Organophosphorus Pesticide Residues in Tomatoes: a Case of Mlali and Doma Wards in Mvomero District, Morogoro

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Abstract

Extensive pesticide use poses a significant challenge to Tanzania's horticulture industry, particularly affecting the leading tomato producer, the Morogoro region. This study focused on assessing organophosphorus pesticide residues in tomatoes from Mlali and Doma divisions in the Mvomero district of Morogoro. A total of 40 samples were collected from both farms and markets for analysis, utilizing Gas Chromatography with Mass Spectrometry (GC-MS) for extract analysis. Statistical Analysis Software (SAS) Version 9.1 was employed for data analysis. The study identified six organophosphorus pesticides, with concentrations in the following order: pirimiphos methyl > diazinon > fenitrothion > dimethoate > profenofos > chlorpyrifos. Pirimiphos methyl levels ranged from 3.32±0.03 to 9.53±0.05 µg/kg in Mlali samples and 0.20±0.01 to 6.33±0.03 µg/kg in Doma samples. Chlorpyrifos was detected in the lowest concentrations across all samples. Interestingly, higher pesticide levels were found in market samples compared to those from farms, suggesting potential misuse post-harvest and during distribution. This misuse could have severe consequences for tomato consumers, including both carcinogenic and non-carcinogenic effects. The study underscores the importance of educating farmers on the proper use of pesticides and the potential adverse effects resulting from their misuse. Addressing this issue is crucial for ensuring the safety of tomatoes in the market and protecting consumers from harmful effects associated with pesticide residues.

Keywords: Tomatoes, pyrimiphos methyl, analytes, gas chromatography, hazardous effects

Introduction

In numerous countries globally, tomatoes are a staple in diets, renowned for their widespread cultivation and marketing as fresh vegetables. Renowned for their health benefits, tomatoes contain antioxidants like ascorbic acid, vitamin E, flavonoids, phenolic acids, and all four carotenoids: alpha (α), beta (β), lutein, and lycopene (Bhowmik *et al.,* 2012; Schiavon *et al.,* 2013; Ware, 2017, Mahugija *et al.,* 2021;). Additionally, they serve as rich sources of essential minerals such as iron, magnesium, phosphorous, calcium, and zinc, contributing to bone structure and strength (Salehi *et al.,* 2019). Moreover, tomatoes play a role in safeguarding the body against cancer and other diseases (Hlihor *et al.,* 2019; Li *et al.,* 2021).

In Tanzania, tomatoes thrive in temperate regions, notably the Southern and Northern highlands, emerging as a prominent vegetable in terms of production and consumption (Victoria *et al.,* 2017). Accounting for 57.9% of all vegetable production, tomatoes hold a significant position in Tanzanian agriculture, with the Morogoro region leading as the largest producer at 9.2% production capacity, followed closely by Kagera, Tanga, Mwanza, and Iringa (MALF, 2016; Mwatawala *et al.,* 2019). Despite their agricultural significance, tomatoes face challenges in the form of plant diseases and insect pests, causing up to 45% global crop loss (Kolani *et al.,* 2016).

To combat these issues, the Tanzanian tomato growers resort to extensive pesticide use, particularly organophosphates, due to their broad applicability across various crops (Gambacorta *et al.,* 2005; Menezes *et al.,* 2006; Kiwango *et al.,* 2018; Han-ming *et al.,* 2019; Mahugija *et al.,* 2021). However, this widespread pesticide usage poses a significant problem for the horticultural sector, leading to unacceptable pesticide residues and associated health risks,

including cancer, non-cancer, and endocrine-disrupting effects (Keikotlhaile *et al.,* 2010; Nicolopoulou-Stamati *et al.,* 2016; Kariathi *et al.,* 2016; Mahugija *et al.,* 2017; Kiwango *et al.,* 2018;). The study aims to address this issue by evaluating organophosphorus pesticide residues in tomatoes from Mlali and Doma divisions in the Mvomero District, Morogoro. By understanding the types and quantities of pesticide residues in tomatoes, the study seeks to contribute to increased knowledge and the prevention of pesticide misuse among small-scale horticulture producers.

