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ORIGINAL ARTICLE

Determination of Organophosphorus Pesticide Residues in Cabbages from Bindura Market Place by Solid Phase Extraction and Gas Liquid Chromatography

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ABSTRACT
The study investigated presence of organophosphate pesticides residues in cabbages sold at Bindura market place. Gas Chromatography-Nitrogen Phosphorus Detector (GC-NPD) coupled to solid phase extraction on florisil column (500mg/8ml) cartridges was used to determine organophosphate pesticides levels. Percentage recovery was above 76%. Limit of detection and quantification was 0.1ng/ml and 2.35ng/ml respectively. Dimethoate was the mostly detected pesticide. The highest level, 0.81 ± 0.02 was obtained from Aerodrome and the lowest level, 0.02 ± 0.00 was recorded for Magobo cabbages. Overally a total of 51 pesticides residues were detected in 180 samples corresponding to detection rate of 28%. The implicated organophosphate pesticides include; acephate, dimethoate; malathion, chlopyriphos-methyl and monocrotophos. All the levels obtained in the present study were far below the maximum recommended safety limits showing that farmers were sticking to recommended waiting times before harvesting their cabbages. Although levels recorded are low however presence of more than one pesticide residue type is worrisome because of a possibility of synergic tendencies. Usually safety limits are for a single pesticide and not for multiple pesticides.
***Key words**: organophosphorus pesticides, cabbages, solid phase extraction, gas chromatography*

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INTRODUCTION

Organophosphorus pesticides (OPPs) are widely applied in pre and post harvest treatment to control diseases in vegetables such as cabbages [1]. Applied pesticides have been found to persist over the season such that they may appear in vegetables meant for human consumption [2]. Presence of pesticides in vegetables has health implications. They can accumulate in the blood and adipose tissue and reach toxic levels [3,4]. Humans are exposed to organophosphate pesticides via inhalation, absorption through the skin and mostly by ingestion of contaminated vegetables and fruits [5]. Inside the body OPPs bind with enzyme cholinesterase at a synapse and paralyze enzyme activity resulting in elevated acetylcholine [6]. Consequently red blood cells and serum cholinesterase cease to function. Exposure to high levels of OPPs results in acute intoxication characterized by poisoning symptoms such as gastro-intestinal upsets, bronchospasms and urination [7]. In pregnant mothers high level exposure cause spontaneous abortion and fetal death. Previous studies have found Hodgkin's lymphoma in farm employees and cancers in their children as a result of OPPs exposure [8] European Protection Agency (EPA) reports that all OPPs are toxic to the nervous system [9, 10].

Park et al., [11] investigated organophosphate pesticides in agricultural produce and found that they were not free from pesticides. Wen et al., [12] also observed persistence of organophosphate pesticides in agricultural produce. In most developing countries like Zimbabwe there are no strict control measures to regulate the use of pesticides. Although importation and licensing of pesticides is usually regulated by the government selling and buying is often not controlled such that consumers are free to buy and use pesticides at will. Pesticides residues screening on foods is often not mandatory. Due to shrinking economy most people in developing countries ventured into market gardening and most sell their produce at markets such as Bindura Market place. Most of these farm produce would not have passed

through pesticides residue screening regimes. In such a situation researches dedicated to determine pesticide residues levels in such products become most crucial thereby providing the rationale behind this study. Pesticides of interest in this study are the most frequently used; acephate, monocrotophos, dimethoate, malathion and Chlopyriphos-methyl determined by interviewing twenty farmers per each sampling day for five days Table 1. Participation in the survey was voluntary.

