



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

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Authors' contributions

This work was carried out in collaboration among all authors. Author MNV designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. All authors managed the literature searches, read and approved the final manuscript

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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 QuEChERS method. Results showed that pesticide residue levels from greenhouse tomatoes was higher compared to open fields, markets and consumers. Alpha-cypermethrin level in greenhouse tomatoes (0.0871 ± 0.0087 mg/kg) was significantly ($p < 0.01$) higher than from consumers (0.0218 ± 0.0061 mg/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 are 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.

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 ranging from 1150 - 1800m above sea level. Tomatoes grow in a wide range of soils as long as the drainage and physical soil structure is good although the best production is on more fertile soils. Optimum pH is between 5.0 - 7.0 and temperatures between 20° - 27°C. The crop requires a minimum of and regular supply of 600 mm well distributed rainfall during the growing season [3]. Tomato crop 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]. Kenya is among the 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 a 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 can cause massive losses if not controlled [4]. An increase in demand within the country has forced farmers to rely on use of pesticides in order to increase production. However, excessive and improper use of these pesticides results in contamination of the produce and the environment [12,13].

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 (Fig. 1). The scheme which has about 51,444 households, a density of 341 people per km² within an area of 516.7 km². lies between latitudes 0.540° and 0.788° South and longitudes 37.228° and 37.497° East (Fig. 1). Mwea irrigation scheme has a moderately uniform topography that stretches over the flat land [14]. The scheme lies along the basins of rivers Nyamindi and Thiba which supply the irrigation water favorable for the production of tomatoes and other crops. There was need to carry out this research in the scheme in order to fill in the knowledge gaps by comparing pesticide residue levels in tomatoes from production to consumption points.

2.2 Sampling, Packaging and Submission to the Laboratory

Tomato samples of one kilogram each were randomly picked in triplicates from open fields, greenhouses, markets and consumers and thoroughly mixed to form a 3kg composite sample. One kilogram sample was randomly picked from each composite sample and wrapped in sterilized aluminum foil. It was placed in a self-sealing polythene bag, labeled by indicating the origin and date of collection, placed

in a plastic container and transported the same day to Kenya Plant Health Inspectorate Services (KEPHIS) laboratory in polyurethane cool-boxes containing dry ice. A total of thirty five samples were collected, ten each from open fields, markets and consumers, and five from greenhouses. After checking to ensure the tomatoes were fresh and not rotten, they were received in the laboratory and each given a traceability code that showed the source and date of submission. The samples were stored in a cold room at a temperature of -18°C prior to extraction the following day to stop degradation of the pesticide residues that could lead to reduction of their 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 blending each sample, the chopper and blender were thoroughly cleaned with distilled de-ionized water to remove contaminants and rinsed twice with acetone (99%) to remove pesticides or any other contaminants from the previous sample. Extraction and analysis of the homogenized supernatant was done using the Quick, Easy,

Cheap, Effective, Rugged and Safe (QuEChERS) method [15].

A 50 ml 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, 50 µl (0.05µg/g) of malathion D10 (10 ppm) for the liquid chromatography-mass spectrometry (GC-MS) were each added. Acetonitrile, 10ml ±0.2ml, solvent used for extraction was added into the mixture in each tube then vigorously shaken by hand and vortexed using Wiemix-VM-10 machine for one minute. Pre-mixed extraction salts (6.5g) was added into the sample mixture in the vortexed tube. The pre-mixed extraction salts containing (4g ± 0.2g) anhydrous magnesium sulphate, 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 shaken by hand, vortexed for one minute and centrifuged using a universal 320 R centrifuge for five minutes at 3700 revolutions per minute (rpm) to separate the liquid and solid portions of the sample extract. The liquid portion was taken for sample cleanup.

