

Full Length Research Paper

Pesticide residues in locally produced grape wine in Tanzania: a case study of Dodoma urban and Bahi districts

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The aim of this study was to assess the level of pesticide residues in locally produced grape wine in Tanzania. Fifty samples of grape wine from different locations in Dodoma urban and Bahi district were analyzed to determine the presence of 49 pesticides using the quick, easy, cheap, effective, rugged and safe (QuEChERS) multi-residue extraction, followed by gas chromatography-tandem mass spectrometry (GC-MS/MS). Twenty-two pesticides were detected among the 49 pesticides analyzed. The selected 49 pesticides was based on its use in grape cultivation which were reported by grape farmers in the study location which includes fungicides, insecticides and herbicides. The pesticides whose concentrations exceeded the maximum residue levels (MRL) were: Pyroquilon, ethofumasate, chlorobeb, azobenzene and cycloate in 38, 33, 46, 14 and 1 wine samples, respectively. Of the samples analyzed 9 (18%) contained one pesticide, 8 (16%) contained two different pesticides, 23 (46%) contained three different pesticides 8 (16%). The results indicated the occurrence of pesticide residues in grape wine produced in Dodoma urban and Bahi districts, Tanzania, and pointed to an urgent need to develop comprehensive intervention measures to reduce potential health risk to consumers.

Key words: Pesticides residues, grape wine, food safety, maximum residue levels (MRL).

INTRODUCTION

Grapes (*Vitis vinifera*) belong to the *Vitaceae* family and are one of the world's most important economic fruit crops (Kocher and Nikhanj, 2019). This crop has many uses as it can be eaten raw or can be used for the manufacture of wine, jam, juice, jelly, grape seed-extracts, raisins, vinegar and grape-seed oil (Kalimang'asi et al., 2014; Grimalt and Dehouck, 2016). Approximately 4,744 tons of grapes were produced in Tanzania in the year 2018 while the largest producer of

grapes in the world for the year 2018 was China with 13,494,811 tons (FAOSTAT, 2020). Grape production is the mainstay for many farmers in the Dodoma region, including Dodoma city, Bahi, Chamwino and Kongwa districts (Kalimang'asi et al., 2014). The excellence of wine depends on the quality of grapes and to obtain high-quality wines, grapes at the correct stage of ripeness and free from parasites must be used (Caboni and Cabras, 2010). In addition, the quality of wine

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depends on the vinification process, the geographical origin of the grapes as well as the varietal composition of the grape wort; therefore, grapes traceability is important in quality control and suppliers' information (Espineira and Santaclara, 2016).

There is increasing interest in health and safety aspects associated with pesticide use and the presence of their residues in processed foods and beverages (Lian et al., 2010). Pesticide use in viticulture is a major issue for grape protection, increased productivity and wine quality (Martins et al., 2011). Wine has beneficial effects on human health, if moderately consumed, including prevention of heart and circulatory diseases, favorable to the fight against obesity, provides greater longevity and quality of life, creates barriers to the development of dementia, the meal accompanied by wine results in a better digestion, anti-infective effect, can prevent blindness, have anti-inflammatory action and can alleviate lung diseases (Wurz, 2019).

However, wine may also contain components, which have detrimental effects on human health such as pesticide residues, as a result of their use in viticulture and those that can remain on grapes after harvest and may be transferred to the wine (Grimalt and Dehouck, 2016; Cepo et al., 2018). Grey mould (*Botrytis cinerea*), powdery mildew (*Erysiphe necator*) and downy mildew (*Plasmopara viticola*) are responsible for serious yield loss in the wine sector resulting in significant commercial losses (Weng et al., 2014). In addition to an important crop yield loss, these diseases can also reduce the wine quality by providing an unstable colour, oxidative damages, premature aging, unpleasant flavours, and clarification difficulties (Rodriguez et al., 2020). The correct use of these phytosanitary products has no adverse effects on public or environmental health, but indiscriminate treatment applied without respecting the safety periods or the recommended doses may result in residues being present in the grapes used for winemaking these fungicides may be passed on to the must and then to the wine during fermentation (Vaquero-Fernandez et al., 2012).

