 5 and 6. On the other hand, different IC_{50} values as a result of *in-vitro* exposure of pirimiphos-methyl, profenofos and carbaryl in plasma are presented in Table 5 while brain homogenate values are shown in Table 7.

Comment [u4]: Where is it

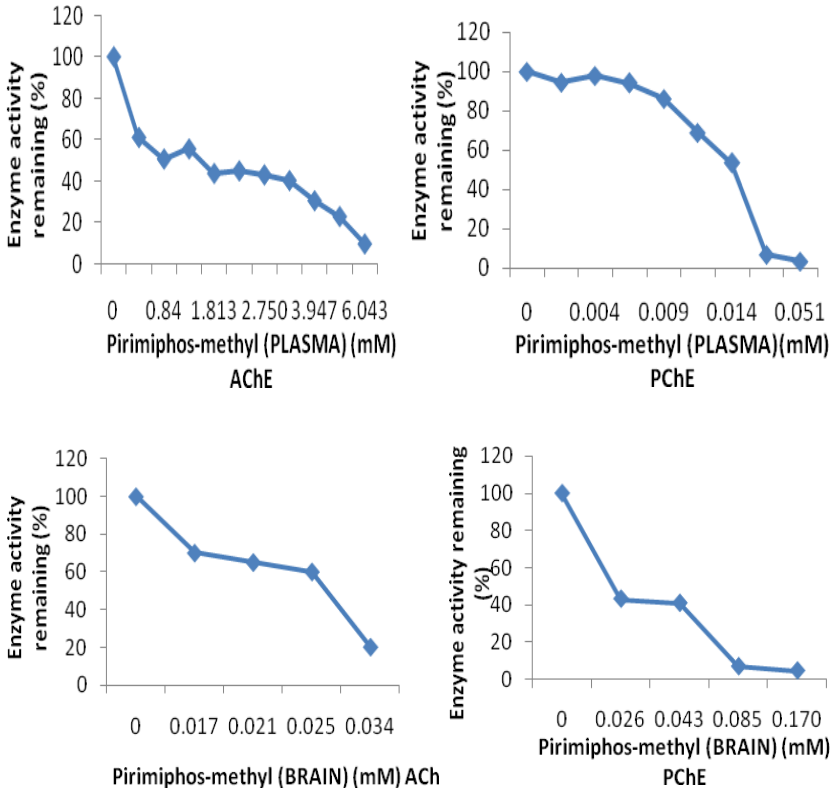

Figure 5: The *in-vitro* effects of Pirimiphos-methyl on Acetylcholinesterase and Pseudocholinesterase activity in plasma and brain homogenate of *O. niloticus*

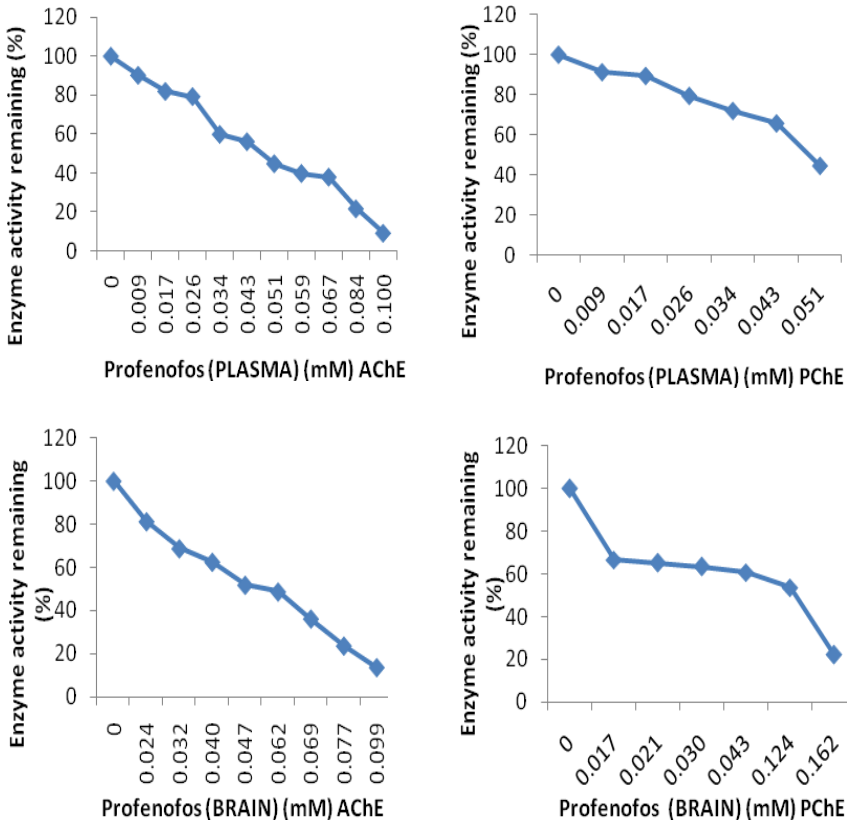

Figure 6: The *in-vitro* effects of Profenofos on Acetylcholinesterase and Pseudocholinesterase activity in plasma and brain homogenate of *O. niloticus*

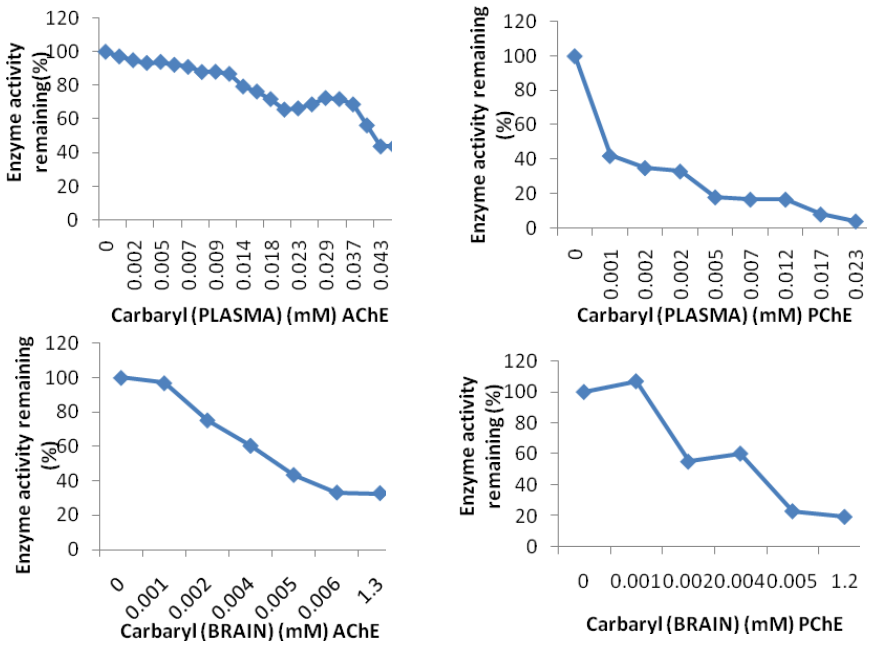

Figure 77: The *in-vitro* effects of Carbaryl on Acetylcholinesterase and Pseudocholinesterase activity in plasma and brain homogenate of *O. niloticus*

From the results of IC_{50}, arrangement of effectiveness among pesticides studied was profenofos 0.031mM > pirimiphos-methyl 0.630mM > carbaryl 1.801mM, for AChE in plasma, and Cabaryl 0.045mM > profenofos 0.091mM > pirimiphos-methyl 0.153mM, for PChE in plasma (Table 5). Cabaryl 0.004mM > pirimiphos-methyl 0.005mM > profenofos 1.307mM, for AChE in brain, and pirimiphos-methyl 0.007mM > profenofos 0.031mM > cabaryl 0.743mM, for PChE in brain (Table 5).

