PESTICIDES RESIDUES AND MICROBIAL CONTAMINATION OF TOMATOES PRODUCED AND CONSUMED IN KENYA

BY JOSEPH HONORE NGUETTI (B.Sc. UY1, M.Sc. PUCA)

A THESIS SUBMITTED IN FULFILMENT OF THE REQUIEREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN FOOD SAFETY AND QUALITY

DEPARTMENT OF FOOD SCIENCE, NUTRITION AND TECHNOLOGY

FACULTY OF AGRICULTURE

UNIVERSITY OF NAIROBI

2019

DECLARATION

This thesis is my original work and has not been presented for a degree in any other institution
Joseph Honore Nguetti
SignDateDate
This thesis has been submitted for examination with our approval as University supervisors
Prof. Jasper Kathenya Imungi
Department of Food Science, Nutrition and Technology, University of Nairobi
SignDateDate
Prof. Michael Wandayi Okoth
Department of Food Science, Nutrition and Technology, University of Nairobi
SignDateDateDate
Prof. Eric Simon Mitema
Department of Public Health, Pharmacology and Toxicology, University of Nairobi
SignDateDateDateDate
Prof. Joseph Wang'ombe
School of Public Health, University of Nairobi
SignDateDate



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Faculty of Agriculture

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To our Lord God Almighty Savior and Redeemer, you have started and you have completed. Glory is your name ever and forever in Jesus Christ!

May you dwell in my heart and fill it with love (Eph 3: 17 - 19) in Jesus Christ of Nazareth!

To my mother Assen Machia Florine: For all your pain and endless support. God has been with you powerfully for me to come to this level. May He *bless* you forever!

To my children Monkam Nguetti Wilfred Blair, Nkemi Nguetti Joseph Orane Nobel, Berhanu Nguetti Mary and their mother Julienne Ngadjieu for your warm love and psychological support during this training. You have been so strong and encouraging by the grace of the Lord Almighty in the name of Jesus Christ of Nazareth!

To my elder brother Sanam a Mbang Faustin: Your initiative and readiness to support will never be forgotten. May God bless you abundantly!

To all my brothers and families, checking on me and encouraging me have been worthy. I magnify the name of the Lord Jesus Christ of Nazareth to have given you as family.

To my late first born elder brother, your happiness to see me coming to Nairobi to achieve this goal is fulfilled by the grace of the Holy Lord and your return to Our Heavenly Father was so painful but, this was God's plan. May you rest in peace in Jesus' name!

ACKNOWLEDGEMENT

I sincerely thank my supervisors Profs J.K. Imungi, M.W. Okoth, S.EMitema and J. Wang'ombe for taking me through this journey and mostly for their time and guidance to the end of this training. I also thank Prof W.F. Mbacham in Cameroon for his support.

I thank: The European Commission for the scholarship through AFIMEGQ program, I have been able to complete the degree I have looked for years; PCPB for experts' consultation and provision of chemical standards; KEPHIS for pesticide residues analyses; PHPT and the lab of microorganisms analyses of the DFSNT of CAVS- University of Nairobi.

I thank the academic staff of DFSNT and the technical staff.

Finally, I wish to each and everyone that is not mentioned in the column but played a role however small in supporting me to accomplish this task.

May the Almighty God bless you all!

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ABBREVIATIONS AND ACRONYMS

ADI: Acceptable Daily Intake
DAILY: Disability Adjusted Life Years
E. coli: Escherichia coli
EU: European Union
FAO: Food and Agriculture Organization of the United Nations
GCMS: Gas Tandem Mass Spectrometry
GDP: Gross Domestic Product
ICBN: International Code of Botanical Nomenclature
KEPHIS: Kenya Plant Health Inspectorate Service
LCMS/MS: Liquid chromatography coupled with Mass Spectrometry
LOQ: Limit of Quatitation
MRL: Maximum Residue Limit
NCDs: Non-communicable diseases
OCPs: Organochlorines Pesticides
OPPs: Organophosphate pesticides
PHI: Preharvest interval
PCPB: Pest Control Products Board
QuECHERS: Quick, Easy, Cheap, Effective, Rugged and Safe
SDGs: Sustainable Development Goals
SPSS: Statistical Package for Social Sciences
WHO: World Health Organization

ABSTRACT

Tomato is one of the most cultivated and consumed vegetables in the world. The main production in Kenya is by small scale farmers who may not comply with the prescribed pesticides practices. Similarly, the environment or postharvest handling may be the source of bacterial contamination. Freshly consumed tomatoes are thus likely to contain high pesticide residues levels and bacterial loads. This study was designed to determine on-farm pesticide practices, pesticide residue levels, bacterial load and consumers' awareness on potential presence of pesticide residues and bacteria on fresh tomatoes sold in markets.

A cross-sectional survey with tomato farmers using a semi-structured questionnaire was randomly administered to 52 participants in Mwea. A number of 240 tomatoes samples were collected twice a month from three open-air markets and two supermarkets from January to June 2017 in Nairobi for pesticide residues and bacterial analyses. For further studies, sampling of freshly prepared tomato for salad from three restaurants and three hotels of the four stars levels and above randomly selected in Nairobi was also conducted. A total of six samples was collected for pesticide levels and bacteria load analyses. Lastly, consumers' awareness of pesticide residues and bacteria on fresh tomato was assessed using a semi-structured questionnaire administered to 101 households in Kangemi in Nairobi.

E. coli and *Salmonella* presence on tomato were detected using pour and spread plate's methods respectively followed by enumeration and biochemical characterization. QuECHERS followed by GCMS tandem LCMS/MS were used for detections. Data from surveys (farmers and consumers) were analyzed using SPSS and tested at 95% level of confidence.

About 90 % farmers were males, 46% were 36 to 49 years old and 4% had never attended any school. Around 93% spray pesticides once a week on-farms, 77% observe at least 7 days pre-

harvest period, 6% spray at postharvest and education had a negative correlation (p= -0.003) on on-farms pesticide use. About 48.5% of tomatoes analyzed had pesticide residues of which 27.27% were single, 21.21% multiple and 6.06% and 1.51% had levels above EU and Codex MRLs respectively.

Tomatoes had 96% of *E. coli* load and the same samples had 20% of non-typed *Salmonella* spp. About 50% of *E. coli* had levels above recommended load $(\log_{10}cfu.ml^{-1} > 2)$. Freshly prepared tomatoes for salad were free of *Salmonella*. Single and multiple pesticide residues were detected of which, omethoate (0.08 mg.kg⁻¹; LOQ 0.02 mg.kg⁻¹; MRL 0.01 mg.kg⁻¹) and dimethoate (0.04 mg.kg⁻¹; LOQ 0.02 mg.kg⁻¹; MRL 0.01 mg.kg⁻¹) had levels above EU MRLs. Consumers' awareness on enteric bacteria load had no significant difference (p= 0.083) between males and females. However, the sociodemographic characteristics significantly influenced consumers' awareness (p< 0.05) on pesticides presence on tomato. About 86% and 74% of consumers knew pesticide and bacteria can be found on tomato (p= 0.000) respectively.

The study concludes that, farmers in Mwea were conversant with pesticides, and most of them observe good practices. Single and multiple residues were detected in samples and very few were above EU and Codex MRLs. Most fresh tomatoes sold in open air markets and supermarkets in Nairobi contain *E. coli* above acceptable load and some samples contain *Salmonella*. Consumers were slightly more aware of health effects of bacteria than with pesticide residues. The level of education influences the awareness of consumers on pesticide health effects thus, information, education and communication are necessary.

CHAPTER ONE: GENERAL INTRODUCTION

1.1 BACKGROUND INFORMATION

Tomato or *Lycopersicon esculentum Mill* is one of the most cultivated and consumed vegetables in the world. In Kenya, many varities of tomatoes (Roma VF, Cal J, Fortune Maker, Money Maker, Tanzanite and Monyalla, Anna F1) are marketed for consumption and production is mainly by small scale farmers. The crop is ranked first in horticulture (Mbaka et al., 2013) and the vegetable is produced all over the country in open lands and greenhouses. Eighty per cent of the produce is consumed locally (Wachira, 2012) in stews, raw in salad including the traditional salad "Kachumbari" used as accompaniement for barbecue meat. Some production areas including Kiambu, Machakos and Kajiado (Mutuku et al., 2014), Loitoctock, Nakuru and Mwea supply the vegetable to the Nairobi markets. The vegetable has lots of health values including prevention from different types of cancer, diabetes and provides other nutrients such as vitamins and phytochemicals to the human body (Esra et al., 2010; Dias, 2012).

With progress in agricultural sciences, many cultivars resistant to pests and diseases have been produced. Cultivation of the vegetable is currently extended in dry and wet season. Irrigation is one of the methods vastly used to maintain production all year round. The success in raw tomato production however is still heavily dependent to pesticide use in farms. Due to chemicals use in farms, tomato's scarcity in markets is under control- making the use of pesticides an asset for food security. Although these chemicals are useful for fresh tomato production, they can be a potential threat to consumers' health when they are incorrectly used in farms (Mutuku et al., 2014). Pesticides are now a serious public health problem and have probably contributed to the increase in the prevalence of chronic diseases. As such, these chemicals increase the government's burden of diseases and play against the sustainable development goals.

Tomato can also be a reservoir for *E. coli* and *Salmonella* spp whose sources may be related to the seeds, environment, soil, postharvest and handling. Information on bacterial load is not always available to consumers. A few may be aware but, a large number of the population unaware is at risk of developing diseases related to tomato consumption. Previously, bacteria transmitted by food have been responsible for more than 70% of fatal cases (Thi, 2007). In 2010, 31 global food hazards recorded caused 600 million foodborne illnesses and 420,000 deaths (WHO, 2015). In the year 2,000 for instance, the USA recorded 2.1 million deaths due to illnesses related to diarrhea. The disability adjusted life years (DALY) from food illnesses is estimated to have triggered 76 million diseases. This for instance has led to 325,000 hospitalizations and 5,000 deaths in the USA (Thi, 2007).

The presence of microbiological pathogens in fresh vegetables sets tomato the second cause of infection after the leafy vegetables (FAO/WHO, 2008). According to WHO (2015) hazardous food with dangerous microbes, viruses, parasites or chemical substances causes more than 200 diseases – ranging from diarrhoea to cancers. Information on the extent of harvested and marketed tomato with pesticide residues and enteric bacteria is therefore important for documentation.

1.2 PROBLEM STATEMENT

According to studies, tomato cultivated and highly consumed in Kenya might contain pesticide residues at postharvest due to pesticides misuse during farms production. Also, soils in which the produce is cultivated and water used for irrigation during production could contain pathogens from the environment. In Nairobi and elsewhere in the country, tomato is eaten in different dishes as fresh salad and cooked in various stews and side dishes. Improper use of pesticides onfarms, failure to adhere to pre-harvest intervals (PHI) and possible use of uncertified pesticides may expose consumers to pesticide residues.

Similarly in Kenya, postharvest handling of tomato from farms to urban markets is generally difficult due to insufficient good roads, cost of shipping and poor storage facilities in markets (Sigei et al., 2014) which might end up with cross-contaminations of pesticide residues and bacteria on the crop. Despite this reality, little is known on pesticide residue levels and bacterial loads of fresh tomato sold in domestic markets especially in Nairobi where majority of tomato nationally cultivated is sold at good price.

It is thus important to assess the safety of tomato sold in markets of Nairobi with regard to pesticide residues and enteric bacteria to evaluate the level of consumers' exposure.

1.3 JUSTIFICATION

This study has been designed for the determination of *E. coli*, *Salmonella* and pesticides residues. *E. coli* and *Salmonella* were prioritized because they are common pathogens found in food (Razzaq et al., 2014). As well, they are present in environment, water for irrigation, untreated manure (Rodney, 2006; Orozco et al., 2007, Armendáriz et al., 2015) that might be used by growers as well as their potential contamination during postharvest handling (Brendan et al., 2013; Liu et al., 2018). Moreover, species of *E. coli* are known for their destructive effect of the intestinal mucosa and, for their ability to cause watery diarrhea bursting to severe dehydration and death between the 1st and 3rd day of ingestion (Delaware, 2010). *Salmonella* was chosen because of its capability of causing bloody diarrhea and for its rapid manifestation starting from 8 hours after ingestion of the bacteria to 72 hours (Krittika and Gi-Hyung, 2012).

Pesticides were chosen because it is reported that their use by farmers is not in compliance with the standard of practices and they are pointed as responsible of some chronic diseases. They are a positive tool in agriculture contributing to food security and achievement of some Sustainable Development Goals (SDGs). Findings from this study will be useful to government and consumers for the control of pesticides by farmers to lower residue levels in harvested produce.

1.4 OBJECTIVES

1.4.1 Overall Objective

To assess on-farm pesticide practices, pesticide residues levels, bacterial load and consumers' awareness on potential presence of pesticide residues and enteric bacteria on fresh tomato marketed in Nairobi.

1.4.2 Specific Objectives

- 1. To evaluate the knowledge and on-farm pesticides practices in a tomato growing area
- 2. To establish the levels of pesticide residues; *E. coli* and *Salmonella* loads in tomato sold in some markets of Nairobi
- 3. To establish the level of pesticides residues, *E. coli* and *Salmonella* load in fresh tomato prepared for salads in restaurants.
- 4. To determine consumers' awareness on potential pesticide residues and bacteria presence on tomato marketed in Nairobi

1.4.3 Hypotheses

- 1- Tomato farmers use pesticides according to good agricultural practices
- 2- Pesticide residues levels in tomatoes sold in some markets of Nairobi are below MRLs and tomato sold and consumed in Nairobi is not loaded with *E. coli* and *Salmonella* spp
- 3- Freshly prepared tomato for salad sold in restaurants and hotels of Nairobi is free of pesticide residues, *E. coli* and *Salmonella*
- 4- Consumers are aware of their exposure to pesticide residues and enteric bacteria

CHAPTER TWO: LITERATURE REVIEW

2.1 GLOBAL PERSPECTIVE OF TOMATO PRODUCTION AND UTILIZATION

2.1.1 Production

Tomato because of its importance in diet and even curative health benefits has raised much curiosity among researchers around the world. The crop is second in the worldwide productivity of vegetable with 458.2 million ha used for farming and 32.8 tons/ha sent in markets (Abdulkareem et al., 2015). The vegetable has attracted lots of interests in science and new tomato varieties continue to be developed by plant breeders and geneticists (Scott, 2012). Chauhan et al. (2011) in India revealed that; intake of tomatoes for 2-3 days compared with zero days is associated with significant reduction in mortality at 48% as well as risk of death associated with diarrhea. Tomato contains the P3 substance which prevents platelets clot and curbs death from heart diseases and strokes (Tarla et al., 2015). Srinivasan (2010) in Taiwan noted that, tomato is an important vegetable in Asia and Africa offering 79% of the global farming area and producing more than 65% of global produce. In 2008, the world largest producers of tomato included China (33.8 million tons), USA (12.5 million tons), Turkey (11.0 million tons), India (10.3 million tons) and Italy (6.0 million tons) (Naandanjain, 2012). Romero-González and Verpoorte (2008) declare- the highest farming countries of the world including Egypt produced 87% of the overall raw fruit. These quantities of raw tomato production are achieved through integration of modern agricultural tools (pesticides, manure, irrigation) necessitating good agricultural conduct to reduce pesticides presence on food in tables. Without control, ready-to-eat vegetables might end up with presence of heavy metals, bacteria and pesticide residues harmful to humans' health.

2.1.1.1 Varieties of tomatoes grown

Tomatoes can be divided into two different varieties including the determinate and indeterminate. The determinate variety has the potential for self-growth whereas the indeterminate type needs to be oriented because cannot sustain itself.

The determine varieties are the bushy tomatoes which usually gather their fruits around the tree and ease therefore the time for harvest. This category grows up to a self-sustainable height (Ivors et al., 2010). The handling and shipment are easier for this cultivar because they are hard and are more preferred for consumption in non-cooked dishes like salad. This type is mostly required by retailers because attracts more the customers. In the USA, the determinate tomatoes are mostly cultivated for shipping and not for local consumption (Rutledge et al., 2015). With such peculiarity, latest development of the fruit has focused their interest on varieties that respond better to such characteristics. On contrary, indeterminate type is a low growing type that produces its fruits at the same time with plants' flowers. This group seems more resistant as it produces flowers and fruits during the plant's lifetime and generally stops if only curb by an agent (Ivors et al., 2010). This class of tomato is more soft at hand and cannot easily remain in bar when sliced. The variety is not more requested by consumers and is picked in case either the determinate is unavailable or when the cost of the determinate is high. This type tends to remain as natural because it is not in the range of the modified or improved species. It is rarely considered in shipment and is mostly consumed locally due to hard conditions for storage and handling. It gets ripe within a long period of time and thus, cannot contribute to generate quick benefits needed in modern times. With the challenges of feeding the current and future populations, plant breeders are always seeking new varieties of high quality of plants to maximize production quantitatively and qualitatively to fulfil consumers' needs. Under this

trend, varieties of raw tomato are being devised such as the purple tomato dubbed the "Indigo Rose" with high quantities of antioxidants (Scott, 2012). As well, these innovations are reliable, can be reproduced and transplanted and factors as growth conditions, cultural practices, seasonal effect (of which biotic and abiotic factors can be more or less and even varying), storage and postharvest processing (de Vos et al., 2015) are being mastered. It might therefore be interesting to know whether determinate and indeterminate tomatoes harbor the same level of pesticide residues and bacterial load at postharvest.

2.1.1.2 Challenges in tomatoes production

According to Rutledge et al. (2015), the farming of tomatoes is a sensitive activity assorted with various constraints. The atmospheric conditions are significant in raw tomato business and can end up in both profound losses and deep harm for farmers. In the meantime, the requisites for production, grading, follow up from growth to harvest, packaging and transportation remain some of the points to overcome when involved in the production of the good. These aspects seem to be the determinants that should be well known in order to achieve good results and successful ends of efforts. As the purpose of using the tomato depends on the end product needed by consumers, the fruits are now harvested at different stages of its maturity. Relatedly, some markets are highly demanding on the red vegetable while some insist on those slightly pink when start showing colorful parts. With this evolution on the storage or consumption of the fruit, retailers are now accepting or have preference on green tomatoes when still nice at sight, not attacked by bacteria and pests or not threatened by abiotic and climate conditions. They prefer them at such stages of maturity to have them getting ripe in the shelves for economic purposes. However, despite this motivation, it appears that farmers who harvest green tomatoes use ethylene to hasten the color and the maturity of the fruit (Rutledge et al., 2015). Also, retailers

without knowledge on postharvest use of pesticides can receive green tomatoes and spray them to protect from pests and diseases of postharvest.

Many challenges are to overcome in order to get good tomato quality as required in markets. Apart from pests and diseases, transportation and handling, tomato in farm can face ecological influences (de Vos, 2012) as the physiological disorders (Ivors, 2010). These include blossomend Rot (seen as a black point on the bottom of tomato due to low level of calcium); blossom drop (the fruit is phosphorous deficient, has much moisture, too much nitrogen); puffiness (due to either high concentration of nitrogen or unbalanced ratio between nitrogen and potassium); catfacing (due to high variation of temperature either cool or extremely hot); fruit cracks (causing highest damages on green and maturing tomato exposed to much sun during wet season) and gray wall (marked by black colour on the skin of the vegetable) (Ivors, 2010).

Aside this, peculiar compulsory ways for harvesting are required to preserve the quality of the fruit and maximize the packing for good profits. The fruits should be removed at adulthood to safeguard the highest nutritional value and have it red naturally (Ivors and Sanders, 2010). They should be taken off at the mature green level usually when a white star is seen on the blossom end of the tomato. This clearly states that, the ripening process can proceed either naturally (Ivors and Sanders, 2010) or through application of ethylene gas (Rutledge, 2015). This is preferred because of easy handling and shipping allowing best and high quality production. Three different classes of tomato including the "extra" class, class I and class II exist (Codex standard, 2008).

Extra class is tomato of highest value with firm flesh, good shape (uniformity in size), appearance and well bred. Their colour when ripe must be satisfactory. Class I is tomato with constant size, free of flaws and no black appearance. Tomatoes classified as class 1 are those

with minor faults in profile, shortcoming in colour, not completely smooth on skin and with unimportant damages. Tomato Class II is those excluded from extra class and class I. They have imperfect forms, no good skin and may have superficial healed wounds. Such classification encourages farmers to abandon indeterminate variety and focus on determinate on which they might easily misuse pesticides for more return on investment. They might be doing this because they don't have the right knowledge on pesticides residues and human health.

2.2 MARKETING

2.2.1 Market Requirements for Raw Tomato

The quality of tomato required in markets is set above the different challenges raised during the production including pre and post-harvest of the vegetable. Codex standard (2008) for raw tomatoes has picked out four commercial types for commercialization including the round, ribbed, elongated and cherry. Minimally, the tomatoes must be whole, sound (with no affected or deteriorating point), clean on skin (free of any dirt), attractive at sight, bright, new in appearance, healthy and; with no green sign spot. In addition, the flavor, color, form and texture (tough skin, firm flesh, pericarp tissues) are useful details when classifying tomatoes (Yara, 2017). The color of a tomato qualified as extra class varies between orange, pink, red or white. Achieving these determinants to address the needs and quality requirements of markets may oblige the harvesting of green tomato to ease the shipment and reduce losses. This is mostly done when farmers and retailers have good storage conditions and when the farming area is in a remote place.

Measures to provide quality of the crop include its protection with chlorine gas, thiabendazole, calcium hypochlorite, calcium chloride (CaCl₂), 1-methylcyclopropene (1-MCP) and sodium hypochlorite (Arah et al., 2016; Rutledge, 2015). This precaution is advised because tomato at post-harvest is still exposed to other diseases including alternaria, buckeye rot, gray mold, soft

rot, sour rot and bacterial soft rot. In case mature green tomato is harvested, ethylene ripening gas (Rutledge, 2015) can be used during storage to hasten the ripening process and meet the market's demand. These chemicals are without side effects on human health when used properly. In addition, no related human health consequence or exposure has been cited in research. They are normally used for preparation of drinking-quality water (WHO, 1996). This information should be shared within the value chain of tomato industry to lessen the use of pesticides at postharvest.

2.2.2 Marketing Requirement for Marketing Good Quality of Tomato

Tomato depicted as a well-known ingredient of daily cuisine and one of the most cultivated good in terms of value and volume (Ivors, 2010; Heuts and Mol, 2013) in the world remains a very sensitive produce to care of. Post-harvest caring from farm to the end destination (retail market and household) is the big challenge. Heuts and Mol (2013) in the Netherland studied what good tomato is. Specifically, their survey focused on the real value of tomato and they studied the valuing practices including the marking of goods and bads. They consulted sellers, consumers, chefs, farmers, processors and developers able to speak accurately on the quality of tomatoes bought and ready to consume. Empirically, they concluded that, the quality of tomato considered as good start with its financial cost in the markets defining its significance. It continues with handling, the variety cultivated, the genuineness and the sight appearance considered as indicators for texture and flavor. Though the survey was conducted in the Netherlands, it reflects the customers' habit buying tomatoes in open-air and supermarkets as noticed in Nairobi. This customers' behavior on quality requirement has an influence on middlemen shipping tomato from the farming areas to various markets. As a feedback of customers' choice, they are likely to impose this to farmers whom at their turn may end up misusing pesticides in crops while using untreated manure to come out with best tomatoes at sight. Unawareness of potential practices of farmers by consumers may not allow them to develop precautions lessening levels of vegetables contamination and this might constitute a route of health infection.

2.2.3 Marketing of Fresh Tomato in Kenya

If tomato production in farms necessitates farmers' time, physical hard work, irrigation and use of pesticides for instance, selling the produce at postharvest remains another big deal to sort out. Sigei et al. (2014) in their study of challenges and ways to ameliorate affordability of tomato in Kenya remind that, ways to improve the business of the produce are curbed by some hurdles. These include inaccessibility to markets mainly causd by insufficient and poor roads leading to farming areas or to farms influencing on transport cost. These two impediments have a direct impact on cost of the good in markets creating fluctuations. Other barriers in the chain of tomato include insufficiency or absence of modern facilities for storage, and also markets lobbies already shaped by dealers. Some of these obstacles have been probably slowing down the business while poor storage of the crop degrades its safety (Sigei et al., 2014) and thus creates ways to contaminants.

2.2.4 Post-harvesting for Fresh Tomato

To reduce losses from farms to markets, the chain of post-harvest including farmers, middlemen and retailers has to be cautious in handling (packaging, shipping and storage). Being a critical control and sensitive interval between farmers and consumers, post-harvest management is very tricky and seems to have been given less attention in research. Arah et al. (2016) accordingly argue that greater than 95% of agricultural focus goes to food production while less than 5% is allocated to post-harvest studies very useful for safety analyses, consumers' health infections prediction and prevention. It can therefore be said that, the removal of produce from farms and provision to the customers targeted in food security has been neglected and constitutes a hindrance to the achievement of some SDGs. The utility for different postharvest research have already been depicted. Some foodstuffs are cultivated in inaccessible areas and possibilities for supply in markets are low (Arah et al., 2015) while others are grown in accessible places and have less loss. With such probabilities and potential others, post-harvest is challenging mainly when Ramaswamy (2015) reports 25 to 30% produce losses at this level. With such amount of losses, the safety of the produce becomes also crucial within this section when the steps of handling are poorly or inadequately suitable to the crop.

It is following the difficulties and challenges within this step that number of scientists including Bourne, Spurgeon and Sigaut came out with the postharvest process realism. They depicted the intelligence or the real step by step useful to understand the post-harvest system. This includes the graphic design of the pipeline of food by Bourne in 1977, the flow diagram of Spurgeon in 1977 and the web of food of Sigaut in 1979 (FAO Corporate Document Repository, 2017). Spurgeon defined postharvest or "hidden harvest" as a pipe engulfing provision of the produce from the moment and area of harvest to the period and lieu of eating (Grolleaud, http://www.fao.org/3/AC301E/AC301e00.htm). The full process should minimize losses, increase efficiency and get high return of the global activity. Achieving this safely in order to meet the return on investment leads to intelligence of crops' management from pre-harvest to ready-to-eat in households. More attention and investment should be given to postharvest management of tomato and its outcomes will inform on preharvest management which is a determinant of human's health protection. Knowledge of this process may contribute to improve pesticides use in tomato farms. The flow of good in the diagram of Bourne (1977) reveals for instance five main steps from harvesting to entry of the mouth (consumption) within which contamination and quality loss can occur. They include:

- 1- Pre-processing during which the fruit is exposed to braking and rejects, excessive trimming
- 2- Shipping from which the fruit may go through spillage, bruising/hurting, breakage, damage and leakage caused by environmental conditions such as heat and frost
- 3- Storage that may come with abrasion/scratch of other pests and diseases (insects, rodents, birds, molds, bacteria, growth, decomposition and over ripening) deriving from rain and humidity
- 4- Processing and packaging during which contamination can occur expressing inefficiency, excessive peeling, trimming and the polishing
- 5- Marketing that comes with contaminated product which is unsafe food that has loss both the quantity and quality from farms

This diagram was modified by Ramaswamy (2015) to meet the current environment. It engulfs: harvesting, preparation or packaging, cold storage, shipping and distributing, retailing, household cooking and ingesting

Depending of the maturity, if mature green tomato is harvested (because of extreme distance); it is advisable to store at a warm temperature above 55^{0} F to maintain the right ripening temperature. Below this temperature, the enzyme lycopene responsible of the red color will not achieve all the steps of development (Rutledge, 2015). Consequently, the fruit will loss the quality requirements defined for instance for "extra" class according to Codex Standard. Once ripe, the fruit can be stored between 45 and 50^{0} F but, the humidity ought to be high to keep the

vegetable aloof from swiveling deriving from loss of water. More detailed information should be provided on this process to ascertain the safety and quality of the produce before retailing in markets. Studies focusing on this management sould be encouraged and this should include analysis of chemical residues levels and bacterial loads on the produce.

If tomato is not well handled from harvest to consumption, drastic effects as injuries, breakage can damage the quality and shelf of the good.

Harvesting: If done by hand, lots of care should be taken to keep good quality appreciated by consumers and recommended by Codex standard. Mature green tomato is advised so as to allow ripening and senescence/ageing to proceed. It prevents the fruits from damages and losses. Environmental conditions and weather should be considered. The task is preferably done either early morning or late afternoon with less heat at a temperature of 13 to 20^oC. In case harvested with heat, the vegetable should be precooled to lose excess of heat and set the best conditions described above. A potential and more practical way may be the use of ice in a clean water using decontaminators cited above. Also, an on-farm hut can be adopted as a shade for tomato against heat (Arah et al., 2016).

Precooling after harvest: It is a relevant step to include when removing the good from the farm. It decreases and stops development and microbial colonies action, slowdown metabolic process, breathing level and production of ethylene. It controls both the temperature and humidity within and surrounding the good. Combination of these steps delays the progress of lycopene, keeps water thus the good shape of the fruit and deterioration.

Cleaning: If done on-farm, microbial colonies are removed or reduced. Evidence of contaminated vegetables with *Salmonella*, *hepatitis A virus*, *Cryptosporidium* and *Cyclospora* and transmission to consumers has been cited in India (Arah et al., 2016). Thus, preventive

methods are required to avoid further transmission before distribution to retailers. This step also prevents from decay by dwindling the bacterial load on the surface of the produce and thus, delaying any diseases development which might still occur later on during shipping.

Grading: this allows the differentiation between the best (extra class), the middle grade (class 1) and the lower grade (class 2). This step shows the importance of understanding the chemical reactions that might start from a lower quality to contaminate the middle and high quality. For instance, the damaged or diseased fruits can release a quantity of ethylene that might hasten the ripening process of the best ones. Or some diseases may be transmitted to the good ones and develop so as to start the putrefying procedure. As well, grading stands for the asset of distinguishing in terms of size, maturity level, colour and senescence. These two processes seek to control the quality and shelf life of the vegetable. It is advisable for farmers and retailers to write down the grading and sorting standards.

Packaging: It is a preparation for shipment/transport and prevents the produce from mechanical wound, poisoning or transmission of infection from another good. Unfortunately, common packaging locally encompasses wooden boxes, woven palm baskets, cardboard boxes, nylon bags, polythene bags without secured protection needed by the produce.

Storage: It is a delicate phase in postharvest. Chances for moisture growth are considerable. The normal conditions of temperature and pressure are not favorable for a long term keeping. For short term preservation, tomato can be kept at ambient conditions in case the warehouse is ventilated to lower or cool down heat concentration from respiration (Arah et al., 2016). It has been asserted that, breathing increases the temperature which interacts with the environment and accelerates the continuity of biochemical and chemical reactions potentially leading to ripeness, senescence and spoilage of fruit. Storage of tomato in a warehouse should not include the

presence of other or different fruits and vegetables. Presence of a mixture of good can liberate ethylene gas that can accelerate the ripening and senescence of another like tomato (Ramaswamy, 2015).

Transportation: It stands as the last point to proceed with the good to the consumers. Depending of the distance between the farm and the point of distribution, changes can occur on the goods following some primary causes of losses (biological and microbiological, chemical and biochemical, mechanical, physical and physiological) (Bourne, 1977; Ramaswamy, 2015). As well, the layout of the good and absence of appropriate containers such as refrigerated vans are shortcomings that hasten the destruction of the good. The state of the road (ondulations), vibrations, quality of packaging are some factors responsible of losses in quantity and quality due to mechanical degradation.

For the quality of material and practices attached to postharvest handling of tomatoes, farmers might be much at ease during production and might not necessarily misuse pesticides in farms. Such facilities hardly found in the sub Saharan countries should be encouraged to release farmers' from preharvest and postharvest stress. This may certainly play a key role in on-farm pesticides management. Tomato at postharvest might not be that much contaminated with pesticides residues for instance because farmers will probably improve their on-farms practices.

2.2.5 Post-harvesting for Fresh Tomato in Kenya

Tomato at posthavest in Kenya is managed by horticultural players including farmers, collecting wholesalers, middlemen, transporters, local authorities and cities wholesale distribors waiting in towns (Ministry of Economic Affairs, Agriculture and Innovation and Research Solution Africa, 2015). It is a well organized business chain settled around horticulture nationally and tomato singularly. Different marketing stages exist. Growers can retail directly in harvested areas to

increase their benefits. But due to huge seasonal productions and inadequate or lack of facilities and premises for postharvest management, wholesale gatherers usually stand as first buyers ensuring zero postharvest losses to growers. They usually travel all over the farming areas to assemble enough produce before arrival of middlemen who are second buyers and shippers to towns. As the growing areas are spread all over the country, collectors recruit some local purchaser partners to ease and maximize their activities. Once produces are collected in accessible places by agent buyers, wholesale collectors arrive in farming areas just to pile the produce in lorries for shipment to cities (Ministry of Economic Affairs, Agriculture and Innovation and Research Solution Africa, 2015).

Distribution in towns is the business of wholesale distributors specialized in urban marketing and dissemination. In order to avoid wasting time to retail the whole package collected in farms; wholesale collectors usually sell to urban wholesale distributors and rush back for new collections. Most often, urban distributors have contracts with green grocers, retail centers as supermarkets, food business dealers as restaurants and hotels (Ministry of Economic Affairs, Agriculture and Innovation and Research Solutions Africa, 2015). As well, they understand well what to sell to open air markets retailers and how to sell the produce packaged in special baskets. Such organisation might have been having an impact on the quality and safety of the produce at postharvest. The chain of traders under the requirement of consumers or food dealers might misuse pesticides in farms to protect the crop. Also, growers might strongly use manure without having the technology for treating it before application in tomato farms. Those two points might be the potential sources of unsafe tomato in markets.