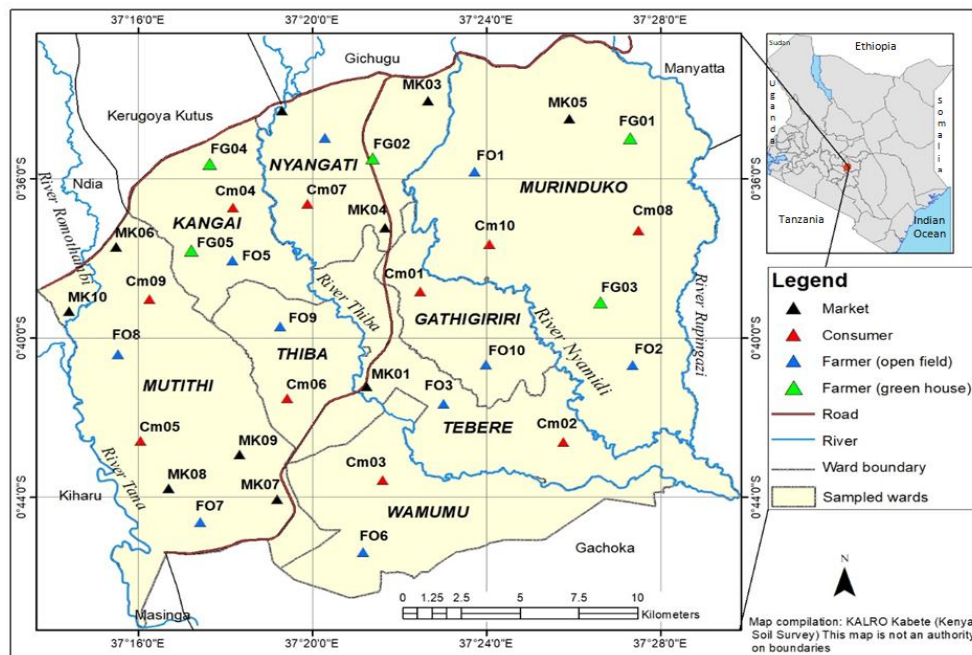


Fig. 1. Sampling points (wards) in Mwea Irrigation scheme
 FO= Farmer open field, FG= Farmer greenhouse, MK= Market, Cm=Consumer

2.4 Sample Clean-up and Analysis

Four, 4 ml sample portions of the liquid sample extracts containing the pesticides were each pipette into 15 ml centrifuge tubes. Two sample portions were taken for LC-MS/MS and the other two for GC-MS analysis. A standard mixture, 20 μ l (0.02 μ g/g), of each targeted pesticide was added to obtain the calibration curves for the LC-MS/MS analysis. QuEChERS multi-residue method for the analysis of pesticide residue levels in low-fat products was used for analysis. For sample analysis, 10 μ l of formic acid (10 μ l per ml of sample) and 60 μ l of D-sorbitol (30 μ l per sample) were added to each separated liquid sample extract portion in 15 ml centrifuge tube. After one minute vigorous shaking, 500 μ l of mixture was pipetted into a 1 ml auto sample vial and 5 μ l of the procedural injection internal standard dimethoate D6 (10 ppm) added. It was diluted by adding 495 μ l 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, 50 μ l (0.05 μ g/g) of standard mixtures were prepared and used for the calibration of GS-MS machine. Triplicate 500 μ l of each sample extract was pipette from each sample mixture into a 1 ml auto sample vial, concentrated to near dryness under a gentle stream of white spot nitrogen gas, and 500 μ l of GC-MS pesticide solvent 2, 2, 4-Trimethylpentane (Iso-octane) was added, vortexed and analyzed in GC-MS machine for 42.5 minutes at room temperature below 60° - 300°C. Any sample which was detected with pesticide residue levels was re-analyzed to confirm the result.

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-SL 8CB-15 m, 0.25 mm internal diameter (id), 0.25 μ m film and polar column DB-1701-15 m, 0.53 mm internal diameter (id), 0.5 μ m 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 calibrated concentrations would be about the same, the compounds identity was ascertained by their peaks. The resolution and identification were also confirmed using relative retention

times obtained by measuring the retention time of each standard analyte.

