ASSESSMENT OF ANTICHOLINESTERASE CONTAMINANTS IN RUVU RIVER USING CHOLINESTERASE BIOMARKER IN AFRICAN SHARPTOOTH CATFISH

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A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN COMPARATIVE ANIMAL PHYSIOLOGY OF SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA.

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ABSTRACT

A cross sectional study was conducted to assess the extent of exposure of anticholinesterase contaminants in Ruvu river using cholinesterase biomarker in African Sharptooth Catfish. Questionnaire interviews were used to collect sociological data from 200 respondents, also Ellman's method and 5, 5'-dithiobis-2-nitrobenzoic acid chromophore was used to establish cholinesterase activities in plasma and brain of 40 *Claria gariepinus*. The sociological findings showed that all respondents (100%) use pesticides in agriculture and 62.5% were males with primary level of education (69%). Organophosphates pesticides were mentioned to be mostly used in the area. With regards to the proper use, awareness on the effects and disposal of pesticides, it was observed that most farmer know how to use the pesticides and were aware of the effect of pesticides in human and pests but not to the environment. Also mishandling and poor disposal of pesticides and their leftovers were reported. There was no significant difference between the levels of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) in plasma. AChE and BChE activities in brain showed insignificant difference ($P > 0.05$) among fish from the study sites and the control. The brain AChE activities were significantly lower ($P < 0.05$) in fish from Ruvu Darajani compared to the control. This study revealed differences in AChE and BChE activities in the study area. Such differences could be ascribed to the environmental contamination due to agrochemicals used by the farmers around Ruvu river basin.

DECRALATION

I, **Tumwesige Mshobozi Katto** do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.

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DEDICATION

This work is dedicated to my beloved parents Mrs. Mwl Katto Tinkasimile and the late Mwl. Katto Tinkasimile for their diligent efforts in building up my education foundation.

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LIST OF ABBREVIATIONS AND ACRONYMS

ACh	Acetylcholine
AChE	Acetylcholinesterase
ANOVA	Analysis Of Variance
BChE	Butyrylcholinesterase
DMR	Duncan's Multiple Range Test
DTNB	5, 5'-dithiobis-2-nitrobenzoic acid
G	Gauge
GLOWS - FIU	Global Water for Sustainability Program - Florida International University
H0	Null hypothesis
Ha	Alternative hypothesis
MJNUAT	Mwalimu Julius K. Nyerere University of Agriculture and Technology
OHCEA	One Health for Central and Eastern Africa
OP	Organophosphate
SPSS	Statistical Package for Social Studies
SUA	Sokoine University of Agriculture
TNBC	Tanzania National Business Council
TPRI	Tropical Pesticide Research Institute
URT	United Republic of Tanzania
URTNC	United Republic of Tanzania National Census

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Agriculture plays a major role in the economy of Tanzania. The country has a total land area of 945 087 km^2 out of which the total land mass is 886 126 km^2 and water bodies 58 961 km^2 (TNBC, 2009; URT, 2009). The total land suitable for agriculture is estimated to be 620 227 km^2 (70 %) of total land area. It is further estimated that, out of 620 227 km^2 which is suitable for Agriculture, 440 000 km^2 is arable land, out of which only about 100 000 km^2 (23 %) is currently utilized for crop production. Agricultural activities practiced by more than 73% of the country population (Kimaro and Hieronimo, 2014).

Like other developing countries in the world, Tanzania has been experiencing rapid human population growth. As of 2002 census, the population was reported to be 37 million (URTNC, 2002) and has currently increased to 50 million. This increase in human population goes along with increased food demand. In order to increase crop production to meet this high demand most of the land-use has been directed towards permanent annual or multiple cropping (Mati, 2005). Apart from the high increase in agricultural activities there are also high occurrences of pests and diseases that have led to increased indiscriminate use of pesticides and other agrochemicals. For example, a high level of pesticide use in smallholder vegetable farmers has been observed in Northern Tanzania (Ngowi *et al*., 2007). Also poor soil fertility has been observed and this has caused increase in usage of organic and inorganic fertilizers by farmers (Mati, 2005; Salami *et al*., 2010).

The indiscriminate application of these chemical compounds could lead to adverse effects in non-target organisms. Bocquene and Galgani, (1998) pointed out by that pesticide compounds such as Organophosphates (OPs) and carbamates are toxic to non-target organisms in both terrestrial and aquatic environment as they have ability to inactivate the group of cholinesterase enzymes (ChE) which are butyrylcholinesterase (BChE) and acetylcholinesterase (AChE). Acetylcholinesterase is present in most animal species and is responsible for the hydrolysis of the neurotransmitter acetylcholine (ACh) into choline and acetic acid. Thus, when AChE is inactivated by organophosphorus or carbamate compounds, the enzyme is no longer able to hydrolyse ACh and the concentration of ACh in the pre-synaptic junction of the nerve remains high. This leads to continuous stimulation of the muscle or nerve fiber, resulting in tetany and eventually paralysis and death of the organism (Massoulie *et al*., 1993). AChE is commonly found in nervous tissues, brain, red blood cells, and muscle tissues, while BChE is found in blood plasma, liver, and pancreas, where its physiological role is not well defined (Bocquene and Galgan, 1998). However two enzymes differ by just one amino acid in the esteric site and both are inhibited by organophosphates and carbamates compounds (Bocquene and Galgan, 1998).

Non-target organisms such as fish are inevitably exposed to these compounds and/or their degradation products through environmental contamination or occupational use in air, water and food. These pollutants cannot be easily detected by chemical analysis because of their relative short half-life in the environment, thus the use of biomarker in particular cholinesterase activities are alternative method that may provide links between the presence of chemicals in the environment and their biological effects in organisms as quoted by Mdegela *et al.* (2010) from Chambers *et al.* (2002) and Reinecke, (2006). The presence of AChE has been demonstrated in a variety of tissues of aquatic organisms including brain, adductor muscle and gills of shellfish and abdominal muscle of crustaceans. Highest activities were found in the brain and muscle of most fish species and in the muscle of prawn (Frasco *et al.,* 2010). Thus, aquatic organisms are potential for use in assessing the environmental fate of contaminants with anticholinesterase activity (Mdegela *et al.*, 2010).

1.2 Problem Statement and Study Justification

Due to climate change and unreliable rain, most farmers in Tanzania are forced to engage in agricultural activities in river basins and along other water resources (Lema *et al.,* 2014). The Ruvu river basin is among the river basins with high agricultural activities accompanied with usage of pesticides which disquieting on the environmental and public health concern as there is a risk of increase contamination of these pesticides and their residues to water. The analysis of these pesticides levels in water using chemical methods is difficult as most have very short half-life. Therefore the use biomarker is the alternative method.

In Tanzania the use of biomarker in assessing the chemical environmental contamination is limited. Thus, this study aims at using cholinesterase biomarker in African Sharptooth Catfish to assess the extent to which the aquatic environment is contaminated with anticholinesterase compounds. The findings of the study will assess the handling and usage of pesticides in the area and how these may contribute to the environmental contamination. In additional the findings will add up to the limited science available on the use of biomarker in assessing the environmental contamination in the country caused by anticholinesterase compounds.

1.3 Objectives

1.3.1 Overall objective

The overall objective of this study was to assess the extent of contamination and exposure of fish (*Claria gariepinus*) to anticholinesterase compounds in Ruvu river.

1.3.2 Specific objectives

i. To assess the farmer's knowledge and practices related to pesticide contamination in Ruvu river.

ii. To determine the extent of exposure of African sharptooth catfish (*Claria gariepinus*) in Ruvu river with anticholinesterase contaminants.

1.4 Research Hypothesis

H0: There is no significant difference in farmer's knowledge and practice related to pesticides contamination in Ruvu river.

Ha: There is significant difference in farmer's knowledge and practice related to pesticides contamination in Ruvu river.

H0: There is no significant difference in exposure of African sharptooth catfish in Ruvu river with anticholinesterase contaminants.

