Original Research Article

Comparison of Pesticide Residue Levels in Tomatoes from Open Fields, Greenhouses, Markets and Consumers in Kirinyaga County, Kenya

ABSTRACT

The study was carried out to determine and compare pesticide residue levels in tomatoes from Mwea Irrigation Scheme. Thirty five tomato samples of Rambo variety randomly collected from open fields, greenhouses, markets and consumers were analyzed using Easy, Cheap, Effective, Rugged and Safe (QuEChERS) method. Results showed that the level of pesticide residue from greenhouse tomatoes higher that from open fields, markets and consumers. Alpha-cypermethrin level in greenhouse tomatoes (0.0871±0.0087mg/kg) was significantly ($p<0.01$) higher than from consumers (0.0218±0.0061mg/kg) while difenoconazole from greenhouse tomatoes (0.2597±0.0522 mg/kg) was significantly ($p<0.05$) higher than from the open field (0.0295±0.0014 mg/kg). Carbendazim level in greenhouse (1.2341±0.1667 mg/kg) tomatoes was significantly ($p<0.001$) higher than from open fields (0.0596±0.0178 mg/kg), markets (0.1160±0.0490 mg/kg) and consumers (0.0494±0.0155 mg/kg). Imidacloprid in greenhouse tomatoes (0.1446±0.0086 mg/kg) was significantly ($p<0.001$) higher than from the markets (0.0236±0.0019 mg/kg) and consumers (0.0170±0.0017 mg/kg). High pesticide residue levels in tomatoes is a health concern for consumers. Enforcing the food safety laws, enhancing farmer training on safe use of pesticides and creating awareness on pesticide risks would promote production of uncontaminated crops consumed locally.

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Keywords: Tomato, pesticide, residue level, open field, greenhouse, market, consumer

1. INTRODUCTION

Tomato (Lycopersicon esculentum Mill) is an important vegetable grown globally and in Kenya. Its popularity as a commercial crop is on the rise compared to other cash crops. The crop is among vegetables mainly grown in open field and greenhouse production systems globally [1;2]. The crop grows well in areas with altitudes that range from 1150 and 1800m above sea level. For optimum production, tomatoes require deep, medium textured loamy or sandy loam, fertile, well drained soils with a pH between 6.0 and 7.0, 3 to 4 months warm, clear and fairly dry weather and temperatures between 20° to 27°C. Tomatoes need 600 mm of well distributed rainfall over the growing period [3]. Tomato ranks second in importance among the produced vegetables (after potatoes) in terms of production volume and value; placing Kenya among the top African producers [4]. The crop accounts for about 7% and 14% of the total production for horticulture and vegetable production respectively [5;6].

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Kenya is among top tomato producers in Sub Saharan Africa, with a production of over 400,000 tons in an area of over 20,000 ha [7;4]. Kirinyaga County leads (14%) in production followed by Kajiado (9%) and Taita Taveta (7%) [6].

Actual yields remain below the maximum attainable levels with Sub Saharan Africa recording an production that is below the global average [8]. Despite efforts to improve tomato production by introducing modern technologies such as greenhouses in Kenya, productivity declined from 22.4 tons in 2011 to 17.9 tons in 2015 and 16.9 tons in 2016 [9]. Deviations persisted in 2018 with an average yield of 12 tons/ha against a potential yield of 30.7 tons per ha [4]. The low productivity is associated with the inability of farmers to fully utilize available technologies and other factors such as reduction of land availability for agricultural production due to huge population growth, soil degradation and intensified land fragmentation. High poverty levels combined with other factors limiting production have made it difficult for farmers to increase production [10;11]. High pest and disease infestation and nutrient deficiency are major setbacks in tomato production of which if not controlled can cause great losses [4]. Increased demand in Kenya has necessitated an increase in production forcing farmers in Mwea to rely heavily on pesticides to control pests and diseases since no marketable produce can be harvested from untreated crops. However, excessive and improper use of these pesticides results in contamination of the produce and the environment [12;13].

