PESTICIDE CONTAMINATION AND EFFECTS OF PEELING ON PESTICIDE RESIDUES IN TOMATOES FROM THE MARKETS. A CASE STUDY OF IRINGA MUNICIPALITY

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ABSTRACT

Tomato is the most consumed horticultural crop in the world due to its economic and nutritional benefits. The crop is very susceptible to pest infestation making the use of pesticides during production inevitable. Consumption of tomatoes contaminated with pesticide residues has been associated with both chronic and acute health risks to human. This study was aimed at assessing awareness and pesticide residues in tomatoes from selected markets in Iringa region of Tanzania together with assessing effects of peeling on reduction of pesticides. Seven pesticides (Profenofose, Chlorpyrifose, Cypermethrine, Hexaconazole, Lambda cyhalothrin, Endosulfan and Chlorothalonil) were analyzed in tomatoes by a using Gas Chromatography tandem mass-mass with time of fight (GC-MSMS-TOF) after extraction with quick, easy, cheap, effective, rugged and safe (QuEChERS) methodologies. The results showed that all sellers were aware on pesticides contamination in tomatoes and none of them applied pesticides prior to sell. Peeling tomatoes reduced pesticide contamination by 35—100% depending on the type of pesticides and the original pesticide levels before peeling. Thirty-four percent (34%) of samples didn't comply with EU MRLs on ʎ- Cyhalothrin, (28%) chloripyrifos, (25%) hexaconazole and (22%) chlorothalonil. All the samples had lower health risk index, however cumulatively continuing using tomatoes with low level of pesticides will lead to increased concentration in the system. Therefore, the proper use of pesticides is encouraged to minimize residues in the agricultural products that will reduce health risks upon their consumption.

DECLARATION

I, Ludege Yolla Dominicus do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution for a degree award.

______________________________ ____________________

Ludege Yolla Dominicus **Date**

(MSc. Food Quality and Safety Assurance)

The above declaration is confirmed;

_________________________ ____________________

Dr. Beatrice Mgaya Kilima **Date**

(Supervisor)

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DEDICATION

This work is dedicated firstly and foremost to my God, Lord Jesus Christ, then to my wife Emakulata O. Mkindi and my beloved children Emilio and Emiliana who constantly conducted their prayers for me to complete this study successfully, to my mother Magreth Kimata and the entire family of my father the late Dominicus Ludege who have always been my basis of inspiration and encouragements.

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LIST OF ABBREVIATIONS AND ACRONYMS

ADE	Average Daily Exposure
ADI	Allowable Daily Intake
EU	European Union
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization Statistics
GC	Gas Chromatography
GCLA	Government Chemist Laboratories Authority
HI	Health Index
HPLC	High Performance Liquid Chromatography
HRI	Health Risk Index
IARC	International Agency for Research on Cancer
LOD	Limit of Detection
LOQ	Limit of Quantification
MRL	Maximum Residue Limits
GC-MS/MS	Gas Chromatography Tandem Mass Spectrometry
NBS	National Bureau of Statistics
PCA	Principal Component Analysis
QuEChERS	Quick, Easy, Cheap, Effective, Rugged and Safe
TOF	Time of Flight
WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Tomato (*Solanum lycopersicum L.*) is a plant species from the Solanaceae family, which originated from the Americas (Lind*a et al*., 2016). Tomatoes are good sources of iron, phosphorus, calcium, magnesium and zinc which help the body to build and maintain bone structure and strength (Saleh*i et* al., 2019). It is an important source of vitamins A, C and contain all four major carotenoids, i.e. alpha (α) and beta (β) carotenoids, lutein and lycopene (Bhowmi*k et* al., 2012). Tomatoes can protect the body against risks of cancer such as prostate, gastric and colorectal (*Li et al*., 2021); reduce the blood glucose level, protect the eyes and the heart (Hliho*r et* al., 2019).

Tomato production in Tanzania is basically in the temperate areas including Southern and Northern highlands (Victori*a et al*., 2017). According to NBS (2012), number of household that grow tomatoes represent to about 2% of all total number of household involved in agriculture. In Tanzania Mainland, Morogoro Region had the largest area planted of tomato (9.2 %), followed by Kagera (9.0%), Tanga (8.7%), Mwanza (8.4%) and Iringa (8.4%).

There is intensive application of pesticides in tomatoes production because the crop is highly susceptible to infestation and diseases (Kiwango *et al.*, 2018). It is estimated that as much as 45% of the world’s crop is destroyed by insect pests and plant diseases (Kolan*i et al*., 2016a).

Improper use of pesticides, inappropriate dosage, lack of adherence to pre-harvest interval, use of a mix of pesticides in a single spray and generally not following good agricultural practices can result to unacceptable pesticide residues in vegetables like tomato (Kiwango *et al*.,2018; Mahugija, 2017).

The presence of chemical residue is a concern because pesticides have been associated with a wide variety of human health hazards, ranging from acute impacts such as headache, vomiting and diarrhoea to chronic impacts like cancer, reproductive harm and endocrine disruption (Santarelli *et al*., 2018; Mahugija, 2017). Risks are prominent in underdeveloped countries where around 99% of the pesticide-related diseases or deaths occur (mostly among farmers) even though these countries only account for 25% of the global pesticide use (Akte*r et* al., 2018). However a greater intake of fruits and vegetables with low-pesticide-residue was associated with a lower risk of Coronary Heart Diseases (Chi*u et al*., 2019).This study therefore aims at assessing the levels of pesticide residues in tomatoes at markets in Iringa municipality.

1.2 Problem statement and Justification

Tomato is a short duration crop and gives high yield, and susceptible to pests and diseases (Andrad*e et al*., 2011). This necessitates farmers to apply chemical pesticides in order to protect crops from damage and losses. Improper applications of chemical pesticides result to pesticide residues in the produce (Mahugija, 2017).

Pesticide residues in food pose a serious risk to consumers'. Infants; children and adults can be exposed to these pesticides by consuming contaminated food produce (Bhanda*ri et* al., 2019). For example, the maximum acceptable amount of profenofos pesticide residue in tomatoes is 10 mg/kg; above 10 mg/kg is risk to the health of consumers. In recent years studies have proved that organophosphate pesticides are mutagenic, carcinogenic, cytotoxic, genotoxic, teratogenic and immune-toxic (Ki*m et al*., 2017). In some case, it has been suggested that diseases such as acute neurological toxicity, neuro development impairment, cancer, allerges and reproductive disorders may be related to pesticides exposure (Qin *et al.*, 2015).

