PRESENCE OF SELECTED ORGANOPHOSPHORUS PESTICIDE RESIDUES IN RAW CUCUMBER AND TOMATO SAMPLES IN TAMAN UNIVERSITI, JOHOR

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Abstract

Consumption of raw vegetables is common among Malaysians due to its nutritional values as well as anecdotal evidence supporting its medicinal properties. Although periodical analyses of pesticide residues on imported vegetables have been performed by the relevant authority, the same has not been amply emphasized on the locally produced vegetables and those that are available at food outlets, rendering the possible threat towards human health. In this research, the possible presence of diazinon, chlorpyrifos and dimethoate residues in two commonly eaten raw vegetables viz. cucumber and tomato conveniently sampled from four food outlets within Taman Universiti was investigated using gas chromatography with electron capture detector (GC-ECD) preceded by the Jabatan Kimia Malaysia-validated liquid-liquid extraction method (JKM F0702). Samples of organic cucumber and tomato were used as controls. Once detected, the confirmation was done using gas chromatography with mass spectrometer (GC-MS). Results of this research revealed that presence of diazinon, chlorpyrifos and dimethoate residues were not detected in all the samples of cucumber and tomato sampled from the selected permanent food outlets. The fact that two unknown compounds were found in both of the organic cucumber and tomato samples eluted at 2.25 minutes and 3.58 minutes, respectively, while an unknown compound eluted at 3.47 minutes was evident in one of the tomato samples included in this research, expanding the spectrum of pesticide residues in these vegetables for future studies deserves consideration. Although the concentrations of diazinon, chlorpyrifos and dimethoate found in those vegetables appear to be within the acceptable levels for human consumption, absence of possible health threats for human may not be construed, considering the limited samples and organophosphorus pesticide residues included in this research.

Keywords: Organophosphorus pesticide residues, cucumber and tomato, raw vegetables, Johor Bahru, Malaysia

INTRODUCTION

In view of the increasing pattern of income and the awareness about the importance of balanced diet, consumption of vegetables is gaining popularity among Malaysians [1]. In contrast to the preference of eating cooked vegetables prevalently observed among Chinese and Indians, several types of vegetables are commonly eaten fresh among Malays [2] considering anecdotal evidence supporting its medicinal benefits [3]. Frequent infestation of pests viz. insects, fungi, slugs, snails, unwanted plants or microorganisms [4] has been considered as the main restriction factor in achieving sustainable yield in food production among countries with tropical climate [5] and the use of chemical pesticides is deemed crucial in controlling such infestation from damaging vegetable products, considering high demand for vegetables [6, 7].

While vegetables may be contaminated by pesticides at different stages of food production prior to consumption, it has also been indicated that environmental conditions such as rain and wind would lead to differences in the dispersal of pesticides during application [8]. Despite facilitating good quality of vegetable products as well as increasing productivity [9], theexcessive application/inappropriate utilization of pesticides may lead to contamination of raw food supply and agriculture related side-products and those would prove detrimental towards human health such as respiratory, myocardial and neuromuscular transmission impairments [8] as well as cancers [10].

Since the consumption of raw vegetables such as cucumber and tomato in daily meals is customary in many parts of Malaysia including Johor, issue pertaining to its safety merits consideration. Although general inspection has been routinely performed by the relevant authority on random samples of imported vegetables upon entry of consignments, the same has not been amply emphasized on the locally produced vegetables and those that are available at food outlets, rendering the possible threat towards human health. Hence, this present cross-sectional research aimed at ascertaining the presence of organophosphorus pesticide residues in such vegetables as cucumber and tomato that were sold as complete dishes at permanent food outlets within Taman Universiti, Johor Bahru acquires significance in revealing the current status of contamination by diazinon, chlorpyrifos and dimethoate in those vegetables and its health implication among populations in Johor, Malaysia. Given all the above information, this present research was designed at investigating the level of compliance between the concentrations of diazinon, chlorpyrifos and dimethoate residues in those vegetables in Taman Universiti, Johor Bahru with that of the prevailing Maximum Residue Limits (MRLs).