Grapes are usually harvested and directly used for follow-up fermentation without washing or other treatment to reduce pesticides residues, this may lead to their presence in wine offered commercially or public consumption, therefore, their determination in grape wine is designed to ensure the safe consumption of the important beverage in the community (Jiang et al., 2009).

In order to ensure food safety for consumers and protect human health, many organizations and countries around the world have established Maximum Residue Limits (MRLs) for pesticides in food commodities (Cepo et al., 2018). The MRL is the maximum level of a pesticide residue (expressed in mg/kg) which is legally permitted in food or animal feed (Jallow et al., 2017). The MRLs which specify the

maximum concentration of a pesticide that can exist in certain agricultural commodities were regulated by many nations to promote good agricultural practice (GAP) (Lekei et al., 2016). Furthermore, current agricultural practices are based on the wide use of chemical pesticides that have been associated with negative impacts on human health, wildlife and natural environment (Goulson, 2013; Nicolopoulou-Stamati et al., 2016). This, in turn, raises concerns among consumers and producers due to possible health hazards as well as the impact of these residues on the sensory quality of wines. These facts emphasized the need for continuous monitoring of pesticide residues in wine (Cepo et al., 2018).

The study aims to establish the levels of pesticide residues in locally manufactured wine, particularly in the leading producer region Dodoma, in order to determine its quality and safety. This study will serve as a basis for awareness creation to farmers, consumers, processors and other stakeholders and enable the government to regulate the sector and advocate use of best practices and prevent economic losses.

MATERIALS AND METHODS

Sample collection

Fifty bottled grape wine samples were collected from all 15 wine processors, branded (with label) and unbranded (without label sold in bulk) in Dodoma urban (capital of Tanzania) and Bahi district in Dodoma region, these districts were selected purposively due to high production of grapes. Approximately one liter of each branded and unbranded wine 36 red wines and 14 white wine from small, medium and large scale industries was packed into sterile amber plastic containers, sealed, coded, stored at -2°C and transported to the Tanzania Bureau of Standards laboratory in Dar es Salaam. The samples were stored at -2°C for further analysis.

Sample extraction

Quick, easy, cheap, effective, rugged and safe (QuEChERS) method (Anastassiades et al., 2003; Jiang et al., 2009; Pazzirota et al., 2013; Wang and Telepachak, 2013) with some modifications were used to extract pesticide residues from the samples. 10 ml of sample was transferred into an empty 50 ml centrifuge tube polytetrafluoroethylene (PTFE), followed by 10 ml of acetonitrile (Romil Ltd The Source Convent Drive Waterbeach Cambridge Gb-Cb259QT) and vortexed for 1 min using Stuart auto vortex Mixer (Stuart Scientific Co. Ltd-England). A blank sample was fortified with 10 µl of standard pesticides.

A sachet made up of 4 g of magnesium sulfate anhydrous, 1 g of sodium chloride 1 g of trisodium citrate dihydrate, and 0.5 g of disodium hydrogen citrate sesquihydrate was added (Quercher Extract Pouch, En Method shaken and vortexed for 1 min, then centrifuged for 5 min at 4,000 rpm using Centrifuge 5810R (Eppendorf Ag Co. Ltd Hamburg, Germany).

Sample clean up

Exactly 7.5 ml of the supernatant (upper layer) was transferred

into 15 ml PTFE, then 750 mg of MgSO₄ (Surechem Products Ltd Suffolk, England) and 150 Primary Secondary Amine (PSA) was added into PTFE tube. The mixture was then vortexed for one minute then centrifuged at 4,000 rpm for 5 min. Then 5 ml of extracted solution was added into a test tube and evaporated to near dryness under nitrogen at the temperature below 42°C. Then reconstituted to 1 ml with toluene and homogenized for 5 s and transferred into autosampler vials.

Blank sample preparation

A blank sample was prepared using distilled water which was prepared using Evoqua Water Technologies PTE LTD Farrernberg-Germany) and also wine from South Africa which was free from pesticide residues used as black and the same procedures for extraction was followed.