The results for *in-vivo* exposure of pirimphosmethyl in AChE and PChE activity reactions are shown in Figure 7 to 10. A considerable inhibition of AChE and PChE activities in plasma and brain homogenate were observed, as a result of exposure of

O. niloticus to pirimiphos-methyl at a concentration of 0.16 µM.

The fish which were exposed to pirimiphos-methyl at the highest dose of 0.16 µM showed changes in clinical signs. The observed signs included: reduced opercula movements, erratic swimming, restlessness, lethargy and loss of swimming equilibrium, decreased behavior of chasing each other, and searching for food. There were no differences on the extent of clinical signs manifestation among those exposed fish, which showed changes in clinical signs. After 24 hours of exposure, no pirimiphos-methyl-exposed fish died (Table 6.). The total length, standard length and weight measurements of *O. niloticus* were taken before taking blood and brain homogenate sample for analysis (Table 7). The inhibition percentage of AChE and PChE actions in plasma and brain homogenates as a result of *in-vivo* exposure to pirimiphos-methyl is shown in Table 8.

Table 6: IC50 of AChE and PChE activity following *in-vitro* exposure of plasma and brain homogenate by Pirimiphos-methyl, Profenofos and Cabaryl in *O.niloticus.*

Pesticide	IC_{50} (mM)			
	Plasma		Brain	
	AChE,	PChE	AChE	PChE
Pirimiphos-methyl	0.630	0.153	0.005	0.007
Profenofos	0.031	0.091	1.307	0.031
Cabaryl	1.801	0.045	0.004	0.743

Table 7: Body measurements of *O.niloticus* used in the experiment.

Group	Dose(µM)	n	Total length(cm)	Standard length(cm)	Weight(kg)
G1	0.04	6	12.62±2.276	11.28±2.058	30.75±12.63
G2	0.08	6	13.35±1.721	11.08±1.42	35.25±13.31
G3	0.16	6	13.34±1.593	11.38±1.304	34.31±11.22
G4	NP	5	14.2±0.7583	11.8±0.9083	38.13±8.558
G5	BL	4	14.7±0.2449	12.13±0.25	44.73±3.851

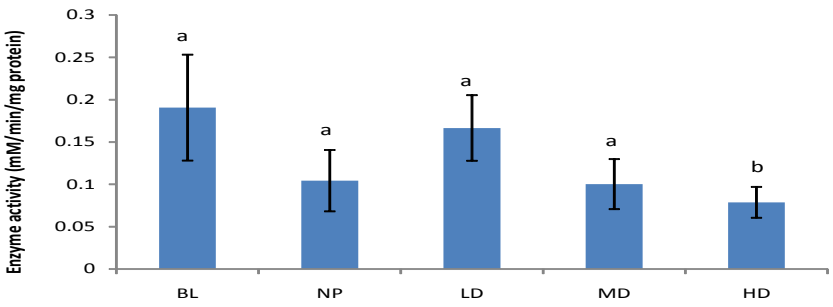

Figure 8: The *in-vivo* outcome of Pirimiphos-methyl pesticide on AChE actions in plasma in *O. niloticus.* Error bars represent the standard error of the mean (SEM) at 95% confidence interval.

Key: BL=Blank, NP=No pesticide, LD=Low dose, MD= Medium dose, HD= High dose. A letter superscript different from BL indicates significant difference ($p<0.005$).

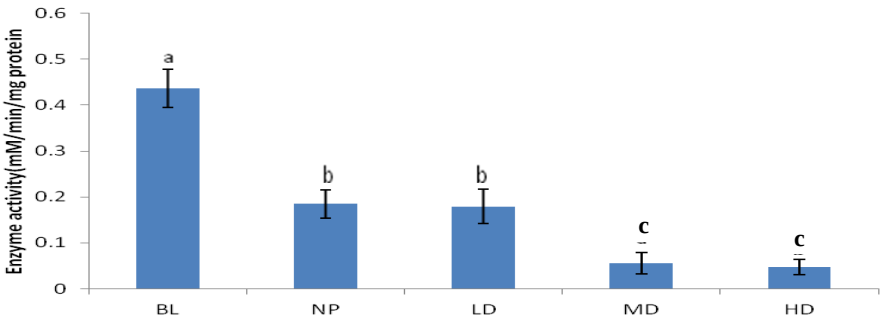

Figure 9: The *in vivo* effect of Pirimiphos-methyl pesticide on PChE actions in plasma in *O. niloticus.* Error bars represent the standard error of the mean (SEM) at 95% confidence interval.

Key: BL=Blank, NP=No pesticide, LD=Low dose, MD= Medium dose, HD= High dose

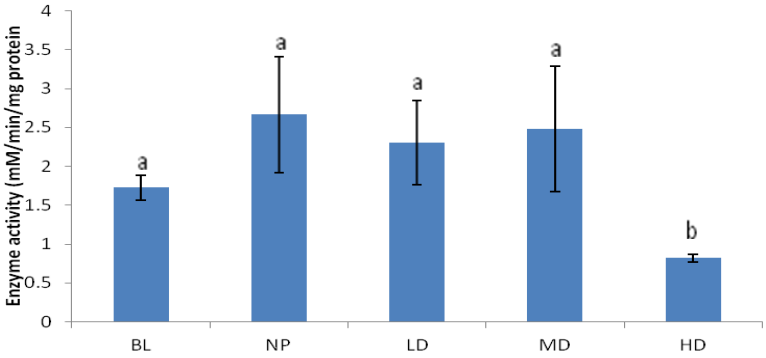

Figure 9: The *in vivo* outcome of Pirimiphos-methyl pesticide on AChE actions in brain homogenates in *O. niloticus.* Error bars represent the standard error of the mean (SEM) at 95% confidence interval.

Key: BL=Blank, NP=No pesticide, LD=Low dose, MD= Medium dose, HD= High dose. A letter superscript different from BL indicates significant difference ($p<0.005$).

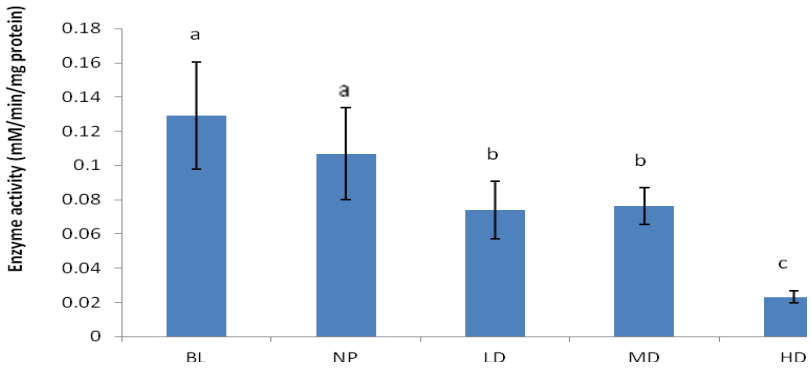

Figure 10: The *in vivo* outcome of Pirimiphos-methyl pesticide on PChE actions in brain homogenates in *O. niloticus.* Error bars represent the standard error of the mean (SEM) at 95% confidence interval.

Key: BL=Blank, NP=No pesticide, LD=Low dose, MD= Medium dose, HD= High dose.

Table 8: *In vivo* inhibition of Acetylcholinesterase and Butyrylcholinesterase /Pseudocholinesterase in plasma and brain from *O.niloticus* after 24 hrs waterborne exposure to Pirimiphos-methyl (the values are percentage inhibition calculated from blank (unexposed control).

Pesticide	**Dose**	**Plasma (% inhibition)**		**Brain (% inhibition)**	
		AChE	PChE	AChE	PChE
Pirimiphos-methyl	0.04µM	10	52	48	40
	0.08µM	48	85	50	42
	0.16 µM	53	90	50	75

CHAPTER FIVE

5.0 DISCUSSION

5.1 Socio-demographic Characteristics of Fish Farmers

In the current study most of the fish farmers in the study area were old people being 56% (n=25) with the age above 51 years. This was attributed to experience farmers got from the past aqua farming, as well as youth migration from rural to urban looking for employment. The youth group claimed that fish farming was not paying faster, .Their view point is contrary to the results reported by Agboola, (2011), whereby most (42.2%) of the respondents were at the age of 41- 50. This implied that the majority of the respondents were still in their active age. According to Adesiji *et al.* (2009) most of the youth are migrating from rural to urban areas to look for social amenities, employment, improve their way of life and peer pressure.