Studies conducted in countries such as Togo, Nigeria, Kenya and Zambia the samples of Vegetable tested for the presence of pesticides were reported to be contaminated with pesticides and some of samples had high levels of pesticide residues (Kolan*i et al*., 2016b, Mwanj*a et* al., 2017, Inond*a et al*., 2015). Iringa Municipality hosts markets where by tomatoes are among the agricultural products sold in the markets. Tomatoes come from different areas of Iringa where the use of pesticides is a common practice. In spite of the risks associated with pesticides, up to date limited studies have been conducted on the levels of pesticide residues in tomatoes from the markets in Iringa municipality.

The status of pesticides pollution in Tanzania shows that little studies have been conducted on pesticide in Iringa (Elibariki and Maguta, 2017). According to Mtashobya and Nyambo (2014) the levels of pesticide residues in tomatoes from tomato growers in Kilolo district reported high levels of endosulfan residues. Also, a study on levels of pesticides residues in vegetables from Dar es Salaam markets showed that the levels of pesticides were above the Maximum Residue Limits (MRL) (Mahugija, 2017). So there is a need to conduct the study to assess the levels of pesticide residues in tomatoes in order to a certain the quality and safety to consumers. Assessment of pesticide residues in tomatoes from these markets could give the reflection of the contaminations in the ready to eat products in the market.

1.3 Objectives

1.3.1 General objective

The overall objective of this study is to assess the levels of pesticide residues in tomatoes from markets.

1.3.2 Specific objectives

i. To assess the awareness on pesticide residues in tomatoes and handling practices of tomatoes among sellers in the market

ii. To determine the levels of pesticide residues in tomatoes from markets

iii. To determine the effect of peeling on levels of pesticide residues in tomatoes

CHAPTER TWO

2.0 LITERATURE REVIEW

2. 1 Tomato production

According to FAO data for vegetable production, Tanzania ranked from the twentieth in 2000 to fifteenth position in 2009. Actually, through that period, Tanzania remained in the top 20 vegetable producers in the world. The highest bulk of the vegetables produced in Tanzania tomato is the single most leading vegetable crop. It is estimated that, the area planted with tomatoes in Tanzania is 26,612 ha. Tomatoes contribute the highest percent of harvested quantity (314,986 tons 64%) to the total harvested quantity of vegetables (Mutayoba, 2018). In the year 2019 the quantity of tomatoes produced in Tanzania was approximately 627,788 tons (FAOSTAT, 2020).

2.2 Tomato plant diseases

Tomato is the target of more than 200 pests and diseases. Normally, diseases are treated by the use of pesticides, which however cause several impacts, such as: very harmful to the environment contributing to the climate change; in the health of people which consume the final product. Some common pests of tomatoes are hornworms, tomato fruit worms, tomato pinworms, stinkbugs, whiteflies and leaf miners. Plant diseases are one of the most limitation factors to tomato production. The most common diseases include bacterial, virus and fungal diseases (Fuentes *et al*., 2016).

2.3 Pesticides

Pesticides are chemicals or mixtures of chemicals that are mainly used in agriculture or in public health protection programs in order to protect plants from pests, weeds or diseases, and humans from vector-borne diseases, such as malaria, dengue fever and schistosomiasis. The typical examples of pesticides are Insecticides, fungicides, herbicides, rodenticides, and plant growth regulators (Nicolopoulou *et al.*, 2016; Elibariki and Maguta, 2017). The use of pesticides in agriculture is necessary to guarantee the world wide food supply, which has increased in volume due to the growing population demand for food. However, the over use of these compounds and lack of application of good agricultural practices can generate residues in the final products, causing potential health hazards for consumers due to their high toxicity (Alcântar*a et al*., 2019).

Pesticides are classified according to their chemical classes such as organochlorines, carbamates, pyrethroids and organophosphates. Some pesticides that are used in tomatoes are chlorpyrifos, dioctyl sodium succinate, deltamethrin, dimethoate, fastac, alphacypermethrin, fenvalerate, lambda cyhalothrin, azadirachtin, copper hydroxide**,** mancozeb, and profenofos (Victori*a et al*., 2017).

2.4 Route of contamination of pesticides

The route of pesticides into human being is variable and can be originated from occupational activities, agriculture and household use that can also indirectly contaminate food or food products. For pesticide applicators, dermal route was found to be the most common and effective route (Anderson and Meade, 2014) as the result of spill, splash or spray drift of which the pesticides are absorbed through the skin. Other route of pesticide exposure includes through the respiratory route for volatile components, eye due to spill on mixing and/or spraying; or oral especially mislabeling of pesticide bottles (Ki*m et al.*, 2017).

2.5 Effects of pesticide use on human health

Pesticides may be associated to a range of diseases including cancers, leukemia, asthma, diabetes, Parkinson's disease, cognitive effects and many others (Ki*m et* al., 2017). Different studies have shown relationship between pesticide exposure to risks/ incidence of cancer such as thyroid cancer in male pesticide applicators (Lerr*o et al.*, 2021), colon cancer (Mart*in et al*., 2018), bladder cancer (Koutro*s et al*., 2009), hematopoietic cancers (Merh*i et al*., 2007), breast cancer (ElZaem*ey et al*., 2013), lung cancer (Ler*ro et a*l., 2015), brain tumours (Provos*t et al*., 2007) and many other disorders apart from cancer such as disruption of spermatogenesis and sperms (Mehrpou*r* e*t* al., 2014). The risk of health hazards due to pesticides exposure depends not only on how toxic the ingredients are but also on the level of exposure. In addition, certain people such as children, pregnant women or aging populations may be more vulnerable to the effects of pesticides than others (Ki*m et* al., 2017).

2.6 Maximum residue limits (MRLs) of pesticides

Governments and International organizations around the world regulate the use of pesticides by establishing the MRL for pesticides in food commodities to evaluate food safety and avoid risks to human health (Hamma*d et al*., 2017). The MRL is the upper legal levels of the concentrations for pesticide residues (expressed in mg/kg) in or on food or feed and the lowest possible consumer exposure to protect vulnerable consumers. It is not expected to be exceeded in any food if the pesticide was applied in accordance with directions for its safe use. If a pesticide residue is found to exceed the MRL in a given foodstuff, the food commodity is said to be adulterated because it exceeds an illegal amount of the residue (Mahugija, 2017). For example the MRL for dithiocarbamate (Mancozeb) in vegetable like tomato is 2 mg/Kg of tomato. In its excess, the manganese contained in dithiocarbamate fungicides is neurotoxic. In general, dithiocarbamates are considered to have very low acute mammalian toxicity with effects such as eye irritation, skin rashes, scratchy throat, sneezing, and inflammation of the nose. However, its associated chronic effects include endocrine disruption, alteration of immune system response, developmental defects in children, and Parkinson disease (Atuhair*e et* al., 2017)

CHAPTER THREE

3.0 METHODOLOGY

3.1 Study Areas

Tomato samples were collected in Iringa Municipality in Iringa Region in Tanzania. This location was purposefully selected because it hosts markets where by tomatoes are among the agricultural products sold in its markets. Tomatoes come from different areas of Iringa region districts where the use of pesticides is a common practice.

