2020-08

Biomonitoring of pesticides exposure and the fate of pesticides use among smallholder vegetable producers in Tanzania

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BIOMONITORING OF PESTICIDES EXPOSURE AND THE FATE OF PESTICIDES USE AMONG SMALLHOLDER VEGETABLE PRODUCERS IN TANZANIA

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A Thesis Submitted in Fulfillment of Requirements for the Degree of Doctor of Philosophy in Life Sciences of the Nelson Mandela African Institution of Science and Technology

Arusha, Tanzania

August, 2020

ABSTRACT

This study assessed drivers of increased and changing patterns of pesticide use, levels of Acetylcholinesterase (AChE) inhibition, associated health effects and co-exposure risks of pesticide residues and bacterial contaminants in fresh vegetables. A total of 613 vegetable samples were collected from Arusha, Kilimanjaro, Manyara, Dar es Salaam, Morogoro and Iringa, regions. Binary probit models were used to analyze factors fostering increased pesticide use, determinants of pesticide exposure and risks of co-exposure. Significant results were accepted at $p < 0.05$. Results revealed that most farmers (88.9%) were unaware of pesticide safety practices. Compared with previous studies, there was increased trend in pesticide use (58.4%), which was accompanied by changing pesticide formulations. The number of crops grown ($p = 0.002$), pesticide mixing ($p = 0.012$) and region ($p = 0.001$) contributed positively to likelihood for increased pesticide use. Smallholder farmers were found to be occupationally exposed to pesticides, where exposed farmers had significantly lower AChE levels. The number of exposure symptoms (14.10±7.70) was higher in exposed than unexposed farmers. Self-reported symptoms were also confirmed to correlate with lower AChE and the use of personal protective equipment did not significantly reduce exposure. Women, younger and older farmers, underweight, overweight, and obese farmers were at increased risk of pesticide exposure. Moreover, locally produced fresh vegetables were highly contaminated with pesticide residues, 47.5% had detectable levels of pesticide residues, 74.2% of which recorded average residue levels above Codex Maximum Residue Levels (MRL) standards. Multiple pesticide residues were also detected, these included organophosphates (95.2%), organochlorines (24.0%), pyrethroids (17.3%) and carbamates (9.2%), all constituting the main detected pesticide residues. Consequently, bacterial contamination of fresh vegetables was also evident, with prevalence of bacterial contamination being high (63.2%). Enterobacter (55.6%), *Pseudomonas aeruginosa* (32.4%), *E. coli* (28.2%), Citrobacter (26.8%), *Klebsiella oxytoca* (14.8%) and Salmonella (7.7%) were isolated. 46.4% of tested samples were positive for both pesticide residues and bacterial contaminants. Vegetables from farms (60.7%) contained more bacterial contaminants while vegetables with pesticide residues were about twice more likely to be contaminated with bacteria (OR: 2.231; 95% CI: 0.501, 8.802). Findings from this study also showed extensive use of pesticides, bacterial contamination and exposure among small holder farmers. The observed exposure risks pose short and long-term effects on health of both farmers and general population. The contamination levels of pesticide residues and bacterial contaminants could also be perceived as a serious health problem, as most fresh and vegetables recorded values of pesticide residues far above the Maximum Residue Limits (MRLs) with pathogenic bacteria isolated in higher proportions. Maximum Residue Limits were higher in most vegetables that were consumed raw or semi-cooked. There is therefore an urgent need to develop pesticide monitoring and surveillance systems at farmers' level by educating farmers and promoting the use of greener pesticides to mitigate the health effects of pesticides and bacterial contaminants.

DECLARATION

I, Jones Ackson Kapeleka, do hereby declare to the Senate of Nelson Mandela African Institution of Science and Technology that this thesis is my own original work and that it has neither been submitted nor being concurrently submitted for degree award in any other institution.

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CERTIFICATION

The undersigned certify that they have read and hereby recommend for examination of the thesis entitled “Biomonitoring of pesticides exposure and the fate of pesticides use among smallholder vegetable producers in Tanzania”, in fulfillment of the Degree of Doctor of Philosophy in Life Sciences at the Nelson Mandela African Institution of Science and Technology

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ACKNOWLEDGMENTS

I am very much grateful to the Tanzanian Government through my employer, Tropical Pesticides Research Institute (TPRI) for granting me a study leave to pursue my PhD at the Nelson Mandela Institution of Science and Technology. I am very much thankful to my supervisors, Prof. Patrick Ndakidemi, Dr. Elingarami Sauli Nkya, and Prof. Omowunmi Sadik for their unfailing support, guidance, encouragement from the very beginning to the end of my study. I'm grateful for the support from CREATES Centre of Excellence for sponsoring my research. I also acknowledge the Acting Director of TPRI, Dr. Margret Mollel for fully providing lab space and utilization of the GC-MS machine, analytical standards and other chemical reagents for the analysis, detection and quantification of pesticides residues. I also appreciate Dr. Osmund Ndomba, the pathologist from TPRI for technical support and allowing utilization of laboratory equipment for isolation, culturing and identification of bacterial contaminants.

I am grateful for the kind support provided and participation of District Agricultural officials and Market Managers from Arusha, Kilimanjaro, Iringa, Morogoro, and Manyara together with their respective village Agricultural Extension Officers for their ready contribution with sample collection from the markets and farms. I most sincerely thank the management of the National Medical Research Institute (NIMR) for providing ethical clearance to undertake this study, more specifically on the biomonitoring of exposure.

My heartfelt appreciations are extended to Tanzania Horticultural Association (TAHA) and Rikolto East Africa for funding two major aspects of this study, pesticides residue and bacterial contamination. Specifically, I appreciate Ms. Jacquline Mkindi (CEO-TAHA), Regional Director of Rikolto Mr. David Leyssens, the Country Manager Mr. Kain Mvanda. the Senior Agribusiness Advisor Mr. Shukuru Tweve as well as Mr. Eric Mwesigwa from TAHA for their unfailing logistical support that made fieldwork successful.

DEDICATION

This work is dedicated to the Highest God Jah Rastafari, ever living, ever loving, ever sure

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LIST OF ABBREVIATIONS AND SYMBOLS

AChE	Acetylcholinesterase
AOAC	Association of Official Analytical Chemists
BMI	Body Mass Index
CA	Carbamates
CREATES	Centre for Research , Agricultural Advancement, Teaching Excellence and Sustainability
DAP	Dialkyl phosphate
DDT	Dichlorodiphenyltrichloroethane
DEDTP	Diethyl dithiophosphate
DEP	Diethyl phosphate
DETP	Diethyl thiophosphate
DMDTP	Dimethyl dithiophosphate
DMP	Dimethyl phosphate
DMTP	Dimethyl thiophosphate
EFSA	European Food Safety Authority
EPA	Environmental protection agency
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
GAP	Good Agriculture Practices
GC-MS	Gas Chromatography-Mass Spectrometer
GC-MS	Gas Chromatography-Mass Spectrometer
HCB	Hexachlorobenzen
HCH	Hexachlorocyclohexane
HPLC	High Performance Liquid Chromatography
LOD	Limit of Detection
LOQ	Limit of Quantification
MRL	Maximum Residue Levels
NA	Nutrient Agar
NIMR	National Medical Research Institute
OPs	Organophosphates
PSA	Primary and Secondary Amine
ROS	Reactive Oxygen Species

RSD	relative standard deviation
TAHA	Tanzania Horticultural Association
TPRI	Tropical Pesticides Research Institute
TSA	Tryptic Soy Agar
TSB	Tryptic Soy Broth
WHO	World Health Organization
XLD	Xylose Lactose Deoxycholate

CHAPTER ONE

INTRODUCTION

1.1 Background of the problem

Globally, pesticides use has increased extensively in agricultural production to prevent and control pests, diseases, weeds and other plant pathogens as farmers' efforts to reduce or eliminate yield losses and get high product quality (Eskenazi *et al.*, 2008; Sanborn, Cole, Helena & Bassil, 2004). In developing countries, mostly African based, carbamates, organophosphates and pyrethroids are the most used families of pesticides (Pastor *et al.*, 2003). The use of these pesticides for insect control is mandatory in many cases and will remain so until future developments permit increased reliance on safer methods. Most benefits of pesticides are based on direct crop returns and do not include indirect health, environment and economic costs associated with them (Pimentel, 2005). Pesticide consumption is estimated, to be two million tons per year. Europe alone accounts for 45%, of the global pesticide consumption, USA accounts for 25% while other parts of the world consume 25% of pesticides (De, Bose, Kumar & Mozumdar, 2014). In Africa, pesticides use accounts for 2 – 4% of the global pesticide use with a market value of 31 billion US Dollar (Williamson, Ball & Pretty, 2008). The average active ingredient/hectare (ai/ha) use of pesticides estimated at 1.23 Kg a.i/ha is low in Africa compared to those of Latin America and Asia estimated at 7.17 and 3.12 Kg a.i/ha for Latin America and Asia respectively (Repetto & Baliga, 1996).

In Tanzania, pesticides used in livestock and agricultural sectors are estimated to be 81% of total pesticides volumes, while 18% is used in the public health sector and 1% is used in other areas including protecting buildings from damage caused by insect pests (Agenda, 2012). By 2016, Tanzania had registered more than 1043 different insecticides formulations, 570 herbicides and 507 different types of fungicides, mainly used in vegetable production (TPRI Gazette, 2016). These pesticides are highly used in areas where coffee, fruits and vegetable farming are practiced. Smallholder vegetable farmers depend heavily on the use of these pesticides for the control of different pests and diseases (Lekei, Ngowi & London, 2014). This is probably because they believe that the only solution to pest problems is to spray more frequently and to use different types of pesticides (Dinham, 2003).

Despite the beneficial effects associated with the use of pesticides, many of these chemicals may pose potential hazards to humans and nature (Undeger & Basaran, 2005). Excessive uses of chemical pesticides result in pest resistance to pesticides and dangerous diseases to humans (Pimentel & Burgess, 2014). According to the (Environmental protection agency [EPA], 1992) human pesticide poisonings and illnesses constitute the highest price paid for all pesticide use. Exposure to pesticides can range from mild skin irritation to congenital disabilities, tumors, genetic changes, blood and nerve disorders, endocrine disruption and even coma or death (Alavanja, Hoppin & Kamel, 2004; Huen *et al.,* 2012). Exposure to pesticides significantly increases genetic damage, whereby tissues are damaged at the chromosomal level, causing a significant increase in chromosomal and chromatid-type aberrations, chromosome breakage and/or mitotic spindle alterations, along with other nuclear abnormalities, such as pycnosis, karyolysis and karyorrhexis (Bolognesi, 2003; Gómez‐Martín *et al.,* 2015). Reducing exposure and other pesticides related costs demand critical identification of hazardous exposure risks and quantification of mechanism for toxicity (Kapka-Skrzypczak, Cyranka, Skrzypczak & Kruszewski, 2011).

Occupational and environmental pesticides exposure had been linked to malignancy, damage in DNA and disruption of enzyme activity, developmental, reproductive, neurodegenerative, respiratory and metabolic diseases (Ali *et al.,* 2018; Connolly *et al*,. 2017; Gangemi *et al.,* 2016; Hayat, Afzal, Aqueel, Ali & Saeed *et al.* 2018; Mostafalou & Abdollahi, 2017). Likewise, exposure to these toxic substances adversely affects human blood cells, liver, and the peripheral nervous system (Guytingco, Thepaksorn & Neitzel, 2018; Hu *et al.,* 2015). The presence of pesticides in the environment, is also linked to providing support to the growth of pathogenic bacteria, hence increasing co-exposure risks to pesticides and pathogenic bacteria (Naphade, Durve, Bhot, Varghese & Chandra, 2012).

Acetylcholinesterase (AChE) is an enzyme involved in rapid hydrolysis of the neurotransmitter acetylcholine, thereby catalysing termination of impulse transmission in numerous cholinergic pathways in the central and peripheral nervous systems (Tougu, 2001). Organophosphates pesticides exhibit toxicity by interacting with the enzyme, hence forming a covalent bond with the serine of the catalytic site resulting in an extremely stable enzyme-inhibitor complex (Enz & Floersheim, 1997). These pesticides are substrate analogues to ACh, hence they enter the active site like natural substrates, covalently binding to serine hydroxyl group, and in the acetylation process, they are split and the enzyme is phosphorylated (Colovic, Krstic, Lazarevic-Pasti, Bondzic & Vasic, 2013). This, phosphorylated enzyme cannot hydrolyze the neurotransmitter. Therefore, biomonitoring of enzyme activity provides the mechanistic exposure effects and the extent of internal dose of the toxic substance absorbed by the body tissues. Biomonitoring of pesticide exposure provides a high degree of confidence in predicting the potential for adverse effects in an individual or population-based on marker levels (Liu *et al*., 2006).

Poor and injudicious use of pesticides had been reported among smallholder farmers in Tanzania (Kiwango, Kassim & Kimanya, 2017; Lekei et a*l.,* 2014; Ngowi, Mbise, Ijani, London & Ajayi, 2006; Nonga, Mdegela, Lie, Sandvik & Skaare, 2011) contrary, biomonitoring studies had focused on farmers in traditional crops including coffee and commercial farm workers in tea and flower industries (Kapeleka, Lekei & Hagali, 2016; Mrema, Ngowi, Kishinhi & Mamuya, 2017; Mwabulambo, Mrema, Ngowi & Mamuya, 2018; Ngowi, 2002). Comparative biomonitoring of pesticides exposure using unexposed (control groups) in uncontrolled smallholder vegetable production had not been well documented. The aim of this study was therefore, to assess and quantify pesticides exposure among smallholder vegetable producers through comparative acetylcholinesterase levels between exposed and unexposed individuals, derive the determinants of increased exposure risks, drivers of increased pesticides use, co-exposure risks and relationship between pesticides residues and pathogenic bacterial growth in vegetables produced by smallholder vegetable producers in Tanzania.

1.2 Problem statement

In general, when pesticides are applied to the environment, the general population is exposed to their residues due to physical and biological degradation of pesticide products in the air, water and food (Bhalli, Khan & Nasim, 2006; Bolognesi, 2003). As a result, both farming and non-farming populations have been occupationally and environmentally exposed due to excessive use of pesticides in their areas (Latif, Sherazi, Bhanger & Nizamani, 2012; Mathur, Agarwal, Johnson & Saikia, 2005). Pesticides exposure among smallholder farmers in Tanzania have been reported mainly in commercialized cash crops such as cotton, tea and coffee (Kapeleka *et al.,* 2016; Mrema *et al.,* 2017; Mwabulambo *et al.,* 2018; Ngowi, 2002). Major areas reported include pesticides handling practices and acute poisoning resulting from exposure to cholinesterase-inhibiting organophosphates and carbamate insecticides (Lekei *et al.,* 2014; Mwabulambo *et al.,* 2018; Ngowi, 2002).

Pesticides solution provide a suitable environment for the survival and growth of human pathogens, such as *L. monocytogenes*, *E. coli*, *Salmonella and Shigella spp* (DuPlessis, Korsten, Buys, Pillay & Taylor, 2015; Ng, Fleet & Heard, 2005). These pesticide solutions are reported to be a source of microbial contaminants due to their chemical composition which could either stimulate or inhibit bacterial growth. Despite this, there are very few studies on the co-occurrence of pesticides and microbial contaminants in fresh vegetables (Amoah, Drechsel, Abaidoo & Ntow, 2006; Santarelli *et al.*, 2018).

Although the exposure assessments done can be considered significant, and information is available on the effects of pesticides used, there is limited information on quantified biomarker exposure assessment to multiple pesticides. Likewise, much had been done in documenting health effects of pesticides in the environment, yet there is scanty information on the comparative biomonitoring of occupational and environmental pesticides exposure and the resultant effect on pesticides use on pathogenic microbial communities. This study therefore, aimed at critically assessing comparative pesticides exposure using unexposed groups (control), dynamics of pesticides use and elucidates the causal link between pesticides use, human exposure and growth of pathogenic bacteria contaminants in smallholder vegetable production in Tanzania.

1.3 Rationale of the study

This, to the best of our knowledge, is the first study of its kind to evaluate the comparative AChE activity among smallholder farmers occupationally exposed to pesticides using unexposed control group in deriving association between AChE inhibition and self-reported symptoms of pesticides exposure. The information generated from this study provides scientific evidence on the level of exposure, hence prompt farmers to adopt safe use practices and use of safer and less toxic pesticides in vegetable production. Likewise, the results from this study will provide critical inputs to policy makers on the improvement of horticultural subsector by addressing and developing strategies to control important issues related to pesticides residues and microbial contamination of fresh vegetables, in effort to revamp the horticultural subsector in the country. Furthermore, the study provides useful information for food safety and other stakeholders in redressing the improvement of fresh vegetable value chain, changing consumers' behaviour in enhancing commercialization, supply and consumption of safe vegetables.

1.4 Objectives

1.4.1 Overall objective

The general objective of the study was to assess pesticides exposure through biomonitoring of acetylcholinesterase (AChE) activity and establish fate of pesticides use among smallholder vegetable producers in Tanzania.

1.4.2 Specific objectives

(i) To determine changing patterns and drivers of increased pesticides use among smallholder vegetable producers.
(ii) To assess comparative acetylcholinesterase (AChE) activity between exposed farmers and unexposed individuals and associated health effects.
(iii) To determine levels of pesticides residues and bacterial contamination of vegetables produced by smallholder production producers.
(iv) To assess co-exposure risks from pesticides residues and bacterial contamination of vegetables produced by smallholder producers.

1.5 Research questions

(i) Which are the most frequent pesticides used, and what are the dynamics and changing patterns of pesticide use and practices among smallholder vegetable producers?

(ii) Is there any difference in levels of acetylcholinesterase (AChE) activity between exposed farmers and unexposed individuals?
(iii) What are the levels of pesticides residues and bacterial contamination of vegetables produced under smallholder production systems?
(iv) What is the extent of co-exposure risks and is there any association between pesticides residues and bacterial growth in vegetables produces by smallholder farmers?

1.6 Significance of the study

This study provides information on the extent of occupational and environmental exposure to pesticides in uncontrolled smallholder farming systems. It also provides insights into co-exposure risks among farmers and consumers by deriving the association between pesticide residues and bacterial contamination of vegetables, bridging the knowledge gap on biomonitoring exposure to pesticides and the fate of pesticide application in smallholder vegetable production systems. The findings herein will derive policy recommendations for developing pesticide monitoring and surveillance systems to monitor and control pesticides to ensure sustainable vegetable production system in a manner that minimizes pesticide exposure while effectively reducing the levels of pesticide residues and bacterial contamination of vegetables under smallholder production systems.

1.7 Delineation of the study

Biomonitoring studies involve the collection and analysis of blood samples. Cultural beliefs attached to the collection and analysis of blood samples affected the recruitment of farmers and non-exposed individuals in the study. Both farmers and unexposed individuals each signed a written consent form for blood test and participation in the research. Furthermore, awareness and sensitization meetings with village government officials, health and extension officers in respective villages were done with farmers to explain the objectives of the study. Culture sensitivity was addressed by the use of local medical personnel in respective village health facilities in the collection of blood samples as well as undertaking health survey on exposure symptoms. The study focused on smallholder producers in uncontrolled farming systems and hence the findings cannot be generalized to the entire smallholder farming population in other field crops including maize, wheat and perennial crops such as cotton and cashew nuts where pesticides are extensively used in the production process.

CHAPTER TWO

LITERATURE REVIEW

2.1 Dynamics and changing practices of pesticides usage

2.1.1 Overview of pesticides

Pesticides are extensively used in agricultural production to prevent and control pests, diseases, weeds, and other plant pathogens (Eskenazi *et al.*, 2008; Sanborn *et al.*, 2004; Sankoh, Whittle, Semple, Jones & Sweetman, 2016). They are chemicals with unique properties designed to be toxic to pests, constituting a heterogeneous category of chemicals specifically designed for the control of pests, weeds or plant diseases. Their application remains the most effective and accepted means for the protection of plants from pests and has contributed significantly to enhanced agricultural productivity and crop yields (Bolognesi, 2003).

Pesticides are intended to kill living organisms and are harmful to human if not used properly (Van Der Hoek, Konradsen, Athukorala & Wanigadewa, 1998). Farmers depend heavily on the use of these pesticides for control of different pests and diseases (Damalas & Koutroubas, 2016). Their use had increased in the recent past due to their rapid action (Latif, Sherazi & Bhanger, 2011b; Mattah, Mattah & Futagbi, 2015). However, these toxic substances can contaminate the environment and pose risks to both humans and the ecosystem (Ruiz-Guzmán, Gómez-Corrales, Cruz-Esquivel & Marrugo-Negrete, 2017). Different pesticides formulations, including insecticides, herbicides and fungicides, are being used in developing countries (Ngowi *et al.,* 2006; William, 2008; Vikkey *et al.,* 2017).

The government of Tanzania through Tropical Pesticide Research Institute (TPRI) Act No.18 of 1979 has defined pesticides as any matter of any description (including acaricides, arboricides, herbicides, insecticides, fungicides, molluscides, nematicides, hormonal sprays, and defoliants) that are used or intended to be used, either alone or together with other material substances for the (a) control of weeds, pest and disease in plants, or (b) control of the external vectors of veterinary or medical diseases and external parasites of man or domestic animals, or (c) protection of any food intended for human or animal consumptions.

Pesticides can be classified into different groups according to their purposes, that is, insecticides, fungicides, herbicides, acaricides, rodenticides, nematicides, and plant growth regulators. They can also be classified according to their chemical composition, such as organophosphates, organochlorines, carbamates, pyrethroids, sulfur and urea (Lee, Park, Lee, Oh & Ko, 2017).

Generally, the main chemical families of pesticides across the farming population in developing countries remain the same. Carbamates, organophosphates and pyrethroids are the most widely used families of pesticides in both developed and developing countries (Dari, Addo, & Dzisi, 2016; Gundogan *et al.,* 2018; Ngowi *et al.,* 2006; Pastor *et al.,* 2003; Ramirez-Santana *et al.,* 2018; Vikkey *et al.,* 2017). These chemical families constitute the major environmental contaminants due to their repeated use.

The current technology development of nanotechnology provides the key solution to human and environmental exposure to pesticides. The green synthesis of nanopesticides which is cheap and environmental friendly does not require the employment of highly toxic chemicals (Benelli, Pavela, Maggi, Petrelli & Nicoletti, 2017). Despite the use botanicals for nanosynthesis being cheaper and effective application of green technology, it had not been widely adopted mostly in developing countries where the use of highly hazardous pesticides prevails. Bio-pesticides are effective in controlling insect pests as they are active against a variety of insects, fast penetrating and no toxic residues in the treated products.

The development and use of green pesticides had been reported much in developed and to a small extent in developing countries (Nnamonu & Onekutu, 2015). Green pesticides, though not well applied in developing countries, can also prove effective in agricultural situations, particularly for organic food production (Benelli *et al.,* 2017; Kola, 2011; Mossa, 2016; Nnamonu & Onekutu, 2015; Qian, Lee & Cao, 2010). Low level application of green pesticides in developing countries is fuelled by low level support from governments in these countries and the fact that few commercial companies invest in the development of commercialized formulations and hence the botanical pesticide market has not grown in a comparable way to the botanical medicine market (Nnamonu & Onekutu, 2015).

Farmers are unaware of the health and environmental implications of pesticide use and knowledge on safe use are limited. This ultimately leads to pesticides misuse and negative impact on the environment and the health of the farmers (Jallow, Awadh, Albaho, Devi & Thomas, 2017; Sankoh *et al.,* 2016). Farmers in diverse production systems are using a wide range of pesticides. Chlorothalonil and metalaxyl/mancozeb constitute the majority of used fungicides products, while lambda-cyhalothrin, cypermethrin, profenophos endosulfan, chloropyrifos, dimethoate, triadmenol and triadimefon constitute the majority of insecticides used among most farmers in Tanzania (Mtashobya & Nyambo, 2014; Ngowi, 2002).

A study conducted in Brazil showed that farmers use organophosphate pesticides, including disulfoton, chlorpyrifos, acephate and dimethoate (Jardim & Caldas, 2012). Furthermore, herbicides such as glyphosate and paraquat, fungicides, including triazoles and dithiocarbamate, are reported to be used (Silvério *et al.,* 2017). Increased pesticides application by farmers is a result of the influence of a wide range of pests and diseases including birds, rodents, insects, root rot and other organisms that reduce farm yields (Sankoh *et al.*, 2016). Insecticides, fungicides and herbicides constitute the main types of pesticides used by the farmers in developing countries (Damalas & Khan, 2017; Ngowi *et al.*, 2006).

For example, it has been reported that 40 and 43 different types of pesticides were used in vegetable farming in Tanzania and Ghana, comprised of mainly insecticides, fungicides and herbicides (Ngowi *et al.*, 2006; William, 2008). But farmers may use any pesticide product in controlling pesticides. Their desire to eliminate crop pests drive them to use pesticides not registered for use, as well as banned pesticides for control of crop pests and diseases (Dari *et al.,* 2016; Diop *et al.,* 2016), thus increases health and environmental risks of exposure to highly hazardous pesticides (Jallow *et al.*, 2017).

The use of botanicals and non-conventional methods for pest control is not common among vegetable smallholder farmers in developing countries. Nevertheless, some farmers use cultural methods, including crop rotation. Other common alternatives include manual uprooting of affected plants and the use of wood ashes (Mtashobya & Nyambo, 2014).

Poor pesticide handling and haphazard pesticide spraying have been associated with contamination of food products with hazardous chemicals from pesticides. A huge quantity of distributed pesticides suggests a high potential for human exposure, food contamination, health injuries and illness (Lekei *et al.,* 2014; William, Gijzen, Kelderman & Drechsel, 2006). In a study to assess farmers' knowledge and practices concerning pesticide exposure, the association between high poisoning symptoms and failure to calibrate their spraying equipment suggested that poor application practices can result in higher pesticide residues in freshly consumed fruits and vegetables (Lekei *et al.*, 2014). It is undeniably that pesticide use has increased agricultural productivity, but excessive uses of these toxic chemicals result in increased pest resistance to pesticides and environmental contamination, resulting from improper disposal of empty pesticides' containers, spillover pesticide drifts and volatilization (Keikotlhaile & Spanoghe, 2011).

In most cases, farmers use high pesticide concentrations above the recommended rates on pesticides labels, determined by excess amount of pesticides mixed in a mixing container, with increased frequency of applications and mixing of several pesticides together for better control of different pests and/or diseases. This practice adversely affects the environment and soil micro and macro fauna (Damalas & Khan, 2017; Wilson & Tisdell, 2001). This may further lead to the presence of pesticide residues in harvested food, which raises health concerns to consumers because pesticides are known to have potential harmful effects to the health of other non-targeted organisms (Gilden, Huffling & Sattler, 2010).

2.1.2 Characteristics of smallholder vegetable and pesticides use practices

Smallholder vegetable production is characterized by minimal productivity in small scattered plots. High pesticide use dominates the production systems. Agricultural typology in vegetable based agroecosystem is a year-round production through irrigation (Mtashobya & Nyambo, 2014). Knapsack is the most popular spraying equipment used in pesticides application, though few farmers use motorized sprayers (William *et al.,* 2006). Farmers do not wait until a certain pest has been identified, but spray as a preventive measure before any visible damage to crops by pests was observed. The spraying frequency ranges from once every three to five days, which is excessive by any agricultural standard (Van der Hoek *et al.,* 1998).

In spite of numerous benefits, the use of pesticides brings substantial hazard because of their high biological activity (Damalas & Koutroubas, 2016; Pimentel & Burgess, 2014). In developing and under-developed countries, the situation is worse because safety and regulatory guidelines on pesticides handling are hardly practiced (Ali *et al.,* 2018) and most farmer in these countries are not adequately informed about the hazards associated with the chemicals (Ngowi *et al.,* 2006; William *et al.,* 2006). Exposure risk is predicted to be high in developing countries due to unsafe pesticides handling practices (Hayat *et al.,* 2018). This is because government extension programs encourage the use of pesticides with minimal consideration of their effects in the environment and health risks associated with consuming pesticides residues in food products (Abate, Van Huis & Ampofo, 2000). Large quantities of pesticides used in both small and large scale farming systems result in indiscriminate accumulation of pesticides in food chain, human matrices and the environment (Lekei *et al.,* 2014; Quansah *et al.,* 2016). But due to lack of basic knowledge on pesticides, farmers' decisions on what pesticides to use and how to use them do not have a bearing on health or safety of food produce and environment (Guytingco *et al.,* 2018).

Excessive use of pesticides is a burden in multiple ways as farmers pay more for the pesticides, while on the other hand, increased pesticide usage develops resistance in pests, thus making them more destructive to the crops (Abdullah, Brobst & Pervaiz, 2004). There is no uniformity in pesticides use practices across the farming population. Different factors influence the choice of each product depending on the kind of crop, weather conditions, pests, etc. Pesticides are usually sprayed in combination, and the efficacy of one may mask the other in the mixture. Mixing of pesticides with high concentrations and increased frequency of application are fostered by the farmers' desire to have rapid knockdown of pests (Damalas & Koutroubas, 2016; Pastor *et al.,* 2003).

2.1.3 Prevention and control on usage of pesticides

In most developing countries, farmers' awareness on disposal and management of pesticides is low (Diop *et al.,* 2016). Poor pesticides use and associated health and environmental risks can be minimized through effective extension system to build farmers' capacity and monitor pesticides use at farm level. The role of extension is to train farmers, coordinate, supervise and monitor the pesticides management cycle at farm level and to ensure farmer's adherence to pesticides safe use and handling practices (Damalas & Eleftherohorinos, 2011). Most pesticide leftovers and wastes are left in the farm, homes or dumped directly onto the land or into water, increasing risk of exposure (Yang *et al.,* 2014). Likewise, pesticides over use are a common practice which further increases the risks of adverse effects to the environment (Jallow *et al.,* 2017; Singh *et al.,* 2011). Though some pesticides can be used with relatively low health risk upon proper adherence to pesticide labels, some pesticides are highly toxic and their use requires special precaution (Ogg, Hygnstrom, Bauer & Hansen, 2012). But because pesticide usage will continue to be an important component in agricultural production, there is a great potential to make its use as effective as possible to minimize health, economic and environmental costs (Pimentel & Burgess, 2014).

Poor extension services, farmers not well informed through agricultural input providers and low general knowledge on pesticide usage are attributed to overuse of different pesticides (Damalas & Eleftherohorinos, 2011). But due to lack of efficient means of controlling pests, excessive and injudicious use of pesticides is a common practice among smallholder farmers, leading to human and environmental exposures. Pesticides exposure had been associated with an increase in the incidence of diverse health effects (Jacobsen-Pereira *et al.,* 2018).

The WHO regulatory framework on International Code of Conduct on Pesticide Management for forbids using pesticides for a purpose except that which is prescribed on the label. Pesticides users are therefore, required to use personal protective equipment (PPE), proper application equipment, as well as safe disposal of empty containers with the objective of protecting health of users and the public, and the environment (WHO, 2015). Governments are required to exercise authority to ensure provisions to monitor workers' health and enforce provisions affording protection and reducing risk from pesticide use. Unlike regulatory authorities in developing countries, pesticides use and marketing is highly regulated by a large body of EU legislation. They cannot be allowed to get to the market or used without prior authorisation to prevent the use substandard products. In the US, strict measures are developed and implemented under EFSA to evaluate each active substance used in plant protection products. Regulation of Plant protection products is first and foremost managed by framework regulation (EC) No 1107/2009 (Ansell, 2008).

2.2 Biological monitoring and genotoxic effects of pesticides exposure

2.2.1 Biomonitoring of pesticides exposure

Human biomonitoring is the direct measurement of people's exposure to toxic substances in the environment by measuring the substances or their metabolites in human samples, such as blood or urine (Sexton, Needham & Pirkle, 2004). It involves measurement of the parent compound or its metabolites in human biological samples in order to identify and quantify the internal exposure to specific chemicals (Koureas, Tsezou, Tsakalof, Orfanidou & Hadjichristodoulou, 2014). Biological monitoring provides a useful tool to identify certain biological effects associated with chronic health outcomes. It can estimate the genetic risk deriving from an exposure to a complex mixture of chemicals (Bolognesi, 2003). Biological monitoring of exposure to pesticides is aimed at the estimation of internal dose based on the fate of the compound in human body (Koivunen, Gee, Nichkova, Ahn & Hammock, 2007).

Biomonitoring in human populations exposed to pesticides is a useful tool to estimate the genetic risk from an integrated exposure to complex mixtures of pesticide (Liu et *al.,* 2006). Genetic risk of pesticide exposure is positively associated with increased DNA damage among exposed individuals (Singh *et al.,* 2011). Individuals with occupational exposure to pesticides, including field workers, mixers, loaders, appliers through direct contact, provide most affected individuals and a good opportunity to study the adverse health consequences of pesticide exposure (DaSilva *et al.,* 2008). Comparative assessment of acetylcholinesterase activity (AChE) and resultants health effects of pesticides provide the proxy to genotoxic effects of pesticides exposure among farmers.

Currently, the major biomonitoring approach among agricultural workers is the measurement of acetylcholinesterase levels in pesticide applicators and handlers (Muniz *et al.,* 2008). For instance, the AChE activities among coffee workers assessed during spraying and non-spraying period were comparable giving no suggestion of decreased AChE in exposed farmers, whereas about 30% commercial tea workers had AChE below the limit, suggesting occupational exposure to pesticides (Kapeleka *et al.,* 2016; Ngowi, Maeda, Partanen, Sanga & Mbise, 2001). Likewise, about 27% of flower and onion pesticide applicators in Arusha had an acetylcholinesterase level below the limit value suggesting that exposure to pesticide was evident (Mwabulambo *et al.,* 2018).

However, data from one study in one particular occupational setting cannot be used to draw conclusions on genetic risk in another occupational setting (Naravaneni & Jamil, 2007). This is because populations exposed to pesticides are rather specific due to different lifestyles, nutritional habits, climatic and environmental conditions, and are exposed to different mixtures of pesticides. The use of biomarkers helps to evaluate potential exposures to pesticides as well as predicting the effects to human health (Arshad *et al.,* 2016).

Biological monitoring approaches can be categorized into four main types; direct measurement of unchanged pesticides in biological matrices, determination of metabolites in biological matrices, quantification of biological effects related to internal dose (acetylcholinesterase activity) and measurement of macromolecule adducts combined with target or non-target molecules (DNA and hemoglobin adducts). The level of exposure and the amount of pesticides absorbed in human body can be determined through well-conducted biomonitoring studies (Paustenbach & Galbraith, 2006).

Biomonitoring is therefore, the direct measurements of environmental chemicals, their primary metabolites, or their reaction products (such as DNA-adducts) in people, usually in blood, urine, milk, sweat or an expired breath specimens of an exposed individual. It provides a more accurate reflection of internal dose resulting from pesticide exposure, in contrast to environmental monitoring which can only indicate the level of external contamination (McKinlay, Plant, Bell & Voulvoulis, 2008).

The use of biomarkers in estimating pesticides exposure provides a critical quantification of exposure and its effects in human body. A biomarker is an indicator signalling events in biological system or sample (Anwar, 1997). It is a measurement of a molecular or chemical substance or event in a biological system. Biomarkers include detection of environmental substance itself or its metabolites in urine or blood, changes in genetic material, and cell death (Anwar, 1997; Győrffy, Anna, Kovacs, Rudnai & Schoket, 2007; Jacobsen-Pereira *et al.,* 2018). The parent pesticides compounds can be monitored directly in blood products instead of their metabolites, which are usually measured in urine. Blood measurements provide an estimation of the dose available for the target site, allowing for prediction of dose-response relationships (Mathur *et al.,* 2005). Pesticide exposure can be measured by evaluating the cholinesterase activity in the blood. A different method for evaluating pesticide exposure is to quantify the levels of urinary dialkyl-phosphate (DAP), which is an organophosphorus metabolite (Lee *et al.,* 2017). Genotoxic biomarkers like DNA damage data along with AChE levels are important parameters for determining farmer's health who are exposed to pesticides in any situation (Naravaneni & Jamil, 2007).

Six dialkyl phosphate (DAP) metabolites are the most commonly measured general biomarkers of OP insecticides. These metabolites reflect exposure to OP but do not verify the presence of a particular OP compound. The six common DAP metabolites measured are dimethyl phosphate (DMP), diethyl phosphate (DEP), dimethyl thiophosphate (DMTP), dimethyl dithiophosphate (DMDTP), diethyl thiophosphate (DETP), and diethyl dithiophosphate (DEDTP). The primary metabolites of OP pesticides, DETP and DEDTP, are genotoxic under metabolic conditions, and with additional metabolism, could produce secondary metabolites that could exert specific hepatic genotoxicity (Vega, Valverde, Elizondo, Leyva & Rojas, 2009).

N7- methyldeoxyguanosine (N7-MedG) has been shown to be a robust biomarker for exposure to methylating agents, because of its reported inefficient elimination from DNA. Significantly increased N7-MedG levels indicate a genotoxic alkylating effect of pesticide exposure (Gmez-Martin *et al.,* 2015). Techniques that measure DNA damage (e.g., detection of DNA adducts) provide a powerful tool in measuring environmental effects.

Cytogenetically visible damage in human chromosomes can be detected as sister chromatid exchanges (SCEs) or as micronucleated cells.

Biomarkers for some pesticides exist in blood, serum, semen, ovarian follicular fluid, amniotic fluid, umbilical cord blood, breast milk, meconium, and urine (Gilden *et al.,* 2010). While many studies have indicated increase in the frequency of micronuclei (Costa *et al.,* 2007; DaSilva *et al.,* 2008; Remor *et al.,* 2009) another study (Pastor, Gutiérrez, Creus, Cebulska-Wasilewska & Marcos, 2001) did not show any significant increase in the frequency of micronuclei (used as biomarkers of genetic damage) in neither peripheral blood lymphocytes nor epithelial buccal cells, indicating a lack of clastogenic and/or aneugenic effects related to the particular pesticide exposure.

2.2.2 Genotoxic effects of pesticides exposure

Genotoxicity is defined as a destructive effect on a cell's genetic material (chromosome, DNA, or RNA) affecting its integrity (Shah, 2012). It describes the property of chemical agents that damage the genetic information within a cell causing mutations which may lead to cancer (Nagarathna, Wesley, Reddy & Reena, 2013). Genotoxins are mutagens, and exposure to mutagenic chemicals generally results in increased risk for developing tumors, hormonal changes, DNA damage, and changes in ovaries and eggs which may lead to different types of cancer (Arshad *et al.,* 2016; Li *et al.*, 2015; Srivastava *et al.*, 2012).

