ASSESSMENT OF PESTICIDE RESIDUES IN HARVESTED TOMATO FRUITS AT MAKAMBAKO TOWN COUNCIL IN NJOMBE REGION, TANZANIA

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A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN APPLIED TOXICOLOGY OF SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA.

2021

ABSTRACT

In modern agriculture, pest infestation has posed a great challenge to farmers. The use of pesticide has eventually become an important tool to ensure quality and quantity of crop yields. However, such chemicals might have residual impacts to consumers. This study was conducted to assess the extent of pesticide residues in harvested tomato fruits at Makambako Town Council in Njombe region, Tanzania. Specifically the study aimed at: (i) investigating adherence to recommended manufacturer's instructions on pesticide application among tomato farmers (ii) determining level and frequency of occurrence of selected pesticide residues in tomato fruits and (iii) characterizing dietary risks associated with consumption of fresh tomato fruits containing pesticide residues. Forty seven (47) farmers were interviewed on pesticide types and their application using structured questionnaire. Forty two (42) fresh tomato fruits for laboratory analysis were sampled from interviewed farmers who were at harvesting season. QuEChERS (CEN) Method 15662.5 was employed for pesticide extraction and analyzed by Gas Chromatography Mass Spectrometer. It was found that all respondents mixed more than one pesticide in a single spray tank without adhering to recommended mixing procedures. The average withholding period was 5 days, below the recommended 7 days for mixture of mancozeb and metalaxyl which were commonly used fungicides at the study area. Eighty three percent of the respondents exceeded the mixing concentration of pesticide above the recommended mixing ratios. Residues of chlorpyrifos, profenofos, gamma cyhalothrin and cypermethrin were alternatingly detected in 78.51 % of analyzed samples. The average concentrations of residues were 0.014, 0.056, 0.003 and 0.2 mg/kg for chlorpyrifos, profenofos, gamma cyhalothrin and cypermethrin, and were all below Codex MRLs of 1, 10, 0.3 and 0.2 mg/kg respectively, as per FAO/WHO guidelines. The maximum residue concentration was 0.718 mg/kg for cypermethrin, which was above the Codex MRL of 0.2 mg/kg. Profenofos was the most frequently detected pesticide, occurring in 60 % of samples. The hazard indices for the selected pesticide indicate no potential health hazards to general population due to lifetime consumption of fresh tomato fruits from the study area. The study recommend regular training to farmers on good agricultural practices through extension officers and pesticide regulatory authority. Further research on pesticide residues and dietary risk assessment is recommended for other pesticide commonly used at the study area.

DECLARATION

I, Jansen Stanslaus Bilaro, do hereby declare to the senate of Sokoine University of Agriculture that this Dissertation is my own original work done within the period of registration and that it has neither been submitted before nor being concurrently submitted to any other institution.

__________________________ ____________________

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The above declaration is confirmed by;

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Dr. Benigni Temba Date

(Supervisor)

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Dr. Silvia F. Materu Date

(Co – supervisor)

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ACKNOWLEDGEMENT

I thank our almighty God for his mercy and by blessing me with this opportunity to advance my academic career and for the life gift he gave to me.

I am so grateful to my supervisors, Dr. B.A Temba from the Department of Veterinary Physiology, Biochemistry and Pharmacology (SUA) and Dr. Silvia Materu of the Department of Biosciences (SUA) for their valuable guidance throughout the entire period of my research work.

I am sincerely indebted to the office of the Government Chemist Laboratory Authority (GCLA), the Chief Government Chemist (CGC), Board of directors and GCLA management for this opportunity given to me and for financial support of my studies. I thank the staff of Toxicology and Food Laboratories at GCLA for the support they offered to me during laboratory work.

Last but not least, I appreciate the good cooperation I received from the staff of Makambako Town Council and for permitting my study to be conducted within their administrative territory. I personally thank Town Agricultural Officers, Mr. Telesphory J. Danda and Mr. Peter Munguyampa for their support during interviews and sample collection from tomato farmers.

DEDICATION

I dedicate this work to my beloved parents, my father Jackson Bilaro and my mother Catherine Mafwimbo who raised and nurtured me. This dedication also goes to my family, my wife Dorice and our children Deon and Jessie for their prayers, encouragement and their patience throughout the period of my study.

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LIST OF ABBREVIATIONS AND SYMBOLS

ADI	Allowable Daily Intake
aHQ	Acute Hazard Quotient
ARfD	Acute Reference Dose
cHQ	Chronic Hazard Quotient
DDT	Dichlorodiphenyltrichloroethane
EDI	Estimated Daily Intake
ESTI	Estimated Short – Term Intake
FAOSTAT	Food and Agriculture Organization Corporate Statistical Database
GC MS	Gas Chromatography Mass Spectrometer
GCB	Graphitized Carbon Black
GCLA	Government Chemist Laboratory Authority
GEMS	Global Environmental Monitoring System
HI	Hazard Index
HPLC	High Performance Liquid Chromatography
HQ	Hazard Quotient
JMPR	Joint Meeting on Pesticide Residue
LC MS	Liquid Chromatography Mass Spectrometer
LOD	Limit of Detection
LOQ	Limit of Quantification
MRLs	Maximum Residue Limits
MTC	Makambako Town Council
NBS	National Bureau of Statistics
ppm	parts per million
PSA	Primary Secondary Amine
QuEChERS	Quick Easy Cheap Efficient Rugged and Safe
SAGCOT	Southern Agricultural Corridor of Tanzania
SIDO	Small Industries Development Organization
SPE	Solid Phase Extraction
SPSS	Statistical Package for Social Sciences
TPRI	Tropical Pesticide Research Institute
UCP	Universal Crop Protection
VCP	Villa Crop Protection

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

A pesticide is any substance or mixture of chemical or biological ingredients intended to repel or kill pests such as insects, rodents, fungi and unwanted plants. They also include substances used to regulate plant growth (FAO, 2014). Pesticide are extensively used in modern agriculture and are effective and economic way to improve yield quality and quantity, so as to ensure food security for the ever growing population around the globe (Chu *et al.*, 2019; Sharma *et al.*, 2019). The world pesticide use for agricultural purpose has increased from 2.3 million metric tons in the year 1990 to 4.1 million metric tons in the year 2018 (FAO, 2021). China is the leading pesticide consumer worldwide with annual average use of 1.4 million tons followed by United States at 406 684.38 tons (FAO, 2021).

According to (Sharma *et al.,* 2019), approximately 45% of the annual food production worldwide is lost due to pest infestations. Therefore, effective pest management by using wide range of pesticide is required to control pests and to increase crop production. Despite of the economic importance, the indiscriminate and extensive use of pesticide in food crops remains a challenge to environmental and public health all over the world (Jallow *et al.,* 2017). In Tanzania, small scale farmers have been mishandling and misusing pesticide without full understanding of their impact on human health and the environment (Ngowi *et al.*, 2007). The use of unregistered pesticide, inappropriate dosage, lack of adherence to pre-harvest interval, use of banned pesticide, inappropriate combination of pesticide and mixing of pesticide in a single spray is commonly practiced by Tanzania farmers (Ngowi *et al.*, 2007; Nonga *et al.*, 2011). Mixing of more than 5 pesticide in a single spray tank prior to application is well documented (Ngowi *et al.*, 2007). A study by (Kariathi *et al.*, 2016) in Meru district has reported that farmers sometimes do use higher concentrations of the pesticide in a single spray than the recommended ratios. Improper pesticide use and application practices on crops may contribute to accumulation of residues in food (Kariathi *et al.*, 2016). The left behind residues in and on fruits and other food matrices may cause direct or indirect toxic effects such as carcinogenicity, mutagenicity, teratogenicity and interference with endocrine system (Abdelbagi *et al.*, 2020).

Organophosphorus insecticides such as chlorpyrifos are acetylcholinesterase enzyme inhibitors, with the point of action being the peripheral nervous system (William Hughes, 1996). Pyrethroid insecticides such as cypermethrin cause many health hazards resulting into physiological impacts, neurotoxicity, reproductive and molecular toxicity (Eraslan *et al., 2016)*. Chronic exposure to cypermethrin may lead to the decrease in testicular and epididymal sperm count in mammals (Ahmad *et al.,* 2012). Extensive studies on the adherence to the recommended pesticide application among tomato farmers as well as ascertaining the levels of their residues in the harvest is crucial as it will provide baseline scientific data for regulatory authorities and to the public in general for decision making.

1.2 Problem statement and study justification

Presence of unacceptable high levels of pesticide residues in food matrices such as vegetables is of public concern to the producers, governments and consumers due to their potential harmful health effects (Kiwango *et al.,* 2018; Rajabu *et al.,* 2017). Worldwide the indiscriminate and overuse of pesticide have led to unacceptable high levels of pesticide residues in foods including vegetables (Kariathi *et al.,* 2017). In Tanzania, these malpractices in pesticide application has been attributed by limited knowledge of good farming practices, safety on pesticide handling, and failure to correctly interpret language on labels (Ngowi *et al.,* 2007; Nonga *et al.,* 2011). A study by (Kapeleka *et al.,* 2020) conducted in selected regions of southern highlands, northern and coastal zones in Tanzania showed that, 74.2 % of 613 vegetable samples had pesticide residues above the recommended MRLs for tetramethrin, pirimiphos, permethrin, endosulfan, carbaryl, profenofos, chlorpyrifos and dieldrin, most of which were in tomatoes, onions, watermelons, cucumbers, Chinese cabbages and sweet pepper. Although studies on assessment of pesticide use in farming and their residues in tomato fruits have been conducted in some farming areas of Tanzania (Kapeleka *et al.,* 2020; Kariathi *et al.,* 2017; Mahugija *et al.,* 2021; Ngowi *et al.,* 2007), there are no available data of the same for Makambako, a highly productive and emerging tomato farming and trading centre in the southern zone of Tanzania (MUVI-SIDO, 2009). At peak production periods during the dry seasons, the wholesale market in Makambako receives approximately 70 tons of tomato daily and 28 tons during low production in the wet season (MUVI-SIDO, 2009). Despite being one of the hotpots for tomato production and trade, the current status of pesticide application practices among farmers and pesticide residue levels in the tomato fruits at Makambako, is not well known. Therefore, this study aimed at assessing the adherence to the recommended pesticide application safety practices among farmers at

Makambako town. The study will also determine levels of residues of commonly used pesticide in tomato fruits and their associated dietary risk to consumers. It is anticipated to provide baseline information for policy makers and regulatory authorities to make decisions for the safety of the general public.

1.3 Study objectives

1.3.1 Main objective

To determine the pesticide residue levels in harvested tomato fruits at Makambako town council in Njombe region, Tanzania.