2.3. UTILIZATION

Tomato is highly consumed in salad; meals and it is also processed mainly due to its nutrientand contribution to health

2.3.1 Importance of Tomato in Human Health

Tomatoes contribute to a healthy and well-balanced diet and are excellent source of vitamins B, C, E, iron, folic acid, potassium, and secondary metabolites such as b-carotene, lycopene, and phenolic compounds. They are rich in minerals, essential amino acids, sugars and dietary fibres. Yellow tomatoes have higher vitamin A content than red. But the red tomato fruit contains lycopene enzyme demonstrated as very efficient against carcinogenic substances (Shankara et al., 2005). Although these are known, de Vos et al. (2015) insist, other useful compounds may be detected, extracted and analysed depending of the chosen platform. Lycopene is responsible of the red to pink colors seen on tomatoes, pink grapefruit and other fruits and vegetables. Processed tomatoes are the richest sources of lycopene dietary. The enzyme is described by scientists as containing antioxidant with beneficial properties helping to prevent, reduce evolution and decrease cancer diseases and others (stroke, coronary, heart diseases and bone health for instance) (Nasir et al., 2015; Chauhan et al., 2011; Kanwar, 2011; Ganesan et al., 2012; Agarwal and Rao, 2000). It is thus worthy to analyse the safety of tomato from markets in Nairobi to inform farmers, consumers and policy makers on the status of the produce intensely eaten in different dishes.

Tomato diets	Fresh tomato	Tomato cooked	Tomato sauce	Tomato paste	Tomato soup	Tomato juice	Ketchup
Lycopene content (mg/100g)	0.9-4.2	3.7-4.4	7.3-18.0	5.4-55.5	8.0-10.9	5.0-11.6	9.9-13.4

 Table 2.1: Content of lycopene in tomato (Chauhan et al., 2011)

2.3.2 Tomato in Prepared Meals

Tomato is consumed in different forms including steam, boiled tomato or stew, raw in salad and processed. More and more studies are discouraging boiling vegetables before eating and are rather encouraging steaming to preserve nutrients useful to good health and diseases prevention as diabetes (Muthike et al., 2018). Other studies encourage consuming tomato sauce that provides cis-isomers lycopene mainly present in human serum (Nuray et al., 2007). Relatedly, Stahl and Sies (1992) and, Choksi and Joshi (2007) argue that, boiling tomato for an hour with corn oil increases the level and bioavailability of lycopene in juice. With such a difference, it may be useful to study the level of presence of some microorganisms and pesticide residues in ready-to-eat tomato to assess how safe are consumers.

In Kenya, vegetables are mostly steamed in households and restaurants to preserve useful nutrients (Muthike et al., 2018). Though this alignment, there is still an increase of non-communicable diseases among consumers in the country. This seems to show that, vegetables might be harboring other contaminants as pesticide residues and heavy metals randomly infecting consumers' health. As vegetables are always steamed before consumption, it might be interesting to analysed the safety of vegetables steamed with focus on pesticides residues and bacterial presence to know to what extend those contaminants are discarded during processing.

2.3.3 Processing of Tomato

Tomato is not sold only as raw fruit. About 30 to 40 million metric tons of the processed good are produced yearly in the markets (de Vos, 2015). The produce is therefore expanded in terms of billions of dollars in industry. The vegetable is a significant source of vitamins and enzymes as lycopene, quercetin, kaempferol and myricetin. These carotenoid and flavonoids are the main destructive elements for several diseases. They are anti-mutagenic, anti-carcinogenic, anti-inflammatory and anti-allergic nutrients (Tokuşoğlu et al., 2003; Tarla et al., 2015). For these reasons, it is useful to assess the safety of processed tomato related to bacterial and pesticide residues presence. This allows knowing whether the vegetable is still providing the same virtues to consumers without infecting them with pesticide residues deriving from insufficient good agricultural practices. It also allows to appreciate the processing practices of industries as well as their awereness on potential decontamination of tomato with pesticide residues.

In Kenya, tomato is no only consumed boiled, raw in salad or cooked. Industries as Njoro canning, True Food, Premier food (Sigei et al., 2014), Damalex, Tamu, Peptang and Ken are processing the crop in the country. Recently in Kilifi County, an agreement of cooperation for a joint venture between a domestic company and a United Emirates-based food processing firm was signed for processing around 500 tonnes of fresh tomato daily (Bylanes, 2016). Some types of processed tomato found in Kenya include tomato ketchup, tomato sauce inter alia (Sigei et al., 2014) dried tomato, powder, juice, paste, jam, and puree.

Canned tomatoes are sealed tomatoes in a can without skins; some contain ingredients, like calcium chloride, salt, and fructose corn syrup. Diced tomato are chopped into small pieces and canned. They are usually packed with tomato juice, calcium chloride and citric acid. Tomato puree is peculiar in for his quality and texture standing between the cruched tomato and tomato

paste and is thicker in viscosity. Tomato paste is usually found in small cans, has less water compared to fresh tomato and is darker in colour (Kitchn, 2015). Tomato juice is a beverage with lots of vitamins, mineral and antioxidants specifically lycopene (Bauer, 2019). It is made with ripe tomato and can be mixed with leaves, sugar, salt, and pepper. Tomato concentrate is any type of concentrated tomato from which water has been removed. Ingredients as salt, lemon juice, sodium bicarbonate and spice can be added. Dried tomatoes are much tougher, with less flavor; they are pale, dehydrated and do not look fragrant with the usual intensified taste of fresh ones (Onge, 2019). Maybe tomato processed by industries can be either free of bacteria and pesticide residues or can contain less residues of pesticide probably at levels below MRLs. However, studies can be taken to show exactely what process tomato related to pesticides and bacteria presence is.

2.4 SOME FARMING AREAS IN KENYA AND THE VARIETIES OF TOMATO CULTIVATED AND SOLD IN NAIROBI

Literature indicates that a large proportion of horticultural produce consumed in Nairobi is grown in the neighboring counties of Kiambu, Machakos and Kajiado (Mutuku et al., 2014). For tomato, the major producing areas include Mwea, Nakuru, Meru, Nyeri and Taita Taveta (Wachira, 2012). Also, visit to supermarkets revealed that, the tomatoes found vending in Nairobi come from diverse counties both from the vicinity and long distance. From the neighboringhood (50 - 150km), producers of tomatoes are found in Kikuyu Kilimambogo, Mbeere, Kithimani, Muthurwa and Thika. From areas above 300km from Nairobi, production areas will be found in Loitoktok (Rombo and Kimana), Nyahururu, Kinamba, Limuruti, Subukia and Isiolo which reinforce the supply of the crop the city of Nairobi. The increase in production is attributed to the extensive adoption of high yielding varieties in open-field such as Roma VF,

Cal J (Kamongo), Fortune Maker, Money Maker, Rio-Grande, Onyx, Eden, Tanzanite and Monyalla, and in greenhouses like Anna F1 or indeterminate varieties like Kentom, Marglobe, Monset and Nemonneta (Wachira, 2012). In addition, other varieties of tomato such as Asira, Mirere, Rambo, commando and Safari revealed by the field visit are also cultivated and contribute to the chain of tomato supply in the markets. The business of the raw produce is still increasing indefinitely. The vast introduction of newly fashioned types of produce engulfing the snack, vine and cherry already exists. Other types with change in colour such as the orange, the Indigo Rose or purple, striped and the yellow (Scott, 2012; de Vos et al., 2015) are either being hosted or still to come soon. Some families depending on farming activities With the socioeconomic challenges raised by capitalism, families depending on vegetables' farming might have have raw tomato production as the major source of income. With consumers mostly requesting the best crops in markets, farmers might be misusing pesticides in farms to protect tomato and have it sold at good price to middlemen.

2.4.1 Tomato Farming in Kenya

Tomato in Kenya is mostly cultivated in open-fields, through rain fed and irrigated agriculture both for local consumption and export. Tomato is cultivated in all counties within the altitudes 1150m and 1800m above the sea level. Over 300,000 farm families earn the major part of their income through horticulture (Mutuku et al., 2014). In 2011, the area under tomato production was estimated at 19,000 ha, from which 600,000 metric tons were produced generating an income of KES 14.2 billion (Mbaka et al., 2013). Since 2007, tomato farming in Kenya has been extended to greenhouses aiming at improving the supply in quality and quantity all year round for local consumption and export. The varieties of tomato recommended for production in greenhouses are the hybrids because they are able to resist to diseases better than the older openpollinated types (Hochmuth, 2015). Though introduction of greenhouses, tomato consumers might still be exposed to bacterial presences (*E. coli*, *Shigella* and *Salmonella* for instance) bound to environmental contamination when the produce is eaten raw without washing (Kunyanga et al., 2018). Due to potential biotic factors as diseases attacks in greenhouses, farmers might highly use pesticides to protect the crop in order to maximize returns on building the greenhouse and planting the vegetable.

Tomato cultivation is practiced everywhere in Kenya. The produce stands as the example that might follow other vegetables and food production nationally at the time when the world has elaborated strategies for food everywhere and to all. Some localities such as Kaliluni are reported to have 400 tomato farmers (Mutuku et al., 2014). Tomato is an instrument for development in Kenya for its socioeconomic value and contribution to roll back poverty.

Socioeconomically, tomato stands as an important mean of cash crop for small and mediumscale farmers (Shankara et al., 2005). The produce has the potential for increasing incomes in rural areas, improving standards of living and creating employment opportunities (Wachira et al., 2012) independently to the season. Politically, the horticultural component plays a role nationally for its adherence to the vision 2030 of the Country, and internationally for its structural dynamism participation in the world trade. Since the last two decades, horticulture in Kenya is introducing US\$300,000,000 annually (Nyakundi et al., 2010). This has made horticulture led by tomato to emerge as a major export industry that has joined tourism and tea as the top three foreign exchange earners for Kenya (Masinde et al., 2011). Therefore, looking to fulfill the needs of the families under the rampant poverty in farming areas, growers might misuse pesticides in tomato farms. By using water for irrigation to produce tomato useful in food security, farmers may be contaminating the vegetable with bacteria unwillingly. Under this economic progress, fruits and vegetables exports of Kenya have found some little concerns with the European Union currently requesting to control pesticide residues level on each commodity.

2.5 PESTICIDES

The pesticides are any kind of substance, or mixture of different substances of chemical or biological components manufactured to prevent, repel, kill or control any pest or vector, disease, and which contribute for the regulation of plant growth (FAO/WHO, 2014).

Pesticides are also chemicals designed under some specific and precise aims successively for growth regulations, defoliants desiccants and fruit thinning agents. They prevent for the premature fall of fruits, and they are substances applied to crops either before or after harvest to prevent deterioration during storage or transport. (Zacharia and Tano, 2011). Recent studies in Mexico by Garcia et al. (2012) reveal that- 20,000 chemical trade were recorded in 1998 by the US Environmental Agency Protection as pesticides.

The term "pesticides" is a generic name including different existing chemicals with diverse use. They are differentiated according to their utility and final objective on specific commodities of agriculture. Pesticides include insecticides, herbicides, fungicides, rodenticides, wood preservatives, garden chemicals and household disinfectants (Zacharia and tano, 2011). Some pesticides are particularly designed for horticulture of which some are also categorized into classes such as organophosphate, organochlorine, pyrethroids and carbamates. Their residues can accumulate in blood and adipose tissue and contaminate the hosting body (Shasha et al., 2014). Most of these chemicals are not biodegradable, they bioaccumulate (Bhoke, 2012; Shasha et al., 2014), can cause chronic illnesses through concentration in food as vegetables at a rate over the MRL.

2.5.1 Organophosphate Pesticides (OPPs)

The organophospate or organophosphorous pesticides are synthetic derivatives of the phosphoric, phosphorothioic or phosphonothioic acids components encompassing one of the molecules of esters, amides or thiol (Stoytcheva and Zlatev, 2011). In Zimbabwe for instance, they are widely sprayed as pre and post-harvest treatment to manage diseases in crops such as cabbages (Shasha et al., 2014). Studies on this group of pesticides vary with areas, effects on human health and environment. For example, they are more adopted in the USA and Europe because of their structural diversity disclosing their physicochemical and biological properties. Their life time is shorter in the environment; they do not show any bioaccumulation and biomagnifications. These characteristics have made OPs to be more endorsed for application in farming in industrialized countries. Currently, they are mostly used in the world and their metabolites are widely spread on populations (Eleršek and Filipič, 2011) and biodiversity. In 1999, they represented 37% of the chemicals in utilization in the world and 72% of products in application in the USA (Stoytcheva and Zlatev, 2011). Despite this, these chemicals remain a threat to humans. Through inhalation, absorption via skin and consumption of contaminated vegetable as tomato, they inhibit the cholinesterase enzyme in various parts of the nervous system and then cease nervous transmission between this location and the rest of the body (Dieter and Don, 1990). This inhibition curbs the functioning of the red blood cells and cholinesterase serum and rather increases the production of acetylcholine which proliferates in the blood stream (Shasha et al., 2014). Studies show that the toxic effect of some OP compounds start after 24 hours of application on the pests. The toxicity increases over the time after 48 and 72 hours (Nasr et Hoda, 2013).

OPPs are severe producers of many diseases deriving from their misuse. Intoxication by OPPs manifests as poison in the body during which symptoms as gastro-intestinal upsets, bronchospasms and urination are predominant. Their effect on pregnant women starts from abortion to fetal death; other consequences include Hodkin's lymphoma in the farmers and cancer on children (Stoytcheva and Zlatev, 2011; Shasha et al., 2014).

2.5.2 Organochlorine Pesticides (OCPs)

Organochlorine pesticides are chlorinated hydrocarbons greatly used between the farming for pests and diseases control (CDC, 2009). Kang and Chang (2011) in Republic of Korea studied OCPs in human serum and reported that, everybody has them in their body regardless to age, gender, socioeconomic status and country. These chemicals and their related metabolites can be seen in blood or tissue, even though the levels of contact differ according to factors. The mostly residue found in human population globally is p,p'- DDE whose half-life in human body is estimated at more than 7 years (Kang and Chang, 2011). The hazardous nature of OCs is the level of their high toxicities, chemical and biological stabilities, and their ability to accumulate in fatty tissues of living organisms. This association instills the development of breast cancer in women due to anti androgenic and estrogenic properties (Omwenga, 2013). Explicitly, this chemical class is an organic compound embedded with chlorine. They are characterized by their stability against decomposition/degradation through simple biological or biochemical processes due to their firm bond carbon-chloride. Their solubility is extremely weak in water while it is intense in hydrocarbon (Gourounti et al., 2008). They are known as long-lasting, compounds with bioaccumulation and biomagnifications characteristics along the nutritional chain due to their lipophilic properties (Kuet and Seng, 2003).

OCPs are poisonous for the environment and augment the risk of affecting human health by inhalation in air, ingestion in food, water and skin infiltration (Codru et al., 2007; Gourounti et al, 2008; Ruiz-Suárez et al., 2014). In Japan, they were found at a higher rate in air, rivers and water sediments. In Mexico, residues of DDT were found in the blood plasma of old people, children and women especially in breast milk in which the main metabolite dichlorodiphenyldichloroethylene (DDE) was found (Imo et al., 2007; Ruiz-Suárez et al., 2014). Their utilization can generate serious side effects on human and animals and for this reason, they have been banned in many industrialized countries including Japan (between 1970-1980), Europe and the United States of America to be replaced by OPs which are less persistent in the environment (Kuet and Seng, 2003; Ruiz-Suárez et al., 2014).

In spite of their restriction in the first world, Kang and Chang (2011) assert that countries in development are still using a quantity at around 4000 to 5000 tons annually for pests' control. They come to aquatic environment through direct run-off, leaks, washing of equipment and lack of attention for the disposition of empty containers (Codru et al., 2007). In order to try to understand the severity of their persistence in the environment, it worth to remind that, despite their prohibition in Europe and Japan, they were still found for instance 25 years later in canal waters in Germany recently (Imo et al., 2007).

Number of chronic diseases is pointed as able to be generated by OCPs through long exposure. For long term exposure, potential diseases include cancer development for both male and female, reproductive effects, behavioral and neurological and genotoxic effects. As well, exposure to low doses of OC is able to raise potential risk to diabetes type 2 (Gourounti et al., 2008; Ruiz-Suárez et al., 2014). During long exposure, OCs may also damage liver, kidney, central nervous system or neurotoxicity, thyroid and bladder (Delaware, 2010). Exposure to these chemicals can show up early. Different health contaminations are noticed in human including developmental toxicity (prenatal and neonatal period), and influence on hormone (endometrisios, infertility). These effects are probably related to the possibility of these compounds to interfere at some levels of some hormones, enzymes, growth factors and neurochemicals from where they release key genes (such as cytochrome P-450 1A1 gene) enrolled in metabolism of steroids (or sex hormones) and xenobiotics or undesired substances in human body (Gourounti et al., 2008)

2.5.3 Diversity of Pesticides against Losses in Tomato Farms and Human Health's Threat

Due to the high existing varieties, pesticides and other agrochemicals used in tomato farming are rampant. They are intensively and incorrectly used by the farmers under the incessant pressuring economic basic needs. This is also motivated by the demand from local consumers, probably processors and exporters from Nairobi and other cities of the country. Different kinds of pesticides (organophosphate, pyrethroids, carbamates and organochlorine), even the prohibited ones are still in used around the country and in the neighboring areas providing tomato sold and eaten in Nairobi. Some of them among others are dithane 45, ridomil, antracol, karate, bestox, daconil, decis, cyclone, shothane, milraz, thiovit and ortiva. Among them are still some restricted insecticides such as mocap and dimethoate (banned for the use on fruits and vegetables). Mutuku et al. (2014) clarifies for both latter that, the prohibited ones are still applied in areas at 0.5% (for mocap) and 1.9% (for dimethoate) by some growers. Despite the negative impact of the insects repellent on health, pesticides are still sprayed differently in the tomato farms. Some tomato growers spray every week, others spray every two weeks but, high number of growers (98.6%) sprays pesticides frequently in the farms (Nyakundi et al., 2010; Ngowi et al., 2007). They poisoned the environment and jeopardize the life of the populations. "In 1986, the World Health Organization (WHO) reported that 1 million cases of pesticide poisoning were reported annually

which resulted from farm use. Approximately, 200,000 (20.0%) of these cases resulted in death often attributed to improper handling and management of pesticides by farmers who lack adequate knowledge of the common guidelines for safe use of pesticides (Tandi et al., 2014)". While the concern keeps on increasing, Nyakundi et al. (2010) state that, the challenges facing the horticultural industry and tomato specifically in Kenya are to produce pest free products, which are also pesticide residues free. The only way to come out of the puzzle is to adopt natural pest control methods (Nyakundi et al., 2010; FAO, 2015).

2.5.4 The Code of Conduct for Use of Pesticides

The international community knows the usefulness of pesticides for the current world and its challenges bound to the increase of the human population in need of permanent food. The joint partnership FAO/WHO has prescribed the regulations of these chemicals seeking to control the growth and protection of food in farms for the purpose of food security. Food supply without pesticides in farming would not be enough and humans would lack good health and even peace. Though their contribution in raw food production, Elersek and Filipič (2011) in their studies of mechanisms of pesticides toxicity in Slovenia found that, for an annual application of more than 4 million tons of pesticides around the world, only 1% of this huge quantity thrown in the environment has an effect on the targeted pests.

Pesticides have double faces; properly used they are of high contribution to human life due to their great participation in the production of food and fiber in farms. But on the opposite side, wrongly utilized and inappropriately controlled on what they are destined for, they cause serious harm on human and his broad environment. It is from this international conscience that the FAO (1991) designed the code to raise the awareness of the utility and safe use of these helpful but sensitive chemicals to people and societies. The code shows a peculiar focus for developing countries pointing out the necessity to enforce effective control over pesticides (FAO and WHO, 2014) recognized by the first world as with acute toxicity- thus remain under control and regulation (Stoytcheva and Zlatev, 2011). This has been for example greatly adopted and integrated in the USA by the EPA specialized in chemical limits tolerance in food and, by the Department of Agriculture FSIS in charged for the regulatory enforcement and monitoring of these chemicals in precise areas (Zrostlfkova et al., 2002). Together with the registration and control scheme of pesticides developed by the joint partnership FAO/WHO (2014) the EPA and FSIS have put together their expertise to maximize the benefits of use of pesticides for pests control in both agriculture and public health. With such a partnership, they have spared humans, animals and environment from the negative and risky endpoint of synthetic chemicals (WHO and FAO, 2014; FAO, 1991).

2.5.5 Pesticide Regulation and Registration in Kenya

Pesticides are important tools in modern farming and at the time food security is an issue supported by international organizations and governments. Some have been either banned or restricted for use and under this scheme; governments were called to regulate chemicals use. Previously in East Africa, pesticides were regulated by the Pesticides Control Act of the East African Community until 1977. In 1982, the Pest Control Product Act was adopted in Kenya and the Pest Control Products Board was given mandate to regulate imports and exports; production, distribution and use of chemicals for pests and diseases control (Ngaruiya, www.pcpb.or.ke).

According to the legislation in Kenya, no synthetic chemical product can be imported nor sold within the national territory without PCPB's approval. According to the regulations LN 46/1984 of registration, every product approved should be packaged and labelled on the basis of the governing Act. The authority regulates the final product, conventional chemicals, the quality of

active ingredients, compounds improved or changed in the product, the chemical characteristics of a product to which the compound has been added such as wetting substances and adjuvants. Registration of any product comes after provision of experimental label and a copy of experimental label and technical information. Approval comes after insurance of safety to the public, animal and environment by the Board. Other criteria for registration include quality assurance, efficacy and economic value of the product. Products adopted go through experimental trials for biological efficacy led by accredited institutions selected by the Board such as the Kenya Agricultural Research Institute (KARI). Products adopted are supported by successful trial reports to PCPB and a full registration issued for 3 years renewable every 2 years.

However, temporal registration can be issued previous to scientific or technical information completion. Also, product registered can be suspended or deregistered if wrong information was provided including new information indicating the unsafeness of the product or the premises from which the product is formulated, manufactured or kept do not meet the adequate expectations (Ngaruiya, <u>www.pcpb.or.ke</u>).

2.5.6 Pesticides Import in Kenya for Use in Farms

Diseases and pests constraining tomato cultivation in Kenya include insect pests (Heliothisarmigera, Bemisiatabaci, Thrippalmi); fungal diseases [early blight (Alternaria solani), late blight (Phytophthora infestans), Fusarium Wilt; bacterial diseases (bacterial Wilt, bacterial Spot) and virus diseases (Leaf Curl, Spotted Wilt). They have imposed the use of pesticides unfortunately associated with significant public health hazard, causing short illness (headaches and nausea), and chronic diseases (cancer and endocrine disruption) impacts (Nyakundi et al., 2010; Mutuku et al., 2014). The import of pesticides in Kenya oscillates between 8,832 tonnes in

2009/2010 (European commission, 2013) and 7,000 metric tons annually (Mutuku, et al., 2014). They are a collection of insecticides, fungicides, herbicides fumigants, rodenticides, growth regulators, defoliators, proteins, surfactants and wetting agents (Nyakundi et al., 2010). But, for the whole quantity of pesticide imported, 40% equivalents of 2,900 metric tons are insecticides. This has made its cost to account for 50% of the total of the pesticide imported (Nyakundi et al., 2010). Availability of these chemicals and desire of farmers to make good deals at postharvest can lead production of tomato with pesticide residues above MRLs.

2.5.7 Practice of Use of Pesticides in Tomatoes in Kenya

2.5.7.1 Pesticides control in the national boundaries: The challenges for the legal authority

Following the recent progress of the international cooperation with partners, the government of Kenya has established the Kenya Plant Health Inspectorate Service (KEPHIS). The organ analyses pesticide residues on food in Kenya and surveils pesticides used on vegetables. Though this surveillance, studies reveal the use of non registered pesticides in the national boundaries. Some enter illegally while others are coming under the auspices of international aid system (Raini and Kulecho, 2008). For this reason, pesticides control and homologation in Kenya can be challenging. May be, in addition to the registered pesticides by the Pest Control Products Board (PCPB) of Kenya, others might still be out of control of the legal authority. Probably, some are used by some small scale farmers in the country. As such, pesticide in Kenya may be rampant. In Tanzania, Ngowi et al. (2007) found that eight pesticides, out of 42 pesticides used by small scale farmers were unregistered for general use. Observations as such are pertinent at the time when the chain of global and sub-regional exchange including information across borders has increased. From this practice, it may be possible to find a residue of pesticide not registered for use in Kenya on tomato marketed in Nairobi.

2.5.7.2 Pesticides use in Kenya and limits for applications in tomato farms

Tomato growers in Kenya have received several capacity building interventions for the improvement of tomato production. This has been done through international sponsorship such as the Crops Protection Programme aiming to reduce the impact of key pests on tomato, and ameliorate the crops production and quality (Sarah, 2006). Despite this intervention, pesticides management by small growers is still a challenge in Kenya. Mutuku et al. (2014) stress pesticides overspray and produce harvested before the withdrawal period. Some use unauthorized pesticides, and even when they use the allowed ones, they spray with judicious doses and harvest before the period is over (Nyakundi et al., 2010; Ngowi et al., 2007).

Tomato cultivation in Kenya as elsewhere in Africa and worldwide is subject to biotic challenges. They cause crucial losses in farms, threat the farmers' welfare and play against the first three sustainable development goals (SDG) (Osborn et al., 2015). Consequently, they lessen the increase of agricultural productivity supporting the security of food in Kenya expressed by President Uhuru (Kenyatta, 2013). These studies reveal farmers practices but have not given details on contamination of the produce in markets for a specific period of time.

2.5.7.3 Compliance with MRLs in harvested tomatoes: Chemicals residues levels in tomato

Tawiah (2011) in Ghana assessed the level of pesticides residues on tomato cultivated in an area of Ashanti region. The study showed that, organochlorine chemical residues were between 0.00079 and 40.97 μ k/kg. This result highlighted that, only Aldrin residue was at a concentration 0.00079 μ k/kg under the Maximum Residue Limit (0.01[μ k/kg]) recommended by the WHO. Other organochlorine deposits found were beyond their recommended or acceptable levels. In analyses done in Egypt on raw, processed, cuticular and subcuticular tomatoes by Abou-Arab (1999), lindane (0.003), HCB (0.009), heptachlor epoxide (0.008), dieldrin (0.006) and DDT (0.083 mg/kg) were found in vegetables studied. Corroborating some of the results from Abou-Arab (1999), Zhang et al. (2007) described that, the use of some detergents, the time given for washing, refrigerating and cooking also influence and reduce the quantity of pesticides residues in vegetables such as cabbage.

In Kenya, Mutai et al. (2015) reported that, the vegetables consumed in Nairobi contain mainly the presence of residues of organophosphates and pyrethroids at 42%. Manduu (2015) analyzed levels of residues of Chlorpyrifos and Dimethoate in french beans marketed and found that, Chlorpyrifos level (0.05 mg kg⁻¹) on the preharvested day complied with the MRLs of Codex Alimentarius and EU. He also found that, analysis of Dimethoate and Chlorpyrifos on all French beans samples collected from open air markets and supermarkets were below the detection limits. Similarly, Musila (2010) in a study of Chlorothalonil on Snow peas, French beans and Passion fruits in Nairobi found that, its residues varied from 0.01 mg kg⁻¹ in banana to 70 mg kg⁻¹ in dry chili peper under an Acceptable Daily Intake of 0.03 mg kg⁻¹. These studies assessed levels of some pesticides residues in some fresh produces mostly destined to export (Musila, 2010) and showed levels variations of pesticides. They were sometimes below and sometimes above MRLs. Their results stand as indices for a study of presence of pesticide residues on tomato sold in markets for domestic consumption in Kenya and Nairobi specifically. These results were not delineated to show the levels and limits of pesticides residues in tomatoes

2.5.7.4 Pesticides management in tomato farming

Tomatoes are fruits loaded with all kinds of health benefits mostly concentrated on lycopene. Its cultivation meets lots of challenges with pests and diseases that have imposed the use of pesticides. Mutuku et al. (2014) assessed the use of pesticides and its application practices in tomato. They found that, pesticides used for the protection of tomato against insects, weeds,

diseases, rodents and other pest in agriculture in Kenya are of great benefit as they decrease crop losses and insure good harvests. However, pesticides thought to be the solution with the unique goal of protecting crops from biotic challenges have shown its side effects on human health through infection, poisoning, and environmental contamination. Many studies around the world decry human health destruction by these chemicals. Different reasons explain this effect on human. Despite the regulation and control, pesticides and other agrochemicals are rampant in tomato cultivation in Kenya for instance (Mutuku at al., 2014).

Different kinds of limitations in the use of pesticides such as insufficient knowledge and low educational level of the tomato growers (Tandi et al., 2014) are part of the main cause justifying the high risk of tomato contamination and its impact on humans. Musebe et al. (2014) pointed out the lack of farmer knowledge in pest control together with misinformation that had led to inappropriate use of chemicals. This included the application of large quantities of chemicals in tomato cultivation as noticed in Cameroon by Fontem et al. (1999). Here, the growers practiced intensive pesticidal spray to limit losses caused by pests and diseases. In some areas, the use of banned products, incorrect preparations of mixture and dosages, abnormal dosage of chemical, mixing of various products with different active ingredients and toxicity classes- able to cause incompatibility and phytotoxicity (Rutledge et al., 2015) and then, the lack of awareness of preharvest intervals are also part of the problem increasing the risk of contamination of consumers.

2.5.8 Methods of Pesticides Analysis

Gas chromatography and liquid chromatography are instruments mostly known in pesticide residues analysis in fruits and vegetables. Varieties of liquid chromatography may include thin layer chromatography (TLC), high performance liquid chromatography (HPLC) and ultra performance liquid chromatography (UPLC) (Serháti and Szőgyi, 2012). Semi-volatile and volatile compounds are analyzed in gas chromatography. Also, the instrument is useful for analysis of compounds with stable temperature at their considering time.

Numbers of methods of pesticide residues analysis in fresh produces exist. Gas chromatography has been coupled respectively to mass spectrometry to form gas chromatography mass spectrometry (GCMS), to electron capture detector to form gas chromatography electron capture detector (GC-ECD) and to nitrogen phosphorous detector to form gas chromatography nitrogen phosphorous detection (GC-NPD) (Hammad et al., 2017). GC-ECD was for instance used in OPPs (quinalphos, malathion, chloropyrofos, profenofos, diazinon) and OCPs (chorothanoil, alpha-endosulfane, beta-endosulfan) residues analyses in cucumber, tomato and strawberry (Serháti and Szőgyi, 2012; Hammad et al., 2017). Chemical analysis in wine and grapes targeting azole pesticide residue showed efficacy with offline dispersive solid phase extraction (DSPE) tandem gas chromatography positive chemical ionization mass spectrometry (GC-MS/PCI). In the same line, liquid chromatography has been combined with mass spectrometry (LCMS/MS) and the instrument was used in detections of 450 pesticides in a short period of time (Mastovska et al., 2017). These methods are mostly used for analysis of pesticide residues in fruits, juices and vegetables. Others may include the low-pressure gas chromatography time of flight mass spectrometry (LP-GC/TOFMS) used for analysis of 150 chemical residues in orange, tomato, lettuce and potato. Pesticide residues (cypermethrin and permethrin) were also analysed in pear juice using an ultrasonic-assisted dispersive liquid-liquid microextraction (UA-DLLME) in combination with gas chromatography flame ionization detection (GC-FID). Other recent methods include the use of primary secondary amine (PSA) matrix solid phase dispersion coupled to GCMS-SIM (selected-ion monitoring) mode from which 346 pesticides multiresidues were detected in grapes (Serháti and Szőgyi, 2012). Among these instruments, GCMS tandem

LCMS/MS were more appropriate for this study as they allow analysis of both volatile and nonvolatile compounds using a multiresidue standard of 98 pesticide residues.

2.5.8.1 Pesticides residues analysis in fresh produces

The joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture (FAO/IAEA, 2007) defined three major steps for pesticides analysis including extraction, cleaning and determination for residues analyses worldwide. The method of analysis of pesticide residues in raw fruits has evolved due to "technical advancement upgrading from the most complicated primary idea to a simple application. Better understanding opening floor to amelioration, Anastassiades and Lehotay (2003) have developed and presented the characteristics of a simple method for multi-residues chemical examination. The method is fast (includes few steps), easy to implement, cheap, low solvent is consumed, nontoxic for the users and their milieu, selective, sharp, precise and reach good results for a broad analyte spectrum. It has simplified analysis which has always been more consuming in time, funds, space, personnel and equipment.

Numbers of multi-residue processes have been devised for residues analysis in food. Kuet and Seng (2003) reported the extraction of six OC pesticides in vegetables using acetone and dichloromethane. The cleaning was done using SPE SAX/NH₂ and determination was achieved using a Gas Chromatography equipped with Electron Capture Detector. The recoveries for the compounds varied, they were able to find residues not classified as OCs. However, their results revealed the efficiency of sample washing with SPE sorbent. This has allowed mentioning the advantages of the SPE method in which low solvent is consumed, no cross-contamination, short scrutiny time, no usage of unsafe solvent and the possibilities for the method to be robotic. Dasika et al. (2011) studied chemical residues on fruits and vegetables using the liquid chromatography coupled with mass spectrometry (LC-MS/MS). Analysis started with the QuECHERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method using acetate buffering (AOAC official method 2007.01) for sample preparation and clean up and the process combined two parallel methods for qualitative and quantitative results. Seeking for the synchronized analysis of OP and OC in animal fat, Zrostlíkova et al. (2002) developed and optimised a rapid and easy analysis process, utilizing pulsed flame photometric (PFPD) and micro-electron capture detection (µECD) in gas chromatography for the analysis of 40 residual analytes. The method uses GPC to decouple fats from pesticides without further clean-up before the rugged analytical step. Recoveries for good number of compounds analysed ranged from 60-70% with 10-20% RSD. They found that, the approach (GC/PFPD + μ ECD) was rapid; cheap; requested less instruments, labor, space and is effective in replication. In addition, the method provided consistent results over time and is rugged. Shasha et al. (2014) analyzed OPPs in cabbages; they prepared samples using ethyl acetate, sodium hydrogen carbonate and anhydrous sodium sulphate. After centrigation, the supernatant was clean-up and preconcentrated using a solid phase extraction requesting permanent sorbent control and pesticides detections was done in Gas Chromatography equipped with a nitrogen phosphorus detection (GC-NPD).