The study indicated that fish farming is principally done by adults over 51 years, which is likely to be attributed by experience and rural-urban migration for employment (Adesiji *et al.*, 2009). Unlike the adults, the youth group claims that the returns from fish farming were slow (Agboola, 2011). Furthermore, the current study shows that the majority of the respondents had primary level of education. Meanwhile education is an important factor in influencing management and the adoption of any technology. At this level, most people especially in Tanzania have poor understanding of English language. Due to this, the respondents might not be able to read or understand pesticide and other agrochemical label and instructions on use since are written in English. Hence this leads to mishandling and misuse of pesticides as observed in the study area. Also, respondent had no training relating to

contamination of fish ponds with pesticide like pirimiphos-methyl and other agrochemicals and training in good agricultural practices (GAP) which among other things include the proper use and safe handling of pesticide and agrochemicals.

These results are comparable to the published findings by Nonga *et al* (2011), which explain about poor education background among the users of pesticides and other agrochemicals. In the study by Nonga *et al.* (2011), the number of primary school leavers of the respondent was 84% higher than 68.0% reported in the present study. In addition, similar finding were reported by Mekonnen and Agonafir, (2002), and was attributed to language barriers, in communities with primary education, were reported in Ethiopia, among pesticides sprayers and farmers. The related findings were reported by (Agboola, 2011) that 13.3 % had primary education. The number is less and contrary to the present study, whre by most of the aqua farmers 43.3% had tertiary education most of whom were civil servants either (active or retired), teachers, medical doctors and a host of other professionals. This is an indication of high literacy level that may be required for effective management of fish farms. Although nowadays some of the pesticides like pirimiphos-methyl , and other agrochemicals their instructions are written in both languages that is English and Kiswahili, it is recommended that fish farmers be trained on the proper use and handling of pesticides and other agrochemicals and good agricultural practices and continues, if possible to put the instructions of the pesticides and others agrochemical in Swahili which is the national language to reduce mistreatment and mishandling of this chemical . Therefore this will prevent human, animals, aquatic and environmental health.

Field survey indicates that majority 64.0% (n=25) of the respondents who practiced fish farming were males. One of the reasons is generating income within the family since male are responsible for it. The finding was in agreement from previous studies by Agboola (2011) whereby 95.6% of the respondents were male while the female constitute 4.4%. This shows the extent of gender sensitivity on occupation like fish farming. This could be attributed to the fact that agricultural production is faced with a lot of risk and uncertainties and women are risk averse. So this is the result of drudgery that aquaculture business is involved in.

Most of the aqua farmers 96.0% (n=25) were married. The distribution of marital status confirms that fish farming activities in the study area attract mostly adults, whose main activity for their wellbeing is farming. The reason is that it adds income to the family. This is contrary to the findings by Gamal *et al*. (2008), who found the major reasons that the figure is expected to enhance is the use of more family labour in the fish farming operations thereby leading to reduction in the use of hired labor among the people in the study area.

The present study revealed that respondents' family size is between seven and above people 36.0% (n=25) were practicing fish farming more compared to other groups, where as 45.5% (n=11) were the family size between seven to nine people from Langali and 42.9% (n=14) were between ten and above people from Mkindo village, because high household size results from extended family. The size of the household has influence on socio-ecological resilient of the household. Large number of household would be able to provide the labour that might be required by performing different activities including fish farming. These are comparable to the finding of

Gamal *et al*. (2008), who revealed that majority of the fish farmers dependents number with the highest proportion (68.8%) is 1-20 members. The results imply that the lowest range of family size has the highest proportion. It is still an indication that family labor would be used intensively. Therefore, if serious commitment is shown from the family labor, it is expected to lead to higher productivity in fish farming in the area.

5.2 Fish Farming and Contamination of Fish Ponds with Pirimiphos-methyl

Field surveys in the present study indicate that some of the respondents in Langali and Mkindo villages were actively involved in subsistence farming and fish farming as their major dependable rural livelihood. In addition, some of the fish farmers and villagers were involved in small-scale irrigated farming. This was common on small pieces of land. The majority of the respondents both in Langali and Mkindo villages kept *O. nioticus*. The availability of market was the main reason for keeping *O. niloticus*. It was found that market for *O. nioticus* was high due to the demand from the consumers, but also *O. nioticus* was among the species which show some resistance to varying environmental conditions particulary when subjecting them to live in most fresh waters, like lake, dam or river waters with the exception of marine waters (Kiwale, 2003). The findings of the present study agree with that of Eira *et al*. (2008) who found that tilapia, catfish and carp are the most commonly cultured fish species in the tropics. Furthermore, according to Fitzsimmons and Naim (2010), tilapia is a commonly raised fish throughout the world, second only to Carp. In 2009 more than 3 million metric tons of tilapia was raised. Basically, it was because tilapia thrives in warm tropical areas. It is a good fish for resource for poor farmers to grow

because tilapia are: easy to raise, fast growing and tasty, able to eat many types of foods and are low on the food chain, highly disease resistant, able to reproduce easily, hardy and can tolerate poor water quality conditions.

The present study revealed that the majority of interviewers 88.0% (n=25) used maize bran and vegetables to feed fish. The remaining few of the respondents used maize bran and food remnants. In fact, the inclusion of vegetables on animal feeds was based on their availability in the study area (Tacon, 1999). It appears that the use of pesticides to store maize and control vegetable pests is a common practice for fish farmers from Mkindo and Langali villages. The frequency of use of pesticide was lower than what was reported in the previous studies by Fianko *et al*. (2011) where there were 78% of pesticides users. These results are also comparable to the published findings by Ntow *et al.* (2006) who found higher uses of herbicides by vegetable farmers in Ghana. Also according to Nonga *et al*. (2011), the uses of pesticides were rampant with limited or no knowledge on the possible effects to the environment. The increased use of commercial pesticides which apart from increasing crop production have long term negative effects on Fauna and flora. In that observation, chemical pesticide like pirimiphos-methyl and others are the common practices used to control pests and diseases in crops cultivated in Tanzania, since most of the pesticides used in the study area were in Class II. This depicts that they have moderate hazardous effects to the environment. That is why most insecticides were in the group of organophosphate and pyrethoids which are easily degradable in the environment and public health.

5.3 *In-vitro* and *In-vivo* Effect of AChE and PChE in Plasma and Brain Homogenates of *O.niloticus*

Both the *in-vitro* and *in-vivo* studies show a clear difference in effectiveness of various pesticides on activities of AChE and PChE in plasma and brain homogenates. It was determined in plasma and brain homogenates of tilapia fish *O.niloticus* (Porte and Albaigés, 2001). The *in-vitro* detection of 0.004, 0.005 and 1.307mM of carbaryl, pirimiphos-methyl and profenofos respectively in brain homogenates of *O.niloticus* agrees with the results of Mdegela *et al.* (2010) in brain homogenates of *Clarias gariepinus*, where he established IC_{50} of 0.002, 0.003, and 0.003 µM for pirimiphos-methyl, carbaryl, and profenofos respectively. The IC_{50} values found in the present investigation were higher than the reported values by Mdegela *et al.* (2010) in C. gariepinus and also greater than the reported values by Dembe´le´ *et al.* (2000) in *Cyprinus carpio L* with the following concentration 0.4, 19 and 19 µM as IC50 for carbaryl, chlorfenvinphos and diazinon, respectively. Although the species and organophosphate especially of Dembe´le´ *et al*. (2000) differ in the sensitivity of brain AChE activities from this study, the IC_{50} seen in the present work reveals that *O.niloticus* is also useful bioindicator fish species for assessment of organophosphate and carbamate pesticide exposures. According to Zahavi *et al*. (1971), the reasons behind the species' differences in inhibitory potency have been reported to be the result of stearic exclusion of the inhibitor from the active site of the enzyme.