Materials and methods

Study sites

The research was undertaken in the expansive Mvomero District within Morogoro, specifically encompassing the Mlali and Doma Wards. Morogoro boasts a substantial cultivation of tomatoes, with over 19,195 hectares dedicated to this endeavor, particularly dominating in Mvomero and earning the distinction of being Tanzania's premier tomato-growing region. Positioned in the northeast of Morogoro region at coordinates 6 ˚14' 8.2212' South and 38 ˚ 41' 37.4928' East, Mvomero stands as the largest district in the Morogoro region. The focal points of this study were the Doma and Mlali wards within the Mvomero District (Fig. 1).

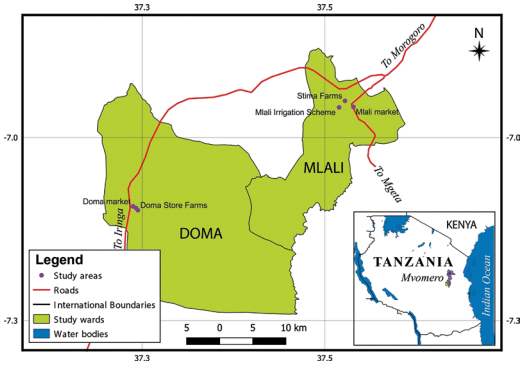

Figure 1: A map showing the locations of study sites

Sampling study design and sample size

We implemented a cross-sectional study design to examine the pesticide presence in tomatoes. Our sampling strategy involved gathering tomato samples from both farms and markets situated within the designated study sites. Specifically, we collected a total of 40 tomato samples directly from farms and markets within the study area for subsequent pesticide analysis. Our focus was on freshly harvested tomatoes that were prepared for sale.

In the Mlali Ward, we obtained samples from five farms and five markets, while in the Doma Ward, samples were collected from seven farms and three markets. To preserve the integrity of the collected tomato samples, each one was carefully enveloped in aluminium foil, subsequently placed in polyethylene bags, and then stored in an icebox. This ensured their proper preservation during transportation to the laboratory for detailed analysis.

Laboratory analysis

We executed a series of procedures encompassing sample preparation, analysis, and identification of organophosphorus pesticides on the chosen samples.

Selection of reference standards

In this study, we employed certified reference standards with a purity level exceeding 99% for the identification and quantification of analytes. Specifically, for the group of organophosphorus pesticides, our reference standards included profenofos, chlorpyrifos, pirimiphos methyl, fenitrothion, dimethoate, and diazinon. To create working standard solutions, we diluted these reference standards in cyclohexane, ensuring accurate representation. Subsequently, these solutions were stored in a freezer, with concentrations spanning from 0.293 to 4.367 µg/mL.

Sample preparations and extraction

The initial step involved taking approximately 100 g of fresh tomato samples from Mlali and Doma. These samples were meticulously chopped with a clean stainless-steel knife and subsequently homogenized through grinding using a clean mortar. Replicate aliquots were then extracted for pesticide analysis.

For each aliquot, 20 g was precisely weighed and combined with 50 mL of dichloromethane in a glass bottle, which was securely stoppered with a screw cap. The sealed bottles underwent

a 30-minute treatment in an ultrasonic bath. Following this, the supernatant was carefully transferred into an E-flask, and the residue was rinsed with 10 mL of dichloromethane. The rinsing supernatant was then combined with the initial extract in the E-flask.

To eliminate any residual moisture, 20 g of anhydrous sodium sulfate was introduced into the E-flask containing the sample. The contents were swirled until the sodium sulfate freely floated. The resulting extract was filtered through a glass wool plug into an evaporating flask to facilitate concentration. Subsequently, the extract underwent evaporation under vacuum conditions, reducing its volume to approximately 2 mL using a rotary evaporator at 40°C, rendering it ready for the clean-up process.