From all these procedures, QuECHERS was adopted in this study for its simplicity in chemical residues extraction and cleaning. The method uses kits which are availabale, affordable and fast in samples extraction and cleaning. An average of 10 extracts can be ready for instrument's analysis in a period of 30 minutes by a single investigator (UCT, 2011). The method allows working in a clean environment and is not time consuming, requires less space and personnel to prepare samples for analyses. QuECHERS is intensively used in North and South America, Asia; but is scarcely used for pesticides in Africa. Dominguez et al., (2014) in Chile; Zeying et al., (2015) in China; Lehotay and Maòtovská (2005) used QuECHERS for different analyses.

2.6 ENTERIC MICROORGANISM ASSOCIATED WITH FRESH TOMATOES

The enteric bacteriaceae family is a mixture of pathogenic and non-pathogenic types living in the intestine of humans and animals (WHO, 2019). The class contains number of different kinds of germs including among others *Campylobacter* spp., *Listeria* spp., *Salmonella* spp., *Clostridium* spp, *Yersinia* spp., *Vibrio* spp., *Bacillus cereus*, and *Clostridium perfringens*. These agents are found in poultry or domestic animals (Thi, 2007). Colin et al. (2015) asserted, *Salmonella* and *E. coli* are able to colonize the interior of fruits and vegetables to become endophyte. For Marvasi et al. (2013), human pathogens such as enteric bacteria can infect the fruits and vegetables at any stage of their growth. In this study, only *E. coli* and *Salmonella* contamination were analyzed.

2.6.1 E. coli and Salmonella Load

2.6.1.1 E. coli

E. coli is an engulfing broaden name of a colony of different bacteria which vary from the pathogenic to the non-pathogenic ones. Several types of the germ are inoffensive and their pathogenicity starts with the production of venom dubbed "Shiga toxins" that create diseases. Illnesses deriving from *E. coli* include diarrhea, kidney damage, dysentery, urinary tract infections, septicemia and neonatal meningitis (Thi, 2007). These bacteria are commensal and usually contribute to the functioning of the digestion and regulation of the hosting body. For instance, they curb strongly the development of the wild bacteria and synthesize vitamins useful for the good health of their hosts. However and despite these specificities, four to five species including enteropathogenic, enteroinvasive, enterotoxigenic enterohemorrhagic and enteroaggregative *E. coli* (Thi, 2007) are dangerous for human and animals health.

Enteropathogenic *E. coli* (EPEC) provokes watery diarrhea for both infants less than one year old and adults. The disease can last for over 2 weeks, may lead to serious dehydration and

becomes fatal. Infection of this pathogen in adults is manifested through diarrhea, nausea, vomiting, abdominal cramps, headache, fever, and shivering. The inception of the illness from this type is between the 1^{st} and 3^{rd} day or from 17 to 72 hours.

Enteroinvasive *E. coli* (**EIEC**): This type contrast with other pathogens that usually damage the tissue of the body such as *Salmonella* and enterohemorragic *E. coli*. In this case, the bacterium penetrates and starts the destruction of the intestinal mucosa. Its symptoms include: shivering, fever, headache, muscle pain, abdominal cramps, and abundant diarrhea. The deriving illness noticed is the bloody diarrhea. Inhalation of a large quantity of the bacteria (104 to 105 cells) is required for the manifestation of the illness. The body starts experiencing the symptoms 8 to 24 hours after ingestion of the agent.

Enterotoxigenic *E. coli* (**ETEC**): It is the serotype less recognized causing either diarrhea of cholera illness mostly in zones deprived of hygiene and unappropriate clean water (Thi, 2007). Variety of ETEC lives in epithelial cells which can be fibrillary or not. Its infection engulfs unfriendly abdominal contractions followed by bloody or watery diarrhea. In some cases, infected people may suffer from watery diarrhea (Jafari et al., 2012). ETEC can provoke kidney insufficiency in kids usually called hemolytic uremic syndrome. It stands as the main reason for kidney malfunction in children that obliges dialysis which severally in most cases become fatal. Other symptoms from the pathogen enroll disruption of the central nervous system that may lead to blood clots in the brain of infected persons turning to mortality. The latency or dormancy of this specie turns around 3 to 9 days.

Enterohemorragic *E. coli* (EHEC): Its peculiarity is the ability to produce the Shiga-like toxins allowing the clinical manifestations. The germ may infect the large intestine and express itself through watery diarrhea (Delaware, 2010) which progressively turns into bloody runny stools

due to ulcer followed by pus and wounds of the bowel. The presence of the pathogen in vegetables reflects ingestion of the good contaminated with cattle feces. EHEC shows the highest tolerance of acidity compared to other enteric bacteria germs. This germ is of high threat for kids under five years. The mostly found specie of EHEC in the Western World is the serotype O157:H7 (Welch, 2006).

Enteroaggregative *E.coli* (EAEC) This category of the agent is free of enterotoxin or Shigatoxins. The bacterium is responsible of a watery diarrhea which follows the stomach contractions. It is characterized by a long lasting and severe runny stomach. Its pathogenicity is badly known (Welch, 2006)

2.6.2 Salmonella spp.

Salmonella is a dynamic infectious bacterium; an important threat involved in foodborne diseases actually known as a real public health matter in both the western and southern worlds (Carrasco et al., 2011, Pires et al., 2014). For Heaton and Jones (2007), the agent is mostly responsible of diseases related with raw and fresh vegetables associated with practices of human and their behavior. Previous studies by Sivaplasingham et al. (2004) in the USA showed that of 54% of outbreaks associated with known germs, 60% derived from pathogens and 48% were related to *Salmonella* infections. The pathogenic germ is one of the frequently agent pointed as source of food related diarrhea.

Biochemically, the bacterium is a non-spore-forming, Gram-negative, a facultative anaerobic bacterium able to decrease nitrates and ferment or change the chemical components of glucose. Morphologically, the genus is covered all over with uniformly distributed flagella. Although *Salmonella* enterica and *Salmonella* bongori are the current known species, its serotypes have

gradually grown up to 2500 (Thi, 2007). In the developing areas, the bacterium is mostly transmitted through contaminated vegetable, meat, water and human to human.

Different species of the bacterium both the symptomatic and non-symptomatic have been found. The physical manifestations detected for the symptomatic specie include fever, diarrhea, abdominal pains, nausea, vomiting and frisson causing dehydration and headaches (Krittika and Gi-Hyung, 2012). They mostly appear within the first week after the ingestion of the infected food. Precisely, the symptoms in the human body are noticed between 8 to 72 hours following the inhalation of the bacteria and can persist for 3 to 5 days (Krittika and Gi-Hyung, 2012). Later after the signs of its symptoms, the organism can be found in the feaces of the infected body (Nyenje and Ndip, 2013). Many cases of Salmonellosis infections have been reported in the western world as in the USA with 500 dramatic cases, 1.4 million infected people and at least 16,000 disabled bedded people. Millions of other salmonellosis cases are reported yearly in the world and result in thousands of deaths (Krittika and Gi-Hyung, 2012). These effects have placed the bacteria as a major challenge for the public health (Nyenje and Ndip, 2013). Progressively, developing measures for the control of such pathogens help in the improvement of the saving related to the economy of health. Wegener et al. (2003) studies in Denmark show for this fact that, the principles for control of *Salmonella* contribute to the savings of the Danish society by U.S. \$25.5 million.

For a total of 2274 intestinal infections illnesses recorded in England and Wales from 1992 to 2006, 4% were related to consumption of raw vegetables (Little and Gillespie, 2008). In Mexico and USA, records of fatal cases were related to fresh produce as tomato. The USA singularly recorded 15 confirmed deaths from contaminated fresh produce caused by *Salmonella*, hepatitis A and *E. coli* O157:H7 (FAO and WHO, 2008). Diarrhea, a common symptom of foodborne

disease (Havelaar et al., 2010) able to be caused by unsafe tomato is responsible of 6% of deaths in Kenya (Ministry of Devolution and Planning, 2013).

Many light and neglected cases of stomach infections that people fill very often can be attributed to *Salmonella*. These infections are only considered and cured when the colony of bacteria increases. *Salmonella* is a threat for the low and middle income countries where the populations regularly face co-infections with other poverty related diseases as malaria or tuberculosis for instance; or non-communicable diseases such as cancer or diabetes.

2.6.3 Bacteria of Importance in Harvested Tomatoes

Several fatal cases pointing pathogens found on fresh vegetables like tomato have been reported in countries as the USA with data accounting for foodborne diseases. These fresh vegetables related diseases damage the health of populations deepening therefore the level of poverty of poor people. Food related illnesses induce by those organisms are an increasingly important public health problem (WHO, 2002). Some pathogenic microorganisms frequently found in vegetables analysis in Kenya include Typhimurium Salmonella (responsible for typhoid), Staphylococcus, Shigella, Klebsiella (responsible of pneumonia), Listeria, Vibrio cholarea (Cholera and parahaemolyticus), Escherichia coli and Enterobacteria. However, it is important to mention that studies on fresh vegetables in the world are revealing the emergence of new serious pathogens such as entero haemorrhagic (WHO, 2002) in the food chain of fresh vegetables. This assertion points therefore the need for profound investigations in fresh vegetable and particularly tomato highly sold and consumed in Nairobi. It also includes regular capacities building and update in the system involve in tomato management and evolution. Studying E. coli and Salmonella in this study may contribute to update the policy makers, retailers and consumers on the status of the vegetable and the level of exposure from consuming it.

Focus on presence of *E. coli* and *Salmonella* in vegetables ready for consumption have been largely done done in the USA (Deering et al., 2015). In Kenya Analysis of bacterial presence on fresh produces as Kachumbari (Gitahi, 2012; Mbae et al., 2018) and mixture of fresh produces seeking to explain the origin of diarrhoeal episodes (Waithaka et al., 2014) have also been conducted. Interest of these studies contributed to both the goal seeking to offer to all Kenyans a superior quality of life (Kenyatta, 2013) and the willing to provide equitable, affordable and quality health to all citizens (Ministry of Devolution and Planning, 2013). Aside this, the present study contributes to understand the trend of tomato production in Kenya and some specific challenges leading to bacterial contamination that might be facing the producers in farming areas as well as the whole chain of the industry.

Analyses have been done on tomatoes for diseases' outbreaks (Finn et al., 2013, Pierangeli et al., 2014; Thilini et al., 2016; Brandl et al., 2013) and contamination with *Salmonella* and *E. coli* (Gu et al., 2011; Orozco et al., 2008; Razzaq et al., 2014) reported through fauna, irrigation water, soil, runoff, manure and workers (Islam et al., 2004; Jablasone et al., 2005; Orozco et al., 2008; Berger et al., 2010). Although sanitation and sanitization measures (Cummings et al., 2001; Harris et al., 2001; Warriner et al., 2003; Wright et al., 2017; Holden et al., 2017), fresh crops are still recorded in outbreaks (CDC, 2005; Warriner, 2005; Greene et al., 2008; Hanning et al., 2009; Scallan et al., 2011; Brendan et al., 2013; Liu et al., 2018). These finding stands as justification of analysis of some enteric bacteria in tomato sold and consumed in Nairobi.

These findings have an economic impact noted for instance in Mexico and USA where fatal cases related to fresh produce as tomato were recorded. The USA singularly recorded 15 confirmed deaths from contaminated fresh produce caused by *Salmonella*, hepatitis A and *E. coli* O157:H7 (FAO and WHO, 2008). The total cost of bacterial food related disease in 1989 in the

USA economy was estimated at US\$ 6,777,000,000. In Kenya, the government spends 5.4% of its GDP on health which is equivalent to 4.6% of its national expenditures (Ministry of Devolution and Planning, 2013). Diarrhea, one of the most common symptoms of foodborne diseases (WHO, 2002) able to be caused by unsafe tomato harboring potential pathogens is responsible of 6% of deaths in Kenya (Ministry of Devolution and Planning, 2013). These results justify why bacteria analysis could be of interest interest on tomato highy consumed raw in salad by the Kenyan populations.

2.7 SOME METHODS OF MICROORGANISMS ANALYSIS IN TOMATOES

2.7.1 Molecular Markers

Molecular techniques are major tools for the analysis of microorganisms from food and other biological substances (Firas and Abdulkareem, 2015; Idress and Irshad, 2014). The techniques provide ways to screen for a broad range of agents in a single test (Firas and Abdulkareem, 2015). The technique has been adopted as the rapid diagnostic test of species, strain detection and awareness of threats from biological substances such as food plants. Specifically, a genetic marker is a sequence of gene or DNA carrying an identified position on a chromosome related to a peculiar gene (Firas and Abdulkareem, 2015). The molecular approaches differ from other conventional techniques with respect to discriminatory power, reproducibility, simplicity in the usage and understanding. Also, the markers lead to exact genetic information needed and precise information of the specie studied (Firas and Abdulkareem, 2015). Some of the methods currently available for microorganisms' analysis include the polymerase chain reaction (PCR).

2.7.2 The Polymerase Chain Reaction (PCR) Analysis

Presented for the first time by Saiki (1989), the method has evolved to become one of the most adopted and applied in microbiological sciences. It is among the current fastest latest method of detection of microorganisms used nowadays in the laboratories of analysis. It saves time than the conventional methods. The PCR method has allowed the detection of most enteric bacteria previously unknown such as *Campylobater* and *Lactobacillus*. From the inception, other efficient PCR detection methods have been generated by researchers for different analytical purposes (Babalola, 2003).

2.7.3 Enteric Bacteria Culture Method

The simplest method for the detection and counting of bacteria present in vegetables include the pour plates for *E. coli* and spread plates for *Salmonella*. This requires media preparation, aerobic plate count, enumeration and isolation. Media for preparation consist of a variety of nutrients depending of the agent sought. This may include MacConkey Agar, Salmonella-Shigella agar, Methyl red voges-proskeur broth, E.C Broth, Agar-Agar, Brilliant E.C, Xylose Lysine Deoxy-cholate Agar, Violet Red Bial Dextrose, Lysteria selective agar, Baird Parker. These media are prepared following the instruction from the manufacturers. Some media are sterilized in the autoclave at 121⁰C for 15minutes while others may not follow the same process. XLD for *Salmonella* does not require autoclaving for instance. Rather, this medium is sterilized by boiling through the flame (Eni et al., 2010; Amoah, 2014). This method was chosen for the availability of medium of targeted specie and for the afforadility of material needed to achieve the analyses of the bacteria targeted in this study.

Bacterial culture for detection followed by confirmatory tests for *E. coli* and *Salmonella* were chosen for this study. Media, equipments, material needed for culture, identification and

confirmation during analysis were easily available. The media for these methods were affordable and easy to prepare; the practice was not time consuming and was simple in application, reproducible and results obtained after 24 hours following incubation time were reliable.

CHAPTER THREE:

KNOWLEDGE AND PRACTICES OF PESTICIDES USE BY THE TOMATO FARMERS IN MWEA REGION, KENYA

ABSTRACT

In Kenya, lots of studies on pesticides use in tomato cultivation decry their misuse maybe due to non adherence to prescribed application procedure. This study was conducted to assess the knowledge on-farm pesticides practices in a tomato growing area. A cross sectional study using a semi structured questionnaire to assess farmers' knowledge and practices on pesticides use in tomato farms was conducted in Mwea Region in February and March 2017. The questionnaire previously pretested in Kamulu was administered to 52 farmers randomly selected in Mwea East and West. The sociodemographic characteristics, farmers' knowledge on pesticides use in tomato farms and some corelations were investigated and data was entered in MS excel and analysed using SPSS. About 99% of farmers were men; 46% of farmers were between 36 to 49 years old; 39% and 39% attended primary and secondary schools; 15% and 4% had tertiary and university levels of education. Nearly 69% of respondents knew pesticides through other farmers; 31% through agrovets, extension officers and agricultural experts. Up to 56% farmers knew pesticides names through colleagues, 44% got them from agrovets, agricultural officers and chemical companies and 98% farmers used pesticides approved by the government. Up to 93% spray pesticides once a week in farms and 77% observe at least 7 days pre-harvest interval. A negative correlation (p= -0.295) was found among farmers with training on pesticides use and farm sizes. About 85% agreed that waiting for the preharvest time is farmers' local knowledge in the region. Only, 6% of farmers spray pesticides in post-harvest period while 85% said that, the preharvest period is to avoid having pesticides in harvested produces. The farmers demonstrated good knowledge of pesticides usage practices potential meaning that, tomato sold in Kenyan markets may be safe for consumption.

Published as: J.H. Nguetti, J.K. Imungi, M.W. Okoth, S.E. Mitema, W.F. Mbacham, J. Wang'ombe (2018). Assessment of the knowledge and use of pesticides by tomato farmers in Mwea Region, Kenya. African Journal of Agricultural Research, 13(8): 379-388

3.1 INTRODUCTION

From the archaic to the modern agriculture, pesticides have been used in farms to control crops' pests and diseases. Many pesticides are currently produced (Garcia et al., 2012) and this might be confusing to the tomato farmers. Some of these pesticides may remain as residues (Musebe et al., 2014) such that, food eaten is likely to contain these chemicals on the harvested product. Under this scheme, current food is therefore likely to contain chemicals and contaminated diet may be seen as a health hazard to consumers. Pesticides adopted for crop protection become harmful as early depicted in the fifties (Peshin et al., 2009; Amuoh et al., 2011; Nunifant, 2011; Pujeri et al., 2015). The situation is worsened when farmers have little or incomplete knowledge which is likely to lead to chemical misuse on crops (Inonda et al., 2015).

The intensive use of pesticides in tomato production (Asante et al., 2013) seems to favour production of good quality of produce for markets to generate good revenue for farmers and vendors. However, if the use of pesticides is not controlled, these benefits could be lost by production of food that endangers consumers' health and cause diseases spanning from diarrhea to cancer in humans (WHO, 2015). To reduce the burden of disease from pesticides ingestion, the European Union (EU) has designed the maximum levels of pesticides residues for individual crops.

In Kenya, lots of studies on pesticides use in farms decry their misuse probably due to non adherence to prescribed application procedures. In an assessment of synthetic chemicals use in vegetable farming domestically consumed, Inonda et al. (2015) found mainly organophosphate and pyrethroids pesticides residues during dry season. The investigators argued that, farmers' adherence to preharvest period can reduce chlorpyrifos residues at 99%. Together with Mutuku et al., (2014); they decried pesticides misuse in farming. This has lead investigations to say that

in Kenya, pesticides use in tomato production is characterized by limited knowledge on chemical practices (Nyakundi et al., 2010) with regard to when to apply, the preharvest interval and availability of produces in markets (Mutuku et al., 2014; Inonda et al., 2015). Postharvest use, mixing different pesticides before spraying and even use of banned chemicals have been reported in crops production (Nyakundi et al., 2010; Dankwah, 2014; Mutuku et al., 2014). The present study assessed the knowledge and practice of pesticides use by tomato farmers in Mwea Region.

3.2. STUDY DESIGN AND METHODOLOGY

3.2.1 Study Design

A cross sectional study using a semi structured questionnaire to assess farmers' knowledge and practices on pesticides use in tomato farms was conducted in Mwea Region in February 2017. A total of 52 tomato farmers were randomly selected and interviewed. The semi structured questionnaire administered was previously pretested in Kamulu and improved to be easily understood by respondents in order to get the expected outputs. Mwea was chosen because it is one of the four major tomato producers in Kenya and the study covered the whole farming area partitioned into Mwea East and West.

3.2.2 Methodology

3.2.2.1 Study setting

The study was conducted in Mwea region situated in Kirinyaga County in the central province of Kenya. Mwea was purposively selected because it is a region of small-scale tomato farmers partitioned into Mwea East and West. The population in Mwea is at around 150,000 persons. An estimate of 73% of the population is fully engaged in agriculture (Mwangi, 2014). Tomato production is a major business utilizing more than one-third of the total cultivated land of the

Sub-county. The Sub-county is among the four major production areas of tomato in Kenya (Mueke, 2014). The agricultural activities have profoundly transformed the region structurally and economically. Water flowing in the rivers is from Mount Kenya; this has always been of great interest for farming tomato in the area. An estimate of 73% of the population is fully engaged in agriculture; 13% are casual laborers, 7% are involved in business and 3% are formal employees (Mwangi, 2014).

Mwea is situated at about 100 km in the south east of Nairobi City. The Sub-county is located at latitudes 37°13'E and 37°30'E and longitudes 0°32'S and 0°46'S. The Sub-county is known as a tropical area with a semi-arid weather, the average annual temperature is approximately 23 to 25°C. This temperature differs by 10°C between the minimum noticed in June and July and the highest seen from October to March. The region is positioned at a high altitude at around 1,800m above the sea and at 50km south of the Equator (Muuru, 2009). Its climate is both cool and sunny; this provides natural good conditions for farming. The region has an annual average rainfall fluctuating between 1,000mm and 1,800mm (Ndiiri et al., 2013). The location of Mwea Sub-county is shown in the map of figure 4.1

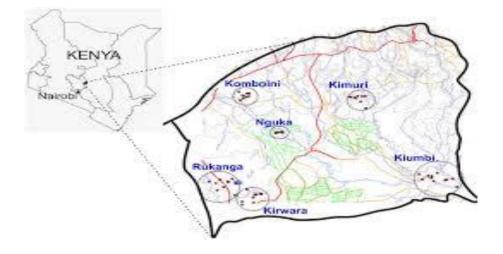


Figure 3.1: Map of Mwea Sub-county in Kirinyaga county Kenya

3.2.2.2 Sample size calculation

The size of the tomato farmers in Mwea was estimated at around 1,000 for both Mwea east and west. But, the sample size calculation was restricted to the Fisher formula used by Mutete (2005). In this case, the tolerance limit was set at 10% (0.1) significance.

$$nf = \frac{Z^2 Pq}{d^2}$$

n = The minimum sample size

Z = Standard normal deviation 1.96 equivalent to 95% confidence interval

P = Proportion of all the agricultural farmers in Mwea 73%. But in this case, the estimate for the farmers cultivating tomato in Mwea is 50%

$$q=1-P$$
 \implies $q=1-0.5$ \implies $q=0.5$

d= Tolerance limit set at 10% (0.1) significance due to the accuracy needed for this survey.

 $n = \frac{1.96^2 (0.5 \times 0.5)}{(0.1)^2} = \frac{3.8416 \times 0.25}{0.01} = \frac{0.9604}{0.01} = 96.04 \approx 96$

Knowing the number of farmers involved in agriculture in Mwea, the formula Yamane developed in 1967 was applied.

 $n = N/1 + N.e^2$ where $\begin{cases} n = \text{sample size} \\ N = \text{Overall number of people involve in agriculture in Mwea} \\ e = \text{Error of sampling} \end{cases}$

This formula of Yamane was modified for a situation where the tomato farmers are less than 10,000 as done by Rwanda (2015). The estimated number of farmers exclusively cultivating tomato was estimated at 200.

The formula becomes

 $nf = \frac{n (1+n)}{N} \begin{cases} -nf = \text{Desired sample size} \\ -n = \text{sample Size for and estimated population (96)} \\ -N = \text{Estimated population of the tomato farmers (200)} \\ 96(1+96) \end{cases}$

We finally obtain: $nf = ---- = 46.56 \approx 47$

200

By adding 10% attrition (+ 4.7 \approx 5), the desire sample size was finally: nf = 47 + 5 = 52

3.2.2.3 Sampling procedure

Preliminary field reconnaissance visit to Mwea was done for meeting with some Agricultural Extension Officers for more information on the farming site. This included, the average number of tomato farmers, pesticides regularly used, the contribution of the business in the area and the challenges faced by the tomato farmers, farmers knowledge on pesticides and their practices on pesticides use. Information was obtained through literature review and random semi-structure questionnaire to some researchers who conducted studies in the area. The Pests Control Product Board (PCPB) and Kenya Plant Health Inspectorate Service (KEPHIS) were visited for meeting with some experts. Some agrovets were interviewed in Nairobi to understand their implication in the management of pesticides with tomato's farmers. The tomato's agribusiness in Kenya was also studied by meeting with some middlemen and tomato retailers.

The questionnaire was pretested in Kamulu and improved according to observations and findings after meeting with some tomato farmers. Some questions were discarded while others were added to fit with farmers understanding. An effort to obtain close or same results if the questionnaires are to be reproduced was made. A Masters student was recruited during this field work for translation from English to Kiswahili and vice versa. The study was conducted in February and March 2017. Two key enumerators were recruited in Mwea and trained to administer the questionnaire. Consent and voluntary participation was always obtained from interviewees after introducing the aim of the study. The enumerators were requested to collect data equally from both sides, Mwea East and Mwea West. The simple random sampling or design effect was included during farmers' recruitment and conversations. Data were collected from the peasants who only cultivate tomato during all seasons. The number of farmers to

interview was obtained on the basis of information given by the Agricultural Extension Officers and also from literature review.

3.2.2.4 Study tool

A semi-structured questionnaire was designed for the collection of data from tomato farmers. The data collected from tomato farmers included sociodemographic characteristics, knowledge on pesticides use, experience in tomato farming, practices of pesticides use, farm size and annual income.

3.2.2.5 Data collection procedure

The questionnaire targeted the sociodemographic (gender, age, level of education) characteristics of respondents. The socio demographic helped to know whether farmers were mostly males or females; their age range (old people, middle age or young); if agriculture in this region is an activity abandoned to non-educated or if it is an inclusive activity gathering together illetrates and literates. Also, farmers' knowledge on pesticides and their practices on pesticides used were covered by the study. The aim was to know where farmers get their information on pesticides. As well, the questionnaire was to guide on whether information farmers have was appropriate for pesticides use, whether it was useful for good agricultural practices in farms and alignment with the government's recommendation. Pesticides practices in this case included farmers' experience in pesticides' use in tomato farms; the number of times pesticides are sprayed in farms during a season, preharvest period and potential postharvest spray. Observation during interviewed was also considered as part of study. This help to understand whether farmers' answers and practices matched together.

3.2.2.6 Data analysis

The Statistical Package for Social Sciences (SPSS) software was used for data analysis. The data was entered and cleaned. The means were used to get the averages of farmers and standard deviation to measure the dispersion. The descriptive statistics were used to generate the picture of farmers and their knowledge on the use of pesticides in tomato farms. The linear regression contributed to show that the middlemen use the level of education of farmers to buy the tomato. The Bivariate correlation based on Pearson was used to measure the association between two variables. The level of significance was tested at 95% confidence.

3.3 RESULTS

3.3.1 Sociodemographic Characteristics of the Farmers

The sociodemographic characteristics reveal that most of farmers had been to school and most participants were male farmers with little number of females (10%) in the activity. Majority of farmers had at least primary (38.5%) and secondary (38.5%) level of eductation. These sociodemographic characteristics are shown in Table 3.1

Demographic			
characteristics	Variables	Frequencies (N)	Percentages (%)
Gender	Male	47	90.4
	Female	5	9.6
Age	18-28	4	8
	29-35	12	24
	36-49	23	46
	50-above	11	22
Level of education	Never attended school	2	3.8
	Primary level	20	38.5
	Secondary level	20	38.5
	Tertiary level	8	15.4
	University level	2	3.8

Table 3. 1: De	emographic c	characteristics	of	farmers
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3.3.2 Respondents of Knowledge and its Source on Pesticides Application

The knowledge of farmers on pesticides, experience on tomato farming and pesticide use, pesticide practices, the relationship with colleagues, farm size and annual income assessed are presented in Table 3.2. About 92 % of farmers use pesticides approved by PCPB, 98 % were aware of the usefulness of pesticides in tomato farms and 100 % knew how to use pesticides on farms. Many farmers (77%) observed the PHI and majority (56%) had more than 6 years of experience in the use of pesticides. About 85 % knew the preharvest withdrawal period 4 % followed the manufacturer's instructions and the finding reflects a weak (2.2%) contribution of agricultural extension officer. A total of 77 % of farmers said that, they should spray at least 7 days before harvesting and few farmers (2%) mentioned the manufacturers' instructions.

		Frequencies	
Question	Answer	(n)	Percentage %
First time was board on	Training	15	28.8
First time you heard on pesticides for tomato protection	Agrovet	3	5.8
pesticides for tomato protection	Farmers	34	65
	Single source of		
The source from which farmers	information	36	69.2
know how to use pesticides	Multiple sources of		
	information	16	30.8
Pesticides approval by the Pest	Yes	48	92.3
Control Products Board (PCPB)	I never know	1	1.9
Awareness of the usefulness of	Yes	50	98
Pesticides	No	1	2
Knowledge of the use of	Yes	51	100
pesticides in tomato farms	No	0	
	Always reminded by		
	the extension officer	1	2.2
	It is written on the		
	instruction of the		
7 days of pre-harvest interval	manufacture	2	4.3
and local knowledge	It is the culture in the	•	
	area	39	84.8
	Depends of the day of		
	market	3	6.5
	Others	1	2.2

Table 3.2: Farmers' knowledge on pesticides use in tomato farms

	Able to give some		
Names of the mostly used	names	49	96
pesticides in tomato farming	Unable to give any		
	name	2	3.8
Knowledge of names of pesticides use in farms: The	Other farmers	30	56
influence of other farmers	Agrovets	21	44
	2 years	8	15.4
Years of experience in	5 years	6	11.5
pesticides in tomato farms	6 years	4	7.7
-	More than 6 years	29	55.8
	Every 7 days	39	75
Interval of pesticides spray in	Every 14 days	5	9.6
tomato farm	Every 9 days	1	1.9
	No timing	7	13.5
	14 days before	1	15.5
	harvesting	4	7.7
	At least 7 days before		
	harvesting	40	76.9
Quality of social interaction	4 days	4	7.7
between farmers and the PHI	Depends of the		
	intruction of the manufacturer	1	1.9
	Others: 2 days; 3 to 4	1	1.9
	days; 6 days	3	5.8
Pesticides spray in post-harvest	Yes	3	5.8
period	No	46	88.5
Middlemen have preferences	Yes	48	92.3
between yellow and red tomato	No	4	7.7
	0.25 to < 1 acre	29	56.9
	1 to < 2 acres	12	23.5
Farm size	2 to $<$ 3 acres	3	5.9
	3 to < 4 acres	5	3.9
	4 to 5 acres	5	9.8
	[5,000 - 59,000]	14	31.8
A	[60,000 - 199,000]	17	31.5
Annual income	[200,000 - 599,000]	20.4	24.1
	[600,000 - 1,200,000]	2	3.8
We we are the interface we activite of			
Were you trained on pesticides'	Yes	32	62.7

3.3.3 Association between Farmers' Experience and Knowledge of Pesticide Used

A correlation between farmers experience in tomato farming and knowledge of names of pesticides use is shown in Table 3.3.

In order to know whether the number of years spent in pesticides use had an influence on the knowledge of names of pesticides used in tomato farms, a bivariate correlation was assessed between the two variables and a positive association (p=0.025) was found between them using a Spearman's rho coefficient correlation.

			Number of years you have used pesticides	Knowledge of names of pesticides used in tomato farms
Spearman's rho	Number of years you have used pesticides	Correlation Coefficient	1	0.025
		Sig. (1-tailed)		0.433
		Ν	50	50
	Knowledge of names of pesticides used in tomato farms	Correlation Coefficient	0.025	1
		Sig. (1-tailed)	0.433	
		N	50	52

Table 3. 2: Association between knowledge of pesticides' names and farmers' experience

3.3.4 Periodicity for Pesticides Application in the Tomato Farms

A correlation between the number of times of pesticides spray and farmers training is shown in Table 3.4.

An association between the number of time farmers spray pesticides on-farm, their training on the use of pesticides and farm size was found. Results revealed a weak and negative correlation (p=-0.295) between farmers' training and farm size when tested at 95% level of significance. Their farm size might dictate the timing of pesticides spray whether they are trained or not because of the socioeconomic challenge of the modern world.

			Number of times of pesticides spray	Were you trained on pesticides use?	Farm size
	Number of times	Correlation coefficient	1	0.169	0.005
Spearman's rho	of pesticides spray	sig.(1-tailed)	•	0.128	0.486
		N	48	47	47
	Were you trained	Correlation coefficient	0.169	1	-0.295*
	on pesticides use?	sig.(1-tailed)	0.128	•	0.019
		N	47	51	50
	Forma size	Correlation coefficient	0.005	-0.295*	1
	Farm size	sig.(1-tailed)	0.486	0.019	•
		Ν	47	50	51

Table 3. 3: Correlation between farmers' training and farm size

Correlation is significant at the 0.05 level (1-tailed)

3.3.5 Association between Level of Education and Preference of Middlemen

An association between the level of education and the preferences of the middlemen was found as shown in Table 3.5.

The variables level of education and preference of middleme correlate positively (p=0.000) and allow establishing the kind of potential relationship that might be existing between farmers and middlemen. It is possible that, middlemen contribute enormously to the misuse of pesticides in tomato farms as they use the level of education of farmers to make the choice of the produce they want to buy.

		Level of education	Do middlemen have preference
	Level of education		1
	Do middlemen have		
Pearson	preference		
Correlation	Level of education		
	Do middlemen have		
	preference	1	
	Level of education		0.000
	Do middlemen have		
Sig (1 toiled)	preference		
Sig.(1-tailed)	Level of education		
	Do middlemen have		
	preference	0.000	
	Level of education		48
	Do middlemen have		
Ν	preference		
1 N	Level of education		
	Do middlemen have		
	preference	48	

Table 3. 4: Correlation between level of education and preference of middlemen

3.3.6 Correlation between the Annual Income and Farm Size

An association between annual income and farm size found in Table 3.6 is revealing that, the annual income is related to the size of the area on which farmers have invested (p < 0.01).