Standard preparation

Individual pesticides solution was prepared by 10 mg of each standard pesticides purchased from (Sigma- Aldrich Co. Ltd. Steinheim am Albuch - Germany) were dissolved in 10 ml acetone and stored at -20°C, then intermediate Standard solution was prepared by 100 µl stock standard solution which was brought to 10 ml volumetric flask mixed with acetone and stored at 2°C. Working solution was prepared by 10 µl which was dissolved to 10 ml acetone and six levels of an intermediate standard solution of each pesticide were prepared to maintain the same matrix concentration for the preparation of calibration curve and stored at 2°C.

Gas chromatography-tandem mass spectrometry (GC-MS-MS) conditions

An Agilent 7010 GC/MS Triple Quad (Agilent Technologies Co. Ltd. Waldbronn-Germany) with a 7693 Autosampler with a capacity of 150 samples at a time was used for the analysis of pesticide. GC-Column –J&W HP-5MS UI (15 m × 0.25 mm i.d. × 0.25 µm film thickness) was used for GC separation, with helium (99.99%) as the carrier gas at a constant flow rate of 1.5 ml/min. The column inlet temperature was initially at 60°C (hold for 1 min), increased to 120°C at rate of 40°C/min, then to 310°C at a rate of 5°C/min, the total holding time was 40.50 min. Quantification and results calculation were done by mass hunter software using the following formula.

Concentration of each analyte (mg/L) = Concentration from curve × dilution factor

Where by, concentration from curve = Peak area of the analyte/Peak area of internal standard.

Linearity was studied in the range 0.5 - 100 mg/L with five calibration points (0.5, 10, 25, 50, 75 and 100 mg/L) by matrix-matched standard calibration which were spiked with the corresponding volume of the working solution. Linear calibration graphs were constructed by least-squares regression of concentration versus relative peak area (analyte/IS) of the calibration standards. Linearity values, calculated as determination correlation coefficients (R²), were in the range 0.986-0.999 (Table 1).

Pesticides analysis

Wine samples were analyzed at the Tanzania Bureau of

Standards (TBS) quality control laboratory, Dar es Salaam. The samples which were stored at -2°C were equilibrated to room temperature (25°C) before analysis.

Statistical analysis

Data were analyzed using R- version 3.5.0 (2018). Analysis of variance (ANOVA) was used to test significant differences on the pesticide residues concentration (mg/L) amongst the brand, color of wine and among the categories of processors (Large, Medium and Small Scale). Statistical Package for Social Sciences (IBM SPSS® Version 20) was used to test compliance of pesticide residues detected to European Union Database MRL.

Method validation

Percentage recovery of pesticides

Recovery studies were performed to examine the efficacy of extraction and clean up. Grape wine samples were spiked with known concentration of the pure pesticides standard solution and extraction and clean-up were performed as described earlier in the process of sample extraction.

$$\% \text{ Recovery} = \frac{\text{Spiked} - \text{Unspiked Conc}}{\text{expected Conc}} \times 100\%$$

The sensitivity of the method was obtained by determining the percent recovery of pesticides by calculating the percentage recovery of pesticides spiked samples and unspiked samples. Accuracy was evaluated in terms of recovery, and the satisfactory recoveries were from 70 to 110% indicating the suitability and good performance of GC-MSMS). To test the performance of an analytical method, the following criteria have to be considered: pesticide recoveries should be in the range 70-120%. The precision of the method was studied; intra- and inter-day variations were estimated and expressed as relative standard deviation (RSD) of the signals or peak areas for each analyte following an analysis of 0.1 mg/L standard working solution injected five times consecutively on the same day and injected five times over four consecutive days. The results in Table 1 showed that inter-day variation of peak areas for 49 pesticides were in the range of 1.26- 10.24%, and intra-day variations of 1.68-14.22% (Vaquero-Fernandez et al., 2012; Machado et al., 2016).

Limit of detection and quantification

The qualitative pesticides were based on the retention time of peak and abundance ratios of the selected ions for each pesticides. Limit of detection (LOD) and limit of quantification (LOQ) was determined based on the signal to noise ratio of quantifier transition of all analyte. The retention time, precursor and product ion, LOD, LOQ of the analyzed pesticides on the samples are presented in Table 1.