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2. METHODOLOGY

2.1 The study area

The study was conducted in the eight wards; Gathigiriri, Tebere, Kangai, Wamumu, Murinduko, Nyangati, Mutithi and Thiba of Mwea irrigation scheme in Kirinyaga County, Kenya (Figure 1). The scheme has about 51,444 households, a density of 341 people per km2 within an area of 516.7 km2. The area lies between latitudes 0.540o and 0.788o South and longitudes 37.228o and 37.497o East (Figure 1). Its topography is relatively uniform and stretches over the flat land on the outskirts of Mt. Kenya [14]. The scheme is well supplied with irrigation water from Nyamindi and Thiba Rivers which favours tomato production. Mwea irrigation scheme was considered appropriate for the study to fill in the knowledge gaps on information on the variation of pesticide residue levels in tomatoes from production to consumption.

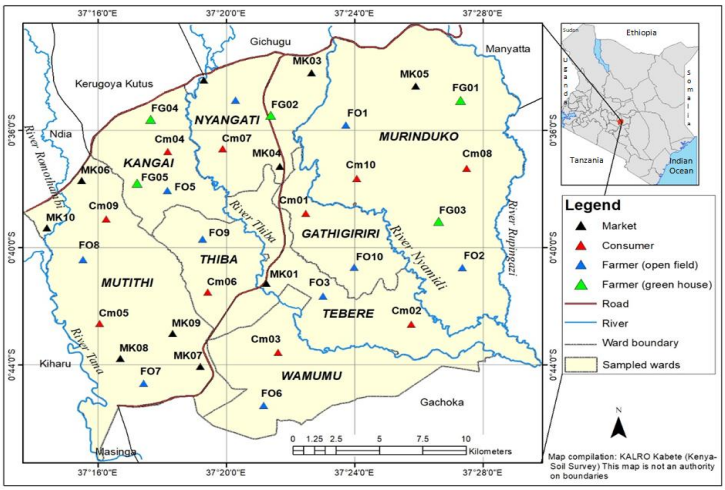

Figure 1: Map of Mwea Irrigation Scheme showing sampling points (wards).
FO= Farmer open field; FG= Farmer greenhouse; MK= Market; Cm= Consumer

2.2 Sampling, packaging and submission to the laboratory

One kilogram tomato samples each were randomly picked in triplicates from open fields, greenhouses, markets and consumers then thoroughly mixed to form a 3kg composite sample. One kilogram sample was picked randomly from each composite sample, wrapped in sterilized aluminium foil, placed in a self-sealing polythene bag, labelled, placed in a plastic container and stored temporary in polyurethane cool-boxes containing dry ice. They were transported to Kenya Plant Health Inspectorate Service (KEPHIS) laboratory on the same day. Thirty five samples were collected; 10 each from open fields, markets, consumers and 5 from greenhouses. After checking to ensure tomatoes were fresh in terms of water quantity and not rotten, they were received in the laboratory through filling a sample submission form. Samples were each given a laboratory traceability code that showed the source and date of submission after ensuring that all samples had been labelled from the field by indicating the origin and date of collection for traceability. The samples were stored in a cold room at a temperature of -18ºC prior to extraction to stop degradation of the pesticides that leads to reduction of pesticide residue levels.

2.3 Processing, Extraction and Separation

Each 1kg tomato sample from the cold room was chopped into smaller sizes using a Stephen chopper then homogenized by a wiring blender to get a uniform sample. After chopping each sample, the chopper and blender were thoroughly cleaned with distilled deionized water to remove contamination from the previous sample and rinsed twice with high purity acetone (99%) to remove pesticides or any contaminants from the previous samples. Extraction and analysis of the homogenized supernatant was done using the Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) method as in [15].

A 50ml single use extraction polyethylene tube was rinsed twice with high purity acetone (99%) to remove any contaminants and dried before use. Ten grams of each homogenized sample was weighed in duplicate in the tube using calibrated ADAM AFP 200100 LC analytical balance. Two Internal standards for quality control check, 50µl (0.05µg/g) of Malathion D10 (10ppm) for the liquid chromatography-mass spectrometry mass spectrometry (LC-MS/MS) and 5µl (0.005µg/g) of Dichlovos D6 (10ppm) for the gas chromatography mass spectrometry (GC-MS) were each added. Acetonitrile, 10ml ± 0.2ml, solvent used for extraction was added into each tube, vortexed using Wiemix-VM-10 machine for 1 minute and 6.5g of pre-mixed extraction salts (4g ± 0.2g anhydrous magnesium sulphate anhydrous, 1g ± 0.05g sodium chloride, 1g ± 0.05g trisodium citrate dehydrate and 0.5g ± 0.03g disodium hydrogen citrate sesquihydrate) were added. The mixture was vortexed for 1 minute and centrifuged using a universal 320 R centrifuge for 5 minutes at 3700 revolutions per minute to separate liquid and solid portions of the sample extracts. The liquid portion was taken for sample clean-up.