		Annual Income	e
		of farmers	Farm size
Pearson Correlation	Annual Income of farmers	1	0.673**
	Farm size	0.673**	1
Sig. (2-tailed)	Annual Income of farmers	•	0.000
-	Farm size	0.000	
N	Annual Income of farmers	44	44
	Farm size	44	44

Table 3. 5: Corre	elation between	annual income	and farm size
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**Correlation is significant at the 0.01 level (2-tailed)

3.3.7 Gender, Age, Level of Education and Annual Income on Awareness of Pesticide Use

An association between the farmers' knowledge and sociodemographic characteristic is shown in Table 3.3

			Means scores ±	
Demographic	Variable	n	SD	P-value
Gender	Male	47	90.92±0.495	0
Genuer	Female	5	9.075±0.262	0
	22 - 35	16	31.8 ± 0.080	
Age	36 - 50	25	51.2±0.218	0.061
	51 - 70	9	17.0 ± 0.000	
	Primary	20	37.37±0.484	
	Secondary	20	38.87±0.491	
Education	Tertiary	8	16.25 ± 0.585	0.003
Education	University	2	3.47±0.176	0.005
	Never attended School	2	4.02±0.530	
	[5,000 - 59,000]	10	28.5±0.395	
Annual	[60,000 - 199,000]	17	40.5 ± 0.225	0.091
income	[200,000 - 599,000]	11	26.2±0.389	0.091
	[600,000 - 1,200,000]	2	4.8 ± 0.000	

 Table 3. 6: Mean scores of farmers' knowledge of pesticide with their sociodemographic characteristics

It was found that; mature people '51 to 70 years old' seemed to be less knowledgeable, followed by the age interval '22 - 35 years old' and lastly respondents from [36 to 50 years old] who's scored a bit higher than the others.

An association of farmers' knowledge of pesticides with their socidemographic characteristic showed that males were significantly knowledgeable than females (p = 0.000). The knowledge on pesticides usage does not influenced of farmers by age (p = 0.061). A negative correlation (p = -0.003) between the level of education and potential farmers' pesticides practices in tomato farms was observed. Annual income (p=0.091) does not correlate with knowledge on pesticides use on-farms.

3.4 DISCUSSION

Sociodemographic characteristics of respondents

Involvement of many males in tomato farming in this community could be linked to some factors like intensive labor as reported similarly by Dankwa (2014) in Ghana or the amount of cash provided by tomato at postharvest. Probably, many families get through sales of tomato considerable amounts that contribute to the families' welfare all year round mainly due to irrigation farming highly noted during the survey. Males were mostly responding to the questionnaire. Maybe, the culture of the community requires men to step forward on behalf of the family in any given occasion involving the family like in this study. As rooted in African culture, females cannot take the lead of an activity when the husband is fully connected. Maybe, reasons elucidating the utility of men may also engulf the needs for good organizational skills, quite number of workers, financial input and attention to details (Rutledge et al., 2015).

These findings confirm studies in India by Pratibha et al. (2015) and, in Cameroon by Tarla et al. (2015). The study contrasts the report of Ayandiji and Omidiji (2011) in Nigeria who did not observe a great difference (51% males versus 49% females) among gender in their survey.

Most farmers from this region can read and write. This appears to be an asset for the use of pesticides in tomato farming by farmers. Easy communication can be established in both English and Kiswahili for the majority during capacity building and, easy translation can be done to the lowest of non-educated farmers. For the minimum who have never attended any school, it is expected that information received by the literates on pesticide used in tomato farms can easily be transmitted to those unable to read and write. These results contradict with the study done in

Nigeria by Ayandiji and Omidiji (2011) who conducted postharvest tomato losses among farmers and found that, 83% had primary level of eduction.

The first time farmers heard about pesticides for protecting tomatoes in farms

The sourcing for pesticides varies in this study. Multiple source of information (agrovets, agricultural expert, agricultural extension officer and during training) is a possibility for accurate, different or confusing information. The single source of information may stand as erroneous, improper and non-updated information. This can be confusing in some points as the pre-harvest interval observed by farmers. Farmers may be dealing among themselves either due to unavailability of agricultural extension officers (too few to serve all the farmers) as noticed in Vietnam by Huynh (2014) or because, the evidence of the good production seen from fellows' farms may influence their behavior and increase their reliability on other farmers.

Also, agricultural officers may be favoring some areas or farmers while neglecting others. Tawiah (2011) in Ghana revealed complaints that, the agricultural agents follow and advise some tomato farmers on the basis of prepaid or post-paid contracts. This practice is unfortunate as the farmers may end up spraying pesticides in farms according to inadequate information and finally produce unsafe tomato harming the health of consumers. This confirms that, the farmers are not trained at same level and consequently do not have the same understanding of the use of pesticides. This may be of health risk to consumers at postharvest mostly if they are not aware of pesticide residues presences in fresh tomatoes sold in markets and the ways of reducing them before consumption in stews and freshly prepared tomatoes. Pesticides use in tomato farms here may therefore be confusing among farmers themselves. This confusion raises the necessity for the harmonization of information leading to appropriation based on the same official knowledge. These observations contradict both the findings from Tawiah (2011) in Ghana where 48% of cultivators received their first information from other farmers and; from Bandara et al. (2013) in Sri Lanka where 48.9% knew pesticides for the first time from neighbors and 51.1% got the first information from multiple sources (Extension Officers, Farmers Cooperative and Dealers). However, observation of this study confirms the report from from Jamali et al. (2014) in Pakistan where 25% deal with multiple sources of information.

The source from which farmers know how to use pesticides

Agrovets are the outlets for farmers to buy pesticides and this gives them the legal authority among farmers. The extension officers and industries (which usually promote their chemicals) were ignored by the participants. The source for pesticides use seems appropriate but requires clarifications on the quality of information provided by the agrovets.

The chemical shops known as "agrovets" are business oriented and may not have enough time to train or transfer information required for the best use of pesticides to the beginners in tomato farming. If not reminded, agrovets may assume that, tomato farmers buying chemicals already have appropriate knowledge before ordering. This can be crucial as pesticides mismanagement by farmers is decried by a number of studies in Kenya (Mutuku et al., 2014; Tandi et al., 2014).

Assessment of pesticides use by farmers and their source differ and reveal several limits worldwide. Wasudha et al. (2015) in Surinam found for instance that, farmers knew pesticides use in vegetable by knowledge received from parents, other farmers and pesticides shops. Such practices may either be accurate or contain missing information and gaps for the new generation of farmers. Periodic follow up and capacities reinforcement should be inserted in strategic plans of governments. These can include information such as the last spray and withdrawal period;

essential actions while spraying and after spraying; recommended pesticides to use in tomato farms and; potential health risks and exposure from misuse of pesticides in crops, environment and on farmers.

Above this, the chemical shops might not be qualified enough to provide accurate, complete and pertinent information to farmers for the best use of pesticides in tomato farms. If trust should be on agrovets, criteria and evidence for the level of education of personnel in agrovets shops should be defined; a roadmap established and, quality assessment and audit adopted by the government. Such strategy or measures can be applied worldwide in countries with economies in transition or, in developing countries. These observations are consistent with both the studies done by Tarla et al. (2015) in Cameroon where farmers rely on chemical vendors. Also, they confirm the work from Jamali et al. (2014) in Pakistan where 81% of farmers receive knowledge through traders.

Knowledge of pesticides approved

Farmers were very aware to use pesticides approved by the Pest Control Products Board (PCPB). This indicates a good sign of communication between farmers themselves and between farmers and agrovets. This also reveals the quality of pesticides regulation, management and distribution within the country by the authority.

The high level of regulatory requirement found in the present study is however not common. For instance, such knowledge is not the same in some sub-Saharan African countries (Tarla et al., 2015). The misappropriation of recommended pesticides by the legal authority might have led farmers in some areas to consider pesticides as an instrument that helps to produce more tomato (OO et al., 2012). The current finding agrees with the findings in Pakistan by Jamali et al.

(2014), but contradicts those reported by Tarla et al. (2015) in Cameroon where 69.9% of the farmers were not aware of the relevance of pesticides approval by the government and; only 15% knew that they had to look at the registration number before getting pesticides needed from the agrichemicals shops.

Awareness of the usefulness of Pesticides in tomatoes

Only few (2%) of the respondents were not aware of the importance of pesticides in tomato farms. Considering that 98 % of farmers were aware of importance of pesticide use, it can be said that, it is a common tool for tomato farming in this area. Paiboon and Tikamporn (2014) argue that, awareness is the response based on previous experience and related to the effects that happened and which lead to be conscious of the situation. It thus becomes useful to interrogate the quality of awareness claimed by farmers on the usefulness of pesticides in tomato farms.

Based on their source of information, the usefulness of pesticides in farms goes from mouth to ear and spread easily among farmers. The information content in such a chain might decrease, be distorted, be incomplete and contain incorrect advice leading to malpractices. This may lead farmers into wrong, inappropriate, indecent and invalid use of synthetic chemicals in tomato farms. Additionally, the relevance of the content released may depend on who shared the information, the place where it was given, the status and mood of the person at the moment of sharing. As well, the quality of content of the message received previously by the informant, the level of understanding of the listener and his/her capacity of transmitting or applying the previous information received. The reliability of the speaker as perceived by the listener also affects the effectiveness of the information received. Regarding this, simple additional information for a better understanding and good practice of the use of pesticides is needed. These findings corroborate with the finding by Wasudha et al. (2015) in Surinam who reveal that, 100% of the farmers were aware of the usefulness of pesticides

Knowledge of the use of pesticides in tomato farms

All farmers knew how to use pesticides in tomato farms. Nonetheless, studies of chemical use in tomato farms in Kenya have revealed numerous shortcomings in the practices of farmers (Nyakundi et al., 2010; Mutuku et al., 2014).

The claim of good knowledge of use of pesticides in tomato farms is questionable. Probably, a step by step procedure may reveal some flaws sustained by improper practices adopted or applied by some cultivators. This may include: spray following the direction of wind, no break for cigarette smoking before ending with spray, changing cloths and washing them, taking a shower before eating and smoking; disposing empty containers of pesticides in the farms after usage and not throwing everywhere as seen in many farms, starting another task within the farms without taking a bath and changing the cloths (Paiboon and Tikamporn, 2014). These findings contradict those from Pakistan by Jamali et al. (2014) who found many irregularities on the practices of the farmers in the use of pesticides in vegetables.

Seven days pre-harvest interval and local knowledge

The use of pesticides in this region seems to be considered by farmers as a local indigenous knowledge. As such, farmers have insufficient knowledge on the synthetic chemicals being used in tomato farms motly at the time when insects' resistance to pesticides is currently decried (İnci and Ikten, 2017). In spite of this, farmers of this community are still stuck to culture which does not follow the dynamism of insects' pest depicted as more and more resistant to synthetic

chemicals. This trend might lead into pesticides resistance and their misuse causing tomato contamination and health risk exposure. The farmers of this community seem not to be aware of the potential health's exposure for those consuming tomato contaminated with chemical residues above the maximum limits. They have no idea on pesticide residues in post-harvest tomatoes and its effect on consumers' health (Shashi et al.; 2016). They apply pesticides and wait because they found it rooted in the community. The pre-harvest interval here is not a precaution for the production of safe vegetable for human consumption. If the farmers' knowledge is uplifted, almost all the farmers will follow the rules. These findings are in agreement with those of Wasudha et al. (2015) who found that, 100% of farmers in Surinam knew the pre-harvest time through parents' experience and culture.

Most commonly used pesticides in tomato farming

Most farmers (96.2%) knew names of some pesticides versus few (3.8%) who could not give any name reveals farmers' interest in their duty to protect the crops against pests and diseases. This assertion is supported by the Spearman's rho correlation coefficient (p= 0.025) between farmers' experience and knowledge of names of pesticides use in tomato farms. But still, details on some key aspects such as the safety/care in the way chemicals are used, the quality of protective clothing of farmers (rubber boots, impermeable trousers, waterproof coverings for instance) (Matthews et al., 2003), the number of times to spray from planting to harvesting and, the withdrawal period are important. The observations in the present study are in agreement with the findings of Matthews et al. (2003) in Cameroon where pesticides listed by the farmers were classified as frequently and uncommonly used.

A large number of farmers purchase their pesticides based on concrete evidence in tomato farms. Probably, they witness the status of the produces in farms before asking the names. Although it seems logical for farmers to do so, this result may also be explained by the inability for some farmers to read properly the labels on the containers of pesticides or, their incapacity to remember the names of the pesticides due to illiteracy (Nyirenda et al., 2011; Tarla et al., 2015). This may be the source of different pesticides use in farms from planting to harvesting which can lead to multiple pesticide residues presences at various levels on tomatoes sold in markets.

About 67.3% of the farmers indicated that, the agrovets always promote new powerful chemical products. Farmers are most likely to rely on each other based on the success or the best yields witnessed in the neighboring farms as also shown in India by Pratibha et al. (2015). They learn how others have overcome some difficulties so as to harvest good tomatoes. Before ordering for a pesticide, farmers probably have the reason and expected outcomes from the brand ordered since the most important fact in tomato farming may be the amount of cash to be received from the middlemen at postharvest. The results in the present study support both the finding from Jamali et al. (2014) who reported that, the knowledge for chemicals spray in farms has a variety of origins and, Tarla et al. (2015) who noted that farmers order their chemicals through advices from other farmers, suppliers and Agricultural extension agent.

Years of experience in pesticides use in tomatoes farms

Farmers with less experience in this community have two years and those with high experience have at least six years. Tomato farming has been embraced by the community for quite some time. As such, practices as pesticides use in farms may have become a routine for most of these farmers. Despite this, a dual situation for this category is envisaged: on one hand, the large number of this group seems an asset for use of pesticides in tomato farming. On the other hand, this set can be a source for malpractices in case they did not have appropriate skills for pesticides management. They may have transferred incomplete or improper knowledge to the young generation and, the socioeconomic fallouts will still be harmful for families and society as depicted by Huynh (2014). The necessity to understand the level of pesticides attained by the elders is relevant.

These results are consistent with those from Wasuhda et al. (2015) in Surinam where most respondents (66.7%) and Mispan et al. (2015) from Malaysia where majority of the farmers (60%) have a lot of experience in tomato farming and use of pesticides.

Interval of pesticides spray in tomato farms

A big percentage of farmers (75%) follow the same rule of spraying pesticides every week in farms meaning that, a routine has been established in chemicals application in farms in this community. Although high proportion follows the same rule, the minority may have developed unsafe thoughts on the meaning of pesticides and its role in farms. For instance, 1.9% thinks that synthetic chemicals spray is important for good yields and returns. They may also believe in addition that, the quantity of pesticides wrap in the containers is not enough to cover the surface of the farms for pests and diseases control (Shashi et al., 2016). A bivariate correlation using the Spearman's rho (1-tailed) showed a negative association (p=-0.295) between the farm size and farmers trained on pesticides use. This may indicate that, training on pesticides use may not influence on the practices of farmers depending on their farm sizes.

Institutions in charge of national approval of chemicals in developing countries should be equipped with laboratories for quality control of the chemicals before recommendation. These results agree with those from Mutuku et al. (2014) in Kaliluni – Kenya where 86.1% spray weekly , Lutap and Atis (2013) in Ilocos- Philippines with 90% of weekly application, and in India by Shashi et al. (2016) who found that, 60% of farmers spray pesticides in a weekly basis

in their farms. But, they contrast with those by Wasudha et al. (2015) in Surinam where 50% of farmers spray pesticides twice per day- early in the morning and late in the afternoon.

Pesticides spray in post-harvest period

About 5.8% continue with post-harvest spray of pesticides to protect the produce. This may be explained by the need for making a good deal with the middlemen and the fear of postharvest diseases (*alternaria*, *buckeye rot*, *gray mold*, *soft rot*, *sour rot* and *bacterial soft rot*) attacking the crops (Rutledge, 2015). Other reasons may include: Difficult access to the markets and worries of returns on investment to meet the financial households' needs. Postharvest spray of pesticides on tomato also signifies insufficient knowledge of farmers. They ought to use chlorine gas, thiabendazole, calcium hypochlorite, calcium chloride (CaCl₂), 1-methylcyclopropene (1-MCP) and sodium hypochlorite (Arah et al., 2016; Rutledge, 2015). This finding confirms the finding in India by Pratibha et al. (2015) revealing postharvest by 16% of farmers and in Ghana by Dankwah (2014) who reported 6% of farmers spray pesticides on cabbages while harvesting.

Level of education, preference of middlemen and farm size as determinants of pesticides use in tomato farms

The level of education plays a great role in tomato farming and the middlemen are the assessors determining the welfare of tomato farmers in the farming areas. A correlation between preferences of the middlemen and the level of education was statistically significant (p= 0.000). It can then be said with confidence that, the preference of the middlemen depends on the level of education of the farmers as 92.3% of farmers pointed that, middlemen have preference between tomatoes.

There was a strong correlation between the annual income and the farm size (p=0.000) in which the middlemen still play a key role by determining the variety of tomato they prefer at postharvest. It can confidently be asserted that, the regression explains the independent variable (farm size) on the variability of the dependent variable (annual income). This may show that, more investment in tomato farming may lead to more benefits. This can then be related to the intensive use of pesticides for the return on investment as the middlemen are unpredictable on their choice. This may lead to the misuse of pesticides if farmers do not have good knowledge on potential repercussions on consumers at post-harvest. The Pearson correlation coefficient applied to income and farm size discloses a strong positive association (r= 0.673). This shows that, farmers may likely misuse the chemicals whether they were trained or not. Also farmers may misuse pesticides though they were trained and this can persist if they are not fully aware of their potential negative aspects in post-harvest. This trend reveals the influence of poverty or the need to generate enough income on tomato farms through the use of pesticides. This finding correlates with the work of Nyakundi (2010) who said farmers cultivate tomato in order to address the socioeconomic needs

Influence of gender, age, education and annual income on knowledge of pesticide use

Male farmers had good knowledge on the use of pesticides compared to females (p= 0.000). This knowledge was not influenced by age from which no significant difference (p= 0.061) was found among age intervals. The level of education of participants in understanding the concern of tomato contamination with pesticides was an important factor and was negatively significant (p= -0.003). Respondents with secondary education level seemed to be more conversant with pesticides followed by farmers with primary level of education, then tertiary and those who never attended any school. These results revealed that, the level of education influences good agricultural practices. Farmers' annual income is not absolutely bound to knowledge of pesticides use. The earning of farmers at postharvest is probably random as middlemen are unpredictable in their choice and decision making during the purchase of the produce. No

significant corelation was found in association between knowledge of pesticides and annual income (p= 0.091). Higher knowledge of pesticides use came from farmers earning between [200,000 - 599,000] ksh/year followed by those in the interval of [5,000 - 59,000]. Those with high income [600,000 - 1,200,000] might not be followers of good agricultural practices as they might have a huge size of cultivated land from which they invest a lot (time and finance). From their investment, they only expect high return on investment and might probably be misusing pesticides on-farm to protect the crops in an absolue manner. It can finally be said that, farmers who invest more on tomato farming are more likely to misuse pesticides on-farm depite their level of education and age because their target is on return on investment.

This work is in agreement with the one done in Nigeria by Sanzidur and Chidiebere (2018) who studied the use of pesticides in food crop production. They concluded that, farmers who invest more in farms mostly look for high profit making and, they are more bound to modern agricultural tools as pesticides because they are looking for high production

3.5 CONCLUSION

Most farmers have good knowledge on pesticide use in tomato farms; majority was aware of pesticides approval by the pest Control Products Board. Most farmers practice the preharvest interval as recommended by the manufacturers. Farmers are able to transfer skills on pesticides use among each other and a strong collaboration does exist among them.

CHAPTER FOUR: PESTICIDES RESIDUES LEVELS IN TOMATO MARKETED AND CONSUMED IN NAIROBI METROPOLIS

ABSTRACT

Studies on pesticide levels in vegetable production have been done but little is known on tomatoes sold for consumption in Kenya. This study sought to determine pesticide residues on whole and skins of tomatoes sold in Nairobi and determined the presence of either single or multiple pesticide residues on tomatoes. The study was done from January to June 2017 from three open-air markets and two supermarkets. A total of 240 tomato samples were collected during the study period of which 66 pooled samples comprising 60 and 6 extractions for analyses of whole and skins of tomatoes respectively were done. QuECHERS method followed by a multi-residue standard of 98 pesticide residues using GCMS tandem LCMS/MS were used. About 14 residues were detected during the study period; only 9.09 % and 1.51 % of the samples were above EU and Codex MRLs respectively. Pesticide residues on skins of tomatoes were significantly more (p < 0.05) compared to whole tomatoes. Forty nine pesticides were detected in whole tomatoes and skins had 10 additional totaling 59 residues. The dry months of January, February and March had more pesticides (n= 4) above EU MRLs in tomatoes compared to the wet months of April and May (n=1). Fenamiphos (0.19 mg/kg) and acephate (0.47 mg/kg) levels on the skin were above EU MRLs whereas only dimethomorph (0.03 mg/kg) levels on whole tomatoes were above EU MRL. About 48.5 % of all tomato samples analyzed had pesticide residues of which 27.27 % were single pesticide molecules whereas 21.21 % were multiple residues. Carbendazim, profenofos and azoxystrobin were mostly detected and their levels were below both MRLs. This study shows that most tomatoes sold in Nairobi have pesticide levels below EU and Codex MRLs. Data from this study indicates that most farmers comply with good agricultural practices in pesticides use.

4.1 INTRODUCTION

Tomato is a vegetable with nutritious values cultivated worldwide and is usually eaten fresh in salads, cooked with meals and processed as various products. However, tomato cultivation requires pesticides use on-farm to control damages by pests and diseases (Wasudha et al., 2015). As a consequence, some of the pesticides may leave traces of residues (Musebe et al., 2014) in the produce sold in markets (Hammad et al., 2017). There can therefore be serious issue when pesticides are not used as recommended (Pujeri et al., 2015; Hammad et al. 2017), making tomato a potential vector of harmful residues that can impact negatively on consumers' health. In Kenya, several studies on pesticides use in farms and their residual levels on postharvest crops have been conducted. Manduu (2015) studied the importance of pesticides in farming and found that, food production in Kenya may decrease from 15% to 80% if synthetic chemicals are not applied in farms. However, some reports on chemical use in farms indicate misuse (Mutuku et al., 2014; Manduu, 2015) whereas Musila (2010) reported that, fresh crops marketed in urban areas contain more pesticide leftovers compared to those sold in rural areas. Musebe et al. (2014) conducted a survey on the use of pesticides in tomato farms in Kenya comparing farmers trained on integrated pest management (IPM) and those untrained and observed that, growers with IPM knowledge use few synthetic chemicals than untrained ones. Manduu (2015) investigated pesticides used by farmers on French beans and reported the presence of fifteen chemicals. Oyugi (2012) in a study evaluating farmers awareness of potential health effect of pesticides use on-farms and their measures of protection found that, about 75% were aware of exposure to chemicals use in farms. However, 81% used no protective clothes while spraying the chemical substances in farms. Other studies on laboratory analyses have also provided a number of useful information in food safety. Musila (2010) in a study evaluating chlorothalonil fate on snow peas

and passion fruits observed levels below EU MRL on snowpeas but residues above EU MRL on passion fruit. Oyugi (2012) in his report on pesticide residues in vegetables alternated with tobacco found that, kales had acephate MRLs above the EFSA levels though methomyl's levels were below the prescribed MRLs by the Codex Standards.

Pesticides residues in tomato can bio-accumulate in blood, serum and adipose tissues (Imo et al, 2007; Kang and Chang, 2011; Bhoke, 2012; Shasha et al., 2014; Ruiz-Suárez et al, 2014) and become hazardous for consumers' health. Their health effects is the result of xenobiotics in bodies outcomes of pesticide residues discharged and interferences with hormones, enzymes, growth factors and neurochemicals in key genes of metabolism (Gourounti et al, 2008). Humans' organophosphate pesticides intoxication for instance includes gastro-intestinal upsets, bronchospasms and high urination. Hodkin's lymphoma and cancers may be observed in farmers and children while abortion, fetal death and breast cancer may occur on exposed pregnant women (Stoytcheva and Zlatev, 2011; Shasha et al., 2014; Omwenga, 2013). Similarly, cancers and toxicities (prenatal and neonatal period, endometriosis, infertility) developments; behavioral, neurological, genotoxic, risk of diabetes type 2; damages of liver, kidney, central nervous system or neurotoxicity, thyroid and bladder are potential effects of long exposure to low doses of organochlorine pesticides (Gourounti et al, 2008; Ruiz-Suárez et al, 2014).

The objective of this study was therefore to determine some pesticide residues present on whole and skins of tomatoes sold in some parts of Nairobi region and compare their levels with maximum residue limits (MRLs) standards. It also determined the presence of either single or multiple pesticide residues on tomatoes.

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4.2 MATERIALS AND METHODS

4.2.1 Study Design

A cross sectional study for pesticide residues analyses in raw tomato sold in Nairobi was done from January to June 2017 covering dry (January, February, March) and wet (April, May, June) months. The wet months in Nairobi corresponds to the period with abundant tomato of good quality sold at a low price by retailers in open air markets and supermarkets. Dry months correspond to the period with less tomato of good quality at sight sold at a high price in markets. The criteria for fresh tomatoes sampling included: Red and firm, without disease, no blemishes and no soft or melted part. Sites chosen for sample collection represented retail supermarkets and retail open air markets.

4.2.2.1 Study setting

The study was set out in three retail open-air markets $(OAM_1, OAM_2 \text{ and } OAM_3)$ and two retail supermarkets $(SM_1 \text{ and } SM_2)$ located in Nairobi Metropolis (Figure 4.1).

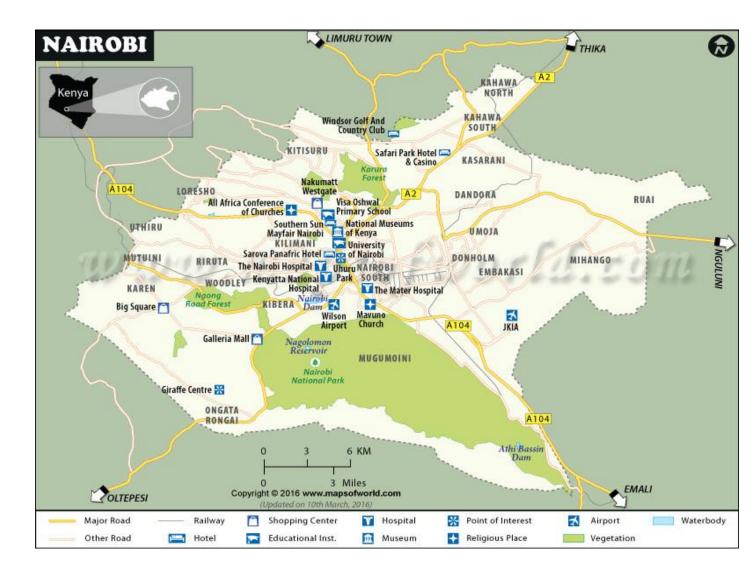


Figure 4.1: Map of Nairobi showing the five sites selected for tomato sampling

4.2.2.2 Sample size calculation

The sample size for tomatoes collection was calculated according to the formula of Fisher as used by Omwega (2013) on fish.

According to the formula, the sample size is calculated as:

$$n = \frac{Z^2 p. q}{d^2}$$

Where:

n= Desired sample size

p= Proportion of the probability in the large population of fresh tomato estimated to contain pesticides residues and enteric bacteria at 50% or (0.5)

q = Proportion of tomato expected to be polluted. It is obtained from (1-p) = (1-0.5) = 0.5

z = Probability of type 1 error set at (1.96) corresponding to 95% confidence interval

d= Tolerance limit set at 10% (0.1) significance due to the accuracy needed for this study.

n =
$$\frac{1.96^2 (0.5 \ge 0.5)}{(0.1)^2}$$
 = 96.04 \approx 96

The formula of fisher was modified for a situation where the vendors were less than 10,000 as done by Rwanda (2015). The estimated number of raw tomato vendors in this case was 200 retailers in the open-air markets

The formula becomes

$$nf = \frac{n (1+n)}{N} \begin{cases} -nf = \text{Desired sample size} \\ -n = \text{sample Size for and estimated population (96)} \\ -N = \text{Estimated population of the tomato retailers (200)} \end{cases}$$

We finally obtain:
$$nf = \frac{96(1+96)}{200} = 46.56 \approx 47$$

By adding 10% attrition (+ 4.7 \approx 5), the desire sample size was finally: nf = 47 + 5 = 52 samples

4.2.2.3 Sampling procedure

Tomatoes were collected twice a month from January to June 2017 with the first sampling done between the 1st and 10th day of the month while, the second sampling was done between the 23rd and 28th day of the month. About one kilogram of tomato samples from each open-air market were randomly collected from four different vendors at different areas, packed and labeled as one sample. Similar sampling procedure was done in super markets in four different areas of the display in boxes. Thus, the total number of tomato samples collected from the different sites was 240 during the study period of which 66 samples were pooled out for samples preparation. Both samples of tomatoes collected from open air markets and supermarkets were used for analysis of pesticides residues in whole and skins of tomato samples. The tomato samples collected were placed in cool boxes and taken to the fridge of the laboratory of Toxicology.

4.2.2.4 Samples preparation

4.2.2.4.1 Whole tomato preparation

Eight tomatoes from each labeled sample from various sites during the first and second collections of the month were pooled, chopped and blended as done by Jallow et al. (2017) before pesticides extraction. Comparison of pesticide residue presence in whole and skins of tomatoes was done once a month either on the first or second collections of the month during the study period and thus, a total of six compared analyses between whole and skins of tomatoes were done during the investigation period. The area for preparation of tomatoes was cleaned and sterilized using acetone between preparations of different samples. Sterile water, aluminum foil, cutting board, tongs, spatula, gloves and stainless steel knife were used. A total of 60 blended samples from the various sites were prepared for analyses of whole tomatoes during study period out of the 240 samples collected from the various sites.

4.2.2.4.2 Skin tomato preparation

A similar number of 8 tomatoes from each labeled sample from either the first or second collections of the month were picked, skins peeled and thereafter blended for extraction as done by Abou – Arab (1999). Similar aseptic techniques were employed as for whole tomatoes. Thus, a total of six blended skin samples out of the 240 samples collected from the different sites were used for pesticide analysis on tomato skins during the study period.

4.2.3 Material and Equipment

Weighing balance (ADAM AFP-2100LC), spatula, centrifuge tube (50ml), gloves, vortex (WiseMix VM-10, Daihan Scientific Co., Ltd), centrifuge (Universal 320R Centrifuge, Hettich

Zentrifugen), marker, pipettes, vials, Nitrogen gas (Fabricated Nitrogen evaporator, gas from Gaslabs), automated shaker (Spex sampler prep 2010, GENO GRINDER), flask, rotary evaporator (Buchi Labotechnik AG Switzerland), GCMS (Agilent Technologies 7890A GC, coupled with 5975 inert Mass Selective Detector);and LCMS/MS (Agilent Technologies 6460 Triple Quad LC/MS coupled with 1290 Infinity II Liquid Chromatography) were used.

4.2.3.1 Reagents for extraction, cleaning and analysis

Extraction salt, acetone and acetonitrile were HPLC grade, isooctane, internal standards (Dichlorvos and malathion d10 for quality control check and effectiveness of sample extraction method in GC-MS and LC-MS/MS respectively were used. Lindane and Dimethoate d6 for quality control check on the performance of GC-MS and LC-MS/MS instruments respectively were also used). The internal standards were spiked on unknown samples, blank, control and calibration standards. The responses of these internal standards were then plotted on a control chart and monitored on daily basis; LC water (from LiChroSolv Merck KGaA 64271 Darmstadt Germany); multi-residues standard for reading 98 pesticide residues (from Dr. Ehrenstorfer GMbH Lboratories, Germany) were used.

4.2.3.2 Sample extraction, cleaning and pesticide residues detection

The "Quick, Easy, Cheap, Effective, Rugged and Safe" (QuECHERS) method was applied as done in China by Zeying et al. (2015) for extraction and cleaning. No peculiar pesticide residue was targeted. A multi-residue standard of 98 pesticide residues was used for detection using Gas Chromatography-Mass Spectrometry (GCMS) combined with Liquid Chromatography tandem Mass Spectrometry (LCMS/MS) as done by Jallow et al. (2017) in Kuweit. Instruments (GCMS and LCMS/MS) were combined to detect volatile and non-volatile compounds respectively.

4.2.3.3 Extraction and clean-up for GC and LC analyses

Ten grams (10 g +/-0.1 g) of each blended and frozen tomatoes together with blank matrix for GC (1 tube for spike and 1 for control) and same for LC were placed into 50 ml centrifuge tubes. A volume of 10 ml of acetonitrile was added in each tube prepared and then vortexed all for 1min. A quantity of 6.5 g extraction salt was added. All tubes were spiked with internal standard to control the level of recovery of analytes as described by Hammad et al. (2017) in Sudan. Quantities of 5 μ l (50 ppb) of lindane and dimethoate d6 were injected to GC and LC samples respectively and tubes were shaken in an automated shaker. They were then centrifuged for 5 minutes at 4,000 rpm and thereafter 500 μ l of aliquots were transferred to two sets of vials labelled for GC and LC. Solvents exchange in GC vials using nitrogen gas for evaporation and reconstitution of vials with 500 ml of isooctane was done for both GC and LC samples pending analysis.

4.2.3.4 Calibration solution for GC and LC

Calibration solution for GC: A quantity of 5 ml of supernatant from the tube of control was transferred to a sterile round bottom flask tube previously rinsed with acetone. Acetonitrile was exchanged for solvent control using rotar evaporator. About 5 ml of isooctane was transferred in the flask and vortexed to mix the solvent with the matrix. An amount of 500 ml of the supernatant was transferred into a vial. A multi-residue standard of 1 ppm was prepared from 10 ppm and was used to prepare serial calibration standards (900 ml of the control was pipetted and 100 μ l of the standard was added to get 1000 ml). Serial preparation of 10 ppb; 50 ppb and 200 ppb were done by dilution for the instrument's calibrations. For the lowest calibration, 10 μ l was pipetted from the multi-residue standard, 985 μ l of control and 5 μ l of internal standard lindane were added. For medium calibration, 50 μ l was pipetted from the multi-residue standard; 945 μ l

of control and 5 μ l internal standard of lindane were added. For the maximum calibration, 200 μ l was obtained from the standard previously prepared, 795 μ l of control and 5 μ l of lindance were then added.

Calibration solution for LC: A quantity of 500 μ l of supernatant was transferred from the centrifuge tube to a vial. A volume of 495 μ l of LC water was added. Then, 5 μ l of the internal standard dimethoate was added and the content vortexed. About 900 ml of LC water and acetonitrile were equally pipetted (50:50) and a quantity of 100 μ l of the standard was added to get 1000 ml (1 ppm). Serial preparation of 10 ppb, 50ppb and 200 ppb were done as described in the case of GC calibration above. In this case, 5 μ l of internal standard dimethoate d6 were used to replace lindane used in the case of GC.