RESULTS AND DISCUSSION

Pesticide residues in grape wine

Results indicated that, twenty-two pesticides were detected among the 49 pesticides analyzed (Figure 1).

Table 1. Parameters for the simultaneous determination of 49 pesticide residues in grape wine by GC-MS/MS.

Pesticides	LOD (mg/L)	LOQ (mg/L)	Retention time (min)	Precursor ion	Production	R ²	Precision RSD (%)		Recovery
							Intra day	Inter day	
Azobenzene	0.002	0.008	13.864	77.0	51.0	0.997	2.36	4.32	78.30
Butafenacil	0.004	0.01	33.762	331.0	180.0	0.998	6.72	7.38	82.46
Chlormephos	0.004	0.01	9.719	121.0	65.0	0.998	4.75	4.46	88.72
Chloroneb	0.003	0.009	11.426	206.0	191.1	0.999	6.78	8.22	90.20
Chlorothalonil	0.004	0.009	17.862	263.8	168.0	0.987	14.22	10.24	89.24
Chloropropylate	0.003	0.008	25.856	139.1	111.0	0.988	5.38	5.04	75.32
Chlorpyrifos	0.004	0.009	21.094	197.0	169.0	0.997	3.68	7.54	94.42
Cloquintocet-mexyl	0.003	0.009	29.987	192.0	190.0	0.987	9.25	7.86	74.27
Cyanofenphos	0.002	0.008	27.263	169.0	141.1	0.989	8.23	8.78	80.06
Cycloate	0.004	0.009	13.828	83.0	55.1	0.996	9.84	10.13	96.58
Cyfluthrin I	0.003	0.009	34.401	162.9	90.9	0.987	1.68	1.26	77.82
Cypermethrin III-beta	0.003	0.008	34.458	163.0	91.0	0.986	7.56	9.62	92.16
Cyphenothrin II	0.004	0.008	31.401	181.0	152.1	0.995	2.35	4.79	86.48
Cyproconazole	0.003	0.01	25.820	139.0	111.0	0.986	6.37	7.24	110.46
o,p'-DDT	0.004	0.008	24.771	235.0	165.2	0.997	9.14	9.84	84.82
DEET	0.002	0.009	11.383	119.1	91.0	0.997	4.78	6.82	82.63
Demeton-S- Methyl-sulfon	0.003	0.008	19.400	169.1	125.1	0.987	7.82	9.46	72.78
Deltamethrin	0.004	0.009	37.861	252.9	93.0	0.996	10.22	9.88	92.78
Diclobutrazole	0.002	0.009	25.270	269.8	158.9	0.991	5.68	5.32	86.40
Dichloroaniline	0.003	0.008	9.595	161.0	90.0	0.986	4.74	7.66	74.86
Diphenamid	0.004	0.008	21.788	167.1	165.1	0.986	8.45	8.25	91.76
Diphenylamine	0.004	0.01	13.803	168.0	167.2	0.987	7.69	9.64	75.34
Endosulfan I	0.003	0.008	22.094	197.0	169.0	0.995	6.65	4.76	82.74
Esfenvalerate	0.004	0.009	36.644	167.0	125.1	0.999	9.36	9.88	76.90
Etaconazole	0.003	0.01	24.757	173.0	145.0	0.991	2.58	4.74	78.26
Ethofumesate	0.003	0.01	19.669	161.0	105.1	0.986	7.44	9.42	82.23
Ethoxyquin	0.002	0.008	14.978	202.1	145.1	0.996	9.16	10.22	86.43
Flamprop-isopropyl	0.004	0.008	26.278	105.0	77.1	0.988	7.06	8.96	78.96
Fenvalerate II	0.005	0.009	36.644	167.0	125.1	0.999	5.67	7.09	85.82
Hexaconazole	0.002	0.008	24.269	231.0	175.0	0.986	9.12	9.84	90.56
Leptophos	0.004	0.008	30.432	171.0	77.1	0.987	5.71	9.04	78.09
Malaoxon	0.003	0.01	19.343	126.0	99.0	0.998	8.25	7.78	86.75
Metalaxyl	0.002	0.008	19.766	234	146.1	0.996	6.54	6.47	112.26
Mevinphos	0.003	0.009	9.976	125.0	109.0	0.991	9.34	7.46	76.54
Metazachlor	0.003	0.008	20.943	133.1	132.1	0.987	3.68	5.44	87.66

Table 1. Contd.