Four local markets of Iringa Municipality were purposefully selected for collection of tomatoes in August 2020 the dry season of the year. The samples were collected from Mashinetatu, Ruaha, Mlandege and Kihesa markets in Iringa region. All these markets are common selling places for tomatoes and the tomatoes come from different districts of Iringa Region-(Figure 1).

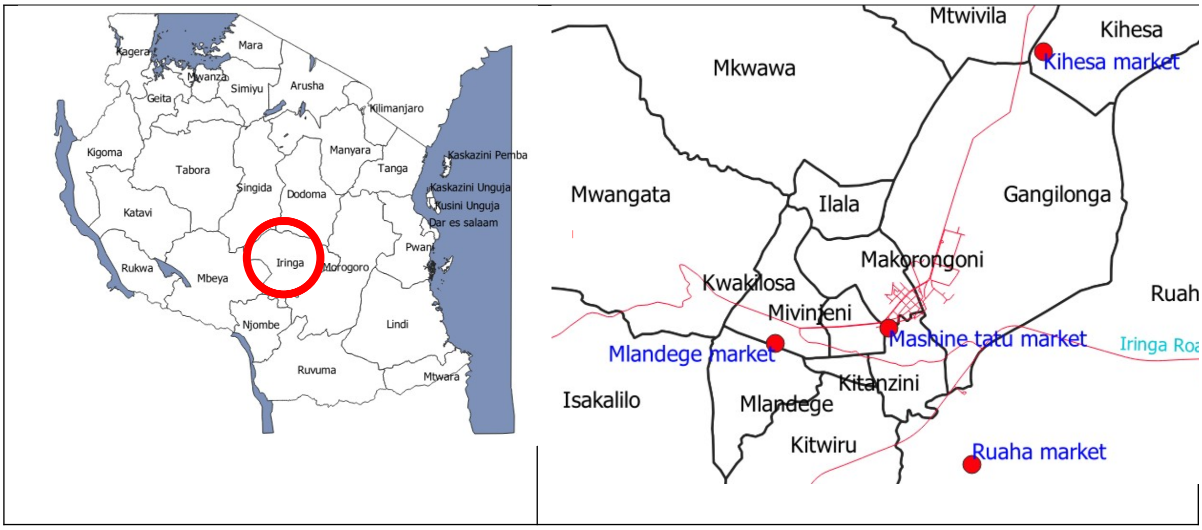

Figure 1: A Tanzanian map locating Iringa region (left) and locations of sampling points (right)

3.2 Sampling and sample handling

Sample size was estimated using a simple sampling equation in which the finite-population correction factor was ignored (Thompson, 2012) as shown in Equation 1 below.

$$n = \frac{z^2 p(1-p)}{d^2}$$

....

.....................1

Where; n is sample size, z is the upper point of the normal distribution at given confidence level, for this study a 95% confidence level = 1.96 at and d = acceptable error (the precision/ estimation error) set at 0.173 for this study and p is the population proportion where by its maximum level was chosen = 0.5. Thus, 1.962 x 0.5 (1 – 0.5)/ 0.173 = 32.

In total, 32 samples from four markets (8 from each market) were collected from retailers, each sample weighing approximately 1 kg, with 10 tomatoes of approximately 100 g each. Tomatoes were purchased from designated markets, at each market, tomato samples were selected by randomly picking from different randomly selected eight sellers. In the markets, immediately after picking, each sample of 1 kg tomatoes was packaged in polyethylene bag, tightly sealed, labeled, and sampling bags were perforated in order to avoid sweat from building up that would otherwise wash away the residues on the surface of tomatoes. The samples were then packed into the cool iced box and transported to GCLA where they were stored at frozen temperature until analysis of samples was conducted. At the laboratory, samples were stored frozen prior to analysis.

3.3 Pesticide residues awareness assessment

Interview was conducted for assessing general awareness on pesticides in tomatoes followed by collection of samples from them. A total of 32 tomato sellers from four markets were selected randomly and interviewed; a sample questionnaire is shown in Appendix 1

3.4 Chemical and reagents

Reference standards of profenofose, chlorpyrifos, chlorothalonil, endosulfan, cypermethrin, hexaconazole and lambda cyhalothrin were obtained from Sigma- Aldrich Company Limited, Steinheim am Albuch – Germany with 99.9% purity. The above-mentioned standards were stored in a freezer at -18 ºC. All solvents used were of analytical grade or similar quality. The solvents used acetonitrile, dichloromethane and ethyl acetate were of HPLC grade. The ethyl acetate used was analytical grade. Other reagents like anhydrous (Mg_2SO_4) and sodium chloride (NaCl), trisodium hydrogen sesquihydrate were obtained from Fischer Chemicals in the United Kingdom.

3.5 Sample preparation

3.5.1 Pesticide extraction and clean up

Extraction and cleanup of samples for determination of pesticide residues in tomato samples were done by using a quick, easy, cheap, effective, rugged and safe (QuEChERS) according to Golge and Kabak (2015) with minor modification. About half kilogram of unwashed tomato samples were cut into small pieces using a knife and homogenized by a mechanical blender (Lyons, Model: FY-309, China). Briefly, a 10 g of a sample was weighed with analytical balance (Shimadzu, 03070369, Japan) into a 50-mL polypropylene centrifuge tube. Exactly 10 mL of acetonitrile was added and the mixture was mixed by vortex (Heidolp, PN541, Germany) then centrifuged for 5 minutes with 4000 rpm (Hettich, Universal 320, Germany). Thereafter, 4 g anhydrous magnesium sulfate, 1 g sodium chloride, 1 g trisodium citrate dihydrate, and 0.5 g disodium hydrogen citrate sesquihydrate were added, and the tube was vortexed for 1 minute, then centrifuged at 4000 rpm for 5 minutes. A 6 mL aliquot of the supernatant was transferred to a 15-mL polypropylene centrifuge tube containing 150 mg of primary secondary amine **(**PSA) and 900 mg of anhydrous magnesium sulfate and 15g Carbon (Wenaty *et al.*, 2019).