In the present work, it is demonstrated that carbaryl was the most and pirimiphos-methyl the least potent inhibitor of AChE activity in brain, and also the investigation

from this study showed that carbaryl was the most and profenofos the least potent inhibitor of PChE activity in brain. This is due to the *in vitro* assays conducted with pirimiphos-methyl, profenofos and carbaryl pesticides. The significant difference in inhibition of acetylcholinesterase and butyrylcholinesterase by pirimiphos-methyl and carbaryl may be due to phosphorylation or carbamilation of esterase site, serine hydroxyl group of the enzyme. Thus, the anticholinesterase potency depends largely on the phosphorylating or carbamylating ability of either organophosphate or carbamate ester respectively. In addition, in the current work *in vitro* exposure showed carbaryl to be more effective than pirimiphos-methyl and profenofos. The result was the same as previously reported observation, whereby carbaryl was more potent than chlorfenvinphos and diazinon (Mora *et al*., 1999; Dembe´le´ *et al*., 2000; Mdegela *et al*., 2010) in *C. gariepinus*, Furthermore *in vitro* exposure showed cabaryl was more effective than methylparathion in *in vitro* studies of AChE activities in gill tissues of *Mytilus galloprovincialis*.

Similarly, *in vitro* exposure in the present work showed carbaryl to be more potent than pirimiphos-methyl and profenofos for PChE in brain. In view of the fact that carbamates being more potent than OPs in *in vitro* studies (Çokugras, 2003), the reason may be due to irreversibility of organophosphates and reversibility of carbamates inhibitors to AChE and PChE. The irreversibility of enzyme activity in Organophosphate is due to aging of the complex enzyme in which the structural changes are imposed with covalent modifications. Also, the N-methyl carbamate esters cause reversible carbamylation of the acetylcholinesterase enzyme, allowing accumulation of acetylcholine, the neuromediator substance, at parasympathetic

neuroeffector junctions (muscarinic effects), at skeletal muscle myoneural junctions and autonomic ganglia (nicotinic effects), and in the brain (CNS effects).

There are two main reasons for using fish cholinesterase as biomarker. The first concern is on the availability of this source. In 2009, the world fisheries and aquaculture production was 145.1 million tones, and most of the fish waste reused comes from tissues other than those that provide ChEs (FAO, 2010). Furthermore, in the recent study different concentrations of pirimiphos-methyl tested inhibited the brain AChE and BuChE activity and dose–effect relationships were detected. According to the FAO (2007), 20% inhibition of brain AChE activity is considered the endpoint to identify the no observed-adverse-effect-level (NOAEL) in organisms, while signs and symptoms appear when AChE is inhibited by 50% or more. Death occurs above 90% inhibition. In fish, the relationship between AChE inhibition and mortality is not clear because some species are able to survive with high percentages (90–95%) of brain enzyme inhibition (Fulton and Key, 2001; Ferrari *et al*., 2004a, Ferrari *et al*., 2004b and Ferrari *et al*., 2007).

Also in this sense, some authors have established that 50% of AChE inhibition could indicate intoxication or poisoning (Dembélé *et al*., 2000). Cholinesterase inhibition of more than 70–90% at sublethal concentrations of organophosphates and carbamates has been observed in fish species such as common carp (Gruber and Munn, 1998). Meanwhile, Wright and Welbourn (2002) reported that reduction of brain AChE activity by 20% or more in birds, fish or invertebrates indicates exposure to OPs or carbamate pesticides, and a 50% or greater reduction is indicative of a life-threatening situation.

In this finding, 0.04 µM inhibited brain AChE activity by 48% following *in vivo* exposure to pirimiphos-methyl and 0.16 µM inhibited brain AChE activity by 50%. Furthermore 0.04 µM inhibited brain BuChE activity by 40% following *in vivo* exposure to pirimiphos-methyl and 0.16 µM inhibited brain BuChE activity by 50%.

The level of AChE and PChE inhibition observed revealed that *O. niloticus* is also a useful fish species in assessing contaminants in aquatic environment by OPs and carbamates and other anticholinesterase pollutants. In other previous finding, exposure of *Oreochromis mossambicus* to Monocrotophos organophosphate at 51.5 µM inhibited brain AChE activities by 40 % (Rao, 2006). Chandrasekara and Pathiratne (2007) found that 50% inhibition of brain AChE activity after exposure of *O.niloticus* to 0.011µM in Chlorpyrifos. Chandrasekara and Pathiratne (2005) also observed a 52% inhibition of brain AChE after exposure of *Cyprinus carpio* to 0.97 µM in Trichlofon organophosphate.

The findings from this study demonstrate the sensitivity of brain AChE and BuChE activity in *O. niloticus* fish following *in vivo* exposure to OPs and carbaryl pesticides; they also reveal the greater in inhibition of brain PChE than AChE activities. The findings are in line with Caio Rodrigo Dias Assis *et al.,* (2011) who found that for pesticides with larger acyl chains or higher lipophilic characteristic (for which only a small fraction reaches the target tissues), BChE can be more sensitive than AChE. Also in this study, the inhibition in plasma is greater than in brain as a result of *in vivo* exposure of *O. niloticus* in pirimiphos-methyl. The finding from this work found that AChE inhibition in brain is higher than AChE in plasma. in addition, PChE inhibition in plasma is greater than in brain; this shows that AChE

in brain is sensitive than AChE in plasma as a result of *in vivo* exposure of *O. niloticus* in pirimiphos-methyl. This view is supported by Cero´n *et al.,* (1996); Sancho *et al*., (2000)) who found that brain tissues are the sensitive tissues in assessing inhibitory responses resulting from carbamates and OPs exposure. On the other hand, according to Akman *et al.,* (2009) AChE is predominant in brain and muscle tissues, whereas BChE presents mostly in the liver and plasma.

In the current study the concentrations of pirimiphos-methyl had an impact in AChE and BuChE activities in *in vivo* study. Thus the clinical signs observed in fish exposed to pirimiphos-methyl were in fish groups that were exposed at 0.16 µM. These concentrations cause a reduction of AChE and PChE activities in plasma and brain homogenates. Other previous results by Sandahl *et al*. (2005) show inhibition of AChE activities in fish with clinical sign, like reduced spontaneous swimming rate, low swimming rate during feeding, latency to first strike, and total food strikes. Also, another previous work of Murthy *et al*. (2013) shows clinical sign after inhibition of AChE and BuChE activities in fish, the behavioral effects seen includes: increased stress, swimming ability, which in turn can reduce the ability to feed, interrupt schooling behavior, disruption of schooling behavior is thought by some researchers to be a classic method for examining sublethal effects of pesticides because the effect is so common. Other clinical signs are seeking sub-optimal water temperatures, and inhibition normal migration. Other results by Olufemi *et al*. (2008) found restlessness, increased reaction to exogenous stimuli, incoordination of movement and postural orientation before death of fish. Additionally, the clinical signs seen in this work are likely to be related to failure of energy production, and

thus affecting respiratory system producing nervous signs with decrease acetylcholinesterase activities or release of stored metabolic energy, which might have lead to severe stress and sometimes death of the fish.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

From this study, it can be concluded that the majority 56% of respondents of the age above fifty one years were practicing fish farming more than any other age group. Sixty four percent (64.0%) of the respondents who practiced fish farming were males and 96.0% were married. The distribution of marital status confirms that fish farming activities in the study area attract mostly adults, whose main activity for their wellbeing is farming. Moreover, on individual villages more than a half of the respondents attained primary education, and were practicing fish farming more than any other level of education. The study further revealed that respondents family size between seven and above people were practicing fish farming. It is possible that high household size results from extended family. Furthermore, the study sought to ascertain the type of fish kept on the study area. It was found that majority of the respondents both in Langali and Mkindo villages kept *O. nioticus* and the reason for keeping it was the availability of market. The study also shows that the majority of respondents used maize bran and vegetables to feed fish. The remaining few of the respondents used maize brain and food remnants. Moreover, they used pirimiphos-methyl pesticides when storing maize and spraying in vegetables during cultivation while very few use famazeb.

The results from this study have established the sensitivity of AChE and PChE/ BuChE activities in plasma and brain homogenates of *O.niloticus* subsequent in vitro and in vivo exposure to sub-lethal concentrations of OPs (pirimiphos-methyl

and profenofos) and carbaryl for in vitro and pirimiphos-methyl for in vivo studies. These findings propose that AChE and BChE in *O. niloticus* are potential biomarkers for assessment of environmental contamination resulting from anticholinesterases.