Methoxychlor-p,p'	0.004	0.01	29.819	227.0	141.1	0.996	7.44	6.72	88.75
Nitrapyrin	0.003	0.009	10.367	194.0	133.0	0.997	9.18	10.06	91.64
Permethrin II- trans	0.004	0.01	33.107	182.9	168.1	0.994	2.34	2.78	71.06
Pentachlorobenzene	0.004	0.009	11.751	249.9	215.0	0.997	4.06	8.56	74.88
Phorate –sulfoxide	0.003	0.008	18.777	96.9	64.9	0.987	11.08	9.74	92.84
Phosmet	0.003	0.01	29.317	160.0	77.1	0.986	6.76	8.94	78.96
Piperophos	0.004	0.009	29.618	320.0	122.0	0.987	9.16	8.26	82.44
Pyrazophos	0.005	0.009	30.029	221.0	193.1	0.988	7.66	8.48	76.98
Pyroquilon	0.003	0.008	16.048	129.9	77.0	0.986	2.66	4.36	80.98
Sulfotep	0.004	0.008	15.290	201.8	145.9	0.999	7.68	9.12	72.86
Tefluthrin cis	0.004	0.009	17.858	177.1	127.1	0.999	9.84	8.36	91.64
Tetramethrin I	0.002	0.009	29.427	164.0	77.1	0.996	4.76	9.14	88.72
Thiometon	0.003	0.008	13.935	125.0	79.0	0.987	3.62	7.98	95.84
Tebuconazole	0.004	0.01	27.514	125.0	89.0	0.986	8.48	8.22	78.66

These included: mevinphos, thiometon, diethyl-m-toluene (DEET), chloroneb, diphenylamine, pyroquilon, metalaxyl, Chlorpyrifos, endosulfan 1, o, p'-DDT, cryproconazole, tebuconazole, deltamethrin, cypermethrin-beta, cyanofenophos, ethofumesate, metazachlor, azobenzene, cycloate, phosmet, chlorpropylate and chlormephos. Among the detected pesticides, there were five pesticides that exceeds the MRL according to the European Union pesticides specification of grape wine. These pesticides included pyroquilon whose concentration exceeded MRL in 38 wine samples, ethofumesate exceeded MRL in 33 samples, chloroneb exceeded MRL in 46 sample, azobenzene exceeded MRL in 14 samples and cycloate exceeded MRL in 1 sample.

Among the 50 wine samples analysed 9 (18%) contained one pesticide, 8 (16%) contained two different pesticides, 23(46%) contained three different pesticides 8 (16%) contained four different pesticides among those exceeds MRL

(pyroquilon, ethofumesate, chloroneb, azobenzene and cycloate). The incidence of having multiple pesticide residues in grape wine sample was reported in different studies. The study conducted by Cus et al. (2010) on pesticide residues and microbiological quality of bottled wine indicated that, among 25 wines, two wines did not contain residues of pesticides analyzed, eight wine samples contained residues of one pesticide, four wine samples contained the residues of two pesticides, seven wine samples contained the residues of three, and four wine samples contained the residues of four pesticides.

Also the study conducted by European Food Safety Authority in the year 2018, on pesticide residues in food, it was reported that, 1,317 samples of wine (red or white) made from grapes were analyzed, where 768 samples (58.3%), had no quantifiable pesticide residues, while 549 samples (41.7%) contained one or several pesticides in quantified concentrations

and multiple residues were reported in 263 samples (20%); up to 10 different pesticides were reported in an individual wine sample (EFSA, 2018). The incidence of having multiple pesticide in grape wine were also reported by (Duca et al., 2012; Esteve-Turrillas et al., 2016).

Presence of multiple pesticides in grape wine might be due to unfavourable weather conditions most commonly occurring in geographical area which favours the development of pests and diseases in the vineyards, so more intense application of different types of pesticides are generally required in order to safeguard grape quality, which eventually may result in greater fungicide residues in wines (Esteve-Turrillas et al., 2016; Jallow et al., 2017).