The tube was vortexed for 1 minute and centrifuged at 4000 rpm for 5 minutes. The supernatant was concentrated to dryness by a rotary evaporator (Biotage Model, German) operating at a temperature of 43°C and reconstituted by 500 µL of dichloromethane before injection into the GC-MS/MS.

3.5.2 Gas chromatography instrument conditions

The chromatographic analyses were performed on Gas chromatograph coupled with triple quadrupole time of flight (Agilent technologies, 7890B, Germany) operated with Mass Hunter software (7000D, Germany) for data acquisition and processing; the GC was connected to an auto-sampler (Agilent 7693A) column oven compartment and TOF MS/MS detector (Agilent 7000D Series). Capillary flow technology 2-way splitter with one port capped – used for back flushing the analytical column and retention gap Pneumatic Control Module, Helium plumbed to 2-way splitter (PCM) PCM pressure 4.0 psi during run, 60.0 psi during backflush. Connections between retention gap and 2-way splitter retention gap 2.0 m × 0.25 mm, Siltek deactivated fused silica tubing (Restek, Bellefonte, PA), connections between inlet and analytical column by using an Ultimate Union to couple the retention gap to the column Restrictor 80 cm × 0.15 mm deactivated fused silica tubing (Agilent), connections between the 2-way splitter.

Table 1: Instrument condition used

Column	Agilent J&W HP-5ms UI 15 m × 0.25 mm × 0.25	Ionization mode	Electron ionization mode (70 eV)
Inlet liner	Helix Single taper	Transfer line temperature	280 ºC
Injection mode	Splitless	Ion source temperature	250 ºC
Carrier gas	Helium (1.2 mL/min)	Quadrupole temperature	280 ºC
Injection volume	1µL	Mode	Multiple reaction mode
Oven temperature program	70 °C (1 min), 25 °C/min to 180 °C (3 min), 6 °C/min to 280 °C (13 min)	Collision gases	Helium (2.3 mL/min) Nitrogen (1.5 mL/min)
Mass detector	Agilent 7000D Series		
Library used	Willey 2011 & NIST 2017	Threshold	10

3.6 Analytical quality control

3.6.1 Preparation of pesticides standard solution for linearity

Accurately, 180 µL of pesticides namely profenofose, chlorpyrifos, endosulfan, cypermethrin, hexaconazole and lambda cyhalothrin were accurately pipetted into 2 mL vial using a micropipette and 1620 µL of ethyl acetate was added to give pesticide mix. Standard solution with concentration 10 ppm was made for the calibration curve that has the concentration ranging from 0.03125 ppm to 1 ppm.

3.6.2 Limits of Detection (LOD) and Limit of Quantification (LOQ)

To maintain the quality of analytical results, solvent blanks and standards were run. The limits of detection (LOD) were calculated as concentrations whose peaks were three times the peaks of signal to noise (S/N) ratios, whereas the corresponding limits of quantification (LOQ) were calculated as concentrations using the peaks which were ten times the peaks of signal to noise ratios (Saadat*i et a*l., 2013).

3.6.3 Recovery of pesticides in tomato samples

In order to check the accuracy of the experimental method, pesticides recovery from tomatoes were evaluated with "spiked" samples. A single homogenized tomato sample was injected with three levels of different concentrations namely 0.025, 0.05 and 0.1 mg/kg of each mentioned pesticides. The extraction and clean-up methods were conducted as discussed above. The sensitivity of the method used in the extraction of pesticides from samples of tomatoes was obtained by determining the percent recovery of pesticides spiked samples and un-spiked samples.

$$\text{Recovery (\%)} = \frac{\text{Concentration of the spiked sample} - \text{Concentration of unspiked sample}}{\text{Expected spiked concentration}} * 100\% \quad .....2$$

3.7 Risk assessment

The procedure for assessing health risk upon consumption of tomatoes was adopted according to Hlihor et al., (2019) in all steps including hazard identification, dose response relationship, exposure assessment and risk characterization. Risk assessment upon consumption of the tomatoes that were sampled in this study was based on percentage contribution of the detected pesticide residues to Hazard Risk Index (HRI) as shown below.

$$\mathrm{HRI} = \frac{\mathrm{EDI}}{\mathrm{ADI}} * 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots.3$$

Results with HRI exceeding 100% indicate a risk potential (Chun and Kang, 2003).

Where by EDI is estimated daily intake upon consumption of tomatoes per day (mg/kg bw per day). ADI is acceptable daily intake. Average consumption of tomatoes per day for a Tanzanian were estimated to be 258 g from two days recalls on tomatoes consumption (Kariat*hi et* al., 2016) while the average Tanzanian body weight was estimated to be 55 kg (Tungara*za et al*., 2011). Dose response relationship was assessed based on the values of ADE to EDI

$$\mathrm{EDI} = \frac{\text{Tomato consumption} * \text{pesticide concentration in tomato}}{\text{Body weight}} \quad \ldots\ldots\ldots\ldots\ 4$$

In addition, on assessing longtime exposure the average daily dose (ADD) was calculated based on the following equation

$$ADD = \frac{C * IR * EF * ED}{BW * AT} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ 5$$

Where: *C* is the pesticide residue level (mg/kg), *IR* is the tomatoes consumption rate (g/day), *BW* is the body weight (kg), *EF* is the exposure frequency (365 days/year), *ED* is the exposure duration = 60 annums, *AT* is the average exposure time for non-carcinogens (exposure days within whole lifetime =21900days).

The hazard quotient for non-carcinogenic effects for each pesticide was calculated as;

$$\mathrm{HQ_i} = \frac{\mathrm{ADD}}{\mathrm{ADI}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 6$$

Since tomatoes have been shown to be contaminated with multiple pesticides the cumulative health hazard (HI) was used to assess whether the produce might pose health risk upon consumption or not by equation 7 bellow

$$\mathrm{HI} = \sum_{i=1}^{n} \mathrm{HQ}_i \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 7$$

If *HI* has a value higher than 1, the pesticide residues in tomatoes can be considered a risk to consumers, while if *HI* has a value lower than 1, the pesticide residues are considered to be in an acceptable limit with no risk to human health (Hliho*r et al*., 2016).