1.3.2 Specific objectives

i. To investigate adherence to recommended manufacturer's instruction on pesticide application among tomato farmers.
ii. To determine level and frequency of occurrence of selected pesticide residues in tomato fruits.
iii. To characterize dietary risks associated with consumption of fresh tomato fruits containing pesticide residues.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Tomato production and consumption

Recent data show that about 180.7 million tons of fresh tomatoes (*Lycopersicum esculentum*) are grown in an area of 5.03 million hectares worldwide, making it the second important vegetable crop next to potato (FAO, 2021). In the years 2018/19 the global tomato consumption was 38.3 million metric tons (estimated raw material equivalent) (Branthôme, 2018). About 12 % of the global tomato production in the year 2019 was contributed from African region (FAO, 2021). In Tanzania, tomatoes are grown almost across the country, significantly by small scale farmers in the southern and northern highlands, whereby an estimated annual production of 627 788 tons in the year 2019 were recorded (FAO, 2021). Apart from being an important source of income, tomato is also one of the most important edible vegetable crop in Tanzania (Illakwahhi & Srivastava, 2017).

2.2 Diseases of tomato and pesticide use in Tanzania

Tomato is one of the important economic horticultural crops and plays an important role in agricultural vegetable production and trade in the world (Liu & Wang, 2020). But like other vegetable crops, tomato is affected by various diseases and pests. The major tomato diseases include late blight, a very destructive fungal disease caused by *Phytophthora infestans*, wilt, crown and root rot caused by *Fusarium* species, and bacterial leaf spot caused by *Xanthomonas campestris* (Singh *et al.*, 2017). Major insect pests of tomato include thrips, whitefly, tomato fruit worm, leaf miner, leaf hopper, aphid, mites and mealy bug (Illakwahhi & Srivastava, 2017).

In Tanzania, many tomato farmers, including those from Makambako struggle with control of tomato leaf miner (*Tuta absoluta*) which is locally named in native swahili language as *"Kanitangaze"*. *Tuta absoluta* has become a worldwide pest of economic importance and it accounts for about 80 – 100 % production loss if left unchecked (Illakwahhi & Srivastava, 2017). Pesticide are therefore indispensable in modern agriculture and they have consistently revealed their worth through increased agricultural productivity, reduced insect-borne, endemic diseases and protection as well as restoration of plantations and forests (Grewal *et al.,* 2017; Tudi *et al.,* 2021). Without the use of pesticide, there would be a 78 % loss on fruit production, a 54 % loss on vegetable production and 32 % loss of cereal production in the world (Tudi *et al.*, 2021). Despite of the mentioned economic benefits of pesticide, it is important for farmers to adhere to their recommended application, so as to ensure that, their residues are kept within acceptable limit levels in food matrices.

2.3 Pesticide management, residues in tomato and health benefits of tomato fruits

In Tanzania, all pesticide formulations are to be registered in accordance to the Plant Protection Act (No 13) of 1997 and the Plant Protection Regulations of 1998, prior to their use. Tropical Pesticide Research Institute (TPRI) is responsible for registering and regulating all pesticide formulations that are to be used in Tanzania. Among the registered pesticide formulations for control of diseases in tomato and other vegetables are fungicides (propineb, chlorothalonil, mancozeb and metalaxyl), and insecticides (chlorpyrifos, alpha - cypermethrin, deltamethrin, lambda - cyhalothrin, profenofos, carbaryl and carbosulfan) (TPRI, 2020).

Registration and certification of such formulations is valid for five years for full pesticide registration and two years for provisional pesticide registration. The Plant Protection Act (No. 13) of 1997 also requires registrant to adhere to the code of conduct for the proper handling and use of pesticide for the purpose of protecting human health, animals, ground water and the natural environment. The FAO and WHO have developed international standards on pesticide residues in food for the purpose of protecting public health and to facilitate trade of food products across countries, these standards are administered by the Codex Alimentarius Commission (WTO, 2021). Among other things, the Codex Alimentarius commission has established the Maximum Residue Limits (MRLs) of pesticide in foods including vegetables and fruiting vegetables such as tomatoes, okra, eggplant, chilli pepper, bell peppers, paprika and strawberry tomato (FAO/WHO, 2021).

The Codex has 189 members made of 188 member states and 1 member organization (the European Union) and Tanzania has been a member state since 1972. It is a requirement by the Codex that, no any food listed under the commission shall contain more than the established MRL or EMRL (in mg/kg) of the pesticide residue at the point of entry into another country or at the point of entry into trade channels within a country (FAO/ WHO, 2021).

Although there are well established national and international pesticide regulatory requirements for controlling pesticide residues in food products, several studies have shown presence of higher levels of pesticide residues in tomato which exceeded the recommended MRLs. For instance, a study by Atuhaire *et al.* (2017) have reported higher levels of mancozeb above MRL of 2 mg/kg in 47.4 % and 14 % of Ugandan farm and market tomato samples, respectively. In Ghana, analysis of organophosphate residues in tomato indicated that, chlorpyrifos had extreme residue level of 10.76 mg/kg, above the MRL of 0.5 mg/kg (Essumang *et al.,* 2017). A study conducted in Dar es Salaam, Tanzania by Mahugija *et al.* (2017) revealed that, chlorpyrifos represented the highest concentrations in marketed tomato samples, with concentrations up to 2340 $\pm$ 60 ng/g which were 1.1 to 4.68 times greater than the MRL of 500 ng/g set by FAO and WHO. Assessing safety of fruit vegetables, such as tomatoes is of public health importance as they form part of many food recipes and tomatoes contain antioxidant substances which play important role of neutralizing free radicals associated with a number of degenerative diseases and conditions in human (Raiola *et al.,* 2014; Yazdizadeh Shotorbani *et al.,* 2013). Epidemiological studies have found that, the observed health effects of tomato are due to the presence of different antioxidant molecules such as carotenoids particularly lycopene, ascorbic acid, vitamin E and phenolic compounds (Frusciante *et al.,* 2007). These bioactive compounds have been associated with reduced risk of inflammatory processes, cancer and chronic non communicable diseases such as cardiovascular diseases (hypertension, coronary heart diseases) and obesity, (Raiola *et al.*, 2014).

2.4 Farmers exposure to pesticide in Tanzania and associated health effects

Pesticide in Tanzania are extensively used for various pest controls in agriculture (Lekei *et al.,* 2014). The extensive use of these chemicals suggest a high potential for direct and indirect human exposure that arise from unsafe handling and poor application practices among the artisan farmers (Kariathi *et al.,* 2016; Ngowi *et al.,* 2007; Nonga *et al.,* 2011). The inadequate knowledge on the hazardous nature of pesticide and insufficient adherence to the usage and precautionary instructions as stated on pesticide labels have been associated with increased exposure to pesticide among farmers in Tanzania (Mrema *et al.*, 2017). Acute toxicity studies have linked pesticide exposure with adverse health symptoms such as sneezing, itching, difficulties in breathing, nausea and sore eyes (Mrema *et al.,* 2017). However the major concerns for Tanzania farmers are the long-term exposures that are linked to chronic pesticide health effects such as reproductive impairments, diabetes, hypertension and cancer (Mrema *et al.,* 2017). Similarly, exposures to pesticide through contaminated food is also a concern to farmers and public given the evidence of higher levels of pesticide residues above MRLs in food matrices (Kariathi *et al.,* 2016; Mahugija *et al.,* 2021 and 2017b).

Dietary exposure from ingestion of contaminated foods like vegetables is the primary route of exposure of most pesticide and has been shown to be up to five times higher than other means of exposure like inhalation and drinking of contaminated water (Kariathi *et al.,* 2017). Chronic exposure to pesticide is associated with several birth defects, hepatotoxicity, endocrine disruption, infertility and various forms of cancer (Cecchi *et al.,* 2012; Chiu *et al.,* 2015). Health risk indexes greater than one, for chlorpyrifos, ridomil and permethrin were reported by Kariathi *et al.* (2016) in Meru district, which indicated potential health risk through life time consumption of contaminated fresh tomato fruits obtained from such areas.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Description of the study area

3.1.1 Location of the study area

Makambako town council is one of the six districts of Njombe region in Southern Tanzania. It is bordered to the north and east by Mufindi district, to the south by Njombe rural district and to the west by Wanging'ombe district. The district lies between 33˚05'E and 35˚08'E and 8˚ 08'S and 9˚08'S. The council experiences mild and sunny weather throughout the year with maximum temperature in September and October while minimum temperature occurs in between June to August. With exceptional of very few small hills, the council is relatively homogeneous with gently plains intersected by seasonally flooded valley bottom soil. Makambako town is strategically located at the junction of the Iringa to Mbeya and Iringa to Songea highways, the former also leads to Tunduma town, a Tanzania border to Zambia. According to the 2012 Tanzania National Census, the population of Makambako district was 93 827 people and the regional population was projected to grow at a rate of 2.1 annually in the year 2020 (NBS, 2021). Administratively, the Council has one division, twelve wards, fifty four streets, fourteen villages and sixty seven hamlets (MTC, 2016). Error: Reference source not found shows the location of Makambako town in Njombe region and the sampling stations.

3.1.2 Selection of the study area

Commercial farming is one among the major economic activities at Makambako. The main cash crops produced at Makambako are tomatoes and sunflower (MTC, 2016). Makambako is also one of the main tomato collection centers visited by traders from regions like Mbeya, Dodoma, Morogoro, Dar es Salaam as well as Zanzibar and neighboring countries of Zambia and Malawi (Fintrac Inc., 2019; MUVI-SIDO, 2009). Therefore it is important to assess the current tomato farmers' pesticide application practices at Makambako area, any malpractices during handling, analyze any residues in tomatoes fruits and to evaluate the associated human dietary risks.

Five wards namely; Utengule, Lyamkena, Majengo, Kivavi and Mji mwema (Error: Reference source not found) were selected for the study, based on their accessibility during rainy seasons, availability of tomato farms and tomato production being the main economic activity. A total of 14 villages were visited within the five wards based on the same criteria.