Likewise, mutagenic and cell death potential of pesticides through increased frequency of micronucleus and other nuclear abnormalities had been associated with chronic exposure to pesticides (Adad *et al.,* 2015). Pesticide exposure is also associated with significant increases in chromosomal aberrations and sister chromatid exchange, providing suggestive evidence of genotoxic effects induced by pesticides (Ruiz-Guzmán *et al.,* 2017). Pesticides are capable of inducing alterations on cell proliferation kinetics. Exposure to pesticides therefore, induces the acceleration of cell cycle and increased mitosis (Gómez-Arroyo, Díaz-Sánchez, Meneses-Pérez, Villalobos-Pietrini & DeLeón-Rodríguez, 2000). Continuous and sub-lethal exposure to complex mixtures of pesticides may result in single and double strand breaks of DNA, oxidative stress and crosslinks (Jacobsen-Pereira *et al.,* 2018). Occupational exposure to mixture of organophosphorus pesticides (OPs) (pirimiphosmethyl, chlorpyrifos, temephos and Malathion) may cause DNA damage, decreased AChE activity, and hepatic and renal toxicity (Singh *et al.,* 2011). Cancer and neurological disorders have been associated with oxidative stress and DNA damage resulting from pesticide exposure (Kisby *et al.,* 2009). The mutagenic and carcinogenic nature of these toxic chemicals is explained by the formation of DNA adducts (Rusiecki *et al.,* 2017). Moreover, pesticide exposure induces oxidative stress by depleting intracellular glutathione and increasing Reactive Oxygen Species (ROS) production as a result of metabolism process of pesticides. This produces more toxic metabolites harmful to cells (Sabarwal, Kumar & Singh, 2018). Metabolic activities of pesticides within human bodies are considered an important underlying cause of mutations leading to cancer (Gómez-Martín *et al.*, 2015; Hernandez *et al.*, 2013; Singh *et al.*, 2011). The DNA damage is considered as an important genotoxicity biomarker and is clearly one of the underlying causes of mutations leading to cancer (Gómez-Martín *et al.*, 2015). Exposure and resultant effects of pesticides are summarized in Table 1.

Table 1: Effects of exposure to pesticides reported from different countries

Category of people tested	Analyzed biological matrices	Results	Reference
160 male paddy farmers exposed to organophosphorus pesticides and 160 control group (Malaysia)	Buccal mucosa cell and the peripheral blood	Exposed farmers to mixtures of organophosphorus pesticides had significant increase of DNA damage	How *et al.* (2015)
129 tobacco farmers exposed to pesticides and 91 non-exposed (where)	Peripheral blood lymphocytes:	Exposed farmers had significantly increased nuclear plasmatic bridge, micronucleus and nuclear bud frequencies	Kahl *et al.* (2018)
46 non-exposed controls and 81 soybean workers occupationally exposed to pesticides from Brazil	Exfoliated buccal cell samples	Micronuclei, nuclear buds and binucleated cells in individuals exposed to pesticides were reported	Benedetti *et al.* (2013)
Vineyard workers exposed to pesticides with control group (Brazil)	Binucleated lymphocytes and peripheral leukocytes	High rate of Micronuclei and DNA damage in pesticide-exposed individuals	Da Silva *et al.* (2008)
Children living in agricultural areas (where)	Peripheral blood lymphocytes	Higher Micronuclei frequencies, nuclear buds, and apoptotic cells were recorded in exposed children	Ruiz-Guzmán *et al.* (2017)
50 rural workers exposed to pesticides, and 46 controls from the same city (Brazil)	Peripheral blood lymphocytes	Peripheral blood lymphocytes of exposed individuals had significantly higher DNA damage	Pereira *et al.* (2018)
Greenhouse Non-smoker horticulturist (Italy) and control group	Peripheral blood lymphocytes	DNA adducts significantly higher in floriculturists	Andre *et al.* (2007)
38 exposed workers in pesticides industry and 20 control (unexposed	Peripheral blood lymphocytes	Workers with long working period had significant DNA damage	Arshad et al. (2016)
154 rice, corn, and double-crop farmers and 60 non-farmer control (Thailand)	Urine and blood samples	DNA damage and Acetylcholinesterase activity between farmers and non-farmers were not different. No statistical significance was found in the dialkyl-phosphates levels, among all farmers	Hongsibsong, Sittitoon, & Sapbamrer (2017)
240 men from an infertility clinic (where)	Urine and sperm	Increased sperm DNA fragmentation and decrease of sperm concentration Increased urinary concentration of 3-PBA	Ji *et al.* (2011)
70 occupational workers exposed to mixture of pesticides with same number of healthy subjects as controls (India)	Peripheral blood lymphocytes Hepatic and renal function	DNA damage, decrease in Acetylcholinesterase activity, hepatotoxicity and nephrotoxicity detected from exposed farmers	Singh *et al.* (2011)

Exposure to pesticides exhibits increased level of DNA damage even if no detectable amounts of pesticides are seen in the blood serum because pesticides exhibit toxicity by binding specific areas in the DNA (Kasiotis *et al*., 2012). Oxidative stress induced by organophosphorus pesticides interferes with the functioning of different organs and tissues due to the accumulation of oxygen free radicals in erythrocytes and other cell, resulting in tissues damage (Lukaszewicz-Hussain, 2010; Mecdad, Ahmed, ElHalwagy & Afify, 2011). This reactive oxygen species (ROS) has been assumed to be the mechanism linking pesticides exposure to increased risk of diseases development, including cancer and neurodegenerative diseases (Srivastava *et al*., 2012). It has potential to cause injury to organs including liver, brain and pancreas which may result in impaired metabolism of protein, lipids and carbohydrates (Jacobsen-Pereira *et al*., 2018).

DDT and its metabolites had been found to induce DNA damage in peripheral blood mononuclear cells. Comparison of blood levels of hexachlorobenzen (HCB) and total DDT in 159 women with breast cancer and 250 presumably healthy controls from the villages of Punjab, India showed that mean levels of total DDT and HCB were significantly higher for breast cancer patients than controls (Mathur *et al*., 2005). Studies from Brazil using Comet Assay and Buccal micronucleus cytome assay revealed DNA damage in soybean workers with increased occurrence of cells with micronuclei, nuclear buds and binucleated cells, as well as cell death (Benedetti *et al*., 2013; Jacobsen-Pereira *et al*., 2018). Genetic alterations due to mutagenic and non-mutagenic processes caused by pesticides may occur within cells (Jacobsen-Pereira *et al*., 2018). Pesticides have also been associated with the modulation of the gene expression at the level of non-coding RNAs, histone deacetylases and DNA methylation patterns signifying their role in epigenetics (Sabarwal *et al*., 2018).

Occupational exposure to pesticides is associated with increased sperm DNA damage (Ji *et al*., 2011; Jurewicz *et al*., 2015; Saad-Hussein *et al*., 2017), which may cause infertility among men. Some pesticides have endocrine disrupting chemicals with the ability to disrupt the chemical messenger’s system in the body or can mimic hormones, resulting in reduced fertility, male and female genital, thyroid gland abnormalities, and immune suppression and general interference in hormonal signalling resulting in various types of cancers. The potential of these chemicals to mimic hormones and thereby disrupt endocrine system is a particular concern in humans. They can bind and activate numerous hormone receptors and act agonistically to the natural hormone action or bind receptors without activating them hence blocking the receptors and inhibits their normal action (Ji *et al*., 2011; Lacasana *et al*., 2010; Sabarwal *et al*., 2018).

Andre *et al*. (2007) assessed the level of DNA adducts between open-field and fruits growers, where the mean DNA adduct levels were significantly higher among open-field farmers compared with fruit growers. A significant increase of DNA adduct level was observed exclusively among farmers at the time of heavy pesticide use compared with non-exposed period. Furthermore, it was concluded that, occupational exposure to some OPs induces highly significant increase in the level of DNA damage in occupational workers than control subjects (Singh *et al*., 2011).

It is evident from literature reviewed that both occupational and environmental exposure to mixtures of pesticides adversely result in increased DNA damage, as measured in the peripheral blood lymphocytes of exposed population (Ali *et al*., 2018; Gundogan *et al*., 2018; Intranuovo *et al*., 2018; Jurewicz *et al*., 2015; Singh *et al*., 2011). Though several genotoxicity studies (Table 1) have been conducted in developed countries providing information on the subject matter, but there is scanty information in developing countries, mostly African based, where pesticides have been used extensively over years. In this regard, the extent of genotoxic effects in uncontrolled smallholder production systems could be alarmingly high due to evidences of poor pesticides use and handling practices in most developing countries. This therefore, demands critical assessment of genotoxic effects of pesticides use and quantification of DNA damage induced by exposure to complex mixtures of pesticides.

2.3 Toxicity of organophosphate and carbamate pesticides against acetylcholinesterase activity

2.3.1 Toxicity of pesticides

Pesticides exposure has been found to have profound effects on the nervous system. It is associated with a range of symptoms as well as deficits in neurobehavioral performance and abnormalities in nerve function (Alavanja *et al*., 2004). Pesticides usually disturb the physiological and biochemical activities of lymphocytes and erythrocytes. Their residues persist in adipose tissues for long periods and are found to be endocrine disrupters, inhibiting many enzymes and causing immune suppression (Latif *et al*., 2012).

Pesticides exposure is the root cause of some diseases to humans including cancer, respiratory diseases, skin diseases, endocrine disruption, and reproduction disorders (Alavanja *et al*., 2004; Gangemi *et al*., 2016). These pesticides can accumulate in the body over time with different chemicals having different effects on exposed individuals. The effects can range from mild skin irritation, tumors, genetic changes, blood and nerve disorders, endocrine disruption and even coma or death (Toshima *et al*., 2012; Watts, 2012).

2.3.2 Toxicity due to organophosphate, carbamate, pyrethroid and organochlorine pesticides

Organophosphate and carbamate pesticides exert their toxicity by interfering with the normal function of acetylcholine hydrolysis, a necessary task for synaptic response and an essential neuro-transmitter in the autonomic and central nervous system (Dasgupta, Meisner, Wheeler, Xuyen & Lam, 2007; Rao & Jyothsna, 2016). Therefore, the presence of cholinesterase inhibiting chemicals prevents the breakdown of acetylcholine leading to excessive acetylcholine in the nervous system resulting in the failure of breaking up acetylcholine (Dasgupta, Meisner, Wheeler, Xuyen & Lam, 2007). Over accumulation of acetylcholine (Ach) further leads to subsequent overstimulation of cholinergic receptors thereby disrupting neurotransmission (Rathish *et al*., 2018).

Exposure to OPs displays symptoms including headache, dizziness, nausea, vomiting, pupillary constriction, excessive sweating, tearing, and salivation. More severe cases involve development of muscle weakness and muscle twitches, changes in heart rate, and bronchospasm and can progress to convulsions and coma (Alavanja *et al.*, 2004; Damalas & Eleftherohorinos, 2011; Gilden *et al*., 2010; Mostafalou & Abdollahi, 2017; Vivien *et al*., 2013). These symptoms are a consequence of overstimulation of postsynaptic cholinergic receptors following inhibition of acetylcholinesterase by OPs (Suratman, Edwards, & Babina, 2015). Exposure to OPS is also associated with changes in mood and can cause sensory disturbances as well as cognitive effects such as memory loss, language problems and learning impairment (Rohlman, Anger & Lein, 2011). They may also induce adverse health effects including cancer, and effects on reproduction, immune or nervous systems (Bolognesi, 2003).

Organophosphorus compounds used in agriculture account mainly for the bulk of acute poisoning cases (Van der Hoek *et al*., 1998). However, the effects of pesticides on human health are more harmful depending on the toxicity level of the chemical, length and magnitude of exposure (Battershill, Burnett & Bull, 2008). Therefore, the use of these agricultural chemicals without necessary protection may also lead to alterations in the genetic material and the possible development of various types of tumors (Bhalli *et al*., 2009). Furthermore, they may induce adverse health effects including; effects on reproduction, immune or nervous systems. It is believed that non-Hodgkin's lymphoma, multiple myeloma, soft tissue sarcoma, lung sarcoma, pancreatic, stomach, liver, bladder and gall bladder cancer, parkinson disease and reproductive outcomes among others, are a result of human exposure to pesticides from environmental contaminants (Pastor *et al*., 2003).

Neurological, gastrointestinal, dermatological and respiratory manifestations are associated with acetylcholine inhibition due to organophosphorus exposure (Patel, Syamlal, Henneberger, Alarcon & Mazurek, 2018). Exposed farmers with depleted acetylcholinesterase activity exhibit significant respiratory illness, including allergic rhinitis and asthma, as well as lung function impairment as a result of long term exposure to organophosphorus (Fareed *et al*., 2013; Quansah *et al*., 2016). Strong evidences exist on the association of pesticides exposure with asthma, bronchitis and lung cancer (Mostafalou & Abdollahi, 2017; Patel *et al*., 2018; Ye, Beach, Martin & Senthilselvan, 2016).

Carbamate pesticides had been associated with the initiation and facilitation of pathological immune processes which result in immunotoxicity. This is effected through induction of mutations in genes coding for immunoregulatory factors and modifying immune tolerance (Dhouib *et al*., 2016). Exposure to dithiocarbamate fungicide, Mancozeb leads to significant induction in the frequency of chromosomal aberrations and micronuclei, confirming that exposure to carbamates can induce genotoxicity in humans (Srivastava *et al*., 2012).

Synthetic pyrethroids had become a main pesticide owing to growing pest resistance to organophosphorus pesticides. Pyrethroids and respective metabolites have been detected in urine samples from the general population indicating a widespread to pyrethroids (Quansah *et al*., 2016; Roberts & Karr, 2012; Saillenfait, Ndiaye & Sabaté, 2015). Exposure to pyrethroids had been linked with reproductive disorder and infertility in men. Significant positive correlation between urinary metabolite, 3-phenoxybenzoic acid (3-PBA) concentration and sperm DNA fragmentation reported signifies that exposure to pyrethroids has a negative impact on sperm DNA integrity and semen quality in men (Ji *et al*., 2011; Jurewicz *et al*., 2015; Koureas *et al*., 2014; Toshima *et al*., 2012).

The molecular toxicity of pyrethroids is the induction of metabolites that are more likely to interact with the cellular estrogen receptors (Ji *et al*., 2011). Pyrethorids may mimic estrogens or inhibit estrogen action. Furthermore, steroid hormones including progesterone and estradiol are also inhibited by pyrethroids (Lukaszewicz-Hussain, 2010). It is presumed that pyrethroids exhibit sperm DNA damage as a result of their hydrophobic nature and small molecular size, enabling it to pass and reach the nucleus of spermatogenic cells binding the DNA through the active group of its group (Ji *et al*., 2011; Saillenfait *et al*., 2015; Toshima *et al*., 2012).

Organochlorine pesticides are commonly found in human adipose tissue, water, sediments, and aquatic biota (Chopra, Sharma & Chamoli, 2011). Dichloro-diphenyl-trichloroethane DDT and its metabolites (γ-HCH, β-HCH, p,p′-DDE, p,p'-DDT, β-endosulfan, endrin aldehyde) were detected in blood sample of residents in Brazil where DDT was used in vector control (Ruiz-Suárez *et al.*, 2014). Likewise, human biomonitoring studies in the United States indicate that most people have detectable levels of DDT in their bodies despite the fact that DDT was banned from use in the United States in 1972 (Gilden *et al*., 2010). Organochlorine pesticides and polychlorinated biphenyls had also been recently reported in human breast milk in Tanzania among health lactating mothers suggesting that the mothers have been exposed to different sources of organochlorine pesticides though their use of had been banned in Tanzania (Müller *et al*., 2017).

Organophosphorus, pyrethroids, carbamates and organochlorines pesticides have been described as genotoxic pesticides because they generate free radicals that react with cell membranes and initiate the process of lipid peroxidation (Benedetti *et al*., 2013). Higher concentration of pesticides in the blood of individuals with higher genetic damage implies that pesticides bioaccumulation is responsible for genotoxic effects (Hayat *et al*., 2018). Pesticide exposure is also reported to induce subtle biochemical liver toxicity and adversely affects blood cells and the central and peripheral nervous system (Hernandez *et al*., 2013; Hu *et al*., 2015).

The general population living in areas with high pesticide use have increased risk for cancer (Kapka-Skrzypczak *et al*., 2011; Parrón, Requena, Hernández & Alarcón, 2014). Environmental exposure to pesticides may therefore, be risk factor for different types of cancers. Long-term exposure leads to accumulation of pesticides residues in the blood, oxidative damage and impairment of immune functions by initiating and facilitating pathological immune processes. This is enhanced due to their affinity to combine with the proteins of the blood plasma and induction of mutations in genes coding for immuno-regulatory factors. This modifies immune tolerance, altering of enzymatic reactions and damaging the DNA by producing derivatives or active form metabolites (Corsini, Sokooti, Galli, Moretto & Colosio, 2013; Dhouib *et al*., 2016; Martínez-Valenzuela *et al*., 2009; Mecdad *et al*., 2011).

Different pesticides and their metabolites have been frequently detected in urine samples from the non-occupational exposure population, confirming widespread environmental exposure of children and adults to one or more pesticides (Saillenfait *et al*., 2015). Non-occupational environmental exposure to pyrethroids has also been associated with negative impact on sperm DNA integrity and semen quality (Ji *et al*., 2011). Infant exposure to pesticides in the agricultural areas had been reported to causing cytogenetic damage and adverse effects on cognitive and neurodevelopment in children (Koureas *et al*., 2014; Mie, Rudén, & Grandjean, 2018; Ruiz-Guzmán *et al*., 2017). Blood organophosphate pesticide has been also reported in mothers and newborns living in agricultural communities (Huen *et al*., 2012). Occupational exposure to a mixture of pesticides (organophosphates, carbamates, pyrethroids) have been linked with fatal death, hormonal changes, DNA damage, birth defects and abnormal sperm, ovaries and eggs (Eskenazi *et al*., 2008; Gangemi *et al*., 2016; Hongsibsong *et al*., 2017). The effects of pesticides exposure and resultant health effects are summarized in diagram below (Fig. 1).

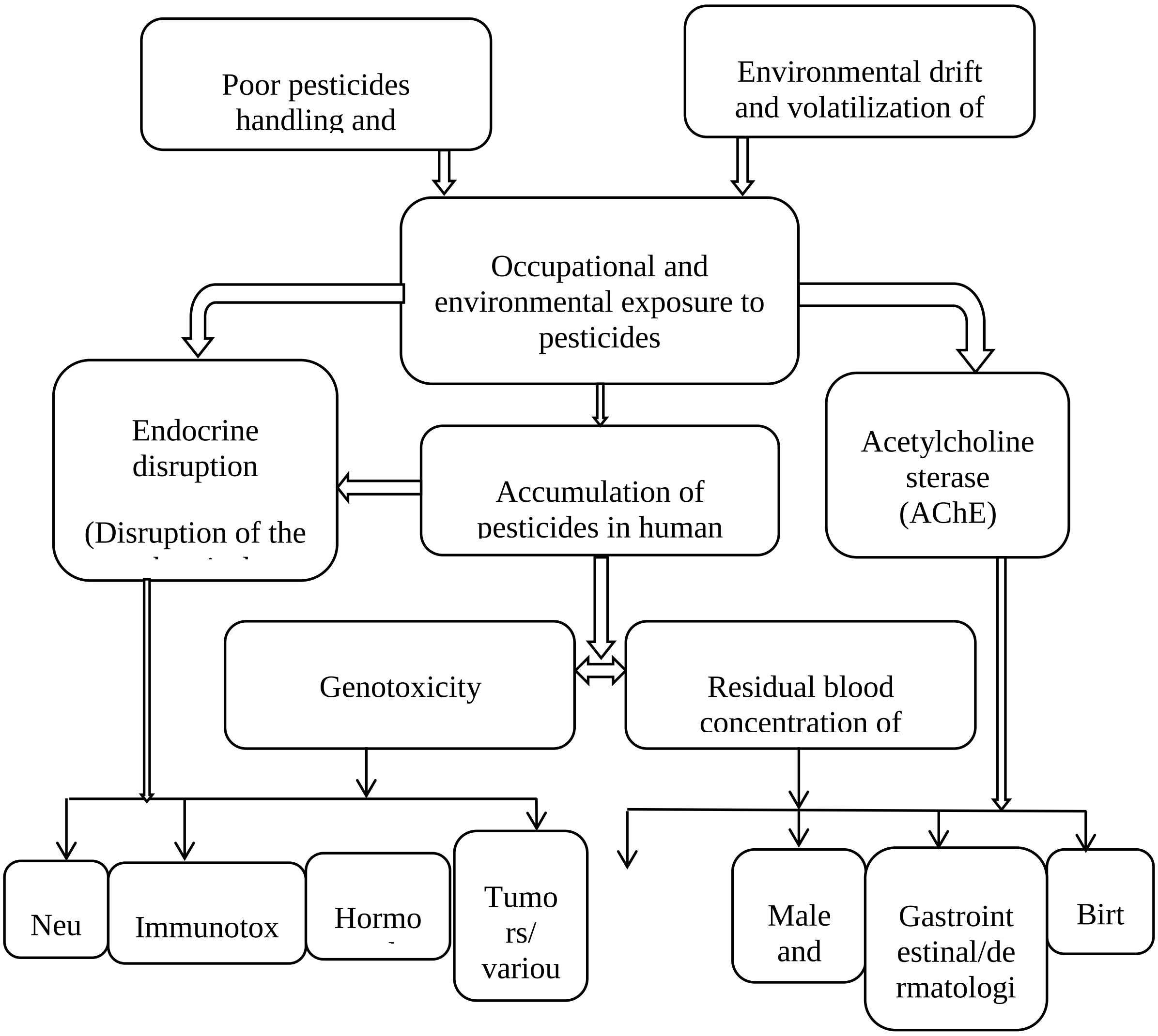

Figure 1: Effects of pesticides exposure

Neurotoxicity can also result from high-level exposure to pesticides including organophosphates (OPs), carbamates, organochlorines, fungicides and fumigants (DaSilva *et al*., 2008). Organophosphorus pesticides like monocrotophos, profenofos, chlorpyrifos and acephate not only act as genotoxic agents but also affect several other biochemical pathways (Prabhavathy Das, Pasha & Jamil, 2006). Farmers are more likely to develop leukemia, brain, prostrate and skin cancer and non-Hodgkin's lymphoma than the general population (Mathur *et al*., 2005).

Pesticides exposure accounts for bioaccumulation of pesticides in human tissues, which ultimately increases risks of DNA damage. The assessment of DNA damage in Pakistani agricultural workers revealed that individuals with longer comet tail lengths showed higher concentration of pesticides in their blood when checked through HPLC. Pesticides are responsible for causing DNA damage in individuals exposed to these chemicals (Bhalli *et al*., 2009).

Generally environmental pesticide exposures have been associated with detrimental health problems to the general populations including respiratory health, especially on lung function and hematological alterations (Fareed *et al.*, 2013; Quansah *et al*., 2016; Ye *et al*., 2016). The general population living within or near agricultural settings where pesticides are used have high levels of environmental exposure, raising the morbidity and mortality rated due to pesticides exposure (Ramirez-Santana *et al*., 2018; Suratman *et al*., 2015). Environmental exposures to organophosphates, pyrethroid and organochlorine pesticides are directly linked to genotoxicity of human lymphocytes, impaired lung function, poor semen quality, pregnancy outcomes, reproductive hormones and sperm DNA damage among men (Jurewicz *et al*., 2015; Perry, Venners, Barr & Xu, 2007; Remor *et al*., 2009; Saillenfait *et al*., 2015; Srivastava *et al*., 2012; Toshima *et al*., 2012; MingYe, Beach, Martin & Senthilselvan, 2017). Exposure to organophosphorus pesticide is also associated with adverse neurobehavioral effects in humans (Rohlman *et al*., 2011).

2.4 Environment fate of pesticides use

Pesticides are among the most important environmental pollutants (Matisova & Hrouzkova, 2012). Environmental pollution with pesticides exacerbates a serious burden of disease among the population living in agricultural settings (Aprea, 2012). This is due to their widespread presence in water, soil, the atmosphere, workplaces, homes, schools and agricultural products (Blair, Ritz, Wesseling & Freeman, 2015; Gilden *et al*., 2010; Matisova & Hrouzkova, 2012). Their use has shown significant impacts in the environment due to salient effects on both the microbiota in the soil and poisoning of both aquatic and terrestrial life. Pesticides can move through the air and eventually end up in other parts, such as soil or water (Chopra *et al*., 2011; Kim, Kabir & Jahan, 2016).

Pesticides hold unique position among environmental contaminants due to their high biological acute and chronic toxicity (Bhalli *et al*., 2006). These pesticides are associated with reduction of species diversity in the soil, as well as the total biomass of these biota and contribute to population decline in animals and plants by destroying habitats, reducing food supplies and impairing reproduction (Kegley, Neumeister & Martin, 1999). Organochlorine pesticides are persistent in the environment and may find their way to contaminate ground water, surface water, food products, air and soil, which may eventually affect human beings through direct contact with water, food and air (Jamil, Shaik, Mahboob & Krishna, 2005). There are differences in the level environmental exposure across different farming systems depending in the variations of agricultural practices and farmers' working behaviours (Wong, Garthwaite, Ramwell & Brown, 2018).

Excessive use of pesticides had led to an accumulation of a huge amount of pesticide residues in the food chain and drinking water as well as the environment (Sankoh *et al*., 2016) all of which lead to a substantial health hazard for the current and future generations due to uptake of these toxic compounds (Naphade *et al*., 2012). But owing to their importance in agriculture, vector control, and structural protection, pesticides will continue to be used and will therefore, be present in the human environment (Alavanja *et al*., 2004). Pesticides easily find their way into soils, where they may be toxic to arthropods, earthworms, fungi, bacteria, and protozoa (Bhanti & Taneja, 2007). These small organisms are vital to the ecosystems, as they dominate both the structure and function of ecosystems, breaking down organic matter, and enabling the vital chemical elements to be recycled (Pimentel & Burgess, 2014). Pesticides, such as organophosphates, carbamates, and pyrethroids, are potential hazards for the environment and non-target organisms (Perry *et al*., 2007).

Pesticides are released into the environment through various means such as direct application at fields or accidental release leading to occasional contamination of a wide range of water and terrestrial ecosystems (Baishya & Sharma, 2014). Pesticides residues, including profenofos, triazophos, chlorpyrifos, cypermethrin, and malathion had been found in soil samples from vegetable farms (DelPrado-Lu, 2015). This leads to decreased ecological functioning, degradation of natural vegetation, decreased number of biological species, and depletion of fish resources (Liu, Yin, Liu & Wu, 2012).

The use of pesticides decreases the general biodiversity in the soil. Nitrogen fixation for example, which is required for the growth of higher plants, is hindered by pesticides in the soil through interference of pesticides with flavonoid signaling from leguminous plants on nitrogen fixing soil bacteria (Potera, 2007). Environmental exposure from pesticides affects the natural enemies of crop pests, hence, farmers resort in the use of additional and sometimes more pesticides in efforts to sustain crop yields (Pimentel, 2005). In addition to destroying natural enemy populations, the extensive use of pesticides has often resulted in the development and evolution of pesticide resistance in insect pests, plant pathogens and weeds (Pimentel, Zuniga & Morrison, 2005). Broad spectrum pesticides such as organochlorine, organophosphorus and carbamate insecticides, destroy both pests and beneficial organisms indiscriminately hence disturbing the natural balance of the ecosystem (Gangemi *et al*., 2016; Pimentel *et al*., 2005)

Persistent organochlorine pesticides have been found in the environment from agricultural production area. For example, sediment and biota samples from the rivers flowing in the Indian ocean in the coastal marine area of Dar es Salaam in Tanzania were found to contain quantifiable levels of dieldrin, p,p'-DDT, p,p'-DDE, p,p'-DDD, o,p'-DDT and γ-HCH (Mwevura, Othman & Mhehe, 2002). Traces of pesticides residues (31.4%) had been reported in ready to eat vegetables (Kiwango *et al*., 2017) while pesticides residues and their metabolites were detected in 95.8% of tomato and watermelon samples from Dar es salaam markets (Mahugija, Khamis & Lugwisha, 2017). Furthermore, pesticides had been reported the sediments in cassava, eucalyptus and cashew leaves (Marco & Kishimba, 2006) indicating environmental pesticides exposure.

Likewise, high concentrations of HCHs, DDTs, endosulfans, heptachlors, aldrin and methoxychlor in surface sediments from Wetland conservation area in China indicated that they originated from different contamination sources (Liu *et al*., 2012). Moreover, honeybees and wild bees which are vital for pollination of fruits, vegetable and other crops are affected by most insecticides used in agriculture which are toxic to these pollinators. Wild birds and other mammals are also damaged and destroyed by pesticides (Pimentel *et al*., 2005).

Pesticides had been reported to impart environmental concerns in the destabilization of ecosystem, reducing natural processes in the improvement of soil fertility and control of crop pesticides. The toxicity of pesticides in the environment inhibits the natural process of environmental detoxification through biodegradation process which involves the use of microbes or plants (Singh, 2008). This raises a major concern on environmental, public and health to smallholder farmers directly involved in the vegetable subsector due to significant impact on environment and human health as manifested in human diseases, destabilization of ecosystem and reducing natural process in improvement of soil fertility and control of crop pests (Naphade *et al*., 2012). Decreasing the biological diversity of soil micro and macro fauna, decreasing species diversity and disrupting natural ecosystem had been adversely noted to be fuelled by pesticides use (Potera, 2007).

2.5 Determinants of farmers' exposure to pesticides

Exposure to pesticides can occur via inhalation, ingestion, direct contact, or across the placenta. Biomarkers currently exist for detecting some pesticides in blood serum, semen, ovarian follicular fluid, amniotic fluid, umbilical cord blood, breast milk, meconium and urine (Anwar, 1997; Gilden *et al*., 2010). For exposed workers, absorption through the dermal pathway is the most important route of uptake and not the respiratory system as is commonly believed (Anwar, 1997).

The general population is exposed to the residues of pesticides, including physical and biological degradation products in air, water and food (Bhalli *et al*., 2009; Bolognesi, 2003). Although indications of health effects from pesticides are applicable to the general population, workers employed in pesticide production and farmers who use pesticides are at particularly high risk for the potential health effects of pesticides and hence warrant particular concern and special protections (Gilden *et al*., 2010). Studies have shown that both the farming and non-farming populations have been occupationally and environmentally exposed due to excessive use of insecticides for pest control in their areas of cultivations (Latif *et al*., 2012; Mathur *et al*., 2005).

Most farmers do not take precaution by utilizing protective gears during pesticides handling and application thus subjecting them to high risk of exposure thorough direct contact with pesticides (Damalas & Khan, 2017; Lekei *et al*., 2014; Ngowi *et al*., 2006; William *et al*., 2006). Effective use of protective equipment significantly reduces the health hazards from pesticides. Workers in the municipality of Ankara (Turkey) who had taken the necessary individual safety precautions had less DNA damage than those who had taken no precautions (Gundogan *et al*., 2018). In their study (Undeger & Basaran, 2005) reported that the increase in the number of highly damaged cells were significantly noted in workers without protection compared to those using protective gears. Since the use of pesticides is indispensable especially in the tropics, farmers education will significantly influence the decision to spray and choice of the use of personal protective equipment (PPEs) (Van Der Hoek *et al*., 1998).

Education level have a positive influence on the choice of protective clothing, observing re-entry period and proper disposal of empty pesticide containers (William, 2008), as higher proportion of farmers who had only basic education is reported to indiscriminately using pesticides. Inadequate knowledge and broad information regarding the application of pesticides from government extension services predispose farm workers to pesticide exposure resulting from inappropriate usage, disposal and use of special protective equipment (Latif *et al.*, 2012).

When individuals are exposed to mixtures of pesticides, it is difficult to predict the final effect because of the interaction that could occur among the involved agents as whether potentiating or antagonizing the effect (Naravaneni & Jamil, 2007). Certain pesticides are capable of chemically interacting when combined in mixtures, mainly because the metabolism of one chemical can affect the other (Das, Shaik & Jamil, 2007). An in vitro study on genotoxicity induced by pesticide mixtures showed that higher concentrations could cause significant DNA damage with individual pesticides while very low concentrations of their binary mixtures could bring about the same effect (Das *et al.*, 2007). The genotoxic damage resulting from pesticides exposure is associated with inappropriate or general lack of protective measures taken by the workers. Farmers and pesticides attendants therefore, need to be educated about the potential hazards of using pesticide cocktails and the importance of using protective measures (Grover *et al.*, 2003).

Pesticides storage among smallholder farmers also fosters exposure to toxic substances. In the study on pesticides handling among coffee farmers, in northern Tanzania (Ngowi, 2002) coffee farmers more often displayed unlabeled pesticides containers and missing instructions, while cotton pesticides were reported to be stored in bedrooms, near food as well as open fires and presence of pesticides leftovers in place. These pesticides handling practices increase the risks for exposure by farm workers and their families to pesticides.

Agricultural activities were reported to influence the extent of exposure (Bolognesi, 2003) especially for people involved in preparing and spraying pesticide mixture and are considered to be at higher risk. Personal pesticide exposure in both occupational and residential settings is influenced by both the pesticide application characteristics and personal behaviour (Alavanja *et al.*, 2004). Smoking also increases the level of exposure among farmers and pesticides workers (Bhalli *et al.*, 2009). Likewise, an impact analysis of individual characteristics (age, prescription medicine, alcohol consumption, and smoking) on DNA damage, found that only smoking influenced both hematological parameters and DNA damage level (Andre *et al.*, 2007).

Exposure duration likewise increases risks of detrimental health effects. A significantly increased exposure was observed in pesticide manufacturing workers with more than 10 years exposure (Bhalli *et al.*, 2009). Age was found to have a non-significant effect on exposure

(Naravaneni & Jamil, 2007) but they found a negative correlation between exposure effects measured by DNA damage and length of exposure to pesticide in farmers. These differences are accounted by the fact that biomonitoring studies on populations exposed to pesticides are specific, because different populations have different lifestyles, nutritional habits, climatic and environmental conditions, and are exposed to different mixtures of pesticides (Bhalli *et al*., 2009).

Individuals' genetic variability in the enzymes that metabolize agricultural chemicals affects the exposure effects of pesticides among exposed farmers (Coskun, Coskun, Cayir, & Ozdemir, 2011; Lozano-Paniagua *et al*., 2016). When these enzymes are not efficient in detoxification, metabolic products accumulate, contributing to the carcinogenic process (Liu *et al*., 2006). Individuals with null genotypes for GSTM1 and GSTT1, as well as carriers of PON1 192RR genotype, might be at increased genotoxic alkylating effect after exposure to pesticide (Liu *et al*., 2006).

Several studies (Adad *et al*., 2015; Gómez-Martín *et al*., 2015; Lacasana *et al*., 2010) have shown that individual susceptibility plays a critical role in the response to pesticide exposure and therefore, determining the onset or absence of clinical symptoms, as well as acute poisoning. Polymorphisms of key metabolism enzymes such as paraoxonase 1 (PON 1) are directly associated with individuals' sensitivity to pesticide exposure. These polymorphisms affect the efficiency of protein and its catalytic function for different substrates, and they result in the differential response regarding the incidence of DNA damage in individuals exposed to organophosphates (Lozano-Paniagua *et al*., 2016).

Pesticide exposure can be controlled for farmers and population at large by strictly controlling the access to pesticides, to minimize their use and replace highly toxic pesticides with those of low toxicity (Lee *et al*., 2017). Despite the fact that most developing countries do not have, or cannot effectively enforce, regulations that protect individuals from the adverse health effects of pesticides (Van Der Hoek *et al*., 1998), their governments should advance information technologies that make it less costly to monitor pesticide use and make self-reporting requirements politically and economically feasible (Sexton, 2007). Regulation and legislation in relation to pesticides is therefore essential. The transfer of responsibility for application of pesticides to licensed professionals constitutes another attempt at reaching first-best solutions. Those professionals who provide both diagnosis and cure of pest problems should be educated and informed about all aspects of pesticide use and can be held liable for certain aspects of mismanagement (Sexton, 2007).

2.6 Concentration of pesticides in biological matrices and their effects

Exposure to pesticides results in pesticide and/or their metabolites to remain in human bodies. Presence of organophosphorus pesticides in blood means that they do persist in the body for good amount of time (Latif *et al*., 2011b; Rao & Jyothsna, 2016). Insecticides, including chlorpyrifos and endosulfan are the predominant pesticides found in blood samples (Latif *et al*., 2012). High concentration of carbamates and organophosphorus insecticides inhibit the enzyme acetylcholinesterase by reversible carbamylation and irreversible phosphorylation, respectively (Stenersen, 2004).

Occupational exposures to pesticides cause accumulation of these toxic substances in the body influencing significant changes in hematological parameters (Fareed *et al*., 2013; Hayat *et al*., 2018). Pesticides bioaccumulation involves the buildup of these toxic chemicals as a result of continuous absorption of chemicals in the body at the rate at which the body cannot metabolize and excrete (Kim *et al*., 2016). This is accelerated by persistent use of pesticides that do not break down into safer constituent parts but rather remain intact over prolonged periods of time becoming readily accessible to the human body. Bioaccumulation can occur via inhalation, ingestion, dermal contact, or across the placenta (Gilden *et al*., 2010).

Several studies had reported the presence of parent pesticides compounds in human blood, urine, breast milk, semen, adipose tissue, amniotic fluid, infant meconium and umbilical cord blood. Bioaccumulation of these chemicals in the body over time had been linked to birth defects, tumors, genetic changes, blood and nerve disorders, difficult in conceiving, and even coma or death (Ali *et al*., 2018; Hayat *et al*., 2018; Jun, Bajgar, Kuca & Kassa, 2015). Table 2 summarizes studies on the presence of pesticides in human matrices.

Residual blood concentration of pesticides may lead to abnormal enzyme activities affecting their biochemical activities (Hayat *et al*., 2018; Singh *et al*., 2011). They can act as weak hormones, thereby interfering organisms' balance of natural endogenous hormones, such as estrogens, androgens, and thyroxine (Gundogan *et al*., 2018; Matisova & Hrouzkova, 2012). Likewise, chronic exposure to organophosphorus impairs glucose homeostasis and cause insulin resistance and type 2 diabetes (Bolognesi & Holland, 2016; Lasram, Dhouib, Annabi, El Fazaa & Gharbi, 2014). Allergic effects from pesticides exposure are a result of long term bioaccumulation of pesticides in exposed individuals (Kumar, Patheran, Saini & Kumar, 2012).