6.2 Recommendations

Considering fish farming in the study area fish production is economically prizing and profitable. It is able to create employment; add to income and improving the standard of living of the people. Based from the findings of this study, the following recommendations are made:

i. Adequate training programme on fish production should be organized for fish farmers in the study area for dissemination of research findings to fill the gap. Since most of the fish farms were owned by individuals who had little access to finance. Therefore, the government's participation in fish farming should be encouraged in the area to improve the quantity of fish available for consumption.

ii. Again, since fish farming in the study area is male dominated, females need to be encouraged to participate in fish farming in the area as a means of adding to their income and improving their standard of living.

iii. Fish farmers should be organized into alarming groups such as cooperative to enjoin economies of scale in the purchase of inputs and sale of output. The arrangement of the cooperative should also be done towards ensuring labour availability.

iv. It is recommended that social amenities and services such as, effective communication system, good roads and health care, bank and insurance etc

should be improved to prevent youth to move to the urban Areas. Rural-urban float has a extraordinary impact on agriculture and fish farming production because it brings an extreme decrease in the proportion of those engaged in agriculture and fish farming, leaving mostly the old people. There is also an increase in the cost of hired labour because of the insufficiency of able youth in the villages. However, majority of the respondents are attracted to the cities because of good social amenities. That is why, they push the youth out of the rural area to the urban city.

v. Adequate training programme on fish production should be organized for fish farmers in the study area for dissemination of research findings to fill the gap. Since most of the fish farms were owned by individuals who had little access to finance. Therefore, the government's participation in fish farming should be encouraged in the area to improve the quantity of fish available for consumption.

vi. Additionally, the other study is needed in order to make easy the interpretation of depressed AChE and BuChE in terms of mortality, negative effects for the physiology of the organisms, neurological disorders and changes on behavior.

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REFERENCES

Adesiji, G. B., Omoniwa, V., Adebayo, S. A., Matanmi, B. M. and Akangbe, J. A. (2009). Factors associated with the youths' rural-Urban drift in Kwara State, Nigeria. *Interdisciplinary Journal of Contemporary Research in Business* 1(8): 231 – 245.

Agboola, W. L. (2011). Improving fish farming productivity towards achieving food security in Osun State, Nigeria: A Socioeconomic Analysis. *Annals of Biological Research* 2(3): 62 – 74.

Akman, O. E., Clement, R. A., Broomhead, D. S., Mannan, S., Moorhead, I. and Wilson, H. R. (2009). Probing bottom-up processing with multistable Images. *Journal of Eye Movement Research* 1(3): 1 – 7.

Assis, C. R. D., Bezerra, S. and Jrin, C. (2011). Pesticides in the modern world - pests control and pesticides exposure and toxicity assessment. [http://www.intechopen. com/books/pesticides-in-the-modern-world-pests-controland-pesticides-exposure -and-toxicity-assessment/fish-cholinesterases-as-biomarkers-of-organophosphor us-and-carbamate-pesticides] site visited on 2/9/2014.

Balarin, J. (1979). *Tilapia: A Guide to Their Biology and Culture in Africa.* University of Sterling, Scotland. 56pp.

Cero´n J. J., Ferrando M. D., Sancho, E., Gutierrez-Panizo, C. and Andreu-Moliner, E. (1996). Effects of diazinon exposure on cholinesterase activity in different tissues of European eel (*Anguilla anguilla*). *Ecotoxicology Environment Safety* 35: 222 – 225.

Chambers, J. E., Boone, J. S., Carry, R. L., Chambers, H. W. and Straus, D. L. (2002). Biomarkers as predictors of health and ecological risk assessment. *Human Ecology Risk Assessment* 8: 165 – 176.

Chandrasekara, H. U. and Pathiratne, A. (2005). Influence of low concentrations of Trichlorf on on haematological parameters and brain acetylcholinesterase activity in common carp. *Cyprinus carpio. Aquaculture Research* 36: 144 – 149.

Chandrasekara, L. W. H. U. and Pathiratne, A. (2007). Body size-related differences in the inhibition of brain acetylcholinesterase activity in juvenile Nile tilapia (*O.niloticus*) by chlorpyrifos and carbosulfan. *Ecotoxicology and Environmental Safety* 67: 109 – 119.

Çokugras, A. N. (2003). Butyrylcholinesterase: Structure and physiological importance. *Turkish Journal of Biochemistry* 28(2): 54 – 61.

Coppage, D. L. and Mathew, E. (1974). Shorten effect of organophosphate pesticides on cholinesterase of estuarine fishes and pink shrimp. *Bulletin Environmental Contamination. Toxicology* 11: 483 – 487.

Coppage, D. L., Mathews, E., Cook, G. H. and Knight, J. (1974). Brain acetylcholinesterase inhibition in fish as a diagnosis of environmental poisoning by malathion pesticide. *Biochemistry and physiology* **5:** 536 – 542.

De la Torre, F.R., L. Ferrari and A. Salibian (2002): Freshwater pollution biomarker response of brain acetylcholinesterase activity in two fish species. *Comparison Biochemistry Physiology* 131: 271 – 280.

Dembelé, K., Heubruge, E. and Gaspar, C. (2000). Concentration effects of selected insecticides on brain acetylcholinesterase in the common carp (*Cyprinus carpio* L.). *Ecotoxicology and Environmental Safety* 45: 49 – 54.

Durkin, P. R. and Follansbee M. H. (2004). *Control Eradication Agents for the Gypsy Moth Human, Health and Ecological Risk Assessment.* Syracuse Research Corporation, New York. 301pp.

Eira, C., van Eer, A., van Schie, T. and Aldin, H. (Eds.) (2003). *Small- scale Freshwater Fish Farming*. Agromisa Foundation, Wageningen. 48pp.

Ellman, G. L., Courtney, K. D., Andres, V. J. and Featherstone, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemistry and Pharmacology* 7: 88 – 95.

FAO (2004). *Monitoring Progress towards the World Food Summit and Millennium Development Goals*. State of Food Insecurity in the World, Rome, Italy. 120pp.

FAO (2007). *Pesticides in Food Report 2007 Plant Production.* Protection Paper No. 191. Food and Agriculture Organization, Rome, Italy. 68pp.

FAO (2010). *The State of World Fisheries and Aquaculture.* Food and Agriculture Organization, Rome, Italy. 197pp.

Ferrari, A., Venturino, A. and D'Angelo, A. M. P. (2007). Muscular and brain cholinesterase sensitivities to azinphos methyl and carbaryl in the juvenile rainbow trout *Oncorhynchus mykiss. Comparative Biochemical and Physiology* 146: 308 – 412.

Ferrari, A., Venturino, A. and Pechen de D'Angelo, A. M. (2004). Time course of brainCholinesterase inhibition and recovery following acute and subacute azinphosmethyl, parathion and carbaryl exposure in the goldfish (*Carassius auratus*). *Ecotoxicology Environmental Safety* 57: 420 – 425.

Comment [u5]: Check it information not complete

Fianko, J. R., Lowor, S. T., Donkor, A. and Yeboah, P. O. (2010). Nutrient chemistry of the Densu River in Ghana. *Environmentalism* 30(2): 145 – 152.

Frtitzsimmons, K. and Naim, S. (2010). *Tilapia 2009 State of the Industry*. Tilapia Session, San Diego, Washington, USA. 85pp.

Fulton, M. H. and Key, P. B. (2001). Acetylcholinesterase inhibition in estuarine fish and invertebrates as an indicator of organophosphorus insecticide exposure and effects. *Environmental Toxicology and Chemistry* 20(1): 37 – 45.

Gamal, EL-N., Ahmed, N. and Kareem, R. O. (2008). Economic analysis of fish farming in Behera Governorate. *Conference 8th International Symposium on Tilapia fish in Aquaculture, Egypt*. pp. 693 – 710.

Gruber, S. J. and Munn, M. D. (1998). Organophosphate and carbamate insecticides in agricultural waters and cholinesterase (ChE) inhibition in common carp *(Cyprinus carpio)*: *Archives of Environmental Contamination and Toxicology* 35: 391 – 396.