2.4 Sample Clean-up and Analysis

Four, 4 ml portions of the liquid sample extracts containing pesticides were each pipetted into 15ml centrifuge tubes. Two sample portions were taken for LC-MS/MS and the other two for GC-MS analysis. A standard mixture, 20µl (0.02 µg/g), of each targeted pesticides was added to obtain the calibration curves for the LC-MS/MS analysis. QuEChERs multi-residue method for analysis of pesticide residues in low-fat products was used for analysis. For sample analysis, 10 µl of formic acid (10 µl per ml of sample) and 60µl of D-sorbitol (30µl per sample) were added to each separated liquid sample extract portion in 15ml centrifuge tube. After 1 minute vigorous shaking, 500µl of mixture was pipetted into a 1ml auto sample vial and 5µl of the procedural injection internal standard dimethoate D6 (10ppm) added. It was diluted by adding 495µl of High Performance Liquid Chromatograph (HPLC) water, vortexed and taken for analysis using Liquid Chromatography technique with triple quadruple mass detector (LC-MS/MS Agilent 6430) for 30 minutes at room temperature. For the GC-MS analysis, 50µl (0.05 µg/g) of targeted pesticides, standard mixtures were prepared and used for the calibration of GC-MS machine. Triplicate 500µl of each liquid sample extract was pipetted from each sample mixture into a 1 ml auto sample vial, concentrated to near dryness under a gentle stream of white spot nitrogen gas, then 500µl of GC-MS pesticide solvent 2, 2, 4-Trimethylpentane (Iso-octane) was added, vortexed, and analysed in GC-MS machine for 42.5 minutes at a temperature between 60-300ºC.

2.5 Identification and confirmatory tests

Where, many compounds, including co-extracts interfered with retention times, their identities were confirmed by running the samples on two different (non-polar and polar) columns with different stationary phases. Non polar column CP-SIL 8CB-15 m, 0.25 mm internal diameter (id), 0.25µm film and polar column DB-1701-15 m, 0.53 mm internal diameter (id), 0.5µm film or GC-MS were used for confirmation. Whenever retention times of the substances and standards agreed on both columns and the GC-MS and the calculated concentrations would be about the same, the compound's identity was ascertained by their peaks. The resolution and

identification was also confirmed using relative retention times obtained by measuring the retention time of each test standard analyte.

2.6 Limits of detection and quantification

The lowest concentration of the analytes that the analytical process can reliably detect is referred to as the limit of detection (LOD). Based on the relationship between the lowest detectable analytes signal Sd, the field blank Sb, and the variability in the field blank (σb) the estimation of LOD is given by equation 1. LOD can be defined as the analyte concentration which gives a gross signal exceeding Sb by Kd units of σb..

At LOD, $Sd = Sb + Kd\ \sigma b$ (Equation 1)

Where a value of three is assumed for Kd (Kd=3)

For the estimation of limits of quantification (LOQ) as given by equation 2, the quantification (Numerical estimations of the amount) of the concentration of the analyte is considered reliable if the corresponding gross signal (Sq) is:

$Sq = Sb + Kt\ \sigma b$ (Equation 2)

Where a value of 10 is assumed for Kt so that at least one figure of the results is significant.

3. RESULTS

3.1 Pesticide residues in tomatoes from open fields, greenhouses, markets and consumers.

Eleven different pesticides were detected in all tomatoes from the open field, greenhouse, market and consumer. The greenhouse had the highest number and percentage (7, 63.6%) followed by open field (5, 45.5%) market and consumer had (3, 27.3%) each (Table 1).