Detection of parent organophosphorus pesticides in blood, urine, human milk, saliva had been performed to confirm exposure of the farming population. For example, chlorpyrifos and endosulfan residues were detected in the blood of non-agro professional volunteers in Pakistan, signalling environmental exposure due to the massive use of pesticides in the area (Latif *et al*., 2012). These pesticides have been reported as developmental neurotoxicant, persistent developmental disorders, malformation and micronucleus formation, and maternal toxicity which specifically target immature brain leading to a range of childhood cancers and hepatic and renal toxicity (Li *et al*., 2015; Singh *et al*., 2011; Watts, 2012).

Environmental and non-occupation exposure to organophosphorus had been reported in various studies. Children in agricultural areas exhibit cytogenetic damage and pesticides metabolites in urine and blood provide evidence of pesticides environmental exposure (Ruiz-Guzmán *et al*., 2017). Likewise, respiratory symptoms linked to environmental pesticide exposures including coughing, airway irritation, wheezing, and airway infection in children had been reported (Ming Ye *et al*., 2017).

Table 2: Concentration of pesticides and their metabolites in human biological matrices

Category of people tested	Analyzed biological matrices	Results	Concentration of analyte (min-max)	Reference
Exposed farmers in Pakistan	Human blood serum	Chlorpyrifos, endosulfan, 1,1,1-trichloro-2,2-bis (p-chorophenyl) eth-ane (p,p'-DDT) and parathion residues detected	Chlorpyrifos (0.10-0.37) mg.kg^{-1} Endosulfan,(0.15-0.30) mg.kg^{-1} p,p'-DDT (0.0-0.20) mg.kg^{-1} Parathion (0.0-0.31) mg.kg^{-1}	Latif *et al.* (2012)
Children living in agricultural areas in Colombia	Urinary	Detectable levels of Atrazine concentrations and its metabolites were recorded	Atrazine (ATZ) (13.0–25.5) μg/g creatinine Atrazine desiso-propyl (ADI) (0.0-14.7) μg/g creatinine Atrazine desethyl-desisopropyl (ADDI) (0.0-190.6) μg/g creatinine	Ruiz-Guzmán *et al.* (2017)
Expectant (Pregnant) mothers upon delivery	Umbilical cord blood	Chlorpyrifos was detected	Chlorpyrifos (nondetectable-1.15ng ml^{-1})	Tan & Mohd (2003)
Farmers exposed to pesticides in open fields and unexposed control (Pakistan)	Blood serum	Cypermethrine, cyhalothrine, deltamethrine and endosulfan pesticides residues detected in blood serum.	Cypermethrine* Cyhalothrine* Deltamethrine* Endosulfan*	Bhalli *et al.* (2009)
Pesticide applicators and farm workers working in the fruit orchards	Urine samples	Urinary organophosphorus metabolites were significantly higher in farm workers and applicators when compared with controls.	DMP(10-24) ng/ml DEP (4-10)ng/ml DMTP (60-90) ng/ml DMMTP (10-15)ng/ml DETP (2-4) ng/ml	Kisby *et al.* (2009)
38 exposed workers in pesticides industry and 20 control (unexposed	Whole blood	Quantifiable levels of malathion were detected in the exposed	Malathion (0.01-0.31 mg.l^{-1}).	Arshad *et al.* (2016)
138 (69 exposed and 69 control) cotton picking Women, Pakistan	Blood samples	Residues of three pesticides: cyhalothrin, endosulfan, and deltamethrin significantly higher in the serum samples of the exposed group compared with the unexposed	Cyhalothrin (1.04 ± 0.38) ppm Endosulfan (0.54 ± 0.22) ppm Deltamethrin (1.07 ± 0.52) ppm	Ali *et al.* (2018)
40 work tasks involving glyphosate and fluroxypyr (Republic of Ireland,)	Urine samples	Pesticide urinary concentrations of glyphosate and fluroxypyr were higher than those reported for environmental exposures	Glyphosate (0.17-5.33) μg.l^{-1} Fluroxypyr (0.04-2.74 μg.l^{-1}	Connolly *et al.* (2017)

***Concentrations not indicated**

Women picking cotton with bare hands in Pakistan were found with cyhalothrin, endosulfan, and deltamethrin in serum samples indicating continuous accumulation of pesticides in the blood (Ali *et al*., 2018). Another study reported the presence of carbosulfan, profenofos, cypermethrin, endosulfan sulfate, and chlorpyrifos-methyl in blood samples of occupationally exposed agricultural workers (Hayat *et al*., 2018).

Bio accumulation of these chemicals in the body over time culminates in a range of effects including skin irritation, respiratory disorders, birth defects, tumors, genetic changes, blood and nerve disorders, endocrine disruption, and even coma or death (Watts, 2012). Insecticides are the predominant pesticides found in blood samples (Latif *et al*., 2012). These included chlorpyrifos and endosulfan. Chlorpyrifos is one of the most widely used organophosphorus pesticide that has been reported as a developmental neurotoxicant which specifically target the immature brain (Mathur *et al*., 2005). Presence of organophosphorus pesticides in blood means that they do persist in the body for a long duration (Mathur *et al*., 2005). Human biomonitoring studies in the United States indicate that most people have detectable levels of dichloro-diphenyl-trichloroethane (DDT) in their bodies, despite the fact that DDT was banned from use in the United States in 1972 (Gilden *et al*., 2010). Study in Pakistan found presence of chlorpyrifos and endosulfan residues in the blood of non-agro professional volunteers. This may be due to the massive use of these pesticides since last couple of decades (Latif *et al*., 2012).

Despite available evidence of pesticides exposure and presence of pesticides residues in the biological matrices for many studies (Table 2), there is still limited information on the extent of exposure and level of concentration of toxic substances in biological matrices of smallholder farmers from developing countries. This necessitates studies to analyze and quantify pesticides body burden for the smallholder farmers working in uncontrolled pesticides environment in developing countries mostly found in Africa. Focus should be on establishing the causal link between pesticides exposure and growing cases of cervical and breast cancer, prostate cancer and increasing trends of non-communicable diseases in developing countries.

2.7 Presence of pesticides residues in fresh vegetables and other food products

Pesticides use in vegetable production poses a public health concern due to poor pesticides handling practices. Studies have shown that smallholder farmers use pesticides indiscriminately (Lekei *et al*., 2014; Ngowi *et al*., 2006; Vikkey *et al*., 2017), which increases the risks for contamination of vegetable produce with pesticides residues while non-occupational exposure to pesticides may occur through ingestion of residues in food (Saillenfait *et al.*, 2015).

In Tanzania like other countries, pesticides residues had been reported in vegetables (Kiwango *et al.*, 2017; Mahugija *et al.*, 2017). Considering the frequency of pesticide residues detected in food commonly produced and consumed, a wide range of horticulture produce produced from developing countries do not fetch the EU and regional market as products imported from developing countries to the EU market as were found to have higher prevalence of residues exceeding the MRL (EFSA, 2016).

Pesticides residues had been reported in various food materials (Bai, Zhou, & Wang, 2006; Baker, Benbrook & Benbrook, 2002; Darko & Akoto, 2008; Diop *et al.*, 2016; Kiwango *et al.*, 2017; Wu *et al.*, 2017). Majority of foods purchased in supermarkets in the US had detectable levels of pesticide residues (Baker *et al.*, 2002). Pimentel (2005) reported that up to 5% of the foods tested in 1997 contained pesticide residues that were above the FDA tolerance levels. Despite these foods violating the US tolerance of pesticide residues in foods, they were consumed by the public as the food samples were analyzed after the foods were sold in the supermarkets. People are therefore exposed not only through spraying but also consumption of vegetables that are contaminated with pesticides (Van der Hoek *et al.*, 1998). Fresh fruits and vegetable constitute the most frequent pesticide contaminated foods (Chen *et al.*, 2011).

In Jordan, 32% of fruits and vegetables had detectable levels of pesticides with thiamethoxam hexaconazole, propargite, clofentezine, propiconazole, myclobutanil and pyridaben residues violating MRLs according to European regulations (Algharibeh & AlFararjeh, 2019). In China, over 40% of food samples were reported to contain detectable levels of pesticides residues with procymidone, some banned or restricted pesticides including HCB, DDT and carbofuran detected. Furthermore, 3.88% exceeded the maximum residue limits (MRLs) (Wu *et al.*, 2017). Likewise, 48.3% of samples tested in Brazil were found to contain pesticides residues (Chen *et al.*, 2011; Jardim & Caldas, 2012). Vegetable samples from India were also reported to be contaminated with pesticides with malathion, methyl parathion and chlorpyriphos residues but the concentration of these pesticides were well below the MRLs established (Bhanti & Taneja, 2007). Pesticides residues, including cypermethrin, dichlorvos, dimethoate, parathion-methyl, pirimiphos-methyl and parathion were reported in Chinese markets with cereals, vegetables, and fruits having concentrations' mean levels exceeding the MRLs (Bai *et al*., 2006; Chen *et al*., 2011; Wu *et al*., 2017).

2.8 Measures to prevent and control pesticides exposure

In preventing consumer exposure to toxic chemical substances, Maximum Residue Levels (MRLs) are therefore established. Maximum Residue Levels MRLs are the upper legal levels of concentration for pesticide residues in food or feed based on good agricultural practices and ensure the lowest possible consumer exposure (FAO, 2007). The EU-harmonized MRLs had been set for more than 500 pesticides with a default MRL of 0.01 mg/Kg, a level equal to limit of quantification (LOQ) achievable with analytical methods used for MRL enforcement (EFSA, 2016). But meeting these international food safety requirements has become a major challenge for fresh produce export sector in many developing countries (Dureja, Singh & Parmar, 2015). There is general lack of designed programs to improve pesticides usage, regulation and management on vegetable crops to ensure and maintain export compliance, grower and consumer safety and environmental integrity (Karungi, Kyamanywa, Adipala & Erbaugh, 2011).

Pesticides residues may be present in organically produced vegetables, implying that pesticide residues can be found in food materials even when pesticides are used in accordance with recommended rates under Good Agriculture Practices (GAP) (Baker *et al*., 2002; Uysal-pala & Bilisli, 2006). This is because persistence pesticides application results in accumulation of toxic substances in the soil, and most leafy vegetables tend to absorb organochlorine and other pesticide residues from the soils and translocate them into edible crop tissues (Baker *et al*., 2002). Pesticide residues had been found in strawberries, onions, cucumber, lettuce, cabbage, okra, pepper, tomatoes, beans, oranges and lemons grown under organic farming (El-Nahhal, 2004; Hanson, Dodoo, Essumang, Blay & Yankson, 2007; Hussain, Masud & Ahad, 2002).

Presence of pesticides residues in food produce had been reported from both developing and developed countries (Baker *et al*., 2002; Bhanti & Taneja, 2007; Chen *et al*., 2011; Darko & Akoto, 2008; Jardim & Caldas, 2012; Kiwango *et al*., 2017; Mahugija *et al*., 2017; Mtashobya & Nyambo, 2014).. In Ghana for example, 42% of tomato samples and 10% of eggplants had pesticides residues (Darko & Akoto, 2008), whereby methyl-chlorpyrifos, ethyl-chlorpyrifos, and omethioate in tomatoes and methyl-chlorpyrifos, ethyl-chlorpyrifos, dichlorvos, monocrotophos and omethioate in eggplant exceeded MRLs. In a recent study from Ghana, (Forkuoh, Boadi, Borquaye & Samuel, 2018), the levels of aldrin and gamma-hexachlorocyclohexane (HCH) exceeded the maximum residue limits in analyzed fruit samples and the estimated health risk indicated that they could pose potential toxicity to the consumer.

Large proportion of food samples from developing countries are reported to constitute pesticides residues above the MRLs (Neff *et al.*, 2012), whereby over 60% were reported to contain pesticides residues above MRLs. On the other hand, only 3% of food samples from EU had pesticides residues above MRLs (EFSA, 2016) and up to 5% of the foods tested in the US contained pesticide residues that were above the MRLs levels (Pimentel, 2005). Despite the alarming rate of pesticides residues in developing countries like Tanzania, consumers are not well informed on the adverse acute and chronic effects of pesticides resulting from consumption of contaminated food products (Ecobichon, 2001).

Poor use and application of pesticides in different parts of the world, are assumed to foster contamination of food materials with pesticides residue (Kiwango *et al*., 2017; Latif *et al*., 2011b). In this regard, even consuming pesticides residues below the established tolerances cannot not be assumed safe because continuous consumption of contaminated vegetables with low levels of residue can accumulate in the body receptors, thereby resulting in long terms chronic effects (Bhanti & Taneja, 2007).

2.9 Co-occurrence of pesticides residues and microbial contamination

Extensive accumulation of pesticides residues and metabolites in the soil at high levels affects microbial strains that have a great degrading potential on organophosphorus and other toxic compounds in the environment (Singh, 2008). Some pesticides, including 2,4-D and carbofuran, can serve as a carbon source, hence supporting growth of some microorganisms (DuPlessis *et al*., 2015). Several bacterial and fungal strains have significant ability to carry out the degradation of pesticides in the natural process of removing soil contaminants. Pesticides may therefore present a suitable environment for the survival and growth of pathogenic bacterial species (Ng *et al*., 2005). Pesticides with organophosphates and carbamates as their active ingredients are reported to support the growth of pathogenic microbe growth (DuPlessis *et al*., 2015).

Bacterial belonging to genus *Enterobacter, Bacillus thuringiensis, Pseudomonas putida, Stenotrophomonas maltophilia* and *Rhodococcus erythropolis* had been found to able to degrade pesticides (Ibrahim, Amin, Hassan & El-Sheikh, 2015; Lovecka *et al*., 2015; Singh, 2008). Furthermore, Aspergillus *niger* had been reported to degrade endosulfan and lindane, while *Trichosporon spp* had the capacity to degrade Chlorpyrifos (Iqbal & Bartakke, 2014). The organism can degrade pesticide and utilize it as a carbon source for growth (DuPlessis *et al*., 2015). Presence of pesticides in the environment can support the survival and growth of bacterial species including species of *Pseudomonas, Salmonella* and *Escherichia coli* (Ng *et al*., 2005). Pesticide residues adsorbed to inorganic matter has also been proposed as another factors which influence the availability of the microbial activities in the environment (DuPlessis *et al*., 2015).

Rivers, dams, lakes, boreholes and streams constitute the main sources of water used for irrigation at production level. Owing to unhygienic farm environment in rural setting where most vegetable production is undertaken, faecal matter, soil and other contaminants including sewage overflow can introduce foodborne pathogenic bacteria in water sources which can affect the quality and safety of agricultural water (DuPlessis *et al*., 2015).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

This study was undertaken in major smallholder growing agro ecological zones as well as major consumption area in Tanzania including southern highlands (Iringa), northern corridor (Arusha, Manyara and Kilimanjaro) and coastal zone (Dar es Salaam and Morogoro). Dar es Salaam and Morogoro municipal were mainly targeted for the consumption (market), while the other regions were both production and consumption.

3.2 Study design

The study employed a multifaceted approach in the sampling and collection of data. A cross section study design was used in the assessment of pesticides usage in 385 smallholder farmers who practice vegetable production. In biomonitoring of pesticides exposure, a subsample was drawn from this sample with matched control group from the same geographical and socioeconomic settings and comparative analysis of Acetylcholinesterase (AChE) inhibition done across demographic and anthropometric variables. The assessment was undertaken during high spraying seasons. From the 385 farmers, a sub sample of 90 exposed farmers was selected for the AChE test and health assessment and 90 unexposed individuals were recruited. High volumes of pesticides were observed in Ngarenanyuki and Kilolo compared to other areas, hence provided the most possible area for exposure assessment.

3.3 Sampling and sample size calculation

Purposive sampling (Kothari, 2004) was employed in selecting regions and districts with high vegetable production in different agro ecological zones. Random sampling was used to select wards, villages and household to be included in the study sample. A total sample of 385 was used in this study. The sample size was calculated based on the following formula for determination of minimum sample size (Kothari, 2004);

$$n = \frac{Z^2 \mathrm{p}(1 - \mathrm{p})}{\mathrm{M}^2}$$

Where; n = Sample size, Z = % point of the standard normal distribution which is 1.96 in this case corresponding to 95% confidence level, M = marginal error which is 5%, p = expected proportion of the respondents taken as 50%, = 0.5, q = 1-p. 50% was used in the sample size calculation since if the proportion of the population (p) is unknown, p = 0.5 assumes maximum heterogeneity of the population (i.e. a 50/50 split which is the percentage picking a choice or response) (Kothari, 2004; Mundfrom, Shaw, & Ke, 2005);

$$n = \frac{(1.96)^2 \text{ X } 0.5\ (1-0.5)}{(0.05)^2} = 384.16$$

$$\approx 385$$

3.4 Samples type, collection and analysis

3.4.1 Sample collection

A total of 613 samples from 17 horticultural crops produced by smallholder farmers were analyzed for pesticide residues, out of which 250 were also tested for microbial contamination. Samples were randomly purchased from market places, farmer fields during harvesting period and along with highway selling points. A subsample of 250 (189 from markets and 61 from farm fields) was analyzed for bacterial contamination due to the homogeneity of samples. Homogeneity of samples and location from the farms reduced the proportional of samples. Likewise, only samples collected from areas where samples could reach the TPRI lab within 4 hours of sample collection were considered for the assessment of bacterial contamination. Samples were taken in sterile polyethylene bags which were placed in iced packed cool boxes and transported to the laboratory. Samples were selected according to FAO recommended guidelines on sampling for pesticide residue analysis (FAO, 2007), a sampling method for determination of pesticide residues, where each whole fruit, vegetable, or a bunch of vegetables were taken to form a unit, except where these were very small. The minimum weight for the small and medium-size sample was 1 Kg and that for large size product was 2 Kg.

3.4.2 Chemicals and reagent

Fifty-two (52) certified pesticide standards with purities between 99.0–99.9% were obtained from the official registrant companies of specific pesticides in Tanzania. Tetramethrin standard was obtained from Star Import & Export, Tanzania, Pirimiphos–methyl, Permethrin, Profenofos, and Lambda Cyhalothrine from Syngenta Crop Protection Ag, Switzerland; Cabaryl from Bayer Environmental Science, Triadimefon from Meru Agro Tours &

Consultant Tanzania, Dimethoate from Sapa Chemical Industrial Ltd, Chlorpyrifos and Bifenthrin from Balton Tanzania Ltd-Tanzania, Oxyfluorfen from Bayer East Africa Ltd-Kenya, Malathion from Tanzania Crop Care Limited, Tanzania while Propanil was obtained Suba Agro-Trading and Engineering Co.Ltd –Tanzania. Solvents at HPLC grade, including acetonitrile, acetic acid, and acetone, salts of analytical grade such as anhydrous magnesium sulphate, sodium sulphate, and sodium acetate; Primary and Secondary Amine (PSA), were locally sourced from Immuno Lab Supplies, Dar es Salaam, Tanzania.

3.4.3 Sample preparation and extraction for pesticides residues analysis

Sample preparation and extraction were done following the Association of Official Analytical Chemists (AOAC) official method (QuEChERS Protocol), an extraction method for pesticide residues in foods by acetonitrile extraction and partitioning with magnesium sulfate, applicable for pesticides in fruits and vegetables (Anastassiades, Lehotay, Štajnbaher & Schenck, 2003; Lehotay, 2007). Fresh vegetables analyzed for pesticide residues included onions, watermelons, tomatoes, sweet paper, Chinese cabbage, African nightshade, carrots, amaranths, kale, Ethiopian mustered, African eggplants, eggplant, green beans, cabbage and okra. Samples were processed soon after collection and transported to the laboratory at 4 ºC, stored at this temperature until they were analyzed.

Briefly, a whole unwashed 200 g sample was homogenized through chopping into small pieces, grinding, and blending. This is because, according to the CODEX Guidelines on Good Practice in Pesticide Residue Analysis, pesticides may tend to collect in the stem area of fruits and on the top of vegetables. Therefore vertical sections must be cut through the stem and centre of fruits, and the top and centre of vegetables should be chopped and homogenized for analysis (FAO, 2001). Exactly 15 g of the sample was transferred in a 50 mL centrifuge tube, and 15 ml of ethyl acetate was added. An internal standard, 100 µL, was added and vortexed for 1 min, then 5 g $MgSO_4$ and 1.5 g sodium acetate were added, vortexed to 1 min, and centrifuged at 4000 r.p.m for 10 min. A supernatant layer (3 mL) was transferred in a 15ml centrifuge tube, 300mg $MgSO_4$, and 150 mg Primary and Secondary Amine (PSA) added, vortexed for 1 min and centrifuged again at 4000 r.p.m for 10 min. A final supernatant layer (1.5 mL) was transferred into G.C. vials and injected in GC-MS for the detection of pesticide residues.

3.4.4 Standard preparation for pesticides residues

Individual pesticide standard stock solutions were prepared in acetone to a final concentration of 20.0 mg/L and stored at -20 ºC. The standard mixed component solution was then prepared by diluting each primary standard solution with acetonitrile with 1% acetic acid (1:10 v/v mL). This was then used for spiking extracted fruit and vegetable samples. A 100 µL of 1 mg/mL heptachlor was used as an internal standard to ensure the accuracy of the GC-MS response.

3.4.5 Gas chromatography-mass spectrometry system and operating conditions

The Agilent 7890A Gas Chromatography-Mass Spectrometer (GC-MS), which is equipped with 7693 auto-sampler coupled with a 7000B triple quadrupole M.S. system was used in the detection and in quality assurance of pesticides residues. A fused silica DB35 capillary column, 30 mm long with 0.25 mm internal diameter and 0.25 µm film operating at a range of 50 ºC to 360 ºC was used with the internal temperature set at 50 ºC for 1 min, constantly raised to 150 ºC at a rate of 50 ºC per minute, followed by 280 ºC at a heating rate of 5 ºC per min and held for four minutes. The injector temperature was 250 ºC and a carrier gas was helium (99.9%) at a flow rate of 1.2 mL min-1 split less injection. The injection volume was 1µL at a pressure of 43.193 Psi. The MS ion source temperature was 250 ºC operated in full scan mode at a scan range of 50 - 550 ºC atomic mass unit.

3.4.6 Recovery, quantitative evaluation and detection limits

The method performance for the quantification of the concentration of pesticide residues in fresh vegetables widely produced and locally consumes was validated according to the European Commission guidelines for pesticide analysis (FAO, 2001). This was done by determining recoveries, Limit of Detection (LOD), Limit of Quantification (LOQ), precision, and linearity. Recovery was performed by analyzing a mixture of standard pesticides in blank vegetable samples at different known concentrations of 0.1, 0.5, 1 and 1.5 mg/Kg in triplicates. A 15 g homogenized sample was spiked with pesticide mixture standard solution and allowed to equilibrate for 3 hours prior to extraction. Extraction and analysis were done according to the procedures described previously. Calibration curves constructed from the concentration and peak areas of the chromatograms obtained with standards were used to calculate recovery values. The mean recoveries ranged between 75% and 115%, with an average of 94%. Precision was determined by calculating the relative standard deviation

(RSD) of the lowest concentration that could show linearity in blank vegetable samples. The relative standard deviations (RSD) obtained was below 10% with an average of 7.7%.

Linearity was determined by analyzing a mixture of pesticide standards at different concentrations ranging from 0.005 - 0.02 mg/Kg. The area of the corresponding peak in the sample was then compared with that of the standard. Specificity and validity of the method was monitored by running control blank vegetable samples simultaneously, in which no chromatographic peak was observed at the same retention times of target pesticides which indicated non-occurrence of interferences. Analyses were carried out in triplicates and the mean concentrations based on the number of samples that tested positive for each sample calculated. Limits of quantification for the method were calculated by considering a value 10 times that of background noise while detection limits were found by determining the lowest concentrations of the residues in each of the matrices that could be reproducibly measured at the operating conditions of the G.C. using a signal-to-noise (S/N) ratio of 3. The calculated limit of detection limits (LOD) ranged from 0.002 – 0.006 mg/Kg, while the Limits of Quantification (LOQ) ranged from 0.002 to 0.016.

3.5 Blood sample collection and handling for acetylcholinesterase (AChE) Tests

The assessment of acetylcholinesterase (AChE) inhibition was conducted among selected exposed farmers and control groups from Kilolo (Iringa) and Ngarenanyuki (Arusha). These two areas were purposely selected based on extensive use of synthetic pesticides in vegetable production. Vegetable farmers were randomly selected from the list of households provided by respective village government officers. The sample was chosen based on the proportion of farmers involved in smallholder vegetable production.

Inclusion criteria included individuals who were occupationally involved in pesticides handling and working in a sprayed field and had sprayed during the last week before the survey or had weeded/harvest field sprayed with pesticides during the same period. Exclusion criteria consisted individuals not involved directly in any handling pesticides or not working in pesticides related activities. Excluded were also those individuals who reported any known conditions that could influence levels of AChE, such as those previously diagnosed with hyper/hypotension, diabetes, anemia and those under medication/vaccination during the time of the survey. The control group was purposely selected based on the criteria that none of them had been exposed to agrochemicals during the study period or previous occupationally exposure to pesticides and matched with age, sex and other demographic variables. This group included office employees and shopkeepers living in the same region as exposed individuals.

The estimated study sample for biomonitoring of exposure was based on previous studies which indicated that a sample size of 30 farmers would be sufficient to detect a difference in cholinesterase activity between farming and non-farming groups and that of 90 yields power over 80% (Cotton, Edwards, Rahman, & Brumby, 2018; Neupane, Jørs & Brandt, 2014). A total of 29 individuals from the control groups did not meet the inclusion criteria and therefore, were removed from the sample. Hence, a sample size of 61 unexposed individuals with similar social economic characteristics was used for controlling confounding factors and draws a comparative conclusive statement on the extent of pesticides exposure in smallholder vegetable production.

3.6 Data collection and health survey

A semi-structured questionnaire containing both closed and open-ended questions was administered to participants during the cross-sectional survey. The questionnaire used in previous studies (Ngowi *et al*., 2006) was used with minor modifications to suit the current research. This improved questionnaire was further pretested among 20 individuals from one village in the study areas, which was finally removed from the sample. Collected information included pesticides used, handling practices, frequency of application, areas sprayed, use of PPEs and exposure risk behaviours. Demographic information and farmer habits and life styles (age, gender, alcohol consumption and smoking) were also collected.

Data on used pesticides were grouped according to their active ingredients, and classified using the five WHO Acute Toxicity Hazard Categories, namely Extremely Hazardous (Class Ia), Highly Hazardous (Class Ib), Moderately Hazardous (Class II), Slightly Hazardous (Class III) and unlikely to present acute hazard (CLASS U) (WHO, 2015). Self-reported exposure symptoms to organophosphates (OPs) and carbamate (CA) pesticides were collected through health survey using a questionnaire with random list of 38 different symptoms typical to OPs and CA exposure. Anthropometric measurement (height and weight) were also taken to determine the Body Mass Index (BMI), which was calculated and categorized using four WHO criteria for underweight, normal, overweight and obese. Field observations were done to observe pesticides mixing, handling, type of PPE used and disposal methods of empty pesticides containers.

3.7 Blood collection and determination of acetylcholinesterase (AChE) inhibition

Collection of blood was carried out according to the procedures explained by Cotton *et al.* (2018) and Neupane *et al*. (2014). Erythrocyte Acetyl cholinesterase Test Mate Photometric Analyzer kit (Model 400) was used to test the cholinesterase inhibition (AChE activity standardized against whole blood haemoglobin) based on manufactures' standard methodology (EQM, 2003). In brief, alcohol was used to wipe fingers which were then air-dried for about 30 seconds. A capillary blood sample of 10 µL was collected using a finger prick sterile lancing device, immediately put into the assay tube. Distilled water was used to dissolve AChE erythrocyte cholinesterase reagent and inserted into the analyzer. Haemoglobin, AChE and Q readings were recorded. This haemoglobin adjusted erythrocyte acetylcholinesterase activity (Q) was measured in (U/g Hb) and used to describe the levels of exposure.

3.8 Assessment of bacterial contamination

Out of the 613 fresh vegetable samples collected, 250 were apportioned aseptically in sterilized sampling bags and transported to the lab in sterilized cool boxes within 4 hours and processed soon after arrival. Samples were homogenized using a pre-sterilized blender, and the sample mixture was filtered through a filter paper to get a clear filtrate. About 5 mL of the filtrate was inoculated in the tryptic soy broth (TSB) enrichment broth and incubated for 24 hours at 35 ºC. After growth, each sample was streaked onto selective and differential agar plates [MacConkey and Xylose Lactose Deoxycholate (XLD)] agar and incubated for 24 hours (Saima *et al*., 2018). Pure bacterial colonies were isolated and sub-cultured in nutrient agar (NA), tryptic soy agar (TSA) (Ruangpan & Tendencia, 2004) and incubated for the other 24 hours at 35 ºC. Identification of the bacterial strains was done using biochemical identification tests for common gram-negative bacteria isolates including Simmons Citrate Agar, Lysine Iron Agar, Urea Agar Base, Triple Iron Agar, and Sulphur Indoor Motility Agar (Abdallah, Mustapha, Gambo & Ishaq, 2014; Saima *et al*., 2018).

3.9 Data analysis

Statistical analysis of data was done using SPSS 22.0 computer software. Descriptive statistics such as frequencies, percentages, mean, and standard deviations were performed to summarize the characteristics for the study population, and results are presented as (Mean ± Standard Deviation). Association of risk behaviours, including smoking, eating, and use of PPEs, BMI, and haemoglobin adjusted erythrocyte acetylcholinesterase activity (Q) was done using Chi-square. One-way ANOVA test was used to determine factors influencing haemoglobin adjusted erythrocyte acetylcholinesterase activity (Q) among exposed and control subjects, which was dichotomized into high and low inhibition. The cut-off point was set at one standard deviation SD below the population mean, i.e., at the 25.2 U/g Hb. Student t-test was used to compare the significant difference in the levels of exposure between farmers and control groups. Binary logistic regression analysis was used to determine the critical explanatory factors for AChE inhibition as well as explanatory factor to co-occurrence of pesticides and bacterial contaminants in vegetables produced in smallholder vegetable production. Significant level for the results was accepted at $p < 0.05$.

Determinants of farmers' changing patterns in increased pesticides use were determined by regressing the levels of pesticides use (dependent variable) on a set of demographic and handling practices using a binary probit model. The purpose of this model is to estimate the probability that an observation with particular characteristics falling in one of proposed categories (Khan & Damalas, 2015). Thus, binary probit model was used to predict the probability that farmers will resort in increased use of pesticides based on specific predictors. The region was included in the model to ascertain possible differences in farmers' level of pesticides use with respect to their geographical location.

The regression model used to estimate the determinants of increased pesticides use (IPU) is given by:

$$IPU= (\beta0 + \beta1GF + \beta2AF + \beta3EL + \beta4NC + \beta5FS + \beta6PE + \beta7MP + \beta8AI + \beta9FA + \beta10SP + \beta11PPE + \beta12PL + \beta13RF + \mathcal{E})$$

Where:

IPU= Increased Pesticides Use, GF = Gender of Farmer, AF = Age of Farmer, EL = Education Level, NC = Number of vegetable Crops farmer grows, FS = Farm Size, PE = Perception on the Effectiveness of Pesticides, MP = Mixing Practices, AI = Access to Information on Pesticide Use, FA = Frequency of Pesticides Application, SP = Source of Pesticides, PPE = Use of Personal Protection Equipment, PL = Read Pesticide Label, RF = Region of the farmer, $\mathcal{E}$ = unknown parameters.

Binary logistic regression analysis with the outcome variable, the probability of having low AChE level, adjusted for age, BMI, working experience with pesticides, working hours, average area sprayed and breaking period was used to determine the critical explanatory factors for AChE inhibition. Significant level for the results was accepted at $p < 0.05$. Taking these factors into account, the following logistic regression model with dummy variables to control for any individual differences was developed.

Pesticides Exposure = f (Age, BMI, WEP, AAS, CHP, SBP, Ɛ)

Where; Pesticides Exposure is the measure of AChE inhibition indicated by low or high Q level, as dichotomized at one SD below the mean Q, i.e., at the 25.2 U/g Hb. For the explanatory variables, Age is the age category of farmers, BMI is the WHO Body Mass Index categories, WEP is the working experience with pesticides, AAS is the average area sprayed per day in acres. Furthermore, CHP is the contact (working) hours with pesticides, SBP is the spraying break period before embarking on another intensive spraying season, and Ɛ are the unknown variables.

3.10 Ethical clearance

Ethical clearance was obtained from Tanzania’s National Institute of Medical Research (NIMR) with Reference No. NIRM/HQ/R.8a/Vol.IX/2742. Both farmers and unexposed individuals each signed a written consent form for a blood test and participation in the research.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results

4.1.1 Changing patterns and drivers of increased pesticides use among smallholder vegetable producers

(i) Geographical and demographic information

The survey to assess pesticides usage was done in three vegetables production regions namely; Arusha (46.8%), Iringa (29.1%) and Kilimanjaro (24.2%). A total of 385 farmers were interviewed from four districts; Kilolo (29.1%), Arumeru (28.6%), Hai (24.2%), and 18.2% Karatu, from which 20 villages were visited (Table 3), covering the two main vegetable production agro ecological zones, namely; northern and southern zones. Dar es Salaam and Morogoro were omitted in production but included in the consumption areas.

Table 3: Geographical coverage (N=385)

Variable	Description	n	%
Region of respondent	Arusha	180	46.8
	Iringa	112	29.1
	Kilimanjaro	93	24.2
District of respondent	Kilolo	112	29.1
	Arumeru	110	28.6
	Hai	93	24.2
	Karatu	70	18.2
Village of respondent	Kimashuku	63	16.4
	Ngabobo	58	15.1
	Mtitu-Manimbi	56	14.5
	Ihimbo	40	10.4
	Olkung'wado	38	9.9
	Qangded	31	8.1
	Mbuga Nyekundu	23	6.0
	Uwiro	14	3.6
	Barazani	14	3.6
	Modio	10	2.6
	Muhimbili	8	2.1
	Sonu	5	1.3
	Roo	5	1.3
	Kiboyeye	5	1.3
	Shirimatunda	5	1.3
	Mtitu-Ngugi	2	0.5
	Mtitu-Magharibi	2	0.5
	Itengule	2	0.5
	Image	2	0.5
	Kambi ya Simba	2	0.5

Smallholder vegetable production was found to be dominated by males (77.9%) compared to females (22.1%). Likewise, middle aged and youths were found to be highly involved in smallholder vegetable production, as over 64% of all smallholder vegetable producers aged between 25 to 44 years. Majority (79.4%) of smallholder farmers had primary education level while only 13.5% had attained ordinary level secondary education (Table 4).

Table 4: Demographic information of respondents (N=385)

Variables	Description	n	%
Gender of respondent	Male	300	77.9
	Female	85	22.1
Age category of respondent	15-24 years	15	3.9
	25-34 Years	122	31.7
	35-44 years	127	33.0
	45-54 Years	96	24.9
	55-64 Years	21	5.5
	65 and above	4	1.0
Highest education level attained	Never gone to school	24	6.3
	Primary education	305	79.4
	Ordinary level Secondary education	52	13.5
	Advance level Secondary education	3	0.8

(ii) Pesticides usage among smallholder vegetable producers

Characteristics of smallholder vegetable production

The results show that 80.8% of farmers grow tomatoes while onions is grown by 35%, cabbage 27.1% and 17.1% sweet peppers (Fig. 2). Other vegetable crops grown by smallholder vegetable producers include Chinese cabbage, Nightshade, Kale, Amaranths, African egg plants, Cucumbers Okra and Carrots.

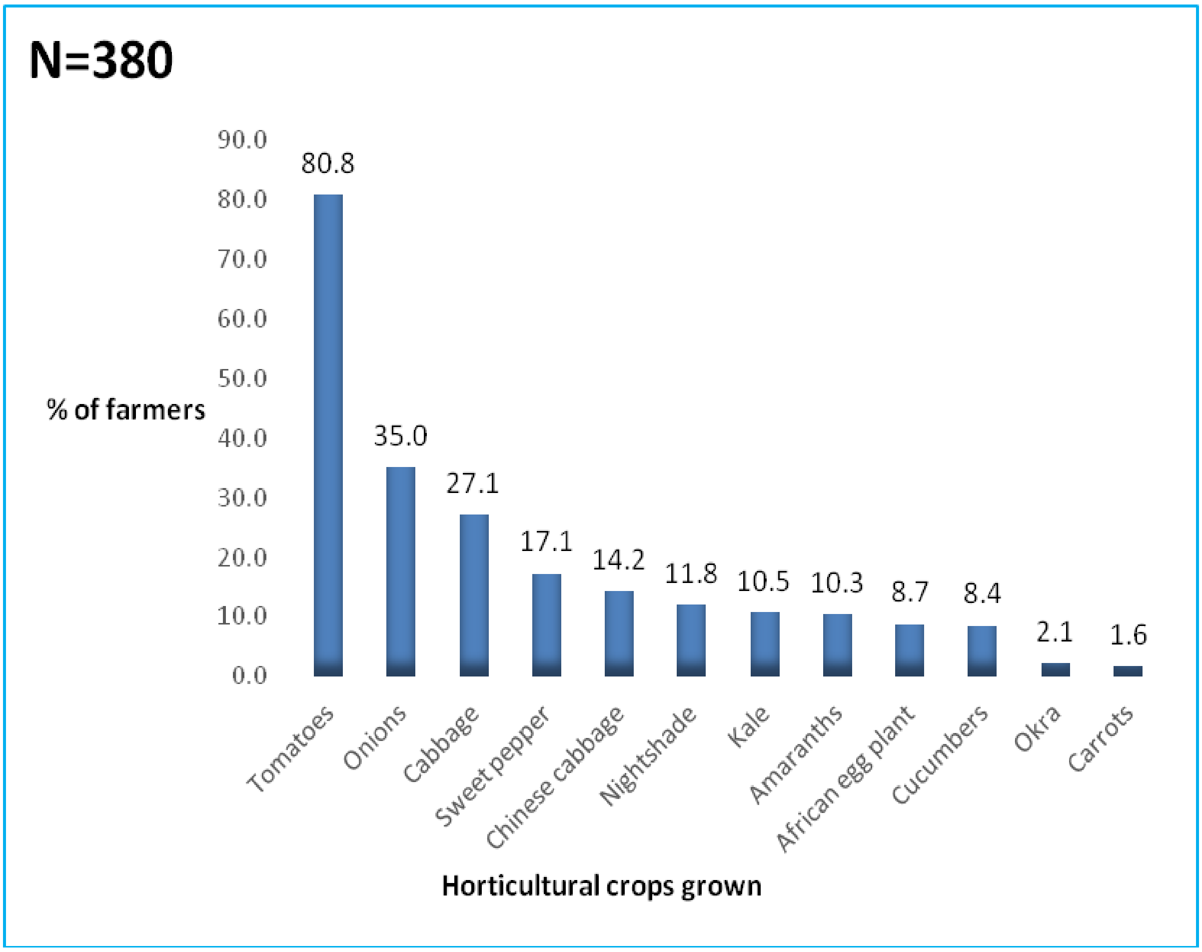

Figure 2: Proportion of respondents with the type of vegetables grown

Smallholder vegetable production is characterized by limited area under production. The average area under production per household was found to be 1.24 acres with no remarkable differences in all the regions surveyed (Table 5).