Table 1. Mostly applied OPPs in Bindura farming area

Vegetables origin	Pesticide used	Frequency of quote (%)
Musana	acephate dimethoate	77 90
Manhenga	Monocrotophos dimethoate	55 83
Supa	Dimethoate	33
Madziwa	Dimethoate malathion	67 22
Glendale	Malathion Dimethoate	56 78
Matepatepa	Dimethoate Chlopyriphos- methyl	34 57
Shamva	Chlopyriphos- methyl Dimethoate	88 72
Chipadze	Dimethoate Chlopyriphos- methyl	66 35
Chiwaridzo	Dimethoate	67
Aerodrome	Dimethoate	44
Shashi	Dimethoate Chlopyriphos- methyl	67 68
Magobo	Dimethoate	89

MATERIALS AND METHODS

Chemicals and materials

Acephate, dimethoate, monocrotophos, Malathion, Chlopyriphos- methyl standards (95 %) were provided by Kutsaga tobacco research Station, Harare, Reagents, ethyl acetate, acetone, sodium hydrogen carbonate, anhydrous sulphate were all analytical grades bought from SkyLabs, South Africa

Sample collection and storage

Cabbages were bought basing on place of origin. One cabbage was bought every day until day five. The five cabbages were then processed to make a composite sample. Samples were packed in separate polythene bags, sealed and labeled with a unique sample identity and placed in an ice chest box. All samples were brought to the lab and were stored at 5^0C.These samples were then extracted and analyzed within 24hrs from the time of their collection.

Sample preparation

Fresh vegetable samples were thoroughly shredded and homogenized (for this only the edible portions were included, whereas bruised and/or rotten parts were removed). Twenty grams of the sample was macerated in 40ml of ethyl acetate. 5.0g of sodium hydrogen carbonate and 20.0g anhydrous sodium sulphate were added to remove moisture and further macerated for 3 minutes using the ultra-turax macerator (blender). The samples were then centrifuged for 5 minutes at 3,000 rpm to obtain the 2 phases. The supernatant was then transferred to a clean graduated cylinder (25ml) to measure its volume. This was then taken for clean-up and pre-concentration using solid phase extraction.

Solid phase extraction

A solid phase extraction was carried out using SPE columns. The florisil column (500mg/8ml) cartridge [13] was conditioned with 5 ml of a mixture solution of acetone: n-hexane (3:7, v/v) through the column. During the conditioning and sample loading steps, the sorbent was never allowed to dry. The extract column was fitted with 20-port vacuum manifold and a receiving flask was placed under the column to collect the eluate. The sample was loaded under vacuum at flow rates of 5ml min^{-1}.After extract had passed; the column was dried by vacuum aspiration. This was done under increased vacuum for a period of 30 minutes. The eluted mixture was then concentrated to 1 ml using a rotary evaporator (Bucchi type) and then dried. This was then dissolved in 1 ml of ethyl acetate. Pesticides were then quantified using GC-NPD [14].

Gas Chromatography nitrogen phosphorus detection (GC-NPD) Analysis

GC (the Hewllet-Packard model 5890 II) equipped with an NPD detector with a split less injector at 250^0C and the injection volume of 1 µL together with an auto sampler model 7673. A GLC capillary column-DB-

17 was used. The detector temperature was set at 280 ^{0}C with carrier gas and makeup gas as nitrogen with a 2.0 ml/min and 30 ml/min flow rate respectively, hydrogen at 5 ml/min and air at 80ml/min respectively employing the split less mode with temperature program, 100^{0}C for 1 min, 100^{0}C-150^{0}C at 30^{0}C/min,150^{0}C for 2 min, 150^{0}C-205^{0}C at 3^{0}C/ min,205^{0}C-260^{0}C at 2^{0}C /min, and 260^{0}C for 1min [6]. Typical chromatogram for standard analytes obtained is shown in Fig 1 below. Analyte identification within the samples was based on chromatographic retention times i.e. if any of the targeted pesticides was found to match peak identity with standards e.g. Figs 1 and 2.Quantitation was based on peak area. Calibration curve method was used for quantitation. Regression equation for Acephate, dimethoate, monocrotophos, Malathion, Chlopyriphos- methyl were y = 6.792x + 29376, R^2 = 0.994, y = 47.50x + 72257, R^2 = 0.998, y = 6.778x + 59736, R^2 = 0.994, y = 3.675x + 45912, R^2 = 0.996, y = 4.903x + 67754, R^2 = 0.994 respectively. The limit of detection was 0.1 ng /ml while limit of quantification was 2.35 ng /ml. All tested percentage recoveries were above 76.20 ± 4.53%.