All pesticides that were not detected were removed during data analysis therefore twenty-two pesticides was used for further statistical analysis such as ANOVA. The study revealed that there was significant difference at ($p < 0.05$) on pesticide concentration when comparing

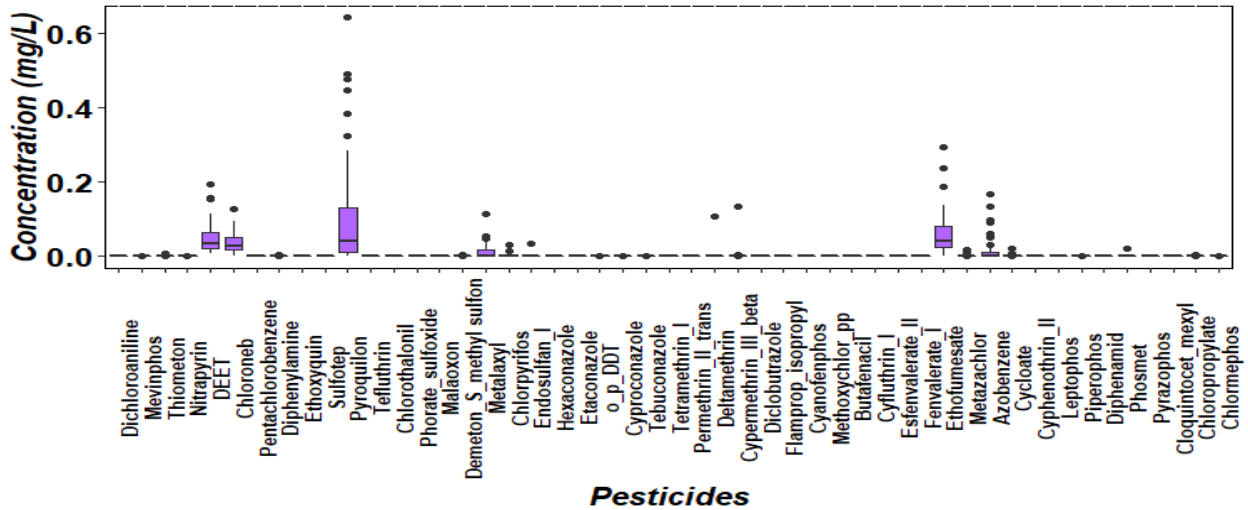


Figure 1. Box plot showing all 49 analyzed pesticides.

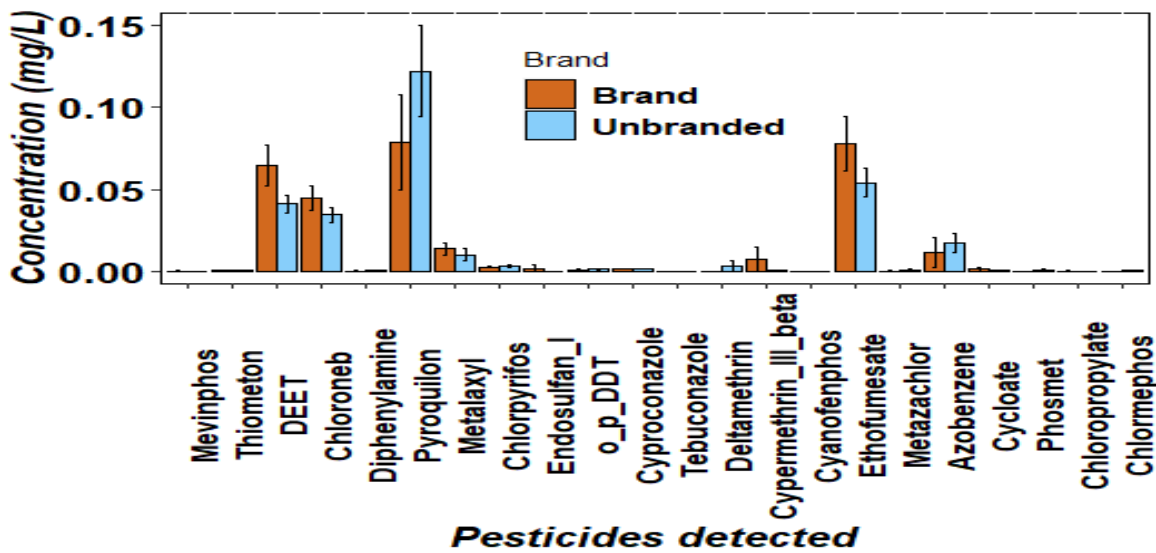


Figure 2. Bar plot showing concentration of pesticides according to brand and unbranded.