Table 5: Acreage of production

Variable	Description	Farm size under production in acres			
		Minimum	Maximum	Mean	Std Deviation
Region of respondent	Arusha	0.25	5.00	1.26	0.87
	Kilimanjaro	0.50	3.00	1.30	0.63
	Iringa	0.25	5.00	1.17	0.84
Total		0.25	5.00	1.24	0.81

Important pests in smallholder vegetable production

Tomatoes were found to be affected by a wide range of pests and diseseas. According to the respondents, *Tuta absoluta* (89.5%) is the main pest affecting smallholder tomato production in the study area followed by thrips (47.6%), late blight (45.6%) and aphids (12.5%), fruitflies, armyworms were also pests affecting tomato productions (Fig. 3).

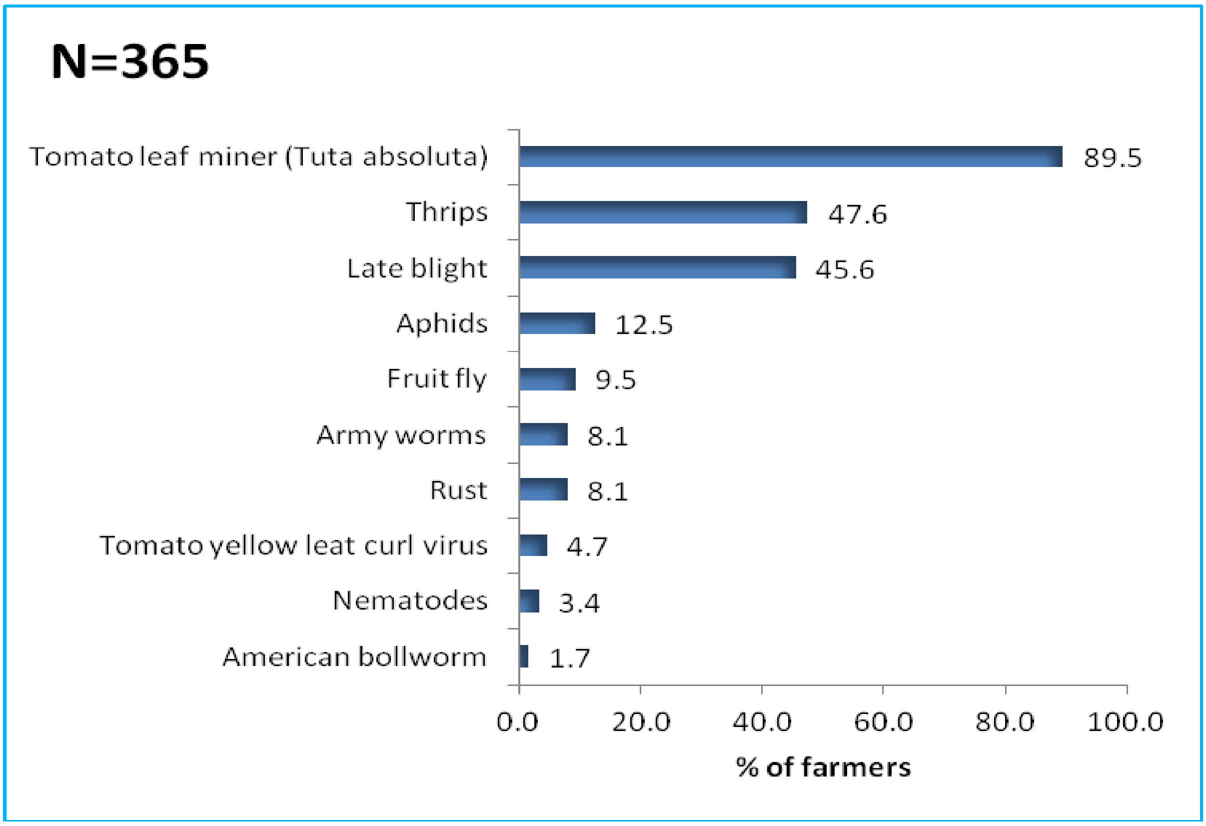

Figure 3: Prevalence of pests affecting tomato production

Onions were reported to be affected with fewer pests compared with tomatoes. Figure 4 shows that the main pests affecting onion production are aphids (55.3%), thrips (31.4%), army worms (21.4%) and fungal diseases (10.1%).

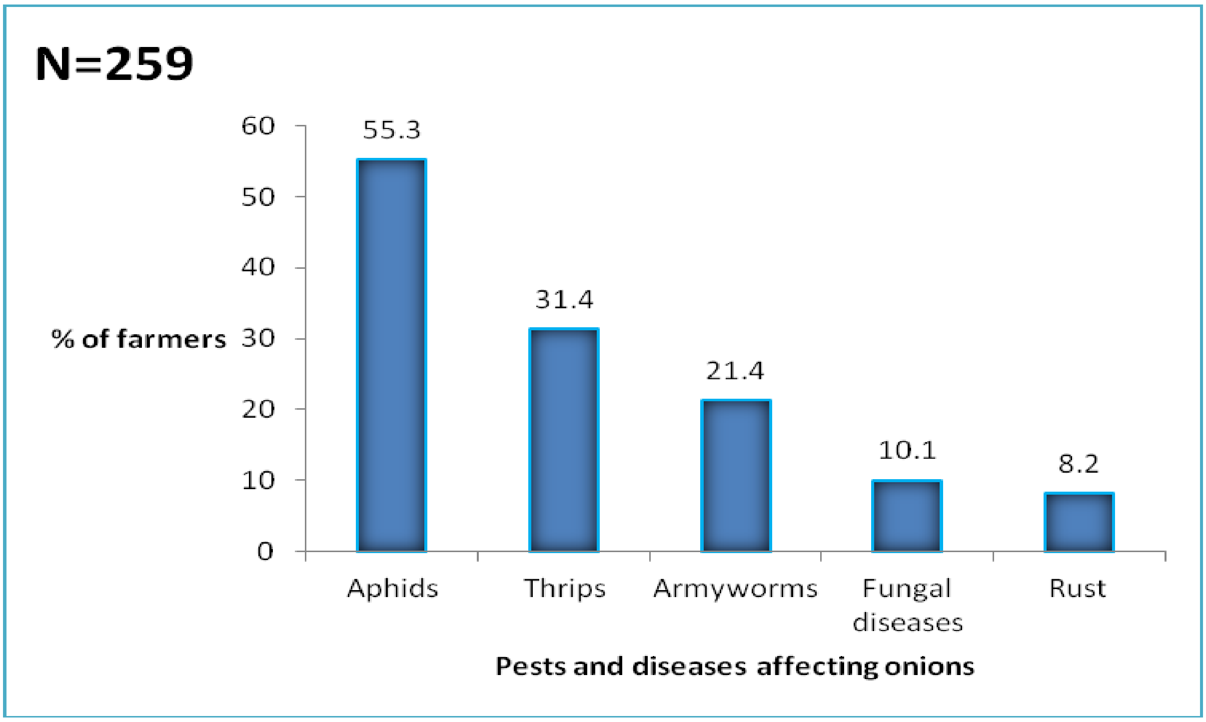

Figure 4: Prevalence of pests affecting onion production

The important pests in carrot production were reported to be leaf rust (64.7%), cutworms (52.9%) and *kimamba* (41.2%) as shown in Fig. 5. Others were fungal diseases as well as white flies.

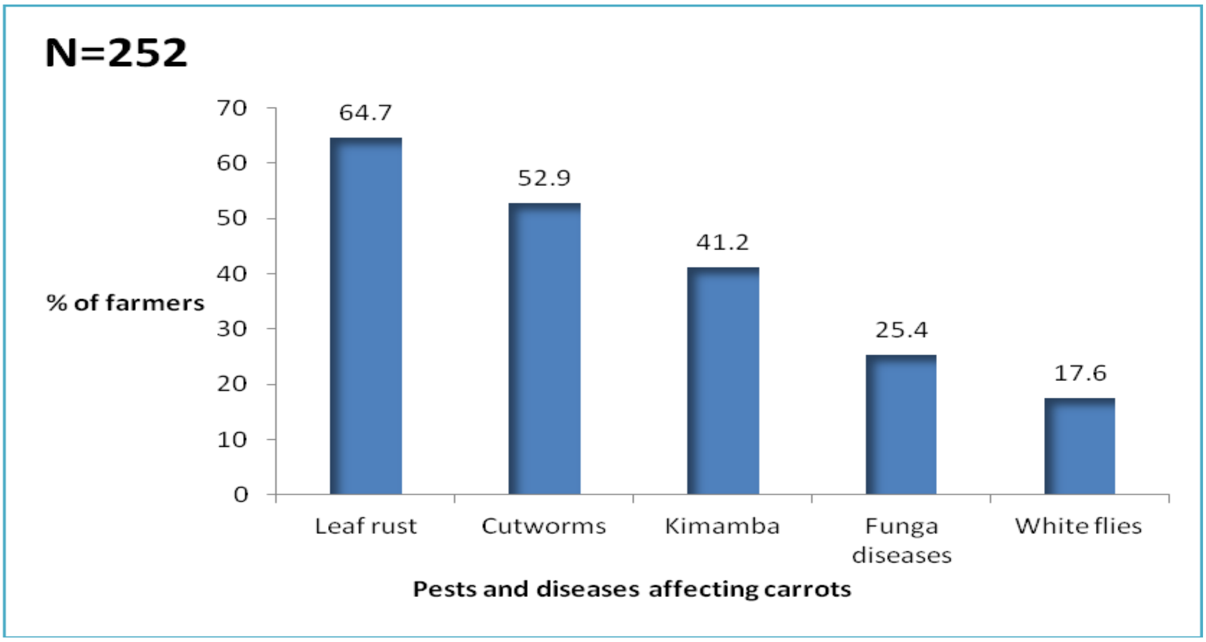

Figure 5: Prevalence of pests affecting carrot production

(iii) Pesticides use in vegetable production

Almost all farmers reported to use pesticides (99.1%) in fresh vegetable production. Some farmers used organic/farm yard manure (53.2%) and inorganic fertilizers (32.3%) in vegetable production (Fig. 6).

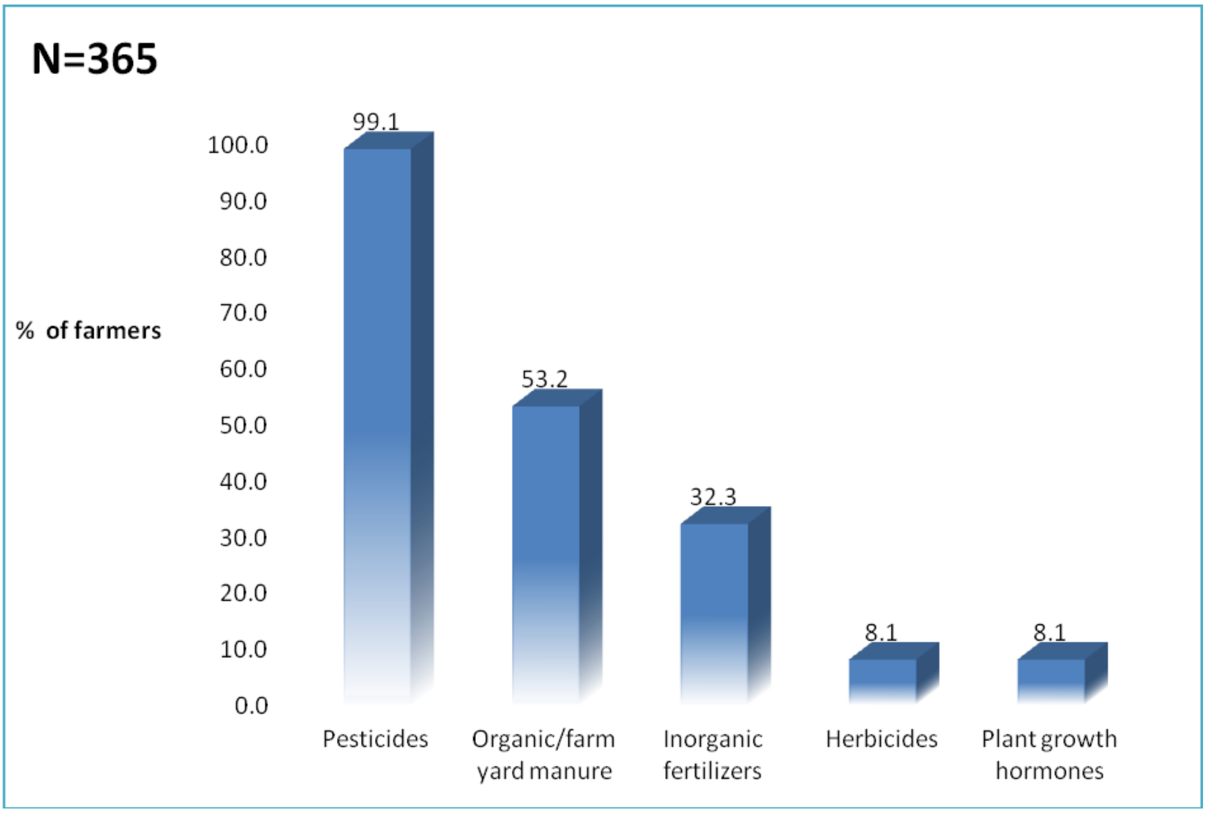

Figure 6: Agrochemical used in fresh vegetable production

The majority of farmers (92.6%) reported that pesticides were highly used in tomatoes, while 90% said in cabbage, 80% in cucumbers, 66.7% in onions, 62.5% in African eggplant and sweet pepper. Other vegetables with high pesticides use were kale, Chinese cabbage and amaranths.

(iv) Source of water for vegetable production

Most farmers (62.5%), reported to use river/stream as the main source of water for vegetable production while 40.8% depend mainly on water rainfall and 10.8% tap water (Fig. 7).

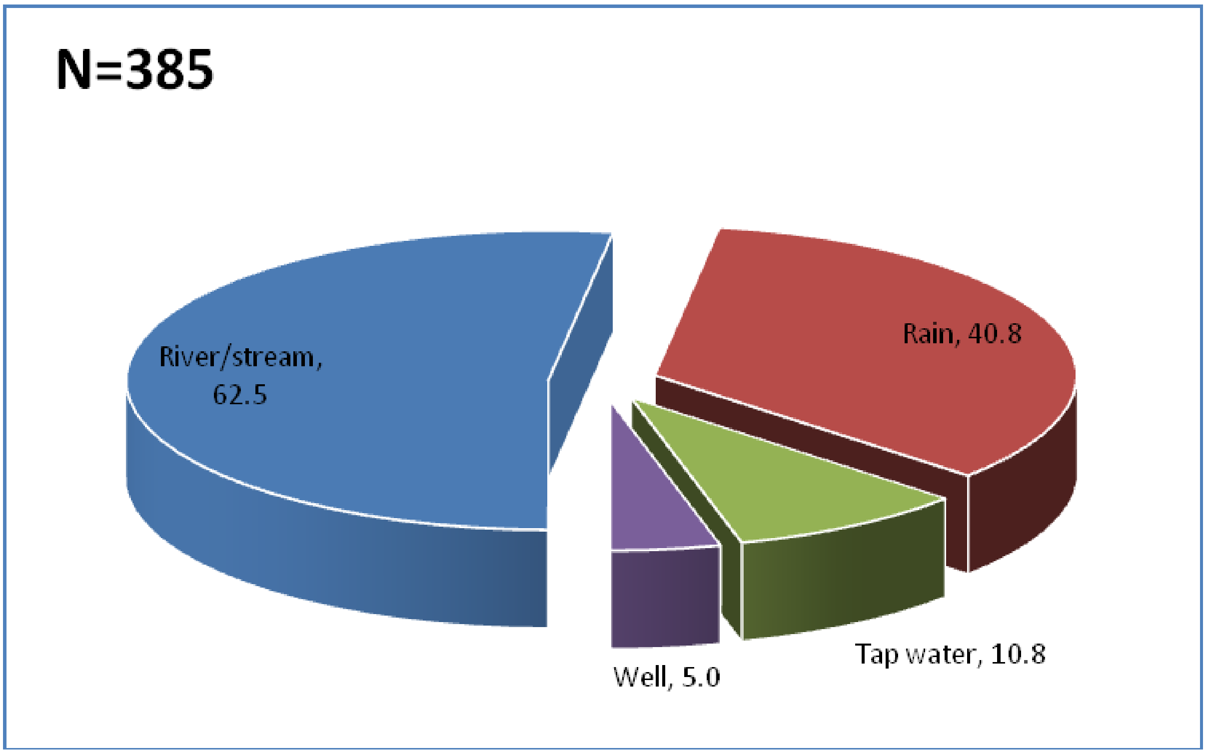

Figure 7: Proportion of respondents using sources of water for irrigating vegetables

(v) Farmers access to agricultural extension services

The results show that 88.6% of all smallholder vegetable producers interviewed had not received agricultural extension services on pest control in the past 3 years. Likewise, 88.9% had not received any advice on pesticides safety from extension personnel, few farmers reported to receive information on pesticides use from pesticides sellers (Table 6).

Table 6: Proportion of farmers accessing extension services on pesticides use (N=378)

Variable	Description	n	%
Have you ever received agricultural experts' advice on pest control?	No	335	88.6
	Yes	43	11.4
Have you ever received experts' advice on pesticides safe use?	No	336	88.9
	Yes	42	11.1
If yes, name the source?	Pesticides sales agents	19	47.5
	Extension officer	18	45.0
	NGO's	3	7.5

(vi) Types of pesticides, use and handling practices

The rate of pesticides use had increased in the recent past (58.4%). It was revealed that majority of farmers (71.2%) mix more than one pesticide during spraying. None of the farmers was observed mixing pesticides as per instructions from the pesticides label, which increases the risk of exposure to both farmers and final consumers of fresh vegetables produced (Table 7).

Table 7: Perception on pesticides use and handling practices (N=365)

Variable	Description	n	%
State of current pesticides use	Had increased	213	58.4
	Had reduced	89	24.4
	Remained virtually the same	60	16.4
	I don't know	3	0.8
Do you mix more than one pesticide during spraying?	Yes	257	71.2
	No	104	28.8

The results showed that, farmers mixed three to four different types of pesticides with Kilimanjaro having the highest followed by Arusha and Iringa being the least. Table 8 shows that, 46.9% of all smallholder vegetable producers mixed up to four different pesticides in one mixing container during spraying, 29.6% mixed three pesticides while 11.1% mixed up to five different pesticides.

Table 8: Number of pesticides mixed during spraying (N=243)

		Region of respondent						Total	
Variables	Description	Arusha		Kilimanjaro		Iringa			
		n	%	n	%	n	%	n	%
Number of pesticides in a pesticides cocktail	Two	14	14.9	6	9.5	4	4.7	24	9.9
	Three	19	20.2	21	33.3	32	37.2	72	29.6
	Four	52	55.3	36	57.1	26	30.2	114	46.9
	Five	7	7.4			20	23.3	27	11.1
	Six	2	2.1			4	4.7	6	2.5

Reasons for pesticides mixing were different among farmers with 32% reported to minimize the spraying costs, 28.1% increasing pesticides effectiveness and 26.1% mixed for controlling all pests at once (Fig. 8).

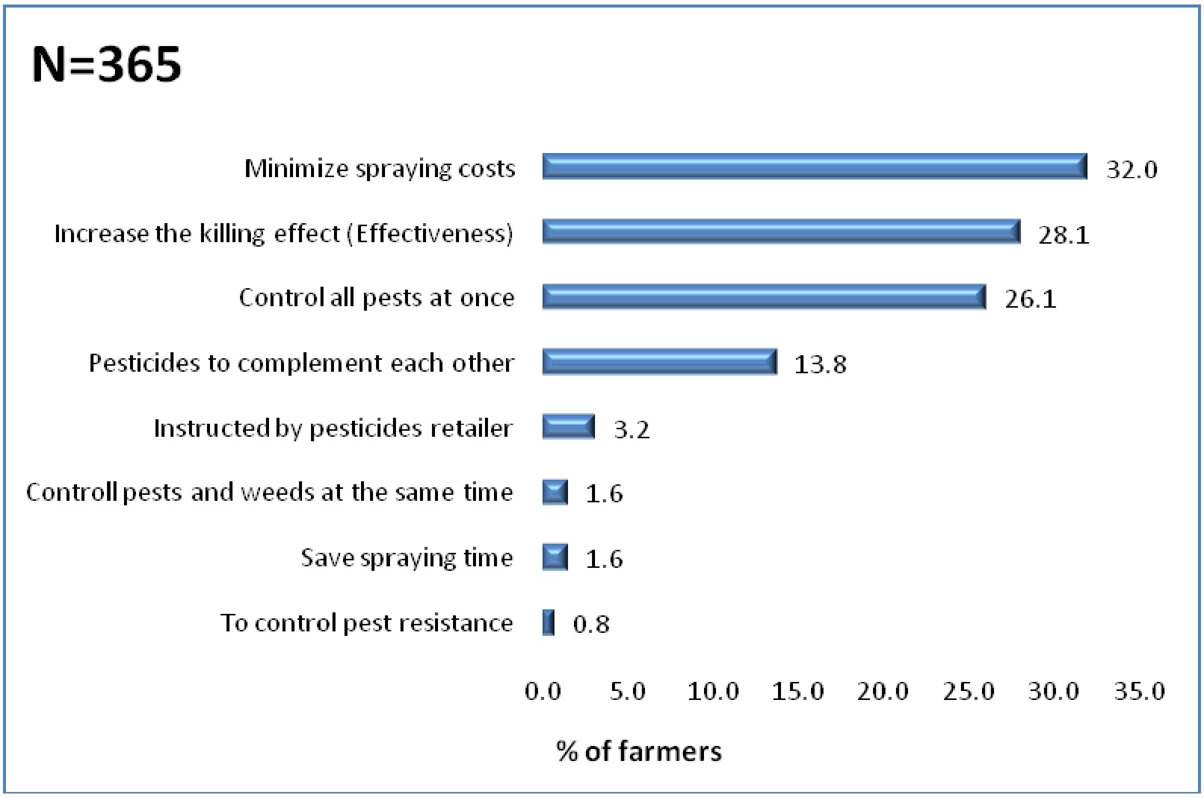

Figure 8: Reasons for pesticides mixing

Almost all farmers (99.7%) reported that the driving force for spraying of pesticides was the presence of pests. Poor pesticides quality (52.9%), counterfeit pesticide products (28.9%) and farmers lacking proper pesticide application practices (28.1%) were the also main challenges reported to smallholder farmers (Table 9).

Table 9: Reasons for pesticides and main challenges in pesticides use (N=375)

Variable	Description	n	%
Main drive for pesticides spraying	Presence of pests	374	99.7
	When neighbor sprays	1	0.3
Are pesticides effective in controlling pests	Yes	218	58.4
	No	155	41.6
Pesticides shortcomings	Poor quality (Insects don’t die when sprayed)	64	52.9
	Fake products in the market	35	28.9
	Poor use methods	34	28.1
	Sometime they control sometime not	8	6.6
	Pesticides inhibits flowering of tomatoes	5	4.1
	Expired pesticides sold in the market	5	4.1
	Pests develop resistance	2	1.7
	Plants dry after spray	1	0.8

4.1.2 Pesticides volumes used in vegetable production

Majority of farmers (67.8%) use drums (215 L) in mixing pesticides while spraying, on the other hand, 32.8% of all farmers interviewed use knapsack (17.5 L) for mixing pesticides.

The results showed high volumes of pesticides use per acre in the areas surveyed. On average of 3 spray men sprayed an acre of land in an average of 5 hours (Table 10).

Table 10: Pesticides volumes used in vegetable production

Variable	Equipment used in mixing					
	Drum			Knapsack		
	Minimum	Maximum	Mean	Minimum	Maximum	Mean
Tomato farm size in acres	0.25	5.00	1.21	0.25	2.00	0.97
Number of drums/knapsacks per day	1.00	12.00	4.27	1.00	50.00	14.14
Spraying hours/day	1.00	12.00	5.06	1.00	12.00	4.48
Number of spray men	1.00	10.00	3.33	1.00	5.00	2.44
Number of spraying days	1.00	20.00	2.72	1.00	3.00	1.04
Onion farm size in acres	0.25	5.00	1.08	0.25	1.00	0.55
Number of drums (215l) per day	1.00	16.00	4.59	1.00	8.00	3.33
Spraying hours/day	1.00	12.00	5.12	1.00	9.00	2.77
Number of spray men	1.00	13.00	2.71	1.00	8.00	1.59
Number of spraying days	1.00	24.00	1.79	1.00	1.00	1.00

(i) Pesticides used in tomato production

A total of 60 different pesticides were found to be used in tomato production with 29 different pesticides formulations. The main pesticides used include Belt 480 SC (45.2%), Wiltigo Plus 50EC (24.3%), Milthan Super 800WP (22.3%), Wilcron 720EC (21.6%), Dudu-all 450EC (19.9%), Farmerzeb 800WP (18.9%), and Supercron 500 EC (18.3%) as shown in Annex I. Mancozeb (71.4%), Profecron (58.1%), Flubandiamide (45.2%), a combination of Cypermethrine+Chloropyrifos (27.9%), Abamectin (27.2%), Emamectin Benzoate (24.3%), Metalaxyl+Mancozeb (22.3%) and Profenos (13.6%) were the main pesticides formulation used in tomato production (Table 11).

Table 11:Pesticides formulations sprayed in tomato (N=301)

Variable	Description	n	%
Chemical names of pesticides sprayed on tomatoes	Mancozeb	215	71.4
	Profecron	175	58.1
	Flubandiamide	136	45.2
	Cypermethrine+Chloropyrifos	84	27.9
	Abamectin	82	27.2
	Emamectin Benzoate	73	24.3
	Metalaxyl+Mancozeb	67	22.3
	Profenos	41	13.6
	Selecron	41	13.6
	Idoxacarb	34	11.3
	Emamectine + Acetamaprid	32	10.6
	Chlorothalonil	28	9.3
	Endosulfan	26	8.6
	Cypermethrin+Imidaclopid	25	8.3
	Lambdacyhalothrin	24	8.0
	Chlorpyrifos	22	7.3
	Copper Oxychloride	22	7.3
	Sulphur	17	5.6
	Chlorantraniliprole	9	3.0
	Paraquat	6	2.0
	Pirimiphos-Methyl	6	2.0
	Fenoxaprop-P-ethyl	5	1.7
	Spinosyn A and B	5	1.7
	Triadimefon	4	1.3
	Malathion	4	1.3
	Deltamethrin	2	0.7
	Hexaconazole	2	0.7
	Fosetyl Aluminium	2	0.7
	Clofentezine	2	0.7

Multiple responses allowed

The main chemical families for the groups of pesticides used in tomato production were found to be organophosphorus (97.6%), carbamates (54.1%), and substituted benzene (34.6%), combination of pyrethroid + organophosphorus (28.8%), avermectin (28.1%), combination of carbamate+acylalanine (22.9%) and dithiocarbamate (19.5%) as shown in Fig. 9.

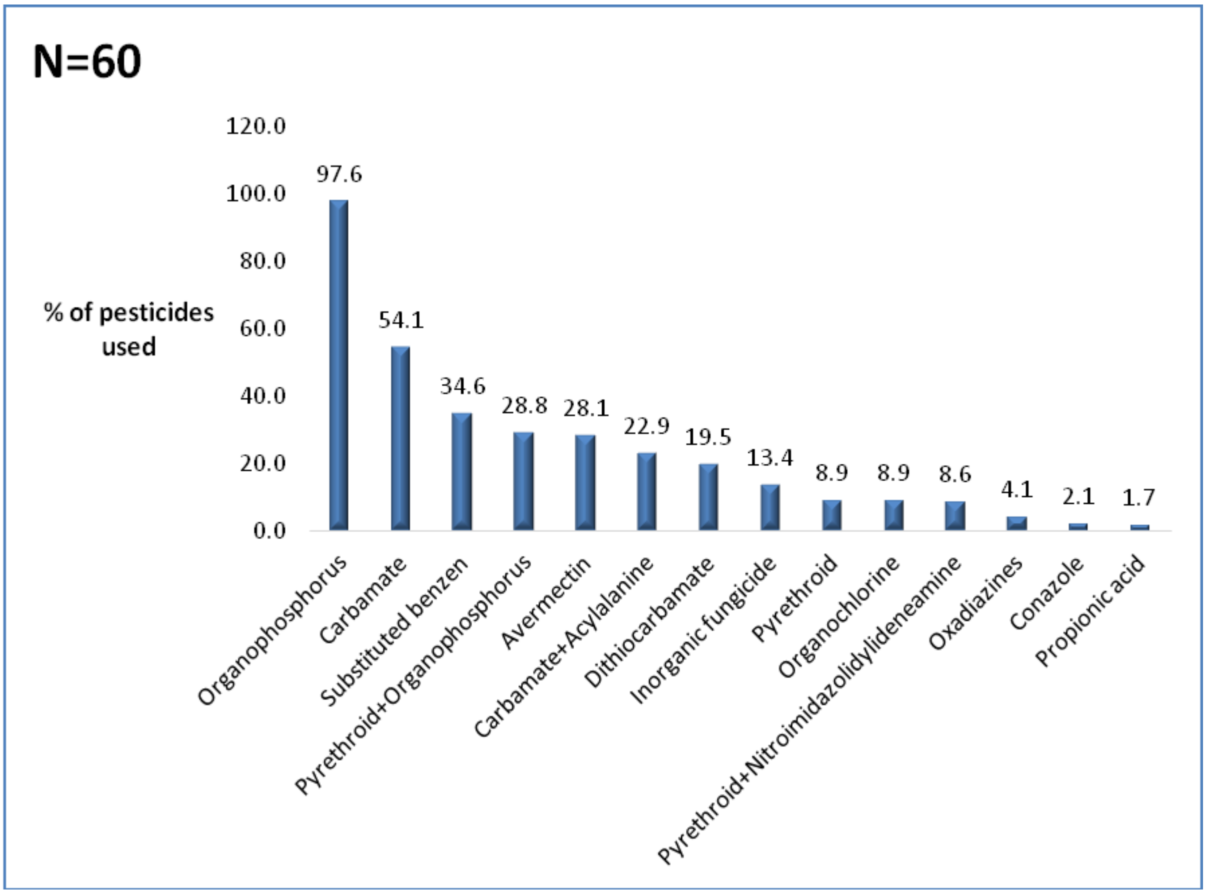

Figure 9: Chemical families of pesticides used in tomato production

Majority of pesticides used in tomato production have full registration category (87.5%) while few (8.1%) were not registered by Tropical Pesticides Research Institute (TPRI) to be used in the country. On the other hand, 59.4% of all pesticides used in tomato production fall under Class II (Moderately hazardous) of WHO hazard classification of pesticides while 24.3% were found to be in Class U (Unlikely to present acute hazard in normal use). Small quantities of extremely hazardous (Class Ia) and highly hazardous (Class Ib) were also found to be used in the production of tomatoes by smallholder farmers (Table 12).

Table 12: Status of Pesticides used in Tomato production (N=301*)

Variable	Description	n	%
Registration status of pesticides used in Tomato	Full registration	151	87.5
	Not registered	97	8.1
	Banned	26	2.2
	Provisional registration	21	1.7
	Restricted registration	6	0.5
WHO Classification of Pesticides used in Tomato	Class II (Moderately hazardous)	55	59.4
	Class U (Unlikely to present acute hazard in normal use)	22	24.3
	Not listed	12	8.8
	Class Ia (Extremely hazardous)	3	4.2
	Class Ib (Highly hazardous)	2	3.3

***Multiple responses allowed**

The results showed that 69.0% of all pesticides used in tomato production are insecticides while fungicides constituted 30.1% and the least used was herbicides. It was further realized that just above half, 55.1% of all pesticides in tomato production are properly used for the target crop pest while a considerable high proportion (44.9%) were wrongly used (Table 13). These included banned pesticides products, unregistered pesticides and pesticides registered for use in other crops such as coffee, cashew nuts and ornamental flower production.

Table 13: Categories of pesticides and usage (N=301*)

Variable	Description	n	%
Categories of pesticides used in tomatoes	Insecticides	818	69.0
	Fungicides	357	30.1
	Herbicides	11	0.9
Farmers use of pesticides	Correct use	618	55.1
	Wrong use	504	44.9

***Multiple responses allowed**

(ii) Pesticides used in onion production

The results from this study showed that 30 different types of pesticides were used in the production of onions with 20 different pesticides formulations. It was further noted that the frequently used pesticides in onion production included Dudumectin, Selecron 720EC, Snowcron, Supercron, Duduall, Profecron, Snowmectine and Wilcron (Table 14).

Table 14: Pesticides frequently sprayed in onions (N=298*)

Varaible	Description	n	%
Pesticides sprayed in onions	Dudumectin 11.2% EC	51	54.3
	Selecron 720EC	40	42.6
	Snowcron	22	23.4

Supercron	20	21.3
Duduall	20	21.3
Profecron	18	19.1
Snowmectine	17	18.1
Wilcron	14	14.9
Belt	9	9.6
Duduba	9	9.6
Dursban	8	8.5
Galligan 720EC	7	7.4
Belaton	6	6.4
Farmerzeb	6	6.4
Thionex	5	5.3
Tarantula	5	5.3
Milthan	4	4.3
Twiga	4	4.3
Snow Plus	4	4.3
Agrocron	3	3.2
Agrofecron 720EC	3	3.2
Ivory	3	3.2
Decis	3	3.2
Victory 72	2	2.1
Ebony	2	2.1
kulumus	2	2.1
Mashal	2	2.1
Abamectine	1	1.1
Duducron	1	1.1
Blue copper	1	1.1

***Multiple responses allowed**

The main pesticides formulation frequently applied in onions were profecron (55.3%), a combination of emamectine + acetamaprid (54.3%), selecron (42.6%), profenos and combination of cypermethrine+chloropyrifos (30.9%), Emamectin benzoate (18.1%) and 16.0% Mancozeb (Table 15).

Table 15: Pesticides formulations frequently sprayed in onions (N=298*)

Variables	Description	n	%
Chemical names of pesticides used in onions	Profecron	52	55.3
	Emamectine + Acetamaprid	51	54.3
	Selecron	40	42.6
	Profenos	29	30.9
	Cypermethrine+Chloropyrifos	29	30.9
	Emamectin Benzoate	17	18.1
	Mancozeb	15	16.0
	Flubandiamide	9	9.6
	Chlorpyrifos	8	8.5
	Oxyfluorfen	7	7.4
	Abamectin	6	6.4
	Triadimefon	6	6.4
	Endosulfan	5	5.3
	Dimethoate	4	4.3
	Profenos+cypermethrin	4	4.3
	Deltamethrin	3	3.2
	Sulphur	2	2.1
	Carbosulfan	2	2.1
	Metalaxyl+Mancozeb	2	2.1
	Copper Oxychloride	1	1.1

Multiple response allowed

The results in Fig. 10 further showed that, organophosphorus pesticides (59.1%) constitute the largest proportion of pesticides used in onion production. Others include a combination of pyrethroid+organophosphorus (14.7%) and substituted benzene (7.6%).

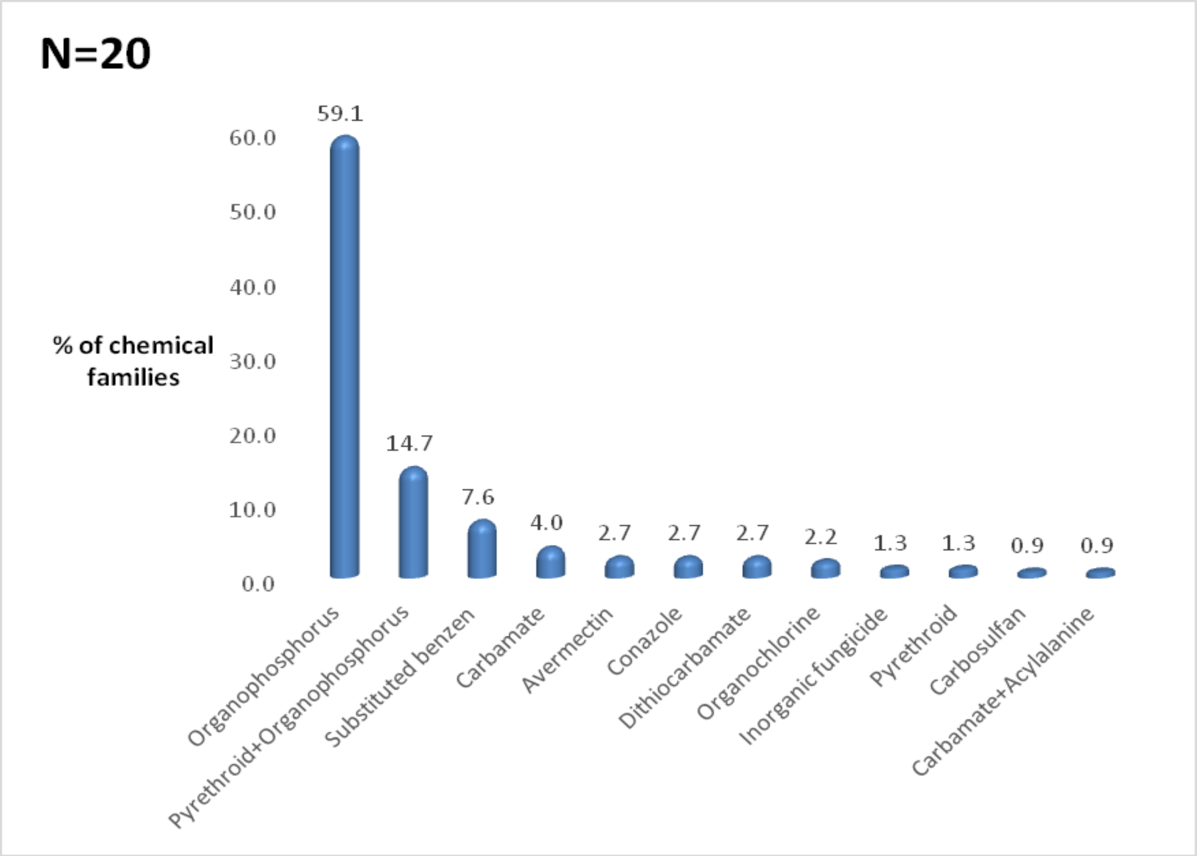

Figure 10: Chemical families of pesticides used in onion production

The results showed that the largest proportion (95.5%) of pesticides used in onion production have a full registration category and 85.5% fall under Class II (Moderately hazardous) WHO hazard classification of pesticides It was further revealed that, insecticides constitute the largest proportion (88.7%) of pesticides, while others include fungicides and some herbicides. On contrary, 69.2% of all pesticides used in onion production are wrongly used and only 30.8% correctly used for the target crop and pests (Table 16).