Ha: There is significant difference in exposure of African sharptooth catfish in Ruvu river with anticholinesterase contaminants.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Pesticides in Agriculture

2.1.1 Overview of pesticides in Tanzania

Pesticides are defined by the Tropical Pesticide Research Institute (TPRI) as "any matter of any description (including acaricides, arboricides, herbicides, insecticides, fungicides, molluscides, nematicides, hormonal sprays and defoliants) used or intended to be used, either alone or together with other material substances) for the control of weeds, pest and disease in plants, or for the control of the external vectors of veterinary or medical disease and external parasites of man or domestic animals or for the protection of any food intended for animal or human consumption" (URT, 1979). Pesticides application in Tanzania has a histrionic increase, as pointed out by Kaoneka and Ak'habuhaya, (2000) that the country imports several tons of pesticides from European and North American countries for both crop and livestock use because of increase in agricultural activities which are challenged by the pests. Also, Ngowi *et al.* (2002) found out that the level of importation of pesticides increased after the liberalization of agrochemicals trade in the country.

2.1.2 Classes of pesticides

Different classes of pesticides such as organochlorines, carbamates, organophosphorus, pyrethroids and atrazines are commonly used in Tanzania (Ngowi, 2002; Nonga *et al.*, 2011). Nevertheless it is estimated that more than 40 different pesticides are used in horticulture of which the most widely used are insecticides (59%), fungicides (29%), with the remaining (12%) being herbicides (Ngowi, 2007). Among the pesticides, organophosphates (OP), carbamates and pyrethroids are commonly used in Agriculture and it’s due to restriction in the use of organochlorines in the country in the early 1990s. According to Nonga *et al.* (2011) the majority of the pesticides used in Tanzania are in class II (moderately hazardous) and a few in class III (slightly hazardous) or U (unlikely to present acute hazard).

2.2 Pesticides Toxicity Development

2.2.1 Target enzymes

Some of the pesticides target the family of enzymes (Cholinesterases; ChEs) which are acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). ChEs are enzymes that hydrolyze the acetylcholine released at central and peripheral sites of the nervous system (Çokugras, 2003). The AChE is synthesized in hematopoietic tissue and is highly present in the brain, endplate of skeletal muscle and erythrocyte membrane, and its main function is to regulate neuronal communication by hydrolysing the ubiquitous neurotransmitter acetylcholine in synaptic cleft (Quinn, 1987; Silman and Sussman, 2005). On the other hand BChE is synthesized in liver and is highly present in blood plasma, smooth muscle, pancreas, adipocytes, skin and heart (Cokugras, 2003). Butyrylcholinesterase is pointed as one of the main detoxifying enzymes able to hydrolyze or scavenge a broad range of xenobiotic compounds like cocaine (Soreq and Zakut, 1990; Cokugras, 2003; Nicolet *et al*., 2003).

2.2.2 Cholinesterase activity inhibition

Cholinesterase can be inactivated by the pesticides of anticholinesterase nature that binds to the active site of the enzyme and inhibit its function. The inhibition of these enzymes leads to accumulation of acetylcholine (Ach) in the synapse resulting in continuous stimulation of the postsynaptic neuron and cholinergic overstimulation (Sancho *et al*., 1998). The common pesticides with high anticholinesterase nature are organophosphorus and carbamates. The inhibition mechanism of carbamates is reversible while that of organophosphorus is irreversible and hence the latter is a good marker of enzymes inhibition, its mechanism involves a nucleophilic attack of the serinic oxygen of cholinesterase active site to the phosphorus atom of OPs. Cleavage of the acidic OP leaving group enables formation of a covalent P-O (serine) bond, thus inhibiting enzymatic hydrolysis of the natural substrate (Mastrantonio *et al*., 2008). Thus, inhibition responses of the enzymes normally vary, depending on the type of pesticide compound used, exposure time, dose, route, water quality and species of the animal (Coppage and Mathews, 1974; Sancho *et al*., 1998).

2.2.3 Other anticholinesterase compounds

The inhibition of AChE from heavy metals, polycyclic aromatic hydrocarbons, detergents, components of complex mixtures of contaminants and other pesticides has been increasingly reported in humans and other animals (Bressler and Goldstein,1991; Goldstein, 1992; Lionetto *et al.* 1994; Jebali *et al.* 2006; Vioque-Fern´andez *et al*. 2007). Also the potential of some metallic ions, such as $Hg^{2+,}$ $Cd^{2+,}$ Cu^{2+}, and Pb^{2+}, to suppress the activity of AChE *in vitro* and/or *in vivo* conditions has been demonstrated in several studies on humans and animals (Costa, 1999; Frasco *et al.,* 2005; Ademuyiwa *et al.,* 2007). Nevertheless, Ademuyiwa *et al.* (2007) also studied the potential effect of Lead on erythrocyte AChE activity during occupational exposure to this metal and suggested that erythrocyte AChE activity could be used as a biomarker of Lead-induced neurotoxicity in occupational exposed subjects. Furthermore AChE activity may also be affected by other pesticides from different chemical families, such as pyrethroids (Reddy, 1994), triazines (Davies, 1993) and paraquat (Szabo, 1992). Additionally, polycyclic aromatic hydrocarbons also are able to inhibit AChE activity in an additive manner together with organophosphate (Jett *et al.,* 1999).

2.3 Pesticides in Aquatic System

Pesticide contaminants affect both aquatic and terrestrial ecosystems. The driving forces for pesticide contamination include drainage patterns, properties of the pesticide, rainfall, microbial activity, treatment surface and rate of application (Maharaj, 2005). In aquatic environment, pesticides are transported through processes such as direct applications, surface runoffs, spray drifts, agricultural returns and groundwater intrusions; either as single chemicals or complex mixtures (Scholz *et al*., 2003). Khan *et al.* (2003) found that most toxic pesticides in urban and agricultural settings were responsible for the deaths of many birds, fish and zooplanktons which are used by fish for food. Though some effects of pesticides only become highlighted after long term exposure, Storrs *et al.* (2004) noted that the survival patterns for early green frogs and late wood frogs are affected only after 24 days of exposure to atrazine. Nevertheless, exposure of pesticides in aquatic organisms such as fish occurs through direct absorption through the skin, uptake of pesticides through the gills and by drinking and feeding pesticide - contaminated water and prey (Storrs *et al.*, 2004).

2.3.1 Measurement of cholinesterase activity in aquatic species

The measurement of cholinesterase activity in aquatic organisms has been shown to be a highly suitable method for assessing exposure to neurotoxic contaminants in aquatic environments. This is due to sensitivity of cholinesterase enzymes to anticholinesterase contaminants and able to be detected at a very low concentration (Galgani *et al*., 1992; Payne *et al*., 1996). Similarly Habig *et al*. (1986) found out that the existence of extremely low thresholds for induction of inhibitory effects on AChE suggests that detection is possible after exposure to low concentrations of neurotoxic insecticides (0.1 to 1 μgl^{-1}). Thus, the use of biomarkers in aquatic species rather than chemical analysis of water are essential in detection of extremely low dissolved chemicals.

2.3.2 *Clarias gariepinus* as a study fish species

Clarias gariepinus is one of the most important tropical freshwater fish, with a widespread geographical distribution ranging from 52ºN to 28ºS. Skelton, (2000) found that the fish have a natural distribution in many parts of Africa and Asia, occurring in habitats with temperatures ranging from 8 to 35ºC and pH from 6.5–8. This fish species has a life span of about 8 years and occurs naturally in contaminated and uncontaminated water bodies also is a benthic species and entirely omnivorous. Therefore, considering its availability, ecological and feeding characteristics, it is considered as an appropriate species for biomarker investigation in aquatic environments in tropical regions (Mdegela *et al.*, 2006a; Braathen *et al.*, 2008). Likewise, the use fish species in biomonitoring of aquatic environment is further supported by Van der Oost *et al.* (2003) who noted that, despite some limitations related to mobility, fish are considered to be the most useful organisms for biomonitoring environmental contamination.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The Ruvu River is one of the major East African Rivers that drain the Eastern Arc Mountains of Ulugulu, with a basin area of approximately 18,000 square kilometers. This basin is typically sub-divided into smaller catchments: the Mgeta, Ngerengere, Upper Ruvu of the Morogoro region, and the Middle and Lower Ruvu in the Coast Region. The main activities taking place at some parts of Ruvu river are agriculture, fishing, gold mining, livestock keeping and charcoal making. Also the GLOWS – FIU, (2014) noted that some of the fish species found in the river include *Claria gariepinus* (Kambale), *Bagrus orientalis* (Katoga, Kitoga), *Eutropis grandis* (Pate), *Petersius conserialis* (Kasa), and *Barbus radiatus* (Kuyu)

3.2 Study Population

The current study targeted the agricultural farmers at the Ruvu river basin especially at Kidimla and Ruvu Darajani sites in Kilangalanga and Mlandizi ward respectively and Kidogozelo and Mtoni sites in Makurunge wards in Kibaha rural and Bagamoyo districts respectively. The selection of the study sites were based on the presence of high agricultural activities and distance from one site to another. Also the criteria for inclusion of household farmers in the study were farming, use of pesticides and willingness to participate in the study.