4.2.3.5 Instruments conditions for analysis

Gas Chromatography-Mass Spectrometry (GCMS) conditions for analysis

Analysis with GCMS was as described by Jallow et al. (2017). A GC (Agilent Technologies 7890A GC), coupled with 5975 inert Mass Selective Detector (MS) and containing a column (Varian CP8912:3044.34623 MF17-10-1 350°C: $30m \ge 250\mu m \ge 0.25 \mu m$) was used for analysis. Sample injection was achieved in a split less mode with an injector temperature of 250° C and with an interface temperature of 250° C. The initial oven's temperature started at a rate of 60° C for 1 min at 40° C.min⁻¹, moved to 120° C at 5° C.min⁻¹ to attain 310° C after 20 min. Helium was used as the carrier gas at a flow rate of 1.2ml.min⁻¹ under an injection port temperature of 280 $^{\circ}$ C. Electron ionization was used at -70 eV under an ion source temperature of 240 $^{\circ}$ C with full detection mode ranging from 50 m/z to 500 m/z. The quadrupole temperature was at 150° C, interface temperature at 230 $^{\circ}$ C; the solvent delay was considered at 4.9 minutes. No pesticide residue was targeted; analysis was done for suspect residues on the tomatoes.

Liquid Chromatography tandem Mass Spectrometry (LCMS/MS) conditions

LCMS/MS screening was achieved using a liquid chromatography (Agilent 1200, Santa Clara, CA, USA) couple to a triple quadrupole mass detector (Agilent 6460) plus an Agilent ZORBAX C-18 column of analysis of 50 mm x 2.1 mm internal diameter and 1.8 μ m particle size. The sheath gas temperature was maintained at 400 0 C, and the sheath gas flow was 12 L.min⁻¹. Deionized water containing 0.1% formic acid (mobile phase A), and acetonitrile and deionized water (95:5, v/v) containing 0.1% formic acid (mobile phase B) were used for the gradient program, which began with 10% B for 3 min and was linearly grown to 90% B after 15 min. The column was then reprogrammed for 20 min to get back to 10% B. The temperature of the column was maintained at 35 0 C, and the volume of injection was 10 μ L with a constant move frequency of 0.6 mL min⁻¹. No pesticide residue was targeted as analysis was done for suspect residues on tomatoes.

4.2.4 Data Analysis

Data for various pesticide residues detected in whole or skins of tomatoes were entered into MS Excel spread sheet with parameters including sites of samples collection, whole tomato and skin pesticide residue levels, single or multiple residues and frequencies of pesticide detections were obtained. Pesticides levels of each sample were recorded as means for the skin and whole tomato. Pesticide residue levels obtained for each sample for whole tomatoes and skins were compared with both EU and the Codex MRLs standard for the respective pesticide to note the number of violative samples in each case

4.3 RESULTS

4.3.1 Pesticide Residue Levels in Whole Tomato from Various Sites per Month

There were no pesticide residue levels detected on whole tomato analysis from SM_1 in January; SM_2 in February, April and June; OAM₃ in June and OAM₁ in March and June (Table 4.1). Pesticide residue levels with more than one chemical residue was found in samples from SM_1 (n= 4), OAM₂ (n= 4), OAM₁ (n= 3), SM_2 (n= 2), and OAM₃ (n= 2). Pesticide residue in whole tomato from SM_1 and OAM_2 were 13 and 11 respectively during the study period. Those from OAM₁ and OAM₃ were 6 each and SM_2 had 5 pesticide residues detected (Table 4.1).

Four to eleven pesticide residues were detected in each month from the various markets during the study period. The months of April and June showed low level of pesticides with four pesticides residues detected from all sites in whole tomato. In the month of May, 11 pesticide residues were detected whereas in the month of February, March and January, 9, 8 and 5 residues were respectively detected (Table 4.1).

Market	January	February	March	April	May	June
	Acetamiprid	Azoxystrobin		Carbendazim	Carbendazim	
OAM ₁		Difeconazole				
		Profenofos				
	Profenofos	Profenofos	Carbendazim	Profenofos	Carbendazim	Azoxystrobin
					Alpha	
OAM ₂					Cypermethrin	Carbendazim
					Chlorpyrifos	Profenofos
					Metalaxyl	
OAM ₃	Profenofos	Profenofos	Carbendazim	Acephate	Carbendazim	
		Carbendazim				
		Carbendazim	Azoxystrobin	Indoxacard	Azoxystrobin	Carbendazim
SM_1		Difeconazole	Imidacloprid		Carbendazim	
		Dimethomorph	Difeconazole		Profenofos	
			Dimethomorph	ı	Acetamiprid	
SM_2	Imidacloprid		Imidacloprid		Cypermethrin	
	Acetamiprid		Acephate			

 Table 4. 1: Various pesticide residues detected in whole tomato from various markets and per month during the study period

Key: OAM_1 , OAM_2 and OAM_3 = Different open air market; SM_1 and SM_2 = Different supermarkets

4.3.2 Pesticide Levels in Whole and Skins of Tomato

Pesticide residue levels in whole tomatoes and skins are compared and shown in table 4.2. Some pesticides, detected on skins of tomatoes were however not found on whole tomatoes (Table 4.2). Acephate (0.5 mg.kg⁻¹) and fenamiphos (0.19 mg.kg⁻¹) detected once in January from OAM₂ samples were above EU MRL standard. However, difeconazole also detected in whole and skin tomato samples is not approved for use in the country.

Month	Market	Whole*	Skin*	$Mean \pm SD$	EU MRL	Codex MRL
Fahmany	OAM1	Car (0.05)	Car (0.02)	0.04 ± 0.02	0.3	0.5
February		Imid (0.01)		-	0.5	0.5
			Acp $(0.5)^1$	-	0.01	1
Ŧ	0.110		Act (0.06)	-	0.5	0.2
January	OAM2	Fen (0.02)	Fen $(0.19)^1$	0.10 ± 0.12	0.04	NA
			Pro (0.3)	-	10	10
		Car (0.03)		-	0.3	0.5
A	04142		Azo (0.04)	-	3	1
April	OAM3		Dif $(0.06)^3$	-	2	0.6
			Pro (0.04)	-	10	10
March	SMO		Azo (0.04)	-	3	1
March	SM2		Dif $(0.06)^3$	-	2	0.6
May	SM2	Cyp (0.06)		-	0.5	0.2
		Azo (0.1)	Azo (0.06)	0.08 ± 0.03	3	1
		Car (0.03)	Car (0.12)	0.07 + 0.06	0.3	0.5
June	SM1	Dif $(0.1)^3$	Dif $(0.21)^3$	0.15 + 0.07	2	0.6
			Imi (0.08)	-	0.5	0.5
			Act (0.09)	-	0.5	0.2

Table 4. 2: Mean ± SD of pesticide residues of skin and whole tomatoes samples during the study period

Key: Acp = acephate; Act = acetamiprid; Azo = azoxystrobin; Car = carbendazim; Dif = difeconazole; Fen = fenamiphos; Imi = imidacloprid; Pro = profenofos; Cyp= Cypermethrin OAM_1 , OAM_2 and OAM_3 = Open air markets; SM₁ and SM₂= Supermarket; NA= Codex MRL not available;

 1 = Pesticide residues with levels above EU MRLs standard; 3 = Pesticide not approved for use;

Pesticide residues levels in $mg.kg^{-1}$, * = the number of samples analyzed from whole and skin of tomatoes was six in each case during the study period

Pesticide residues on six analyses of skins of tomatoes were significantly more (p < 0.05) compared to whole tomatoes where 15 pesticide residues were detected on skins and 8 pesticide residues were detected in whole tomatoes (Table 4.2).

The levels of fenamiphos (0.02 mg kg⁻¹) detected in the whole tomato from OAM_2 in January was below the EU MRL (0.04 mg kg⁻¹) and was safe for consumption because it was below the recommended standard. However, analysis of the skin of the same vegetable revealed a

concentration of the same fenamiphos (0.19 mg kg⁻¹) above EU MRL. Pesticide residues in the whole tomato from OAM₂ in January only showed one pesticide residue (fenamiphos 0.02 mg kg⁻¹; LOQ 0.02 mg kg⁻¹; EU MRL 0.04 mg kg⁻¹). But, skin analysis of the same sample showed the presence of three other residues (acephate, acetamiprid and profenofos) with two residues (acephate 0.47 mg kg⁻¹; LOQ 0.02 mg kg⁻¹; EU MRL 0.01 mg kg⁻¹ and fenamiphos 0.19 mg kg⁻¹; LOQ 0.02 mg kg⁻¹; EU MRL 0.04 mg kg⁻¹) above EU MRLs (Table 4.2). Analysis of whole tomato from OAM₃ in April showed the presence of one pesticide residue (Carbendazim). But, analysis of skin of the same batch showed three more residues (azoxystrobin, difeconazole and profenofos) and increased the overall detection to four pesticide residues on the vegetable (Table 4.2).

4.3.4 Pesticide Residues Levels in Tomato from Various Sites during the Study Period

Forty nine pesticides (Table 4.1 and 4.2) were detected in whole tomatoes samples analyses and another 10 more pesticide residues were detected on the skins of tomatoes (Table 4.2) totaling 59 residues (Table 4.3) during the study period. The months of April and June which had only 4 pesticide residues each in the analysis of whole tomato (Table 4.1) finally had 8 and 9 pesticide residues (Table 4.3) after analysis of skins of tomato. Difeconazole not approved for use in the country was detected five times in the months of February (OAM₁ and SM₁), March (SM₁), April (OAM₃) and June (OAM₁) (Table 4.3). The dry months of January, February and March had more pesticides (n= 4) above EU MRLs in tomatoes compared to the wet months of April and May (n=1).

About 32 samples (48.5 %) of tomatoes out of a total of 66 analyses contained pesticide residues (Table 4.3). Tomato samples with single pesticide residue were 27.27% while those with multiple pesticide residues varying from two to five were 21.21%. Only 9.09 % of samples

analyzed had levels above EU MRLs standards whereas 1.51 % of them were above the Codex MRLs standards (Table 4.3). Pesticide residues in tomato samples with levels above the EU MRLs included acephate, fenamiphos and pesticide residues with levels above the Codex MRL standard was only acetamiprid during the study period (Table 4.3).

	January		nry	Feb	February March		A	April		ay	J	une	
	1st		2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
0.134		-	Act (0.07)	Azo (0.02)	Imi (0.011)	-	-	Car (0.03)		- Car (0.06)	-	-	
OAM ₁		-	-	Dif $(0.03)^3$	Car (0.03)	-	-	-			-	-	
		-	-	Pro (0.08)	-	-	-	-			-	-	
	Pro (0.03)		Fen $(0.31)^1$	Pro (0.15)	-	-	Car (0.08)	-	Pro (0.13)	Car (0.03)	Car (0.02)	Azo (0.02)	
OAM ₂		-	Pro (0.22)	-	-	-	-	-		- Alpha (0.04)	-	Car (0.03)	
		-	Act (0.06)	-	-	-	-	-		- Chlor (0.02)	-	Pro (0.04)	
		-	Acp $(0.47)^1$	-	-	-	-	-		- Met (0.04)	-	-	
	Pro (0.27)		Pro (0.12)	-	Pro (0.03)	-	Car (0.08)	Acp $(0.05)^1$	Car (0.03)	-	Car (0.04)	-	
OAM ₃		-	-	-	Car (0.06)	-	-	-	Azo (0.04)	-	-	-	
		-	-	-	-	-	-	-	Dif $(0.06)^3$	-	-	-	
		-	-	-	-	-	-		Pro (0.04)	-	-	-	
		-			Car (0.03)	Azo (0.02)	Azo (0.03)		Ind (0.02)	Azo (0.03)	Act (0.08)	Car (0.02)	Azo (0.08)
		_	-		Dif $(0.08)^3$		Dif $(0.04)^3$	-		- Car (0.03)	-		Car (0.12)
SM_1		-	-		Dim (0.03)		Dim (0.03)	-		- Pro (0.03)	-		Dif $(0.2)^3$
		-	-	-	-	-	Imi (0.03)	-			-		Imid (0.13)
		-	-	-	-	-	-	-			-		Act (0.09)
SM_2	Imi (0.13)		-	-	-	Imi (0.03)	Acp $(0.09)^1$	-			Сур (0.06)	-	
-	Act $(0.5)^2$		-	-	-	-	-	-			-	-	

Table 4. 3: Pesticide levels (means) (mg kg⁻¹) detected in the 1st and 2nd sample collections during the study period from the

various sites

Key: a- Acp = Acephate; Act = Acetamiprid; Al-Cyp = Alpha Cypermethrin; Azo = Azoxystrobin; Car = Carbendazim; Chlo = Chlorpyrifos; Cyp = Cypermethrin; Dif = Difeconazole; Dim = Dimethomorph; Fen = Fenamiphos; Imi = Imidacloprid; Ind = Indoxacarb; Met = Metalaxyl; Pro = Profenofos;

b- OAM₁, OAM₂ and OAM₃ = Different open air market; SM₁ and SM₂ = Different supermarkets

¹ = Pesticide residues with level above EU MRLs^{; 2} = Pesticide residue above Codex MRL; ³ = Pesticide not approved for use

The prevalence of pesticide residues detected during the study period is shown in Table 4.4. A total of 14 pesticide residues were detected in 66 tomato samples pooled and analyzed during the study period of which 12 and 11 pesticide residues were both below the EU and Codex MRLs standards respectively (Table 4.4). Even though, carbendazim, profenofos and azoxystrobin pesticide residues were mostly detected in frequencies of 15, 11 and 7 respectively their levels were below EU and Codex MRLs standards (Table 4.4).

		EU MRL	Codex MRLs	Number of	number above EU	number above Codex
Pesticide Residues	LOQ			detections/60	MRLs	MRLs
Carbendazim	0.02	0.3	0.5	15	0	0
Profenofos	0.02	10	10	11	0	0
Azoxystrobin	0.02	3	1^{a}	7	0	0
Difeconazole	0.02	2	0.6^{a}	6	0	0
Imidacloprid	0.02	0.5	0.5	6	0	0
Acetamiprid	0.02	0.5	0.2^{a}	4	0	1
Acephate	0.02	0.01	1	3	3	0
Dimethomorph	0.02	1	1.5 ^a	2	0	0
Alpha Cypermethrin	0.02	0.07	0.2	1	0	0
Chlorpyrifos	0.05	0.05	NA	1	0	0
Cypermethrin	0.05	0.5	0.2	1	0	0
Fenamiphos	0.02	0.04	NA	1	1	0
Indoxacarb	0.02	0.5	0.5	1	0	0
Metalaxyl	0.02	0.2	0.5	1	0	0

Table 4. 4: Frequency of various pesticide detected and number of samples above EUMRLs and the Codex MRLs standards

Key: NA = Codex MRL not available; ^a = standard for fruiting vegetables other than cucurbits

4.4 DISCUSSION

Pesticide residue levels in tomatoes were mainly high during the dry months of January, February, March and May. Previous studies on pesticide residue levels in Kenya showed that, rains, air displacement, temperature and humidity are probably some of the factors influencing the presence and levels of chemical residues on vegetables at postharvest (Musila, 2010). April and June had low pesticide residues because of heavy rains in farming areas which could probably have washed the vegetables most of the time farmers sprayed pesticides. With little or no rains in dry months, Castro et al (2016) suggested that sunny periods may contribute to chemicals spreading and sticking on the produce surface and this may be the reason for elevated levels during dry seasons we observed in this study. Our finding is in agreement with the report of Musila (2010) on trend of chlorothalonil leftovers in French beans and snow peas behavior in dry and hot period during which high pesticide residue levels were recorded in Kenya. With frequent and long exposure to sunny days, surfactants of pesticides facilitate wounds formation on the surface of tomatoes (Gu et al., 2011) and make pesticides to adhere much better to skins and even ease their penetration through heads as suggested by Abou-Arab (1999). Scarcity of moisture during dry period increases pests and disease attacks (Rumbidzai et al., 2017) which also increases on-farm pesticides use and thus, their spreading on skins and firm stickiness due to adjuvants (Castro et al., 2016). This may explain Kithure et al. (2014) report on levels of deltamethrin above the Acceptable Daily Intake (ADI) in dry period in their study of pesticide residues in vegetables Kenya. On the contrary, Musila (2010) detected few pesticide residues on fresh vegetables during wet season.

Our finding of more pesticide residues on the skins of tomatoes is in agreement with the report of Abou Arab (1999) who observed high pesticide levels in the skins and recommended skin peeling to reduce pesticide residue levels. These pesticide residues detections on skins of fresh tomato in markets may be related to farmers' malpractices on the use of pesticides during farming (Hammad et al., 2017). Levels of acephate and fenamiphos may suggest that, a consumer of this tomato could be exposed to fifty (50) and five (5) times respectively the acceptable daily intakes of these residues recommended in fresh tomato (Regulation (EC) No 396/2005). Such pesticide residues levels above MRLs standards in a human body may be of health concern (Gourounti et al., 2008) and may contribute to a pesticide related disease such as

cancer in the human body (Stoytcheva and Zlatev, 2011; Shasha et al., 2014; Ruiz-Suárez et al, 2014). The detection of multiple pesticide residues on skins at postharvest in this study and other studies could be the explanation for several studies on peeling tomato while processing (Abou-Arab, 1999; Garcia and Barrett, 2006; Xuan et al., 2014; Graziela et al., 2015). Levels of pesticides observed on the skins of tomatoes in our study concurs with the report of Mbugua (2015) in Kenya who also recommended skin washing to reduce levels of chemical residues on fresh tomatoes.

Several studies have reported the misuse of pesticides in farms in Kenya, Africa and India as well as pesticides' postharvest spray on tomato (Mutuku et al., 2014; Tandi et al., 2014; Himani et al., 2015). Some of the reasons of these practices include prevention from postharvest pests and disease attacks from farms to metropolitans' markets (Rutledge, 2015; Jankowska et al., 2016), insufficient knowledge of good agricultural practices and non-observation of postharvest withdrawal period (Pujeri et al., 2015). Probably, farmers might be unaware of postharvest safety control and this may have led to a situation of pesticide misuse in vegetables' farms. The detection of unregistered pesticide like we observed in the case of difeconazole is similar to the report by Raini and Kulecho (2008) in Kenya who reported illegal trade of pesticides within the country. However, difeconazole is approved for use in Russia against early and late blight in tomato and potato farms though its weak action on targeted diseases (Elansky et al., 2016).

The detection of non-approved pesticide in our study also concurs with the report of Jardim and Caldas (2012) in their study for a period of 10 years in Brazil reporting the use of unauthorized pesticides. Kiwango et al. (2017) in Tanzania who analyzed vegetables detected dichorvos pesticides which is restricted for use in large facilities for maize grain storage. Musila (2010) in

his study in Kenya also reported the presence of chlorothalonil which is not registered on passion fruits production in Kenya.

Single and/or multiple pesticide residues were found in fresh tomatoes sold in some markets in our study. Currently only MRLs of single pesticide residues in fresh vegetable is regulated by some national and international standards (Fernandez-Alba and Garcia-Reyes, 2008; FAO, 2009; Fussell et al., 2016). The presence of multiple residues has raised concerns as their regulation is still not established (Mutengwe et al. (2016). Even in the European Union where countries as Austria, Cyprus and Croatia (EFSA, 2016) have experienced similar concern, consensus is silent. Accordingly, Szpyrka et al. (2015) in Poland recommended surveillance of chemical residues on vegetables between farms and markets. In Denmark, the multiple residues intake by people were evaluated using the Hazard Index method, the sum of residues based on ADI and the Acute Reference Dose (ARfD) and it was concluded that, plurality of residues in vegetables may not present risks to consumers. However, the Netherlands on the same issue of multiple residues concluded after assessing the Critical Crop/Pesticide Concentration (CCPC) that, such contaminated produces are risky and harmful for health on the basis of Regulation (178/2002) of the EC General Food Law (EFSA, 2016). More studies may be required for clarity mostly when Gourounti et al. (2008) suggests that, pesticide residues are known for their interference with growth factors, hormones, enzymes, neurochemicals and key genes which may negatively impact metabolism to harm consumers' health. In researching on pesticide residues, Tago et al. (2014) and Mbugua (2015) reported that, short and long term or constant exposure to pesticide residues consumption even below MRLs in vegetables can lead to gradual bioaccumulation in the body to harm consumers' health in the long term. Thus, consumers of vegetables with pesticide residues are exposed to some undesirable health effects.

Our finding supports the report of Szpyrka et al. (2015) in Poland who analyzed samples of tomato and found 50 % contained pesticide residues. This study also agrees with the report in South Africa by Mutengwe et al. (2016) and in Turkey by Tiryaki (2017) who analyzed fruits and vegetables and found 54.46% and 65% of pesticide residues presence respectively. The finding as well concurs with the report in Brazil by Jardim and Caldas (2011) who analyzed vegetables, fruits, beans and rice and reported 48.3% of pesticide residues presence in samples. The study also corroborates the findings from Austria and Cyprus depicting similar multiple pesticide residue contamination; detection of one or more chemicals in samples and; violation of MRL or non-compliance for consumption due to pesticides above MRLs in analyte (EFSA, 2016). The presence of multiple residues on fresh vegetables from some farms remains a challenge to overcome. A few pesticide residues detected during analysis had levels above EU and Codex FAO/WHO MRLs standards (FAO/WHO, 2019). This may be explained by noncompliance to pesticides use, the nature of the chemical used in farms, insufficient knowledge of pesticide dosage by some farmers as recommended by manufacturers (Mbugua, 2015 and; Tiryaki, 2017) .Our study also concurs with the work done by Mutengwe et al. (2017) on raw vegetables and fruits who reported some pesticide residues above the EU MRLs standard. Musila (2010), however in a similar study during wet season in Kenya from open air markets and supermarkets found that, all pesticide residues detected were below the EU and Codex MRLs standards.

4.5 CONCLUSION

Fourteen different pesticide residues - carbendazim, profenofos, azoxystrobin, difeconazole, imidacloprid, acetamiprid, acephate, dimethomorph, alpha cypermethrin, chlorpyrifos, cypermethrin, fenamiphos, indoxacarb and metalaxyl were detected in tomato samples of which

only acephate, fenamiphos and dimethomorph were above EU MRLs standards while acetamiprid was above Codex MRL standards. Even though carbendazim, profenofos and azoxystrobin were mostly detected, their levels were below both MRLs standards. This study shows that most tomatoes sold in Nairobi have pesticide levels below EU and Codex MRLs. Data from this study indicates that most farmers comply with good agricultural practices in pesticides use in tomato farms

CHAPTER FIVE: E. COLI AND SALMONELLA LOAD OF TOMATO SOLD IN NAIROBI METROPOLIS

ABSTRACT

Tomato is intensively grown and highly consumed in Kenya. However; the vegetable can harbor bacteria and becomes potentially risky to consumers' health. A cross-sectional study was done to establish E. coli and Salmonella loads in tomatoes marketed in Nairobi from January to June 2017. Tomatoes were sampled from three open-air markets (OAM₁, OAM₂ and OAM₃) and two supermarkets (SM₁ and SM₂) twice a month with first collections between the 1st and 10th days of each month while second collections were between the 23rd and 28th days of month. Four samples were always picked per open air market and from four different nooks to cover the whole area. Also, 4 collections per each supermarket were randomly done in boxes used for display. At least 1kg sample was handpicked from each site making together 40 samples monthly from all sites. For analysis, 2 tomatoes were picked per sample and by pooling, 8 tomatoes were set aside as one sample per site. A total of 60 analyses per bacteria were done. Analysis method included washing, isolation, enumeration and characterization. Data were analysed using GenStat and SPSS and thereafter means, standard deviation and level of confidence were determined. Tomato samples had 76% E. coli above the recommended load and the same samples had 20% of non-typed Salmonella spp. The month of January (4.33 \log_{10} cfu.ml⁻¹ >2), February (2.44log₁₀cfu.ml⁻¹ >2) and May (2.8 log₁₀cfu.ml⁻¹ >2) $p \le 0.05$ which were wet months had highest E. coli prevalences while March, April and June wich were dry periods had low presences. E. coli levels were more in open air markets samples (OAM₁ 2.79log₁₀cfu.ml⁻¹; OAM₃3.08 log₁₀cfu.ml⁻¹; Wakulima 2.31log₁₀cfu.ml⁻¹) compared to those of supermarkets (SM₁ 2.07log₁₀cfu.ml⁻¹; SM₂ 1.56log₁₀cfu.ml⁻¹). Fresh tomato in Nairobi harbors more *E. coli* above

recommended load and also contains *Salmonella*; proper disinfection is recommended before consumption.

5.1 INTRODUCTION

Tomato is grown and consumed throughout the world and its consumption improves health (Nelly et al., 2016). In Kenya, tomato is intensively grown; highly consumed in urban areas and has been ranked first in horticulture (Mutuku, 2016). The crop has gained lots of interest due to its vitamins (Romero-González and Verpoorte, 2011) and antioxidants properties (Manach et al., 2004). The fruit is consumed in various forms as fresh, cooked or processed and 25 g are recommended for daily intake.

Tomato can harbor *E. coli* and *Salmonella* from fauna, irrigation water, manure and farmers handling (Orozco et al., 2008; Gu et al., 2011; Razzaq et al., 2014). *E. coli* presence in food is an indicator of potential presence of *Salmonella* (Mensah et al., 2012; Nelly et al., 2016) and other organisms. Some strains of *E. coli* are harmful to human and can cause diseases in human (enteric, nosocomial septicemia, pulmonary, neonatal meningitis, surgical site infections, urinary infection, long-term infirmity and death) (Blattner et al., 1997; WHO, 2015). It was reported that; most infants' diarrheal infections in developing countries are linked to pathogenic *E. coli* (Motarjemi et al., 1993). *E. coli* and *Salmonella* can live in fruits, seeds, leaves and roots of various plants (Jablasone et al., 2005; Berger et al., 2009, Gu et al., 2011) and have been responsible for outbreaks in the advanced world (Finn et al., 2013; Pierangeli et al., 2014; Thilini et al., 2016). Several outbreaks of *Salmonellosis* were linked to fresh fruits and vegetables and in developing world, frequent transmissions are through vegetables, water and human paths (Chang et al., 2013; Wegener, 2003).

In Kenya, several studies on bacterial contamination in fruits and vegetables in postharvest have been done. *E. coli* and *Salmonella* spp were demonstrated in cooked food sold on streets as the raw vegetable salad dubbed *Kachumbari* (containing raw tomato as the main ingredient) (Gitahi, 2012; Mbae et al., 2018). From such findings, tomatoes harboring organisms can pose a serious public health concern (Kutto, 2012; Mugao, 2015; Kunyanga et al., 2018). Another study by Waithaka et al. (2014) hypothesized that; diarrhoeal episodes in Nakuru may be linked to potential intake of contaminated vegetables including tomatoes. Investigation of numbers of samples including vegetables and fruits salad, waste water and drinking water for a year revealed high bacterial presence in vegetables and environment. Most of the studies have been generally on vegetables and few on tomatoes. There is still therefore need to study the presence of *E. coli* and *Salmonella* status of tomato in Nairobi.

5.2 STUDY DESIGN AND METHODOLOGY

5.2.1 Study Design

A cross sectional study for bacterial analysis in fresh tomato sold in Nairobi was done from January to June 2017 covering dry and wet periods. Wet seasons in Nairobi corresponds to the months of April, May and June with abundant tomato of good quality sold at a low price by retailers in supermarkets and open air markets. Dry season corresponds to the month of January, February and March with less tomato sold at a high price in markets. Criteria for sampling of raw tomatoes included: Red and hard, without disease, no scratches and no soft part. Sites chosen for sample collection were three open air markets and two supetrmarkets. Supermarkets selected targeted the retailing points for high and middle social classes whereas open air markets selected targeted middle and lower classes.

5.2.2 Methodology

5.2.2.1 Study setting

The study was carried out in open air markets and supermarkets of Nairobi Metropolis. Three open-air markets (OAM_1 , OAM_2 and OAM_3) and two supermarkets (SM_1 and SM_2) were chosen.

OAM₁ is situated in the Eastern outskirt of Nairobi on Thika high way leading to the Central Region of Kenya. It is a wholesale and retail market receiving its tomato from different farming areas as Kiambu, Mwea, Nyahururu, Meru and Nyeri. OAM₂ is located in the Central town of Nairobi and is a wholesale and retail market receiving tomato from Loitoctock, Machakos, Kajiado, Taita Taveta, Isiolo, Limuruti and Nakuru. OAM₃ is in the outskirt of Nairobi on Waiyaki Way leading to Nakuru and Western Region of Kenya. Tomato retailers from OAM₃ usually buy their produce in OAM₂ and bring back for retail. These open air markets are congested areas as they are also places with other businesses attracting differents customers.

 SM_1 and SM_2 are located in the residential middle class area Westland situated by road in the northwest of the City Centre and are near to Waiyaki Way. They are retailing points mainly for middle and high classes. These are places where shopping is much easier and well organised compared to open air markets. The map of Nairobi Metropolis where tomatoes were sampled is shown in Figure 5.1

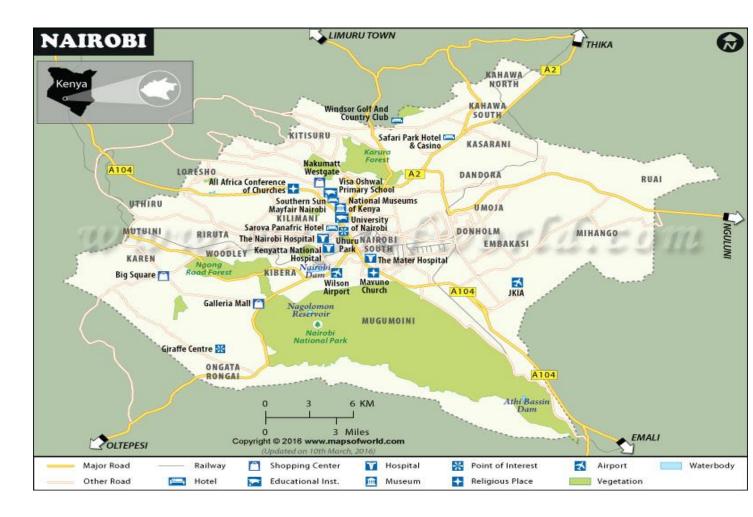


Figure 5.1: Map of Nairobi showing the five sites selected for tomato sampling

5.2.2.2 Sample size calculation of the tomato to collect per batch in the overall markets of

Nairobi

The sample size for tomatoes was calculated according to the formula by Fisher et al. in 1991 as

used by Omwega (2013) on fish.

According to the formula, the sample size is calculated as:

$$n = \frac{Z^2 p. q}{d^2}$$

Where:

n= Desired sample size

p= Proportion of the probability in the large population of fresh tomato estimated to contain pesticides residues and enteric bacteria at 50% or (0.5)

q = Proportion of tomato expected to be polluted. It is obtained from (1-p) = (1-0.5) = 0.5

z = Probability of type 1 error set at (1.96) corresponding to 95% confidence interval

d= Tolerance limit set at 10% (0.1) significance due to the accuracy needed for this study.

$$n = \frac{1.96^2 (0.5 \times 0.5)}{(0.1)^2} = 96.04 \approx 96$$

The formula of fisher was modified for a situation where the vendors were less than 10,000 as done by Rwanda (2015). The estimated number of raw tomato vendors in this case was 200 retailers in the open-air markets

The formula becomes

$$nf = \frac{n (1+n)}{N} \begin{cases} -nf = \text{Desired sample size} \\ -n = \text{sample Size for and estimated population (96)} \\ -N = \text{Estimated population of the tomato retailers (200)} \end{cases}$$

We finally obtain:
$$nf = \frac{96(1+96)}{200} = 46.56 \approx 47$$

By adding 10% attrition (+ 4.7 \approx 5), the desire sample size was finally: nf = 47 + 5 = 52 samples

5.2.2.3 Sampling procedure

Tomatoes were collected twice a month with first collections done between the 1st and 10th days of month while, second batches were collected between the 23rd and 28th days of month. At least 1 kg of fresh produce resulting from four different vendors was always collected in each market. Four (4) samples per open-air markets were randomly picked from four different retailers at four different nooks to cover the whole retailing area. Also, four (4) samples were always collected in each Supermarket in four different nooks following the layout in boxes containing tomatoes. OAM₂ and OAM₁ markets were selected because they are wholesale and retail points.

5.2.2.4 Sample collection and preparation for analysis

Four tomato samples were collected from each open air market and supermarket thus 40 samples of tomato were monthly collected from all sites. Samples collected were placed in a cooler with ice boxes, taken to the fridge of the microbiology laboratory of the University of Nairobi and kept in baskets. They were then prepared for analyses of *Escherichia coli (E. coli)* and *Salmonella* spp in the bacteriology laboratory. Aseptic procedures were keenly followed during analysis of tomato. For analysis, two (2) tomatoes were always picked per sample from each market, pooled as one sample representing the market. Thus, eight (8) tomatoes were always prepared and analysed per market for bacterial assay. Samples from each market were analyzed to determine the presence of bacteria during the study period and also to establish whether bacterial exposure differs between markets and thus, social classes.

5.2.2.5 Biochemical tests

Indole, Methyl red, Voges-Proskauer and Citrate (IMViC) biochemical tests were used for characterization of *E. coli* (Thi, 2007). Deamination of phenolalanine, Sulfite and Indole production in SIM Agar, motility test, decarboxylation of lysine, growth in potassium cyanide, fermentation of glucose and sucrose in triple sugar, urease activity were used for *Salmonella* characterization.

5.2.2.6 Isolation, culture and Identification

E.C Broth Agar medium for *E. coli* and Xylose Lysine Deoxycholate (XLD) Agar for *Salmonella*; Urea Broth and Triple Sugar Ion (TSI) Agar were used and prepared according to the manufacturer's instructions.