3.8 Data Analysis

Descriptive statistics for data on awareness of pesticide contamination from tomato sellers through questionnaires were analyzed by Statistical package for Social Sciences (SPSS) IBM Corporation version 25 (2017). Data for pesticide contamination were analyzed with R Software Version 4.0.3 of 2020 (Team 2012). Shapiro-Wilk normality test, Bartlett test of homogeneity of variances and residual against fitted plots were used to test for normality, homogeneity of variance and independence of variance. Since the data on pesticide contamination found to be not normally distributed i.e. skewed; Kruskal-Wallis rank sum test was used to test for significant effect of sampling sites on levels of each pesticides and its mean separation test were done by pair wise comparisons using Wilcoxon rank sum test with continuity correction. Effects of processing (peeling) on level of pesticide residues were done by unpaired student t-test; alpha level <0.05 were considered significant in all cases.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Awareness on Pesticides Contamination and Handling Practices of Tomatoes among Sellers

4.1.1 Demographic information

The demographic information of the respondents showed that 88% of the respondent were female that were evenly distributed in each market surveyed; 53% were having primary school education where by the level of education were also distributed evenly throughout each market surveyed (Table 2). Only 22% of the surveyed tomato sellers from the four markets were more than 50 years old.

Table 2: Demographic information from the respondents (%) in different markets

Description		Market				Total
		Ruaha	Mashinetatu	Kihesa	Mlandege	
Gender	Male	50.0	0.0	50.0	0.0	4
	Female	21.4	28.6	21.4	28.6	28
Age	18 to 30	45.5	18.2	9.1	27.3	11
	31 to 50	14.3	35.7	35.7	14.3	14

	51 to 60	14.3	14.3	28.6	42.9	7
Education level	Primary school	17.6	23.5	23.5	35.3	17
	Secondary school	33.3	26.7	26.7	13.3	15

Table 3: Contribution of sources of tomatoes.

	Kilolo	Iringa Municipality	Iringa DC
Farmers	4 (33%)	2 (16%)	6 (50%)
Whole sellers	8 (40%)	3 (15%)	9 (45%)
Total	12 (38%)	5 (16%)	15 (47%)

Based on the response from the respondent all tomato sellers (100%) interviewed were aware about pesticides and that they are applied in tomatoes. None of the tomato sellers interviewed in the markets declared to apply/spray any pesticides after purchasing the tomatoes from their clients; this kind of response suggests that pesticides detected in tomatoes originated from the farm only. Nguett*i et al*., (2018) found that only 6% of tomato farmers at Mwea region in Kenya apply/spray pesticides after harvest with the aim of reducing postharvest diseases such as alternaria, buckeye rot, gray mold, soft rot, sour rot and bacterial soft rot that attacking the produce.

4.2 Pesticides contamination in tomatoes

4.2.1 Pesticides contamination in general

The general variability of pesticide contamination in tomato samples are summarized in Figure 2. Profenofos was found to have the highest concentration among the pesticides analyzed with mean of 0.136 mg/kg and the concentration ranged from 0 to 0.683mg/kg. Chlorothalonil was the second that ranged from 0 to 0.539 mg/kg, followed by lamda cyhalothrin that ranged from 0 to 0.143 then chloropyrifos that ranged from 0 to 0.287 mg/kg and hexaconazole ranged from 0 to 0.117 mg/kg while the remaining two pesticides had maximum residues lower than 0.01 mg/kg.

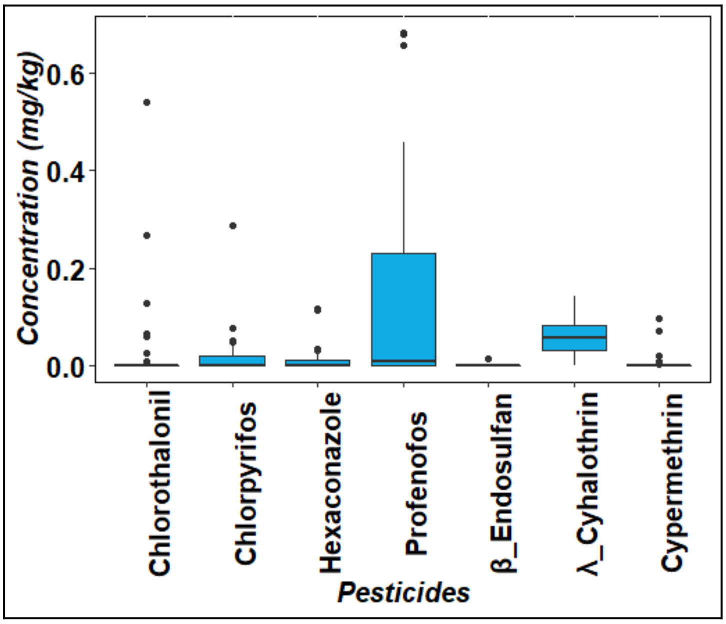

Figure 2: Boxplot showing a general trend of pesticide contamination (mg/kg) in tomato samples (n=32) from four Iringa markets

Other studies on pesticides contamination comparable to this study in tomatoes from Tanzania have been documented such as β-endosulfan, chlorpyrifos and cypermethrin with the highest concentration of 3.81, 0.37, 0.12 and 0.05 mg/kg respectively in Dar es salaam markets (Mahugij*a et* al., 2017), while a study done in Meru district observed chlorpyrifos with the mean concentration of 7.53 mg/kg (Kariath*i et al*., 2016). Additionally, β-endosulfan was observed from western Usambara and Uluguru mountains with the concentration of up to 0.53 µg/kg (Mtashobya, 2017) and in another study, 47.5% of 613 samples were contaminated with 52 different pesticides in different produces including tomatoes from southern highlands (Morogoro and Iringa), northern corridor (Arusha, Kilimanjaro and Manyara) and coastal zone (Dar es Salaam) (Kapelek*a et a*l. 2020). Chilipwel*i et al*., (2021) also found high usage of pyrethroid pesticide (λ – cyhalothrin, cypermetrin and imidachloprid) 31% followed by carbamites (25%) and organophosphates 20.9% (profenofos) in smallholder tomato farmers in the southern corridor of Tanzania. Higher levels were also detected in tomato sample in different parts of Africa such as in Ethiopia 2.5% of the sample were exceeding EU MRLs in profenofos and endosulfan (Loh*a et* al., 2020).

The results obtained from this study found lower values on λ – cyhalothrin (0.143 mg/kg) than the study done in Senegal (0.293 mg/kg) (Dio*p et al*., 2016); lower values of profenofos were observed than the study done in Egypt (0.31 mg/kg) (Ahme*d et* al. 2016) and on chlorothalonyl in Nigeria (<0.2 mg/kg) (Oyeyiol*a et al*., 2017). The differences in the observed values with other researchers are possibly attributed by increasing awareness of farmers and adherence to good agricultural practices.