Sample clean- up

For the sample clean-up process, we adhered to the methodology outlined by Mahugija et al. (2017) and Wenaty *et al.* (2019), utilizing a glass chromatographic column (10 mm i.d. x 32 cm) packed with 3.0 g of frosil and topped with 5 – 10 cm of sodium sulfate. The column underwent a preliminary rinse with 5 mL of cyclohexane. Subsequently, 2 mL of the extract was introduced into the column, followed by elution with 20 mL of cyclohexane and 10 mL of a cyclohexane/acetone mixture at a ratio of 9:1. The resulting eluates were concentrated down to 2 mL using a rotary evaporator, preparing them for subsequent GC – MS analysis.

Blanks, recovery tests and method detection limits

To facilitate "blank" studies, we utilized three pesticide-free tomato samples obtained from an untreated greenhouse garden in Mwanza. Employing identical methods as those for the test samples, we prepared, extracted, and cleaned each control sample concurrently. To assess recovery, known volumes of pesticide standard solutions were spiked into 20 g of each blank sample. Subsequently, these samples underwent extraction and analysis using the same procedures as the treated samples to ascertain recovery levels (Kocourek, 2012; Mahugija *et al.,* 2021).

Recoveries falling within the 70-120% range are typically deemed acceptable according to SANCO (2023) standards. Values outside this range are recommended for correction (SANCO, 2013; EC, 2017; Mahugija *et al.,* 2021), or alternatively, the results for the entire sample set may be discarded. The detection limit for each identified pesticide was established by determining the concentration of the analyte that induced signals three times higher than the background noise level (Kocourek, 2012).

Analysis, identification and quantification

The National Fish Quality Control Laboratory in Mwanza served as the venue for the analyses conducted using a Shimadzu gas chromatograph coupled to a mass spectrometer (GC – MS QP2010 Utra). The column utilized for the analysis was Rtx – 5MS (30 m x 0.25 µm). The temperature program followed a sequence of 90°C for 20 minutes, followed by a 5°C increase to 260°C, held for 5 minutes. The injection mode employed was splitless with a 1 mL injection at 250°C and a purge flow of 3 mL/min. Helium served as the carrier gas, flowing at a rate of 2.17 mL/min, with an internal temperature of 300°C. The mass spectrometer operated in electron impact (EI) ionization mode at 0.2 volts, with an ion source temperature of 230°C, conducting a full scan in the range of 45 – 500 m/z.

Identification of analytes was achieved by comparing retention times and mass spectra with those of reference standards run concurrently under the same conditions as the samples. For quantification, peak heights were utilized, with the mass fragment featuring the highest intensity of the molecular ion selected for this purpose. Quantitative analysis involved establishing calibration curves from measured peak heights of each standard. Series of standard solutions were prepared from 9.078 µg/mL stock solutions into serial dilutions of 0.293, 0.569, 1.142, 2.272, and 4.367 µg/mL. The resulting data points were used to draw best-fit lines of peak heights against their respective concentrations. The linearity of each compound was confirmed by correlation coefficients ($R^{2)}$ falling within the range of 0.967 – 0.9816. Concentrations of compounds in sample extracts were determined

using the equations derived from the calibration curves. The concentration of each pesticide in the samples was obtained by multiplying the concentration from the calibration curve by the final volume of the extract per mass of the sample extracted.

Statistical data analysis

Statistical analysis involved subjecting the measured organophosphorus pesticides data to descriptive statistical analysis, yielding minimum, maximum, mean concentrations, and standard deviations of the detected pesticides. The Statistical Analysis Software (SAS) Version 9.1 was employed for further data analysis. Presentation of organophosphorus pesticide concentrations involved mean ± standard deviation. During data processing, concentrations below the limits of detection (LOD) were treated as zero. A significance level of $p<0.05$ was established for all analyses.

Results and discussion

Percentages Recoveries for the Pesticides Extraction Procedure

Table 1 presents the outcomes of the recovery experiment conducted for the extraction of organophosphorus pesticides. The mean percentage recoveries from triplicate determinations of the extraction parameters ranged from 75.20±0.27 to 92.30±0.31. These results fall within the range of 70% to 120%, a criterion widely accepted as indicative of a flawless extraction procedure, as corroborated by numerous studies conducted globally (Afful *et al.,* 2013; SANCO, 2013; EC, 2017; Wenaty *et al.,* 2019). The findings of the current study affirm the excellence of the extraction technique employed, suggesting its suitability for future pesticide studies of a similar nature. No adjustments to the extraction process are deemed necessary based on these recovery rates.