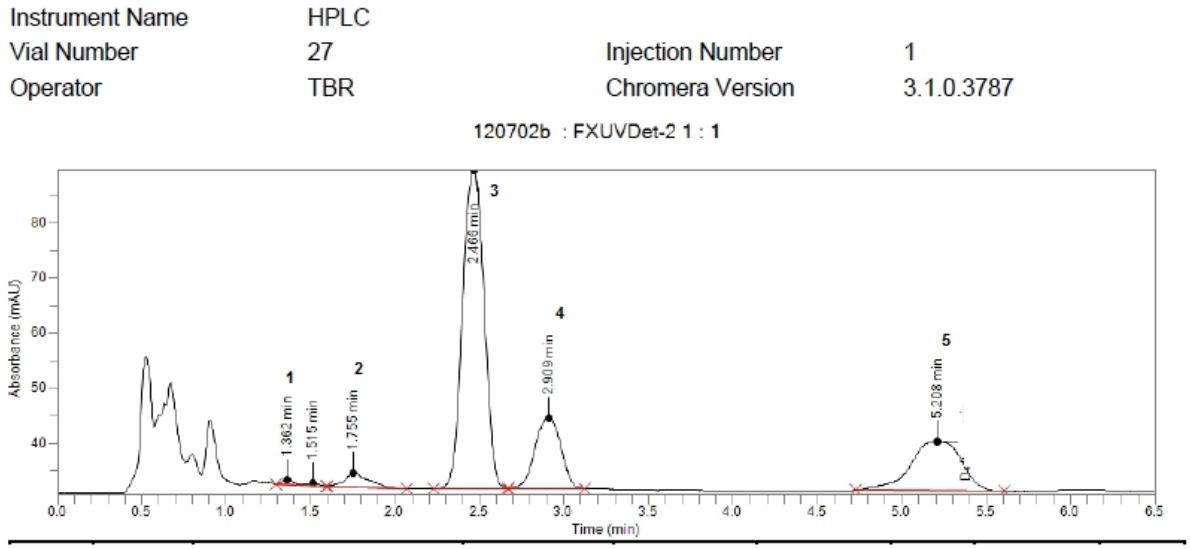

Fig 1. A typical GC chromatogram of standard pesticides, 1 = Acephate, 2 = dimethoate, 3 = Malathion, 4 = Chlopyriphos- methyl and 5 = monocrotophos