Table 16: Status of pesticides used in onion production (N=298*)

Variables	Description	n	%
Registration status of pesticides used in onion	Full registration	279	95.5
	Provisional registration	8	2.7
	Banned	5	1.7
WHO classification of pesticides used in onion	Class II (Moderately hazardous)	177	85.5
	Class U (Unlikely to present acute hazard in normal use)	17	8.2
	Class Ib (Highly hazardous)	7	3.4
	Not listed	6	2.9

Multiple response allowed

(iii) Pesticides used in sweet pepper production

Twenty-three (23) different pesticides were used in sweet pepper production with 16 different pesticides formulations. The frequently pesticides used include Agrithane, Imida C and Dursban (Table 17). The main pesticides formulations used in sweet pepper production include Mancozeb, Profecron, a combination of Cypermethrin + Imidaclopid, Chlorpyrifos, Abamectin and a combination of Cypermethrine + Chloropyrifos (Table 18).

Table 17: Pesticides used in sweet pepper (N=110*)

Variable	Description	n	%
Pesticides used in sweet paper	Agrithan	15	34.9
	Amida C	15	34.9
	Dursban	14	32.6
	Wilcron	8	18.6
	Supercron	8	18.6
	Duduba	6	14.0
	Tarantula	5	11.6
	Mupathion	4	9.3
	Pulsar 5EC	4	9.3
	Belt	4	9.3
	Dudu all	4	9.3
	Abamectin	4	9.3
	Selecron	4	9.3
	Osheten	2	4.7
	Belaton	2	4.7
	Subatex 300EW	2	4.7
	Twiga	2	4.7
	Ebony	2	4.7
	Milthan	2	4.7
	Mupacron	2	4.7
	Acteric	2	4.7
	Wiltigo	2	4.7
	Thionex	2	4.7

Multiple response allowed

Table 18: Pesticides formulations in sweet pepper (N=110*)

Variable	Variables	n	%
Chemical names of pesticides used in sweet pepper	Mancozeb	21	48.8
	Profecron	18	41.9
	Cypermethrin+Imidaclopid	15	34.9
	Chlorpyrifos	14	32.6
	Abamectin	9	20.9
	Cypermethrine+Chloropyrifos	8	18.6
	Lambdacyhalothrin	4	9.3
	Selecron	4	9.3
	Flubandiamide	4	9.3
	Malathion	4	9.3
	Tebuconazole+Triadimenol	4	9.3
	Triadimefon	2	4.7
	Dimethoate	2	4.7
	Emamectin Benzoate	2	4.7
	Endosulfan	2	4.7
	Pirimiphos-Methyl	2	4.7

Multiple responses allowed

Organophosphorus were the major chemical families (93%) used in sweet pepper production. Other chemical families used include carbamate (48.8%), a combination of pyrethroid+nitroimidazolidylideneamine (34.9%), avermectin (20.9%) as well as a combination of pyrethroid+organophosphorus (18.6%). Furthermore, 86.1% of all pesticides used in sweet pepper production have full registration while 12.2% have provisional registration. On the other hand, 67% fall under Class II (Moderately hazardous) of the WHO classification of pesticides, 21.6% under Class U (Unlikely to present acute hazard in normal use) and very few falls under Class Ib (Highly hazardous) as shown in Table 19.

Table 19: Status of pesticides used in sweet pepper production (N=110*)

Variable	Description	n	%
Registration status of pesticides used in sweet paper	Full registration	99	86.1
	Provisional registration	14	12.2
	Banned	2	1.7
WHO classification of pesticides used in sweet paper	Class II (Moderately hazardous)	65	67.0
	Class U (Unlikely to present acute hazard in normal use)	21	21.6
	Not listed	9	9.3
	Class Ib (Highly hazardous)	2	2.1

Multiple responses allowed

Insecticides (76.5%) were the main category of pesticides used in sweet pepper production followed by fungicides (23.5%). Unlike in tomatoes and onions production, 61.1% of all pesticides used in sweet pepper production were correctly used for the target crop, while 38.9% were found to be wrongly used (Table 20).

Table 20: Categories of pesticides used in sweet pepper production (N=110*)

Variable	Description	n	%
Farmer use of pesticides	Correct use	69	61.1
	Wrong use	44	38.9
Types of pesticides used in sweet paper production	Insecticides	88	76.5
	Fungicides	27	23.5

Multiple responses allowed

(iv) Pesticides used in cabbage production

The results further showed that, 29 different pesticides products were used in cabbage production comprised of 20 different pesticides formulations. The main pesticides products used were Belt (41.4%), Ebony, Duduba, Dudu-all, Dudumectin, Tresa, and Thionex (Table 21). The main pesticides formulations frequently used in cabbage production were found to be a combination of cypermethrine+chloropyrifos (47.1%), flubandiamide (41.4%), mancozeb (30.0%), profecron (17.1%), abamectin (15.7%), a combination of emamectine + acetamaprid, endosulfan, and spinosyn A and B (14.3%), respectively (Table 22).

Table 21: Pesticides used in cabbage production (N=60)

Variable	Description	n	%
Pesticides used in cabbage	Belt	29	41.4
	Ebony	19	27.1
	Duduba	18	25.7
	Dudu-all	13	18.6
	Dudumectin	10	14.3
	Tresa	10	14.3
	Thionex	10	14.3
	Wilcron	8	11.4
	Abamectin	6	8.6
	Wiltigo	6	8.6
	Super kinga	5	7.1
	Linkolin	5	7.1
	Ridomil	5	7.1
	Vertigo	5	7.1
	Bajuta	4	5.7
	Snowcron	4	5.7
	Supercron	4	5.7
	Ninja	2	2.9
	Banafos	2	2.9
	Amida C	2	2.9
	Selecron	2	2.9
	Dusban	2	2.9
	Agrocron	2	2.9
	Acteric	2	2.9
	Kulumus	2	2.9
	Farmerzeb	2	2.9
	Sumithian	2	2.9
	Karate	2	2.9
	mupacron	2	2.9
Total		70	264.3

Multiple responses allowed

Table 22: Pesticides formulations used in cabbage production (N=60)

Variable	Description	n	%
Chemical names of pesticides used in cabbage	Cypermethrine+Chloropyrifos	33	47.1
	Flubandiamide	29	41.4
	Mancozeb	21	30.0
	Profecron	12	17.1
	Abamectin	11	15.7
	Emamectine + Acetamaprid	10	14.3
	Endosulfan	10	14.3
	Spinosyn A and B	10	14.3
	Profenos	6	8.6
	Emamectin Benzoate	6	8.6
	Chlorothalonil	5	7.1
	Metalaxyl+Mancozeb	5	7.1
	Fosetyl aluminium+Mancozeb	5	7.1
	Lambdacyhalothrin	4	5.7
	Copper Oxychloride	4	5.7
	Chlorpyrifos	2	2.9
	Sulphur	2	2.9
	Selecron	2	2.9
	Cypermethrin+Imidaclopid	2	2.9
	Pirimiphos-Methyl	2	2.9

***Multiple responses allowed**

Results in Fig. 11 show that the main chemical families of pesticides frequently used in cabbage production includes combination of pyrethroid+organophosphorus (47.1%), organophosphorus pesticides (34.3%), carbamates (27.1%), avermectin, substituted benzene (15.7%) and organochlorines (14.3%).

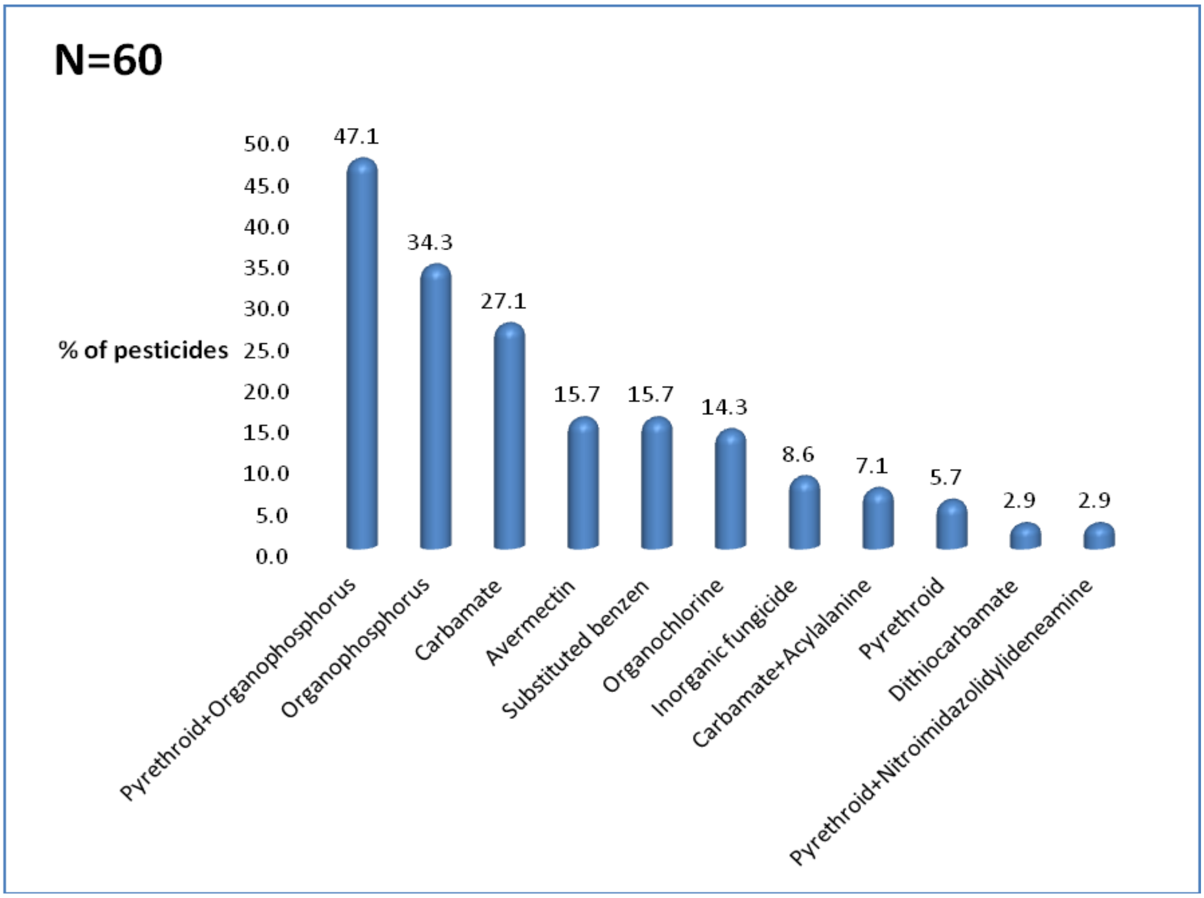

Figure 11: Chemical families of pesticides used in cabbage production

Majority (93.4%) of pesticides used in cabbage production have full registration, while 5.5% were banned. On the other hand, 59.5% of all pesticides fall under Class II (Moderately hazardous) in the WHO classification of pesticides, 19.0% under the Class U (Unlikely to present acute hazard in normal use), while a considerable small amount fall under Class 1a and Ib (Table 23).

Table 23: Status of pesticides used in cabbage production (N=60*)

Variable	Description	n	%
Registration status of pesticides used in cabbage	Full registration	169	93.4
	Banned	10	5.5
	Provisional registration	2	1.1
WHO classification of pesticides used in cabbage	Class II (Moderately hazardous)	72	59.5
	Class U (Unlikely to present acute hazard in normal use)	23	19.0
	Not listed	11	9.1
	Class Ib (Highly hazardous)	10	8.3
	Class Ia (Extremely hazardous)	5	4.1

Furthermore, 70.9% of all pesticides used in cabbages are correctly used for the target crops while 29.1% were wrongly used. However, insecticides (76.8%) were the main pesticides used in cabbage production with fungicides constitute 23.2% of all pesticides used in cabbage production.

(v) Pesticides management and spraying frequencies

The spraying frequency was high. Majority of farmers (61%) spray pesticides once a week while 18% spray twice a week and 12% spray once in two weeks. Likewise, majority of farmers (76.6%) stored pesticides in a pesticide store with few farmers stored them in various unsafe places. Although 58% farmers reported to burn empty pesticides containers, few leave them in the field or use them for home purposes (Table 24).

Table 24: Different methods used to dispose pesticide empty containers (N=368)

Variable	Description	n	%
Frequency of pesticides spraying	Once per week	224	60.9
	Twice per week	67	18.2
	Once in two weeks	44	12.0
	Three times per week	17	4.6
	Twice a month	12	3.3
	Once a month	4	1.1
Where do you get pesticides?	Pesticides Retail shops	337	92.1
	Open markets	17	4.6
	Pesticides wholesale shops	12	3.3
Do you wear PPEs	No	222	60.8
	Yes	76	20.8
	Sometimes	67	18.4
Where do you store pesticides?	Pesticides store	271	76.6
	In the farm	27	7.6
	General store	26	7.3
	Bathroom/toilet	14	4.0
	Hang under the tree	7	2.0
	Kitchen	6	1.7
	Living room	3	0.8

(vi) Drivers of increased pesticide usage

Generally, farmers use increased application rates with high pesticides volumes both in tomato and onion production. The correlation coefficients between increased pesticides use and demographic variables showed that increased pesticides use had a significant positive correlation with mixing more than one pesticide during spraying, number of crops grown consecutively, and region of respondent. On the other hand, increased pesticides use had a significant negative correlation with access to safe use information, perception on effectiveness of pesticides, wearing of personal protection equipment and reading instruction on pesticides labels before use (Table 25).

Table 25: Correlation between increased use of pesticides and demographic variables

Variable	Pearson Correlation	p value
Gender of respondent	-0.119	0.055
Age category of respondent	-0.114	0.065
Highest education level attained	-0.009	0.886
Access to safe use information	-0.142*	0.022
Perception on effectiveness of pesticides	-0.143*	0.022
Mix more than one pesticide during spraying	0.225**	0.000
Number of crops grown consecutively	0.264**	0.000
Do you wear personal protective equipments	-0.258**	0.000
Frequency of pesticides spraying	-0.001	0.982
Region of respondent	0.553**	0.000
Read instruction on pesticides labels before use	-0.183**	0.003

*. Correlation is significant at the 0.05 level (2-tailed).
**. Correlation is significant at the 0.01 level (2-tailed).

The determinants of farmer' increased use of pesticides are presented in Table 26 from the results of the probit regression model. The predictors tested were, region of the farmer, gender, age, education level, number of vegetable crops farmer grows, farm size, perception on the effectiveness of pesticides, mixing practices, frequency of pesticides application, access to information on pesticide use, source of pesticides, use of personal protection equipment and the tendency of farmers to read pesticide label. Among the variables tested, region of the farmer, number of vegetable crops grown and mixing of pesticides significantly contributed to the likelihood of increased use of high levels of pesticides. On contrary, farmers' perception on low effectiveness of pesticides, access to information on pesticides safe use, use of safety measures and reading of pesticides label negatively influenced increased use of high level of pesticides. Other variables including gender, age category, level of education, farm size, frequency of pesticides spray and source of pesticides showed no significant contribution in influencing farmers' decision to excessively apply high levels of pesticides.

Table 26: Drivers lead to increased use of pesticides among farmers

Explanatory variables	Estimate	Std. Error	Sig.	Wald Chi-Square	95% Wald Confidence Interval	
					Lower	Upper
Region	1.488	0.2236	0.000	44.273	1.049	1.926
Gender	0.65	0.2921	0.126	4.957	0.078	1.223
Age category	0.254	0.162	0.117	2.451	-0.064	0.571
Highest level of education	0.107	0.2593	0.68	0.17	-0.401	0.615
Number of crops grown	0.147	0.0468	0.002	9.919	0.056	0.239
Farm size	-0.395	0.2077	0.057	3.616	-0.802	0.012
Perception on effectiveness of pesticides	-0.555	0.2429	0.022	5.221	-1.031	-0.079
Mixing of pesticides	0.592		0.012	6.293	0.129	1.054
Access to safe use information	-0.717	0.4284	0.04	4.205	-1.718	-0.039
Frequency of pesticides spray	-0.2	0.0932	0.116	2.47	-0.329	0.036
Source of pesticides	-0.07	0.3145	0.823	0.05	-0.687	0.546
Wearing of PPEs	-0.349	0.1698	0.04	4.222	-0.682	-0.016
Reading of Pesticides Label	-0.446	0.2136	0.037	4.351	0.027	0.864

(Results of the Binary Probit Model)

4.1.3 Comparative assessment of acetylcholinesterase (AChE) activity between exposed and unexposed individuals and associated health impacts

(i) Socio-demographic information of the subsample

The exposure assessment was done to a sub sample of smallholder vegetable producers from Iringa (71.5%) and Arusha (28.5%) regions. In drawing comparative results of exposure, and controlling confounding factors, exposed farmers (59.6%) and control group (40.4%) were involved in the study. As indicated earlier, the farming population was generally younger. The mean age was (38.74±12.72) years (Mean ± SD). A non-statistically significant difference was observed in age between the exposed and control groups (p = 0.052). Furthermore, men (73.5%) compared with women (26.5%) were involved in the assessment of AChE inhibition (Table 27).

Table 27: Demographic characteristics of respondents (N=151)

Variable	Description	n	%
Region of respondent	Iringa	108	71.5
	Arusha	43	28.5
Category of respondent	Treatment	90	59.6
	Control	61	40.4
Age category of respondent	30-39 years	43	28.5
	20-29 years	35	23.2
	40-49 years	32	21.2
	50-59 years	26	17.2
	60 years and above	9	6.0
	Less than 20 years	6	4.0
Sex of respondent	Male	111	73.5
	Female	40	26.5
For how long have you been working with pesticides?	10 years and above	37	53.6
	5-9 years	19	27.5
	1-4 years	12	17.4
	Less than one year	1	1.4

Anthropometric measurements showed that the mean weight of farmers was (63.2±10.22) Kg while the mean Body Mass Index (BMI) for the exposed group was (22.74 ± 3.23) Kg/m^2 and control group was (23.26 ± 3.38) Kg/m^2 (Table 28). According to the WHO classification of BMI, 71.6% of exposed farmers were normal (18.50-24.99) Kg/m^2, compared with 75.4% control group while only exposed group (6.8%) reported to be underweight (< 18.5 Kg/m^2). The BMI difference between exposed and control groups was not statistically significant ($p = 0.167$).

Table 28: Anthropometric measurements (N=151)

Variable	Category of respondent			
	Exposed		Control	
	Mean	SD	Mean	SD
Weight of respondent in kilograms (kg)	63.27	10.95	63.10	9.14
Body Mass Index in Kg/m^2	22.74	3.23	23.26	3.38
Age of respondent in years	36.16	11.62	42.54	13.41

Smoking was not a common behaviour among farmers. Only 9.2% of exposed farmers compared with 13.3% of the control group were cigarette smokers. Furthermore, 51.1% of exposed farmer drunk alcohol as opposed to 57.4% of control groups. The use of long-lasting treated nets was higher among the exposed (52.1%) as opposed to the control group (47.5%).

Farmers had been working with pesticides for a considerable long period of time. Duration of pesticides handling among farmers shows that 53.6% had been working with pesticides for over 10 years and 27.5% for a period of 5-9 years (Table 29 and 30). Eating and drinking while spraying, were not common behaviours among farmers (77.4% and 72.6%

respectively). About 27% drunk local brew during pesticides spraying. Likewise, smoking was not prevalent (96.4%) during pesticides spraying. However, there was no statistical association in the cholinesterase levels and eating during spraying (p = 0171) or drinking during spraying (p = 0.156).

Table 29: Duration of handling pesticides and pesticides exposure risk practices (N=90)

Variables	Description	n	%
For how long have you been working with pesticides?	10 years and above	37	53.6
	5-9 years	19	27.5
	1-4 years	12	17.4
	Less than one year	1	1.4
Eat while dealing with pesticides	No	65	77.4
	Yes	19	22.6
Drinking while dealing with pesticides	No	61	72.6
	Yes	23	27.4

Table 30: Life style characteristics of respondents (N=151)

Variables		Category of respondent				Total	
		Treatment		Control			
		n	%	n	%	n	%
Use long lasting treated nets	No	43	48.9	32	52.5	75	50.3
	Yes	45	51.1	29	47.5	74	49.7
Smoke cigarettes	No	79	90.8	52	86.7	131	89.1
	Yes	8	9.2	8	13.3	16	10.9
Drink alcohol	Yes	45	51.1	35	57.4	80	53.7
	No	43	48.9	26	42.6	69	46.3

(ii) Pesticides exposure and levels of acetylcholinesterase (AChE) activity inhibition

Pesticides poisoning cases were categorized based on Tanzania poisoning baseline which took into account the excess and frequency of use pesticides in smallholder agricultural production. Acetylcholinesterase (AChE) inhibition was pronounced in 67.8% of the exposed farmers compared to 39.3% in the control group with acute poisoning. The acute poisoning was significantly higher in the exposed farmers compared to none exposed group (p = 0.001). Severe (Table 31) exposure with chronic poisoning was only observed in the exposed farmers (15.6%).

Table 31: Exposure status in relation to Acetylcholinesterase inhibition activity (N=151)

Variables		Category of respondent				Total		t-test
		Treatment		Control				
		n	%	n	%	n	%	p value
Category of pesticide poisoning	Acute poisoning (24.5-31.3 u/gHgb)	61	67.8	24	39.3	85	56.3	p=0.001
	Normal (> = 31.4 u/gHgb)	15	16.7	37	60.7	52	34.4	
	Severe chronic poisoning (< 24.5 u/gHgb)	14	15.6			14	9.3	

Comparative assessment (Table 32) of exposure between men and women revealed that women smallholder farmers were more affected compared with men. Acetylcholinesterase (AChE) inhibition in women who are mainly involved in weeding and harvesting of vegetable crops recorded 26.86±4.95 u/gHgb compared to 28.38±3.49 u/gHgb in men. The difference was statistically different (p = 0.003).

Table 32: Comparative levels of AChE inhibition (N=151)

Variables	Category of respondent							
	Treatment				Control			
	Sex of respondent				Sex of respondent			
	Male		Female		Male		Female	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Cholinesterase inhibition (u/gHgb)	28.38	3.49	26.86	4.95	32.76	4.48	33.08	4.21

(iii) Determinant factors of pesticide poisoning and AChE inhibition

Exposed farmers with low Body Mass Index (BMI) were at risk of exposure. Half (50%) of exposed farmers categorized under the WHO BMI (Underweight (< 18.5 Kg/m^2) showed severe exposure (< 24.5 u/gHgb) compared with only 7.9% of the exposed normal (18.50-24.99 Kg/m^2). Chi square tests showed a statistically significant association between exposure levels and BMI within the exposed group (p = 0.004). Furthermore, AChE inhibition varied with BMI categories. AChE activity was highly inhibited from underweight (26.73±5.56 u/gHgb), overweight (27.32± 4.95 u/gHgb) and obese (21.90 u/gHgb) as opposed to normal health exposed (28.37±3.32 u/gHgb) (Table 33).

Table 33: Relationship between BMI and Cholinesterase depression (N=151)

Variables	WHO BMI classification								Total	
	Underweight (<18.5)		Normal (18.50-24.99)		Overweight (>-25)		Obese (>-30)			
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Cholinesterase depression (u/gHgb)	26.73	5.56	28.37	3.32	27.32	4.95	21.90	.	27.97	3.89

Age of farmers influenced cholinesterase inhibition among exposed farmers. AChE levels were more inhibited in younger exposed farmers aged less than 20 year (23.08±2.84 u/gHgb) and much older farmers aged above 60 years and above (25.20±2.34 u/gHgb) compared with middle aged farmers of 30-39 years and 40-49 years (29.88±3.58 u/gHgb and 28.63±6.34 u/gHgb) respectively. The association between age and inhibition was statistically significant (p = 0.046) (Table 34).

Table 34: Relationship between age categories and Cholinesterase inhibition (N=151)

Variables	Age category of respondent											
	Less than 20 years		20-29 years		30-39 years		40-49 years		50-59 years		60 years and above	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Cholinesterase depression u/gHgb	23.08	2.84	27.74	2.85	29.88	3.58	28.63	4.58	26.55	4.00	25.20	2.43

Farmers exposure was not associated with the use of long-lasting treated nets (p = 0.053) while cigarette smoking behaviour was significantly associated with the levels of pesticides exposure (p = 0.035). Furthermore, there was statistically significant difference in the AChE depression levels between users and non-users of local brew. The AChE activity of users of local brew were significantly low (28.58±4.64 u/gHgb) compared with none users of local brew (31.17 ± 4.52 u/gHgb) (p = 0.001). Likewise, users from the control group (32.02±4.47 u/gHgb) had a slightly lower AChE compared with none users (33.50±4.23 u/gHgb) of local brew, though the difference was not statistically significant (Table 35).

Table 35: Effect of alcohol consumption on the Acetylcholinesterase activity (N=151)

Variables	Category of respondent								Total	
	Treatment				Control					
	Do you drink alcohol?				Do you drink alcohol?					
	No		Yes		No		Yes			
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Cholinesterase depression u/gHgb	29.36	3.89	26.50	3.35	33.50	4.23	32.02	4.47	29.97	4.74

The Analysis of Variance (ANOVA) test showed that, exposure to pesticides did not differ significantly among farmers with different pesticide handling experience (p = 0.737). However, exposed farmers with a working experience of 1-4 years in spraying and handling of pesticides had slightly lower AChE (28.82±2.97 u/gHgb) compared with those worked between 5-9 years (30.69±3.23 u/gHgb) and above 10 years (30.47±5.07 u/gHgb. Although less frequency of application of pesticides (once/week, twice/week and once/month) was associated with low AChE inhibition (30.49±3.83 u/gHgb, 30.08±5.37 u/gHgb, 34.25±8.98 u/gHgb) compared to high frequency of application (three times/week and four times/week) with high AChE inhibition the difference was not statistically significant (p = 0.509) (Table 36).

Table 36: Effect of pesticide spraying frequencies on Cholinesterase activity (N=151)

Variables	On average, how often do you use (spray) pesticides?												Total	
	Once a week		Twice a week		Three times a week		Four times a week		Once in two weeks		Once a month			SD
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	
Cholinesterase inhibition u/gHgb	30.49	3.83	30.08	5.37	28.95	3.39	27.85	3.04	30.98	5.13	34.25	8.98	30.25	4.35

Pesticides contact hours during spraying was relatively high (6.68±2.91 hours), with a single farmer spraying 21.87±18.73 knapsacks per day. Farmer's total active weeks were 12.40±3.48 for the farming duration of 3.38±0.69 months. Cholinesterase inhibition had a positive correlation with the average area sprayed by the farmers (r = 0.242, p = 0.047), while there was no significant correlation between AChE inhibition and number of knapsacks (r = 0.003; p = 0.982), working hours (r = 0.093; p = 0.452) as well as the total number of active weeks (r = 0.118; p = 3.41). Likewise, there was no statistical significance between the levels of pesticides exposure with the frequency of pesticides application (p = 0.543) (Table 37).

Table 37: Farm operation and spraying patterns

Variables	Mean	SD
Average area sprayed/day (acres)	1.35	1.04
Working hours/day	6.68	2.91
Amount (Knapsacks) sprayed/day	21.87	16.73
Intensive working days/week	1.68	0.97
Total number of active weeks	12.40	3.48
Duration of farming season (months)	3.38	0.69

The use of personal protective equipment (PPE) was not common among farmers. Farmers spray pesticides without much safety precaution to avoid direct pesticide contact during spraying. None of the farmers had a complete body protection, most (85.7%) were partially protected (did not use all basic PPE) while 14.3% were completely unprotected. However, the AChE levels was not statistically different between the partially and none protected farmers (p = 0.962), indicating that both are equally exposed.

Gumboots were the only PPE widely used by farmers (83.3%). Nonetheless, farmers in onion production did not wear gumboots to avoid destruction of onion bulbs. Most farmers did not use gloves (92.9%), respirators (95.2%), masks (90.5%), goggles (97.6%) and overalls/overcoats (92.3%) during pesticides handling (Table 38). Owing to poor and inefficient use of PPEs, famers were highly exposed through direct contact with pesticides. Dermal (94.1%), optical (63.2%) respiratory (50%) and oral routes (47.1%) were all reported to simultaneously increase the exposure of farmers to pesticides.

Table 38: Proportion of farmers who use PPEs (N=84)

Variables	Description	n	%
Wear gloves when spraying pesticides	No	78	92.9
	Yes	6	7.1
Wear boots when spraying pesticides	Yes	70	83.3
	No	14	16.7
Wear respirator when spraying pesticides	No	80	95.2
	Yes	4	4.8
Wear mask when spraying pesticides	No	76	90.5
	Yes	8	9.5
Wear goggles when spraying pesticides	No	82	97.6
	Yes	2	2.4
Wear head cover when spraying pesticides	No	78	92.9
	Yes	6	7.1
Wear overall when spraying pesticides	No	12	92.3
	Yes	1	7.7

The results from the logistic regression analysis (Table 39) showed that a unit increase of 1% in age increased risks of lower AChE by 6.7% (Odd Ratio (OR) = 1.067; 95% CI: 0.864; 1.319) while the 1% decrease in BMI increased the probability of risk of having low AChE levels by 86.7% (OR = 0.867; 95% CI: 0.502; 1.496). The decrease in average farm area sprayed per day decreased the probability of farmers having lower AChE levels (OR = 0.001; 95% CI: 0.000; 0.372). Farmers with long working hours had the probability of about three times of having lower AChE levels (OR = 3.497; 95% CI: 1.080; 11.322). The probability of exposure was high (46.6%) among farmers who break less than a month as opposed to those who break for up to two months (7.6%) before embarking on another extensive spraying period [(OR = 0.466; 95% CI: 0.007; 31.497) and (OR = 0.076; 95% CI: 0.000; 12.551)] respectively.

Table 39: Logistic regression analysis for determinants of pesticides exposure

Independent Variables	**B**	**Z-values**	**Sig.**	**Odd Ratios**	**95.0% C.I.**	
					Lower	**Upper**
Age	0.065	0.367	0.045	1.067	0.864	1.319
BMI	-0.143	0.262	0.008	0.867	0.502	1.496
1-4 yrs working with pesticides	-1.369	0.292	0.589	0.254	0.002	36.495
5- yrs working with pesticides	-1.614	0.570	0.450	0.199	0.003	13.147
Average area spread/day	-6.620	5.309	0.021	0.001	0.000	0.372
Working hours/day	1.252	4.363	0.037	3.497	1.080	11.322
Break less than a month before next intensive spray period	-0.763	0.126	0.723	0.466	0.007	31.497
Break for 1-2 months before next intensive spray period	-2.580	0.979	0.322	0.076	0.000	12.551
Constant	-2.792	0.044	0.835	0.061		

(iv) Self-reported symptoms of pesticides exposure

A total of 38 typical symptoms of exposure to pesticides (Table 40) which are specific clinical manifestations of organophosphate and carbamate exposure were observed among farmers. A comparative analysis between the exposed and control indicated a statistically difference in the number of symptoms reported (p = 0.001). Exposed farmers showed more exposure symptoms than control group. The symptoms reported by exposed farmers were (14.10±7.70) compared with the control group (6.48±6.62).

A comparative analysis further indicated that almost all symptoms were statistically significant. However, loss of appetite, lacrimation, loss of consciousness, and vomiting were not significantly linked to exposure. The most reported exposure symptoms which significantly differed from the control group were tiredness (71.6%), fatigue (64.8%), soreness in joints (59.1%), thirst (56.8%), headache and weakness (52.3%, skin irritation (51.1%), salivation and abdominal pain (50.0% respectively).

Table 40: Self-reported symptoms of pesticides exposure (N=151*)

Variables	Category of respondent			
	Treatment		Control	
	n	%	n	%
Tiredness	63	71.6	9	15.5
Fatigue	57	64.8	16	27.6
Soreness in Joints	52	59.1	12	20.7
Thirst	50	56.8	7	12.1
Headache	46	52.3	20	34.5
Weakness	46	52.3	11	19.0
Skin Irritation	45	51.1	10	17.2
Salivation	44	50.0	5	8.6
Abdominal pain	44	50.0	18	31.0
Muscle weakness	42	47.7	14	24.1
Memory loss	42	47.7	17	29.3
Excessive sweating	40	45.5	9	15.5
Blurred vision	40	45.5	18	31.0
Blurred vision associated with excessive tearing;	38	43.2	15	25.9
Eye Irritation	37	42.0	8	13.8
Nervousness	35	39.8	15	25.9
Moodiness	34	38.6	14	24.1
Perspiration	34	38.6	12	20.7
Irritation of the Nose and Throat.	33	37.5	6	10.3
Productive cough	33	37.5	11	19.0
Drooling	31	35.2	12	20.7
Chest pain	31	35.2	5	8.6
Dizziness	30	34.1	14	24.1
Loss of Appetite	28	31.8	22	37.9
Muscle twitches	28	31.8	8	13.8
Red eyes	28	31.8	11	19.0
Nausea	27	30.7	7	12.1
Restlessness	26	29.5	13	22.4
Shortness of breath	24	27.3	6	10.3
Skin rash	21	23.9	3	5.2
Tremor	20	22.7	3	5.2
Lacrimation	20	22.7	13	22.4
Loss of Weight	18	20.5	6	10.3
Diarrhoea	14	15.9	4	6.9
Loss of consciousness	9	10.2	5	8.6
Vomiting	7	8.0	4	6.9
Confusion	5	5.7	3	5.2
Convulsions	3	3.4		

**Multiple response allowed*

Furthermore, 40.9% of exposed farmers showed up to 10-19 exposure symptoms as opposed to 27.6% of the control. Likewise, none of the exposed farmers showed no exposure symptoms while 24.1% of the control reported no exposure symptoms. Likewise, only exposed farmers (27.3%) reported 20 and above exposure signs and symptoms of pesticides exposure (Table 41).

Table 41: Respondent responses on different number of symptoms following pesticide exposure (N=151)

Variables	Description	Category of respondent				Total	
		Treatment		Control			
		n	%	n	%	n	%
Categories of number of symptoms	1-9 symptoms	28	31.8	25	43.1	53	36.3
	10-19 symptoms	36	40.9	16	27.6	52	35.6
	20 symptoms and above	24	27.3	3	5.2	27	18.5
	No exposure symptoms			14	24.1	14	9.6

Exposure symptoms reported were further grouped and categorized based on severity and specific health effects according to Rohlman (2011). Out of 38 symptoms reported, neurobehavioral accounted for 53.9% while others were muscle (13.9%), epithelia/mucosal surfaces (13.5%), respiratory (10.8%) and intestinal (8.3%). Based on severity of exposure, 47.6% of all the signs and symptoms reported indicated mild pesticide poisoning, while 31.4% severe and 21.0% moderate pesticides poisoning.

4.1.4 Levels, types of pesticides residues and risks of dietary exposure

(i) Vegetable sampling sites and locations

Fresh vegetables were collected across both production and consumption areas. Fig. 12 shows that, 42% of the samples were collected from Arusha, 18% from Kilimanjaro and Iringa (18%). The major production was Arusha, Iringa, and Kilimanjaro. Dar es Salaam and Morogoro municipal were mainly targeted for the consumption (market).

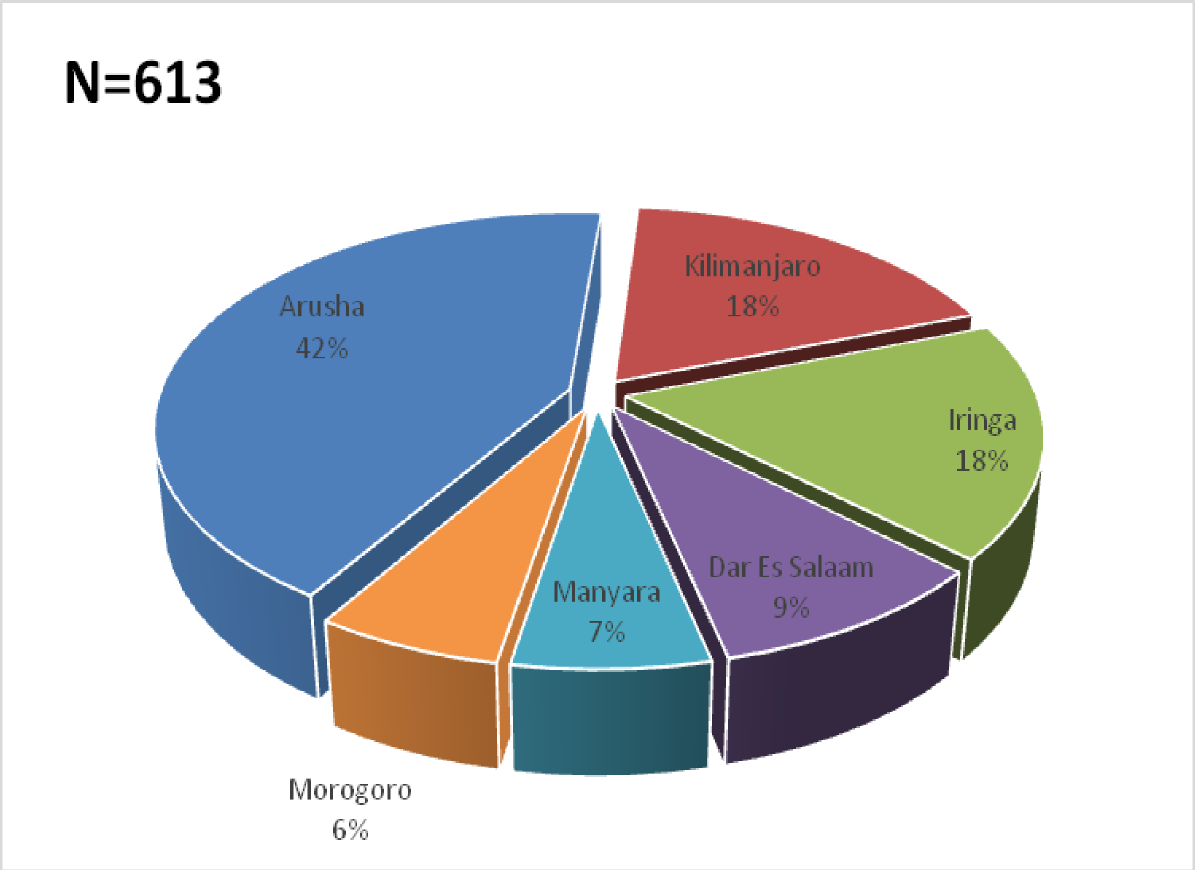

Figure 12: Proportion of vegetable samples collected from each region

Vegetable samples were collected from three sampling sites, whereby 54.3% were collected from market places, 40.1% from the fields during harvesting period and 5.5% from Highways (Table 42).