Pesticide residues in tomatoes from Mlali Ward

Table 2 presents the concentrations of organophosphorus pesticide residues in tomatoes obtained from Mlali Ward. Pirimiphos methyl, in particular, exhibited elevated levels in comparison to other pesticide residues. The concentrations of the pesticides ranged from 4.26±0.02 to 6.97±0.04 µg/kg in tomatoes sourced from farms and from 3.32±0.03 to 9.53±0.05 µg/kg in tomatoes acquired from markets. Notably, pirimiphos methyl overshadowed all other types of pesticide residues in both farm and market samples. The levels of pirimiphos-methyl observed in the present study are notably lower compared to those reported in the prior investigation conducted by Mahugija *et al.* (2021).

In the aforementioned study, the highest concentration of pirimiphos-methyl was determined to be 1.53 mg/kg, and this was detected in 85% of the samples analyzed. The current findings indicate a significant reduction in the presence of pirimiphos-methyl, underscoring potential changes or variations in its usage, application, or environmental factors influencing its prevalence. It is essential to recognize these disparities to comprehend the evolving patterns of pirimiphos-methyl contamination and address any associated implications. In contrast, chlorpyrifos was detected in lower levels in tomato samples from both farms and markets, indicating a distinct pattern of pesticide distribution in the analyzed samples.

Table 1: Percentage Recoveries for the Pesticides Extraction Procedure

Pesticide type	Amount spiked (µg/kg)	Amount calculated (µg/kg)	Recoveries (%)
Profenofos	10.0	9.23±0.21	92.30±0.31
Fenitrothion	10.0	8.12±0.34	81.20±0.92
Dimethoate	10.0	8.35±0.37	83.50±0.64
Diazinon	10.0	7.83±0.14	78.30±0.42
Chlorpyrifos	10.0	7.69±0.26	76.9±0.83
Pirimiphos methyl	10.0	7.52±0.19	75.2±0.27

Table 2: Pesticide residues in tomatoes from Mlali ward (µg/kg)

Sample location		Pesticide Type						Pesticide levels	
		Profenofos	Fenitrothion	Dimethoate	Diazinon	Pirimiphos methyl	Chlorpyrifos	Min	Max
Farm	1	<LOD	<LOD	0.08±0.01	0.26±0.02	4.26±0.02	<LOD	<LOD	4.26
	2	0.06±0.01	0.09±0.01	0.82±0.02	0.22±0.01	6.97±0.03	0.83±0.01	0.06	6.97
	3	<LOD	0.16±0.01	0.08±0.02	0.36±0.02	6.50±0.02	<LOD	<LOD	6.50
	4	<LOD	0.11±0.01	0.09±0.01	0.27±0.01	5.21±0.02	<LOD	<LOD	5.21
	5	0.07±0.02	0.14±0.01	0.12±0.02	0.33±0.02	6.98±0.04	<LOD	<LOD	6.98
Market	1	0.12±0.01	0.29±0.01	0.13±0.01	0.46±0.02	9.53±0.05	<LOD	<LOD	9.53
	2	0.07±0.01	0.14±0.01	0.11±0.02	0.35±0.01	3.32±0.03	0.10±0.01	0.07	3.32
	3	0.09±0.02	0.26±0.01	0.12±0.01	0.40±0.03	7.92±0.04	0.10±0.02	0.09	7.92
	4	0.08±0.01	0.13±0.02	0.09±0.01	0.23±0.03	4.87±0.03	0.11±0.01	0.08	4.87
	5	0.06±0.01	0.21±0.01	0.10±0.01	0.24±0.02	3.94±0.02	<LOD	<LOD	3.94