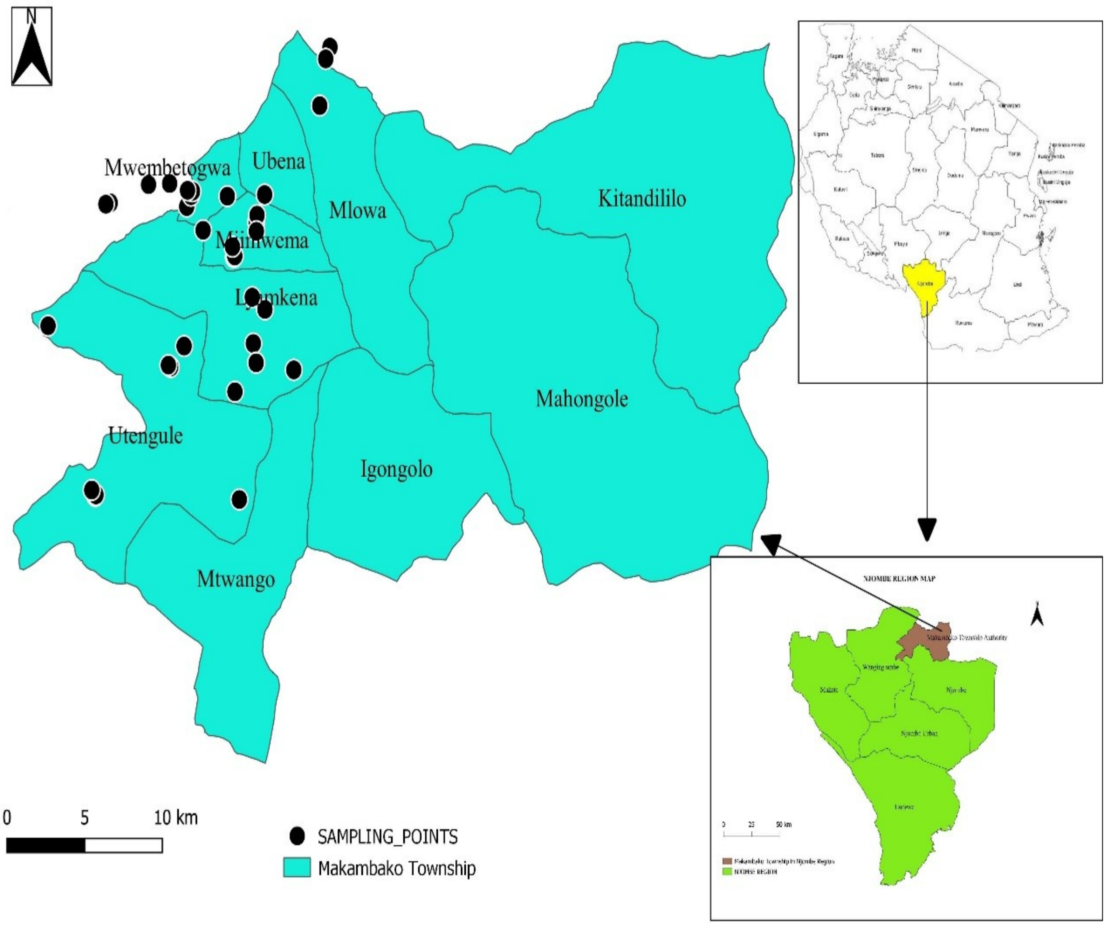

Figure 1: A drawn map of Makambako Town Council showing the study wards and sampling stations in Njombe region of Tanzania.

3.2 Collection of information on socio-demographic and pesticide application practices

Purposive random sampling technique was used to select 47 farmers for interviews with help from district agricultural officers and wards extension officers. Selection of farmers was based on their farm size (≥ 0.25 acres) and tomato farming being one of their main economic activities. A standardized questionnaire with structured and semi-structured questions was used to interview tomato farmers using Magpi+ mobile application version 15662.5 installed in iOS mobile phone. The list of questions asked are as listed in Error: Reference source not found. Face to face interviews were employed to get information on demographic characteristics and pesticide application practices from participants as shown in Error: Reference source not found.

Table 1: Type and details of information collected from farmers

Type	**Details**
Socio-demographic	Gender, age, education level
Tomato production	Farm size, type of farming, experience in tomato farming
Pesticide application practices	Types of pesticide used, frequency of spraying, pre-harvest withdrawal period, efficacy of pesticide, mixing concentration and post-harvest spraying
Consumption of own harvested tomatoes	Fresh fruits, salad or both
Market	Domestic, export

3.3 Tomato sample collection and analysis

Forty two (42) tomato samples were collected from forty two (42) selected tomato farms (1 sample per farm) during harvesting time. Each sample consisted a total of ten (10) medium-sized fresh ripe tomatoes (estimated at 1 kg) as per Codex guidelines (FAO, 1999). Sample collection was done for a period of four days, where each sample was wrapped with aluminium foil, put in polyethene bag and labeled with unique identity number. All samples were transferred to Government Chemist Laboratory Authority (GCLA) for processing on the last day of sampling.

In the laboratory, ripe tomato fruits from each sample were washed separately with tap water to mimic the normal domestic washing process. Thereafter, the tomatoes were left on a clean bench for the water to dry before chopping into small pieces using stainless steel knife and homogenized in a blender. The homogenates were transferred into lidded clean non transparent plastic containers, wrapped with aluminum foil and stored at -20˚C for five (5) days before extraction of analytes as recommended by Codex guidelines (FAO, 1999). Extraction and analysis for all 42 samples were completed within two weeks.

3.4 Preparation of standards and stock solutions

Pesticide standards of profenofos, deltamethrin, cypermethrin, gamma cyhalothrin, chloropyrifos and 4,4 DDT with purity of 95.0 %, 98.0 %, 90.0 %, 95.0 %, 98.0 % and 99.9 %, respectively were used for calibration, spiking and for determination of important parameters such as limit of detection (LOD), limit of quantification (LOQ) and percentage recovery of the method. Acetonitrile, methanol and dichloromethane were all HPLC grade with 99.9 % purity. Formic acid of 98.9 % purity was used for pH control. All standards and solvents were purchased from Anatech Analytical Technology (Nairobi), authorized local dealer for Sigma – Aldrich [Saint Louis, MO 63103 USA].

Teflon centrifuge tubes with pre-weighed buffer salts for extraction/partitioning and for dispersive – solid phase extraction (d-SPE) were obtained from Thermo scientific [197 Cardiff Valley Road, Rockwood TN 37854 USA]. Standard stock solutions of each pesticide were prepared in acetonitrile (ACN) at concentration of 1000 mg/l. A mixed standard solution was prepared at concentration of 10 mg/l from the individual stock solutions. The calibration curve for Gas Chromatograph Mass Spectrometer (GC – MS) analysis was prepared by diluting 10 mg/l of the mixed standard solution to achieve final concentrations of 0.03125, 0.0625, 0.125, 0.25, 0.5 and 1 mg/l in acetonitrile. Calibration curves and chromatograms for selected pesticide are as shown in Error: Reference source not found and Error: Reference source not found respectively. Stock and working solutions were stored at -20 ˚C until use.

3.5 Determination of pesticide residues in tomato fruits

Determination of pesticide residue levels of chlorpyrifos, profenofos, p,p-DDT, gamma cyhalothrin, cypermethrin and deltamethrin was done on forty two homogenate samples of fresh tomato fruits obtained from nine villages in five wards namely, Kivavi, Lyamkena, Majengo, Mji mwema and Utengule in Makambako Town Council. Extraction of pesticide residues was done by using QuEChERS (quick, easy, cheap, effective, rugged and safe) method (BS EN 15662, 2008).

In this method, 10 g of each tomato homogenate sample was added in 50 ml Teflon tube containing a mixture of 4g $MgSO_4$, 1g NaCl, 1 g tri-sodium citrate and 500 mg di-sodium citrate. 10 mL of acetonitrile were added into the tube, shaken vigorously by vortex for 1 minute and centrifuged for 5 minutes at 3000 relative centrifugal field (rcf). Thereafter, 6 ml of supernatant acetonitrile layer were transferred to a dispersive cleanup tube containing 150 mg of $MgSO_4$, 25 mg of primary secondary amine (PSA) and 2.5 mg graphitized carbon black (GCB) [per 1 ml of extract] to remove remaining water, organic acids, proteins and pigments.

The mixture was shaken for 30 seconds then centrifuged for 5 minutes 3000 rcf. One milliliter of supernatant layer was transferred to a 2 mls sterile amber glass vial and acidified with 10µL of 5 % formic acid in acetonitrile and injected into Gas Chromatograph-Mass Spectrometer (GC-MS).

3.6 Quality control and assurance

Quality assurance of the method was done by conducting method performance verification using routine recovery check as per SANTE/12682/2019, (EU, 2019). Pesticide – unsprayed tomato fruits obtained from green houses at the study area were used as blank control samples for recovery tests. Recovery was done by using un-spiked and spiked homogenate of blank tomato fruit samples with mix pesticide standards at five concentration levels of profenofos, deltamethrin, cypermethrin, chlorpyrifos, gamma cyhalothrin and DDT; 0.25, 0.5, 0.75, 1 and 1.25 mg/kg. Spiked samples were extracted and analyzed using similar procedures as stated in subsection 3.5 for the samples. Percentage recoveries were determined by calculations using equation (i) as described by Alam *et al.* (2015). Analysis of pesticide residues in the 42 tomato samples was done in triplicates. The quality control parameters and their values is as presented in Table 2

$$Percentage\,recovery = \frac{CE}{CM} * 100 \qquad \text{(i)}$$

Where CE is the experimental concentration from the calibration curve and CM is the spiked concentration.

Table 2: Quality control data for the method calibration of analytes
Quality control parameters

Pesticide	Retention Time (Minutes)	LOD[a]	LOQ[b]	Recovery %	R^2[c]
Chlorpyrifos	11.203	0.001	0.01	75	0.99998
Profenofos	15.505	0.005	0.01	92	0.99736
p,p-DDT	17.441	0.002	0.01	90	0.99980
γ - Cyhalothrin	20.795	0.002	0.01	80	0.99952
Cypermethrin	23.827	0.002	0.01	104	0.99731
Deltamethrin	26.167	0.003	0.01	86	0.99926

3.7 Limits of quantification and limits of detection

Limits of quantification (LOQ) and limits of detection (LOD) of the method were defined as the lowest concentration of the analytes which could be quantified with acceptable precision and accuracy and the lowest concentration of the analytes in a sample which could be detected but not necessarily quantified, respectively. The LOQ and LOD were determined by using signal-to-noise ratios (S/N) of 10:1 and 3:1 respectively, for each pesticide (Lozowicka *et al.,* 2015).

3.8 GC – MS instrument analysis conditions

Agilent 7890B Gas Chromatography system equipped with a 7000D triple quadrupole mass spectrometer was used for analysis. Both systems were equipped with Agilent 7693A auto sampler. Injection volume was 1 µL in a splitless mode. The inlet temperature was 280 °C and inlet pressure was 18.42 *psi* at constant flow mode.

Chromatographic separations were accomplished using Agilent HP-5ms Ultra Inert column of 15 m length x 0.25 mm internal diameter x 0.25 µm film thickness.

Oven temperature was initially set at 70 °C held for 1 minute then up to 180 °C at 25 ^{0}C/minute held for 3 minutes and up to 280 °C at 6 °C /minute held for 13 minutes. The helium carrier gas flow rate was 1.2 ml/minute. Transfer line and source temperature were 280 °C and 250 °C respectively. MS 1 and MS 2 quadrupole temperature was kept

[a] Limit of detection
[b] Limit of quantification
[c] Correlation coefficient

at 150 °C and ionization mode was electron impact (70 eV). Data were acquired using Multiple Reaction Monitoring (MRM) mode.