Table 1: Pesticide residues in tomatoes from all sampling sites (n=11)

Sampling sites	Name of pesticide residue	Mean Residue level (mg/kg)	Number and proportion	%
Open field farms	Acetamiprid	0.0256±0.0028	5/11	45.5%
	Azoxystrobin	0.0438±0.0039		
	Difenoconazole	0.0295±0.0014		
	Carbendazim	0.0596±0.0178		
	Malathion	0.0315±0.0032		
Greenhouses	Difenoconazole	0.2597±0.0522	7/11	63.6%
	Imidacloprid	0.1446±0.0086		
	Metalaxyl	0.0428±0.0039		
	Dimethomorph	0.0231±0.0025		
	Carbendazim	1.2341±0.1667		
	Thiamethoxam	0.3736±0.0358		
	Alpha-cypermethrin	0.0871±0.0087		

Markets	Acephate	0.0321±0.0032	3/11	27.3%
	Carbendazim	0.1160± 0.0490		
	Imidacloprid	0.0236±0.0019		
Consumers	Carbendazim	0.0494±0.0155	3/11	27.3%
	Alpha-cypermethrin	0.0218±0.0061		
	Imidacloprid	0.0170±0.0017		

Alpha-cypermethrin, carbendazim, difenoconazole and imidacloprid levels in tomatoes detected from more than one sampling point were subjected to Analysis of Variance (ANOVA) followed by Tukey Kramer post hoc at 95% Confidence Interval.

3.1.1 Alpha-cypermethrin

The level of alpha-cypermethrin (0.0871±0.0087mg/kg) in greenhouse tomatoes was significantly ($p<0.01$) higher than from consumers (0.0218±0.0061 mg/kg) as determined by ANOVA at 95% Confidence Interval ($F = 37.748$, $p < 0.01$) (Table 1; Table 2).

Table 2. ANOVA for alpha-cypermethrin in tomatoes from greenhouses and consumers

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.006	1	0.006	37.748	<0.01
Within Groups	0.001	4	0.000		
Total	0.006	5			

3.1.2 Carbendazim

ANOVA (Table 3) indicated very high significant ($p<0.001$) differences of carbendazim level in tomatoes from greenhouses, open fields, markets and consumers ($F = 111.554$, $p<0.001$).

Table 3. ANOVA for carbendazim from open field, greenhouse, market and consumer tomatoes.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.427	3	0.809	111.554	<0.001
Within Groups	0.102	14	0.007		
Total	2.529	17			

Tukey Kramer post hoc test (Table 4) revealed that the level of carbendazim from greenhouse tomatoes (1.2341±0.1667 mg/kg) shown in Table 1 was significantly ($p<0.001$) higher than from the open fields (0.0596±0.0178 mg/kg), markets (0.1160±0.0490 mg/kg) and consumers (0.0494±0.0155 mg/kg).

Table 4. Tukey Kramer post hoc test for carbendazim on tomatoes from open fields, greenhouses, markets and consumers.

Site name		Mean Difference	Std. Error	p-value
Open field	Greenhouse	-1.1746*	0.0738	<0.001
	Markets	-0.0565	0.0602	>0.05
	Consumers	0.0101	0.0522	>0.05
Greenhouse	Open field	1.1746*	0.0738	<0.001
	Markets	1.1182*	0.0738	<0.001
	Consumers	1.1847*	0.0673	<0.001
Markets	Open field	0.0565	0.0602	>0.05
	Greenhouse	-1.1182*	0.0738	<0.001
	Consumers	0.0666	0.0522	>0.05
Consumers	Open field	-0.0101	0.0522	>0.05
	Greenhouse	-1.1847*	0.0673	<0.001
	Markets	-0.0666	0.0522	>0.05

* The mean difference is significant at the 0.05 level

3.1.3 Difenoconazole

The level of difenoconazole (Table 1) from greenhouse tomatoes (0.2597±0.0522 mg/kg) was significantly ($p<0.05$) higher than from the open fields (0.0295±0.0014 mg/kg) as determined by ANOVA at 95% Confidence Interval (Table 5).

Table 5: ANOVA for difenoconazole from open field and greenhouse tomatoes.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.071	1	0.071	8.623	<0.05
Within Groups	0.033	4	0.008		
Total	0.103	5			

3.1.4 Imidacloprid

ANOVA at 95% Confidence Interval (Table 6) showed very high significant difference ($p<0.001$) for imidacloprid level in tomatoes from the greenhouses, markets and consumers ($F= 86.441$, $p<0.001$).