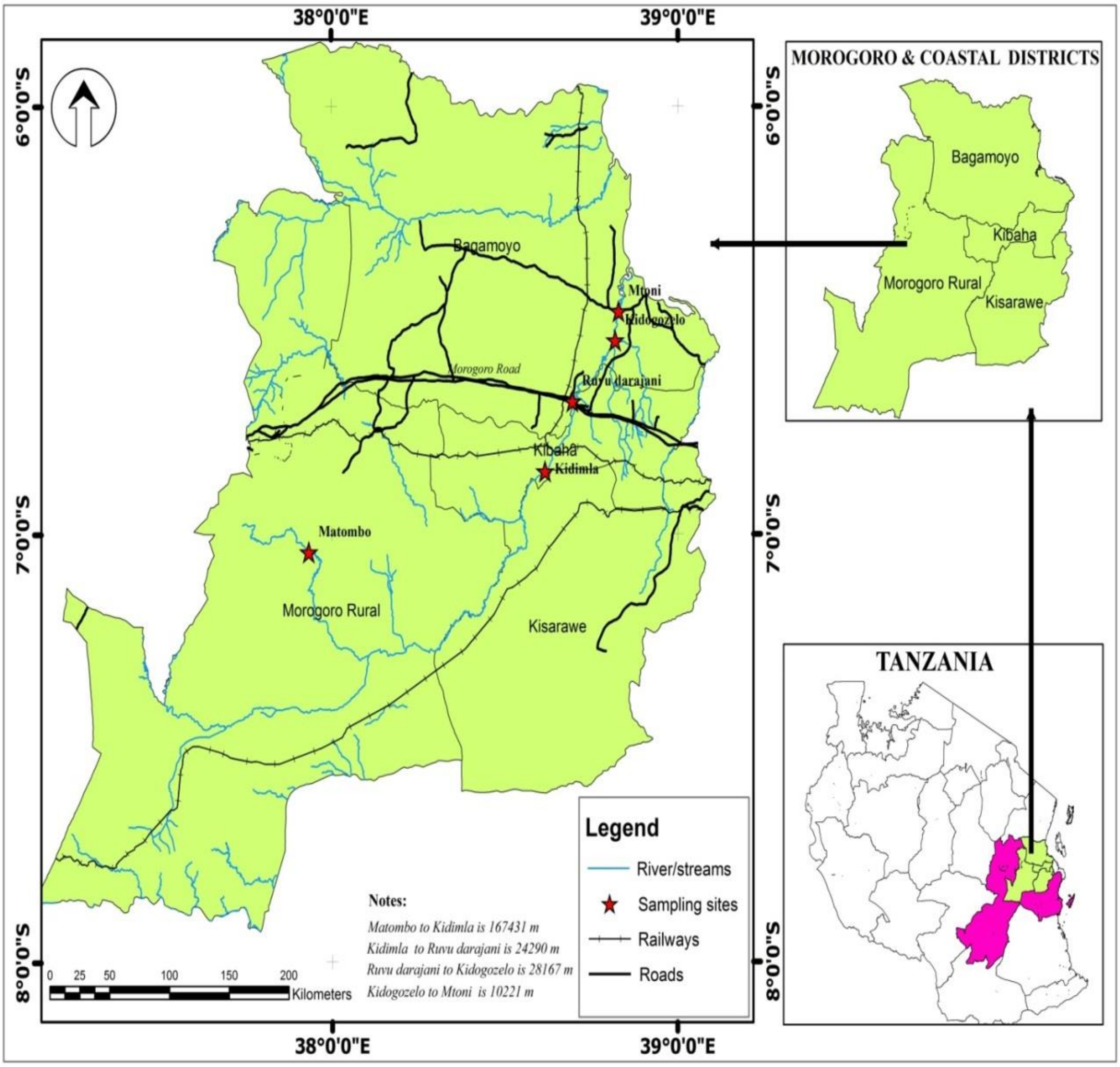

Figure 1: Map showing study/sampling sites and distance from one site to another.

Source: SUA- GIS department, (21/05/2017)

3.3 Study Design

Cross-sectional study (point cross sectional study) was employed so as to investigate associations between risk factors (pesticides use) and the outcome of interest (exposure effects). Thus, well-structured questionnaire was administered once to individuals who met the inclusion criteria to gather information from the study population.

3.4 Sampling and Sample Size Determination

3.4.1 Farmers sampling

The total number of farmers (N=427) were provided by Agricultural extension officers in the study area. The sample size of the study population (farmers) was then calculated according to a formula provided by Yamane, (1967) and a total of 206 farmers obtained. However, due to time and financial constraints, a total of 200 respondents were purposely selected. The formula use 95% confidence level and ± 5% precision.

$$n=N/1+N(e)^{2} \quad \text{………………………………..……………………} \quad (1)$$

Where; n is the sample size, **N** is the population size, **e** is the level of precision

3.4.2 Fish sampling

Fish samples were obtained from the four selected sites which highly involved in agriculture and marked as the hotspot for the anticholinesterase compounds contaminants in the river. The sample size was determined according to the recommendation made by Bocquene and Galgan, (1998) on the minimum number of samples required for assessment of cholinesterase activities (should be six to ten from each site). So with this study a total of 40 fish samples (eight samples per site) were collected from fishermen in four selected sites of the study area (Kidimla, Kidogozelo, Mtoni and Ruvu Darajani) and the control fish samples (eight samples) were collected from Matombo area, the site with low anthropogenic activities associated with pesticides usage (unpublished information).

3.5 Data Collection and Management

3.5.1 Questionnaire survey

Questionnaires were administered to farmers through face-to-face interviews from one respondent to another. The targeted respondents were the farmers who use pesticides or other agrochemicals in agricultural activities. The questionnaires were prepared in english then translated into kiswahili, because it is a national language which is understood by majority of the respondents in the study area**.** Both closed and open ended questions were included in the questionnaire in order to capture information regarding the farmer's demographic information that included farmer's location, age, gender, main occupation, marital status and level of education. Furthermore the questionnaire gathered the information concerning knowledge and the use of pesticides. To complement the questionnaire information gathered from farmers, in-depth interviews were conducted to the key informants (Agriculture and Livestock Extension officers from the study area).

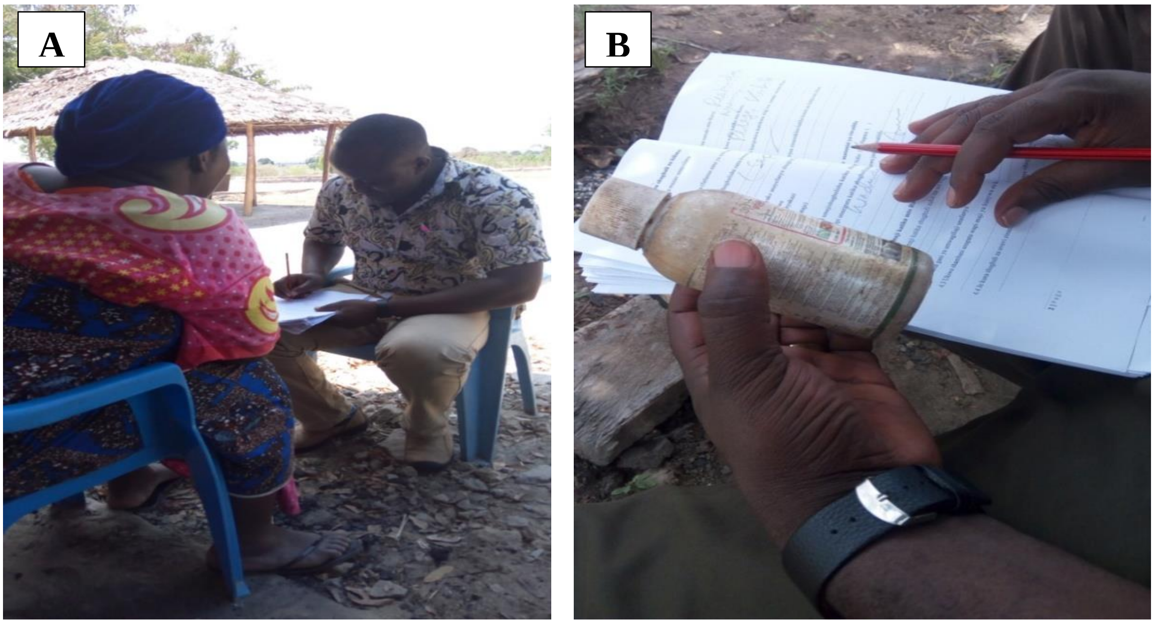

Plate 1: Administration of questionnaire (A); taking important information from the pesticide container (B) used by farmers

3.5.2 Fish samples

Live fish samples were obtained from fishermen from different hotspots in Ruvu river. Similarly, the fish for establishing the enzyme activity baseline (control) were obtained from the area with low anthropogenic activities related to agriculture and pesticide usage at Matombo. These fish samples were well kept in a ventilated tank and transported to Veterinary Physiology, Biochemistry and Pharmacology laboratory at Sokoine University of Agriculture (SUA) for further sample collection and analysis.