Fresh samples pooled per market were vigorously crushed (to mix and homogenize the samples pooled) separately for one minute by hand with glove in a mortar previously cleaned with 70%

ethanol and flamed. Twenty five grams (25g) of raw tomato were weighed using a balance (Mentor Ohaus Corp. Pine Brook, NJ USA - China). They were then placed in different sterile stomacher bags containing 225 ml diluent for homogenization using Stomacher (Stomacher 400 Lab Blender, England) for 2 minutes. Serial dilutions to 10⁻⁵ corresponding to infective dose of typhoidal salmonellosis causing enteric fever (Zubay et al., 2004) were made in sterile dilution bottles. Content of dilution bottles were vortexed for homogenization. A quantity of 0.1ml of each preparation of 10 ml was pipetted and transferred into sterile petri dishes.

For *Salmonella* spp, spread plate method (Wesonga, 2010) was employed to examine the presence and incubation was done at 37° C for 24 hours. Examination of plates with XLD was done by observation of black colonies resulting from non-fermented lactose. Pour plate technique (Waithaka et al., 2014) was employed for enumerating *E. coli* and incubation was done at 45° C for 24 hours.

Enumeration of colonies forming units (CFU) was done using the colony counter (Schneider & Co.AG Vorm. J.E. Gerber & Co, Zurich- Suisse) and the results recorded as log cfu/g (Razzaq et al, 2014). A minimum limit of 10^{-2} CFU (European Commission, 2008) for reporting the results obtained was adopted. The chosen limit corresponds to the highest acceptable concentration of generic *E. coli* in the whole commodity of vegetables analyzed as done by Nelly et al. (2016). The levels of bacterial counts in this study were compared with the EU Commission for Regulation of Food No 2073/2005 for *Salmonella* and *E. coli* as done by Wambui (2016).

5.2.2.7 Biochemical characterization

All the bacterial isolates were confirmed using biochemical tests. *Salmonella* was confirmed using Urea broth and Triple Sugar Iron Agar (TSI), while IMViC biochemical tests were used for *E. coli* (Thi, 2007; Kipkurui, 2011).

5.3 DATA ANALYSIS

Records of the colonies count from monthly analyses were first entered into Excel sheet with all parameters including sites of sample collections in Nairobi. Data analysis focused specifically on *E. coli* as *Salmonella* was rarely detected in samples analyzed. Counts from bacterial contamination were converted into log_{10} CFU/ml as done by Penteado et al. (2016) on analysis of fresh tomatoes marketed in Rio de Janeiro- Brazil, using excel sheet. Data translated were imported into GenStat (General Statistics) to determine the presence or prevalence of *E. coli*. Means of log_{10} CFU.ml⁻¹, standard deviation (±SD), p-values were calculated. The grand mean was used to determine overall bacterial load of samples per market for the study period. Linear regression was used to establish predictive models using the statistical package for social sciences (SPSS) IBM version 20. Significance of level of bacteria per site of collection and per month was considered at 95% level of confidence.

5.4 RESULTS

A total of 60 analyses per pathogen equivalent to 120 for both *Salmonella* and *E. coli* were conducted.

5.4.1 E. coli Presence on Tomato

The month of January had the highest presence (4/5 sites), followed by May (3/5 sites), February (3/5 sites) recorded with high presence \log_{10} cfu.ml⁻¹ >2 sightly lower than May, March (2/5 sites had presence of \log_{10} cfu.ml⁻¹ >2), April (1/5 site recorded high presence of \log_{10} cfu.ml⁻¹ >2) and June (1/5 site recorded high presence slightly lower than April). Lowest presence of samples in June clearly shows the influence of climate variation on tomato infection probably related to the environment (Table 5.1).

5.4.1.1 Ranking of *E. coli* load on tomato per site and month of sampling

E. coli prevalence per site and per month showed OAM₁ market with the highest number (5/6) of most loaded samples (\log_{10} cfu.ml⁻¹ > 2) followed by OAM₃ (4/6), OAM₂ (3/6), SM₁ (2/6) and SM₂ (0/6) (Table 5.1).

Month		OAM ₁	OAM ₂	OAM ₃		SM_1	SM_2
January		3.57 ± 1.37^{gjil}	$2.65{\pm}1.46^{efgh}$	4.33 ± 0.15^{kbj}		$2.60{\pm}0.17^{fghi}$	1.85 ± 0.22^{defg}
February		$2.29{\pm}0.365^{fgh}$	$2.40{\pm}0.175^{\text{fghij}}$	$2.44{\pm}0.227^{\text{defghi}}$		1.62 ± 0.135^{cde}	1.29 ± 0.11^{bc}
March	m	$2.47{\pm}0.1^{\text{defghi}}$	1.70 ± 0.015^{bcd}	$0.0{\pm}0.0^{a}$	Sm	2.21 ± 0.197^{efgh}	0.68 ± 0.042^{b}
April	Oa	$0.52{\pm}0.035^{a}$	1.94 ± 0.06^{bcde}	2.17 ± 0.127^{g}	$\mathbf{\tilde{S}}$	0.93 ± 0.107^{b}	1.40 ± 0.065^{bcd}
May		$2.75{\pm}0.157^{efghi}$	$2.69{\pm}0.067^{\text{ghijk}}$	2.98 ± 0.225^{kl}		1.78 ± 0.092^{bcd}	$1.54{\pm}0.215^{cde}$
June		$2.06{\pm}0.25^{fgh}$	1.11 ± 0.88^{bc}	1.62 ± 0.225^{bc}		1.16±0.137 ^b	1.44 ± 0.093^{bcd}
Grand Mean		2.79±0.775	2.31±0.85	3.08±0.154		2.07±0.153	1.56±0.162

Table 5.1: *Escherichia coli* (log₁₀ cfu.ml⁻¹) load in tomato during the study period

1. Values are mean of two determinations \pm standard deviation

2. Values with different letter in the same column are significantly different at p < 0.05

3. Oam = open air market; Sm = supermarket

There was a significant difference (p< 0.05) in bacterial load between open air markets and supermarkets. Tomato from OAM₁ had more load (5/14) \log_{10} cfu.ml⁻¹ > 2 during the study period. OAM₃ had a load of (4/14); OAM₂ (3/14); SM₁ (2/14) and SM₂ had no load (0/14) (Table 6.1).

About 50% (15/30) of overall tomatoes investigated showed high load of bacteria (\log_{10} cfu.ml⁻¹ > 2); 26.66% (8/30) had moderate levels of contamination (1.30103 < \log_{10} cfu.ml⁻¹ < 2); 20% (6/30) had the lowest load (\log_{10} cfu.ml⁻¹ < 1.301) and 3.33% (1/30) had no bacteria load.

Monthly detection has allowed appreciating climate variation of bacterial presence on tomato. There was a significant diference between months during the study period. Januray, February and May were probably wet in the farming areas and recorded highest prevalences while March, April and June were probably dried periods in the farming areas and had low bacterial presence on tomato. In fact, the months of January and May showed highest *E. coli* prevalence (logcfu.ml⁻¹ >2) with a peaks at 4.33 in January and 2.8 in May. There was no lowest prevalences ($log_{10}cfu.ml^{-1} < 1.301$) during these periods as with other months. Bacteria presences were varying; increases were observed with availability of water in environment and low presences were obtained in absence of water in environment.

5.4.2 Prevalence of Salmonella on Tomato

Salmonella was mostly found in dry season and was observed only in OAM_1 market in wet season (Table 5.3). Analysis showed that, OAM_1 market has the highest load (3 months showed presence) followed by SM_2 (2 months) and SM_1 (1 month) (Table 5.2).

Table 5.2: Salmonella load of tomato in market during the study period

Months	OAM ₁	OAM ₂	OAM ₃	SM_1	SM_2
January	ND	ND	Yes	ND	Yes
February	ND	ND	ND	ND	Yes
March	Yes	ND	ND	Yes	ND
May	Yes	ND	ND	ND	ND

1. OAM= Open air market

2. SM= Supermarket

A total of 20% (6/30) of samples analysed during the period had *Salmonella*. Samples with presence included those from OAM_1 (3/6) or 50%; SM_2 (2/6) or 33.33% and SM_1 (1/6) or 16.66%. Contaminations were recorded in samples of January, February, March and May (Table 5.2). Specifically, one open air market and both supermarkets had Salmonella presence on tomatoes.

5.4.3 Comparison between E. coli and Salmonella Load on Tomato

It was noticed that, *E. coli* load was always higher than of *Salmonella* in most samples where they were found together (Table 5.3).

	January ¹	February ¹	March ¹	May ²
Bacteria	OAM_1	SM_2	SM_1	OAM_1
E. coli (log ₁₀ cfu/ml)	3.145	3.23	7.477	3.636
Salmonella (log ₁₀ cfu/ml)	2.115	2,91	4.477	2.178
1. Dry month				

Table 5.3: Comparison of between E. coli and Salmonella spp load during the study period

1. Dry month

2. Wet month

5.5 DISCUSSION

5.5.1 E. coli Presence on Tomato

Data analysis of *E. coli* showed the comparison per sites and per month. On the basis of ISO 16649- 1 or 2 for pre-cut fruit and vegetables ready to eat, *E. coli* should not be beyond 100 CFU/g as there would be reduction in cooking. This indication is supported by the FSAI stressing that, the colony forming unit of *E. coli* should be lower than 20 (CFU < 20) which is equivalent to 1.30103 in Log₁₀ (< 1.30103). The borderline or marginal limit should be less or equal to 100 CFU (\leq 100, equivalent in Log₁₀ to \leq 2). With colony forming unit greater than 100 (Log₁₀ > 2), the pathogen is most likely to be consumed in food. Thus, the produce is harmful for health and, is a potential source of infection for consumers (European Commission, 2008; FSAI, 2016).

Ninety-six per cent of the tomatoes analyzed during the study period contained bacteria. This level of bacterial load seems normal since it is known that, tomato farming in open field is subject to enterobacteria contamination through fauna, irrigation water, soil, runoff, manure and workers (Orozco et al., 2008). Accordingly, finding high bacterial load of fresh tomatoes with *E. coli* has been linked to presence of the microorganism in contaminated water for irrigation (Lopez-Galvez et al., 2014). This result concurs with the finding of Orozco et al. (2007) in Mexico who found colonies of *E. coli* in hydroponic tomato greenhouses. The observation also

agrees with the work of Penteado et al. (2016) in Brazil who analyzed tomatoes from open air markets and supermarkets with strict hygiene from production to handling and found 1.1% *E. coli* contamination.

By analyzing tomato as whole, observations could not indicate whether, contamination was external or internal. As such, the current finding supports the work of Heaton and Jones (2007) showing presence of enteropathogens like *E. coli* in tomatoes as a way of subsistence. This study further agrees with the findings of Ogundipe et al. (2012) in Nigeria and Nelly et al. (2016) in Canada. Ogundipe et al. (2012) reported that, tomato from Lagos in Nigeria was highly infected with different pathogens and can constitute public health concerns if consumed fresh and Nelly et al. (2016) observed raw vegetables infected with a number of pathogenic agents are a considerable concern for food safety.

Substantial differences were observed at 95% level of confidence within same months and from one market to another. In February for instance, bacterial load from OAM₃ (2.44±0.227 $log_{10}cfu.ml^{-1}$) compared to the one from SM₁ (1.62±0.135 $log_{10}cfu.ml^{-1}$) and SM₂ (1.29±0.11 $log_{10}cfu.ml^{-1}$) were significantly different (p<0.05). This reveals that, there is no similarity of markets within the same period. As such, consumers may be exposed differently due to consumption of tomatoes from different sites. Bacterial load is not bound to sites of collection; it has been linked to different sources of contamination as handling (Kutto et al., 2011), infection in farms by pests (Orozco et al., 2008), from water for irrigation (Deering et al., 2015), seeds (Sheppard, 1998) and soil (Heaton and Jones, 2007). This variation between sites of collection contradicts the work by Pierangeli et al. (2014) in Philipines who found no significant differences between open air markets and supermarkets in analyses of raw produce including tomato. In the present study, open air markets had high presence ($log_{10}cfu.ml^{-1} > 2$) compared to supermarkets which had moderate $(\log_{10}cfu.ml^{-1} > 2)$ and lowest $(\log_{10}cfu.ml^{-1} < 1.301)$ prevalence. These findings are in agreement with the work done in Calabar- Nigeria by Obieze et al. (2010) who observed microbial pollution (including *E. coli*) of vegetables from different markets. Poor hygienic handling by retailers may not be the only source of contamination as some studies have reported possibilities of bacteria living inside the crop (Wright et al., 2017). Accordingly, this finding suggests careful processing when the vegetable is consumed fresh or, thorough cooking as stipulated in food safety standard (FSAI, 2016)

Consumers from OAM₁ might be more exposed to *E. coli* an indicator of fecal contamination (Pierangeli et al., 2014), while those from SM₂ might be less at risk of the bacteria. Maybe, the supermarkets are less infected because tomato displayed in boxes is of high quality. Supermarkets care for the quality of tomato purchased for retailing compared to vendors in open air markets and, they keep standards to meet the level of clientele. As their tomato was always washed and cleaned, probably water used for washing the crop was of good quality and may justify the acceptable level (log₁₀cfu.ml⁻¹ < 1.301) recorded. As well, the supermarkets might manage crops received in a special way before displaying in boxes to keep the image. It worth mentioning that only the best fruits and big in seize were always collected from supermarkets. Maybe the best and biggest tomato at sight (with no disease, no wound and no damage or soft point) might be less contaminated with the bacillus. This assertion agrees with the finding of Franz et al. (2007) in the Netherlands revealing that, crops contaminated with pathogens weigh less than non-infected ones. This might also corroborate the conclusion of Gu et al. (2011) in the USA reporting that, plant growth is slightly affected when infested by enteric bacteria.

Retailers from open air markets rarely displayed best quality tomato on their tables. Probably, they choose the quality mostly affordable by majority of their customers daily in order to minimize losses. This discrepancy between open air markets and supermarkets expresses the difference of social classes and their level of exposure to foodborne diseases.

Decrease of high levels of contamination noticed in March and April seems to reveal water scarcity in farming areas. Probably, less water in environment affects negatively microbial growth in environment, plants and fruits; this therefore reduces microbial internalization in tomato depited in numbers of studies (Zhou et al., 2018). In addition, it can explain why March appears with two sites poorly contaminated (OAM₃: 0.0±0.0; SM₁: 0.68±0.042) while April following the end of dry or beginning of wet season (Kutto et al., 2011) also had weak contamination (OAM₁: 0.52±0.035; SM₁: 0.93±0.107). Contrarily, sudden increment in May reveals the latency and potential of bacteria to multiply and get the highest peak once water is permanently enough in the environment. This confirms the finding of Ogundipe et al. (2012) who reported that, enough moisture in the environment favors microorganisms' growth and their presence on the vegetable. Such information is useful to consumers of raw vegetables as well as providers of ready-to-eat vegetables. This result is useful to understand the periods of high presence and those of low prevalence of bacteria in vegetables. It is worthy to emphasize that, safety measures should be observed all along preparation of ready-to-eat vegetables but specific care should be given during wet seasons. Similar fluctuation of E. coli infection according to climate (before rains, during wet period and after rains with a drop on level of hazard) was recorded by Orozco et al. (2008) in tomato farms in Mexico. They recorded a high incidence of *E. coli* on tomato during rains and low fruits prevalence after rains.

The month of January and May appeared as the most contaminated ones during the study period. Normally, they could have been among the least contaminated ones due to rains which might be washing the skins of the crop. Moreover, retailers had clean and attracting tomatoes during these

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months. Some were even pouring clean water on the vegetable displayed on tables to show the cleanness and beauty of good fruits to attract customers. With these detections means above the ones in dry months, the increase of bacterial load on fruits might be linked to the weather. Rainfall period might respectively be boosting water activity in plants; bacteria multiplication in environment, their attachment to plant and even internalization through different mechanisms increasing their prevalence on/in tomatoes (Cooley et al., 2003; Armendáriz et al., 2015; Wright et al., 2016). Maybe, the level of detection might probably be linked to availability of water that might favor *E. coli* multiplication and movements in the environment including on tomato plants and fruits.

To understand the variability of seasonal occurrence, Cooley et al. (2003) grew *Arabidopsis thaliana* on hydroponic medium together with *S. enterica* serovar Newport and *E. coli*; they reported that, *E. coli* is able to move and invade the whole plant in case of no competition with other bacteria. This was supported by Armendáriz et al. (2015) who stated that, *E. coli* attaches intimately to plants and uses intimin and adhesins to colonize the host. Wright et al. (2016) reinforce the finding and informed that, once on the produce's surface, *E. coli* can find ways to invade internal tissues using the T3SS (*ler*, present on the *LEE1* operon) effector to open stomata of vegetables, live inside and multiply. On the same track, Anat and Sima (2008) showed that *Salmonella enterica* possesses peculiar genes (*yihO*, *bcsA*, *rpoS*, and *agfD*) with ability to attach to a plant and even penetrate the inner side to become endophyte. These mechanisms are probably mostly active during wet seasons.

Our observation during the study allows saying that, high bacterial infections recorded might correspond to wet moments in farming areas. Their increase might be linked to sufficient water favoring biological activity within the plant and fruit. On the contrary, low level of bacteria in

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dry season might correspond on tomatoes to low levels of water ($a_w < 0.85$. $a_w < 0.70$) (Finn et al., 2013) in farming areas and thus, hot weather and water scarcity perhaps reduces bacterial activities in the environment. This may be due to evaporation shortening the level of water needed for the multiplication of bacteria and their potential healthy attachment to the tomato plants. Therefore, dry period might create competition for water between the environment (soil and atmosphere) and tomato plants while reducing bacterial presence in the environment and on tomato plant.

Bacterial load during dry months was below 2.5 in \log_{10} cfu.ml⁻¹ and had its peak at 2.47±0.1 in February. Relatedly, the dry months can be seen as a bacterial reservoir period in the environment ready to multiply from the rains inception. This finding corroborates the work done by Ogundipe et al. (2012) who reported that, an elevated amount of water in the environment favors bacterial growth and presence on raw tomatoes.

5.5.2 Prevalence of Salmonella on Tomato

Salmonella was rarely found during analysis on contrary to *E. coli*. Maybe, its presence was still embryonic and needed more time for maturity and detection (Sheppard, 1998).

According to EN/ISO 6579 for pre-cut fruit and vegetables ready to eat in the market, *Salmonella* should be absent in all units of 25 g of samples analyzed in the laboratory during the shelf-life of the produce. This recommendation is supported by the Food Safety Authority of Ireland (FSAI) stating that, results of *Salmonella* analysis in 25 g should not show any colony forming unit. Any presence should be considered unsatisfactory and thus, hazardous for human consumption (Commission Regulation, 2005; Finn et al., 2013; FSAI, 2016).

This low contamination of *Salmonella* can be attributed to handling since samples were analyzed as whole. The period of collection also influences the load as most contaminations occurred in

dry season (January to May). Tomatoes from supermarkets were always of high and best quality and cleaned; same as some samples from some open air markets. Previous studies on *Salmonella* detection on vegetables in Nairobi showed the level of contamination at 18.8% in water used for washing the vegetable and at 4.5% on the vegetable analyzed (Kutto et al., 2011). Similar results on water used for washing were reported in Spain by Lopez-Galvez et al. (2014). Contamination occurring on tomatoes collected may be a random process that cannot be attributed to specific conditions. Related to period of contamination, dry months may be the periods in which the vegetable is contaminated by the pathogen. Although this does not reveal high exposure of consumers, these findings serve as a warning to food handlers. Studies on the pathogen in Kenya as those conducted in the Peri-Urban farms by Kutto et al. (2011) decried poor agricultural practices and handling as sources of vegetables' contamination.

Worldwide, studies revealed different avenues of *Salmonella* contamination including seedborne, soilborne and plant infection during growth. Others suggest running polluted waters from rains, adulterated water from showers, wrongly converted manure, wounds from pests and adjuvants, human, water of irrigation with unprocessed sewage and airborne contamination (Sheppard, 1998; Heaton and Jones, 2007; Orozco et al., 2008; Gu et al., 2013; Farakos and Frank, 2014; Pierangeli et al., 2014; Deering et al., 2015; Kumar et al., 2017). The present study concurs with the work done by Pierangeli et al. (2014) in Phillipines. They collected 50 samples of tomato from open air markets and supermarkets and found 20% of samples contaminated with *Salmonella*. This study agrees with the work by Finn et al. (2013) indicating that, the pathogen can live long on surfaces and in matrices of food. It is recommended that, further analyses show interest on the inner side of the crop.

5.5.3 Comparison between E. coli and Salmonella Load of Tomato

Salmonella was rarely found in tomato analyzed during the study period unlike to *E. coli*. As such, no correlation between both pathogens could be established. Exceptions were separately found in March where high presence of *E. coli* (7.477 \log_{10} cfu/ml⁻¹) corresponded to strong colonies of *Salmonella* (4.477 \log_{10} cfu/ml⁻¹) in samples from SM₁ and also, high detection of *E. coli* (4.100 \log_{10} cfu/ml⁻¹) corresponded to high presence of *Salmonella* (3.579 \log_{10} cfu/ml⁻¹) on samples from OAM₁. This concurs with the work of Lopez-Calvez et al. (2014) in Spain indicating that, higher presence of *E. coli* on tomatoes analysis corresponded to higher presence of *Salmonella*. This finding futher agrees with the work of Little and Gillespie (2008) in the UK. They analyzed salad ready-to-eat and found inconsistency on human infection with the vegetable during the period of surveillance.

The results of the present study reveal that *Salmonella* presence on tomatoes is random and attention should be paid when preparing raw tomato for salad. The presence of both pathogens seems suggesting that, washing by retailers before seems not providing a safer crop due to potential presence in tissues (Deering et al., 2015).

5.6 CONCLUSION

The study demonstrated that, tomato in Nairobi mostly harbor *E. coli* but also contain *Salmonella*. Tomato collected from OAM₁ market had more of *E. coli* load followed by OAM₃ and OAM₂. Also among the open air markets, *Salmonella* was detected only in samples from OAM₁ while OAM₂ and OAM₃ had none. The Supermarkets were less contaminated with *E. coli* but some samples had *Salmonella* during the study period. Prevalence of *E. coli* was mostly high in January, February and May that had been rainy and; low prevalence was noted in March, April

and June that were dry. *E. coli* presence sometimes occurred with *Salmonella* in some samples in which both pathogens were detected. However, *E. coli* was most prevalent during wet months.

The present study could not clarify where exactly (inside or on the surface or both) tomato was contaminated. Further studies with on the inner side of the vegetable are encouraged since this information may be relevant to tomato retailers, food handlers, consumers and policy makers.

CHAPTER SIX: PESTICIDE RESIDUES LEVELS AND BACTERIAL LOAD IN FRESHLY PEPARED RAW TOMATOES SOLD IN SOME HOTELS AND RESTAURANTS IN NAIROBI, KENYA

ABSTRACT

Tomato is widely consumed in freshly prepared vegetable in food businesses and households. Previous investigations in Kenya reported that, harvested tomatoes are likely to contain pesticide residues and pathogenic bacteria all the way to the food preparation in kitchens. This study sought to evaluate pesticide residues, E. coli and Salmonella in freshly prepared tomato salads from 4 star levels and above hotel and restaurants of Nairobi in May 2018. The City was triangulated and three categories of restaurants picked at the apices of the triangle and each pair to be close together. Samples were picked as take away dishes at weekly interval in a cool box, transported within 4hrs to the laboratory and refrigerated at 4±1°C. Pesticides were extracted using QuECHERS and analysed using a multiresidue standard in GCMS tandem LCMS/MS. E. coli and Salmonella were detected using EC Broth and XLD media respectively. Samples analyzed were free of Salmonella but contained E. coli (1 in \log_{10} cfu.ml⁻¹) at acceptable levels $(1.301 \text{ in } \log_{10} \text{cfu.ml}^{-1})$. Five pesticide residues carbendazim, acetamiprid, indoxacarb, omethoate and dimethoate were detected in four samples and their prevalence varied from single to multiple residues. Overall samples with pesticides were 66.66% and 33.33% had no residues. Only one hotel had a single pesticide while all restaurants' samples had multiple residues. The systemic residues omethoate (0.04 mg.kg⁻¹; LOQ 0.02 mg.kg⁻¹; MRL 0.01 mg.kg⁻¹) and dimethoate (0.04 mg.kg⁻¹; LOQ 0.02 mg.kg⁻¹; MRL 0.01 mg.kg⁻¹) detected in samples from restaurants were above EU MRLs. Freshly prepared tomato collected in this study had no Salmonella and had acceptable levels of E. coli. Single and multiple pesticide residues were detected with some above EU MRLs. Washing and sanitizing applied by the food businesses

reduce bacterial load to acceptable levels in freshly prepared tomato. However, the vegetable can still contain single and multiple residues.

6.1 INTRODUCTION

Tomato is consumed fresh in prepared vegetable salads all over the world. In Kenya, tomato is one of the main vegetables in salads dishes offered in restaurants. Fresh tomato consumption in Kenya is also common in meat roasted in eatery places. Despite high consumption of these dishes nationally, microorganisms loads are little known (Adhiambo, 2016; Mbae et al., 2018) in Kenya as well as pesticides residues levels.

A number of studies have indicated bacteria presence in fuits and vegetables freshly prepared into salad in Kenya. Adhiambo (2016) assessed microbial level in 52 fruits minimally processed for sale in Nairobi streets and found high presence of coliform (log₁₀ 0.08) in salad fruit. Mbae et al. (2018) studied the safety of "Kachumbari" sold alongside Kenyan roads and meat roasting places and reported concerns related to hygiene. In the same line, *E. coli* and *Salmonella* were found in fresh vegetables in Saudi Arabia (Kuddus et al., 2016), Kenya (Kipkurui, 2013) and were reported to be responsible for outbreaks worldwide (Farakos and Frank, 2014). Presence of pesticide residues in fruits and vegetables have prompted call for actions to reduce residues in vegetables consumed in Kenya (Kunyanga et al., 2018). Previous studies by Kithure et al. (2014) who analysed deltamethrin residue in some vegetables in Makuyu reported levels of chemicals above acceptable daily intake (ADI) in dry season.

In Kenya, cancer is the third leading killer after infectious and cardiovascular diseases. On 37,000 new cases, 28,500 expire (MOH, 2017) and some of it could be attributed to ingestion of the chemicals. In Mexico and USA, records of fatal cases were related to fresh produce as tomato. The USA singularly recorded 15 confirmed deaths from contaminated fresh produce caused by *Salmonella*, hepatitis A and *E. coli* O157:H7 (FAO and WHO, 2008). Under this awareness, consumers might not know the extent to which fresh vegetables are safe and hence

might be exposed to harmful residues of pesticides and bacteria. This study was designed to assess bacterial load and chemical residues levels in freshly prepared tomato consumed in eating places of Nairobi.

6.2 STUDY DESIGN AND METHODOLOGY

6.2.1 Study Design

A cross sectional study to assess pesticide residues, *E. coli* and *Salmonella* on freshly prepared tomato was conducted in May, 2018 in three stand-alone restaurants and three hotels restaurants. The two categories of the restaurants taken in Nairobi City were of the same standard. Samples of freshly prepared tomato were randomly taken from these food businesses. Samples were collected as take away dishes in order to facilitate transportation while keeping the serving conditions of those institutions.

6.2.2 Methodology

6.2.2.1 Study setting

The study of raw tomato samples from open air markets and supermarkets of Nairobi has revealed levels of pesticide residues and bacterial load in fresh tomatoes. Further studies were therefore conducted to find out whether freshly prepared tomato consumed in salad in some restaurants of the City might still contain these contaminants. Six restaurants from four stars and above (three stand alone restaurants and three restaurants in hotels) were randomly chosen in the Central Business District (CBD) within Nairobi County located between latitudes 1°16'59"S and 36°49'00"E.

6.2.2.2 Sample procedure

Samples were collected in three different areas of the CBD of Nairobi County following the basis of the four stars levels and above stand alone restaurants and restaurants in hotels. Samples were collected tice a week in an interval of three to four days from each chosen area where restaurants and hotels were identified. This procedure was adopted to avoid or minimize the possibilities to collect freshly prepared tomatoes samples either fom the same market bought on the same day or collecting samples from the same batch bought from the same market. In fact the procedure adopted for samples collection twice a month in some markets of Nairobi was followed.

6.2.2.3 Samples collection

In order to pick the samples, the City was triangulated and three categories of restaurants picked at the apices of the triangle and, each pair selected was to be close together. Samples of salad were picked as take away dishes in sterile containers placed in recyclable paper bags at a weekly interval and they were each time transfered in a cool box. Only two samples were collected from each apice per week and six samples were collected in three weeks. The two samples of each apice were collected in different days with the first collect in the beginning of the week and the second pick three days following the first. They were then transported within 4hrs to the laboratory where they were kept refrigerated at 4 ± 1^{0} C till analysis.

6.2.2.4 Samples processing for analyses

Samples of tomato collected were equally divided in two and each part used for both analysis of pesticide residues and enteric bacteria. Analysis of enteric bacteria was done the following day and pesticide residues were analysed two or three days after collection. A blender cleaned with

acetone was used to prepare samples for pesticide residues analysis and no peculiar pesticide residue was targeted for detection.

6.2.2.5 E. coli and Salmonella load detection

Isolation, culture, Identification and the biochemical characterization of *E. coli* and *Salmonella* followed the method already described in chapter six.

6.2.2.6 Pesticide residues analysis

Samples collected were extracted and cleaned. The material and reagents for both GC and LC analyses and their calibration followed the one described in chapter five. Both GC and LC were combined for an overall detection of pesticide residues in samples collected.

6.3 RESULTS

A total of six (6) samples of tomatoes were analyzed for detection of *E. coli* and *Salmonella* spp in the laboratory of the University of Nairobi. Also, the other six (6) samples were used for analysis of pesticide residues in the laboratory of toxicology.

6.3.1 E. coli and Salmonella Load

Analysis of *E. coli* showed low (1 in \log_{10} cfu.ml⁻¹) and bordering limit (1.301 \log_{10} cfu.ml⁻¹) presence while *Salmonella* spp was not detected (Table 6.1).

Table 6.1: *E. coli* and *Salmonella* from tomato freshly prepared in some restaurants in Nairobi (log₁₀cfu.ml⁻¹).

	Hotel A	Hotel B	Hotel C	R1	R2	R3	
E.coli	ND	1	ND	ND	1.301	ND	
Salmonella	ND	ND	ND	ND	ND	ND	

^{1.} Values are mean of one determination in \log_{10} cfu.ml⁻¹

^{2.} ND = Not detected

^{3.} $\mathbf{R} = \text{Restaurant}$

6.3.2 Pesticide Residues

All samples of freshly prepared tomatoes analyzed from the stand alone restaurants and hotel's restaurants had varied levels of detectable pesticide residues. For instance, no residue was detected from hotels A and B. A single pesticide residue (carbendazim 0.02 mg kg⁻¹; LOQ 0.02 mg kg⁻¹; MRL 0.3 mg kg⁻¹) was detected in hotel C whereas restaurants 1, 2 and 3 had multiple pesticides ranging from three to four chemical residues (acetamiprid; indoxacarb; omethoate; dimethoate). Restaurant 1 had the highest (4) residues' presence while Restaurant 2 and 3 had three detections each. Some pesticide residues like omethoate (0.06 mg kg⁻¹; LOQ 0.02 mg kg⁻¹; MRL 0.01 mg kg⁻¹) and dimethoate (0.04 mg kg⁻¹; LOQ 0.02 mg kg⁻¹; MRL 0.01 mg kg⁻¹) detected were above EU MRLs (Table 6.2).

Table 6.2: Pesticide residues detected in tomatoes freshly served in some Restaurants (mg/kg or ppm), LOQ (Limit of Quantitation) and MRL (Maximum Residue Limit)

Hotel C	Restaurant 1	Restaurant 2	Restaurant 3	LOQ (mg/kg)	MRL (mg/kg)
Car (0.02)				0.02	0.3
	Act (0.07)	Act (0.03)	Act (0.03)	0.02	0.5
	Ind (0.01)	Ind (0.01)	Ind (0.01)	0.02	0.5
	Ome (0.08)	Ome (0.04)	Ome (0.07)	0.02	0.01
	Dim (0.04)			0.02	0.01

Key to the table: Car: Carbendazim; Act: Acetamiprid; Ind: Indoxacarb; Ome: Omethoate; Dim: Dimethoate The overall detection of analyses based on sites collection when merged together was 4 (4/6) (66.66%) detections. Two restaurants in hotels equivalent to 66.66% (2/3) were free of pesticide residues; one or 33.33% (1/3) of analyte had a single pesticide residue. All samples 100% (3/3) from stand alone restaurants had multiple pesticide residues varying from three to four molecules and, all samples 100% (3/3) had pesticide residues levels above EU MRLs. Five (5) different pesticide residues carbendazim, acetamiprid, indoxacarb, omethoate, dimethoate were found in samples analyzed. Dimethoate and omethoate were systemic pesticide residues (Bonnechere et al., 2012; Kelageri et al., 2015) above MRLs.

6.4 DISCUSSION

Data from this study revealed acceptable levels of E. coli load and absence of Salmonella. This may indicate good handling practices including washing, chopping, sanitizing (Faour-Klingbeil et al., 2016) and serving measures of fresh vegetables ready to eat applied by the food service institutions. In fact, handling and processing fresh tomatoes ready to eat in these places were similar; all of them mentioned several washings and sanitization. Though no inspections of kitchens were done, it can be assumed from the observation that, these institutions are well organized and prepare their food under good hygienic conditions (Krsteski and Rockliff, 2012; Pesewu et al., 2014). On the basis of ISO 16649-1 or 2 for pre-cut fruit and vegetables ready to eat, E. coli should not be beyond 100 CFU/g. Similarly, referring to EN/ISO 6579 for pre-cut fruit and vegetables ready to eat in the market, Salmonella should be absent in all units of 25 g of samples analyzed in the laboratory during the shelf-life of the produce. This recommendation is supported by the Food Safety Authority of Ireland (FSAI), stating that results on Salmonella analysis in 25 g should not show any colony forming unit. Each presence should be considered unsatisfactory and thus hazardous for human consumption (Commission Regulation, 2005; Finn et al., 2013; FSAI, 2016).