Dimethoate, diazinon, and pirimiphos methyl were identified in all tomato samples, encompassing both those sourced from farms and markets. Fenitrothion, on the other hand, was present in 80% of the tomato samples from farms and 100% in samples from markets. Profenofos exhibited a detection rate of 40% in farm samples but was identified in 100% of market samples. Conversely, chlorpyrifos was detected in 20% of farm samples, while 60% of market samples displayed detectable levels of this pesticide. Additionally, the occurrence frequency and concentrations of organophosphorus pesticides identified in the current study exhibit a trend of lower values compared to those reported in a preceding study, as detailed by Mahugija *et al.* (2021). In the mentioned study, profenofos was detected in an extensive 90% of the samples, with the highest concentration reaching 18.49 mg/kg. Remarkably, this concentration stands approximately 264 times higher than the levels of profenofos identified in the present investigation. Furthermore, the maximum concentration of chlorpyrifos in the previous study was documented at 9.22 mg/kg, representing an elevation of 11 times compared to the levels recorded in the current study.

This discrepancy in both detection frequency and concentration highlights potential shifts or alterations in the application, usage, or environmental distribution of these organophosphorus pesticides over the period between the two studies. Recognizing these variations is crucial for a comprehensive understanding of the evolving contamination patterns associated with profenofos and chlorpyrifos. This disparity in pesticide detection rates implies that tomatoes procured from markets exhibited higher concentrations of multiple pesticides compared to those from farms. Such a pattern suggests the potential misuse of pesticides, with continued application even post-harvest.

Pesticide residues in tomatoes from Doma Ward

Table 3 displays the pesticide residues found in tomatoes sourced from both Doma farms and markets. All six organophosphorus pesticides were identified in low concentrations. Dimethoate, diazinon, and pirimiphos methyl were detected in tomatoes from all the sampling stations, each exhibiting distinct concentration levels. Similar to the findings in Mlali Ward, pirimiphos methyl demonstrated significantly higher levels compared to other organophosphorus pesticides.

The concentrations of pirimiphos methyl ranged from 0.20±0.01 µg/kg, the lowest detected concentration, to 6.10±0.02 µg/kg, the highest concentration observed in the samples. This suggests a consistent pattern across both wards, emphasizing the prevalence of pirimiphos methyl in tomatoes and highlighting potential concerns related to its usage. As outlined by Mahugija et al. (2021), the levels

of various organophosphorus pesticides identified in the tomatoes within the scope of this study demonstrate a considerable reduction in comparison to those observed in tomatoes sourced from various farms in Iringa. This discrepancy suggests that tomatoes originating from the study areas exhibit a comparatively safer profile concerning residues of organophosphorus pesticides when juxtaposed with their counterparts from Iringa.

The substantial contrast in pesticide levels signifies potential variations in agricultural practices, pesticide application methodologies, or environmental conditions between the two regions. The findings underscore the importance of local factors in influencing the contamination levels of organophosphorus pesticides in tomatoes. By recognizing and understanding these differences, it becomes possible to formulate more targeted and region-specific strategies for pesticide management and food safety enhancement.

markets. Similarly, chlorpyrifos was found in 14% of tomato samples from farms and 33% in tomatoes from markets. This pattern suggests a common practice of likely pesticide application while tomatoes are in the marketing channels.

Each sample in this study contained at least three out of the six types of organophosphorus pesticides assessed. The levels of pesticides were consistently ordered as follows: pirimiphos methyl > diazinon > fenitrothion > dimethoate > profenofos > chlorpyrifos.

Furthermore, the ADI of chlorpyrifos is 0.001mg/kg body weight per day. It is possible that the risk could increase depending on one's daily or weekly intake through contaminated tomatoes. While the mean concentrations of pirimiphos methyl and other pesticides from each sampling site did not exceed the Maximum Recommended Limit (MRL) of 0.5 mg/kg set by FAO/WHO and other competent authorities, the presence of pesticide residues in foods intended for human consumption raises significant

Table 3: Pesticide residues in tomatoes from Doma Ward (µg/kg)