3.5.3 Collection, preparation and storage of samples

Fish was restrained manually and approximately two (2) ml of blood sample was collected directly from the caudal vein by inserting a sterile hypodermic 23G needle through the lateral line at the level of midpoint of the anal fin and immediately transferred into EDTA vacutainer tubes. Collected blood was centrifuged at 3000XG, 4°C, for 15 minutes. Blood plasma was separated and frozen at -21°C until analysis. Thereafter the fish was sacrificed by pithing and decapitating of the head. Fish heads were frozen at -21°C overnight. After overnight storage of the fish heads the whole brain was removed, weighed and immediately homogenized using Potter-Elvehjem homogenizer, in ice- cold 0.1M phosphate buffer pH 8.0 (1:5w: v**)**. Aliquot (3ml) of homogenates was stored at -21°C until analysis.

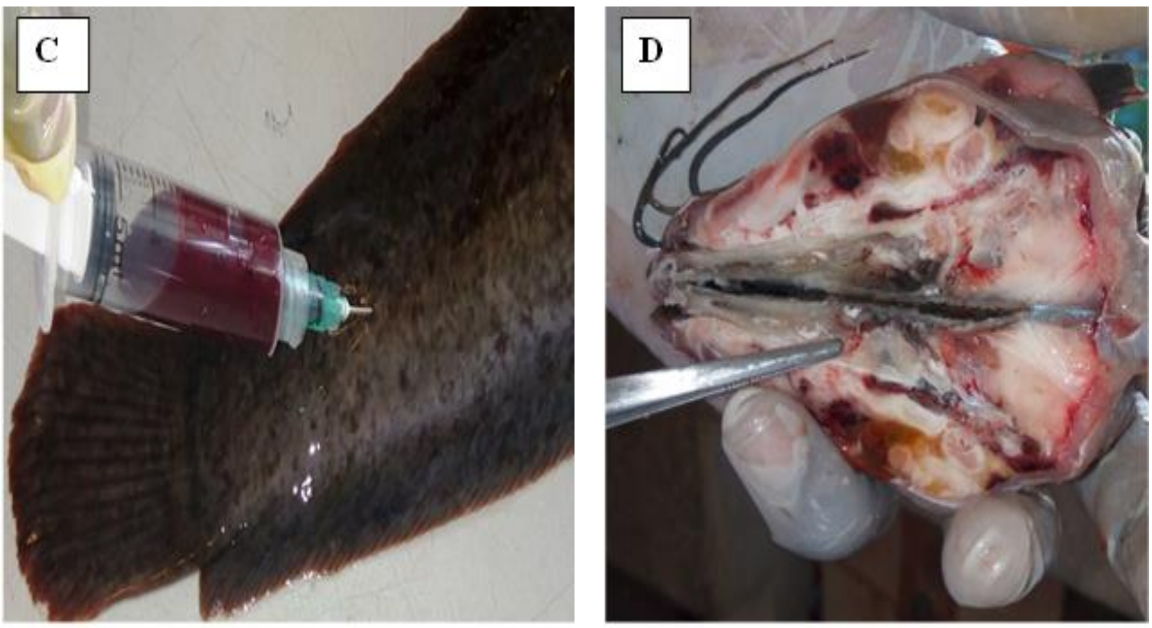

Plate 2: Collection of blood (C) and taking brain sample (D) from *Claria gariepinus*

3.5.4 Protein analysis in plasma sample and brain sample homogenates

The total protein concentration in plasma sample and brain sample homogenates was determined using a total protein concentration determination kit (Micro Lowry, Peterson's Modification) as described by Lowry *et al.* (1951) using lyophilised bovine serum albumin as a standard. All chemical used were obtained from Sigma Aldrich Company LTD and were of analytical grade.

3.5.5 Measurement of AChE and BChE activities in plasma and brain

Measurements of AChE and BChE activities were performed using a spectrophotometric method described by Ellman *et al.* (1961). The activities of these enzymes were determined in a way that 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 5, 5'-dithiobis-2-nitrobenzoic acid (DTNB) chromophore and 25 µL 45 mM acetylcholine iodide or 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 in the spectrophotometer (Cole Parma 1100 series, UNICO, Dayton New 22 Jersey, USA). The readings were done in duplicate in a single sample. Then the activities were calculated using Beer Lambert's Law with molar extinction coefficient of DTNB of 13 600 M^{-1} cm^{-1}. The percentage of enzymes inhibitions were derived by expressing the activity in the suspect samples exposed with pesticides and the activity in base line samples/control group.

3.6 Statistical Data Analysis

Collected data were summarized, coded and verified before the analysis. Statistical Package for Social Studies (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 in the questionnaire data, and also were used to present the laboratory data regarding to the cholinesterase activities into Mean and Standard Deviation. Testing of the means differences among the fish enzymes activities from the study sites and between the study sites and the control site were done using One way Analysis Of Variance (ANOVA) and T test with site as an independent variable. Also Duncan's Multiple Range Test (DMR) was used to compare the means of all four study sites. Significance differences were observed at $P < 0.05$.

CHAPTER FOUR

4.0 RESULTS

4.1 Questionnaire survey

4.1.1 Socio-demographic characteristics of respondents

Socio-demographic characteristic of respondents were characterized by age, sex, marital status and education level, so the outcome of the interviewee composition is indicated in Table1 below;

Table 1: Socio-demographic characteristics of respondents

Characteristics	Categories	Respondent (n)	Percentage (%)
Sex	Male	125	62.5
	Female	75	37.5
Age (Years)	Maximum	78	
	Minimum	18	
Marital status	Single	41	20.5
	Married	140	70
	Widowed	10	5
	Divorced	9	4.5
Education level	No formal education	49	24.5
	Primary education	138	69.5
	Secondary education	11	5.5
	College and high education	2	1

4.1.2 Age and sex of the respondents

The composition of the selected farmers who were involved in agriculture from the study area showed that 62.5% (n=125) of respondents were males and 37.5% (n=75) were females. With regards to age, the minimum and maximum age of the respondent who were involved in the study was 18 years and 78 years respectively. Also the mean age of respondent was 44.3±16.13 years.

4.1.3 Marital status and education level of the respondents

The marital status was characterized into; single, married, divorced and widowed. The majority of the respondents 70% (n=140) were married, 20.5% (n=41) were single, 5% (n=10) were widowed and 4.5% (n=9) were divorced. The distribution of marital status confirms that most agricultural activities are done by married farmers. Also the study showed that most of the respondents had primary education, 69.5% (n=138), whereas 5.5 % (n=11) had secondary education, and 1% (n=2) attended colleges and high education, while 24.5 % (n=49) did not have any formal education. This distribution showed that in Ruvu river basin the agricultural activities are highly practiced by farmers with primary education, while farmers with college and high education are less involved.

4.1.4 Pesticides knowledge sources and categories

All the respondents (100%) had knowledge on pesticides and got that knowledge from extension services and agrochemical shops; and also all reported to apply pesticides to their crops. The main groups of pesticides used included insecticides, fungicides and herbicides which are mostly used in vegetables. The primary sources of pesticides in the farming areas were; agrochemical shops (77.5%) and both agrochemical shops and fellow farmers (22.5%). In almost all study sites commonly used pesticides were organophosphates (73%), carbamates (4%), pyrethroids (5.5%), (organophosphates, carbamates, pyrethroids) (16%), and others (Inorganic copper, acylalanine, aryloxyalkanoic acid.) (1.5%). The fungicides group; Mancozeb, Victory and Glyphosate isopropylammonium (Kalachi®) were mostly used against crop fungi. Isopropylamine salt of glyphosate (Round up®) and 2, 4-dichlorophenoxy acetic acid (2, 4-D Amine) were the commonly used herbicides. Furthermore most of the pesticides were in Class II (moderately hazardous) and a few in Class III (slightly hazardous). Table 2 and 3 present different types and categories of the pesticides used by farmers in Ruvu river basin.