2.6 Limits of Detection and Quantification

The limit of detection (LOD) is the lowest concentration of the analytes that the analytical process can reliably detect. Based on the relationship between the lowest detectable analytes signal S_d , the field blank S_b , and the variability in the field blank (σ_b) the estimation of LOD is given by equation 1 [16]. LOD can be defined as the analyte concentration which gives a gross signal exceeding S_b by K_d units of σ_b .

$$\text{At LOD, } S_d = S_b + K_d \sigma_b \quad (\text{Equation 1})$$

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

For the estimation of limits of quantification (LOQ) as given by equation 2 [16], the quantification (Numerical estimations of the amount) of the concentration of the analyte is considered reliable if the corresponding gross signal (S_q) is:

$$S_q = S_b + K_t \sigma_b \quad (\text{Equation 2})$$

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

3. RESULTS

3.1 Pesticide Residue Levels in Tomatoes from Open Fields, Greenhouses, Markets and Consumers

Eleven different pesticides were detected in all tomatoes sampled from the open fields, greenhouses, markets and consumers. The greenhouses had the highest number (7) of pesticide residues and percentage (63.6%) followed by open fields (5) and 45.5%. Tomatoes from the markets and consumers had 3 (27.3%) each (Table 1).

Alpha-cypermethrin, carbendazim, difenoconazole and imidacloprid levels in tomatoes detected from more than one sampling point (Table 1) 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 ± 0.0087 mg/kg) in greenhouse tomatoes (Table 1) 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 2). The level of alpha-cypermethrin (0.0871 ± 0.0087 mg/kg) in tomatoes from greenhouses and consumers (Table 1) was significantly ($p < 0.05$) below the EU (0.5 mg/kg) and Codex MRLs (0.5 mg/kg).

3.1.2 Carbendazim

ANOVA (Table 3) showed very high significant ($p < 0.001$) differences of carbendazim level in

tomatoes from greenhouses, open fields, markets and consumers ($F = 111.554$, $p < 0.001$). The levels of carbendazim in tomatoes from open fields, markets and consumers (Table 1) were significantly less than the EU and Codex MRLs (0.3 and 0.5 mg/kg respectively).

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

Sampling sites	Name of pesticide residue detected	Mean Residue level (mg/kg)	EU MRL (mg/kg)	Codex MRL (mg/kg)	Number/ proportion	Percentage (%)
Open fields	Acetamiprid	0.0256 ± 0.0028	0.5	0.2	5/11	45.5
	Azoxystrobin	0.0438 ± 0.0039	3.0	3.0		
	Difenoconazole	0.0295 ± 0.0014	2.0	0.6		
	Carbendazim	0.0596 ± 0.0178	0.3	0.5		
	Malathion	0.0315 ± 0.0032	0.02	0.02		
Greenhouses	Difenoconazole	0.2597 ± 0.0522	2.0	0.6	7/11	63.6
	Imidacloprid	0.1446 ± 0.0086	0.5	0.5		
	Metalaxyl	0.0428 ± 0.0039	0.2	0.5		
	Dimethomorph	0.0231 ± 0.0025	1.0	1.5		
	Carbendazim	1.2341 ± 0.1667	0.3	0.5		
	Thiamethoxam	0.3736 ± 0.0358	0.2	0.7		
Markets	Acephate	0.0321 ± 0.0032	0.01	0.01	3/11	27.3
	Carbendazim	0.1160 ± 0.0490	0.3	0.5		
	Imidacloprid	0.0236 ± 0.0019	0.5	0.5		
Consumers	Carbendazim	0.0494 ± 0.0155	0.3	0.5	3/11	27.3
	Alpha-cypermethrin	0.0218 ± 0.0061	0.5	0.5		
	Imidacloprid	0.0170 ± 0.0017	0.5	0.5		

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			

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			

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). The level from greenhouse and open field tomatoes (Table 1) was significantly (<0.01; <0.001) less than the EU and Codex MRLs (0.5 mg/kg).