Table 42: Number of vegetable samples collected from the three sampling areas (N=613)

Variables	n	%
Market	333	54.3
Farm	246	40.1
Highway	34	5.5

(ii) Types of vegetable samples collected

A total of 613`samples comprised of 17 different vegetables were collected and analyzed for pesticides residues. These included tomatoes (29.4%), onions (26.6%) and sweet pepper (11.1%). Other samples collected include kale, cabbage, African night shade, water melons, and carrots, as shown in Table 43. These vegetable samples represent the most common vegetables produced and consumed locally within the production areas and transported to nearby urban and peri-urban markets.

Table 43: Vegetable samples collected (N=613)

Variables	Description	n	%
Name of horticultural crop (Sample type)	Tomatoes	180	29.4
	Onions	163	26.6
	Sweet paper	68	11.1
	Chinese cabbage	26	4.2
	Kale	22	3.6
	Cabbage	19	3.1
	Nightshade	19	3.1
	Water melon	19	3.1
	Carrots	16	2.6
	African egg plant	15	2.4
	Okra	14	2.3
	Egg plant	13	2.1
	Cucumber	13	2.1
	Green beans	7	1.1
	Amaranths	7	1.1
	Ethiopian mustard	7	1.1
	Onion gallic	5	0.8

(iii) Analysis of pesticides residues from vegetable samples

A considerable proportion (47.5%) of all vegetable samples tested for pesticides residues had detectable levels with vegetables obtained from highways having the highest proportion of samples with detectable levels (Table 44).

Table 44: Vegetable samples with pesticides residues based on collection sites (N=613)

Variables		Sampling place						Total	
		Farm		Market		Highway			
		n	%	n	%	n	%	n	%
Pesticides residues test	No pesticides residues detected	132	53.7	177	53.2	13	38.2	322	52.5
	Pesticides residues detected	114	46.3	156	46.8	21	61.8	291	47.5
Total		246	100.0	333	100.0	34	100.0	613	100.0

Regional wise, high proportion of vegetable samples collected and analysed from Dar es Salaam, Iringa and Morogoro had detectable pesticides residues compared to samples collected from Kilimanjaro, Arusha and Manyara regions. Only 17.9% of the samples from Manyara region had detectable pesticide residues (Table 45).

Table 45: Vegetable samples analysed for pesticides residues by regions (N=613)

Variables		Region sample taken												Total	
		Arusha		Kilimanjaro		Manyara		Morogoro		Iringa		DarEs Salaam			
		n	%	n	%	n	%	n	%	n	%	n	%	n	%
Pesticides residues test	No pesticides residues detected	158	61.5	73	66.4	32	82.1	11	29.7	32	29.9	15	26.3	321	52.9
	Pesticides residues detected	99	38.5	37	33.6	7	17.9	26	70.3	75	70.1	42	73.7	286	47.1

The results indicated that, 49.1% of all vegetable samples with detectable pesticide residues were detected to have one pesticide residue, whereas 31.1% had two detectable pesticides residues, and 11.7% had three different types of pesticides residues detected (Fig. 13).

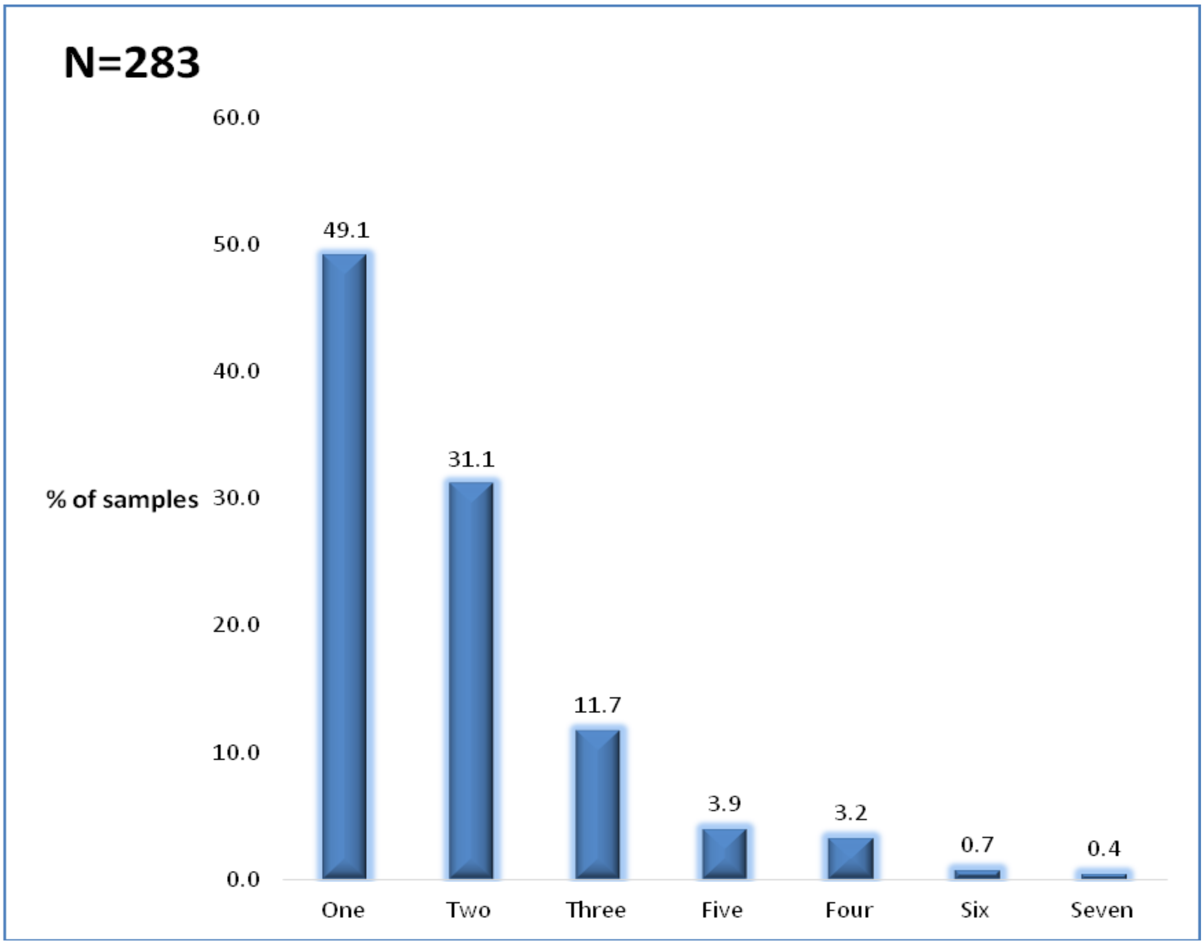

Figure 13: Number of pesticides residues detected per sample

The results revealed no remarkable differences in the proportion of samples with detectable pesticides residues across sampling sites. Table 46 shows that, vegetable samples from all sampling sites have comparable proportions of pesticides residues detected. However, from the same sites proportion of samples with two pesticide residues were 30.8%, 30.3% and 38.1%, respectively.

Table 46: Number of pesticides residues detected based on sampling sites (N=286)

Variables		Sampling sites						Total	
		Farm		Market		Highway			
		n	%	n	%	n	%	n	%
Number of residues	One pesticide residue measured	48	44.9	79	51.0	12	57.1	139	49.1
	Two pesticides residues measured	33	30.8	47	30.3	8	38.1	88	31.1
	Three pesticides residues measures	16	15.0	16	10.3	1	4.8	33	11.7
	Five pesticides residues measured	6	5.6	5	3.2			11	3.9
	Four pesticides residues measured	4	3.7	5	3.2			9	3.2
	Six pesticides residues measured			2	1.3			2	0.7
	Seven pesticides residues measured			1	0.6			1	0.4

(iv) Types pesticides residues detected in vegetable samples

Fifty-two (52) different types of pesticides residues were detected from all vegetable samples collected and analyzed. The main detected pesticides residues included Oxyfluorfen (12.2%), Cyhalothrine (Lambda) (10.1%), Profenofos (9.5%), triadimenol (8.8%), Chlorpyrifos, Cyhalothrin (Gamma), and Triadimefon (8.1%) respectively, Pirimiphos–methyl (7.4%), Endosulfan (Beta) and Carbofuran (6.1%), respectively (Annex II). Samples of chromatograms of pesticides detected from vegetable samples are presented in figures 14, 15, 16 and 17.

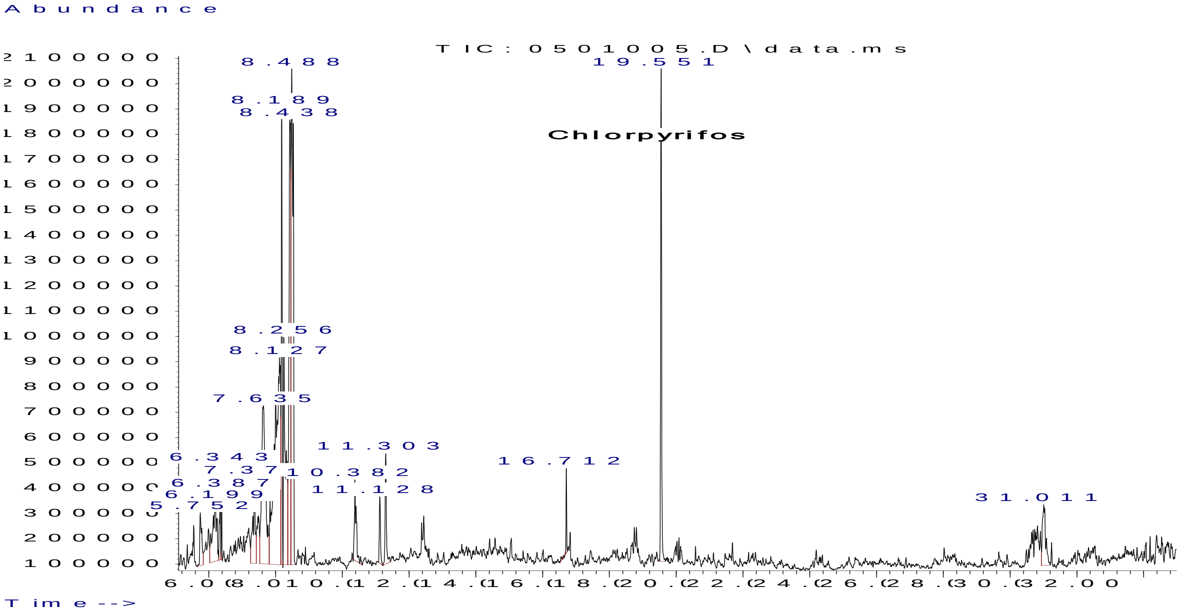

Figure 14: Chromatogram of chloropyrifos pesticides residues in Chinese cabbage

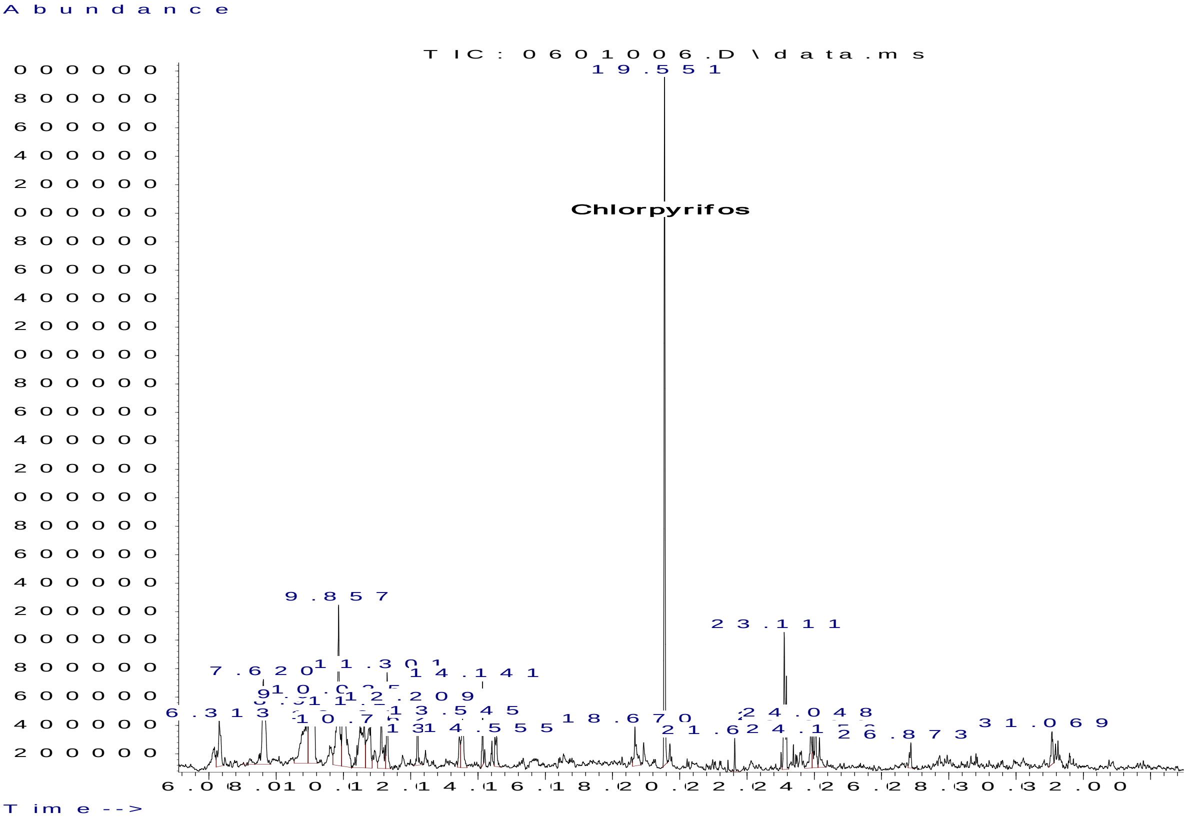

Figure 15: Chromatogram of chloropyrifos pesticides residues in watermelon

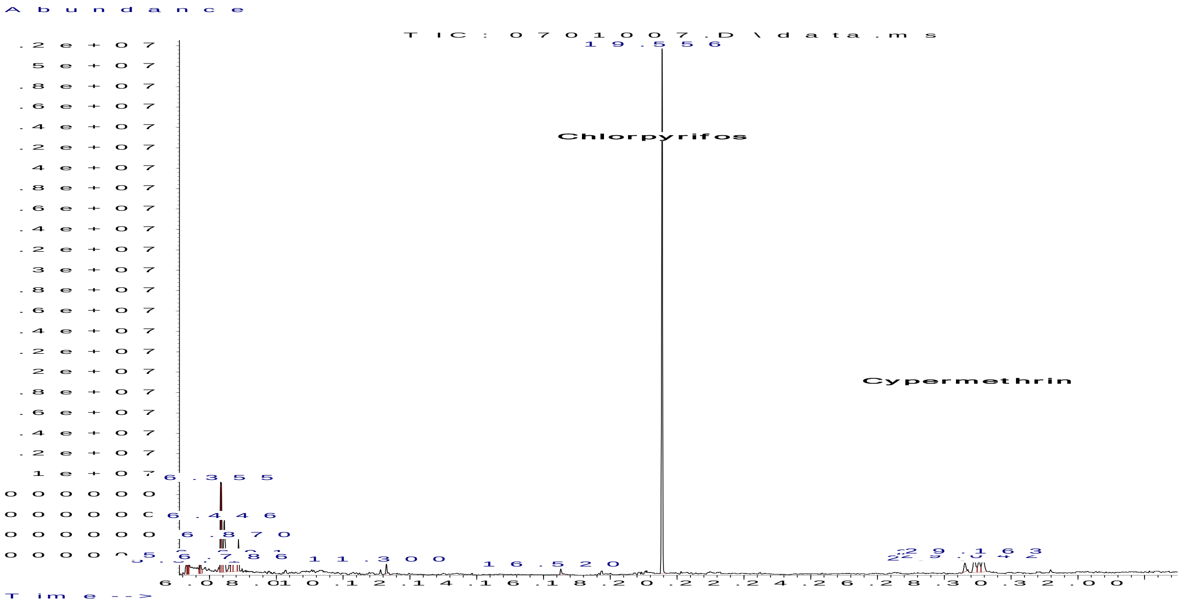

Figure 16: Chromatogram of chloropyrifos and cypermethrin pesticides residues in Kale

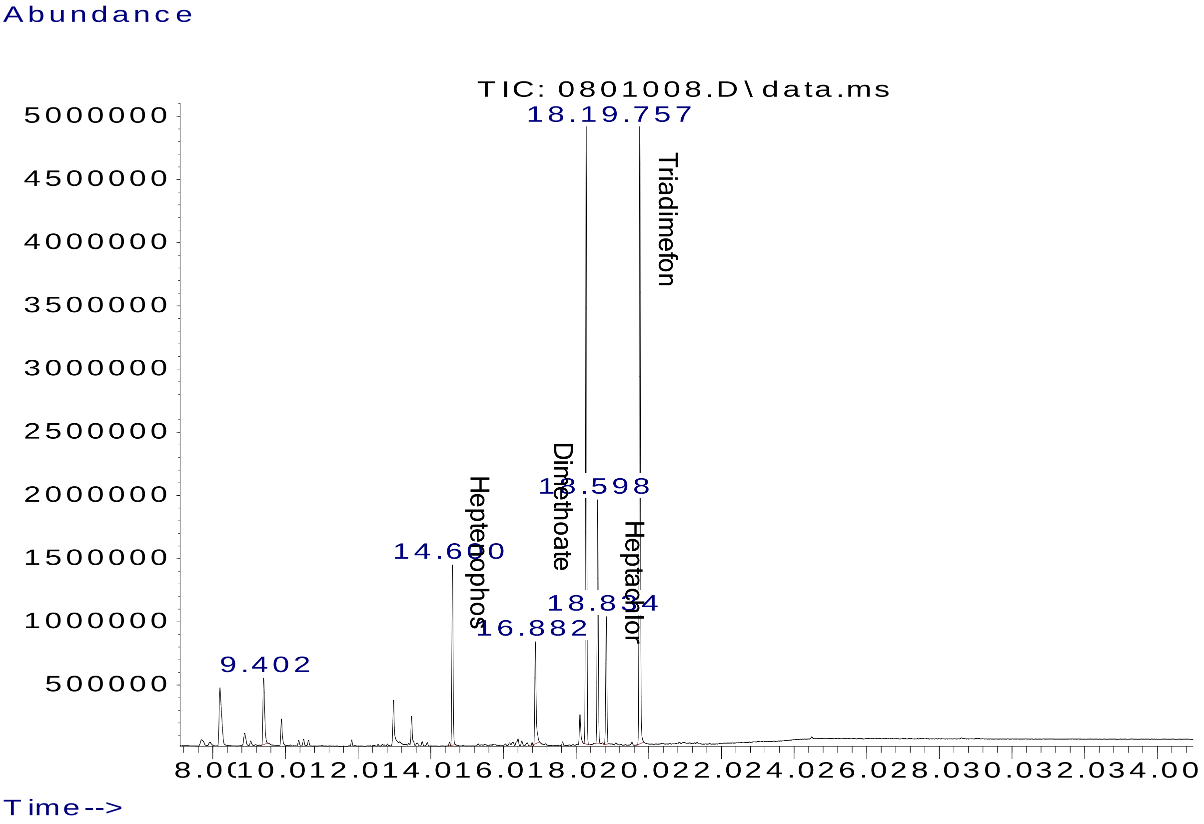

Figure 17: Chromatogram of triadimefon, dimethoate and heptenophos residues in tomato

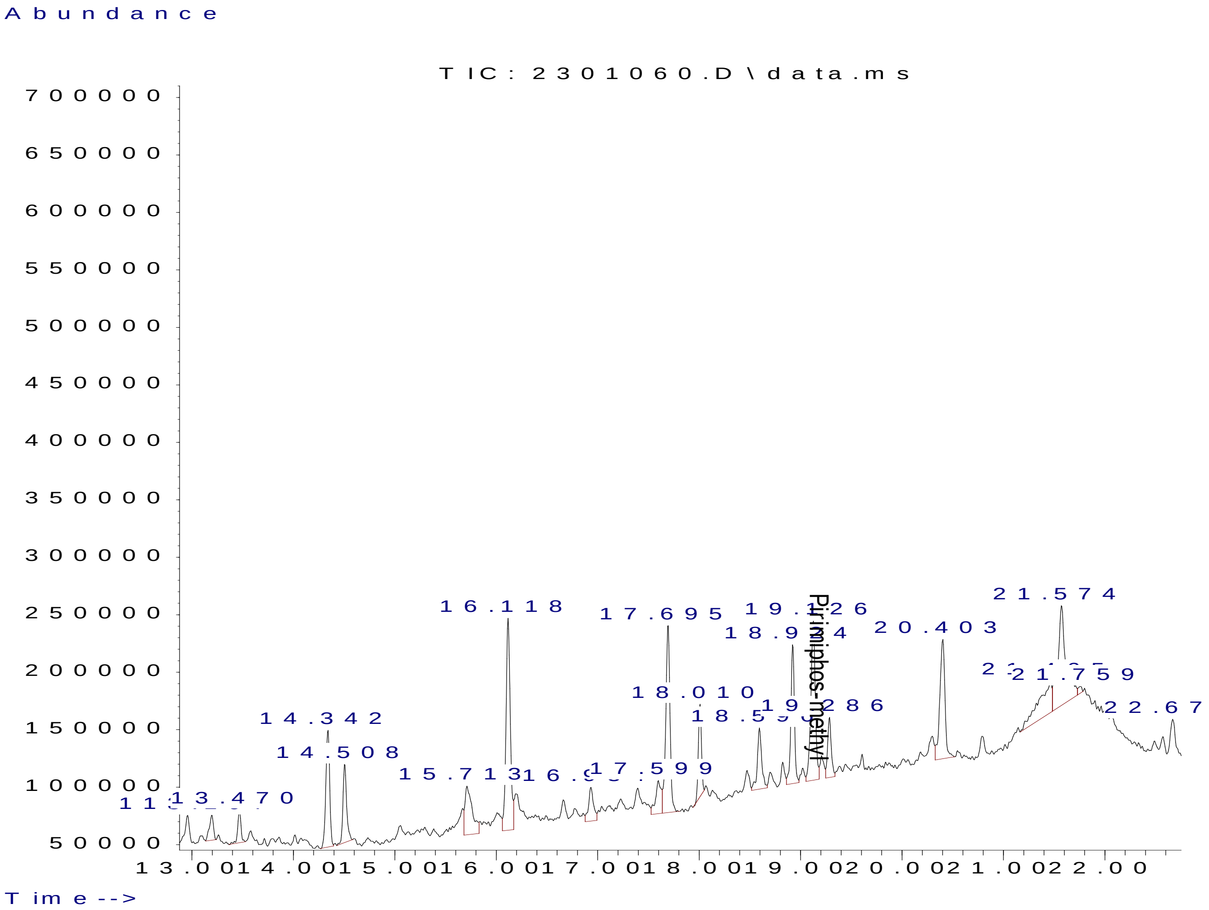

Figure 18: Chromatogram of pirimiphos-methyl pesticides residues in onion

(v) Levels of pesticides residues from vegetable samples

Of the samples that were analysed, 74.2% had average pesticides residues above the Codex MRL standards while 25.8% had residue levels below the Codex MRL standards (Table 47). In addition, samples from the highway recorded the highest (90%) proportion of pesticide residues above the Codex MRL standards (Fig. 19).

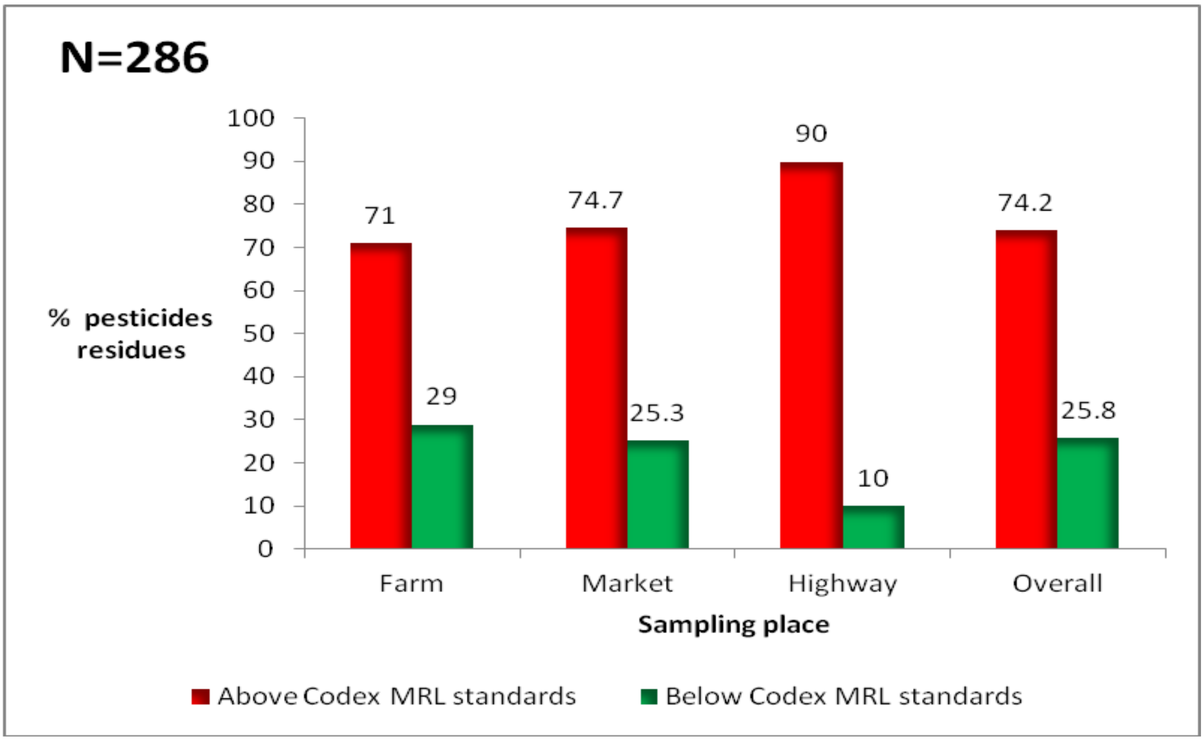

Figure 19: Levels of pesticides residues based on Codex MRL standards

Samples collected from Morogoro (87.8%), Iringa (85.4%) and Kilimanjaro (81.6%) had the highest proportions of vegetable samples with pesticides residues above the Codex MRL standards followed by Dar es Salaam (65.8%), Arusha (65.2%) and Manyara (44.4%) (Fig. 20).

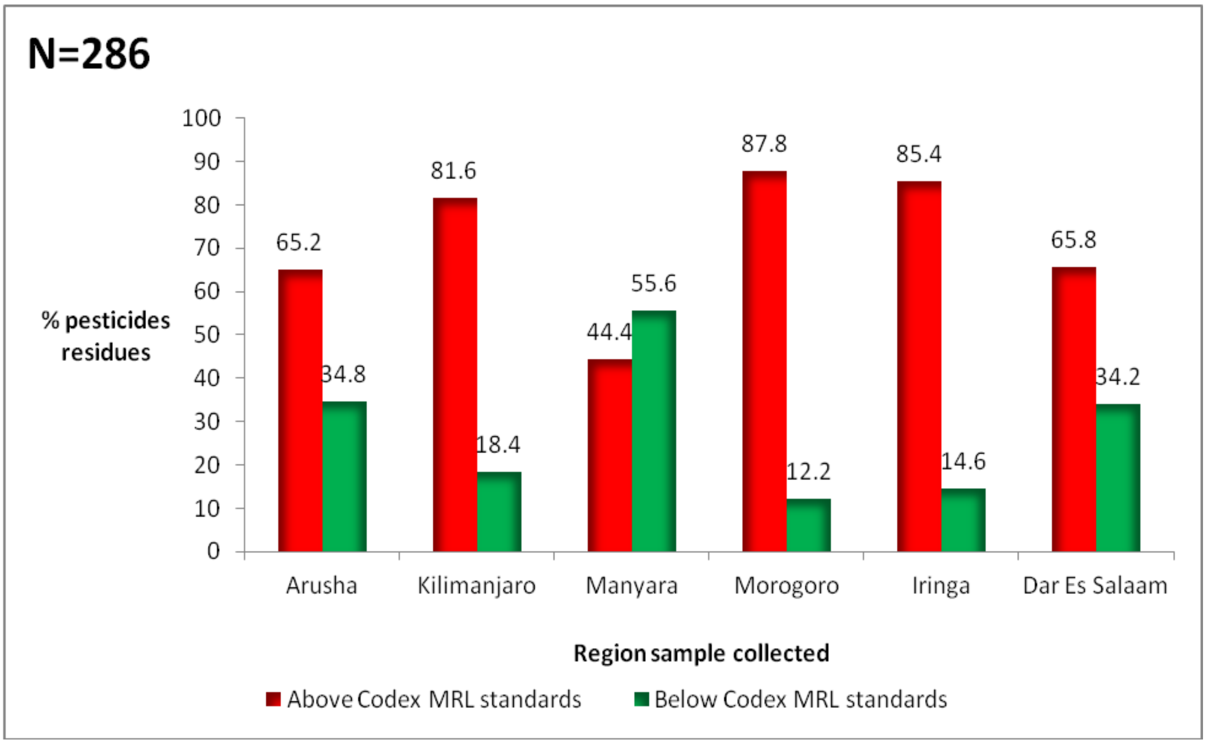

Figure 20: Proportions of vegetable samples with pesticides residues above the MRL

The results showed that onions (2.4537 mg/Kg), water melons (1.3733 mg/Kg), tomatoes (1.2549 mg/Kg) and sweet pepper (1.5068 mg/Kg) recorded the highest pesticides residues according to Codex limits (Table 47).

Table 47: Pesticides residue excess over Codex default limit

		Pesticides residues levels in mg/Kg				
Variables		**Minimum**	**Maximum**	**Mean**	**Codex default limit**	**% excess over Codex default limit**
Name of vegetable crop (Sample type)	Onions	0.0001	2.4537	0.3194	0.01	96.9
	Water melon	0.0001	1.3733	0.2195	0.01	95.4
	Tomatoes	0.0001	1.2549	0.2127	0.01	95.3
	Sweet pepper	0.0001	1.5068	0.1529	0.01	93.5
	Chinese cabbage	0.0016	0.3198	0.0712	0.01	86.0
	Cucumber	0.0008	0.1921	0.0678	0.01	85.3
	Night shade	0.0004	0.1030	0.0592	0.01	83.1
	Carrots	0.0019	0.2492	0.0457	0.01	78.1
	Amaranths	0.0424	0.0424	0.0424	0.01	76.4
	Sukuma	0.0006	0.1476	0.0407	0.01	75.4
	Ethiopian mustard	0.0335	0.0335	0.0335	0.01	70.1
	Egg plant	0.0026	0.0565	0.0284	0.01	64.8
	Green beans	0.0146	0.0192	0.0169	0.01	40.9
	Cabbage	0.0005	0.0215	0.0123	0.01	18.4
	Okra	0.0018	0.0171	0.0095	0.01	
Total		0.0001	2.4537	0.2206		

(vi) Chemical families of pesticides residues detected in vegetable samples

Organophosphates were the most common (95.2%) chemical family of pesticides used in vegetable production with detectable levels of pesticides residues and followed by organochlorine (24%), pyrethroids (17%) and least was chlorophenyl compounds (0.4%) (Fig. 21).

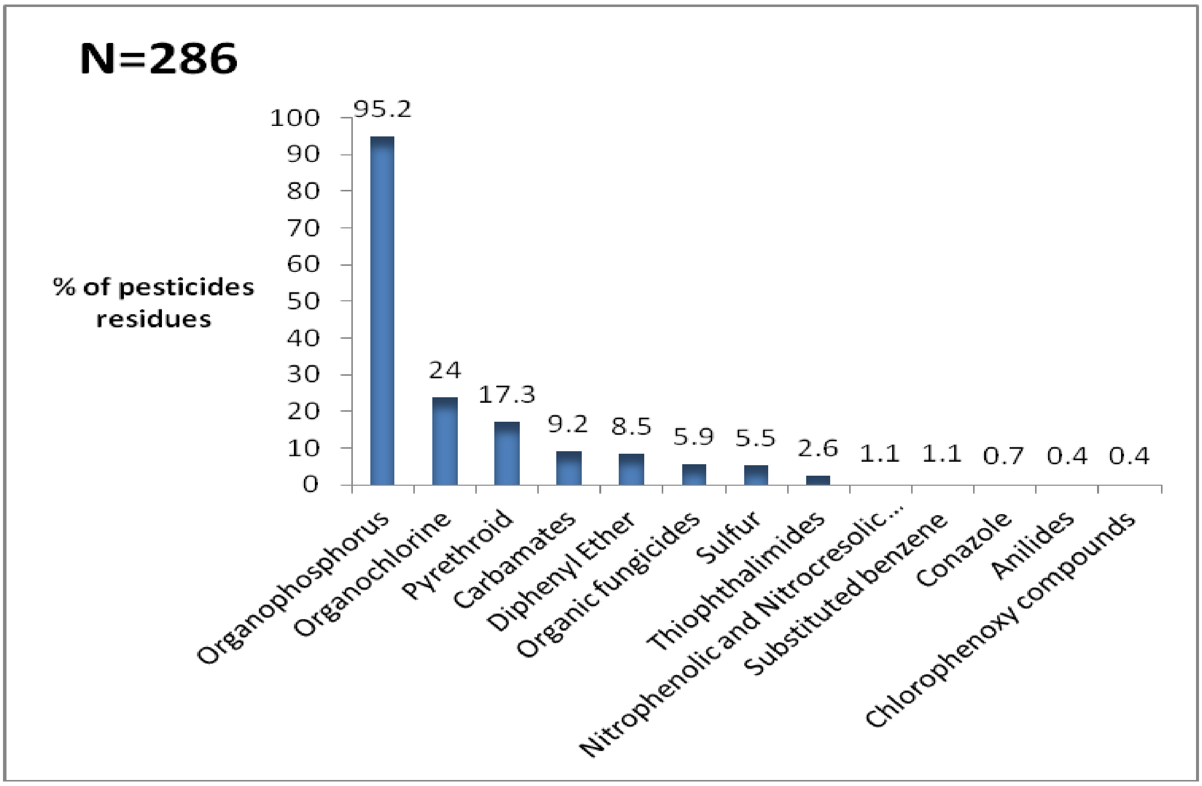

Figure 21: Chemical families of pesticides residues detected in vegetables

4.1.5 Co-exposure risks from pesticides residues and bacterial contamination of fresh vegetables produced by smallholder farmers

(i) Bacterial contamination of fresh vegetables

A subsample of 250 fresh vegetables was sampled for the assessment of bacterial contamination at the lower (farms) and higher (markers) node of fresh vegetables value chain. Fresh vegetables including tomatoes (50.7%), onions (37.1%), kale (33.6%), African nightshade (31.4%), cabbage and amaranths (30%) constitute the largest proportion of fresh vegetables analysed for bacterial contamination (Fig. 22).

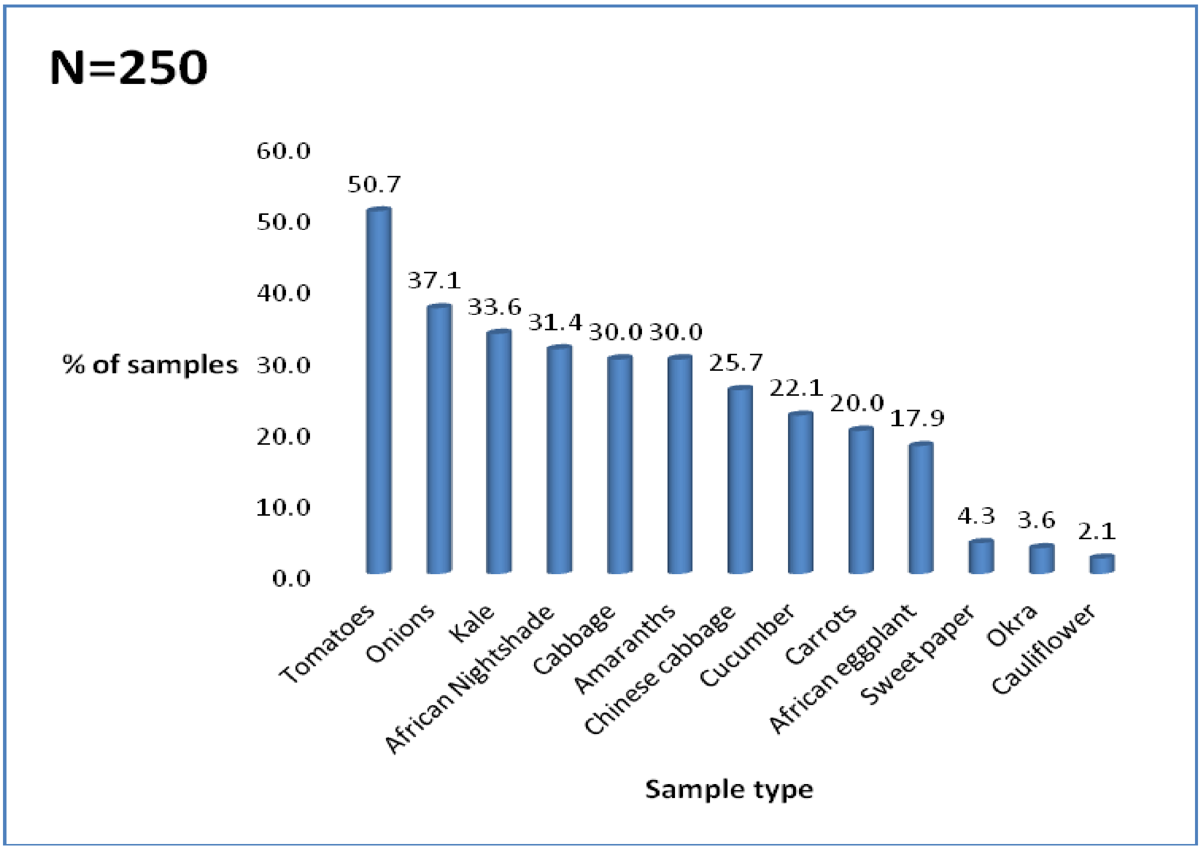

Figure 22: Proportion of fresh vegetables analysed for bacterial contamination

(ii) Prevalence of bacterial contamination

The prevalence of bacterial contamination in vegetables was 63.2%. The market samples were highly contaminated (68.8%) compared with those sampled from farms (45.9%). The results also revealed that, 90.4% of all contaminated vegetables had pathogenic bacteria (Table 48).