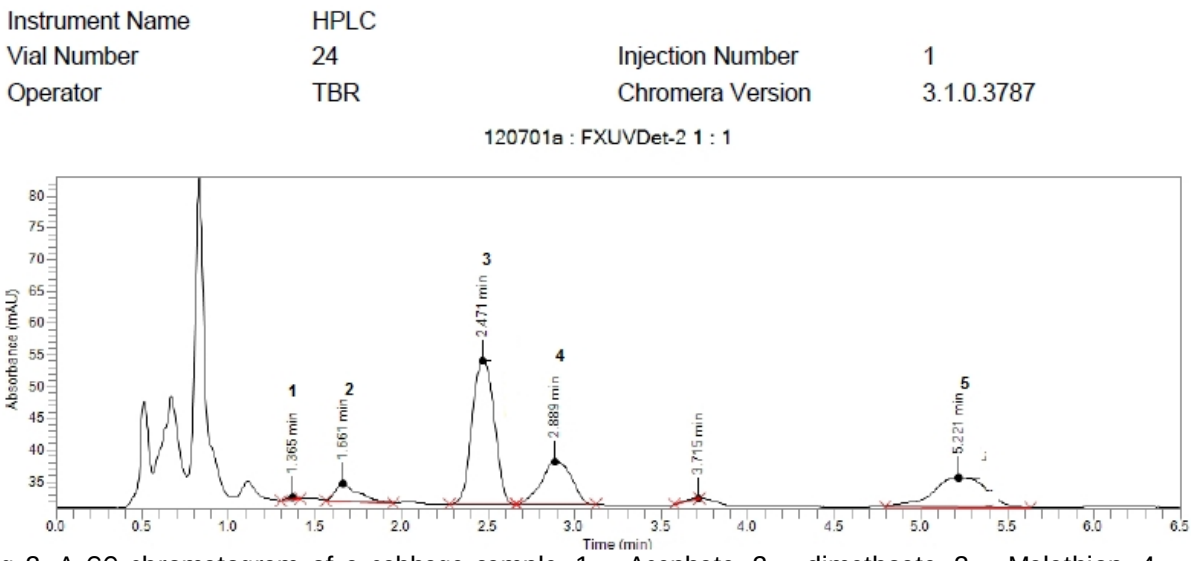

Fig 2. A GC chromatogram of a cabbage sample, 1 = Acephate, 2 = dimethoate, 3 = Malathion, 4 = Chlopyriphos- methyl and 5 = monocrotophos

RESULTS AND DISCUSSION

Figure 1 shows a typical chromatogram obtained for a cabbages sample. The concentration of OPPs in cabbage samples are shown in Table 2. Cabbages from Supa farming area consisted of all the OPPs while they were not detected in cabbages from Madziwa, Shamva and Shashi. Dimethoate was the mostly

detected pesticide. The highest level, 0.81 ± 0.02 was obtained from Aerodrome and the lowest level, 0.02 ± 0.00 was recorded for cabbages from Magobo. A total of 51 residues were detected in 180 samples corresponding to detection rate of 28%. The implicated organophosphate pesticides include; acephate, dimethoate; malathion, chlopyriphos- methyl and monocrotophos. Although organophosphate pesticides residues were detected in some vegetables they were far below the accepted maximum residual limits [16] Low levels obtained in this study imply that famers are sticking to stipulated times before they harvest their crops. Frequent detection of all pesticides in the super cabbage samples is worrisome as this may have health implications through synergistic tendencies [17]. Effects of long term exposure to these low levels of pesticides is usual is not known [17, 18].

Table 2. Concentration of Organophosphates in cabbage samples

Sample Origin	Concentration (mg/kg FW) of				
	Acephate	Dimethoate	Malathion	Chlopyriphos-methyl	monocrotophos
Musana	ND	0.33 ± 0.02	ND	ND	ND
Manhenga	ND	0.41 ± 0.01	0.06 ± 0.00	ND	ND
Supa	0.07 ± 0.00	0.21 ± 0.01	0.10 ± 0.02	0.19 ± 0.01	0.12 ± 0.03
Madziwa	ND	ND	ND	ND	ND
Glendale	ND	0.08 ± 0.00	ND	ND	ND
Matepatepa	ND	0.57 ± 002	ND	ND	ND
Shamva	ND	ND	ND	ND	ND
Chipadze	ND	0.07 ± 0.00	0.03 ± 0.00	ND	ND
Chiwaridzo	ND	0.19 ± 0.02	ND	ND	ND
Aerodrome	ND	0.81 ± 0.02	ND	ND	ND
Shashi	ND	ND	ND	ND	ND
Magobo	ND	0.33 ± 0.04	0.07 ± 0.00	0.02 ± 0.00	ND

FW = fresh weight, ND = not detected

CONCLUSION

All the analyzed cabbage samples consisted of levels that were below the recommended maximum residual limits implying that famers sticked to prescribed times before their cabbages could be harvested for human consumption however presence of low levels of all pesticide residues in some samples is worrisome since effects of chronic exposure to this low levels are not known especially on young children.

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