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). The levels of imidacloprid (Table 1) from greenhouses, markets and consumers were significantly (<0.01; <0.001) less than the EU and Codex MRLs of 0.5 mg/kg. Analysis by Tukey Kramer post hoc test at 95% Confidence Interval (Table 7) indicated that the

level of imidacloprid (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).

4. DISCUSSION

Significantly higher levels of Alpha-cypermethrin, carbendazim, difenoconazole and imidacloprid pesticides in tomatoes from the greenhouses compared to the open fields, markets and consumers in this study could be due to slow degradation in shaded environment unlike in the open fields where breakdown is speeded up by the sunlight, wind and rain. Breakdown is reduced by the netting, shade cloth or other forms/ types of covers in the greenhouse [17]. Due to this, pesticide residue levels in greenhouse crops may be above the allowed maximum residue levels (MRL) even when the recommended waiting period specified on the pesticide label is followed. These results agree with [18] who reported increased occurrence of

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			

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			

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

pesticide residue levels in crops grown in protected environments compared to crops grown in open field conditions. Indiscriminate pesticide use equally attributed to occurrence of pesticide residue levels in tomatoes from production to consumption points. Some farmers in the study intentionally applied higher rates of pesticides to knock down pests and diseases faster and harvested the tomatoes earlier than the recommended period while others did not know how to interpret instructions on the container labels [13]. This may equally leave pesticide residues in crops beyond concentrations considered safe for consumption [19,20,21]. Application of higher pesticide rates can also be due to the higher susceptibility of tomatoes to blights [22]. 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 will reduce negative health effects to the consumer. Consumption of such tomatoes for a long period could be risking the consumer's health [23]. Carbendazim in greenhouse tomatoes which was about 400% higher than the EU and Codex MRLs is a food safety concern to consumers [24]. When carbendazim is absorbed by plants, it accumulates at the end of the food chain because biodegradation process is relatively slow. This possess a serious threat to human health. 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 [25]. Excess carbendazim has been reported to disrupt the human endocrine system and can damage organs such as the mammalian liver, kidneys and the spleen [26,27]. This pesticide is classified by the United States Environmental Protection Agency (U.S. EPA) as Group C possible human carcinogen [25]. Alpha-cypermethrin affects the nervous system and can cause prolonged bradycardia while

difenoconazole could reduce cell viability and inhibit cell proliferation, induce DNA damage and accelerate programmed cell death [28,29] Food safety and nutrition issues interact in determining health outcomes and impact societal livelihoods. Vegetables and fruits play an important role in the nutrition and health of the population by up to 80% of the diets [30,31]. Apart from chronic toxic effects of pesticides, research has shown that soluble sugars in crops such as fruits are easily dissolved in chemical solutions and are continually lost, which affects the nutritive value of food [32,33]. Exposure to pesticides through food is a food safety and health concern worldwide due to related effects on human health [34,35]. Pesticide residue levels in food need to comply with the Maximum Residue Levels (MRLs) which are based on Good Agricultural Practice (GAP). Exceedance of local MRLs is an indication that local GAP is not well followed [36]. There is therefore an urgent need for governments and international organizations to develop effective strategies to reduce pesticide residue levels in agricultural products consumed locally. Such strategies may include strengthening farmers' education regarding GAP and integrated pesticide management (IPM).

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 attributed 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 organizations mandated to evaluate the efficacy of pesticides including Kenya Agricultural and Livestock Research Organization (KALRO) and Pest Control and Product Board (PCPB) should recommend longer PHI for greenhouse use. Studies should also be done to determine the probable dietary exposure and health risks of pesticides such as carbendazim, frequently detected in other vegetables in Kenya.

ETHICAL APPROVAL AND CONSENT

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. Written 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.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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