according to branded and unbranded grape wine (Figure 2). There was high concentration in unbranded wine compared to branded wine especially for Pyroquilon, Chlorpyrifos, o,p'-DDT, Deltamethrin and Azobenzene. According to the survey conducted to wine processors with the branded wine (labeled) they have high production capacity and they undergoes wine clarification and fermentation take long time compared to unbranded wine processors which help to reduce pesticide residues in grape wine. Wine processors of unbranded wine they usually depend on spontaneous fermentation by using natural fermenting yeasts present in grapes and they usually sell their products in bulk

without clarification stage. Wine clarification help to reduce the level of pesticides residues. Among the clarifying substances commonly used in wine (bentonite, charcoal, gelatin, polyvinylpyrrolidone, potassium caseinate, and colloidal silicon dioxide), charcoal allowed the complete elimination of most pesticides, especially at low levels, whereas the other clarifying substances were ineffective (Cabras and Angioni, 2000). Also during the fermentative process, yeasts can cause the disappearance of pesticide residues by degradation or absorption at the end of the fermentation when yeasts are deposited as lees (Caboni and Cabras, 2010). It was also observed that

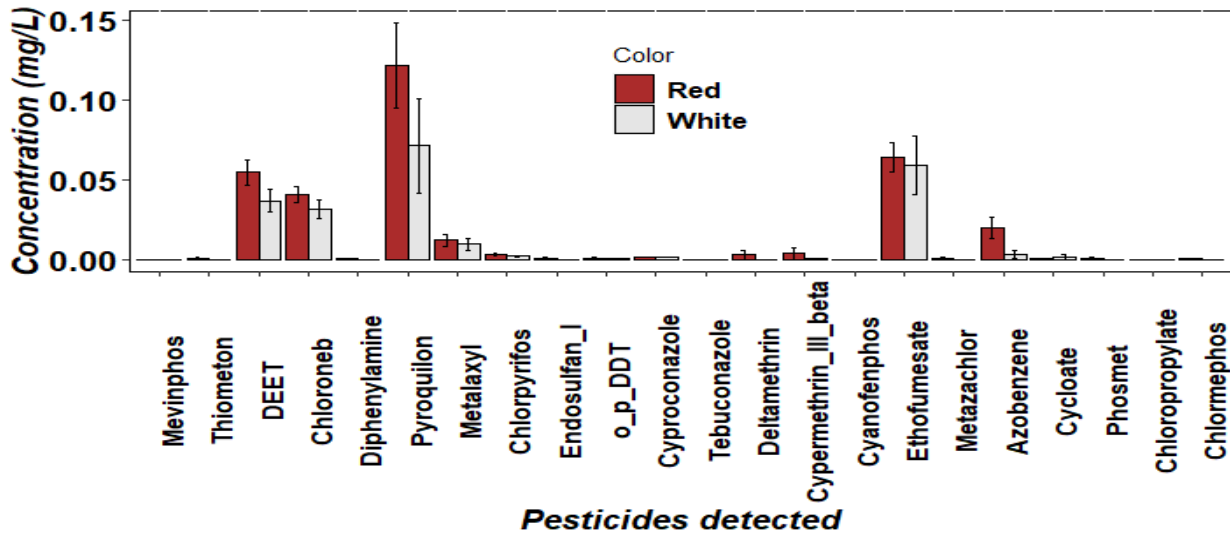


Figure 3. Box plot showing pesticides concentration according to color of wine sample.

deltamethrin, permethrin and fenvalerate were completely degraded after fermentation with *Saccharomyces cerevisiae* that can be attributable to the yeast activity, while the fungicides benalaxyl, folpet, furalaxyl, metalaxyl, iprodione, procymidone, and ofurace remained unaffected (Regueiro et al., 2014). Malolactic fermentation using *Oenococcus oeni* resulted in significant reduction in chlorpyrifos and dicofol concentrations which were reduced by 70 and 30%, respectively, where as the concentrations of chlorothalonil and procymidone diminished only slightly (Bajwa and Sandhu, 2011).