Hart, K. and Pimentel, D. (2002). *Public Health and Costs of Pesticides.* In: (Edited by D. Pimentel, D.), Encyclopedia of Pest Management, New York. 679pp.

Jokanovic, M. and Kosanovic, M. (2010). Neurotoxic effects in patients poisoned with organophosphate pesticides. *Environmental Toxicology and Pharmacology* 29: 195–201.

Kirkwood, B. R. and Sterne, J. A. C. (2003). *Essential Medical Statistics.* (Second Edition) Blackwell Cornwall Publisher, United Kingdom. 56pp.

Kiwale, J. (2003). The Tanzania fish export sector, sector diagnostic. [http://www.tp] site visited on 01/11/2012.

Mdegela, R. H., Mosha, R. D., Morten, S. and Skaare, J. U. (2010). Assessment of Acetylcholinesterase activity in *Claris gariepinus* as a biomarker of organophosphate and carbamate exposure. *Ecotoxicology* 19: 855 – 863.

Mekonnen, Y. and Agonafir, T. (2002). Pesticide sprayers knowledge, attitude and practices of pesticide use on agricultural farms of Ethiopia. *Occupation Medical London* 52: 311 – 315.

Ministry of Livestock and Fisheries Development (2010). *Fisheries Sector Development Programme.* Ministry of Livestock and Fisheries Development, Dar es Salaam, Tanzania. 83pp.

Mora, P., Michel, X. and Narbonne, J. (1999). Cholinesterase activity as potential biomarker. *Environmental Toxicology Pharmacology* 7: 253 – 260.

Murnyak, D. (2010). *Basics of Raising Tilapia and Implementing Aquaculture Projects: A Farmer's Guide to Tilapia Culture*. Heifer Project International, Arkansas, USA. 75pp.

Murthy, R. A. N. and Raju, P. V. (2013). *Safety Evaluation of Plastics Materials for Food Packaging Application Standards.* New Delhi, India. 509pp.

Nicolet, Y. Lockridge, O. Masson, P. Fontecilla-Camps, J. C. Nachon, F. (2003). Crystal Structure of Human Butyrylcholinesterase and of Its Complexes with Substrate and Products. *Journal of Biological Chemistry*. 278: 41141–41147.

Nonga, H. E., Mdegela, R. H., Lie, E., Sandvik, M. and Skaare, J. U. (2011). Assessment of farming practices and uses of agrochemicals in Lake Manyara basin, Tanzania. *African Journal of Agricultural Research* 6(10): 2216 – 2230.

Ntow, W. J., Gijzen, H. J., Kelderman, P. and Drechsel, P. (2006). Farmer perceptions and pesticide use practices in Vegetable production in Ghana. *Pesticide management Science* 62: 356 – 365.

Porte, C. and Albaigés, J. (2001). Residues of pesticides in aquatic organisms. Implications in biomonitoring studies. *Environmental Contamination Toxicology* 1993(26): 273– 281.

Quaqrainic, K. (2007). Training fish farmers in Tanzania. *Aquanews* 22(3): 1 – 9.

Quinn, D. M. (1987). Acetylcholinesterase; enzyme structure, reaction dynamic and virtual transition states. *Chemical Reviews* 87: 955 – 979.

Rahimi, E., Bongadian, M., Rafei, M. and Kazemeini, H. R. (2006). Occurrence of aflatoxin M1 in raw milk of five dairy species in Ahvaz, Iran. *Food and Chemical Toxicology.* 48: 129 – 131.

Rao, J. V. (2006). Toxic effects of novel organophosphorous insecticide (RPR-V) on certain biochemical parameters of euryhaline fish, *Oreochromis mosambicus*. *Pesticide Biochemistry Physiology* 86: 78 – 84.

Rickwood, C. J. and Galloway, T. S. (2004). Acetylcholinesterase inhibition as a biomarker of adverse effect. A study of Mytilus edulis exposed to the priority pollutant chlorfenvinphos. *Aquatic Toxicology* 67: 45 – 56.

Sancho, E., Cero´n, J. J. and Ferrando, M. D. (2000). Cholinesterase activity and hematological parameters as Biomarkers of sublethal molinate exposure in *Anguilla anguilla*. *Ecotoxicology Environment* 46: 81 – 86.

Sancho, E., Ferrando, M.D. and Andreu, E. (1998). In vivo inhibition of AChE activity in the European eel *Anguilla angulla* exposed to technical grade Fenitrothion. *Biochemistry Physiology* 120: 389 – 395.

Sandahl, J. F., Baldwin, D. H., Jenkins, J. J. and Scholz, N. L. (2005), Comparative thresholds for acetylcholinesterase inhibition and behavioral impairment in coho salmon exposed to chlorpyrifos. *Environmental Toxicology and Chemistry* 24: 136 – 145.

Silman, I. and Sussman, J. L. (2005). Acetylcholinesterase; Classical and non classical functions and pharmacology. *Current opinion in Pharmacology* 5: 293 – 302.

Singh, R. N., Pandey, R. K., Singh, N. N. and Das, V. K. (2010). Acute toxicity and Behavioural responses of common carp Cyprinus carpio (Linn) to organophosphate (Dimethoate). *World Journal of Zoology* 5(3): 183 – 188.

Soreq, H. and Zakut, H. (1990). Cholinesterase gene. Maltileveled regulation. *Monographs in Human Genetics* 13: 1 – 102.

Tacon, A. G. J. (1999). *Global Trends and Challenges to Aquaculture and Aquafeed Development in the New Millennium.* International Aquafeed Directory, Uxbridge, Middlesex, UK. 25pp.

United States Public Health Service Commission (1996). Chlorfenvinphos summary. [http:// www.usphs.gov] site visited on 30/10/ 2012.

Walker, C. H. (2001). *Organic Pollutants: An Ecotoxicological Perspective.* Taylor and Francis Publisher, New York. 116pp.

Whittaker, M. (1980). Plasma cholinesterase variants and the anaesthetist. *Anaesthesia* 35: 174 – 197.

Whittaker, M. (1986). *Cholinesterase.* Karger Basel Publishers, New York. 126pp.

Wright, D. A. and Welbourn, P. (2002). Organic compounds. In: *Environmental Toxicology Cambridge Environmental Chemistry.* (Editors by Campbell, P. G. C., Harrison, R. M. and de Mora, S. J.), Cambridge University Press, Cambridge, pp. 355–362.

Zahavi, A. 1971. The social behavior of the White Wagtail *Motacilla alba alba. Journal of Wintering Ibis* 113: 203 – 211.

APPENDICES

Appendix 1: Questionnaire-English version

PART ONE

QUESTIONNAIRE FOR FISH FARMERS

Introduction

Section one; Location. Date…….

$A_{1.}$ Village…….

$A_{2.}$ Ward ……...

$A_{3.}$ District…….

Section two: Respondent characteristics

$B_{1.}$ Respondent name……

$B_{2.}$Sex of the respondents

1. Male ( )
2. Female ( )

$B_{3.}$ What is your age?

1. 21-30 years ( )
2. 31- 40 years ( )
3. 41- 50 years ( )
4. Above 51 years ( )

$B_{4.}$ Marital status

1. Single ( )
2. Married ( )
3. Divorced ( )
4. separated ( )
5. Widowed ( )

$B_{5.}$ What is your education level?

1. No formal education ( )
2. Adult education ( )
3. Primary school education ( )

4. Secondary school education ( )
5. College ( )
6. Others (specify) …….

B_6. What is the size of your family?

1. 1-3 people ( )
2. 4-6 people ( )
3. 7-9 people ( )
4. 10 and above ( )

B_7. Rank the three most important source of income for your household.

1. Livestock keeping ( )
2. Crop farming ( )
3. Fishing ( )
4. Business or selling ( )
5. Paid employment ( )
6. Other ……

Section Three. General knowledge/information on risk factor associated with the use of pirimiphos-methyl and permethrin to the maize.

C_1. Methods used for preserving of maize/maize bran

1. Which methods are you using to preserve maize and other cereals?

……

2. Do you wash your maize after storage? Yes/No
3. Are you using protective gear? Yes/No

If yes mention them……

……

4. What are the methods used for preparing maize bran for fish feeding?

……., ……., ……..., ……...