Table 2: Pesticides used by Farmers in Ruvu river basin

Pesticides type	Trade name	Common name	Chemical group	WHO hazard class
Insecticides	Dimethoate	Dimethoate	OP	II
	Karate	Cyhalothrinlamda	P	II
	Selecron	Profenos	OP	II
	Furadan	Carbofuran	C	Ib
	Rogor	Dimethoate	OP	II
	Dursban	Chlorpyrifos	OP	II
Fungicides	Farmzeb	Mancozeb	C	III
	Victory	Matalaxyl	A	NK
	Blue copper	Coppersulfate	Cu	III
	Ivory	Mancozeb	C	III
	Ridomil	Matalaxyl + Mancozeb	C	II
Herbicides	Round up	Glyphosate	OP	III
	Kalach	Glyphosate	OP	III
	2,4D	2-4 Amine	AA	II

Legend**:** OP-Organophosphate; C-Carbamate; P-Pyrethroid; Cu-Inorganic copper; A-Acylalanine, AA-Aryloxyalkanoic acid; Ib-highly hazardous; II-moderately hazardous; III-slightly hazardous; NK-Not know

Table 3: Categories of Pesticides used by Farmers in Ruvu river basin (N=200)

Agrochemical category	Frequency (n)	Percentage (%)
Organophosphates (OP)	146	73
Both C, P &OP	32	16
Pyrethroids (P)	11	5.5
Carbamates (C)	8	4
Others	3	1.5

4.1.5 Pesticides practices

Information regarding handling, applications and disposal of ineffective pesticides and pesticides containers were gathered during the study. Most respondents (58%) reported to use pesticides more than two times per cropping season. However, higher uses of pesticides were reported during the rainy season. Nevertheless, pesticides application mostly depended on presence of pests in different crops and their potential damages to the crop as well as farmers' perception regarding pest management practices. Most of the farmers (72%) applied pesticides by knapsack sprayers and few farmers (8.5%) used buckets. All farmers washed their sprayers, 26% (n=52) was done in river, 65.5% (n=131) within the farm plots while (8.5%; n=17) at home compound. Furthermore the study observed that (63%; n=126) of the respondents had encountered ineffective pesticides in their farming practice and the disposal of these pesticides was mostly done on farm by burying them (43%) while a few dispose them in rivers (3.1%). Expired pesticides were discarded in the farm by pouring them on the ground or burying (44.3%). Disposal of the empty pesticide containers was done mainly by throwing away on farms (43%; n=83) in river (26%; n=52) while (12%; n=24) dispose them in pit latrines and 19.0% (n=38) dispose them by throwing away in bushes.

4.1.6 Farmer's knowledge towards pesticides exposure

The majority of the respondents 76% (n=152) were aware of the effects of pesticides to the environment and living organisms and pointed out that such knowledge was acquired from agricultural extension officers and some of the agrochemical shops. Nevertheless 16.5% (n=33) of the respondents were unaware of any health hazards associated with the usage of pesticides, while few respondents 7.5% (n=15) pointed out that pesticides have no adverse effects on environment rather than to the target pests (Appendix 1). However the impacts of pesticides exposure reported by most of the respondents who were aware of health effects were eye itching, dizziness, headache, sneezing, skin irritation and stomach upset.

4.2 Laboratory Work Findings

4.2.1 Acetylcholinesterase and Butyrylcholinesterase activities in plasma

The assessment of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activities in plasma samples of the fish were performed in the selected four study sites, namely Kidogozelo and Mtoni in Bagamoyo district; Kidimla and Ruvu Darajani in Kibaha rural District. Also the control/baseline samples were obtained from Matombo site. Results showed that there were no statistical significant differences ($P > 0.05$) on the level of AChE and BChE activities in fish plasma samples among the four sites. When comparing the results from the selected sites and the control site, there was also no statistical significant difference ($P > 0.05$) observed on the level of AChE and BChE activities as shown in Fig. 2 and 3 respectively.

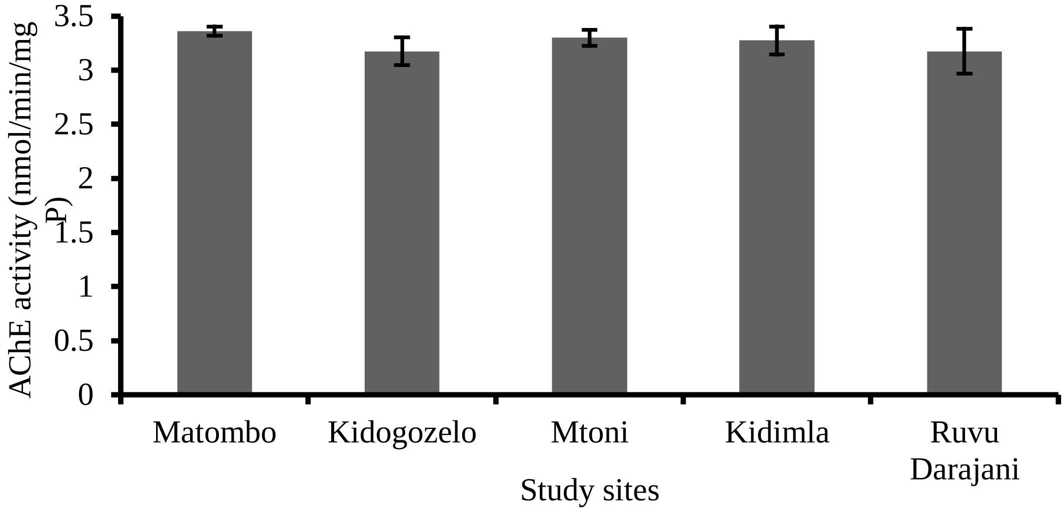

Figure 2: Acetylcholinesterase activities in plasma samples

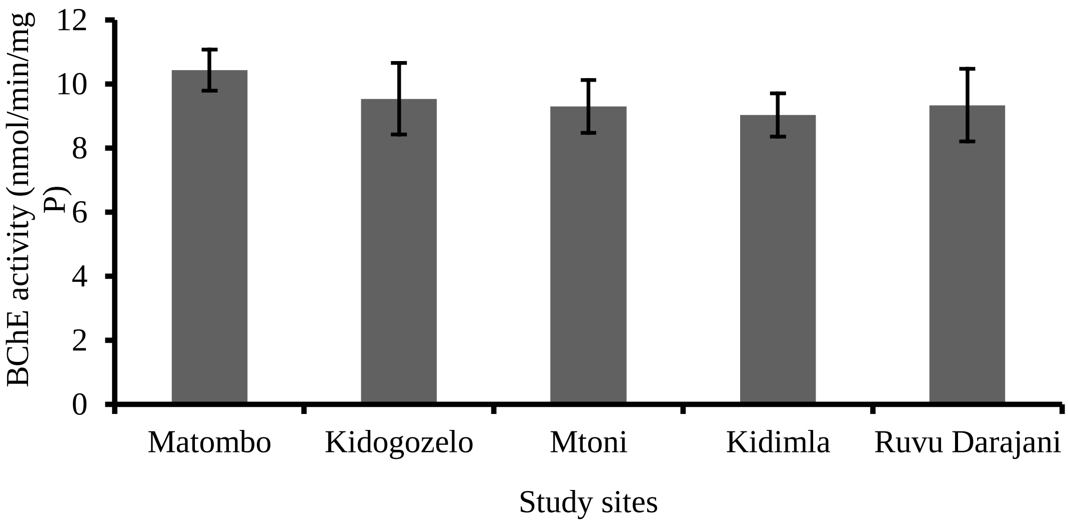

Figure 3: Butyrylcholinesterase activities in plasma samples

4.2.2 Acetylcholinesterase and Butyrylcholinesterase activities in brain

The activities of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) in brain samples were analyzed from the fore mentioned sites as in plasma samples. Generally AChE activities showed no significant difference ($P > 0.05$) among the four selected study sites. However a slightly significant difference of AChE activities were observed between

Kidogozelo and Ruvu darajani with *P* = 0.06. The levels of AChE activities were high at Kidogozelo and low at Ruvu Darajani (Fig.4). The significance (*P* = 0.032) was observed between the AChE activities in fish from Ruvu Darajani and that of the control site. For BChE activities, no significant difference was observed among the fish from the study sites and between the fish from the site and the baseline, the activities of fish from these sites are presented in Fig. 5.