3.9 Statistical data analysis

Data from questionnaire were analyzed descriptively using Microsoft excel and Statistical Package for Social Sciences (SPSS) at significance, and confidence interval levels of 0.05 and 95 % respectively. Data on pesticide residues level were analyzed statistically using one way analysis of variance (ANOVA) at 0.05 level of significance (α = 0.05) and 95 % confidence interval for differences in pesticide residue levels between wards. Pesticide residues level data were recorded in excel and analyzed by IBM SPSS Statistics software version 23.

3.10 Dietary risk assessment

3.10.1 Exposure assessment

Dietary pesticide exposure in (mg/kg of body weight/day) were calculated using equation (ii) and equation (iv), where the values for estimated short term intake (ESTI) and estimated daily intake (EDI) were obtained, respectively.

The acute or short term hazard quotient (aHQ) were calculated according to Chu *et al.* (2019) based on estimated short term intake (ESTI) and the acute reference dose (ARfD) as shown in equation (iii). The chronic or long term hazard quotient (cHQ) were calculated based on estimated daily intake (EDI) and the acceptable daily intake (ADI) by using equation (v) (Chu et al., 2019).

$$ESTI = \frac{The\,highest\,level\,of\,residue\left(\frac{mg}{kg}\right) * Food\,consumption\left(\frac{kg}{day}\right)}{Body\,weight\,(kg)} \qquad \text{(ii)}$$

$$aHQ = \frac{ESTI}{ARfD} * 100\,\% \qquad \text{(iii)}$$

Where aHQ – is the acute hazard quotient, ESTI is the estimated short term intake and ARfD is the acute reference dose for each pesticide.

$$EDI = \frac{Mean\,level\,of\,residue\left(\frac{mg}{kg}\right) * Food\,consumption\left(\frac{kg}{day}\right)}{Body\,weight\,(kg)} \qquad \text{(iv)}$$

$$cHQ = \frac{EDI}{ADI} * 100\% \qquad \text{(v)}$$

Where EDI is the estimated daily intake, cHQ – is the chronic hazard quotient, ADI is the acceptable daily intake of each pesticide.

Tomato consumption value of 24.3 g/day/person was used as per WHO GEMS/Food cluster diets, in which Tanzania is in cluster G13 alongside Kenya, Malawi, Zimbabwe, Mali, Senegal and Sudan, (WHO, 2012). Average of 60 kg body weight for the general population was considered for calculations (Atuhaire *et al.,* 2017). The values for acute reference dose (ARfD) and allowable daily intake (ADI) for each selected pesticide were derived from FAO/WHO Joint Meeting on Pesticide Residue (JMPR), (FAO/WHO, 2012).

3.10.2 Risk characterization

Risk characterization was done by using hazard quotient (HQ) calculated from equation (v) and hazard index (HI) calculated from equation (vi). The HQ was used for assessing the potential risk due to residues by a single pesticide and the HI for mixture risk, by accounting exposure to residues from multiple pesticide with similar mechanisms of action or similar physiological effects, (Bhandari *et al.,* 2019).

$$HI = \sum_{i}^{n} cHQ_i \qquad \text{(vi)}$$

Where HI is the hazard index, $_i$ is an individual pesticide type, n is the number of pesticide with similar physiological effects and cHQ_i is the chronic hazard quotient for individual pesticide.

Hazard quotient (HQ) or hazard index (HI) value of greater than 1 indicated potential health risk for a lifetime consumption of tomatoes containing the measured residue levels of pesticide.

3.11 Limitations of the data

Since the information on pesticide application practices were obtained through face to face interviews in the presence of local agricultural officers and extension officers, this hindered the farmers to open up while responding. To overcome this, each respondent was contacted by phone to clarify and enhance his or her responses. Participant's phone numbers were obtained from the previous interview. Some farmers had difficulties in recalling brand names of some pesticide they commonly use. Therefore consultation with agricultural officers and agrochemical dealers was done to correlate the local names mentioned by the respondents with their respective brands and active ingredients.

CHAPTER FOUR

4.0 RESULTS

4.1 Respondent’s areas of residence

The description of the wards and number of respondents for the study is as shown in Error: Reference source not found.

Table 3: Information about wards, villages and number of farmers visited

Wards	Number of farmers	Percent	Villages	Number of farmers	Percent
Kivavi	11	23	Igangidun'gu	5	11
			Mashujaa	6	13
Lyamkena	15	32	Kiumba	6	13
			Lyamkena	1	2
			Malombwe	1	2
			Muungano	7	15
Majengo	2	4	Majengo	2	4
Mji Mwema	5	11	Chelesi	1	2
			Itebetala	3	6
			Soko la Mbao	1	2
Utengule	14	30	Mawande	6	13
			Utengule	6	13
			Luhota	1	2
			Ikelu	1	2
Total	**47**	**100**		**47**	**100**

4.2 Socio – demographic characteristics of respondents

Error: Reference source not found shows that all 47 respondents were adult with age above 20 years. Majority (91 %) of respondents were male and 9 % were female. Seventeen percent of interviewed tomato farmers didn’t attend formal education and 4 % had college education. Ninety six percent of interviewed farmers own less than 4 acres of tomato planted land, while only 4 % own 5 to 9 acres of tomato planted land in an outdoor rain fed or irrigated plots with 32 % of the farmers having experience of more than 15 years in the work.

Table 4: Demographic characteristics of interviewed tomato farmers

Demographic information	Frequency (n)	Percent (%)
Gender		

Female	4	9
Male	43	91
Total	**47**	**100**
Age group		
20 – 24	5	11
25 – 29	3	6
30 – 34	9	19
35 – 39	8	17
40 – 44	8	17
≥ 45	14	30
Total	**47**	**100**
Education level		
No formal education	8	17
Primary school	30	64
Secondary school	7	15
College	2	4
Total	**47**	**100**
Farm size		
Below 4 acres	45	96
5 - 9 acres	2	4
Total	**47**	**100**
Farming Type		
Outdoor rain fed and irrigation	45	96
Indoor screen house	2	4
Total	**47**	**100**
Experience in Tomato Farming		
≤ 4 years	15	32
5 - 9 years	11	23
10 - 14 years	6	13
≥ 15 years	15	32
Total	**47**	**100**

4.3 Pesticide used and their application practices

4.3.1 Types and brands of pesticide applied on tomato in the study area

Different brands of pesticide were mentioned by farmers as listed in Error: Reference source not found.

Brand name	Active ingredients	Group

Table 5: List of pesticide commonly used by tomato farmers at the study area

Category			
Fungicides	Master kinga	Mancozeb	Dithiocarbamate
		Cymoxanil	Urea
	Linkonil 720 SC	Chlorothalonil 720g/l	Organochlorine
	Linkmil 72 WP	Mancozeb 640g/kg	Dithiocarbamate
		Metalaxyl 80g/kg	Acylalanine
	Multipower Plus	Mancozeb 60 %	Dithiocarbamate
		Cymoxanil 8 %	Urea
		Dimethomorph 10g/l	Morpholine
	Chloroplus 720SC	Chlorothalonil 720g/l	Organochlorine
	Oshothane 80WP	Mancozeb 800g/kg	Dithiocarbamate
	Ivory 80WP	Mancozeb 800g/kg	Dithiocarbamate
	Ridomil gold 68WG	Metalaxyl 40g/kg	Acylalanine
		Mancozeb 640g/kg	Dithiocarbamate
	Korovil 50SC	Hexaconazole 50g/l	Triazole
	Farmazeb 80WP	Mancozeb 80 %WP	Dithiocarbamate
	Bancoffee	Chlorothalonil 70g/l	Organochlorine
	Fungoforce 72 %	Mancozeb 640g/l	Dithiocarbamate
		Metalaxyl 80g/kg	Acylalanine
	Echlonil 720SC	Chlorothalonil 720g/l	Organochlorine
Insecticides	Dudu acelamectin	Abamectin 2 %	Avermectin
		Acetamiprid 3 %	Neonicotinoid
	Snowthunder	Thiamethoxan 30g/l	Neonicotinoid
		Emamectin benzoate 10g/l	Avermectin
	Snowcron 500EC	Profenofos 500g/l	Organophosphate
	Snowmectin 1.6EC	Emamectin benzoate 16g/l	Avermectin
	Agrocron 720EC	Profenofos 720g/l	Organophosphate
	Belt 480SC	Flubendiamide 480g/l	Organofluorine
	Mupacron 50EC	Profenofos 500g/l	Organophosphate
	Sumo	Lambda cyhalothrin	Pyrethroid
	Selecron 720EC	Profenofos 720g/l	Organophosphate
	Wilcron super 250EC	Carbosulfan 250g/l	Carbamate
	Actforce gold	Chloropyrifos 48 %EC	Organophosphate
	Tarantula 1.8EC	Abamectin 18g/l	Avermectin
	Punch 1.8EC	Abamectin 18g/l	

Among the fungicides, dithiocarbamate (mancozeb) and organochlorine (chlorothalonil) were the most used groups of pesticide mentioned by 93% and 75% of interviewed farmers respectively. For control of aphids, whiteflies, thrips, leaf miner *Tuta absoluta* (Meyreck) (*Lepidoptera: Gelechiidae*) and related insects, organophosphate (profenofos) and avermectin (abamectin) insecticides were the most used groups, mentioned by 90% and 86% of respondents respectively.

None of the banned pesticide for use in agricultural crops such as DDT were mentioned by farmers during the interviews.

4.3.2 Using mixed pesticide types in a single spray tank

All 47 interviewed farmers admitted mix two to more different pesticide for application on tomato at their farms. One farmer was seen mixing two brands of pesticide with similar active ingredients in a single sprayer; Snowthunder 40SC [thiamethoxam 30g/l+Emamectin benzoate 10 g/l] and Snowmectin 1.6EC [Emamectin benzoate 16 g/l] and apply on tomato. The farmers mix different pesticide in spray tank based on their individual preferences and experience and there was no specific recipe.

4.3.3 Exceeding recommended concentration of pesticide in spray tank

All 47 farmers agreed that, there are times when they apply pesticide on tomato in their farms and they don’t effectively work against the target pest or they don’t cure the diseases on tomato as expected. Due to that challenge, 83 % of farmers opted to use higher concentration of pesticide than the one indicated on the labels while 17 % of the farmers used different brands of pesticide as an alternative option.