C_2. The Use of pesticides

1. Do you use pesticides or other agrochemical to preserve maize? Yes/No

If yes, what is the name of pesticides? …….....

2. Where are you getting these pesticide/ agrochemicals.....?
3. Do you have any storage facilities for your maize and other cereals? Yes/No

If yes can you mention them……, ……., …….

4. Are you cultivating any vegetables? Yes/No

If yes what are types of vegetables are you growing among these?

a. Spinaches ( )
b. Cabbages ( )
c. Amaranths ( )
d. Carrots ( )
e. Green pepper ( )
f. Potato leaves ( )
g. Legume leaves ( )

Others specify.....

5. Are you using vegetables to feed fish? Yes/No

If yes which type of vegetables? ……..

C_3. Problems/other activities/sources of water during fish farming

1. What problems you face during fish farming?
   a. …….
   b. …….
   c. …….
   d. …….
   e. …….
2. What other activities do you? .....
3. What are the sources of water you are using for vegetables irrigation?
   a. Rivers
   b. Shallow wells
   c. Streams
   d. Pipe water

Others specify.....

5. What are the equipments you use for vegetable irrigation?
    a. watering cans
    b. buckets
    c. tubes
    d. watering channels

Others specify.....

Section Four. Fishing and fish feeding

a. Which types of fish are you keeping.....?

……...

b. Which types of fish farming are you practicing among of this?
    i. Fish farming in natural ponds within your farm? Yes/No
    ii. Fish farming in constructing artificial ponds within your farm? Yes/No

Which type of feeding are you using to feed your fish?

i. Formulated feeds Yes/No
ii. Local feeds Yes/No

Mention other methods you know…….

……...

c. What are types of feeds are you using to feed fish?
    Mention them……...
    …….
d. Which types of equipments are you using for storage of feeds for fish?
    Mention them……
    ……..
e. Which types of methods are you using to feed fish among the following?
    i. Manually feeding……..Yes/No
    ii. Automatic feeder……..Yes/No
f. Do you know anything about the effects of using maize bran contain pesticides? Mention them……...
    ……...

Section five. Attitude towards source of contamination.

Show how you agree or disagree of the following statements in relation to attitude towards sources of contamination of fish ponds.

	ITEM	AGREE	DISAGREE
A. Attitude towards source of contamination of fish ponds			
1	Use of maize bran contain pesticide causes death of fish to fish ponds		
2	Vegetables preserved with pesticides when used to feed fish can cause effects or even death to fish in the fish pond		
3	Water for irrigation contaminated with pesticides when flow to fish ponds can cause effects to fish.		
4	Water from irrigation when contaminate fish farming ponds increases death of fish.		
5	Use of vegetables contaminated with pesticides for fish feeding has nothing to do with contamination of pond water for fish farming.		
6	The use of water from irrigation contain pesticides has nothing to do with contamination of fish farming ponds		
7	It is useful to use maize bran for fish feeding		
8	Use of water for irrigation increase risk of survival of fish		
9	Use of maize bran contaminated with pesticides for fish feeding cause death of the fish in fish pond		
10	Use of water for irrigation has nothing to do with the increase risk of survival of fish		

Interview conclusion

We would like to thank you very much for your time and for this useful information.

Appendix 2: Questionnaire-Swahili version

DODOSO LINALOHUSU WAFUGAJI WA SAMAKI

Utangulizi

Sehemu ya kwanza. Mahali. **Tarehe……**

$A_{1.}$ Kijiji/Mtaa……

$A_{2.}$ Kata…….....

$A_{3.}$ Wilaya…….....

Sehemu ya pili: Sifa za Msailiwa

$B_{1.}$ Jina la Msailiwa…….

$B_{2.}$Jinsia

3. **Mke** ( )
4. **Mme** ( )

$B_{3.}$ Una umri gani?

1. **Miaka 21-30** ( )
2. **Miaka 31- 40** ( )
3. **Miaka 41- 50** ( )
4. **Miaka 51 na zaidi** ( )

$B_{4.}$ Hali ya ndoa

6. **Hujaoa/hujaolewa** ( )
7. **Uko kwenye ndoa** ( )
8. **Mmetarikiana** ( )
9. **Mmetengana** ( )
10. **Mjane/Mgane** ( )

$B_{5.}$ Kiwango cha Elimu

7. **Hajasoma** ( )
8. **Elimu ya watu wazima** ( )
9. **Elimu ya Msingi** ( )
10. **Elimu ya Sekondari** ( )
11. **Chuo** ( )
12. **Elimu nyingine (Fafanua) …….**

B_6. Familia yako ina ukubwa gani?

5. **Watu 1-3** ( )
6. **Watu 4-6** ( )
7. **Watu 7-9** ( )
8. **Watu 10 na zaidi** ( )

B_7. Unafanya kazi gani? Kati ya hizi zifuatazo?

1. Ufugaji ( )
2. Mkulima ( )
3. Mfugaji wa samaki ( )
4. Mfanyabiashara ( )
5. Muajiriwa ( )
6. Nyingineyo…….

Sehemu ya tatu: Uelewa/Habari zihusuzo madhara yatokanayo na matumizi ya Actellic super (pirimiphos-methyl and permethrin) katika kuhifadhia mahindi.

C_1. Njia za kuhifadhia mahindi/pumba

1. Njia gani unazotumia kuhifadhia mahindi pamoja na mazao mengine? Zitaje

…….

…….

2. Je unayaosha mahindi kabla ya kuyatumia? Ndio/ Hapana

3. Je unatumia vifaa vya kuzuia madhara yasikupate? Wakati wa kuhifadhi mahindi na wakati wa uoshaji Ndio/Hapana

Kama ndio vitaje hivyo vifaa ……

4. Je njia zipi unazotumia kutayarisha pumba kwa ajili ya kulishia samaki? Zitaje

……..

……..

……..

C_2. Matumizi ya viwatilifu

1. Unatumia viwatilifu kuhifadhia mahindi? Ndio/Hapana

Kama ndio ni aina gani ya viwatilifu? …….....

2. Ni sehemu gani unapopata hivyo viwatilifu? .....

3. Je unalima mbogamboga? Ndio/Hapana

Kama ndio ni aina gani za mbogamboga unazolima katika hizi zifuatazo?

h. Spinachi ( )

i. Kabeji ( )

j. Mchicha ( )

k. Karoti ( )

l. Pilipili ( )

m. Viazi

n. ( )

o. Maharage/mikunde kunde ( )

Mboga nyinginezo zitaje.....

4. Je unatumia mbogamboga kulishia samaki? Ndio/Hapana

Kama ndio ni aina gani ya mbogamboga?……..

C_3. Matatizo/shughuli zingine/vyanzo vya maji katika ufugaji wa samaki

1. Ni matatizo gani unayoyapata kuhusiana na ufugaji wa samaki?

a.....

b.....

c.....

d.....

e……

2. Ni shughuli gani nyingine unazozifanya?

.....

……

3. Vyanzo gani vya maji unavyotumia kumwagilia?

i. Mito ( )

ii. Visima ( )

iii. Mifereji ( )

iv. Mabomba ( )

4. Vifaa gani unavyotumia kumwagilia mbogomboga?

a. Keni ya kumwagilia (watering cans) ( )

b. Ndoo ( )

c. Mipira (tubes) ( )

e. Vingine vitaje……

Sehemu ya nne: Ufugaji na ulishaji wa samaki

a. Unafuga samaki aina gani? ……

b. Je unatumia aina gani ya ufugaji wa samaki katika hizi zifuatazo?

i. Unaweka samaki katika mabwawa ya asili yaliyoko eneo la shamba lako Ndio/Hapana

ii. Unaweka samaki katika mabwawa yako ya kuchimba Ndio/Hapana

iii. Unalisha chakula kilichotengenezwa kitalamu (fomulated feed). Ndio/Hapana

Taja njia nyingine unazozifahamu…….

…….

…….

c. Ni aina gani muhimu ya vyakula unavyotumia kulishia samaki?

Vitaje…….

……...

……...

d. Vifaa gani unavyotumia kuhifadhia chakula cha samaki? vitaje.....