Sample location		Pesticide Type						Pesticide levels	
		Profenofos	Fenitrothion	Dimethoate	Diazinon	Pirimiphos methyl	Chlorpyrifos	Min	Max
Farm	1	<LOD	<LOD	0.11±0.02	0.40±0.02	0.20±0.01	<LOD	<LOD	0.4
	2	<LOD	<LOD	0.12±0.02	0.32±0.03	5.32±0.60	<LOD	<LOD	5.32
	3	0.08±0.01	0.12±0.02	0.13±0.02	0.41±0.02	0.46±0.02	0.10±0.04	0.08	0.46
	4	0.05±0.01	<LOD	0.11±0.02	0.32±0.02	5.13±0.03	<LOD	<LOD	5.13
	5	0.06±0.01	<LOD	0.08±0.01	0.24±0.02	5.17±0.02	<LOD	<LOD	5.17
	6	0.07±0.02	0.16±0.02	0.12±0.02	0.31±0.02	6.10±0.02	<LOD	<LOD	6.1
	7	<LOD	<LOD	0.07±0.01	0.28±0.02	6.10±0.02	<LOD	<LOD	6.1
Market	1	0.05±0.01	0.13±0.01	0.06±0.01	0.33±0.01	6.33±0.03	<LOD	<LOD	6.33
	2	<LOD	0.17±0.01	0.10±0.01	0.26±0.02	5.28±0.02	<LOD	<LOD	5.28
	3	0.06±0.01	<LOD	0.09±0.02	0.32±0.02	4.67±0.03	0.08±0.01	<LOD	4.67

In samples collected from both Doma farms and markets, dimethoate, diazinon, and pirimiphos methyl were universally detected, exhibiting a prevalent presence across the sampling sites. Profenofos residues were identified in more than 71% of tomatoes from Doma farms and over 66% of tomatoes from the markets. Fenitrothion residues were detected in nearly 29% of the tomato samples from farms and 67% of the tomato samples from

concerns (FAO/WHO, 2013). Studies indicate that these pesticides can form metabolites potentially more toxic than the parent compounds, leading to higher residue levels than MRLs and posing health risks, including cancer, non-cancer, endocrine-disrupting, and teratogenic effects (Van den Dries *et al.,* 2019).

Efforts to assess the effectiveness of washing tomatoes in reducing pesticide residues have highlighted the predominance

of organophosphorus pesticides, with limited efficacy in removal through simple washing (Mahugija *et al.,* 2021). Consequently, tomatoes should undergo multiple washes before consumption, and consumers should be educated on the importance of thorough washing.

The elevated concentrations of pesticide residues, including pirimiphos methyl, diazinon, fenitrothion, and profenofos, could result from inappropriate pesticide dosage, inadvertent pesticide application by farmers, disregard for safe waiting periods, pesticide mixing, and potential illegal pesticide use on harvested tomatoes in the market to extend shelf life (Busindi, 2013; Mahugija *et al.,* 2021). These observations indicate potential risks of pesticide exposure and raise public health concerns for tomato consumers. Similar findings have been observed in other studies within the country, such as elevated levels of chlorpyrifos in tomatoes from Meru District (Kariathi *et al.,* 2016) and Dar es Salaam Markets (Mahugija *et al.,* 2017). Pirimiphos methyl and profenofos residues in raw tomatoes in Tanzania, particularly in the Iringa region, have also been reported (Mahugija *et al.,* 2021). However, the levels of pirimiphos methyl, profenofos, and other pesticides in the present study were notably lower than those found in tomatoes grown in West Sumatra, Indonesia, which reached 8.03 µg/kg (Allen *et al.,* 2016).

Conclusion and recommendations

In fresh tomatoes from Doma and Mlali Wards of Mvomero District in Morogoro region, six types of organophosphorus pesticides were identified at low levels. The levels of pesticide residues in tomatoes from markets were slightly higher than those from farms. Despite the detected pesticide levels not exceeding the Maximum Residue Limit (MRL) set by FAO/WHO and other competent authorities, including the European Union, the presence of pesticides in food items raises concerns for public health. Most pesticides have the potential to be carcinogenic to humans and are associated with various non-carcinogenic effects, in addition to being endocrine-disrupting agents. Consequently, this study emphasizes the need for tomato farmers, sellers, and consumers to minimize the use of toxic pesticides due to the adverse health effects likely caused by these chemicals.

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