There was a significance difference ($p < 0.05$) in pesticides concentration with colour of grape wine (Figure 3). The study showed that red wine has high concentration of pesticide residues compared to white wine this suggest the idea that there might be a correlation between applied winemaking technology, red or white the skin maceration could contribute to higher degree of pesticide residues transferred to wine. Fermentation on the skins, as carried out in red wine production, is likely to lead to higher residue levels in raw wine (Pazzirota et al., 2013). This was also observed in another study which was conducted in Moldovan wine products originated from traditional agriculture which showed that red wine had higher levels of pesticide residues than white wine due to grape skin maceration (Duca et al., 2012; Regueiro et al., 2014). The study revealed that there was no significance difference ($p < 0.05$) on pesticides concentration when comparing to scales of grape wine producers (Figure 4). It was expected that there would be a large amount of pesticides in the wine produced by small producers due to the poor production system. But it was different because nothing was done to reduce the

amount of pesticides before they started production, this was observed during survey conducted to different scale of wine producers.

Conclusion

This study investigated the levels of pesticide residues in grape wine produced in Dodoma urban and Bahi districts (Tanzania). The results indicated that, majority of grape wine samples were contaminated with pesticide residues some of which had their concentrations above the MRL. According to the public health perspective, the observed levels of pesticide residues would pose potential health risks to the public. In order to reduce health and environmental problems there is a need for sensitization to grape farmers, on better pesticide safety use and handling practices and the need for continuous pesticide residues through regular monitoring.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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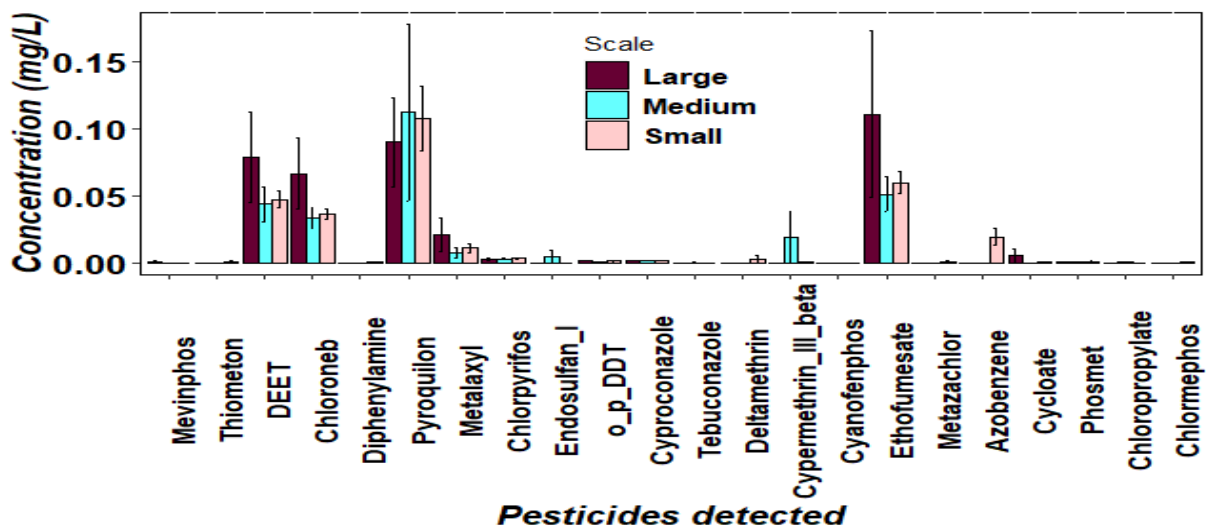


Figure 4. Bar plot showing pesticides concentration according to the scale of produce.

pesticides residues.

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