4.3.4 Pesticide withholding period to harvesting

The average waiting time from last day of pesticide spray to the harvesting day was approximately 5 days. The maximum waiting time among farmers was 7 days and minimum was 2 days. Thirty nine percent (39 %) of farmers observed the pesticide withholding period of less than 5 days. Seventy seven percent (77 %) of the respondents observe pesticide withholding interval of two to six days and only 23 % observed the withholding period of 7 days. Error: Reference source not found shows the summary of the average pesticide withholding periods, maximum and minimum days for each ward.

Table 6: Analysis of the average pesticide withholding period (days) across wards

Ward	Descriptive data					
	n	Mean	STDV[d]	STD_Error[e]	Minimum	Maximum

[d]Standard deviation

[e] Standard error of mean

					(days)	**(days)**
Kivavi	11	5.450	1.695	0.511	3	7
Lyamkena	15	4.800	1.424	0.368	2	7
Majengo	2	5.500	2.121	1.500	4	7
Mji Mwema	5	4.800	1.924	0.860	2	7
Utengule	14	4.710	1.590	0.425	2	7
TOTAL	47	4.960	1.574	0.230	2	7

Error: Reference source not found shows that maximum pesticide withholding period was 7 days, common for all study wards, while minimum was two days which was recorded at Utengule, Mji mwema and Lyamkena wards. At Majengo ward the minimum pesticide withholding period was 4 days, while at Kivavi 3 days were reported. However, there was no statistically significant difference in pesticide withholding period among the five wards in which the study was conducted ($p > 0.05$).

4.3.5 Frequency of pesticide spray in a season among farmers.

The frequencies of pesticide spray in an entire crop season were varying among respondents. Sixty percent of farmers were spraying pesticide on tomato between 10 to 14 times in an entire crop season, and 34 % respondents sprayed more than 15 times. Only 6 % of interviewed farmers sprayed pesticide on tomato for less than four times per season. None of the interviewed farmers agreed to be spraying pesticide on already harvested tomato fruits.

4.3.6 Consumption of fresh/raw tomato among farmers

In Tanzania, tomato fruits are consumed as fresh, cooked or processed in form of sauce, ketchup, chutney and jams (MUVI-SIDO, 2009). Ninety four percent (94 %) of respondents from study area mentioned that they consume raw/fresh tomato fruits harvested from their own farms, 55 % of whom consume fresh tomato in form of salad only and 43 % consumed both as fruit and in salads.

4.4 Pesticide residues in fresh tomato fruits

4.4.1 Pesticide residue levels in tomato samples

Seventy nine percent (79 %) of the 42 tomato fruit samples were found to contain different types of pesticide residues. Chlorpyrifos, profenofos, gamma cyhalothrin and cypermethrin were the only detected pesticide with average residue concentrations of 0.014, 0.056, 0.003 and 0.121 mg/kg respectively and were all below their respective maximum residue limits (MRLs) of 1, 10, 0.3 and 0.2 mg/kg respectively, (FAO/WHO, 2021). The lowest residue concentration detected was 0.002 mg/kg for chlorpyrifos and gamma cyhalothrin and the highest was 0.718 mg/kg for cypermethrin. Deltamethrin and p, p – DDT were not detected in any of the samples. Error: Reference source not found shows a summary of residue concentrations for the detected pesticide in 42 tomato fruit samples which were analyzed.

Table 7: Summary of the residue concentrations for the detected pesticide in tomato fruits as compared to Codex MRL (n = 42)

	Samples	Codex MRL (mg/kg)
Chlorpyrifos		
Samples detected (%)	29	
Average residue concentration (mg/kg)	0.014	1
Range (mg/kg)	0.002 - 0.029	
Samples above MRL (%)	0	
Profenofos		
Samples detected (%)	60	
Average residue concentration (mg/kg)	0.056	10
Range (mg/kg)	0.003 - 0.142	
Samples above MRL (%)	0	
Gamma cyhalothrin		
Samples detected (%)	17	
Average residue concentration (mg/kg)	0.003	0.3
Range (mg/kg)	0.002 - 0.005	
Samples above MRL (%)	0	
Cypermethrin		
Samples detected (%)	26	
Average residue concentration (mg/kg)	0.121	0.2
Range (mg/kg)	0.002 - 0.718	
Samples above MRL (%)	4.76	

Error: Reference source not found shows that profenofos was the most frequently detected pesticide with occurrence in 60 % of samples, followed by chlorpyrifos and cypermethrin at 29 % and 26 % respectively. Gamma cyhalothrin had the least frequency of occurrence, detected in only 17 % of tomato samples.

4.4.2 Average pesticide residue concentrations in tomato per ward

Error: Reference source not found shows that the minimum average residue concentrations for chlorpyrifos (0.012 mg/kg) and gamma cyhalothrin (0.002 mg/kg) were found at Kivavi ward while Lyamkena ward recorded the minimum average residue concentrations of 0.038 mg/kg and 0.004 mg/kg of profenofos and cypermethrin respectively. The maximum average residue concentrations of chlorpyrifos (0.021 mg/kg) at Majengo ward and gamma cyhalothrin (0.004 mg/kg) at Lyamkena ward were detected and both were below the Codex MRLs of 1.0 mg/kg and 0.3 mg/kg respectively. On the other hand, profenofos had the maximum average residue concentration of 0.092 mg/kg detected at Mji mwema ward, which is below the Codex MRL of 10 mg/kg. The maximum average concentration of 0.493 mg/kg for cypermethrin was quantified for tomato samples collected at Majengo ward, which is two-fold higher than the Codex MRL of 0.2 mg/kg.

Table 8: The average residue concentration in tomato per ward

	Average residue concentration (mg/kg)			
	Chlorpyrifos	**Profenofos**	**γ_Cyhalothrin**	**Cypermethrin**
MRL (mg/kg)	**1.000**	**10.000**	**0.300**	**0.200**
Ward				
Lyamkena	0.015	0.038	0.004	0.004
Mji_Mwema	0.000	0.092	0.000	0.144
Utengule	0.014	0.079	0.003	0.007
Majengo	0.021	0.041	0.000	0.493
Kivavi	0.012	0.065	0.002	0.016

The probability values (p – values) for difference between wards tested by ANOVA were 0.618, 0.910, and 0.371 for chlorpyrifos, profenofos and cypermethrin respectively. These values imply that, the mean residue concentrations for the three pesticide were not statistically different between the five wards ($p > 0.05$). However the mean residue concentrations of gamma cyhalothrin in tomato fruit samples were statistically different between the wards with ($p < 0.05$).

4.5 Dietary risk assessment and characterization of pesticide residues in tomato

Dietary risk assessment was conducted for the four pesticide which were detected and quantified from the 47 fresh tomato fruit samples obtained at the study site. The estimated short-term intake (ESTI) and estimated daily intake (EDI) values for residues of chlorpyrifos, profenofos, gamma cyhalothrin and cypermethrin in fresh tomato fruits samples from the study area were all below their respective allowable daily intake (ADI) values of 0.01, 0.03, 0.02 and 0.02 mg/kg, respectively. The other parameters used for dietary risk assessment and their values for each pesticide and groups are as shown in . The values for aHI, cHQ and HI were all below 1.

Group	Pesticide	HRL[f] (mg/kg)	ESTI[g]	ARfD[h] (mg/kg bw)	aHQ[i]	AVRL[j] (mg/kg)	ADI[k] (mg/kg bw)	EDI[l]	cHQ[m]	HI[n]
		Short - term risk				Long - term risk				
Organophosphate	Chlorpyrifos	0.029	1.17E-05	0.100	0.012	0.014	0.01	5.65E-06	0.056	0.132
	Profenofos	0.142	5.73E-05	1.000	0.006	0.056	0.03	2.26E-05	0.075	
Pyrethroid	ỿ_Cyhalothrin	0.005	2.02E-06	0.020	0.010	0.003	0.02	1.21E-06	0.006	0.250
	Cypermethrin	0.718	2.90E-04	0.040	0.724	0.121	0.02	4.88E-05	0.244	

Table 9: Dietary risk assessment of selected organophosphate and pyrethroid residues in fresh tomato fruits

[f] Highest Residue Level
[g] Estimated Short Term Intake
[h] Acute Reference Dose
[i] Acute Hazard Quotient
[j] Average Residue Level
[k] Allowable Daily Intake
[l] Estimated Daily Intake
[m] Chronic Hazard Quotient
[n] Hazard Index

CHAPTER FIVE

5.0 DISCUSSION

Pesticide use has indeed proven to be one of the key solutions in control of varieties of diseases in fruiting vegetables including tomatoes; and therefore they have benefited farmers immensely in terms of quality and quantity of yield, and economically as well.

Several types of pesticide were used by farmers in the study area. Organophosphates (such as, profenofos and chlorpyrifos), pyrethroid (such as lambda cyhalothrin), dithiocarbamates (such as mancozeb) and avermectins (such as abamectin) were the frequent mentioned groups. Other studies in Tanzania have reported usage of similar groups of pesticide in tomato farming. High usage of dithiocarbamate fungicides and pyrethroid insecticides among tomato farmers have been reported in Meru district, Arusha (Kariathi *et al.*, 2016). Organophosphates, pyrethroids and carbamates have been the mostly used pesticide groups by vegetable farmers in northern, central and southern highland regions of Tanzania (Kapeleka *et al.*, 2020; Ngowi *et al.*, 2007). High usage of insecticides such as profenofos, abamectin and chlorpyrifos at Makambako area, can largely be attributed by high prevalence of insect diseases especially tomato leaf miner disease caused by *Tuta absoluta* (Meyreck) (Lepidoptera: *Gelechiidae*).

Majority of pesticide used by tomato farmers at Makambako are clustered under class II of moderately hazardous pesticide as per WHO (2019) guidelines to pesticide classification, (WHO, 2020). Therefore, if recommended procedures of handling and usage are followed, there is low chance of causing acute or chronic health effects to human. Abamectin was the only TPRI (2020) registered pesticide used at the study area which is falling in class Ib of highly hazardous technical grade active ingredients. Hexaconazole and flubendiamide are slightly hazardous class III while mancozeb and chlorothalonil are under U – class which is unlikely to present acute hazards under normal use, (TPRI, 2020; WHO, 2020). All pesticide mentioned by farmers in the study are registered and approved by the TPRI to be used for control of tomato pests and diseases in Tanzania.

Mixing different pesticide types in a single spray tank and applying on tomato was a common practice among farmers at Makambako. There are several risks associated with tank mixing, such as reduction in biological activity due to product antagonism and possible reduction of the final crop yield. Manufacturers of such pesticide provide recommendations and guidelines on application process as shown in Error: Reference source not found. However, farmers sometimes do not follow such guidelines, instead they rely on immediate and effective pest control, thus ending up mixing several brands of pesticide in a single spray. The use of unrecommended pesticide mixtures can have serious impacts on health of consumers due to possible higher residue levels above the MRLs set for tomato fruits, and can also lead to development of pesticide resistant strains of pests and negatively impact the ecological systems (FAO, 2001).