……..

e. Je unatumia njia gani kulishia samaki chakula katika hizi zifuatazo?

i. Kulisha kwa mkono (manually feeding) Ndio/Hapana

ii. Kulisha kwa mashine (automatic feeder) Ndio/Hapana

f. Je unaufahamu wowote kuhusu madhara yatokanayo na matumizi ya pumba yenye viwatilifu? Eleza……...

……

Sehemu ya tano: Uelewa kuhusu vyanzo vya uchafuzi wa mabwawa ya samaki.

Onyesha jinsi gani unakubali au unakataa maneno yafuatayo kuhusiana na uelewa wa vyanzo vya uchafuzi wa mabwawa ya samaki.

	MAELEZO	NDIO	HAPANA
A. Uelewa kuhusu vyanzo vya uchafuzi wa mabwawa ya samaki			
1	Pumba zenye viwatilifu zinapotumika kama chakula cha samaki zinasababisha vifo vya samaki.		
2	Mboga zilizotunzwa shambani/bustanini kwa kutumia viwatilifu, zinapotumika kama chakula cha samaki zinasababisha vifo/madhara kwa samaki.		
3	Maji ya kumwagilia yenye viwatilifu yanapotiririka kuelekea kwenye mabwawa ya kufugia samaki yanaathiri ufugaji wa samaki.		
4	Matumizi ya maji ya kumwagilia yenye viwatilifu yanaongeza vifo/madhara kwa samaki.		
5	Matumizi ya pumba yenye viwatilifu kwa kulishia samaki hayana uhusiano na kusababisha vifo/madhara kwa samaki		
6	Matumizi ya maji ya kumwagilia yenye viwatilifu hayana uhusiano na ongezeko la vifo vya samaki		
7	Utunzaji wa mboga shambani/bustanini kwa kutumia viwatilifu, zinapotumika kama chakula cha samaki hazina uhusiano na kusababisha vifo vya samaki.		

Hitinisho la Usaili

Tunashukuru kwa kupoteza muda wenu kwa kutoa habari hizi muhimu. Ahsanteni.

Appendix 3: Plates

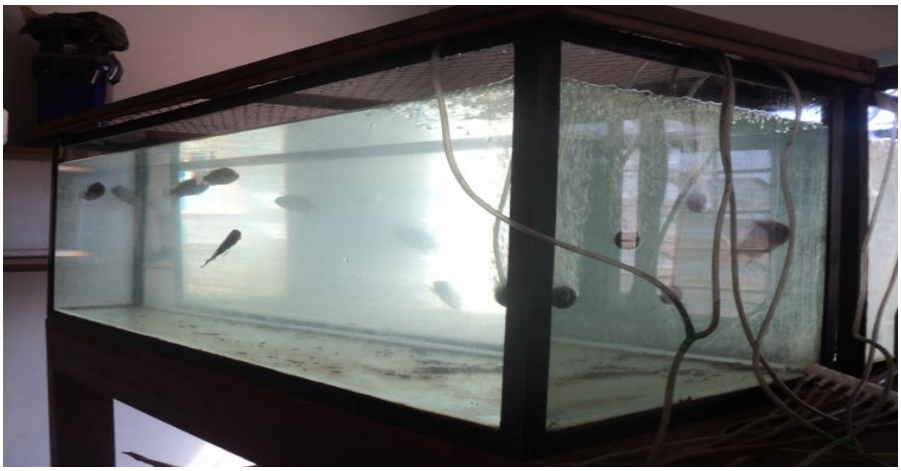

Plate 1: Aquarium Containing *O.niloticus*

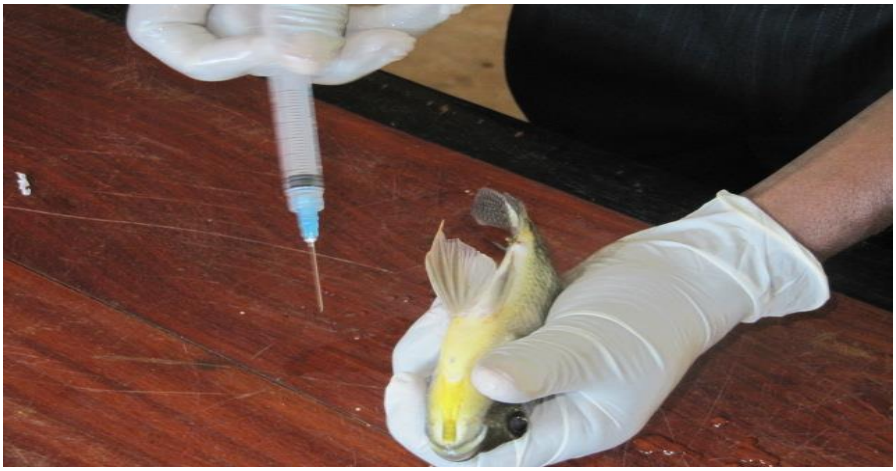

Plate 2: Preparation of *O. niloticus* for Taking Blood Samples

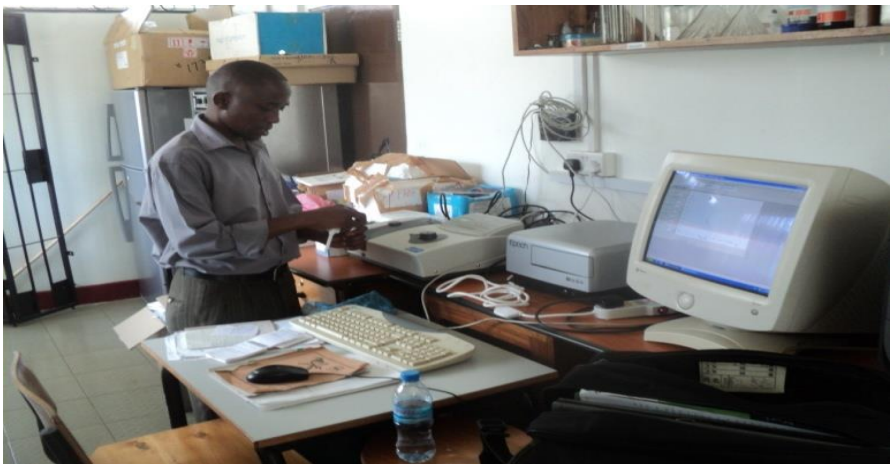

Plate 3: Researcher Analyzing AChE and BuChE in the Blood of *O. niloticus* Using UV-Vis Spectrophotometer.

Appendix 4: Table 9 Experimental set up of *O.niloticus* showing different doses of Pirimiphos-methyl and the fish behaviors

FISH BEHAVIOURS	OBSERVED SIGN	LOW DOSE (0.04µM) at 9:47am				MEDIUM DOSE (0.08µM) at 11:47am				HIGH DOSE (0.16µM) at 1:47 am				MAIZE BRAN WITH NO PIRIMIPHOS-METHYL				BLANK			
	Time	0	6	12	24	0	6	12	24	0	6	12	24	0	6	12	24	0	6	12	24
Stabilization	High	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	Low																				
	No																				
Opercula movement	High	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓			✓	✓	✓	✓	✓	✓	✓	✓
	Low											✓	✓								
	No																				
Erratic movement	High	✓	✓	✓	✓	✓	✓	✓	✓	✓				✓	✓	✓	✓	✓	✓	✓	✓
	Low										✓	✓									
	No												✓								
Searching for food	High	✓	✓	✓	✓	✓	✓	✓						✓	✓	✓	✓	✓	✓	✓	✓
	Low								✓	✓	✓										
	No											✓	✓								
Chasing each other	Normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓			✓	✓	✓	✓	✓	✓	✓	✓
	Low											✓									
	No												✓								
Swimming equilibrium	Normal	✓	✓	✓	✓	✓								✓	✓	✓	✓	✓	✓	✓	✓
	Low						✓	✓	✓	✓	✓	✓	✓								
	No																				
Lethargy	High											✓	✓								
	Low								✓	✓	✓										
	Normal	✓	✓	✓	✓	✓	✓	✓						✓	✓	✓	✓	✓	✓	✓	✓