Table 48: Prevalence of fresh vegetables contamination (N=250)

Variable		**Sampling location**				**Total**	
		Farm		**Market**			
		n	**%**	**n**	**%**	**n**	**%**
State of microbial contamination	Contaminated	28	45.9	130	68.8	158	63.2
	No contamination	33	54.1	59	31.2	92	36.8
Nature of microbes isolated	Pathogenic	24	85.7	118	91.5	142	90.4
	Non pathogenic	4	14.3	11	8.5	15	9.6

(iii) The level of contamination by vegetable types

Sukuma, cabbage, spinach and Ethiopian mustard vegetables were highly contaminated with prevalence of 88.2%, 81.8%, 77.8% and 73.7% respectively. However, okra and sweet pepper had the least prevalence of 33.3% and 31.3% respectively (Fig. 23).

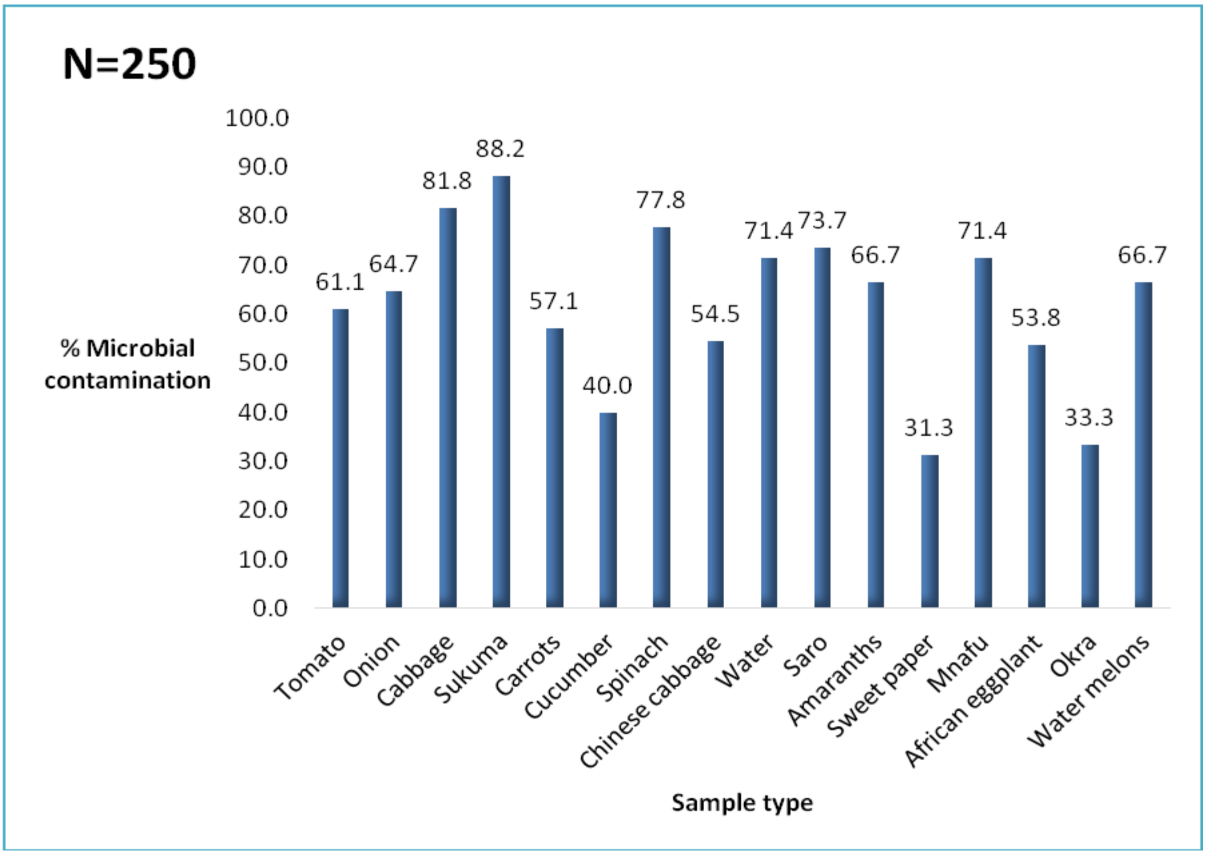

Figure 23: Levels of bacterial contamination of fresh vegetables

Up to four different bacterial strains were isolated from fresh vegetables whereby 46% of the fresh vegetables were found to contain one bacterial strain, 36% had two different bacterial strains while 15% had three different bacterial strains.

(iv) Microbial contaminants of fresh vegetables

The main isolated pathogenic bacteria from the fresh vegetables include, *Enterobacteriaceae* (33.7%) a broader family comprising *E. coli, Shigella, Salmonella* from faecal and urine contaminations, *Pseudomonas aeruginosa* (16.9%) *citrobacter* (14.4%), *E. coli* (14.4%), *Enterobacter* (9.5%), *Klebsiella oxytoca* (6.6%) and *Salmonella* (4.5%). Samples from farms recorded higher *E. coli* contamination (28.1%) compared with the markets (12.3%) while *Salmonella* and *Enterobacter* were isolated from samples collected from the market places only (Fig. 24).

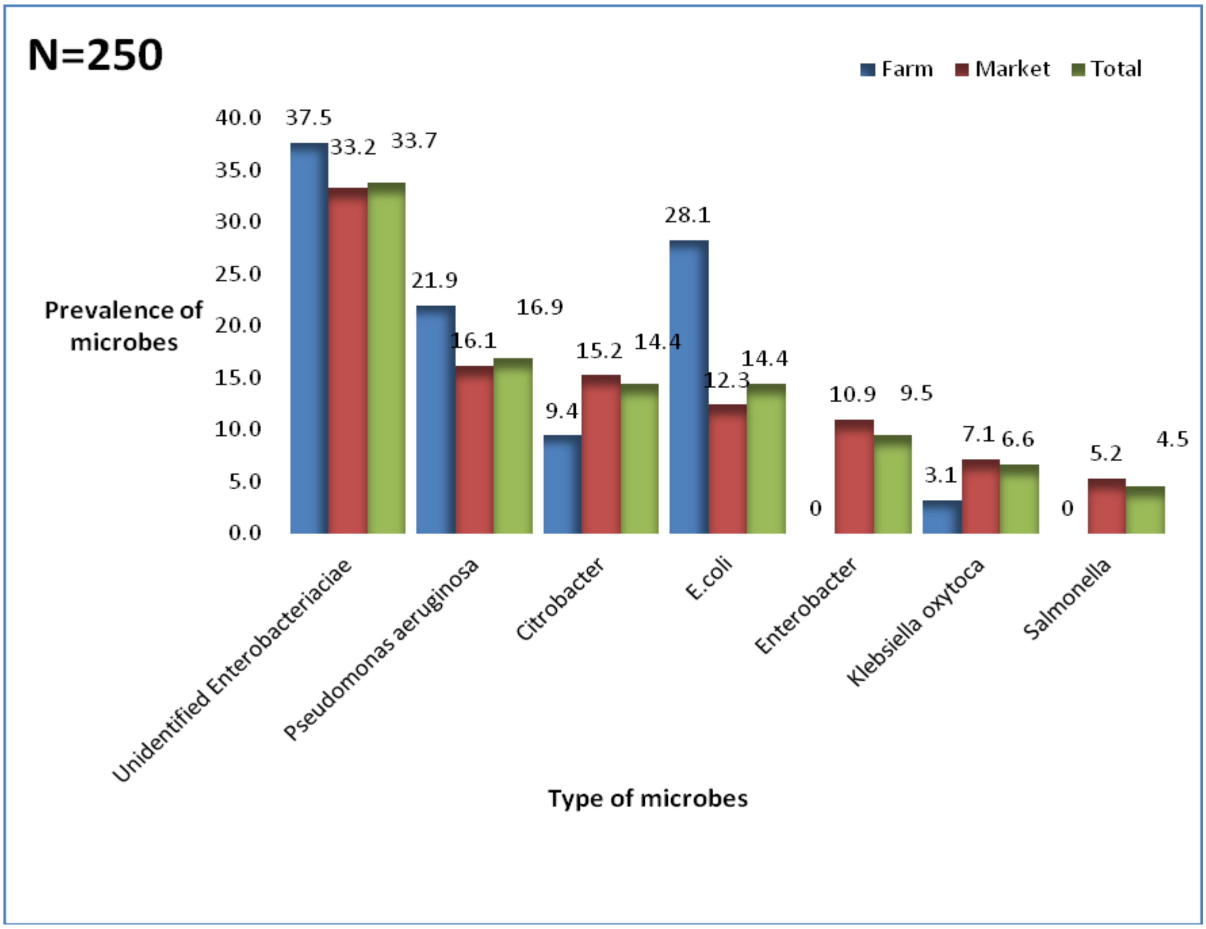

Figure 24: Types of pathogenic bacteria isolated from fresh vegetables

Across the five markets assessed, *Enterobacteriaceae* were predominant contaminants (Table 49). Kilombero and Soko kuu markets reported a broad range of pathogenic bacteria isolated from collected samples. Almost all isolated pathogenic bacteria were present in these markets. Samples from Ngaramtoni (47.6%) and Samunge (27.3%) recorded high *E. coli* contamination. *Salmonella* were isolated from samples collected from Kilombero (5.2%) and Soko kuu (7.7%) while other markets reported no prevalence of *Salmonella.*

Table 49: Bacterial strains isolated by market sites (N=118)

Variables		Sampling site										Total	
		Kilombero Market		Soko Kuu		Ngaramtoni		Samunge		Tengeru			
		n	%	n	%	n	%	n	%	n	%	n	%
Types of microbes	*Enterobacteriaceae*	42	43.3	15	19.2	8	38.1	4	36.4	1	25.0	70	33.2
	Pseudomonas aeruginosa	23	23.7	4	5.1	3	14.3	3	27.3	1	25.0	34	16.1
	Citrobacter	16	16.5	15	19.2			1	9.1			32	15.2
	E. coli	2	2.1	10	12.8	10	47.6	3	27.3	1	25.0	26	12.3
	Enterobacter	6	6.2	16	20.5					1	25.0	23	10.9
	Klebsiella oxytoca	3	3.1	12	15.4							15	7.1
	Salmonella	5	5.2	6	7.7							11	5.2

Sukuma (86.7%), carrots (75.0%), sweet paper (60.0%) and watermelons were highly contaminated with *Enterobacteriaceae*, while spinach (42.9%) and tomatoes (41.7%) were contaminated with *Pseudomonas aeruginosa*. Furthermore, Ethiopian mustard (47.1%), nightshade (40.7%) and Chinese cabbage (23.5%) were contaminated with *E. coli*. *Salmonella* strains were isolated from onion (26.7%), amaranths (16.7%), Chinese cabbage (14.7%) and water samples (4.0%).

The results further showed that, majority of farmers (80.2%) do not store fresh vegetables properly and some heap them on the floor in open space (11.6%), while very few farmers leave them on the ground uncovered. Traders, mostly retailers were found to store fresh vegetables, most of whom keep the on the selling table covered/wrapped with tarpaulins (Table 50).

Table 50: Storage and management of fresh vegetables (N=233)

Variables		Category of respondent				Total	
		Farmer		Trader			
		n	%	n	%	n	%
Where do you store your vegetables?	Do not store (Sell while on farm)	97	80.2			97	41.6
	Left on the selling table (Covered/wrapped)			76	67.9	76	32.6
	In a store/room (normal room)	2	1.7	20	17.9	22	9.4
	Heaped on the floor in open space	14	11.6	3	2.7	17	7.3
	Left on the ground (uncovered/unwrapped)	6	5.0	10	8.9	16	6.9
	In a cold-room	2	1.7	1	0.9	3	1.3
	In a truck			1	0.9	1	0.4

It was further noted that, the risk of cross contamination from other food and non-food materials was minimal among both farmers and traders of fresh vegetables. The results showed that majority (91.7%) do not transport fresh vegetables and other foods and non-food materials, neither mix vegetables with other food and non-food materials during selling/storage (Table 51).

Table 51: Cross contamination from other food and non-food materials (N=230)

Variables		**Category of respondent**				**Total**	
		Farmer		**Trader**			
		n	**%**	**n**	**%**	**n**	**%**
Do you transport vegetables with other food and nonfood materials?	No	113	93.4	98	89.9	211	91.7
	Yes	8	6.6	11	10.1	19	8.3
Do you mix vegetables with other food and nonfood materials during selling/storage?	No	112	94.1	105	96.3	217	95.2
	Yes	7	5.9	4	3.7	11	4.8

The major source of spoilage and contamination of fresh vegetables were identified to be dust and smoke from automobiles (38.8%), dirty market environment (37.8%), lack of storage facilities (32.7%), water used to wash fresh vegetables (26.5%) coupled with high temperature (22.4%) and other potential sources of contamination as shown in Fig. 26.

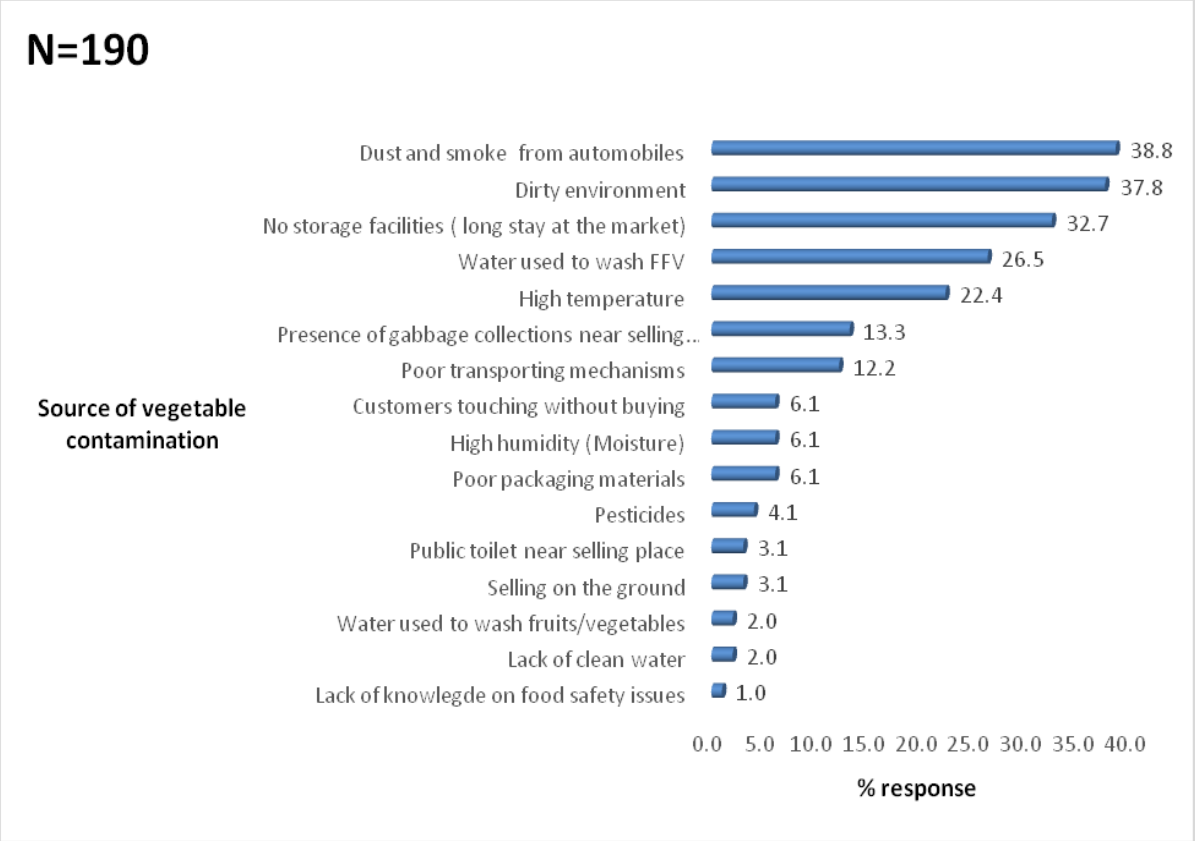

Figure 25: Potential sources of vegetable contamination at marketing level

(v) Environmental hygiene and sanitation

Large proportion (97.5%) of all market places surveyed had a toilet facility as compared with 52.9% of farms surveyed. On contrast, only 36.6% rated the public toilets in the market to be good compared with toilet facilities in farming areas (57.8%). Furthermore, both farming (53.6%) and marketing (44.3%) areas were found to have inadequate hand-washing facilities increasing the risk of faecal contamination of fresh vegetables (Table 52). Additionally, 71.3% of surveyed markets had garbage collection centres, but only 37.0% reported that garbage was collected very often, giving an indication of accumulation of garbage in the marketing areas.

Table 52: Environmental hygiene and sanitation facilities (N=240)

Variables		**Category of respondents**				**Total**	
		Farmers		**Traders**		**n**	**%**
		n	**%**	**n**	**%**		
Is there a toilet at/or near your farming/selling/market place?	Yes	64	52.9	116	97.5	180	75.0
	No	57	47.1	3	2.5	60	25.0
If Yes, how do rate sanitation of the toilet	Very good	8	12.5	5	4.5	13	7.4
	Good	37	57.8	41	36.6	78	44.3
	Average	17	26.6	44	39.3	61	34.7
	Poor	2	3.1	17	15.2	19	10.8
	Very poor			5	4.5	5	2.8
Is there hand-washing facilities at your selling/market place?	Yes	51	46.4	64	55.7	115	51.1
	No	59	53.6	51	44.3	110	48.9
Do you have a garbage collection point at your selling/market place?	Yes			82	71.3	82	71.3
	No			33	28.7	33	28.7
Total				115	100.0	115	100.0
If Yes, how often is the garbage collected	Very often			40	37.0	40	37.0
	Often			38	35.2	38	35.2
	Neutral			8	7.4	8	7.4
	Rare			12	11.1	12	11.1
	Very rare			10	9.3	10	9.3

(vi) Food safety inspection and surveillance mechanisms for fresh vegetables

Farms and market survey revealed weak regulatory enforcement of food safety regulations both at the production and marketing levels. The results showed that, 81.2% of farmers and 65.5% of traders reported that there was no any regulatory tool for controlling food safety at the farming and marketing levels respectively. Likewise, there were no food safety standards targeting fresh at production (78.6%) and marketing (74.4%) levels. Furthermore, there are minimal efforts among handlers of fresh vegetables in ensuring safety both in the production and marketing environment. Above half (52.1%) have no any means of ensuring safety of fresh vegetables. Few farmers (13.2%), and traders (8.3%) reported that, extension officers and health and TMDA officers respectively inspected the farms and hygiene of the marketing environment (Fig. 26).

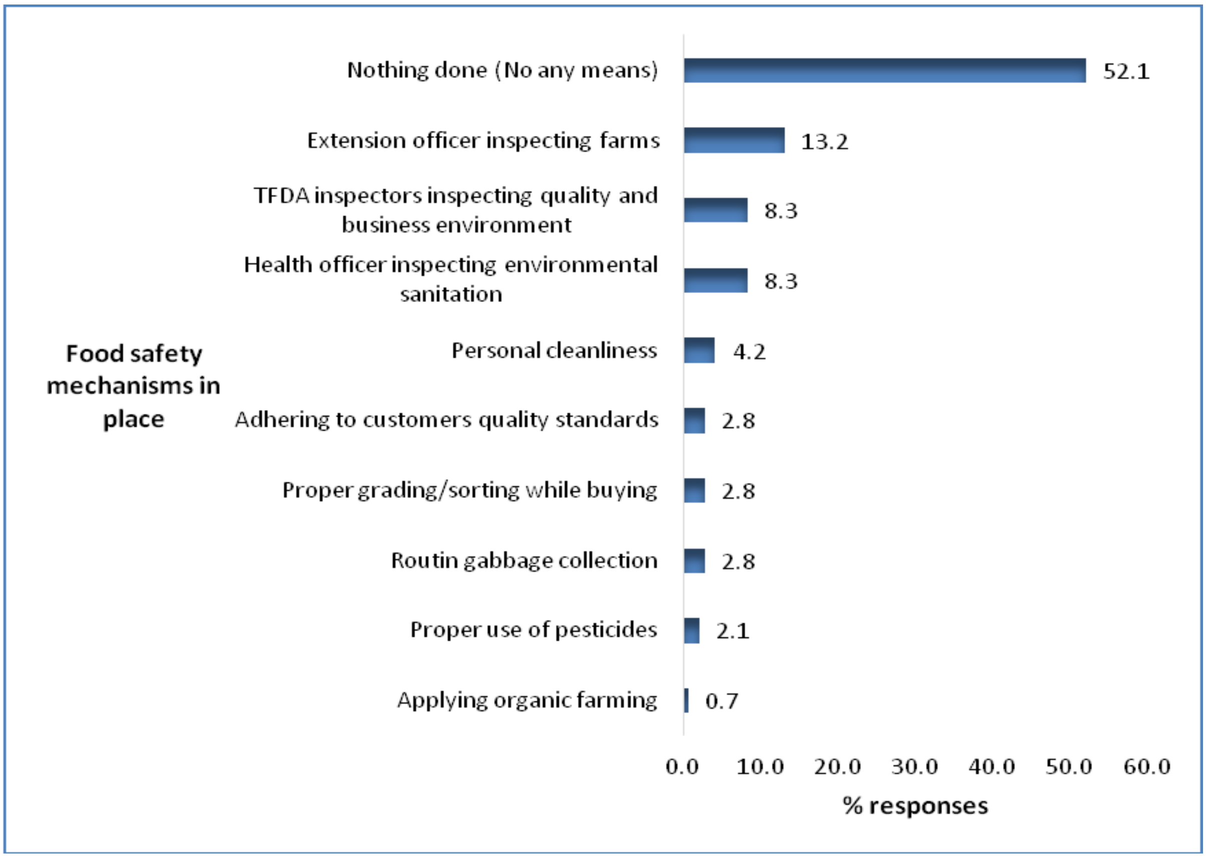

Figure 26: Food safety mechanisms in farms and markets

The level of controlling deterioration and spoilage of fresh vegetables was found to be inadequate among both farmers and traders. Only 20.9% store fresh vegetables in cool and dry places. Other risky handling practices reported are presented in (Fig. 27).

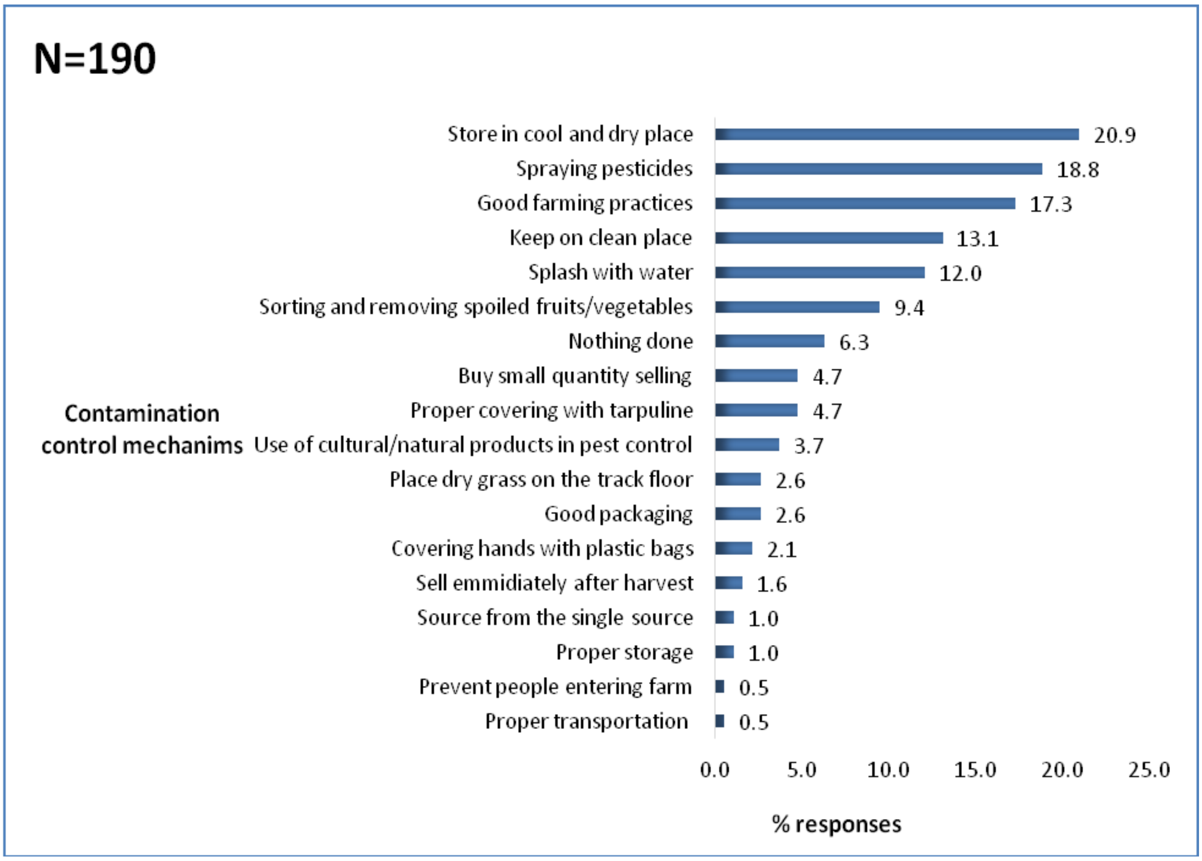

Figure 27: Deterioration and spoilage control of fresh vegetables

(vii) Co-occurrence of pesticides residues and bacterial contaminants

Co-contamination of pesticide residues and pathogenic bacteria was reported in the tested samples. A considerable proportion (46.4%) of fresh vegetables tested positive for both pesticide residues and bacterial contaminants. Vegetables from farms (60.7%) were more contaminated with both pesticides and bacterial contaminants compared to vegetable samples from the market places (41.8%). The difference on vegetable contamination between the two sites was statistically significant (p = 0.010). The number of bacterial pathogens isolated from a single sample differed significantly among market places (p = 0.022), while the difference was not significant for pesticide residues (p = 0.318) in the same locations. Furthermore, the level co-contamination of fresh vegetables was significantly different among the vegetable samples (p = 0.02) with onions (64.7%) and Chinese cabbage (54.5%) being highly contaminated with both pesticides residues and bacterial contaminants compared with carrots (14.3%) and sweet paper (18.8%) (Table 53a and 53b).

Table 53: Co-occurrence of pesticides residues and bacterial contaminants in vegetable samples (N=250)

Variables		**Sampling location**				**Total**	
		Farm		**Market**			
		n	**%**	**n**	**%**	**n**	**%**
Contain both pesticides residues and bacterial contaminants	No	24	39.3	110	58.2	134	53.6
	Yes	37	60.7	79	41.8	116	46.4
Total		61	100.0	189	100.0	250	100.0

Table 54: Co-occurrence of pesticides residues and bacterial contaminants in vegetable samples (N=250)

Variables		**Sample type**					
		n	**%**	**n**	**%**	**n**	**%**
		Tomato		Onion		Cabbage	
State of co-contamination	Contaminated	11	61.1	11	64.7	9	81.8
	No contamination	7	38.9	6	35.3	2	18.2
		Sukuma		Carrots		Cucumber	
	Contaminated	15	88.2	4	57.1	4	40.0
	No contamination	2	11.8	3	42.9	6	60.0
		Spinach		Chinese cabbage		Water	
	Contaminated	14	77.8	12	54.5	15	71.4
	No contamination	4	22.2	10	45.5	6	28.6
		Saro		Amaranths		Sweet paper	
	Contaminated	14	73.7	4	66.7	5	31.3
	No contamination	5	26.3	2	33.3	11	68.8
		African eggplant		Okra		Water melons	
	Contaminated	7	53.8	2	33.3	10	66.7
	No contamination	6	46.2	4	66.7	5	33.3

The binary logistic regression analysis showed that vegetables with pesticide residues were 2.2 times more likely to be contaminated with bacteria contaminants (OR: 2.231; 95% CI: 0.501, 8.802). Likewise, the use of the same wiping cloth/towel in cleaning fresh vegetables increased the likelihood of contaminating fresh vegetables with both pesticides and bacterial contaminants which was about 29% (OR: 1.288; 95% CI: 0.251). The location of vegetables with respect to farm and market place influenced the likelihood of co-contamination of fresh vegetables. The likelihood of co-contamination was 1.8% less for vegetables from the markets as compared with those from the farms (OR: 0.018; 95% CI: 0.112, 0.548). Other factors including water used for irrigation, storage, attending pesticides safe use and hygienic handling of vegetables, and splashing water to freshen vegetables did not significantly influence co-contamination of fresh vegetables (Table 54).

Table 55: Binary logistic regression of factors associated with pesticides and bacterial contamination of fresh vegetables

Variables	B	Wald	df	Sig.	Exp(B)	95.0% C.I for EXP(B)	
						Lower	Upper
Pesticides contamination	3.484	4.379	1	0.036	2.231	0.501	8.802
Storage	0.776	0.917	1	0.338	2.172	0.444	10.622
Water used in irrigation	0.209	0.028	1	0.868	1.234	0.104	14.656
Safe use train	1.117	0.513	1	0.474	3.054	0.144	64.796
Location	-4.030	5.307	1	0.021	0.018	0.112	0.548
Use same wiping cloth	0.253	0.092	1	0.016	1.288	0.251	6.610
Splash water	-0.440	0.232	1	0.630	0.644	0.107	3.868

4.2 Discussion

4.2.1 Pesticides use dynamics and practices among smallholder vegetable producers

Smallholder vegetable production is dominated by the youths. It therefore, provides employment to majority of youths who had little chance for further education. This signifies health risk of exposure to the younger generation in situation where injudicious use of pesticides prevails. Likewise, men, unlike women dominated smallholder vegetable production, reflecting the gender imbalance and male dominance of more economically viable agricultural activities in the study population and possibly in Tanzania. Male dominance in agriculture was also reported in Australia (Cotton *et al*., 2018).

Insects and fungal diseases were a major problem in the production of vegetable crops in small holder vegetable production. Different types of pesticides formulations were continously used by farmers in combination to combat these pests and diseases, posing a threat on exposure and pesticides residues in the produce. This also indicates that farmers, as well as consumers are at high risk of exposure due to mishandling and prevalence of highly hazardous pesticides, as majority farmers have no access to pesticides safe use education. Pesticides malpractices in vegetable production were evident indicating both health and environmental risks of pesticides exposure. This suggests food contamination from the production's points owing to the facts that environmental sanitation is compromised in rural areas and farmers use of high quantities of pesticides and other agrochemicals which may find their way in the water streams used for irrigation.

The use of pesticides in smallholder agricultural production has increased in the recent past. For example, insecticide and fungicides were the main pesticides used in this study. This study revealed that over 60 different pesticides products, comprised of 29 different pesticides formulation were used in vegetable production, and most of which were wrongly used. By 2006, farmers in Tanzania were reported to be using 40 different pesticides products (Ngowi *et al*., 2006). The current study exceeds the number of pesticides reported to be used in vegetable farming in Ghana, whereby 43 pesticides where found to be used in vegetable farming comprised mainly of insecticides, fungicides and herbicides (William, 2008). These increased pesticides use among farmers who are generally not well informed on the pesticides safety and management increases the risk for pesticides exposure.

Farmers mix more than one pesticide during spraying. The main reasons for pesticides mixing reported by farmers were minimizing spraying cost, increasing pesticides efficiency and controlling all pest at once. But mixing practices may lower the effectiveness of the pesticides, as some pesticides are not compatible in the mixture. Mixing of more than one pesticide may also result in chemical interactions between and among pesticides molecules resulting in more severe effects to both farmers and consumers (Aprea, 2012). Pesticides mixing may also be the reason why considerable high proportion of farmers reported lower effectiveness of pesticides in the control of pest. Similar findings of mixing pesticides was reported in Brazil, Pakistan and Nepal, whereby farmers use high concentrations of mixed pesticides (Atreya, Sitaula, Overgaard, Bajracharya, & Sharma, 2012; Damalas & Khan, 2017; Remor *et al*., 2009; Wilson & Tisdell, 2001). Mixing of pesticides is practiced by farmers to have an increased and rapid knockdown of pests (William, 2008). None of the farmers from this study was found mixing pesticides as per instructions from the pesticides label, which increases the risk of exposure to both farmers and final consumers of pesticides residues.

The study unveiled ineffective and poor pest management practices which significantly increased the levels of pesticides residues in vegetable crops. The use of pesticides demands systematic scouting and determination of the economic threshold of pests before pesticides use. Mere presence of pests does not automatically mean spraying of pesticides. Furthermore, mixing of pesticides increases toxicity of pesticides mixtures, which bring about high exposure effect and increased health implications at concentrations much below those of individual treatments (Das *et al*., 2007). Owing to the fact that farmers mix different types of pesticides and their handling practices are not effective, the extent to which the exposure may cause health effects is therefore alarming in Tanzania where pesticides are extensively used.

Most pesticides used in vegetable production fall under Class II (Moderately hazardous) of WHO hazard classification of pesticides. Small quantities of extremely hazardous (Class Ia) and highly hazardous (Class Ib) were also found to be used in the surveyed areas which poses high health risks of exposure to hazardous pesticides chemicals, as these classes of pesticides demand high pesticides management skills to protect both the farmers, consumers and the environment. There is general lack of farmer knowledge and education on the safety of the produce and safe use practices which has an implication on the levels pesticides residues, increased production costs and contamination of the environment with hazardous chemicals resulting from injudicious use of pesticides.

Disposal of empty pesticides containers increased risks of environmental exposure. Empty pesticides containers were left in the field the whole farming seasons and collected from the field and/or open pits in the field during the preceding farming season. Few farmers reported to use the empty containers for domestic purposes. Previous studies (Ngowi *et al*., 2001). Proper procedures are required to be undertaken before burning or even burying in the soil. This endangers the ecosystem, water bodies and the general biodiversity.

The use and application of green pesticides provides a sustainable means for ensuring food safety while protecting both human and environment from detrimental effects of toxic pesticides (Mossa, 2016; Qian *et al*., 2010). Antagonistically, this was not found to be among the remedies for ensuring food safety and sustainability of smallholder vegetable production in Tanzania. This shows low technological development in green chemistry and application of nanotechnology in deriving environmentally and ecologically friendly pesticides in addressing human exposure and contamination of food materials with toxic chemicals. Weak policy and institutional support in the development of greener pesticides as well as the legal framework on safety of locally produced and consumed fresh vegetables may account for lacking application of green pesticides in smallholder vegetable production systems.

The key drivers of farmer's increased use of pesticides as estimated from the probit regression model showed that, region of farmers, number of vegetable crops grown, and mixing practices of pesticides were significantly associated with the farmers' likelihood of using high levels of pesticides. In regions with persistence use of pesticides, farmers are persuaded to continuously use increased levels of pesticides. Farmers pesticides handling is therefore learnt over experience (Damalas & Khan, 2017) which ultimately had become a common practice. This influences farmers to increase more pesticides in efforts to combat crop pests and diseases with the belief that the more pesticides used the more progressive the farmer is.

Most farmers grow more than one vegetable crop consecutively. This had been also found to influence farmers' likelihood of using more pesticides. In efforts to control multiple pests affecting their crops, farmers resort in using high volumes and highly concentrated pesticides mixtures. This threatens both human and environmental health, disrupting natural pest control and predator-prey relationship in the ecosystem. Extensive use of pesticides may also results in the development and evolution of pests resistance to pesticide as reported earlier (Pimentel, 2005). Furthermore, the perception of low effectiveness of pesticides, limited access to information on safe use of pesticides, low use of protective gears among farmers increased likelihood of farmers using pesticides indiscriminately.

Mixing of pesticides during spraying was also found to be the determinant factor for increased pesticides use. Farmers mixing more than one pesticides had been previously reported (Damalas & Khan, 2017). Smallholder vegetable producers from Arusha (55.3%) and Kilimanjaro regions mix more pesticides as compared with farmers from Iringa (30.2%). Poor use and application of pesticides in different parts of the world are assumed to foster contamination of food materials with pesticides residue (Latif, Sherazi & Bhanger, 2011a). High volumes of pesticides use per acre in the areas surveyed may directly be linked to high levels of pesticides residues both in the environment and in food materials.

Pesticides commonly used fall under Class II (Moderately hazardous) of WHO hazard classification of pesticides. These pesticides category had been previously reported to be used among vegetable farmers in Tanzania (Ngowi *et al.*, 2006; Nonga *et al.*, 2011) and in Nepal (Atreya *et al.*, 2012; Neupane *et al.*, 2014). Persistent use of these chemicals poses considerable high exposure risks due to poor pesticides handling practices among smallholder farmers as majority of smallholder vegetable producers have no access to pest control extension services on pesticides use and pests' control.

The study revealed high pesticides spraying frequency among small holder vegetable producers. Most farmers spray pesticides once every week, which is considerably high spraying frequency. Farmers were therefore actively involved in pesticides application throughout the farming season which lasted for three months. On average, farmers spray 4.27 drums of mixed pesticides on an average of 1.21 acre of land in tomato while 4.59 drums of pesticides mixtures are sprayed on an average of 1.08 acre land of onion. This translates in 758.7 litres/acre of pesticides mixture in tomato and 913.75 litres/acre of pesticides mixture in onion field. These levels of pesticides volumes are considerable high suggesting high levels of pesticides residues both in the environment and in food materials, which are likely to cause both acute and long-term health effects. On the other hand, these findings indicate high pesticides exposure among farmers due to very tight spraying schedule.

Likewise, most smallholder vegetable producers do not wear personal protective equipment (PPEs) while handing pesticides signifying high rate of pesticides exposure among farmers. The use of personal protective equipment (PPEs) was not common among the faming population in this study. Most farmers handled and sprayed pesticides without self-protection and the use of gloves, masks, respirators, overalls/overcoats and head covers were not common. Similar findings had been reported previously (Van der Hoek *et al*., 1998). Moreover, the use of PPEs was synonymous with wearing gumboots. Majority of farmers who agreed to be using PPEs only used gumboots. This is contrary to farmers from Australia, majority of whom used gloves (Cotton *et al*., 2018) and those from Nepal (Neupane *et al*., 2014) who mostly used caps. The use of gumboots was common in tomato-based vegetable production. Farmers in onion production did not use gumboots fearing to step on and destroying onion bulbs, which further increases exposure risks among smallholder vegetable producers.