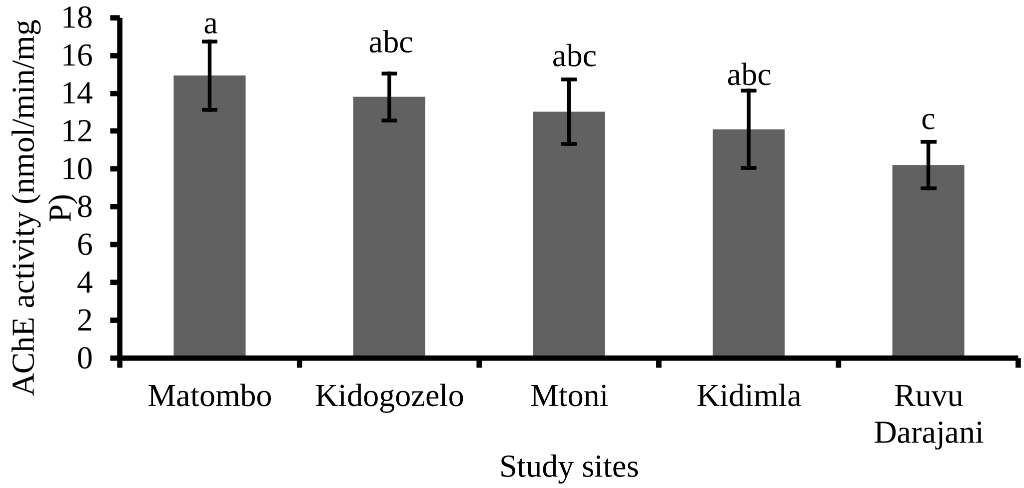

Figure 4: Acetylcholinesterase activities in brain samples. The letter superscript different from control indicates significant difference (*P* < 0.05).

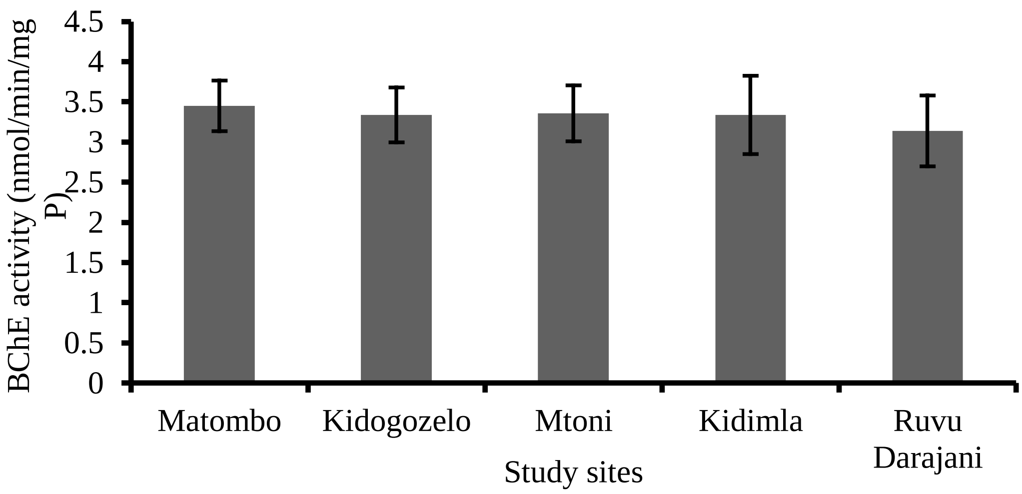

Figure 5: Butyrylcholinesterase activities in brain samples

CHAPTER FIVE

5.0 DISCUSSION

5.1 Socio-demographic Characteristics of the Study Population

The socio-demographic characteristics of the study population namely, age, sex, marital status and education level were gathered during the study and the findings reveal that, farming activities were done by farmer of age ranging from 18 to 78 years, though most age group that involved in farming was 40-65years (76.0%) and 20-39 years (21.0%) were few. The less involvement of young aged community member was reported from adult farmers that they have moved to town/cities looking for other jobs and claiming that farming is not paying as other jobs. Also other farmers added that youth have engaged in motorcycle driving and left farming issues to the adults. These findings are comparable with the work by Ngowi *et al.* (2007) who found high involvement of adult in farming compared to youth.

The current study shows that the majority of the respondents who were involved in farming were mostly male, compared to females and the level of education to most of the respondents were primary education level, few had attended colleges and high education, and some did not have any formal education. This distribution showed that in Ruvu river basin the agricultural activities are highly practiced by farmers with primary education, while farmers with college and high education are less involved. Due to this, the respondents might not be able to read or understand pesticide and other agrochemical label and instructions since very few are written in swahili. Hence this can be associated with mishandling and misuse of pesticides as observed in this study. Similar results were also reported by Nonga *et al.* (2011), who explained that poor education background among the users of pesticides and other agrochemicals contribute to mishandling and misuse of pesticides. Mekonnen and Agonafir, (2002), also reported that pesticides exposure and mishandling was attributed to language barriers in communities with primary education among pesticides sprayers and farmers. Also, it was observed that involvement of married respondents in farming might be the pressure to meet the family needs as a way to generate income.

5.2 Pesticides usage in study population

With regard to pesticides usage in the study population, all the interviewed farmers had a general knowledge on pesticides usage which was acquired from agrochemical shops and extension officers. Again all respondents were applying pesticides to their crops and the primary sources of pesticides in the farming areas were agrochemical shops (77.5%), and among the commonly mentioned pesticides in the study area were in the group of organophosphates (73.0%) such as dimethoate, profenos and chlorpyrifos. The later and other mentioned in Table 3 and 4 follow under class II (moderately hazardous) and few in class III (slightly hazardous). This finding agreed with the study done by Nonga *et al.* (2011) who found out that the highest proportions and quantities of insecticides used were organophosphates and pyrethroids and (90%) of agrochemicals in the farming area of Lake Manyara were from agrochemical shops while majority of the pesticides used by farmers were in class II and a few in class III or U (unlikely to present acute hazard). The study also found that most respondents use pesticides more than two times per cropping season. This was associated with increase of pests as observed in the study area. This finding concur with the previous study by Youdeowei, (1989) who found that yield losses by insects, diseases, weeds and birds are estimated to vary between 10 and 90% in sub-Saharan Africa and this loss became stimuli for farmers to increase usage of agrochemicals in their farming practice.

Additionally, it was noted that there was improper handling and poor disposal of the pesticides containers, expired pesticides and ineffective pesticides. Also cleaning of the sprayers was done at farm plots and few in the river. These findings concur with the previous study done by Mdegela *et al.* (2010) who reported the mishandling of agrochemicals leftovers which were disposed either by throwing away on the farms, burying them or throwing them in rivers. Also majority of the respondents were aware of the effects of pesticides to the environment and living organisms, though few respondents noted to be unaware of health hazards associated with the usage of pesticides in human, environment and to the non-target pests. This is also reported by Nonga *et al.* (2011) who found that majority of the respondents (76.0%) were unaware of the effects of pesticides to the environment. Therefore ignorance of health hazards due to pesticides exposure may cause health risk to the organisms since some of the pesticides are classified to be carcinogenic, cholinesterase inhibitors and others suspected to be endocrine disruptors as reported by Lema *et al.* (2014).

Likewise, the impacts of pesticides exposure reported by most of the respondents in this study who were aware of health effects were eye itching, dizziness, headache, sneezing, skin irritation and stomach upset. Similar findings were also reported by Ngowi *et al.* (2007). Furthermore, the studies carried out in Indonesia (Kishii *et al.* 1995) and in Côte d'Ivoire (Ajayi, 2000) reported that, pesticide applicators tended to accept a certain level of illness as an expected and normal part of the work of farming. Therefore, good pesticides practice should be channelled to farmers as pesticides may put them to the risk of various forms of cancer, birth defects, sterility, damage of liver, kidney, neural organs and deaths as reported by Ngowi, (2002); McCauley *et al.* (2006); Soltaninejad *et al.* (2007); Weiss *et al.* (2007); Aktar *et al.* (2009).