4.2.2 Pesticides concentration within sampling sites

The results on levels of pesticide contamination (mg/kg) within sampling locations and locations where tomatoes are produced showed that the tomatoes sampled at Kihesa market were found to have significantly higher values of profenofos while Mashinetatu was found to have significantly higher values of chlorothalonil, chloropyrifos and ʎ - cyhalothrin ($p<0.05$). Based on face to face interview conducted, tomatoes contaminated with higher values of profenofos were produced at Iringa Municipal and Iringa district while chlorothalonil, chloropyrifos and ʎ - cyhalothrin were produced at Kilolo district.

Table 4: *Pesticide residues (mg/kg) ± SEM in tomatoes in different local markets of Iringa region (n=8)*

	Kihesa	**Mashine tatu**	**Mlandege**	**Ruaha**
Chlorothalonil	0.00 ± 0.00^{b}	0.13 ± 0.07^{a}	0.01 ± 0.01^{ab}	0.00 ± 0.00^{b}
Chlorpyrifos	0.01 ± 0.01^{a}	0.05 ± 0.04^{a}	0.02 ± 0.01^{a}	0.00 ± 0.00^{ab}
Hexaconazole	0.02 ± 0.01^{a}	0.00 ± 0.00^{ab}	0.01 ± 0.00^{a}	0.01 ± 0.02^{a}
Profenofos	0.30 ± 0.10^{a}	0.12 ± 0.09^{b}	0.04 ± 0.02^{c}	0.08 ± 0.05^{c}
ß Endosulfan	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
ʎ Cyhalothrin	0.00 ± 0.01^{a}	0.00 ± 0.01^{a}	0.00 ± 0.01^{a}	0.00 ± 0.01^{a}
Cypermethrin	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.02 ± 0.01^{a}	0.00 ± 0.00^{a}

Means within rows ± SEM with the different letters indicates statistical difference between sampling sites ($p<0.05$)

Improper application of pesticides have been documented by different researchers like the use of counterfeit pesticides (Zikankub*a et al*., 2019), the use of more than one pesticides with different active ingredient (Nong*a et al*., 2011), inappropriate dosage inappropriate pesticides combination (Kiwang*o et* al., 2018); might have been the cause of higher levels of pesticides observed.

4.2.3 Effect of peeling tomatoes on pesticides

The results showed that traditional peeling of tomatoes reduced the levels of pesticides up to 100% for ß - endosulfan and cypemethrin as shown in Table 5. However lower percentage reduction was observed at the samples with significant lower values of pesticide concentration. Lamda cyhalothrin was found to have lower percentage reduction (36%) than all other pesticides analyzed.

Table 5: *Effect of peeling on pesticides concentration (mg/kg)*

Pesticides	**Mean concentration [mg/kg]**		**% Reduction**
	Unpeeled	**Peeled**	
Chlorothalonil	0.034±0.0188[a]	0.007±0.0042[a]	77.6
Chlorpyrifos	0.020±0.0093[a]	0.001±0.0003[b]	97.2
Hexaconazole	0.013±0.0051[a]	0.002±0.0012[a]	77.0
Profenofos	0.136±0.0382[a]	0.008±0.0039[b]	89.8
ß – Endosulfan	0.001±0.0007[a]	0.000±0.0000[b]	100.0
ʎ - Cyhalothrin	0.056±0.0070[a]	0.021±0.0045[a]	35.7
Cypermethrin	0.006±0.0037[a]	0.000±0.0000[b]	100.0

Means within rows with the different letters are statistical significant ($p<0.05$)

Household activities including peeling tomatoes have been found to reduce levels of pesticides by 96% in chlorothalonil (Kwo*n et* al., 2015). Other studies found that most of the pesticide are removed with the peel during peeling and washing tomatoes (Andrad*e et al*., 2015). Other household activities such as washing with either tap water (up 84% reduction, depending on solubility and octanol–water partition coefficient), chemicals (acetic acid or citric acids) or natural extract (87%, 84%, 83% and 64% reduction for dichlorvos, dimethoate, malathion and chlorpyrifos respectively) have been found to reduce pesticide residues in tomatoes (Venkatachalapath*y et al*., 2020).

4.2.4 Correlation between pesticides contamination

Only two pesticides (chlorypyrifos and ʎ - cyhalothrin) were found to have a significantly positive correlation (r^2 = 0.5) among the pesticides analyzed as shown in Table 6. This might imply the use of more than one pesticides or the brands of pesticides used at the study area have more than one active ingredient. Ngo*wi et al*., (2007) found that 75% of small holder farmers of vegetable production in northern Tanzania use a mixture of pesticides in which up to 90% uses mixture of three pesticide in one spray. Also, it has been reported that about 50% of samples from the farm, markets and highway in Tanzania were contaminated with more than one pesticides (Kapelek*a et al., 2020)*.

Table 6: Correlation matrix between amount of pesticides (n=32) in tomato samples from Iringa

	Chlorothalonil	Chlorpyrifos	Hexaconazole	Profenofos	β Endosulfan	ʎ Cyhalothrin	Cypermethrin
Chlorothalonil	1						
Chlorpyrifos	-0.045	1					
Hexaconazole	-0.124	0.018	1				
Profenofos	-0.193	0.191	0.193	1			
β Endosulfan	0.236	-0.048	-0.148	0.140	1		
ʎ Cyhalothrin	0.195	**0.491****	-0.032	0.127	0.201	1	
Cypermethrin	-0.061	0.241	0.142	-0.092	-0.096	0.260	1

**significant correlation at p<0.01

4.2.5 Relationship between samples and sampling sites with the type of pesticides

Relationship between samples and sampling sites with the type of pesticides analyzed in this study are shown in Figure 3. Only 46% of the total variability were explained by the first two PCA components; however, 63% of the total variability of pesticide types and concentration was explained by the first three components. The total variability in the first component was contrast between all samples from Ruaha and few samples from other places on one side that was associated with low pesticides loadings with other samples on other side. On the other hand, the second principle component accounted for 21 % of the total variability and was contrast between samples from Mashine tatu associated with high Chlorothalonyl, β-endosulfan and λ-cyhalothrin loadings on one side while other sample was associated with other pesticides on the other side. More than 50% of the sample were found to relate to each other (concentrated at the origin of the principal components) in pesticide contamination due to their lower values or non-detectable value of the pesticide residues.