4.2.2 Comparative assessment of acetylcholinesterase (AChE) activity with associated health impacts

Occupational exposure to pesticides was evident among smallholder vegetable producers. Exposed farmers had a significantly lower cholinesterase (28.05±3.88 u/gHgb) compared with (32.87±4.36 u/gHgb) control group. This inhibition level is slightly higher than AChE inhibition reported (29.45 ± 3.68 u/gHb) among Indonesian farm workers (Suratman *et al*., 2015). These findings are in agreement with previous studies (Atreya *et al*., 2012; Naravaneni & Jamil, 2007; Neupane *et al*., 2014; Remor *et al*., 2009) which revealed a progressive fall in AChE levels in exposed individuals compared with unexposed individuals. However, studies conducted in Iowa and North Carolina (Hongsibsong *et al*., 2017) as well Australia (Cotton *et al*., 2018) did not establish any statistical difference between the two groups, indicating controlled pesticides management in developed than developing countries.

Decreased level of AChE activity is caused by the enzyme inactivation which is induced by a range of inhibitors including pesticides. This leads to acetylcholine accumulation, hyperstimulation of nicotinic and muscarinic receptors, and disrupted neurotransmission. Therefore, pesticides which interact with the enzyme as their primary target, acetylcholinesterase inhibitors, are discussed in relation to the low level of AChE activity among farmers (Colovic *et al*., 2013).

Organophosphorus and carbamates are the main pesticides used under the current study. Occupational exposure to mixture of these chemicals results in the decreased acetylcholinesterase (AChE) activity as also reported elsewhere (Singh *et al*., 2011), and the use of these agricultural chemicals without necessary protection may lead to alterations in the genetic materials and the possible development of some types of tumors (Bhalli *et al*., 2009). Exposure to pesticide had induced acute as well as severe pesticides poisoning. This is similar to the farmers in Vietnam who experienced acute pesticides poisoning due to occupational exposure to organophosphate and carbamate pesticides (Dasgupta *et al*., 2007). This occupational exposure to mixture of OPs may cause DNA damage, hepatic and renal toxicity (Singh *et al*., 2011). Smallholder vegetable producers are therefore at risk of these health effects of pesticides exposure.

The mostly used organophosphate and carbamate pesticides may therefore be associated with the progressive fall in AChE levels of exposed farmers. Occupational exposure to a mixture of pesticides (organophosphates, carbamates, pyrethroids) and lower AChE levels in exposed farmers are significantly associated with DNA damage, neurotoxicity reactive oxygen stress (ROS), and increased micronuclei frequencies (Bhalli *et al*., 2006; Das *et al*., 2007; Naravaneni & Jamil, 2007). DNA damage have been presumed as mechanisms linking pesticide exposure to health effects including neurological diseases (Kisby *et al*., 2009). Exposure to carbamates and organophosphates had also been associated with fatal death, hormonal changes, birth defects, and abnormal sperm, ovaries and eggs production (Bhalli *et al*., 2006).

Smallholder vegetable farmers are occupationally exposed to different mixture of pesticides. The exposed farmers and control (unexposed individuals) were involved to determine the levels of exposure to pesticides. The use of control groups had been reported in several studies (Bhalli *et al*., 2009; DaSilva *et al*., 2008; Grover *et al*., 2003; Liu *et al*., 2006; McKinlay *et al*., 2008; Naravaneni & Jamil, 2007; Neupane *et al*., 2014). Occupationally exposed farmers were compared with the control group of similar demographic characteristics in drawing comparative results and controlling confounding factors influencing exposure to organophosphate and carbamate pesticides.

The Body Mass Index (BMI) showed no significant difference between the exposed farmers and the control group. However, slight variations were noted among different BMI categories of both the exposed and control groups. Majority of both exposed farmers and unexposed control group had normal BMI (18.50-24.99) Kg/m^2 as categorized by the WHO. The average BMI for the exposed farmers (22.74 ± 3.23) Kg/m^2 was slightly lower than control group (23.26 ± 3.38) kg/m^2. These findings are similar to BMI of exposed farmer (21.41 Kg/m^2) and control groups (25.18 Kg/m^2) from Nepal (Neupane *et al*., 2014). Since obesity is identified as chronic condition of excessive accumulation of body fat that is associated with metabolic complications (Hamouda *et al*., 2019), this accumulation of body fats may accelerate quick absorption of lipophilic organophosphate pesticides thereby increasingly depress AChE activity among exposed individuals. Similarly, a pesticide metabolizing enzymes and biochemical processes involved may be hindered by excess fats and increasing pesticides exposure effects as well. However, studies are needed to validate and evaluate the mechanisms involved and cause-effect relationship between BMI and AChE in exposed individuals.

Pesticides exposure varied with nutritional status of the exposed farmers. Farmers who were either undernourished (underweight) or over nutrition (overweight and obese) were significantly exposed compared with the normal BMI individuals. Nutritional status of the farmers is therefore suggested to be another risk factor for pesticides exposure. Poor feeding habits among rural farming communities can therefore be linked to the exposure status of the farmers. Statistically significant association between AChE inhibition and BMI indicates that, nutritional status of the farming community influences exposure risks of the farming community. Immunotoxicity of pesticides especially carbamates (Dhouib *et al*., 2016) can also be associated to increased exposure among underweight and obese farmer because BMI had been strongly correlated and associated with human immune system (Ilavská *et al*., 2012). Both the underweight and obesity may be immunal-compromised, hence have increased risk of infection (Dobner & Kaser, 2018).

Farmers had been handling pesticides for a considerable long period of time, most over 10 years. They are aware of risk behaviours which increase risks of pesticides exposure and to a large extent avoid them during pesticides handling and management. Smoking, drinking and eating while spraying was not a common practice of the farmers, indicating farmers' awareness on the pesticide's exposure. These farmers' risk behaviours during pesticides handling are different from previous studies which reported that farmers were aware of the standard safety precautions to prevent exposure during spraying but very few, if any, of these preventive measures are actually practiced in the current study (Van der Hoek *et al.*, 1998). Farmers are therefore, vulnerable to exposure health complications. Since smallholder farmers have decreased AChE activity due to exposure to mixtures and organophosphates, carbamates and other pesticides, they are more likely to develop leukemia, brain cancer, prostrate and skin cancer and non-Hodgkin's lymphoma than the general population (Mathur *et al.*, 2005).

In this study, women, who were mainly involved in weeding and harvesting of vegetable crops were more exposed than men. Cholinesterase level was lower among women, suggesting women are more exposed or susceptible to organophosphate and carbamate pesticides. Similar findings were reported in India and Indonesia (Mancini, Van Bruggen, Jiggins, Ambatipudi & Murphy, 2005; Murphy *et al*., 2000). Women being assumed to do less risky jobs and there relative large body surface increases absorption of lipophilic pesticides through their skins because most organophosphates are highly lipid-soluble agents and are well absorbed from the skin (Damalas & Koutroubas, 2016). Although it has been reported that people involved in preparing and spraying pesticide mixture constitute the most exposed groups of farmers (Bolognesi, 2003) the current study revealed women involved in weeding and harvesting vegetable crops being an occupational group at high risk. Weeding and harvesting though perceived to be less risky, re-entry and pre-harvesting intervals which can be explained by the fact that farm workers, mostly women, enter farms soon after spraying and expose them high risk. This may be attributed to lack of pesticides safe use and management trainings (Latif *et al*., 2012).

Some individuals from the control group had significant lower AChE levels. Similar findings were reported in Pakistan where non farmers were found to be exposed (Latif *et al*., 2012). These findings are also similar to previous findings whereby both farming and non-farming populations were occupationally and environmentally exposed due to excessive use of pesticides in their areas of occupation (Mathur *et al*., 2005). These findings indicate environmental exposure to pesticides and exposure through consuming of pesticides contaminated crops, posing a health risks to consumers.

Furthermore, exposure to pesticides varied with age of the exposed farmers. Younger group (less than 20 years of age) and older farmers (above 60 years of age) were significantly exposed compared with middle aged farmers (20-60 years). This suggests the vulnerability and susceptibility of younger and older farmer to exposure of pesticides within their bodies.

This is contrary to the reported study in Benin (Vikkey *et al*., 2017) where the age of the farmers did not significantly influence the level of AChE. Higher exposure among younger farmers can be explained by the fact that pesticides, mostly organophosphates, and carbamates inhibit many enzymes activities (Latif *et al*., 2012). Enzymes involved in the metabolism of pesticides may be highly susceptible to inhibition to the immature immunity (the youth) and compromised immunity (older adults), suggesting that body immunity may be a predisposing factor for vulnerability and susceptibility to pesticides exposure.

In contrary, smoking and eating during pesticides application did not significantly increase exposure among farmers. This is partly because most farmers are aware of the risk behaviours and did not eat and/or smoke during pesticides application. Findings from this study showed statistically significant difference on the level of AChE inhibition between users and non-users of local brew within the exposed farmer groups. Drinking local brew among exposed farmers had lowered AChE levels compared with non-drinkers. This is because alcohol is reported to disturb the functioning of the autonomic nervous system (Haboubi & Thurnham, 1986). Furthermore, alcohol disturbs the structure of water around hydrophobic areas of cholinesterase, possibly causing instability in the enzyme conformation and subsequently decreasing the activity (Fekonja, Zorec-Karlovšek, Kharbili, Fournier & Stojan, 2007).

The duration and experience of working with pesticides among the exposed group did not influence significantly the levels of pesticides exposure measured by AChE depression. However, farmers with a working experience of 1-4 years had slightly lower AChE levels compared with those who had been working with pesticides for over 5 years, suggesting existing knowledge differences between the new farmers and much experienced farmers in handling and management of pesticides. These findings are not in support of previous studies (Singh *et al*., 2011) that reported decreased AChE activity with duration of exposure to different OPs in occupational workers. Lack of exposure difference in this study may be explained by poor pesticides use practices whereby both experienced and inexperienced farmers are equally exposed to mixtures of pesticide deriving similar health effects.

Moreover, there was no statistically significant difference in the levels cholinesterase in relation to pesticides spraying frequency. However, farmers who sprayed 3-4 days a week had a relatively low AChE levels compared with those spraying once a week or once in a month. High spraying frequency ranging from once every three to once every five days had been reported, which is excessively high under any agricultural production standards (Van der Hoek *et al*., 1998). High spraying frequency as observed under the current study where farmers' contact hours and active weeks were relatively high suggests that continuous spraying and contact with pesticides may induce increased risks of pesticides exposure.

The average area sprayed per day by the farmer had a positive influence on the level of exposure. Farmers who sprayed a relatively larger area a day had significantly lower AChE levels ($p = 0.021$). This shows that the increased pesticides contact hours ultimately fosters the rate of dermal exposure from wet cloths and leaking spraying equipment, which were observed in the field, negatively affecting farmer's health by lowering significantly the AChE levels. Similar finding were reported in Nepal where AChE inhibition was found to correlate with number of working hours among farmers (Atreya *et al*., 2012). Likewise, exposure risks are high among farmers who break less than a month because the time required to liberate the enzyme (AChE) from inhibition is more than the time necessary for the synthesis of a new enzyme, which is more than 30 days after exposure (Ecobichon, 2001). Farmers breaking more than a month have more time for the enzyme recovery and metabolic detoxification of OPs. Breaking for a reasonable period before embarking on intensive spraying season can reduce the level of exposure among farmers.

Furthermore, handling practices were observed to increase risks of pesticides exposure under the current study. This may be attributed partly due to poor personal protective equipment (PPEs) or partially used. The use of PPEs did not significantly reduce exposure and thus contradicting other findings (Dasgupta *et al*., 2007) that confirmed the use of protective equipment reduces the risks of pesticides exposure. Farmers who were partially covered were equally exposed as the completely unprotected though effective use of PPEs significantly reduces exposure (Ecobichon, 2001). Poor use of the PPEs may account for their insignificant contribution in reducing exposure among farmers. This calls for a comprehensive awareness through continuous education among producers to enhance protection against pesticides exposure. This is supported by Dasgupta *et al*. (2007) and Undeger and Basaran (2005) who affirmed that, use of PPEs reduces health hazards from pesticides and reduces the risks of pesticides exposure

Typical symptoms of exposure to pesticides which are specific clinical manifestations of organophosphate and carbamate exposure were observed among farmers. Exposed farmers reported more symptoms of exposure to pesticides (14.10±7.70) compared with control group (6.48±6.62). Similar findings were reported in Nepal among vegetable farmers (Neupane *et al*., 2014) and Vietnam with an average of 4 symptoms (Dasgupta *et al*., 2007). However, the symptoms in this current study are far above the average number of symptoms (4.78) reported by farmers and that of control (1.58). Exposed smallholder farmers in Tanzania reported significantly multiple and higher number of exposure symptoms compared with other developing countries (Cotton *et al*., 2018; Naravaneni & Jamil, 2007). Analysis of reported symptoms indicates that almost all were statistically significant when compared to controls. The most reported exposure symptoms which significantly differed from the control group include tiredness, fatigue, and soreness in joints, thirst, headache, skin irritation, excessive salivation and abdominal pain. These symptoms had been described to be typical symptoms of exposure due to OPs and carbamate pesticides (Alavanja *et al*., 2004).

Based on severity of exposure, most of the signs and symptoms reported indicated mild pesticide poisoning and severe pesticides poisoning. Pesticides exposure is associated with a range of symptoms as well as deficits in neurobehavioral performance and abnormalities in nerve function (Alavanja *et al*., 2004). Nevertheless, other studies showed that more severe cases of pesticides exposure was manifested by developing muscle weakness and muscle twitches, changes in heart rate, and bronchospasm and can progress to convulsions and coma (Alavanja *et al*., 2004). Other symptoms were neurobehavioral and those associated with muscles, epithelia/mucosal surfaces respiratory and gastro-intestinal tract.

The main root of exposure was dermal, optical and respiratory, increasing health risks to the farming community. Similarly, absorption through the dermal pathway is the most important route of uptake by pesticides workers (Anwar, 1997). Pesticides exposure, either occupational or environmental results in detrimental human health disorders (Zacharia, Kishimba & Masahiko, 2010).

4.2.3 Levels of pesticides residues and bacterial contamination of vegetables produced under smallholder production systems

Locally produced and consumed vegetables are highly contaminated with pesticide residues, posing a critical threat to the fate and sustainability of smallholder vegetable production and food safety concerns. Fresh vegetables were found to be contaminated with a wide range of pesticides in this study, which increases dietary exposure risks. A total of 52 different types of pesticide residues were detected from all vegetable samples. This number is much higher than that reported from other countries (Bhanti & Taneja, 2007; Chen *et al*., 2011; Wu *et al*., 2017) as compared with 22 pesticide residues detected in vegetable samples from China (Chen *et al*., 2011).

Presence of pesticides residues in vegetables had also been reported in other countries (Chen *et al*., 2011; El-Nahhal, 2004), but the proportion of samples with detectable levels of pesticides in this study are higher than those reported in other developing countries (Bhanti & Taneja, 2007; Chen *et al*., 2011; Wu *et al*., 2017) and much higher than levels of pesticides residues detected from the EU and US pesticides monitoring programs (EFSA, 2016; Neff *et al*., 2012), where the average pesticides residues exceeding established MRL standards in below 2%. This may be attributed to effective pesticides control mechanisms in these countries and poor knowledge of pesticide's safe use and weak institutional frameworks on the judicious pesticide use in developing countries including Tanzania.

Continuous consumption of contaminated vegetables can lead to accumulation of toxic substances in the body causing long term health effects (Bhanti & Taneja, 2007). Organophosphate pesticides, which constitute large proportion of pesticide residues detected under the current study, would pose great threat in human health (DaSilva *et al*., 2008; Dasgupta *et al*., 2007).

Multiples residues were also evident in this study. Up to seven residues were detected in a single sample. Samples containing more than one pesticide (multiple residues) had also been reported from both developed and developing countries (Baker *et al*., 2002; Bhanti & Taneja, 2007; Chen *et al*., 2011; EFSA, 2016). Higher proportion of vegetable samples with pesticide residues along high ways can be explained by the tendency of farmers to over spray pesticides to increase shelf life and improve appearance of the produce to customers. The levels detected in highways may be a result of secondary and/or cross contamination during transportation or storage. This further indicates poor pesticides handling practices and lacking observation of the pre-harvest intervals which suggest higher concentrations of pesticides that do not easily degrade even on transition from the farms to the markets. Failure to observe the pre-harvesting intervals and injudicious of pesticides may account for this as well (Ngowi *et al*., 2006).

These levels of pesticide residues in the sampled vegetable crops are likely to have a long-term effect on the health to consumers, hence immediate actions are required to mitigate the long-term effects of these chemicals. Presence of traces of Sulphur and highly hazardous chemicals in food vegetable samples suggest contamination with pesticides used in cashew nuts, roses and other non-vegetable crops. Likewise, detection of some traces of nicotine signifies unhygienic food handling during harvesting, sorting and at the point of sell.

More than 95.2% of all vegetable samples had organophosphate residues. This is in agreement with other studies that found most common chemical family of pesticides in vegetables are organophosphates (Darko & Akoto, 2008; Lekei *et al*., 2014; William, 2008) unlike Brazil where only 30.8% of all pesticides residues were organophosphates (Jardim & Caldas, 2012). This indicates that almost all that goes in during production (pesticide spraying) comes out as pesticide residues after harvests. This variation might have been results of persistent use of organophosphate pesticides that accumulate in the environment over a good period of time. Organochlorine pesticides were also detected in quantifiable levels, but these pesticides had been banned for agricultural use in Tanzania. Similar residues were also reported in the US, where the use organochlorine pesticides had been banned many years ago. Their presence in food samples suggests some illegal business and/or their persistence in the food chain systems (Baker *et al*., 2002). High proportion (74.2%) of vegetables had concentrations of pesticide residues above the MRLs. This proportion is far above those reported in the EU countries, where 97.4% of the tested food samples fell within the legal limits (EFSA, 2016) Egypt where 81.5% did not have detectable levels of pesticides residues (El-Nahhal, 2004) Brazil where only 3% exceeded MRLs (Jardim & Caldas, 2012) and Pakistan where only 3% exceeded MRLs (Latif *et al*., 2011b). Vegetables produced under smallholder vegetable production poses high public health risks and increased risks of detrimental health effects among consumers as well as farmers who are primary consumers of these vegetables.

The current prevalence of pesticide contamination (47.5%) was lower in this study than the previously reported prevalence of pesticide residues (95.8%) from Dar es Salaam markets (Mahugija *et al*., 2017), where only tomatoes and watermelons samples were analyzed. From this current study, 73.7% of all vegetable samples from Dar es Salaam had detectable levels of pesticide residues. High levels of pesticides in Dar es Salaam can be explained by multiple sourcing of vegetables and direct marketing on the highways which recorded highest levels of pesticide residues. Also, high quantities of vegetables are shipped to the city to curb the high demand due to increased human population.

Generally, tomatoes, onion, sweet pepper, water melons, cabbage and Chinese cabbage recorded high concentration levels of pesticide residues. Consequently, they recorded high excess levels of pesticide residues over a default codex maximum pesticide residue limit of 0.01 mg/Kg of food sample. Similar findings were reported Ghana (Darko & Akoto, 2008) though only small proportion of tomatoes and pepper had levels above MLRs. These levels are much higher than those reported in China (Wu *et al*., 2017) and elsewhere (Chen *et al*., 2011; EFSA, 2016; Neff *et al*., 2012), indicating a significant challenge in sustaining smallholder vegetable production, pesticides safe use and management as well as the quality of pesticides used and supplied in the local market.

Among all pesticides, residues detected, tetramethrin, pirimiphos-methyl, permethrin, endosulfan (beta), and carbaryl recorded high mean concentration in vegetable samples. Other pesticide residues with higher means concentrations included profenofos, bioallethrin, acephate, toxaphene, cyhalothrine (lambda) and trichlorform. Concentration levels detected in this study are much higher as compared with previous studies elsewhere (Bai *et al*., 2006; Chen *et al*., 2011). Similar pesticides detected in vegetables had been reported to be used in smallholder vegetable production systems in Tanzania (Kiwango *et al*., 2017; Lekei *et al*., 2014; Mtashobya & Nyambo, 2014; Ngowi *et al*., 2006; Nonga, Mdegela, Lie, Sandvik & Skaare, 2011), signifying that dietary exposure risks are emanating from pesticides used by smallholder farmers. Higher concentration of these residues may therefore be explained by poor use of pesticides, limited access to pesticides safe use extension education and lack of adherence to pre-harvest intervals (Lekei *et al*., 2014; Ngowi *et al*., 2006; William *et al*., 2006).

The fate of pesticides use in smallholder vegetable production is the culmination of serious health and environmental implications. Pesticides use in controlling crop pests and diseases subjects the general population to pesticides environmental exposure. Significant low levels of AChE among control groups and high levels of pesticides residues in fresh vegetables signify unsustainable smallholder vegetable production systems. Low level of knowledge on pesticide usage and high frequency of pesticide application implies that both human and environmental exposure to pesticides is a serious matter of concern as observed elsewhere (Damalas & Eleftherohorinos, 2011). Locally consumed vegetables are therefore contaminated with pesticides residues, which pose a critical food safety challenge. This further has a negative consequence on the development and commercialization of smallholder vegetable subsector.

4.2.4 Bacterial contamination

Most fresh vegetables produced by smallholder farmers have been contaminated with fecal and other contaminants. A considerable high proportion (63.2%) of samples tested was contaminated with at least one bacterial pathogen. This may be accounted for by possible contamination of water used for watering/irrigation and washing vegetables. Unhygienic market environments in the study area as well as direct movement of pathogenic bacteria from farm to market since some pesticides are presumed support the growth of pathogenic bacteria (Baishya & Sharma, 2014; Ng *et al.*, 2005). This prevalence is higher than that reported in Ethiopia where 48.7% were positive for bacterial contamination (Alemu, Mama & Siraj, 2018).

Pathogenic bacteria contaminants isolated from fresh vegetables included *E. coli* and *Salmonella spp.* These findings are different from the study in Brazil which did not find *Salmonella spp.* in vegetable samples (Maffei, Silveira & Catanozi, 2013). The presence of these microorganisms in fresh vegetables provides critical economic and public health concern (Maistro, Miya, Sant'Ana & Pereira, 2012) that calls for immediate attention along the value chain and establish critical points to prevent such contaminants.

Moreover, *Pseudomonas aeruginosa, Citrobacter, Enterobacter and Klebsiella oxytoca* were also among the identified microbial species in tested vegetables. Among these six identified bacterial species, *Enterobacter* (55.6%) was the commonest contaminant contrary to the previous study in Ethiopia where *E. coli* (31.4%) was the commonest contaminant (Alemu *et al.*, 2018). The prevalence of salmonella (7.7%) is much lower than that reported (24%) from Ethiopia (Weldezgina & Muleta, 2016). On the other hand, the prevalence of *Escherichia coli* (28.2%) was also lower than those reported in 53.1% samples from Brazil (DeOliveira, DeSouza, Bergamini, & DeMartinis, 2011). These pathogens (*E. coli* and *Salmonella spp.*) had been isolated from the vegetable vendors (Mensah, Mwamakamba, Mohamed, & Nsue-Milang, 2012) hence these findings support hypothesis that fresh vegetable vendors are potential sources of pathogenic *Salmonella* and *E. coli*. Likewise, market-related handling, especially where provision for better sanitary standards are inadequate are also reported to be the main source of contamination (Amoah *et al.*, 2006).

These bacterial contaminants are also commonly isolated from fecal and urine samples responsible for a wide range of gastrointestinal disorders (Abdallah *et al.*, 2014), further indicating that smallholder fresh vegetables contains faecal contaminants, which pose high public health concern among consumers. Higher prevalence of *Enterobacter* and detection of *salmonella* from market places may be due to skin contact from customers touching vegetables and environmental contamination (Weldezgina & Muleta, 2016). *E coli* are the major cause of diarrhoea and urinary tract infections, including prostatitis and pyelonephritis. These pathogens cause a range of illnesses, including, respiratory tract, skin, soft-tissues, joints, bones, eyes and the Central Nervous System (CNS) (DuPlessis *et al*., 2015). Consumers of vegetables are therefore at risk of infection from these diseases and other foodborne diseases (FBD), including salmonellosis and cholera.

The production phase, therefore, constitutes the main contamination point of faecal contaminants of fresh vegetables because *E. coli* is a faecal coliform bacterium that is normally excreted in stool (Alemu *et al*., 2018). High risks of faecal contamination may have emanated from people reported to be entering and/or urinating/defecating in the farms. Fertilizers (animal manure), irrigation water, wild animal intrusion, insects, pesticides/fungicides, crop debris, and flooding area also potential sources of microbial contamination at production level (Park *et al*., 2012).

Bacterial isolates from fresh vegetables at production and marketing levels challenge the monitoring and inspection mechanisms employed in ensuring food safety at these points of fresh vegetables supply chain. Owing to increased daily consumption of these fresh vegetables among lower and middle-income dwellers in urban and peri-urban settings where locally produced vegetables are consumed, the fresh vegetable supply chain prompts high public health concern.

4.2.5 Co-exposure risks and Co-contamination of fresh vegetables with pesticides residues and pathogenic bacteria contaminants

Vegetables produced by smallholder farmers had been co-contaminated with pesticide residues and pathogenic bacteria. Pearson correlation test showed a positive, non-statistically significant correlation (r = 0.103, p = 0.2) between the levels of pesticide and bacterial contamination. Few studies on co-contamination of fresh vegetables had previously reported (Amoah *et al*., 2006; Santarelli *et al*., 2018). However, these studies did not provide levels of co-occurrence proportions of pesticides and bacterial contaminants. From the present study, a considerable proportion (46.4%) of fresh vegetables contained both pesticide residues and bacterial contaminants with vegetables from farms (60.7%) being highly contaminated. Statistically significant difference (p = 0.010) in co-contamination of vegetables from farms and markets indicates high health risks to farmers who are both producers and primary consumers of fresh vegetables. There was a statistically significant different among the vegetable samples (p = 0.02) with kale, onions, Ethiopian mustard, nightshade and Chinese cabbage being highly contaminated with both pesticides and pathogenic bacteria which further signifies major public health concerns.

Binary logistic regression analysis showed the association between pesticide residues and bacterial contamination of vegetables. Pesticides residues were more likely to induce bacterial contamination. Excessive pesticides reported among smallholder farmers in Tanzania (Ngowi *et al*., 2006) may account for this increased microbial contamination because pesticides had been found to support the survival and growth of *Pseudomonas*, and *Escherichia coli* (Ng *et al*., 2005). This is in support of the hypothesis that pesticide chemical composition can act as stimulatory substrate for microbial growth (Ng *et al*., 2005). Likewise, pesticide solutions sprayed on agricultural crops in controlling pests and insects, mostly with organophosphates and carbamates as the active ingredients had been reported to provide suitable environment for the survival and growth of human pathogenic microbes, including *E. coli* and *Salmonella* (DuPlessis *et al*., 2015).

Unhygienic handling of vegetables, such as the use of the same wiping cloth/towel in cleaning fresh vegetables increased the likelihood of contaminating fresh fruits and vegetables with both pesticides and bacterial contaminants. This is in line with WHO report that farmers and farm workers may be sources or vehicles for contamination of produce in the growing field as foodborne outbreaks have been attributed to poor hygiene practices of food handlers (WHO, 2008). This current study shows that water used in irrigation, storage practices, attending pesticides safe use and hygienic handling of vegetables, and splashing water to freshen vegetables did not significantly influence co-contamination of fresh vegetables. Therefore, pesticide used in smallholder vegetable production may be perceived as the major source of microbial contamination among many others. High levels of pesticides residues and biological contamination of fresh vegetables found from the analyzed samples may be explained by increased use of pesticides.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

This study highlights evidence of occupational exposure and pesticide residue levels in vegetables. Moreover, it provides nature and determinants of exposure, co-existence of pesticide residue and bacterial contamination of fresh vegetables as a basis for the improvement of pesticide use practices and monitoring. Evidence of poor pesticide uses, mixing practices and increased spraying frequencies and dosage, especially among tomato and onion farmers, have a critical implication on environmental and public health. Locally consumed vegetables are highly contaminated with pesticides residues. Contamination of fresh vegetables with pesticides is therefore a direct result of excessive use of pesticides.

Organophosphate, carbamate and a combination of different pesticides formulations were evidently used and consequently detected in vegetable samples. Over Sixty (60) different pesticides formulations were used by smallholder vegetable farmers. Consequently, fifty-two (52) different types of pesticides residues were detected from all vegetable samples, with those from highway selling points having more pesticides residues compared with samples from farms and markets. The levels of pesticides residues detected were above Codex MRL standards, with banned organochlorines and other highly toxic pesticides were detected in quantifiable levels. These findings indicate a combined health risk of exposure to pesticides and pathogenic microbes which has a huge public health concern, as huge volumes of pesticides currently used end up as pesticides residues in locally consumed vegetables, thereby increasing microbial activities.

Smallholder vegetable farmers are occupationally exposed to mixtures of pesticides. The significant finding from this study on decreased levels of AChE in exposed farmers compared with the control group of unexposed individuals signifies health effects of pesticides exposure. Poor pesticide use, inefficient use of PPEs, improper observation of re-entry and pre-harvest intervals, gender, nutritional status and age of farmers had significant influence on increased risks of pesticides exposure. Remarkable decrease in AChE levels among the control group indicates presence of pesticides in the environment, food chain and water. Increased numbers of exposure symptoms of exposure among non-farming (Control group) showed that the general population was at risk of pesticides exposure.

The presence of pesticide residues in fresh vegetables influenced bacterial contamination, signifying the effects of pesticide use on bacterial contamination of fresh vegetables. A significant association was found between pesticide residues and bacterial contamination. Fresh vegetables locally produced and consumed have traces of faecal and environmental contamination. *Pseudomonas aeruginosa, citrobacter, E. coli, Enterobacter, Klebsiella oxytoca* and Salmonella were isolated from fresh vegetables.

There is weak regulatory enforcement of food safety regulations both at the production and marketing levels. Equally, there are no food safety standards targeting fresh vegetables both at the production and marketing levels. The study revealed excessive use, pesticides misuse and malpractices in onion and tomato production which also poses both health and environmental risks of pesticides exposure. Food safety risks of both chemical and biological contamination are higher in fresh vegetables in markets compared with farms, respectively. High levels of pesticides residues and biological contaminants in fresh vegetables may be explained by increased use of pesticides, lack of food safety standards both at production and marketing levels.

5.2 Recommendations

Since pesticide application will continue to be an important aspect of smallholder vegetable production in low income countries like Tanzania in controlling pests and diseases, strict guidelines (policy) on how these pesticides should be distributed, sold to farmers, used and disposed need to be enforced at all levels. Educating farmers and promoting safe use of pesticides and introduction of greener pesticides in smallholder vegetable production systems is vital. Investing in the green chemistry and utilizing the advancement of nanotechnology in the production of greener pesticides as the sustainable means of managing food safety, human and environmental exposure to pesticides is required.

Mandatory pesticides safe use training offered by TPRI to pesticides dealers should be enhanced and offered at technician (certificate/diploma) level to develop competent skills on pesticides safe use. There is also an urgent need for developing pesticides monitoring and surveillance systems, to monitor and control pesticides use, handling and management at farmer level, to address pesticides exposure.

This study recommends the restriction of pesticides classified as Highly Hazardous Pesticides (HHP) in smallholder vegetable production to control both dietary and occupational exposure to pesticides. There is also a need to develop a comprehensive sustainable vegetable production and food safety control program in addressing critical challenges issues unveiled in the study. Further studies on the antibiotic resistance of identified bacterial strains and determination of effects of pesticide mixtures on bacterial growth are also warranted.

There is an urgent need to establish National Pesticides Risk Based Control Programmes in which food produce with a high prevalence of residues exceeding the legal limit should be listed and included in the control programmes. Policy on regular monitoring of pesticides exposure and residues is vital. Inclusion of pesticides exposure in national health and epidemiological surveys to consider pesticides exposure as a public health concern among the farming population will raise public awareness of pesticides exposure. Studies on genotoxic effects of pesticides exposure should be intensively undertaken. Likewise, long-term environmental effects including the degradation of biological capital of vegetable ecosystems due to pesticides use and its impact on food quality need to be studied extensively.

Further study on genotoxixity effects of herein studied pesticides to smallholder farmers is also warranted.

5.3 Limitation of the study

The study focused exposure to mixtures and organophosphorous and carbamate pesticides which exhibit toxicity by inhibiting AChE levels of exposed individuals. Other possible modulators on AChE inhibition, including lifestyles factors such as smoking, exposure to X-rays, eating habits, inter and intra-individual variations, weather and geographical could have affected the results. Likewise, there are several non-pesticides Ach modulators (agonists and antagonists) which could also affect AChE levels. Matching cases and controls in comparative studies (case-control), to critically conclude that any differences observed were not due to individual physiological differences is another limitation of the study. However, the effects of these limitations were minimized by a critical purposive selection of the control group with matching demographic characteristics and geographical locations, as well as adjusting for demographic and anthropometric characteristics in multivariate analysis. Further study on genotoxic effect of pesticides was not carried out due to limited funding, and this is also warranted in the future.

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APPENDICES

Appendix 1: Pesticides sprayed in tomato

Pesticides sprayed in tomato (N=301)

Variable		n	%
Pesticides sprayed on tomato	Belt 480 SC	136	45.2
	Wiltigo Plus 50EC	73	24.3
	Milthan Super 800WP	67	22.3
	Wilcron 720EC	65	21.6
	Dudu-all 450EC	60	19.9
	Farmerzeb 800WP	57	18.9
	Supercron 500 EC	55	18.3
	Selecron 720EC	41	13.6
	Abamectin 18EC	40	13.3
	Ridomil Gold MZ 68WG	39	13.0
	Ebony (Mancozeb) 80WP	37	12.3
	Profecron 720 EC	37	12.3
	Tarantula 1.8EC	35	11.6
	Ivory 80WP	35	11.6
	Amsac 14.5%SC	34	11.3
	Dudumectin 11.2% EC	32	10.6
	Victory 72WP	26	8.6
	Linkonil 500EC	24	8.0
	Duduba 450EC	24	8.0
	Thionex	24	8.0
	Snowcron 500EC	23	7.6
	Dursban 24ULV	22	7.3
	Mupacron 50EC	18	6.0
	Agrocron 720EC	16	5.3
	Ninja 5EC	16	5.3
	Agrithane 80% WP	15	5.0
	Imida C 344SE	15	5.0
	Blue copper	10	3.3
	Coragen 20SC	9	3.0
	Bajuta Copper 300SC	8	2.7
	Vertigo 1.8EC (Abamectin)	7	2.3
	Sulfarm 80WP	6	2.0
	Kulumus 80%	6	2.0
	Actellic 50EC	6	2.0
	Twigaquat 200SL	6	2.0
	Karate 5EC	6	2.0
	Dume 120EW	5	1.7
	Thiovit Jet WP	5	1.7
	Tracer 480SC	5	1.7
	Amekan C 344EC	5	1.7
	Attakan C 344	5	1.7
	Cobox	4	1.3
	Mupathion 50EC	4	1.3
	Bayleton 25WP	4	1.3
	Bravo	4	1.3
	Sumithian	4	1.3
	Banophos 720EC	2	0.7
	Appolo 50EC	2	0.7
	Oshothane 80WP	2	0.7
	Runner 72WP	2	0.7
	Omex	2	0.7
	Bancofee 720SC	2	0.7
	Xantho 5EC	2	0.7
	Dithan M-45	2	0.7
	Bichlophenical	2	0.7

	KungFu 5EC	2	0.7
	Farmerfose 800WP	2	0.7
	Damka 720SC	2	0.7
	Thiodan	2	0.7
	Decis Forte 100EC	2	0.7

Multiple responses allowed

Appendix 2: Pesticides residues detected in fresh vegetables

Pesticides residues detected in fresh vegetables (N=286*)

Variable		n	%
Pesticides residues found	Oxyfluorfen	18	12.2
	Cyhalothrine (Lambda)	15	10.1
	Profenofos	14	9.5
	Triadimenol	13	8.8
	Chlorpyrifos	12	8.1
	Cyhalothrin (Gamma)	12	8.1
	Triadimefon	12	8.1
	Pirimiphos - methyl	11	7.4
	Dieldrin	10	6.8
	Endosulfan (Beta)	9	6.1
	Carbofuran	9	6.1
	Trichlorform	7	4.7
	Cabaryl	7	4.7
	Sulfur	6	4.1
	Fenothiocarb	6	4.1
	Endrin aldehyde	6	4.1
	Permethri	5	3.4
	Heptenophos	5	3.4
	Vamidothion	4	2.7
	Acephate	4	2.7
	Dimethoate	4	2.7
	Bendiocarb	4	2.7
	Captafor	4	2.7
	Captain	4	2.7
	Empethrine	3	2.0
	Metalaxyl	3	2.0
	Toxaphene	3	2.0
	Nicotine	3	2.0
	Fenitrothion	2	1.4
	Malathion	2	1.4
	Fenobucarb	2	1.4
	Binopacryl	2	1.4
	Pyrethrin	2	1.4
	Prallethrin	2	1.4
	Dinoseb acetate	2	1.4
	Hexaconazole	1	0.7
	Fenthion	1	0.7
	Prarethrin	1	0.7
	Theobromine	1	0.7
	Cypermethrin	1	0.7
	Promocarb	1	0.7
	Propamocarb	1	0.7
	Spiroxamine	1	0.7
	Dinocarb	1	0.7
	Triazamate	1	0.7
	Tetramethrin	1	0.7
	Flumetralin	1	0.7
	Quinoclamine	1	0.7
	Barban	1	0.7
	Chlorothalonil	1	0.7
	Bioallethrin	1	0.7

	Cinerin	1	0.7
	Oxamyl	1	0.7
	Methiocarb	1	0.7
	Anilazine	1	0.7
Total		148	166.9

Multiple response allowed

RESEARCH OUTPUT

Review article: Pesticide exposure and genotoxic effects as measured by DNA damage and human monitoring biomarkers. *International Journal of Environmental Health Research*. https://doi.org/10.1080/09603123.2019.1690132b

Research article: Biomonitoring of acetylcholinesterase (AChE) activity among smallholder vegetable farmers occupationally exposed to mixtures of pesticides in Tanzania. *Journal of Environmental and Public Health*. https://doi.org/10.1155/2019/3084501

Research article: Co-exposure risks of pesticides residues and bacterial contamination in fresh fruits and vegetables under smallholder horticultural production systems in Tanzania. *PLoS ONE*. https://doi.org/10.1371/journal.pone.0235345

Research manuscript: Changing patterns and drivers of increased pesticides use in smallholder vegetable production systems in Tanzania.