5.3 Assessment of Cholinesterase Activities in Fish

In this study, cholinesterase (acetylcholinesterase; AChE and butyrylcholinesterase; BChE) activities were performed in plasma and brain samples in fish as the indicator for the level of contamination of the environment with several anticholinesterase substances such as pesticides and heavy metals. African sharptooth catfish (*Clarias gariepinus)* were used in the study and were obtained from different location of the study area. Different sexes (male and female catfish) were used since cholinesterase enzymes have no sex dependency as reported by Mdegela *et al.* (2010). Also, Galgani and Bocquené (1992) established no differences in the level of cholinesterase enzymes activities between male and female *Limanda limanda* in the Atlantic Ocean. Therefore, it is recommended to use either sex when assessing the cholinesterase enzymes activity and the results can be generalised among sexes within the same species.

5.4 Cholinesterase Activities in Plasma and Brain

Following analysis of cholinesterase enzymes activities, it was observed that the AChE activities were higher in the brain than in the plasma and BChE activities were higher in plasma than in brain. Study done by Frasco *et al*. (2010) also noted highest activities of AChE in the brain and muscle of fish. Akman *et al.* (2009) showed the predominance of the AChE in brain and muscle tissues, whereas BChE mostly predominate the liver and plasma. Furthermore, Jebali *et al*. (2013c) established tissue distribution rank of AChE activity in *Solea solea* fish and noted the highest levels in the brain followed by the gills, kidney and lowest in the liver.

The findings of AChE and BChE activities in plasma and BChE activities in brain showed no statistical significant difference ($P > 0.05$) among the fish from the study sites and between fish from the study sites and the baseline. No statistical significant difference in brain AChE was observed among the fish from the study sites. The significant difference observed in fish from Ruvu Darajani when AChE activities were compared with that of control fish. The level of enzymes activities was found to have a slight different from one site to another. These findings concur with the study done by Bodin *et al.* (2004); Bocquene *et al.* (2004) who found that in the field, several species appeared to have similar AChE activities of the same order of magnitude in different studies/measurements. Again the study conducted to *Mytilus edulis* and *Macoma balthica* from the northern Baltic Sea, found that the mean values of AChE values vary two-fold depending on season, following closely changes in temperature (Leiniö and Lehtonen, 2005). Likewise seasonal variability has also been shown as different responses to natural factors in coastal areas compared to offshore sites (Burgeot *et al*., 2006). Thus to avoid such differences during cholinesterase activities interpretation, samples used in this study were collected during the same season.

5.5 Inhibition of Cholinesterase Activities in Samples from Different Sites

This study showed the highest inhibition of the AChE in the brain compared to the BChE inhibition. Same findings were reported by Kamel and Hoppin (2004) who established that the AChE inhibition is more sensitive than BChE in the case of chronic exposure to organophosphate and reasoned out that AChE inhibition by OPs showed a lower recovery rate compared to BChE and this produces cumulative inhibitory effect on the AChE activity. This observation is also 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 anticholinesterases contaminants.

Moreover, this study found that highest inhibition of the AChE activities in brain (31.7%) were observed in fish samples from Ruvu Darajani site whereas the lowest AChE activities inhibition (7.6%) were observed in fish from Kidogozelo site. In plasma samples the highest inhibition of the AChE activities were observed in fish from Ruvu Darajani and Mtoni sites with the inhibition of 5.4% whereas the lowest enzyme inhibition was observed in samples from Kidogozelo (1.8%). Again the inhibition of BChE activities was highest in fish from Ruvu Darajani site with the inhibition percentage of 13.4 and 9.0 in plasma and brain respectively. These enzymes activities inhibitions are speculated to be associated with mishandling and poor disposal of pesticide, pesticide containers and cleaning of the sprayers as observed in the study site. Findings by Zinkl *et al*. (1987); Busby *et al*. (1989) explained the 20% reduction in AChE activity in fish and invertebrates indicated exposure to neurotoxic compounds. While, Wright and Welbourn (2002) reported reduction of brain AChE activity of more than 20% in birds, fish and invertebrates indicated exposure to OPs or carbamate pesticides, and more than 50% reduction as a life-threatening situation.

Furthermore, FAO (2007) reported that 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. However 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*., 2004 and Ferrari *et al*., 2007). On other hand some authors have established that 50% of AChE inhibition could indicate intoxication or poisoning (Dembélé *et al*., 2000). Gruber and Munn, (1998) reported that cholinesterase inhibition of more than 70–90% at sub-lethal concentrations of organophosphates and carbamates has been observed in fish species such as Common carp.

Generally, the usage and mishandling of anticholinesterase compounds along the river in the study area may cause aquatic environmental contamination and this can be related to the highest inhibition of brain AChE in fish from Ruvu Darajani site where multiple annual cropping accompanied with usage of pesticides were highly observed during the study. This finding is supported by Ozmen *et al*. (2007) who showed that strong relationship between AChE inhibitions in the brain of C*yprinus carpio* collected from Sariyar Dam Lake (Turkey) and the organophosphate pesticides and their residues in water and sediments. In other field investigations using AChE as neurotoxicity biomarker, Lavado *et al*. (2006) reported that AChE was strongly inhibited in the muscle of *C. carpio* sampled from some stations of Ebro river (Spain) highly polluted by organophosphorus, carbamates and heavy metals.

Lastly, it was estimated that less than 0.1 percent of a pesticide that applied reaches target pest and 99.9 % become unintended environmental contaminants to the non-target organisms which may lead to enzyme inhibition, endocrine disruption, and carcinogenic effects (Khan *et al.,* 2003). Therefore from the above discussion, AChE and BChE (Cholinesterase) activities in *C. gariepinus* suggested to be potential biomarkers for assessment of environmental contamination as a result of anticholinesterase compounds exposure.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

From this study, it can be concluded that agricultural activities in Ruvu river basin were done mostly by males with primary level of education. This suggests that farming activities are done by farmers with low level of education, thus related to poor knowledge on all matters associated with pesticide usage. This contributed to inappropriate use, poor handling and disposal of pesticides and their leftovers. Therefore due to mismanagement of the pesticides, the respondents and community in the study area are at high risk of health complications associated with pesticides.

Furthermore, following assessment of cholinesterase activities; acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) in the plasma and in the brain tissue of *Claria gariepinus*, it was noted that the highest inhibition of the AChE activities were observed in fish from Ruvu Darajani and the enzymes activities were also significantly low at the same site as compared to the fish from the control site. This indicates that Ruvu Darajani site is the mostly contaminated site with anticholinesterase compounds than others in this study. These findings further stress that AChE and BChE (Cholinesterase) activities in *C. gariepinus* are used as potential biomarkers for assessment of environmental contamination as a result of anticholinesterase compounds exposure.

6.2 Recommendations

Since most farmers in the study area have poor knowledge on the handling and use of pesticides in agriculture which leads to environmental contamination, the following recommendations are made;

i. Farmer's trainings on efficient and safe use of agrochemicals are needed so as to reduce environmental contamination due to agrochemicals hence minimizes the likely agrochemical undesirable effects.

ii. Since agriculture in the study area is dominated by males, and adult aged 40-65 years, then females and youth need to be encouraged to participate in agriculture in the area as a means of adding to their income and improving their living standard.

iii. Water quality assessment in Ruvu river is needed in order to ascertain quantity of dissolved chemicals as a means of safeguarding aquatic species and public health.

iv. Periodical assessment of AChE and BChE activities are needed to be done in order to ascertain the peak of much environmental contamination due to anticholinesterase compounds.

v. More studies are needed in order to determine other neuro-toxicity effects due to anticholinesterase compounds exposure in *C. gariepinus* and other aquatic species.