All sampling location were found to relate to each other on levels of pesticide residues since their eclipses collide to each other in both principal components. Chlorothalonil and β endosulfan were found to relate to each other and with the samples from Mashine tatu market while profenofos and cypermethrin related significantly to each other and with samples from Mlandege market.

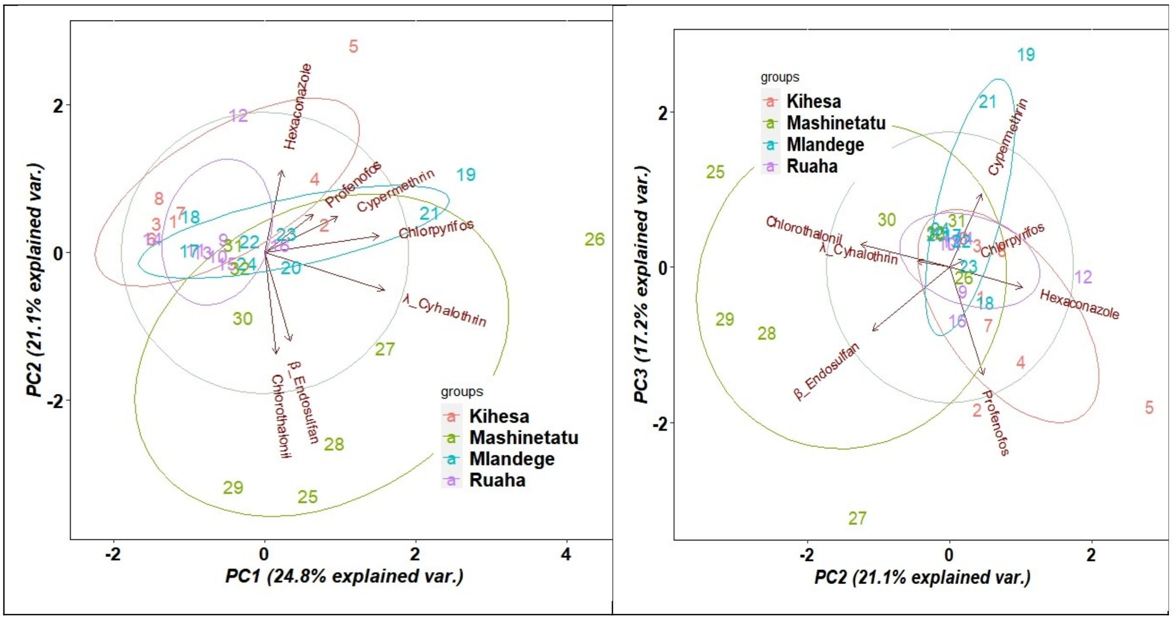

Figure 3: Principal component analysis (PCA) showing relationship between samples and sampling sites with types of pesticides analyzed

4.2.6 Compliance to the MRLs within sampling sites

Eleven samples didn’t comply with EU MRLs on ʎ Cyhalothrin, nine samples didn’t comply with chloripyrifos, eight samples didn’t comply on hexaconazole and seven sample didn’t comply on chlorothalonil. All samples found to comply with EU MRLs on profenofos, β - endosulfan and cypermethrin (Table 7). On average Mashine tatu and Mlandege markets found to have significant higher number of samples that failed to comply with EU regulations on pesticides.

Table 7: C*ompliance with EU MRLs*

Type of Pesticide Frequency	**MRL**	**[mg/kg]**	**Kihesa Ruaha**	**Mashine tatu**	**Mlandege**	
Chlorothalonil	0.01	22 (7)	0.0	62.5	25.0	0.0
Chlorpyrifos	0.01	28 (9)	25.0	50.0	37.5	0.0
Hexaconazole	0.01	25 (8)	37.5	0.0	50.0	12.5
Profenofos	10.0	0 (0)	0.0	0.0	0.0	0.0
β Endosulfan	0.05	0 (0)	0.0	0.0	0.0	0.0
ʎ Cyhalothrin	0.07	34 (11)	12.5	50.0	62.5	12.5
Cypermethrin	0.50	0 (0)	0.0	0.0	0.0	0.0

Higher levels of pesticide contamination in tomatoes above the MRLs might indicate potential risks and concerns for public health upon consumption of these products. Chlorothalonil has been categorized as group 2B (possibly carcinogenic to human) by International Agency for Research on Cancer (IARC) therefore samples with chlorothalonil residues above MRLs might result to health risks upon long time consumption (IARC, 2020).

4.2.7 Health risk assessment

Health risk assessment on pesticides based on health risk index (HRI) upon consumption of the tomatoes analyzed are presented in Table 8. Maximum percentage contribution to HRI was chlorothalonil (31.6%) followed by chloropyrifos (13.6%). On average samples from Mashine tatu market found to have higher contribution to HRI followed by samples from Kihesa market. In all cases no sample was found to contribute more than 100% (a unit in HRI) or HI greater than 1, implies that the levels of pesticides found in the samples couldn't pose health risk upon consumption of these tomatoes in short term. It has been noted that consumption of lower doses of pesticide like the one found in this study might result to variable side effects such as skin rash, allergic dermatitis, itchiness, nausea, headache, diarrhea, abdominal pain, vomiting, nose bleed and eye irritation (Reigart and Roberts, 2018).

Table 8: Health risk assessment based on average percentage contribution to health risk index (HRI) and cumulative health index (HI) in each sampling point (n=8)

Pesticide	ADI [mg/kg bw]	% Contribution to HRI			
		Kihesa	**Mashine tatu**	**Mlandege**	**Ruaha**
Chlorothalonil	0.008	0.00±0.00^{b}	7.48±3.97^{a}	0.66±0.46^{b}	0.00±0.00^{b}
Chlorpyrifos	0.010	0.54±0.35^{a}	2.17±1.65^{a}	1.06±0.51^{a}	0.00±0.00^{a}
Hexaconazole	0.005	2.23±1.27^{a}	0.00±0.00^{a}	1.29±0.35^{a}	1.41±1.41^{a}
Profenofos	0.030	4.63±1.58^{a}	1.92±1.35^{a}	0.70±0.49^{a}	1.27±0.71^{a}
ß Endosulfan	0.006	0.00±0.00^{b}	0.29±0.14^{a}	0.00±0.00^{b}	0.00±0.00^{b}
ʎ Cyhalothrin	0.020	0.62±0.32^{b}	1.94±0.31^{b}	1.67±0.29^{ab}	1.03±0.19^{ab}
Cypermethrin	0.020	0.06±0.06^{a}	0.03±0.03^{a}	0.50±0.33^{a}	0.00±0.00^{a}
Total HI		0.08±0.03^{a}	0.14±0.04^{a}	0.06±0.01^{a}	0.04±0.02^{a}