Similar malpractices were reported in previous studies conducted in Tanzania (Kariathi *et al.,* 2016; Ngowi *et al.,* 2007). The practice of mixing pesticide such as tank mixing is routinely performed to provide practical, economic and agronomic benefits to farmers (Levine & Borgert, 2018). Besides, physical incompatibility among different pesticide can lead to blocking of sprayer’s nozzles and filters (FAO, 2001). Proper pesticide mixtures often require assessments of compatibility and efficacy against target species as well as the possibility of crop damage and ecological aspects (Levine & Borgert, 2018).

Table 10: Recommended application practices for some pesticide used at the study area

Pesticide name	Application rate	Application interval (days)	Withholding period (days)
Mancozeb 600 g/kg + metalaxyl 100 g/kg	270 – 360 g/100L	7 to 10	7
Flubendiamide 720 SC	10 – 15 ml/100L	7 to 14	1
Lambda cyhalothrin 50 EC	7.5 ml/100L	7 to 10	2
Abamectin 18 g/l	60 – 90 ml/100L	28	3
Chlorpyrifos 480 EC	150 – 200 ml/100L	7	4

Sources: (Bayer, 2021; UCP, 2017, 2019; VCP, 2020)

Information such as pesticide application rate, appliction intervals and withholding period are some of the mandatory required information to be present on pesticide labels for the purpose of protecting human health and the environment (FAO, 2014). The withholding periods in **Error: Reference source not found** are established based on dissipation properties, particularly half-life of individual pesticide applied on a given plant following the recommended application practices (Fantke *et al.,* 2014). Therefore, by observing the recommended pesticide application practices including harvesting after the established withholding period, ensures that, the residues on fruits such as tomato are kept within or below the Codex MRL for a specific pesticide (FAO, 2014).

It is clear that, some farmers at Makambako do not observe well the recommended withholding period as per pesticide manufacturer's instructions. Seventy seven percent of respondents observed withholding period of 2 to 6 days which is less than the recommended 7 days for mixture of metalaxyl and mancozeb, commonly used fungicides at the study area. Other study by Kariathi *et al.* (2016) reported 12 % of farmers who were harvesting and sell tomato without observing the recommended withholding period. In Monze district, Zambia, the withholding period observed by majority of vegetable grower including tomato was 1 day and just a few observed 3 or 7 days (Mwanja *et al.*, 2017). These improper pesticide application practices are contributed by limited knowledge among farmers and may have negative impact on human health due to unacceptable levels of pesticide residues on food (Kariathi *et al.*, 2017).

The result of 34 % respondents who were spraying pesticide more than 15 times in the entire crop season is a concern of public health importance. Ngowi *et al.* (2007) reported 15 % of 61 small scale farmers applying pesticide 16 times or more per season in northern areas of Tanzania. Vegetable growers in western Usambara and Uluguru mountains sprayed pesticide once per week and once after 2 weeks during rainy and dry seasons respectively (Mtashobya, 2017). There is clear indication of increased use of pesticide in tomato farming at Makambako and other regions in Tanzania. The repeated pesticide application in tomato farming is more likely associated with residues and exposures compared to other pesticide malpractices (Kariathi *et al.*, 2016). Furthermore, some pesticide such as abamectin and emamectin which were intensively used at the study area have threshold value in frequency of application per season as per manufacturer's recommendations. This is because any insect population may contain individuals naturally resistant to these pesticide groups. Therefore resistant individuals may ultimately dominate the population if these insecticides are used repeatedly (VCP, 2020). The recommended application frequency for abamectin in tomato is not more than 2 times in a single crop season unless the target insect pressure is very high (VCP, 2020).

Ninety four percent (94 %) of respondents from current study area were consuming raw/fresh tomato harvested from their own farms, 55 % of whom consumed fresh tomato in form of salad only and 43 % were consuming both as fruit and in salads. In Tanzania, tomato are consumed as fresh, cooked or processed in forms such as sauce, ketchup, chutney and jams (MUVI-SIDO, 2009). Exposure of pesticide through diet is assumed to be five orders of magnitude higher than other exposure sources like air and drinking water (Claeys *et al.,* 2011; Kariathi *et al.,* 2017). Raw or semi-processed fruit and vegetables are said to contain higher pesticide residue levels compared to other food groups of plant origin (Claeys *et al.,* 2011).

Seventy nine percent (79 %) of all 42 samples analyzed were found to contain different types of pesticide residues. This suggest that, the use of pesticide for control of pest is common compared to other pest control measures such as biological control, crop rotation and integrated pest management measures like the use of modified pest resistant tomato breeds. The frequencies of occurrences of pesticide in analyzed samples were varying for the selected pesticide. Analysis of laboratory results showed frequency of occurrences for profenofos, chlorpyrifos, cypermethrin and gamma cyhalothrin in 60 %, 29 %, 26 % and 17 % of analyzed samples respectively. These results are in line and comparable with previous studies conducted in Tanzania. A study in Dar es Salaam showed detection frequencies of 41.7 % and 33.3 % of chlorpyrifos and cypermethrin respectively in marketed tomato samples (Mahugija *et al.*, 2017). Chlorpyrifos, profenofos, lambda cyhalothrin (stereo isomer of gamma cyhalothrin) and cypermethrin have been detected in washed tomatoes from Iringa at frequencies of 75%, 90%, 50% and 35% respectively (Mahugija *et al.,* 2021). Also chlorpyrifos (33.3%), profenofos (42.9%) and gamma cyhalothrin (33.3%) in locally produced and consumed tomatoes in Tanzania (Kapeleka *et al.*, 2020).

For the four selected pesticide, profenofos, chlorpyrifos and gamma cyhalothrin had levels below their respective maximum residue limits. However cypermethrin residues were found to be higher than the Codex maximum residue limit in 4.67 % of samples analyzed.

A maximum concentration of cypermethrin of 0.718 mg/kg detected in this study is higher compared to a maximum of 0.1 mg/kg and 0.08 mg/kg reported by Lozowicka et al. (2015) and Quijano *et al.* (2016) respectively. Studies in other areas have detected higher levels of cypermethrin in tomato above the Codex MRL. In Dhaka, Bangladesh, a maximum of 0.55 mg/kg of cypermethrin was detected in tomato samples from local markets (Alamgir *et al.,* 2013). A concentration of 3.26 mg/kg of cypermethrin was also detected in washed tomato samples from Iringa, Tanzania by Mahugija *et al.* (2021). The higher levels of cypermethrin in the study area might be attributed by poor pesticide application practices such as shorter pre harvest period and increasing mixing concentrations of pesticide by farmers.

The average pesticide residues levels for profenofos, chlorpyrifos and gamma cyhalothrin were all below the Codex MRL for all samples and for the five wards. However an average of 0.493 mg/kg for cypermethrin at Majengo ward was two-fold higher than the Codex MRL of 0.2 mg/kg. The higher levels of cypermethrin residues in tomato sample from Majengo ward might be largely attributed by individual farmers' pesticide application practices. Although all tomato farmers at Majengo ward were observing the recommended 4 days pre harvest withdrawal period for cypermethrin sprayed in tomato, the practice of increasing mixing concentrations of pesticide in a single spray tank was common among all respondents. Cypermethrin belongs to a group of pyrethroids insecticides. Apart from their use in control of insect pests in agricultural crops, pyrethroids are at the forefront efforts to combat malaria and are also common ingredients of household insecticide and companion animal ectoparasite control products (Soderlund, 2012). Pyrethroids are potent neuropoisons and they act by inducing nerve excitation which occurs as a result of changes in nerve membrane permeabilities to sodium and potassium ions (Narahashi, 1971).

Comparison of the average residue levels for profenofos, chlorpyrifos, and cypermethrin between the five wards showed no statistical significant difference ($p>0.05$), suggesting that, there are similar application practices for the three pesticide across the study area. However the mean residue concentrations in tomato fruits for gamma cyhalothrin were statistically different between the wards with p value of 0.046. This imply that, there is no similarity in the application practices for gamma cyhalothrin pesticide accross the five wards. This is also supported by the fact that, gamma cyhalothrin residues were not detected in two of the five wards where tomato samples were obtained.

The values for the estimated short-term intake (ESTI) and estimated daily intake (EDI) obtained for chlorpyrifos, profenofos, gamma cyhalothrin and cypermethrin are lower compared with their respective allowable daily intake (ADI) in mg/kg body weight per day. This suggest that, consumers are not exposed to unacceptable higher levels of residues of the four pesticide through short-term or daily consumption of fresh tomatoes from the study area. The chronic hazard quotient (cHQ) of 0.056 for chlorpyrifos and 0.075 for profenofos obtained in this study are all below 1 meaning that, long-term consumption of fresh tomato fruits containing measured residues of the two pesticide doesn’t cause potential health risks to consumers. However these values obtained, are higher compared to those reported in Kenya for tomato among adult consumers, (Omwenga *et al.,* 2021). Similar study conducted in Tanzania, reported higher values of hazard risk indexes (HRI) for chlorpyrifos and permethrin than those obtained in this study (Kariathi *et al.,* 2016). The lower values of chronic hazard quotients and hazard indexes obtained in this study might be due to adoption of a relatively lower value of tomato consumption per person from (WHO, 2012) compared to the highest values for tomato consumption of 560 g/day (Kariathi *et al.,* 2016; Omwenga *et al.,* 2021) and 106.9 g/day (Fatunsin *et al.,* 2020).

Lifetime consumption of fresh tomato fruits from study area containing measured levels of residues for the selected pesticide pose no health risks, according to the risk assessment done by this study.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 Conclusion

This study concludes that tomato farmers at Makambako were not adhering to the recommended pesticide application instructions by manufacturers, such as mixing ratios, frequency of spray between one application and another and throughout the crop season, pre harvest withdrawal period after pesticide application as well as compatibility assessment for mixtures of pesticide prior to mixing in one tank.

The residue levels for majority of the commonly used pesticide were below the regulatory maximum residue limits. However, detection of higher levels of cypermethrin above the Codex MRL in this study, indicates the misuse of the pesticide among farmers at Makambako area.

The quantified hazard index values of 0.132 and 0.250 for the selected organophosphorus and pyrethroid pesticide respectively, indicate no potential health risk to the general population through lifetime consumption of washed fresh tomato fruits from the study area, if the WHO (2012) tomato consumption data for Tanzania and an average of 60 kg body weight are considered.