It can be said with such good results that, different legislations on consumers' health protection against unwholesome food existing in Kenya such as the Public Health Act (Chapter 242 of the Laws of Kenya) enforced by the Department of Public Health of the Ministry of Public Health and Sanitation together with the Kenya Bureau of Standards (Mbae et al., 2018) might have structurally influenced the management of these institutions.

The present study concurs with the finding of Faour-Klingbeil et al. (2016) in Lebanon who investigated microbial contamination of ready to eat vegetables from restaurants and found acceptable results. A similar study on microbial load of sliced tomatoes mixed with onions (Kachumbari) from 39 eateries in Kenya by Mbae et al. (2018) showed satisfactory microbial contamination (<20 CFU/g); borderline contamination (20-10² CFU/g) and unsatisfactory (>10² CFU/g) observations on *E. coli* presence in the dish containing ready-to-eat fresh tomatoes. Absence of *Salmonella* in the current study is in agreement with the findings of Mbae et al. (2018) who did not detect *Salmonella* in samples of Kachumbari analyzed. Absence of *Salmonella* from both studies suggests abilities and expertise of these restaurants to prepare fresh tomatoes with zero presence of the pathogen. Absence of *Salmonella* in samples also concurs with the finding of Krsteski and Rockliff (2012) in Australia who analyzed varieties of ready-to-eat food as sandwiches and found no threat related to the pathogen.

Analysis of samples from stand alone restaurants has disclosed more presence of chemicals in freshly prepared tomatoes compared to restaurants in hotels. This result might be explained through the fact that, stand alone restaurants might be having many customers throughout the day ordering for different dishes compared to restaurants in hotels that prepare the buffet from where each customer has a self service or customers help themselves. In light of this, the personnel of stand alone restaurants might always be in hurry to the point of not focusing much enough in handling or processing the vegetable to satisfy the orders. The shortcoming might also increase if some customers express their lack of time. Finding chemical residues in both hotels and restaurants reveals the limits of tomato washing in food service businesses despite the knowledge of professionals serving in these institutions. Maybe, the stand alone restaurants have specific providers of fresh tomatoes and the management ordering for the produce might have

been strict on the quality of tomato to supply. Under this recommendation and by seeking to maintain the partnership so as to always make good deals, the suppliers might have been purchasing only the best quality of tomatoes from farmers. This requirement might be influencing the behavior of growers and might have finally led them to pesticide misuse in tomato farms as depicted in number of studies (Latif et al., 2011; Firas, 2015; Mutai et al., 2015; Kamuri and Basavaraja, 2018).

From this finding, it cannot be said with certainty that, all pesticide residues were detected. Our previous study of the skin alone has shown presence or levels of pesticide residues unseen in analysis of whole tomato. Maybe, more pesticide residues might have been detected if opportunity to collect whole tomatoes ready to be sliced by restaurants was given and analysis of the skin alone done. If this could be the case then, the present study could still concurs with the finding of Abou-Arab (1999) who studied pesticides residues on raw tomatoes in Egypt and found most of them on skins.

It can be said with certainty that consumers of freshly prepared tomatoes in restaurants were exposed to synthetic chemical residues like dimethoate (0.04 mg kg⁻¹; LOQ 0.02 mg kg⁻¹; MRL 0.01 mg kg⁻¹) a pesticide restricted for use on fruits and vegetables (PCPB, 2010). Studies in Mexico and Ghana showed that, washing with tap running water reduces pesticide contamination on fresh vegetables (Perez et al., 2016; Akomea-Frempong et al., 2017). My data might suggest that these restaurants have even been applying the method advised by these researchers and still, pesticide residues were detected. The presence of dimethoate residue in a sample supports the work of Mutuku et al. (2014) who reported that, dimethoate restricted for use in fruits and vegetables in Kenya was still sprayed in farms by 1.9% of growers.

The scarcity of data on pesticide residues in fresh vegetables and their rinsability in Kenya may be a concern limiting provision of safe produces. Even, studies conducted on washing fresh contaminated vegetables and their safety have revealed limits on getting tomatoes free of pesticides residues after washing with different solvents. Andrade et al. (2015) in Brazil revealed in their work that, despite three different washings including water; 10% vinegar and 10% sodium bicarbonate, not all pesticides were removed. Some agrochemicals such as friponil and procymidone could not be cleansed from the skin of tomatoes by water. Previous studies by Abou-Arab (1999) in Egypt reported poor reduction of pesticide residues from the surface of the produce when washed only with tap water. Such findings have generated uncertainties and contradictions (Andrade et al., 2015). Li et al. (2012) in China studied the effect of washing oranges before consumption and found that, washing and immersion in water followed by bubble/boiling for 5 minutes reduces contaminants on surfaces. Đorđević and Đurović-Pejčev (2016) in Serbia stated that, reductions of pesticides remnants on fresh crops (physically undamaged) depend on the type of pesticides, their potential movement (infiltration) on the crop, their water solubility, the nature of solvent, the constitution of the commodity and environmental conditions. Wang et al. (2013) and Subbash et al. (2015) supported the finding and added that, washing to remove pesticide leftover on/in crops is subject to residue location, its physicochemical properties as the lipotropic (affinity for lipids) character, the hydrolytic constant rate, the age of the residue, the octanol/water partition coefficient, the washing technique and the temperature. For Cengiz and Certel (2014) in Turkey, mancozep is significantly reduced on tomatoes when dipped into chlorine solution at 100 mg/ml for 20 minutes. Subhash et al. (2015) in India found significant decline from 29.5 to 99.5% of chlorpyrifos and cypermethrin in okra by washing separately with 1.0% NaHCO₃ (sodium bicarbonate), 0.5% Acetic Acid

(CH₃COOH), 2.0% NaCl and dumping. Yang et al. (2017) in the USA showed an effective drop of levels of non-systemic agrochemicals on the surface of apples when washed with sodium bicarbonate (backing soda or NaHCO₃). But they emphasized that systemic pesticides infiltrating the fruit could not be removed during washing. With simplicity and more clarity, Wang et al. (2013) in China assessed the efficacy of dishwashing liquid available in Chinese markets for the removal of pesticide residues on cherry tomatoes. They found that the commonly used detergents were effective in removal of chlorothalonil and chlorpyrifos from raw tomatoes.

With such a variety and non-standardised methods of removing pesticide residues on vegetables, food business and its professionals may be confused on what to do in order to provide safe fresh produce ready-to-eat. Studies aiming to rate washing of tomatoes using different methods already available will help in adopting the most efficient practice. Consideration of social classes should be included as poverty is a reality that does not allow everyone to get dishwashing liquid from supermarkets for instance.

Professionals in food businesses might be convinced that washing and sanitizing regularly mentioned during sampling are efficient enough to reduce any health threats on freshly prepared produce. They probably think that their practices meet the requirements of the Kenya Bureau of Standards (Mbae et al., 2018) as well as the international standards of food safety such as the Codex Alimentarius. Probably, the absence of a national database standard for levels of pesticide residues in fresh vegetables combined with evaluations of ready-to-eat produces might explain the weak removal of pesticides on/in vegetables. With scantiness of studies in Kenya and sub-Saharan Africa, findings of the present work are disclosing the reality that should be considered by policy makers, scientists for human health protection.

This study supports the work done by Kamuri and Basavaraja (2018) in India. They found that consumers of vegetables in India have no trust on washing practices as a means of consuming vegetables with less pesticide residues contamination.

Comparison of the present results with those for tomatoes collected from open air markets and supermarkets shows that pesticide residues on fresh tomatoes from farms are still consumed in freshly prepared tomato. Some surpluses are still above MRLs and others are present but at acceptable levels. The current work may indicate frequent ingestion of chemical residues by consumers and a potential accumulation of these residues in humans.

6.5 CONCLUSION

Freshly prepared tomato from restaurants in Nairobi analysed in this study had acceptable levels of *E. coli* and had no *Salmonella*. However, tomato samples contained single and multiple pesticide residues and had omethoate and dimethoate levels above EU MRLs. The common pesticide residues detected were acetamiprid, indoxacarb and omethoate.

The washing practices reduce microbial load in freshly prepared tomato from stand alone restaurants and restaurants in hotels at acceptable levels. These practices are not yet able to reduce these chemical residues existing in fresh tomato before consumption Consumers might be accumulating them in organs if the human body cannot establish their metabolism.

Well organized and better equipped stand alone restaurants and some restaurants in hotels are not able to provide freshly prepared vegetables free of synthetic chemicals. As such happens with four stars levels and above restaurants, consumers in households eating the same dish might be highly exposed to pesticide residues and probably to related non-communicable diseases. Studies on pesticide use in tomato farms and their washing should be encouraged.

CHAPTER SEVEN: CONSUMERS' AWARENESS OF THE PRESENCE OF PESTICIDE RESIDUES AND PATHOGENIC BACTERIA ON TOMATOES SOLD IN NAIROBI

ABSTRACT

Tomato is widely cultivated and consumed worldwide for its peculiar virtues, essential vitamins and bioactive elements. Tomato can harbor pesticide residues and bacteria at postharvest. A cross-sectional study using a semi-structured questionnaire was done in 101 households in OAM₃ to assess consumers' awareness on pesticide residues and bacterial presence on fresh tomatoes sold in Nairobi. Consumers' general knowledge on bacteria and pesticides was assessed; the sociodemographic characteristics were used to categorise respondents' knowledge. Data were analyzed using SPSS and some analytical tools as means, standard deviation, the binomial test and bivariate correlation using the Pearson coefficient were used for analyses. Male consumers (64.9 ± 0.483) were more aware of pesticides on tomato (p= 0.037). Consumers of 36 to 60 years old were more knowledgeable (58.3±0.341) than other age groups. Awareness increased with education level at 95% level of confidence (p=0.044). About 86% of consumers' were more conversant with pathogens than with pesticide residues (74%). About 78% of consumers knew pesticides and 97% responded that pesticides were used in farms (p= 0.000). About 91% perceived pesticides as hazardous for human health; 82% said, pesticides use in tomato farms can be dangerous to consumers (p=0.000) and 74% related pesticides used in farms to their presence on tomatoes sold in markets (p=0.000). However, 74% believed that washing provides tomatoes without pesticide residues (p=0.000), 65% mentioned that pesticides used in farms can be present on tomato eaten as salad (p=0.004) and 49% said that pesticide residues can transmit diseases to tomato consumers. Consumers were aware of bacteria and pesticides residues on tomato but, their knowledge was insufficient on the safety of tomato with pesticides. This limit could be improved through information, communication and education while studies on washing fresh tomatoes from markets to get pesticide residues free in households is encouraged.

Accepted for publication as: J.H. Nguetti, J.K. Imungi, M.W. Okoth, S.E. Mitema, W.F. Mbacham, J. Wang'ombe (2019). Consumers' awareness of the presence of pesticide residues and pathogenic bacteria on tomatoes sold in Nairobi on November 20, 2019 by the African Journal of Agricultural Research

7.1 INTRODUCTION

Tomato is widely cultivated and consumed worldwide for its peculiar virtues providing vitamins and bioactive elements (Kariathi et al., 2017). Agudo (2005) and Dias (2012) encourage quotidian intake of vegetables and state that, tomatoes can provide phenolic acid, ascorbic acid and phytochemical compounds protecting against free radical and tumor cells in human body. Viuda-Martos et al. (2013) noticed that, the daily intake of 25 g of the processed tomato is hypolipidemic (decreases the level of lipids in blood). Dias (2012) reported that low consumption of vegetables such as tomato contributes to 11% of stroke and 31% of ischemia heart diseases in the world. Oguntibeju et al. (2013) on the other hand state that frequent consumption of vegetables helps to manage glucose in blood and reduces the incidence of diabetes type-2 and cancers (oropharynx, oesophagus, stomach, colon, rectum, lung cancer, prostate cancer). With such value, the vegetable is currently much diversified phenotypically and genotypically (Vijee et al., 2016). Its efficacy for health protection is being reinforced and the purple tomato or "Indigo Rose" for instance is a new variety with high concentration of lycopene (Scott, 2012). Other improvements are targeting the quality and taste of the crop (Ric et al., 2012).

It has been found that in spite of all these health benefits fresh tomatoes can harbor pesticide residues (Hammad et al., 2017) and pathogens (Liu et al., 2018; Holden et al., 2017).

Pesticides can be found on tomatoes (Elpiniki, 2011; Kiriathi et al., 2017) as they are even misused in farms (Mutai et al., 2015; Kamuri and Basavaraja, 2018). Similarly, potential bacterial pathogens can find themselves on tomatoes through diverse routes including running polluted water from rains, adulterated water from showers, wrongly treated and untreated manure, soil reservoir, wounds from pests and pesticides, irrigation with unprocessed sewage

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(Sheppard, 1998, Heaton and Jones, 2007; Orozco et al., 2008; Gu et al., 2013; Farakos and Frank, 2014). Kithure et al. (2014) studied the seasonal levels of vegetables with pesticide residues in Makuyu- Kenya and detected higher contamination of deltamethrin in dry period than in wet season. Pierangeli et al. (2014) analyzed bacterial contamination of fresh produce from open air markets and supermarkets in the Philippines and found presence of *E. coli* and *Salmonella*.

It is in light of the above that a study was conducted to assess consumers' awareness on the quality of fresh tomato consumed in Nairobi. Specifically, the study aimed to assess consumers' awareness on potential contamination of fresh tomatoes with some enteric bacteria and pesticide residues.

7.2 STUDY DESIGN AND METHODOLOGY

7.2.1 Study Design

A cross sectional study assessing consumers' awareness on exposure to foodborne bacteria and pesticide residues on tomato was done in Kangemi suburb of Nairobi. A total of 101 households were randomly selected and interviewed. A semi-structured questionnaire on fresh tomatoes sold in Nairobi was designed for the collection of data from households. The survey targeted and covered both sides of Kangemi on Wayaki way in order to cover the whole. The questionnaire was pretested in Kawangware to confirm precision, appropriate answers and was reviewed after observations of the first interview.

7.2.2 Methodology

7.2.2.1 Study setting

The study was undertaken in Nairobi region, the capital City of Kenya located in the South-east end of the Kenyan's heartland agriculture. It is the regional headquarters for international organisations and the city produces more than half of the GDP of the country. Kangemi slum located in the outskirt of the City and in a small valley on Waiyaki Way was purposively selected and covered by the survey.

Kangemi is neighboring in the South with Kawangware and four middle class areas, Loresho and Kibagare in North; Mountain View in East and Westlands in West. The low income settlement is within coordinates' 1°16'17.4" S 36°44'36.4"; covers an area of 0.87 km2 and is located at around 10 km from the central business of the Metropolis. The population of the area is more than 100,000 inhabitants with a density of 22,243 dwellers/km² (Cherunya et al., 2015). Kangemi is a multi-ethnic area with a strong informal activity including street food ready-to-eat as "Kachumbari" reflecting the need to feed the growing population (Mwau, 2005; Oyunga-Ogubi et al., 2009).

7.2.2.2 Sample size calculation

The sample size for the households in Kangemi was calculated based on the method of Fisher used by Mwati (2013).

According to the formula, the sample size is calculated as:

 $n = \frac{Z^2 p q}{L^2}$ Where:

p = Proportion of the targeted population to be interviewed for this project (p= 0.5)q = Proportion of consumers expected to be interviewed obtained from (1-p) = (1- 0.5) = 0.5z = Probability of type 1error set at (1.96) corresponding to 95% confidence intervalL= the estimated precision for the margin error (L= 10% or 0.1)

n =
$$\frac{1.96^2 (0.5 \times 0.5)}{(0.1)^2}$$
 = 96 and by adding 10% attrition, n ≈ 105

7.2.2.3 Study tool

A semi-structured questionnaire was designed for data collection form tomato consumers

7.2.2.4 Target population

The lower class population was targeted assuming that, they are less informed on pesticide residues and bacteria presences in fresh tomatoes sold in markets compared to the middle and high classes.

7.2.2.5 Sampling procedure

A preliminary field visit of reconnaissance was done in Kangemi on both side of Wayaki way to decide on how to cover the area during data collection. Six enumerators, all Master's students were recruited and trained to administer the questionnaire in a face to face interview. They were divided in two groups to cover Kangemi on both side of Wayaki Way. The questionnaire was administered during week-ends as described by Okello et al. (2015). A systematic random sampling was applied during household recruitment for interview. The head of the household was always the respondent. In case of absence of the head of the house, the wife was requested for interview or an appointed person by the household was responding to the questions. Consent and voluntary participation were always obtained from the respondents after introduction of the aim of study. The enumerators collected data from 101 elders of each household or from the person appointed by the household.

7.2.2.6 Data collection

The questionnaire targeted the sociodemographic (gender, age, level of education and marital status) characteristics of respondents. The socio demographic helped to know whether households were mostly males or females; if they were mostly old people, middle age or young; if awareness of tomato contamination is known by old, middle age or young people. As well, it was also to know the contribution of education on the issue and whether marital staus was an important factor enhancing the awareness of respondents. The questionnaire also sought to get the overall knowledge of respondents on specific questions on eneteric bacteria and pesticide residues. Some correlations on pesticide residues were assessed in order to measure the level of the perception of consumers on potential diseases able to be obtained by fresh tomato consimption.

7.3 DATA ANALYSIS

The Statistical Package of Social Sciences (SPSS) software was used for data analysis. Answers of each questionnaire were entered into the software. Descriptive statistics were used to generate the sociodemographic characteristics of household for their awareness on exposure to pathogens and pesticides residues in fresh tomato marketed in Nairobi. Respondents' knowledge on pesticide residues and pathogens were coded and differentiated as right or wrong answer and scores one for good answer and nil for wrong ones were allocated. Same procedure was applied on knowledge of potential diseases related to pathogens and pesticides contamination of tomato. Right answers were additionned and converted into percentages. As well, the standard deviation to measure the dispersion was also obtained. The binomial test was applied to assess frequencies of respondent on some specific questions of tomatoes contamination. The bivariate correlation using the Pearson coefficient two-tailed test was used to measure the degree of linkages between

pesticide variables. The mean differences were calculated at 95% level of significance. Fisher's exact test was used to examine the significance of pesticides variables' association.

7.3 RESULTS

7.3.1 Socio Demographic Characteristics of the Population Studied

The survey conducted in Nairobi among 101 respondents showed more females (69/101) participation and majority of respondents (59/101) were between 26 to 35 years old. Very few participants (4/101) had not attended any school and more than half of interviwewees were married. This participation has allowed to measure the reasons why some households have designated people or why some people stood by themselves to take the interview by gender, education and marital status. The percentages of the sociodemographic characteristic was obtained as shown in Table 7.1

Demographic			
characteristics	Variable	Frequency (N)	Percentage (%)
Gender	male	32	31.7
Genuel	Female	69	68.3
	18 to 25	19	18.8
Age	26 to 35	59	53.5
	36 to 53	28	27.7
Marital status	Married	61	60.4
	Single	37	36.6
Level of education	Never attended school	4	4
	Primary school	23	22.8
	Secondary school	43	42.6
	Tertiary level	17	16.8
	University level	9	8.9

Table 7.1: Percentages of demographic characteristics on gender, age, marital status and education

7.3.2 Consumers' General Awareness on Pesticides and Bateria on Tomato and Relations with Other Findings around Contaminated Crops

The overall assessment of enteric bacteria and pesticides residues on tomatoes showed levels of significance. About 74% of consumers knew that, pesticides use in farms can be present in tomato sold in markets, but majority (74%) believed that it is safe to eat fresh tomato from markets after washing. As well, 95% supported that, washing tomato before eating in salad prevents from any diseases. For some consumers, washing stands as the critical control point preventing from any disease infection. An overall knowledge of consumers reflecting pesticides and bacteria knowledge was designed as shown in Table 7.2

Food Safety questions	Responses	Ν	Frequencies %	p-value	
Pesticides					
1. Can fresh tomato cause any disease to someone?	Yes	47	49	1	
	No	48	51	1	
2. Do you know pesticides?	Yes	79	78	0	
	No	22	22		
3. Pests and diseases attack tomatoes in the farms?	Yes	95	94	0	
	No	6	6		
4. Pesticides are done to protect tomatoes in farms?	Yes	96	97	0	
	No	3	3	0	
5. Farmers use pesticides to protect tomatoes in farms?	Yes	99	98	0	
	No	2	2		
6. Pesticides are dangerous for humans' health?	Yes	87	91	0	
	No	9	9	0	
7. Pesticides used in tomato farms can be dangerous for consumers'	Yes	81	82	0	
health?	No	18	18	0	
8. Pesticides used in tomato farms can be present on tomato sold in	Yes	73	74	0	
markets?	No	25	26	0	
9. Pesticides used in tomato farms can be present on tomato eaten in	Yes	65	65	0.004	
salad?	No	35	35		
10. Pesticides used in tomato farms can be present in tomato cooked at	Yes	46	47	0.641	
home?	No	52	53		
	Yes	48	49	0.92	
11. Pesticides can cause diseases to consumers?	No	50	51		
Pathogens					
	Yes	80	79		
12. Do you know pathogens?	No	21	21	0	
	Yes	77	86	0	
13. Pathogens can be found on the surface of tomato?	No	13	14		
	Yes	53	58	0.142	
14. Pathogens can be found inside tomato?	No	38	42		
15. It is safe to eat raw tomato from farms or markets after simple	Yes	73	74	0	
washing?	No	25	26		
16. Tomato eaten in salad can affect human health?	Yes	50	51		
	No	49	49	1	
17. Tomato cooked in food can affect human health?	Yes	35	35		
	No	64	65	0.005	
18. Tomato washing before eating in salad prevents from any disease?	Yes	95	95		
	No	5	5	0	

Table 7. 2: Frequency of food safety knowledge by farmers

7.3.3 Consumers' Awareness on Potential Diseases Related to Pathogens and pesticide Residues in Tomato

About 49% of respondents knew that fresh tomato can transmit any diseases to consumers. They pointed that; raw tomato can transmit diseases as cancers, stomachache and amebiasis. However, for illnesses related to pathogens, 24% pointed stomachache, diarrhea 19% and amabiasis 4%. For sicknesses related to pesticide residues, they indicated stomachache (32%), and cancer (11%) as shown in Table 7.3.

Variables	Answer	Frequence (N)	Proportion (%)		
Diseases related to pathogens contamination					
Can fresh tomato transmit any diseases?	Yes	47	49		
can nesh tomato transmit any diseases:	No	48	51		
Ducontru	Yes	4	4		
Dysentry	No	97	96		
Diarhoea	Yes	19	18.8		
Diamoea	No	82	81.2		
Stomachache	Yes	24	23.8		
Stomachache	No	77	76.2		
Cancer	Yes	2	2		
Cancer	No	99	98		
Diseases related to pe	esticide res	idues contaminatio	on		
Confrach tomata transmit any disassa?	Yes	48	49		
Can fresh tomato transmit any diseases?	No	50	51		
Stomachache	Yes	32	31.7		
Stomachache	No	69	68.3		
Concer	Yes	11	10.9		
Cancer	No	90	89.1		
Headacha	Yes	4	4		
Headache	No	97	96		
Neusee	Yes	2	2		
Nausea	No	99	98		

Table 7. 3: Knowledge of the risk of bacteria and pesticides on tomato by consumers

7.3.4 Influence of Sociodemographic Characteristics on Awareness of Pesticide Residues and Bacterial Organisms in Tomato

Knowledge on pesticide residues and pathogens presences was assessed based on socio demographic characteristics as shown by Table 8.4. Males (65 ± 0.483) had better knolwdge than female (51 ± 0.369); married people (60.2 ± 0.312) had better understanding than single (39.8 ± 0.434) and the level of education was an important factor among the respondents (p< 0.05).

Demographic Variables		n	Knowledge of tomato with pesticides		Knowledge of tomato with pathogens	
			Mean scores±SD	P-value	Mean scores±SD	P-value
Gender	Male	32	64.86±0.48		55.78±0.43	
	Female	69	50.77±0.36	0.037*	42.01±0.39	0.083
Age	18-25	19	49.58±0.45		32.03±0.25	
	26-35	54	57.89±0.23		47.79±0.34	
	36-53	28	58.29±0.34	0.707	49.67±0.54	0.646
Marital Status	Married	56	60.16±0.31		67.16±0.39	
	Single	37	39.75±0.43	0.005*	45.58±0.41	0.26
Level of education	Never attended school	2	24.17±0.26		28.60±0.45	
	Primary	28	35.88±0.65		34.83±0.74	
	Secondary	47	58.77±0.23		55.72±0.35	
	Tertiary	15	71.58±0.45		68.49±0.25	
	University	9	72.19±0.43	0.044*	71.50±0.33	0.068
Grand Mean			53.64±0.38		49.93±0.40	

Table 7. 4: Association between knowledge of pesticides and bacteria and the sociodemographic characteristics of consumers

*Percentage difference significant at 0.05 level

7.4 DISCUSSION

7.4.1 Consumers' Awareness by Association with Sociodemographic Characteristics

7.4.1.1 Consumers awareness by gender

The study had a participation of 68.3% females and 31.7% males. Strong participation of females in this study holds on the fact that, cooking in Africa is naturally a duty destined to women. They were more expected to provide best answers due to the natural social rank given by the society

between men and women in households. Pambo (2013) accordingly reminds that, females are mostly implicated because they are the designers of nutrition schedule and are responsible of food preparation in homes. Although this natural set up, socioeconomic development and its challenges have raised the matter of gender equality. From this reality, participation of men in this study may be justified by number of reasons obliging them to be fully or partially implicated in cooking. The reasons might include unavailability of the female committed to duties generating income, gender equality or good relationship in a couple and obligation to help or contribute in cooking at any time when the need arises. For such reasons, it was useful to have them in the survey.

The present observation is in concurrence with the work of Maschkowski et al. (2010) in Germany who reported 82% female participants in the study of parents' contribution in fruits and vegetables consumption in families. This finding also agrees with the study of Pambo (2013) in Kenya who reported 54.9% of female participation against 45.1% of male in his study of consumers' awareness on fortified sugar.

7.4.1.2 Consumers' awareness by marital status

About 60% respondents were married and 37% were single. Marital status can contribute to understand the awareness of vegetables' contamination and can improve the couples' knowledge through worries as wellbeing, diseases' prevention and family protection. Ambrožič et al. (2016) in Slovenia observed that, women have strong knowledge on viral presence on food than men because they care for their homes and families. Similar report came from Tomaszewska et al. (2018) in a survey that covered Poland and Thailand. Another study from Thailand by Kanang (2012) stated that, men in Bangkok care a lot compared to women on the quality of food purchase for family consumption. Both studies pointed food quality desired by parents in

households no matter the sex when it comes to food provision for families. This behavior is mostly found among married people with children. They request for organic diet to avoid contaminated food with pesticide residues (Davies et al., 1995; Kanang, 2012) to prevent foodborne infections and related disabilities adjusted life years (DALYs). It may be under such consideration that, studies point anxiety and worry of parents generated by their psychological attachment to families while looking for food of houses in markets (Maschkowski et al., 2010; Srinivasan et al., 2015). This attitude was also illustrated by Tanja (2015) in Finland who stated the structural dynamism of consumers and decision making when it comes to buying food. He stressed that, parents buy goods for the family to satisfy the needs as good health under personal, social and psychological factors. Pambo (2013) in Kenya supported the idea especially when he argues that, married people are more aware of food quality and that, households with children care more about the quality of food consumed. This finding also corroborates the work done in Turkey by Erdem et al. (2015) who found that, marital status had an influence on awareness of Halal among respondents.

7.4.1.3 Consumers' awareness by education

About 96% respondents had been to school including primary education, secondary, tertiary and university level. Studies showed that, understanding the worries of food safety requires a great level of education. Respondents with high level of education might be more curious, sensitive, more informed, open minded and interested on such topics. That is probably why Kanang (2012) in Thailand insists that, learned consumers in markets go for organic food for instance. It may be under such thought that, Hassan and Dimasi (2014) in Lebanon decided to assess knowledge on food safety among universities' students. They found that, knowledge grows with the level of education and showed that, the higher the level of education, better the awareness. Kimenju et al.

(2005) in Kenya got similar finding in their study of Genetically Modified (GM) foods and said that, consumers' awareness increases with education.

This survey relates with the study in Turkey by Erdem et al. (2015) on consumers' perception and awareness in consumption of Halal. They got respondents with similar education (5% never attended school, 18% primary level and 47% secondary school) and affirmed that, consumers' knowledge is bound to education. The study also concurs with the survey done in Poland and Thailand by Tomaszewska et al. (2018) who observed that correct answers in their study were frequently given by educated participants.

7.4.1.4 Consumers' general awareness of pesticides and pathogens on tomato and the need of consensus on vegetables harboring pesticide residues

Consumers have good notions on pesticides use in tomato farms and its potential presence on tomatoes sold in markets. Through this knowledge, they are somewhat alerted by the potential threat of pesticide residues on fresh tomatoes. This might help them to observe adequate practice of washing before cooking to reduce the levels of presence during consumption. Though little is known in Kenya on vegetables domestically consumed some results are available. Mutai et al. (2015) reported that, vegetables in Kenyan markets contain organophosphates and pyrethroids at 42%.

The level of knowledge of pesticides found in this study corroborates the work of Bempah et al. (2010) in Ghana. He assessed consumers' knowledge and found that, 70% knew fruits and vegetables contamination with pesticide residues. The finding also agrees with the study of Kamuri and Basavaraja (2018) in India who realized that, 55% of consumers were aware of pesticides use in vegetables farming.

Regarding consumers' knowledge and the safety of tomato ready to eat, 65% say that, pesticides used in tomato farms can be present on tomato eaten in salad (p = 0.004). Also, 74% indicated washing as a practice providing safe tomatoes for consumption (p = 0.0001). As well, 95% of participants disclosed that, washing fresh tomato before eating in salad prevents from disease transmission (p = 0.0001). This stand point can neither be accepted nor rejected. It is believable that, right information can be given if only pesticides' levels compared to MRLs are given in fresh tomatoes ready-to-eat.

This finding is contrary to that of Kamuri and Basavaraja (2018) in India who found that, consumers have no trust on washing practices as a mean providing safe vegetable for consumption and they are restrained on vegetables to buy in markets. They argue that, consumers look for vegetables free of pesticides residues and are willing to spend more on organic crops for health protection. Even their study pointed that, 30% of respondents mentioned long term infection due to consumption of vegetables with chemical residues regardless of levels. This view is supported by Kanang (2012) in Thailand who reported that, consumers in Thailand have adopted consumption of sustainable food and are more attached to green diet or produces free of pesticide residues.

Consumers' awareness seems influenced by the knowledge of washing raw produce for pathogens' reduction to acceptable levels of consumption (Sumonsiri and Barringer, 2014). In fact, of the 26% (25/101) stating that washing cannot provide safe crop to eat, only 3% (3/101) believe the crop can still contain pesticides residues, 2% (2/101) pointed that- it can contain heavy metals and 15% (15/101) designated presence of pathogens and 5% (5/101) remained silent on the question. Even, respondents with tertiary (16.8%) and university (8.9%) levels of education were not able to cover properly the point of chemical residues on raw tomatoes. This

shows that, consumers mostly think of pathogens when cleaning tomatoes from markets and have less understanding on potential pesticide residues presence on the surface of tomato. It is believable that, knowledge on pathogens is well rooted among tomato consumers compared to pesticide residues from farms.

Consumers might have gained knowledge on pathogens through formal education, episodes of sicknesses related to pathogens, information received during diagnoses in health centers, costs of burden (expenditures for treatment for instance), DAILYs and cooking practices transferred by parents from childhood to adulthood. Consumers could not do better than this when studies on pesticides in Kenya focus on vegetables for export (Mutuku, et al., 2014; Mutai et al., 2015) neglecting those consumed locally. It can be assumed that, knowledge on pathogens has been built with progress in science assorted by ways of preventing microbial infection.

Consumers might have either chosen these answers out of any knowledge or, they might be influenced by studies surrounding pesticides use in farms and related critics on side effects. By pointing pesticides as dangerous for human health and that they can be found in tomatoes marketed, they have probably learned from surveys on farmers and pesticides use in Kenya (Nyakundi et al., 2010; Mutuku et al., 2014) and worldwide (Nunifant, 2011; Huynh, 2014; Jamali et al., 2014; Paiboon and Tikampom, 2014, Kariathi et al., 2016). Similarly, respondents might be aware of pesticide multiple residues presence on vegetables demonstrated in South Africa, Sudan, Kuwait and in the European Union (Mutengwe et al., 2016, Hammad et al., 2017; Jallow et al., 2017; EFSA, 2017). Some interviewees might be aware of reports on chemical misuse in tomatoes farms (Latif et al., 2011; Firas, 2015; Mutai et al., 2015; Kamuri and Basavaraja, 2018). By indicating pesticides presence on tomato eaten in salad, they probably knew debates on MRLs adopted for chemical control in farms (FAO, 2009; Elpiniki, 2011; Latif

et al., 2011; Hammad et al., 2017) as well as the EU audit and evaluation outcomes on pesticide residues in Kenyans' fresh crops held in 2013 (European Commission, 2014). In the meantime, establishment of both Pest Control Products Board (PCPB, 2010) and Kenya Plant Health Inspectorate Service (KEPHIS, 2013) by the Kenyan government are some indicators or indices of consumers' knowledge. Lastly, when consumers indicate the safeness of tomatoes after washing, they might be influenced by studies depicting multiple residues on tomatoes ready to eat but pointing them as harmless for human health (Mohammed and Boateng, 2016). Though these probabilities, consumers need updates.