Means within rows ± SEM with the different letters indicates statistical difference between sampling sites ($p<0.05$) according to Turkey's HSD. ADI means acceptable daily intake obtained from WHO/FAO, JMPR (2010—2019)

On average, health risks associated with consumption of tomatoes in the study done in Northern Tanzania found tomatoes to have more than one HRI due high application of pesticides (Kariath*i et* al., 2016). This study found to have similar results on HRI in tomatoes (i.e. HI <1) with other studies such as study done in Chile (Elguet*a et* al., 2020), in Pakistan (Sye*d et al*., 2014) and in Denmark (Jense*n et al*., 2015). It should be noted that the percentage contribution to HRI was estimated from consumption of tomatoes only, if other crops were included the % HRI might have been high. Lower values of HRI was also found in other vegetables such as apricot, strawberry and grape in Aegean region of Turkey (Soyd*an et* al., 2021).

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This study investigated the levels of pesticide residues in tomatoes from the markets. The results indicate that, with exception to two samples, all other samples were contaminated with one or more pesticides where by the profenofos was found to have the highest concentration (mean = 0.136 mg/kg) followed by chlorothalonly then λ - cyhalothrin. About 34%, 28%, 25% and 22% of the tomato samples did not comply with European MRLs on ʎ - cyhalothrin, chlorpyrifos, hexaconazole and chlorothalonil respectively. Samples of tomatoes from Mashine tatu and Kihesa markets were found to have significant higher values of pesticide residues than samples from other markets. In general, on assessing long life health risks upon consumption of these samples in this study, none of the samples were found to have health risk index more than one (1) demonstrating no short term potential health risk upon consumption of these tomatoes. Peeling was found to reduce significantly levels of pesticides by 35—100% depending on type and initial concentration of the pesticides.

5.2 Recommendations

- Pesticides have the potential effects to human health and therefore the responsible regulators should be concerned and address the issue appropriately. The technological advancement on detection of these pesticides in tomatoes shows that there is a misuse of pesticides. This indicates that, there is a need for further monitoring to indicate exactly what quantities of pesticides are used by farmers.

- Pesticides are applied in farms during production of tomatoes; farmer's education on safe pesticide use should be intensified to limit the levels of pesticides residues in tomatoes.

- The regulatory authorities should set up and conduct surveillance program that will focus on the proper use of pesticides in terms of application rates and pre harvesting intervals.

- Processing and preservation including peeling the tomatoes before consumption is recommended for the reduction of pesticide residues in tomatoes as this process contributes substantially to reduce consumer exposure to pesticides.

- This study was limited to only seven pesticides; more research on other pesticides is recommended to acquire adequate information regarding the levels of pesticides in tomatoes. Also, further studies on the levels of pesticide residues in the tomatoes from the farm that will involve the farmers are suggested.

REFERENCES

APPENDICES

Appendix 1: Sample questionnaire on assessing awareness on pesticide residues in tomatoes and handling practices of tomatoes among sellers in the market

A. Personal information

1. Name ……………………………………………………………

2. Location: Ruaha ( ), Kitanzini/ Mashine tatu ( ), Kihesa ( ) and Mlandege ( )

3. What is your sex(Circle the appropriate answer)

i. Male

ii. Female

4. What is your age? (Circle the appropriate answer)

i. Below 18 years

ii. 18- 30 years

iii. 31-50 years

iv. 51-60 years

v. Above 60 years

5. What is the level of education attended? (Circle the appropriate answer)

i. Non formal education

ii. Primary school education

iii. Secondary school education

iv. University education

B. Awareness on pesticide residues in tomato and handling practice of tomatoes

6. Where did you get the tomatoes which you sell to your customers? Tick a correct answer

i) Own farm ( )

ii) Farmers ( )

iii) Whole sellers ( )

7. How long have the tomato stayed since you purchased or collected?...............................

8. Do you know the place where the tomato you sell are cultivated? Yes ( ), No ( )

9. If the answer is yes in 8 above, name the place or area from which your tomatoes are cultivated ………………………………………………

10. Are you aware that tomatoes are attacked by pests and diseases which necessitate the use of Pesticides in tomato? Yes ( ), No ( )

11. Do you apply any pesticide to your tomato to avoid pests and diseases after receiving from Farmers/ Whole sellers? Yes ( ), No ( )

12. If the answer above is Yes, what type of pesticides are used ?…………………………

13. What criteria do you use in receiving the tomato?

i. ………………………………………………………………………………………

ii.………………………………………………………………………………………..

iii.……………………………………………………………………………………….

14. Do you wash the tomatoes after collecting from the suppliers/farmers/farm? Yes ( ) No ( )

15. If the answer from 14 above is yes, why do you wash? (Circle the appropriate answer)

i. To remove dirt from tomato

ii. To remove or reduce amount of pesticide residues contamination

(Thank you for your cooperation)

Appendix 2: Method validation on pesticide determination in tomatoes by GC MSMS

The LOD values for pesticides ranged from 0.001mg/kg to 0.005mg/kg and the LOQ values ranged from 0.01mg/kg to 0.05mg/kg as detailed in Error: Reference source not found below. Accuracy was assessed in terms of recovery, and the satisfactory recoveries ranged from 79 to 105 percent for seven pesticides signifying the suitability and excellent performance of the analytical method. To check the performance of the analytical method, the following acceptable criteria has to be used: pesticide recoveries should range from 70—130% (SANTE 2019). In addition the linearity of each pesticide was greater than the acceptable limits of 0.998 (Christian, 2007).

Summary of retention time, Limit of Quantification (LOQ), Limit of Detection (LOD) and accuracy for the pesticides analyzed

Pesticides	**Retention time**	**M ass to charge ratio**	**LOD**	**LOQ**	**Linearity (r^2)**	**Recovery (%)**
Chlorothalonil	11.18	263.9>167.9	0.003	0.03	0.998	79
Chlorpyrifos	12.66	169.9>168.9	0.001	0.01	0.999	75
Hexaconazole	15.30	214.0>158.9	0.005	0.05	0.999	81
Profenofos	15.58	207.9>63.0	0.005	0.05	0.998	91
β- Endosulfan	16.40	195.0>125.0	0.002	0.02	0.999	90
ʎ - Cyhalothrin	21.25	181.0>152.0	0.002	0.02	0.999	82
Cypemethrin	24.28	181.0>152.0	0.002	0.02	0.999	105