6.2 Recommendations

i. This study recommend extensive training on integrated pest management (IPM) to tomato farmers with focus on good agricultural practice (GAP) like adherence to recommended pre harvest waiting period, frequency of pesticide spraying and mixing concentration ratio. The use of varieties of pesticide formulations among tomato farmers observed at the study area and the detection of residues of different groups of pesticide in tomato fruit samples imply possible exposure to mixtures of pesticide among consumers, but the health risk is low. The dietary risk assessment approach in this study was only for the few selected pesticide used at the study area.

ii. Further dietary risk studies on residues in tomatoes for other pesticide and groups of pesticide are recommended.

iii. Furthermore the study recommend periodic and routine monitoring of pesticide residues in tomato fresh fruits from farms and in markets by pesticide regulators and food safety authorities for the matter of public health. Lastly, good food hygiene practices such as washing tomato fruits with tap water prior to consumption is highly recommended as it can minimize the risk of exposure to unacceptable higher levels of pesticide residues.

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APPENDICES

Appendix 1: Questionnaire

SOKOINE UNIVERSITY OF AGRICULTURE

COLLEGE OF VETERINARY MEDICINE AND BIOMEDICAL SCIENCES

DEPARTMENT OF PHYSIOLOGY, BIOCHEMISTRY AND PHARMACOLOGY

AN INTERVIEW SCHEDULE FOR RESEARCH ON

ASSESSMENT OF PESTICIDE RESIDUES IN HARVESTED TOMATO FRUITS. A CASE STUDY OF MAKAMBAKO TOWN COUNCIL NJOMBE, TANZANIA

BY JANSEN S. BILARO

MSC. APPLIED TOXICOLOGY

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1. PERSONAL RESPONDENT DETAILS

1.1. Full name: (Optional)____

1.2. Interview date. _____/______/___________ (DD/MM/YYY)

1.3. Phone number____

1.4. Ward __________________Village ________________________

1.5. Age interval (Years). **[Please circle your answer(s)].**

a) Below 20

b) 20 – 25

c) 25 – 30

d) 30 – 35

e) 35 – 40

f) 40 – 45

g) Above 45

1.6. Sex: Male __________Female _____________ **[Please tick √].** If college, was it agriculture or related? **YES ______NO ___ [Please tick √]**

1.7. Your highest level of education. **[Please circle your answer(s)]**

a) Primary school

b) Secondary school

c) College

2. QUESTIONS ON TOMATO FARMING AND PESTICIDE APPLICATION PRACTICES

2.1. Which kind of **tomato** farming practice do you conduct?

[**Please circle your answer(s)]**.

a) Rain dependent.

b) Controlled irrigation farming.

c) Both rain fed and irrigation.

d) Greenhouse farming.

2.2. What is the size of your farm (in hectors) in this season? **[Please mention]**

____

2.3. Do you take some of the **tomatoes** you produce for home consumption?

YES ________ NO ________ [Please tick √]

2.4. Do you consume fresh tomatoes sometimes? **YES________NO_____[Please tick √]**

2.4.1. If **YES** in which form(s). **[Please circle your answer(s)]**

a) As whole fruit

b) In salad

2.5. For how long (years) have you been engaging in **tomato** farming? **[Please mention] ___ _________________________**

2.6. Do you use pesticide in **tomato** farming?

YES ________ NO_________ [**Please tick √]**

2.6.1. If **YES**, which brands of pesticide(s) do you use [**Please mention].**

____________________ ___________________ ______

2.7. How do you mix pesticide in spray tank? **[Please circle your answer(s)]**

a) Oneself

b) With help of another person.

2.8. How many times on average do you spray pesticide on **tomatoes** from planting to harvesting [**Please mention**]________________________________

2.9. What is the recommended withdrawal period (days) from last spray to harvesting? **[Please mention]. ___________________________**

2.10. Are there times when you apply pesticide and it doesn't kill the pests?

YES ______________ NO ______________ [Please tick√]

2.10.1. If **YES**, what do you do next? **[Please circle the appropriate answer(s)]**

a) Use different pesticide(s)

b) Increase the mixing concentration(s)

c) Other solution(s). **[Please mention]_________________________**

2.11. Are there times when you mix different pesticide in a single spray tank and apply them? **YES ______________ NO_______________[Please tick √]**

2.11.1. If **YES** how often does it happen? **[Please circle the appropriate answer(s)]**

a) Always

b) Few times

c) Very rare

2.12. Do you spray **tomatoes** with pesticide after harvesting?

YES_________NO_________[Please tick √].

2.12.1. If **YES** how often does it happen? **[Please circle the appropriate answer(s)]**

a) Always

b) Few times

c) Very rare

2.13. To whom/where do you often sell much of your **tomatoes**?

[Please circle the appropriate answer(s)]

Middle men

a) Market vendors

b) Individuals from homes

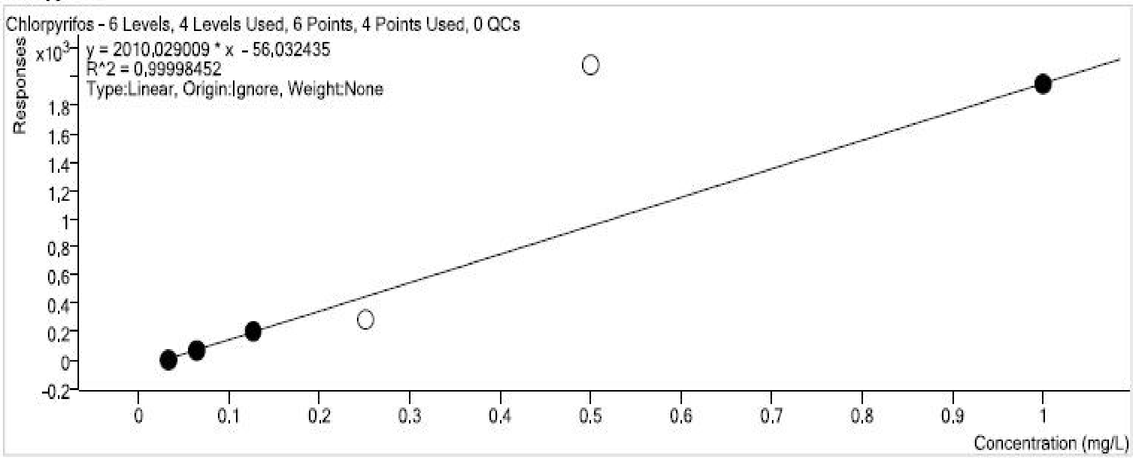
c) Tomato processing industries

d) Outside the country

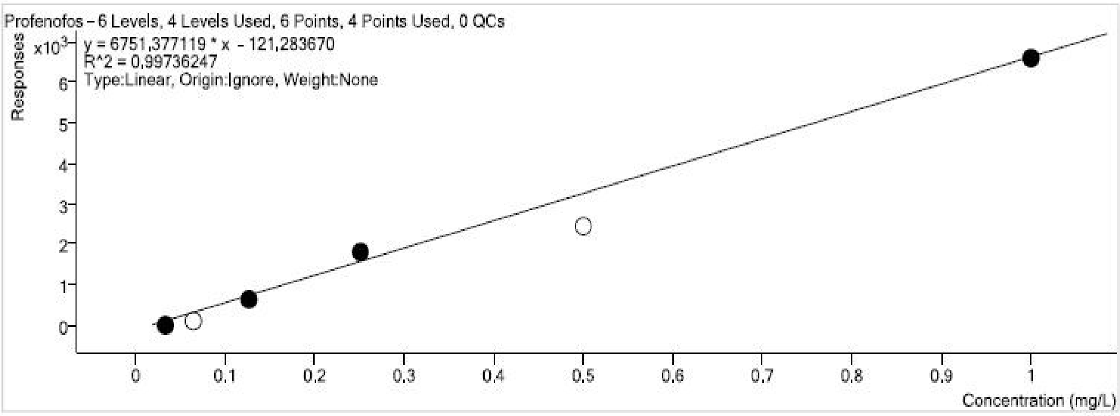
Thanks for your valuable time and responses