Consumers cannot imagine infiltration of synthetic chemicals in tissues of fresh vegetables (Kariathi et al., 2016). They could not also imagine that, pesticides residues on surface cannot be easily removed after washing. From these points, their knowledge is limited and their awareness insufficient. They think pesticide residues are like pathogens which can be reduced to acceptable levels for consumption through washing (Sumonsiri and Barringer, 2014). Washing chemical residues on surfaces of tomatoes seems ineffective. Studies depict surfactants or adjuvants in agrochemicals as containing oil and other water insoluble agents (Castro et al., 2013). Such oily and water insoluble components reduce the value of simple washing for the reduction of pesticide residues to acceptable levels. Thus, this increases exposure and potential health risks of consumers. This point corroborates the work of Bempah et al., (2010) in Ghana who analyzed health risk of chemical residues on tomato and recommended consumers' health protection through constant investigation. Analyses surrounding pesticides use for crops protection and potential contamination of consumers seem to have generated two thoughts; those rejecting potential human's infection versus those arguing human exposure and infection. One of the main arguments standing between both is washing the crop before consumption.

Washing fresh tomato and its inability to transmit diseases to consumers has been demonstrated by number of studies as providing safe vegetables for consumption. Perez et al. (2016) in Mexico agreed with washing of vegetable as a practice preventing any human health infection with pesticides residues because those on the surface are usually removed. Their finding concurs with Akomea-Frempong et al. (2017) in Ghana who found multiple pesticide residues and molecules above MRLs in vegetables ready to eat and concluded that, consumers are not at risk of pesticides related diseases if they wash their crops with running water.

Studies supporting positive effects of washing contaminated vegetables with pesticides residues have been contradicted by a number of researches. Andersson et al. (2014) in France argued that, illnesses generated by pesticides do not manifest instantly after few hours or few days; they appear at long term. This view found support from Kamuri and Basavaraja (2018) in India who analyzed consumers' awareness of pesticides contamination in vegetables. They found respondents pointing health infection by pesticide residues on long term when consuming contaminated vegetables. This implies that, illnesses generated by chemical residues appear long after consumption of contaminated diet. For that reason, consumers or health practitioners cannot trace back the causes of ailments as reported by Ames et al. (1993) in their mutagenesis and carcinogenesis studies. They argued specifically that, effect of synthetic chemical injury is related to human system defense which is also influenced at its turn by previous history of exposure to synthetic chemicals.

Elpiniki (2011) in Greece also contradicted the safety of contaminated produce even after washing with running water. The researcher insisted that, rinsability of vegetables is not bound to solubility and removability of pesticides on crops. He even added with support from Kiriathi et al. (2016) in Tanzania that, pesticides can infiltrate the flesh of crops and washing will not

change their concentrations. This position found support in Brazil by Graziela et al. (2015) who studied effects of washing on contaminated tomatoes with pesticides. They studied the rinsability of tomatoes contaminated with multi residues in households by application of 3 (three) washings on each sample. They concluded after using different solvents (water, sodium bicarbonate 10% and vinegar cleaner 10%) that, all molecules could not be removed. These findings are calling for a need of consensus on pesticide residues on vegetables.

7.4.1.5 Consumers' awareness on potential diseases related to pathogens and pesticide residues in fresh tomatoes

With only 10% pointing cancer as a potential disease related to pesticides residues, consumers have poor knowledge. A number of studies have pointed pesticides use in farms as potential cause of cancer diseases (Ridgway et al., 1978; Pratibha et al., 2015). Perhaps, little official communication has been shared on the topic with consumers and maybe, the knowledge is not yet included in school programs at primary and secondary levels to educate young generations on the issue. Undoubtedly, information is still mostly shared within scientists' communities. Few participants responded to this question which probably was embarrassing to them. But, they seemed more conversant with diseases of pathogens. Insertion of this topic in education program will probably improve consumers' knowledge as reminded by Kanang (2012). He revealed that, educated consumers distinguish organic and potentially contaminated food and show preferences for sustainable diet due to related knowledge (avoiding high levels of chemical residues, standard and labelling, level of safety from label).

This study showed that, pathogens and pesticides are well-known by consumers. However, consumers seem to have more knowledge on pathogens compared to pesticides. This might be from the fact that, pathogens actions in humans are usually sudden, can manifest immediately

after few hours or days following food consumption. On contrary, pesticide residues related action on health is not an instant or short term process rather; it is a long term procedure (Andersson et al., 2014). This might be the reason why European stakeholders rank pathogens as the priority to worry about on fresh produce compared to pesticides (Boxstael et al., 2013).

Also, consumers might be much conversant with pathogens because for a long time, capacities of African populations were built on pathogens. Their capacities were built through information, education and communications on personal hygiene and cooking of food. Pathogens have been taught in schools' programs since primary level. This strategy has been strong enough to inform and educate the populations on the threat. As well, microorganisms are always diagnosed in health centers and this has contributed to build the capacities and knowledge on the issue. On contrary to germs, pesticides use for crops' protection was recently adopted by the FAO for use in farms in the nineties to address the issue of food security (Shaw, 2007). Maybe, the topic is not yet developed enough to share its understanding with laypersons.

7.4.1.6 Influence of gender, age, education and marital status on awareness of pesticide residues in raw tomato

Gender consideration has shown a significant difference (p=0.037) in knowledge of use of pesticides in tomatoes farms. Males had good knowledge on the topic compared to females. This knowledge tended to increase with age though there is no significant difference (p=0.707) among age intervals. Marital status was an important factor on food safety among consumers (p=0.005) at 95% level of confidence. Married people had better knowledge on both pesticide residues and pathogens compared to single who were slightly less aware of the concern. The level of education of participants in understanding the concern of tomato contamination with

pesticides was an important factor and was statistically significant (p=0.044). Answers of awareness came from respondents with higher level of education.

Both variables age and education relate with the work of Ambrožič et al. (2016) in Slovenia who observed that, knowledge of consumers on food safety increases with age. According to contaminants in food, their survey on foodborne viruses reveals that, consumers with higher level of education were much aware of viral food safety than those with low educated. The present study on marital status agrees with the work done by Pambo (2013) in Kenya and Erdem et al. (2015) in Turkey. These researchers found that, marital status was an important factor of food safety in food consumption in households.

7.4.1.7 Influence of gender, age, education and marital status on awareness of pathogens presence in raw tomatoes

On contrary to consumers' knowledge on pesticides use in farms, there was no significant difference on awareness of tomato contamination with pathogenic bacteria on gender (p=0.083), age (0.646), marital status (0.26) and level of education (0.068). However, respondent of 36 to 53 years old had better knowledge followed by respondents of 26-35 years old and lastly the youngest 18-25 years old. Consumers with University level had better understanding followed by tertiary; secondary, primary and those who have never attended school. Although the level of education seemed important, scores obtained for awareness of bacterial load in fresh tomatoes was not significant (p=0.068). This may be justified by the usual practice of washing raw tomatoes before consumption. This does not require any experience, degree or a higher understanding because the habit is rooted within the society and is transferred through generations. High score recorded by respondents of 36 to 53 years old may hold on the fact that, many of them are responsible and probably have families and children. As such, they are used to

cooking and have knowledge for providing safe food in tables to protect the house dwellers from foodborne ailments. The middle age 26 - 35 years old may be on the same track as elders (36-53 years old) and, they might have started building their life experience on safe food and health. The earlier age 18 to 25 years are respondents who have just left the age of teenagers thus; they have less experience in food contamination generally and specifically, vegetables and human's health infection. At this age, some might either start living alone due to studies or job opportunities or, are still in parents' houses. Although they were taught on pathogens in schools, they are not yet concerned on vegetables contamination as their experience in diseases and potential health infection may still be low. In other meaning, they are still not much concerned of where and how they might get infected with food consumed. In one case or another, they are mostly bound to social media (Facebook, Twitter, and WhatsApp) hardly promoting scientific knowledge or raising awareness on issues as this one. From the youngest to the oldest age, the knowledge is acquired progressively and consumers become fully aware with time and experience.

This finding is consistent with the work on consumers' awareness done in Kenya by Pambo (2013) who observed that, consumers' awareness on fortified sugar comes with experience and level of education. The study also corroborates the finding of Malavi et al. (2017) who assessed the practices of food handlers in Kenya and came to similar conclusion.

7.5 CONCLUSION

The study established that, consumers were aware of contamination of fresh tomatoes with pathogens than with pesticides residues. Knowledge on contamination is related to age, level of education, marital status. Consumers knew that pesticides are dangerous to human health and washing reduces their presence on tomatoes. Though respondents knew that, pesticides are dangerous for humans' health and can even be present in freshly prepared salad, they were not able to realise that, pesticide residues presence in salad might be a threat to humans' health. They were convinced that, washing with plain water reduces pesticide residues in raw tomatoes and makes it safe for consumption. This deficiency should be improved through studies on washing contaminated vegetables as well as education, information and communication with consumers.

CHAPTER EIGTH: GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

8.1 GENERAL DISCUSSION

Tomato sold and consumed in Nairobi is contaminated with E. coli, Salmonella and pesticide residues. After processing for eating in salad, bacterial colonies can be reduced to acceptable levels harmless to consumers but, this will not be the case for pesticide residues. The vegetable might still have multiple residues and residues above MRLs which represent a threat to consumers' health. Results seem to indicate that while food security (SDGs, goal 2 and 1) is being positively achieved through provision of enough vegetables such as tomatoes that are good at sight in markets, consumers' health (goal 3) is being gradually adversely affected by consumption of contaminated tomato. It seems therefore that, both goals might not correlate positively. Attaining food security and ensuring healthy lives might not move together as one might always be positive (food security) while the other one (healthy lives) is negative. Thus, good quality of tomatoes at sight seems to be a reservoire of diseases for its consumers. Of course, people have enough tomatoes to consume but, they also have to prepare themselves randomly to potential decline of good health or to health infection due to pesticide residues consumption in the short and long term. This trend seems to be related to income generation in the whole tomato industry. Pesticide use in farms and their rinsability at postharvest are points to improve for the safety of fresh tomato sold to consumers in Nairobi. As noted in the case of enteric bacteria decontamination, food business institutions serving vegetables ready-to-eat would have improved their handling to reduce the levels of pesticides residues below MRLs if concrete measures were available. Unfortunately, scarcity and inaccuracy of a universal procedure for decontaminating pesticide leftover on and in crops remains a concern for these

food businesses seeking to address the needs of rapid urbanization, globalization and their challenges.

During the study, the postharvest spray of pesticides by some farmers (6%) almost coincides with pesticide residues level above MRLs (8%) in samples analysis from markets. Farmers might misuse pesticide in farms due to limited knowledge (Nonga et al., 2011; Mutuku et al., 2014; Tandi et al., 2014; Himani et al., 2015; Nguetti et al., 2018). Also, they might have adopted different strategies to reduce on-farm losses by harvesting the produce mature green and thus fail to respect PHIs (Himani et al., 2015). Though tomato contamination seems to be the farmers' shortcoming, they cannot be blamed alone. The blame goes to the whole economic system bound to modern life and dictated by globalization and capitalism. In trying to address this, Sachiko et al. (2009) in China studied the impact of contract on apples farming linking farmers to supermarkets. Bishay and Tawfig (2008) studied the contribution of high-value raw produce in response to poverty reduction in the Near East and North Africa. Similarly, Wu et al. (2014) in China investigated the environmental and social risk of tomato products in Chinese markets. With such trend, Wu et al. (2014) disclosed that production in agriculture is a tool for short term high profit making leading to food security and particularly food safety threats.

Comparing bacteria and pesticide residues presence on fresh tomato sold in Nairobi, Supermarkets have shown less contamination with bacteria compared to open-air markets. But, open-air markets have less pesticide residues presence compared to supermarkets.

Contamination of raw tomato with pesticide residues should be considered seriously in Kenya as it is of worldwide concern. Gradual exposure of consumers to pesticide related diseases is against social, economic and political development. They are probably slowing down progress in households in which they have appeared and have probably created profound socioeconomic damages. Through DAILYs and related burdens they probably contribute to weaken households, communities and the nation. Unpredictable consumption of pesticide residues in tomato may be seen as national and international chemical weapons that might be contributing to political, economic and social instability. As such, they might be pointed to as indices of immigration decried in the last decades. Improvement of the quality of tomato sold in domestic markets should be considered by policy makers in order to reduce their potential negative effects on consumers' health.

Consuming tomato with pesticide residues and enteric bacteria may lead consumers to double exposure, potential coinfection and thus, high burden of diseases. Such exposure may first lead to expenditures against enteric bacteria infection and then, in the short or long run, the infected or recovering from illness patient may start with new expenditures related to pesticide residues infection. This kind of burden weakens the family or household and may easily lead to strong negative consequences that may contribute to strenghten negative peace while weakening the social security, stability and integrity of the nation. Such scenariot may justify why some people may easily get envolved into riots or open conflict mostly when they have been deprived of their sustenance at their early social time of dependence. Such a situation is against socioeconomic and political development because it hardens the achievement of sustainable development goals of nations witnessing them.

8.2 CONCLUSIONS

Male farmers had good knowledge on the use of pesticides in tomato farms compared to females; most farmers spray pesticides once a week in farms and observed the pre-harvest withdrawal period. A negative correlation was found between farmers with training on pesticides use and the farm sizes and, most farmers agreed that waiting for the pre-harvest time is farmers' local knowledge in the region.

For pesticide residues analysis on fresh tomatoes, fenamiphos detected in whole tomato below MRL was found above MRL on skin. Similarly, acephate not detected in January in whole tomato from Wakulima was detected on skin above EU MRL. Overall analysis of the whole tomato disclosed 40 residues and skins analysis showed 20 additional compounds raising the total to 60 residues. The skin appears the most contaminated area with pesticide residues. The analysis of fresh tomatoes from markets showed that, the vegetable had single and multiple pesticide residues with some above MRLs

For enteric bacteria analysis, fresh tomato had *E. coli* above the recommended load and the same samples had non-typed *Salmonella* spp. High *E. coli* load was recorded during wet months while dry periods had low presence. Levels of *E. coli* were highest in samples from open air markets compared to those of supermarkets.

Samples of freshly prepared tomatoes from some restaurants were free of *Salmonella* but contained *E. coli* at acceptable levels. Single and multiple pesticide residues were detected in some samples of freshly prepared tomatoes and some systemic ones omethoate and dimethoate were above EU MRLs.

An assessment of consumers' awareness on potential presence of pesticide residues and bacterial load on fresh tomato sold in markets showed that, they were more conversant with pathogens than with pesticide residues. Most respondents knew that, pesticides use in farms can be present on tomato sold in markets but that, washing provides tomatoes without pesticide residues. A good number mentioned that, pesticide residues can be present on tomatoes eaten as salad. Consumers were aware of bacteria and pesticide residues on fresh tomatoes but their knowledge was insufficient on the safety of tomato with pesticide residues

Fresh tomatoes sold and consumed in Nairobi tend to be free of pesticide residues, *E. coli* and *Salmonella*. Enteric bacteria found on tomato are easily removed after washing and sanitizing but, pesticide residues might still be present and this is a serious public health concern to consider. Consumers' awareness on pesticide residues on potential contamination of fresh tomato with pesticide residues is insufficient compared to that on enteric bacteria. Studies on washing fresh vegetables to reduce pesticide residues to acceptable levels should be encouraged. With some residues highly above MRLs, this study contributes to understand why pesticide residues in fresh tomatoes might be considered as contributing to some infections. This information should be considered by stake holders and policy makers.

8.3 RECOMMENDATIONS

1. Consider agrovets as part of the system for the use of pesticides in tomatoes farms and therefore, build their capacity accordingly for education, information and communication with farmers.

2. It is advisable to divide pesticides recorded by PCPB into early, mid-term and late use in tomato farms to control contamination on mature produce sold in markets.

3. More efforts from the government and the international community should be added to meet the good agricultural practices of chemicals use in tomato farms leading toward the quality requirements of tomato markets. Under this scheme, the burden of diseases related to pesticide residues may decrease in households and governments and, a sustainable development will rise with healthy people consuming safe tomatoes. 4. Improve consumers' knowledge on pesticide residues contamination through information, communication and education.

5. Encouraged studies on washing raw tomatoes from markets in order to consume tomatoes free of pesticide residues in households.

6. Revise PHIs of some pesticides such as Acephate

7. Azoxystrobin, carbendazim and Profenofos are some of the chemicals to recommend for use in tomatoes farms.

8. Follow the behavior of Acephate, Dimethomorph and Fenamiphos found above MRLs.

9. Seek to understand reasons of multiple contaminations on fresh tomatoes with pesticide residues.

10. Surveillance against the use of pesticides not recommended in Kenya is needed or should be reinforced.

11. Build a national MRLs database for pesticides recommended in Kenya.

12. Encourage studies for a rapid diagnoistic test (RDT) of pesticide residues in vegetables for a better control in local markets

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APPENDIX

Appendix 1: Questionnaire for the survey with farmers Appendix 2: Questionnaire for the survey with households

QUESTIONNAIRE	Code Interviewer	N ₀
SECTION A: QUE	ESTIONNAIRE TO THE FARMERS	
BIODATA OF TH	IE FARMER	
1- Name		
2- Gender: Mal	le □, Female □	
3- Age		
4- Level of edu	ucation: Never attended any school \Box , Prim	ary \Box , Ordinary \Box , advance \Box or
university 🗖]	
5- Marital statu	us: Married \Box , Fiancé (e) \Box , Divorce \Box ,	Widow □, Single □
6- Number of p	people living in the house:	
7- Number of f	females:	
8- Number of r	male:	
9- Any breast f	feeding woman? Yes \Box , No \Box	
10- Number of H	Kids:	

A.I- FARM SIZE IN ACRES

A.1.1- What is the size of your farm? acres
A.1.2- Are you land owner? Yes \Box , No \Box
A.1.3- What is your annual income from the tomato farm?
A.1.4- Do you have other sources of income: Employed \Box , or other business

A.II- EXPERIENCE ON TOMATO CULTIVATION

 A.2.1- How many years have you been cultivating the tomato? Less than a year □; 2 years □,

 3 years □; 4 years □; 5 years □; 6 years □; more than 6 years □

 A.2.2- Which diseases attack the tomato in the farm? List 3 most important ------

 A.2.3- Which pests attack the tomato in the farm? List 3 most important ------

 A.2.3- Which pests attack the tomato in the farm? List 3 most important ------

 A.2.4- What are the varieties of tomato mostly plant in the area? Specify -------

 A.2.5- Which one do you prefer? List 3 ------

 A.2.6- Why do you prefer them? Are preferred by the consumers □, They mature quickly □, They produce more □, The shelf life is high □

A.III- COMMUNITY ENGAGEMENT

A.3.1- Do you know some associations for the tomato cultivators in this town? Yes \Box , No \Box A.3.2- Can you give us the names of those you know? ------

A.3.3- Are you a member of one of them or, do you fellowship with several of them? Yes \Box , No \Box

A.IV- TOMATO PESTICIDES MANAGEMENT

A.4.1- How have you come to know about pesticides for the first time?

a- I saw from the other farmers □, b- Informed at the meeting of the association □, c- we were told during the training by an agricultural expert □, d- we were informed by the agricultural extension Officer □, e- I heard it as a grapevine □ A.4.2-Do you know the usefulness of pesticides on tomato farms? Yes □, No □ A.4.3- Do you know how to use the pesticides in tomato farm? Yes □, No □ A.4.4- From which source do you know how to use pesticides? a- From the other farmers □, b- We were informed during the meeting of the association □, c- we were told during the training by an agricultural expert □, d- We were informed by the agricultural extension Officer □, e- I heard it as a grapevine □, f- From the cooperative shops □, g- From the Agrovet □

A.4.5- For how long have you been using the pesticides in tomato farming? Less than a year \Box ; 2 years \Box , 3 years \Box ; 4 years \Box ; 5 years \Box ; 6 years \Box ; more than 6 years \Box

A.4.6- Were you trained on pesticides use on tomato farming? Yes \Box , No \Box

A.4.7- If yes, who trained you on how to use pesticides on tomato farm? a- I saw other farmers doing \Box , b- The association of tomato farmers \Box , c- I follow the instructions of the Agricultural extension Officer \Box , d- I follow the advice of the Agricultural Expert from the radio \Box , e- The industry

A.4.8- How do you know the names of pesticides to use in your tomato farm?

- a) I was informed by the agricultural extension Officer \Box
- b) I asked to other tomato farmers \Box
- c) We are regularly updated in our association of tomato farmers \Box
- d) The Agrovets present us new chemical products, their efficacy and their names \Box
- e) I always ask to the Cooperative shops \Box

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A.4.9-Where do you buy the pesticides you use in your tomato farm?

- a. Agrovets □,
- b. Cooperative shop \Box
- c. Sometimes, they are donated by the partners \Box

A.4.10- Are the pesticides you buy for tomato farming approved by the PCPB? Yes \Box ; No

A.4.10.1. If yes, how do you know the difference between the approved and non-approved?

a- The recommended ones have a number from PCPB \Box ; b- I rely on the Agrovets \Box ,

c- I always ask the vendor \Box , d- there is no difference \Box

4.11- Can you list the names of the pesticides mostly used in tomato farming? (give them in your priority: N₁, N₂, N₃)

a) -----; d -----; g -----; j -----;

b) -----; e -----; h -----; k -----;

c) -----; f ------; i ------; l ------;

A.4.12. Are there some pesticides highly recommended by the Agricultural Extension

Officer? Yes \Box ; No \Box

A.4.12.1. If yes, do you use them? Yes \Box ; No \Box

A.4.12.2 If yes, can you give some names? ------

A.4.12.3. If no, why? -----

A.4.13- Do you mix different pesticides before spraying in tomato farm? Yes \Box ; No \Box

A.13.1. If yes, which ones? ------

A.4.14- Which instructions for the use of the pesticides do you follow?

a- The instructions from the manufacturer? Yes \Box ; No \Box

- b- The advices of the colleagues? Yes \Box ; No \Box
- c- The advices from the Agrovets? Yes \Box ; No \Box
- d- The advices from the Association of the tomato farmers? Yes \Box , No \Box
- e- The advice from the Agricultural extension Officer? Yes \Box ; No \Box

A.V- KNOWLEDGE ON TOMATO CULTIVATION

A.5.1- Do you do any intercropping in the tomato farm? Yes \Box , No \Box A.5.2- Which crop/s do you intercrop? 1. ----- 2. ----- 2. -----A.5.3- Have you been trained on the best practices for the tomato cultivation? Yes \Box ; No \Box A.5.4- What is the best season to cultivate tomato? Dry season \Box , Wet season \Box , The season doesn't matter \Box , Pesticides and irrigation have made tomato farming easy at any season \Box , A.5.4.1 If the answer is: <u>dry or wet season</u>; why? We apply much pesticides \Box , pesticides applied in tomato farm stays for long time \Box , the diseases and pests are not much \Box

A.VI- SUPPORT FROM THE AGRICULTURAL EXTENSION OFFICER

A.6.1- Do Agricultural Extension Officers visit your farm? Yes \Box ; No \Box A.6.2- Do they regularly give you advises on the use of pesticides on tomato crop? Yes \Box ; No \Box

A.6.c- Who organizes those visits? The Agricultural Extension Officer \Box , The Sub County Agricultural Officer \Box , I request for help \Box , The Agricultural Extension Officer comes even when not expected \Box , The Association of tomato farmers \Box

A.VII- TRAINING ON TOMATO PESTICIDES MANAGEMENT

A.7.1- Have you been trained on the use of pesticides on tomato farms?

Yes □ ; No □

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If yes, how many trainings have you received? 1 \square ; 2 \square ; 3 \square ; more than three \square
A.7.2- Who organized the trainings? The partners \square ; The Industry \square, The Kenya Crop
A.7.3- When (year) was the last training you had? 1 year ago \square; 2 years ago \square;
3 years ago \square; 4 years ago \square; 5 years ago \square.
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A.VIII- PESTICIDES SPRAYING IN TOMATO CROPS

A.8.1- How often (many times) do you spray pesticides in your tomato crops from the time you plant to the time you harvest?

a- Every 2 weeks \Box ;	b- Three times per month \Box ; e- Every 7 days
c- There is no timing \Box ;	d-You spray as you feel your farm may be attacked by
	pests and diseases \Box

A.8.2- Is there any regulation on how to spray pesticides in tomato farm? Yes □; No □;
Don't know □ A.8.2.1- If Yes, How Many Times (Often) Do You Spray Then?
a- Twice a Month □; b- every week □; c- once a month □;
d- Once you can see the fruits on the tomato tree □; e- Once the tomato tree is mature □

A.IX- WITHDRAWAL PERIOD OF FRESH TOMATO FROM FARM

A.9.1- What is the approximate last day or time you spray pesticides on tomato in the farm? a- 14 days before harvesting \Box ; b- 9 days before the harvesting day \Box ; c- At least 7 days before the harvesting date \Box A. 9.1.1- If the answer is at least 7 days before the harvesting date, then ask: How do you know that? a- We were told during the training \Box ; b- It is always reminded by the agricultural extension Officer \Box ; c- It is always reminded during the meeting preceding the planting period of tomato \Box ; d- it is written on the instructions from the manufacturer \Box A. 9.1.2- Why do you wait for these numbers of days? To avoid having pesticides on tomato

after harvesting \Box , I don't know \Box , We were just told to do that during the meeting of the Association \Box , There is no reason for that \Box

A. 9.1.3- Do you spray pesticides on tomato fruits after you have harvested from the farm? Yes \Box , No \Box

A. 9.1.3.1- If yes, why? a- To protect the fresh fruits from diseases and pests \Box ; b- To avoid losses \Box ; c- The middlemen want only protected tomato from here to town \Box , The middlemen are not always present when I am harvesting \Box ,

A.X- POTENTIAL ECONOMIC INFLUENCE ON PESTICIDES USE IN TOMATO FARMING

A.10.a- Relation between the farmers, middlemen and retailers

- Do you have any contract with any middlemen for the sale of tomato? Yes \Box ; No \Box
- Do you receive any instruction from the middlemen on the utilization of pesticides in tomato farm? Yes □; No □
- Do you receive any instruction from the tomato retailers on pesticides utilization in tomato farm? Yes □; No □

A.10.b- Relation between the farmers and tomato exporters

- Do you have any contract with any exporters for the sale of tomato? Yes \Box ; No \Box

Do you receive any advice from the exporters for the utilization of pesticides in tomato farm? Yes □; No □

A.10.c- Relation between the farmers and tomato industries processors

- Do you have any contract with any tomato processor for the sale of tomato? Yes □;
 No □
- Do you receive any advice from the tomato processor for the utilization of pesticides in tomato farm? Yes □; No □

A.XI- ACCESS TO MARKET FOR THE SELLING OF THE FRESH TOMATO

A.11.1- Who mostly buy your tomato? The retailers \Box , Middlemen \Box , Tomato processor A.11.2- Do you have access to the markets for selling your tomato easily? Yes \Box , No \Box A.11.3- Do the middlemen have preference between the yellow or red tomato? Yes \Box , No \Box A.11.3.1- If yes, they prefer the Yellow fruits \Box , they prefer the red fruits \Box , They prefer the mixture \Box

A.11.4- Do they buy them at the same price? Yes \Box ; No \Box

A.11.4.1- Why?

a- To avoid losses \Box ; b- the customers like them most \Box ; c- they are the most requested or welcome by the retailers \Box , d- they are recommended by the middlemen \Box , e- their cost is higher than those already red because they can last for more days in the markets without losses \Box

A.XII- TOMATO FARMING AND SEASONS: SEASONAL APPLICABILITY OF PESTICIDES ON TOMATO CROPS

A.12.1- Do you have a season during which you apply more pesticides in tomato farm than another? Yes D, No D A.12.1.1- If yes, which one? Rainy season D, dry season D A.12.2- For which reason do you do that? a- The chemicals do not stay for long on the crop due to heavy rains D, b- the sun dries quickly the chemical on the crops and ground, then pests and diseases attack easily D; c- there are more diseases attacks D, d- there are more pests attacks D, e- there are more both pests and diseases attacks D, Other (list 2 or 3 reasons) ------

A.XIII- TOMATO PESTICIDES AND HEALTH

A.13.1- Are there any diseases able to be transmitted by raw tomato? Yes \Box , No \Box			
If Yes, which one(s)? Headache \Box , malaria \Box , stomach ache \Box , urination \Box ,			
abortion of pregnant women \Box , deaths of fetus \Box , cancers on adults and children \Box ,			
infertility \Box , diabetes \Box , liver disease \Box , kidney disease \Box , thyroid \Box ,			
influence children and adolescent growth \Box , play against the skills development of children			
or diminish the mental development of children \Box , Diarrhea \Box , None \Box			

THANK YOU FOR YOUR PARTICIPATION, TIME AND CONSIDERATION IN THIS STUDY

QUESTIONNAIRE Interviewer Code: N ₀
SECTION B: QUESTIONNAIRE FOR HOUSEHOLDS
1- BIODATA OF THE HOUSEHOLD
1- Name
2- Gender: Male □, Female □
3- Age
4- Level of education: Never attended any school \Box , Primary \Box , Secondary \Box , Tertiary \Box
or University□
5- Marital status: Married \Box , Divorce \Box , Widow \Box , Single \Box
6- Number of people living in the house:
7- Number of Children:
1- TOMATO SEASONAL PROVISION:
1.1. Which season comes with more and cheap tomatoes in the markets? Wet seasons, Dry
seasons \Box , there is no difference \Box

1.2. Which season comes with less and expensive tomatoes in the markets? Wet seasons \Box , Dry

seasons \Box , there is no difference \Box

2-CONSUMPTION OF TOMATO IN HOUSEHOLDS

2.1. How often do you eat tomato? Everyday \Box , twice per week \Box , Three times a week \Box , at

leat four time a week \Box , I don't know \Box , I don't eat tomato very often \Box

2.2. What are the types of tomatoes you know? Fresh tomato \Box , processed tomato \Box , Both fresh and processed \Box

2.3. Which type of tomato do you prefer for your house? Fresh tomato \Box , processed tomato \Box , both fresh and processed ones \Box , It depends of the means I have \Box

2.3.1. If fresh tomato, then why? It is the cheapest \Box , It is the most available one \Box ; It is easy to cook \Box , It is sweeter \Box , It contains more vitamins \Box , it is natural \Box , It is good for human's health \Box

2.3.2. Can fresh tomato cause any disease to someone? Yes \Box , No \Box

2.3.3. If yes, which ones? Dysentery \Box , diarrhea \Box , stomach ache \Box , head ache \Box , filariosis \Box , diabetes \Box , cancer \Box , Malaria \Box

2.4. Can processed tomato transmit any disease to someone? Yes \Box , No \Box

2.4.1. If yes, which ones? Dysentery \Box , diarrhea \Box , stomach ache \Box , head ache \Box , filariosis \Box , diabetes \Box , cancer \Box

2.4.2. If no, why? It can give diseases \Box , Because farmers use pesticides \Box , It is sealed \Box , Precautions for safety are taken \Box

3. KNOWLEDGE ON PESTICIDES ON TOMATO

3.1. Do you know pesticides? Yes \Box , No \Box

3.2. Do you know that, pests and diseases attack tomatoes in the farms and cause losses to farmers? Yes \Box , No \Box

3.3. Do you know pesticides are used to repel or kill pests and diseases from tomato farms? Yes \Box , No \Box

1.4. Do you know that, farmers use pesticides to protect tomatoes from pests and diseases in farms? Yes \Box , No \Box

3.4. Do you think that, pesticides are dangerous for humans' health? Yes \Box , No \Box

3.4.1. If yes, why? They are poisonous if swallowed \Box ; If they kill pests and diseases on tomato, they can also kill humans \Box ; they can cause malaria \Box , they can cause cancer \Box , they can cause headache \Box

3.5. Do you think that, pesticides sprayed on tomato in farms can be dangerous for consumers' health? Yes \Box , No \Box

3.6. Do you think that, pesticides sprayed in tomato farms can be present on tomato being sold in the markets Yes \Box , No \Box

3.7. Do you think that, pesticides sprayed in tomato farms can be present on tomato eaten in salad? Yes \Box , No \Box

3.7.1. If yes, can you feel/notice its presence on tomato while eating in salad? Yes \Box , No \Box

3.8. Can pesticides sprayed in tomato's farms be present in tomato cooked home? Yes \Box , No

3.8.1. If yes, can you feel the presence of pesticides on tomato while eating in cooked food? Yes \Box , No \Box

3.9. Do you think pesticides can transmit diseases to tomato's consumers? Yes \Box , No \Box

3.9.1. If yes, which diseases? Malaria□, Stomachache□, Tuberculosis□, HIV□, Cancers□, Fever□, Headache□, Nausea□

3.9.2. If No, Why? Because the government has allowed their use in farms, The farmers couldn't have used them in that case \Box , The industries would have stopped producing them, The international community would have requested to stop their use in the tomato farms

4. KNOWLEDGE ON PATHOGENS IN TOMATO

4.1. Do you know pathogens? Yes \Box , No \Box

4.2. What are they? Agents causing illnesses in humans \Box , elements deteriorating human's health \Box , elements of the tomato fruits \Box , friendly organisms \Box

4.3. Do you think that, pathogens can be found on the skin/surface of tomato? Yes \Box , No \Box

4.3.1. If yes, how? It can be transmitted by the customers \Box , it naturally exists on the skin of tomatoes \Box , if hygienic conditions are not met, pathogens will be on the skin \Box , the soil can be contaminated \Box , Others _____

4.4. Do you think that, pathogens can be found inside tomato? Yes \Box , No \Box

4.5. How can you explain that pathogens can be found inside tomato? I don't know \Box , the pathogens can come from the soil \Box , the pathogens can be transmitted by pests \Box , the pathogens can come from the seeds \Box , Pathogens can get in during handling (during packaging, shipping, through wounds) \Box

4.6. Is it safe to eat raw tomato from farms or markets after washing? Yes \Box , No \Box

4.6.1. If no, why? Because tomato from the farms or markets can be infected by pathogens \Box , it can contain other pestcides \Box , it can contain heavy metals \Box

4.7. Do you think that, tomato eaten in salad can affect human health? Yes \Box , No \Box

4.8. Do you think, tomato cooked in food at home can affect human health? Yes \Box , No \Box

4.9. Do tomato washing before eating in salad prevents from any disease? Yes \Box , No \Box

4.10 Do you think that, processed tomato can be infected with pathogens? Yes \Box , No \Box

THANKS FOR YOUR PARTICIPATION IN THIS STUDY SEEKING TO IMPROVE OUR HEALTH THROUGH FOOD!