Table 6: ANOVA for imidacloprid level on greenhouse, market and consumer tomatoes.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.031	2	0.015	86.441	<0.001
Within Groups	0.001	5	0.000		
Total	0.032	7			

Tukey Kramer post hoc test at 95% Confidence Interval (Table 7) revealed that imidacloprid level (0.1446±0.0086 mg/kg) from greenhouse tomatoes was significantly higher than from the markets (0.0236±0.0019 mg/kg) and consumers (0.0170±0.0017 mg/kg) (Table 1).

Table 7: Tukey Kramer post hoc test for imidacloprid in tomatoes from greenhouses, markets and consumers

Site name		Mean Difference	Std. Error	Sig.
Greenhouse	Markets	0.1210*	0.0116	<0.001
	Consumers	0.1276*	0.0116	<0.001
Markets	Greenhouse	-0.1210*	0.0116	<0.001
	Consumers	0.0066	0.0134	>0.05
Consumers	Greenhouse	-0.1276*	0.0116	<0.001
	Markets	-0.0066	0.0134	>0.05

*The mean difference is significant at the 0.05 level.

4. DISCUSSION

Higher levels of alpha-cypermethrin, carbendazim, difenoconazole and imidacloprid in tomatoes from the greenhouses than from the open fields, markets and consumers in this study could be associated with slow degradation in shaded environment unlike in the open fields where breakdown is hastened by the sunlight, wind and rain. Breakdown is reduced by the netting, shade cloth or other forms/ types of covers in the greenhouse [16]. Due to this, pesticide residue levels in greenhouses may be above the allowed MRL even when the recommended waiting period specified on the label is followed. Results agree with Allen et al (2015) [17] who reported increased occurrence of pesticide residues on crops grown in protected environments compared to crops grown in open field conditions. Indiscriminate pesticide use in food crops may equally leave pesticide residues in crops beyond concentrations considered safe for consumption [18;19;20]. It may be safer for the Pre-Harvest Interval (PHI) in greenhouses to be slightly longer than for open field crops. This could prevent the occurrence of high residue levels in the crops from production to consumption points, and thus reduce negative health effects to the consumer. Consumption of these tomatoes for a long period could be risking the consumer's health [21]. Continuous exposure to carbendazim frequently detected in food crops is known to cause chronic effects such as cancer, genetic defects, damage the fertility of people and the unborn child [22]. In addition, carbendazim poisoning may damage organs such as the liver, kidneys and the spleen. This chemical is classified by the United States Environmental Protection Agency (U.S. EPA) as Group C possible human carcinogen [23].

5. CONCLUSION AND RECOMMENDATION

Considerably higher pesticide residue levels were detected in greenhouse tomatoes than from open fields, markets and consumers in Mwea Irrigation Scheme. This was associated to slow degradation in greenhouses, application of higher rates combined with harvesting before the recommended Pre-Harvest Interval. Consumption of such tomatoes is a health risk to the human. The findings of this study showed significantly higher pesticide residue levels in greenhouse tomatoes than from open fields, markets and consumers. It is thus recommended that the County Government of Kirinyaga should enhance farmer trainings on safe use of pesticides and create awareness on pesticide risks. This will help them see the need to embrace and strictly adhere to the manufacturer's application rate and Pre-Harvest Interval indicated on the label. Relevant state organisations mandated to evaluate the efficacy of pesticides (such as Kenya Agricultural and Livestock Research Organization (KALRO), Pest Control and Product Board (PCPB)) should recommend longer PHI for greenhouse use.

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

Authorization for this research was obtained from Kenyatta University (KU) graduate school, KU Ethics Review Committee and the National Commission for Science, Technology and Innovation (NACOSTI). Kirinyaga County Director of Agriculture gave permission to collect data in Mwea irrigation scheme. Informed consent was sought from participants who were assured of confidentiality throughout the study. Those who were willing to participate by signing the informed consent form were recruited to participate in the study. Verbal permission was sought from them to take photographs and their confidentiality was guaranteed by not indicating their names on tomato samples taken for analysis. Participants were also assured that results obtained would be kept confidential and only used by the researcher for the intended purpose. Farmers who were not willing to participate in the study were assured of no victimization from any office.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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