Appendix 2: Calibration curves

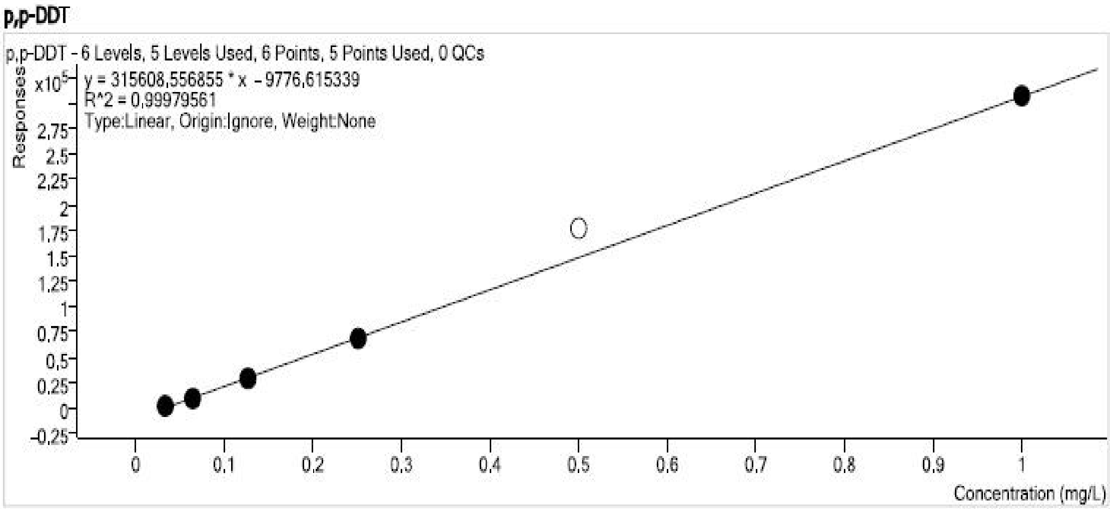
Chlorpyrifos



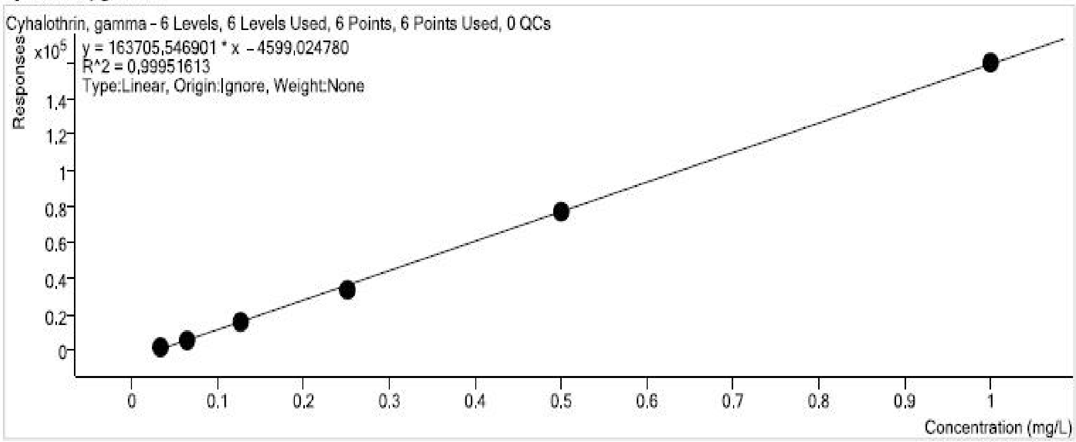
Profenofos



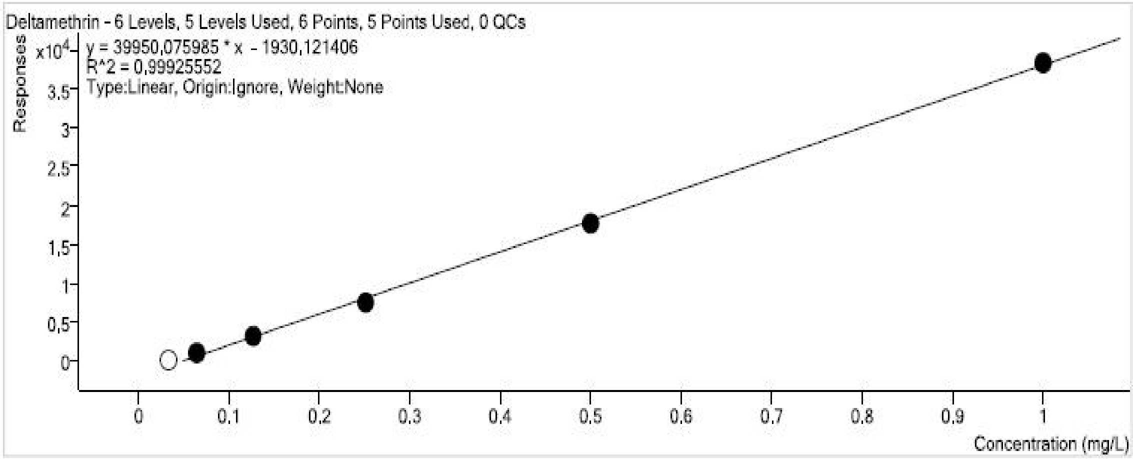
p,p-DDT



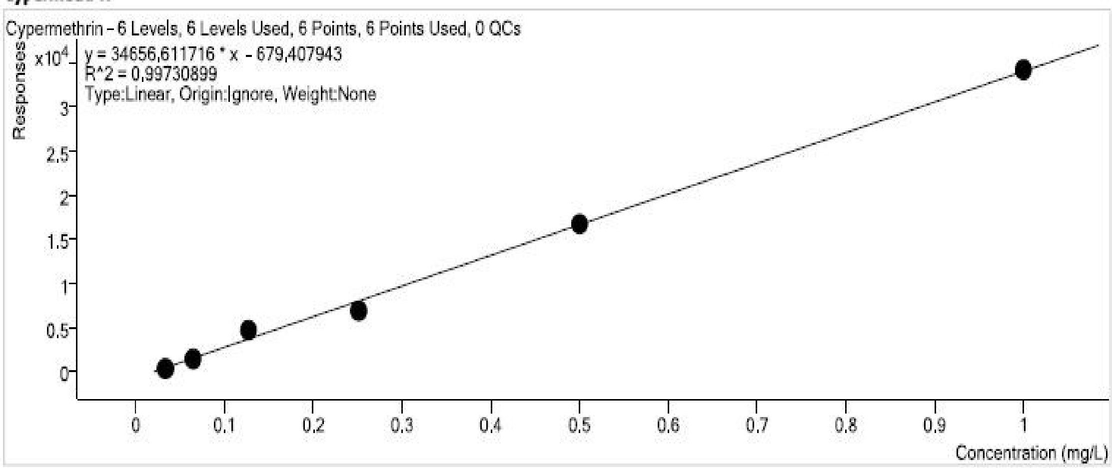
Cyhalothrin, gamma



Deltamethrin

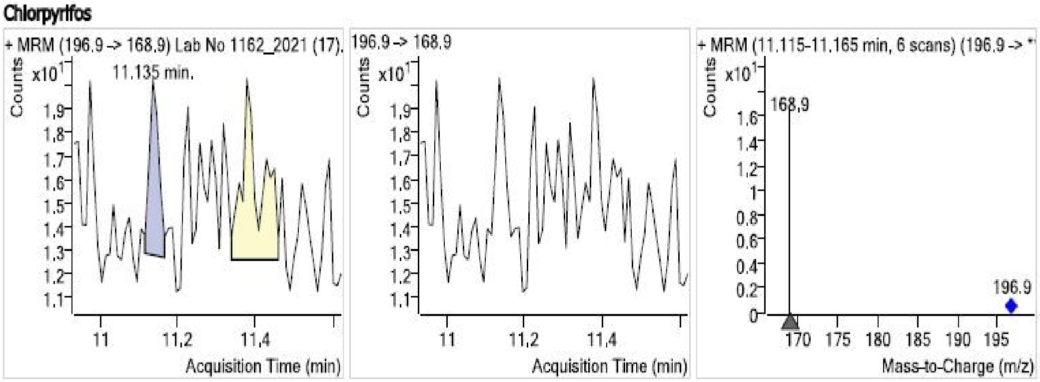


Cypermethrin

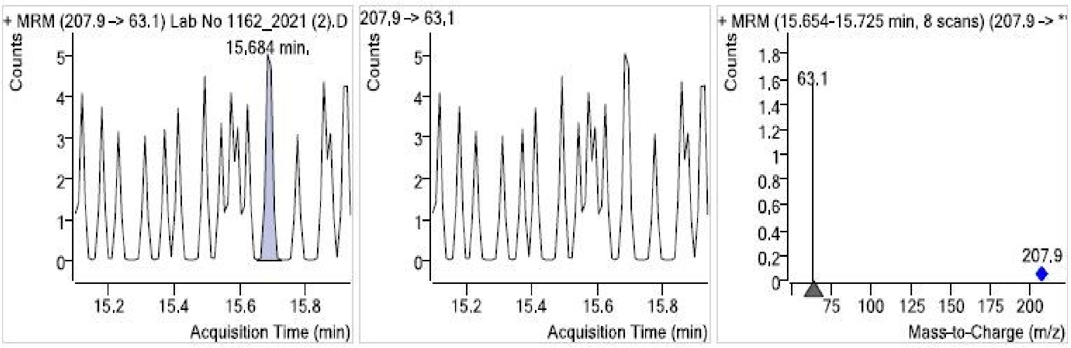


Appendix 3: Chromatograms of analytes in samples

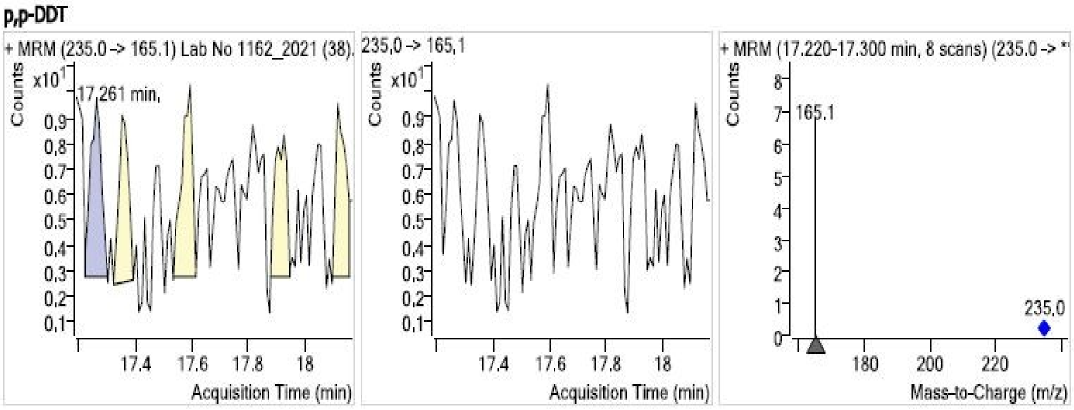
Chlorpyrifos



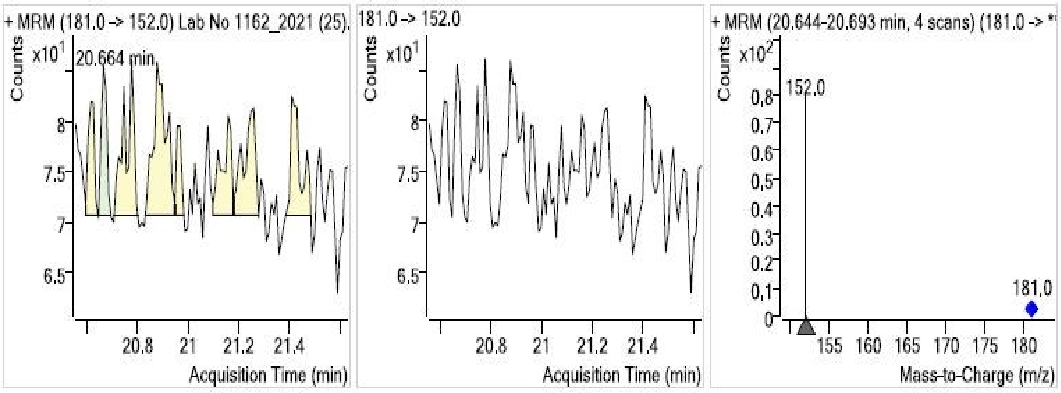
Profenofos



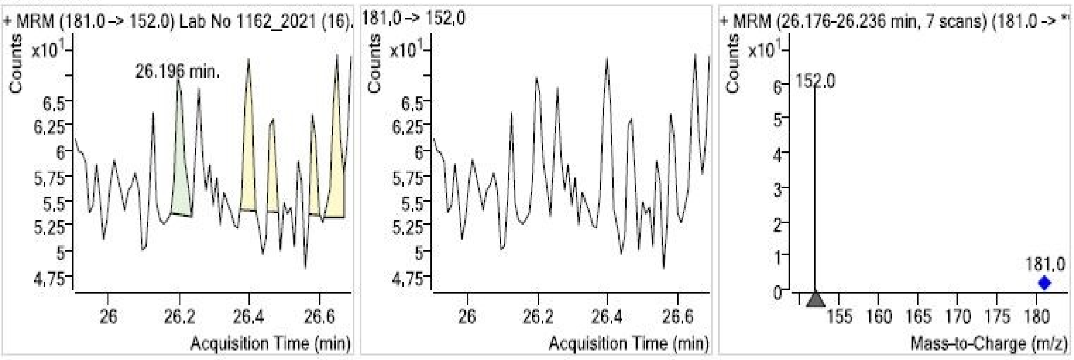
p,p-DDT



Cyhalothrin, gamma



Deltamethrin



Cypermethrin