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APPENDICES

Appendix 1: Farmers knowledge and pesticides practices at Ruvu river basin

Variable	Respondent (n)	Percentage (%)
Pesticides application		
One time per cropping season	41	20.5
Two times per cropping season	43	21.5
More than two times per cropping season	116	58
Pesticides spraying device		
Knapsack sprayer	144	72
Hand sprayer	39	19.5
Bucket	17	8.5
Sprayer washing		
River	52	26
Farm plot	131	65.5
Home compound	17	8.5
Ineffective pesticides disposal		
River	4	3.1
Bushes	35	27.3
Pit latrine	34	26.5
Burying in farm	55	43
Expired pesticides disposal		
River	3	3.1
Pit latrine	25	25.8
Bushes	26	26.8
Burying in farm	43	44.3
Pesticides container disposal		
River	52	26
Pit latrine	24	12
Bushes	38	19
Burying in farm	86	43
Knowledge on Pesticides effects		
Yes	152	76
No	15	7.5
Don’t know	33	16.5

Appendix 2: Questionnaires for agricultural farmers in English version

Introduction:

Dear! You are invited to participate in the interview aimed at gathering information concerning with usage of Pesticides/Agrochemicals in agriculture. **YOU ARE WELCOME!**

1.0: General demographic information

1.1 Record Number…………………….. Date of interview: ___/___/____

1.2 District: ……………………Ward: ……………Street/Village: ………………

1.3 Interviewer's name: ……………………………………………………………….

1.4 Interviewee name……………………………………………………………………….

1.5 Phone number…………………………………………………………………..

1.6 Start time __________ Finish time ________

2.0: Social Economic Characteristics

S/N	Question	Coding answer
2.1	Sex	[ ]Male: [ ] Female
2.2	Age of the respondent (years)	Year of birth / Age…………….………..……
2.3	Highest level of education	1.No formal education: 2.Primary education 3.Secondary education: 4.Others (Specify)
2.4	Marital status	1.Single: 2.Married 3.Divorced 4 Widowed
2.5	What is your main occupation?	……………………………….………………

3.0: The following statements concern the knowledge on handling of pesticides

3.1 What types of crop you are cultivating? A. Maize B. Rice C. Vegetables, others (types)

3.2 At which season you are involved much in farming

3.3 How long you have involved in farming activities

3.4 What are the challenges you are facing in farming

4.0. Water use at Ruvu river basin

4.1 What are the sources of water for agricultural activities?

4.2 What kind of device you are using for irrigation?

4.3 Where do you get water for drinking /cooking when at farm plot?

4.4 Is there fishing activities at Ruvu river

4.5 What type of fishing method is employed?

4.6 What types of fish found at Ruvu river

4.7 Is there any health problems associated with using fish from Ruvu river

4.8 What other activities are taking place at Ruvu river basin

5.0 Knowledge and practices on application of Pesticides

5.1 Do you know pesticides? 1. Yes [ ] 2. No [ ]

5.2 If yes where do you get that knowledge?

5.3 Have you ever applied Pesticides? 1. Yes [ ] 2. No [ ]

5.4 If Yes what types? and for which crop?

5.5 Where do you obtain your pesticides?

5.6 Are they in original container?

5.7 How often you apply the Pesticides on crops

5.8 How do you maintain proper mixing of the pesticides before application?

5.9 Which device you are using for spraying?

A. Hand sprayer B. Knapsack sprayer C. Bucket D. Others

5.10 Are you washing the device above after use? 1. Yes [ ] 2.No [ ]

5.11 If yes where?

5.12 Where do you normally throw the resulted water?

5.13 Where do put the pesticides containers after using them

5.14 Have you ever encountered pesticides that are ineffective? 1. Yes [ ]
2. No [ ]

5.15 If yes in qn 5.14 did you stop using them

5.16 If yes where did you put them?

5.17 Have you encountered expired pesticides?

5.18 Where did you pit them?

5.19 Do you think that pesticides have any adverse effects to aquatic organisms? 1. Yes [ ] 2. No [ ]

5.20 If Yes in qn above, which one do you think so?

THANK YOU FOR YOUR COOPERATION

Appendix 3: Swahili version: Dodoso linalohusu wakulima wa mazao

Utangulizi.

Ndugu! Unakaribishwa kushiriki katika mahojiano yanayohusu uelewa kuhusu matumizi ya viwatilifu , Hivyo ntakuuliza maswali mbali mbali na naomba ushirikiano wako.

KARIBU!

1.0 Taarifa za msailiwa

1.1 Namba ya dodoso…………………….. Tarehe ya kusailiwa ___/___/____

1.2 Wilaya: …………………… Kata……….……….. Mtaa/Kijiji: ………..………

1.3 Jina la msaili: ……………………………………………………….……

1.4 Jina la msailiwa……………………………………………………………..

1.5 Namba ya simu……………………………………………….…………..

1.6 Muda wa kuanza usaili __________ Muda wa kumaliza usaili ________

2.0 Sifa za msailiwa

N	Maswali	Namba ya swali
2.1	Jinsi	[ ]Mme [ ] Mke
2.2	Umri /mwaka wa kuzaliwa	Mwaka……………………………………………
2.3	Kiwango cha juu cha elimu	1. Hajasoma 2. Elimu ya msingi 3 .Elimu ya sekondari 4. Elimu nyingine (fafanua)
2.4	Hali ya ndoa	1. Hujaoa/Hujaolewa 2.Umeoa/Umeolewa 3. Mmeachana 4.Mjane/Mgane
2.5	Kazi yako kuu ni nini?	………………………………….……………..

3.0 Taarifa kuhusu shughuli za kilimo

3.1 Ni aina gani ya mazao unayolima?

[A]. mahidi [B]. mpuga [C]. mbogamboga (fafanua aina ya mboga)

3.2 Ni msimu gani unajishughulisha sana kwenye shughuli za kilimo?

3.3 Ni kwa muda gani umejishughulisha katika shughuli za kilimo?.

3.4 Ni changamoto zipi unazipata katika shughuli zako za kilimo?(taja kwa ukubwa wa changamoto

4.0 Matumizi ya maji katika mto Ruvu

4.1 Unatoa wapi maji katika shughuli zako za umwagiliaji/kilimo

4.2 Ni aina gani ya umwagiliaji unafanya

4.3 Ukiwa shambani unapata wapi maji ya kunywa au kunawa au kupikia

4.4 Je kuna shughuli za uvuvi zinazoendelea katika bonde au katika mto Ruvu

4.5 Mbinu gani za uvuvi zinazotumika kuvua samaki mto Ruvu

4.6 Aina gani ya samaki wanaopatikana kwa wingi katika mto Ruvu

4.7 Je kuna madhara yoyote yaliyowahi kuripotiwa kutokana ulaji wa samaki wa mto Ruvu

4.8 Je ni shughuli gani nyingine unazojua zinaendelea katika bonde au katika mto Ruvu

5.0 Uelewa wa wakulima kuhusu matumizi ya viwatilifu

5.1 Unafahamu viwatilifu ni nini? Ndio [ ] Hapana[ ]

5.2 Kama ndio umepata wapi uelewa juu ya viwatilifu

5.3 Umewahi kutumia viwatilifu? Ndio [ ] Hapana [ ]

5.4 Kama ndio ni aina gani na kwa ajili ya nini

5.5 Unapata wapi hivyo viwatilifu

5.6 Viwatilifu vinakuwa kwenye vifungashio vya aina gani?

5.7 Ni mara ngapi unatumia viwatilifu kwenye shamba

5.8 Unatumia nini kuchanganya viwatilifu kabla ya kupulizia mazao?

5.9 Kifaa gani unatumia kupulizia?

A. Pumpu ya mkono

B. Pampu ya mgongoni

C. Ndoo.

D. Nyinginezo (fafanua)

5.10 Unaosha hicho kifaa baada ya matumizi? Ndio [ ] Hapana [ ]

5.11 Kama ndio uaoshea wapi hicho kifaa?

5.12 Unamwaga wapi maji uliyo oshea kifaa hicho?

5.13. Unaweka wapi vifungashio vya viwatilifu baada ya matumizi?

5.14 Umeshawahi kupata viwatilifu ambavyo havifanyi kazi katika shamba

1. Ndio [ ] 2. Hapana [ ]

5.15 Kama ndio katika uliendelea kuvitumia au uliacha

5.16 Kama uliacha ulivitupa wapi baada ya hapo?

5.17 Ulishawahi kuwa na viwatilifu vilivyoisha muda wake wa matumizi?

1. Ndio [ ] 2. Hapana [ ]

5.18 Kama ndio uliweka wapi viwatilifu vilivyoisha muda wake?

5.19 Unafikiri viwatilifu vina madhara kwa viumbe hai.

1. Ndio [ ] 2. Hapana [ ] 3. Sijui [ ]

5.20 Kama ndio ni madhara gani unafikiri?

ASANTE SANA KWA USHIRIKIANO