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MATERIALS AND METHOD

Research Design

This cross-sectional quantitative research investigated the presence of selected organophosphorus pesticide residues (diazinon, chlorpyrifos and dimethoate) in cucumber and tomato samples served as finished dishes in permanent food outlets within Taman Universiti, Johor Bahru. A preliminary survey was conducted among all the permanent food outlets in Taman Universiti commercial area for identifying the more common vegetables that were served raw. Among the 47 permanent food outlets surveyed, only 10 of them served raw vegetables as finished dishes meant for human consumption; cucumber (90%) and tomato (80%) being the more prevalent. Following the random sampling method, samples of cucumber and tomato were obtained from four of the 10 permanent food outlets, while organic cucumber and tomato samples were included as controls in this analysis. In the laboratory, the samples were pulverized and kept at about 4°C prior to analysis.

Extraction of Organophosphorus Pesticide Residues

This research was conducted in four cohesive phases i.e. (1) qualitative detection of organophosphorus pesticide residues in the sample using GC-ECD, (2) confirmation of the presence using GC-MS, (3) quantification of the observed organophosphorus pesticide residues using GC-ECD and (4) assessment on the compliance of the concentration of organophosphorus pesticide residues with the prevailing safety standards (MRLs) (Food Act 1983 (Act 281) and Regulations, 2013). While the qualitative detection and quantification of organophosphorus pesticides residue using GC-ECD as well as assessment on its compliance with the MRLs were independently performed by the author, confirmation of the presence of such organophosphorus pesticides residue using GC-MS was outsourced to the University Industry Research Laboratory, UniversitiTeknologi Malaysia. During the extraction process, all solvents used were of analytical reagent grade and higher while the pesticide standards (98.5 - 99.8% purity) were purchased from Sigma-Aldrich (Germany). The selected organophosphorus pesticide residues were extracted from a pulverized vegetable sample following the validated extraction method prescribed by the Jabatan Kimia Malaysia i.e. Multiresidue Method of AnalyzingOrganophosphorus Pesticide Residues in Fresh Fruits and Vegetables (JKM F0702) in triplicates detailed below.

Firstly, a specimen (10 g) of the pulverized sample was placed in a clean centrifuge tube followed by the addition of 10 mL of acetic acid in acetonitrile (1% v/v). The mixture was vigorously mixed using a vortex mixer at 2500 rpm for one minute followed by the addition of sodium chloride (1 g) and magnesium sulphate anhydrous (4 g) for inducing phase separation, hence promoting partitioning of the pesticides into the organic layer [11]. The specimen was once again vortex-mixed for one minute followed by centrifugation at 3300 rpm for 2 minutes. Upon completion, the top layer (organic) was filtered through a syringe filter and acetonitrile (100 µL) was added to each of the 500 µL of final extract prior to the injection into GC-ECD and/or GC-MS for analysis.

Organophosphorus Pesticide Residues Analysis by Gas Chromatography

The detection of possible presence of diazinon, chlorpyrifos and dimethoate as well as quantification were done using GC-ECD, while GC-MS was used for confirming the identity of the analytes. The condition for GC-ECD is presented in Table 1. Representative chromatograms for standard solution of dimethoate, diazinon and chlorpyrifos in acetonitrile as well as in organic cucumber sample matrix spiked with those standards at 1 ppm each are presented inFigures 1 (A-B).

Standardization of Method

Standardization of method for analysis of diazinon, chlorpyrifos and dimethoate in cucumber and tomato samples included linearity and calibration curves following the guideline provided by National Poison Centre [12] and Man *et al*. [13]. Stock standard solutions (100 mg/L) for each pesticide were prepared in acetonitrile in 5 mL volumetric flasks. The mixed standard working solutions (10 mg/L) were prepared by diluting all the three standard solutions with the appropriate amounts of acetonitrile. Both the stock solutions and mixed working standard solutions were labeled, wrapped with aluminium foil and stored at 4°C prior to analysis.

The detector linearity for diazinon, chlorpyrifos and dimethoate was assessed by injecting aliquots of those substances in mixed standard solutions prepared in acetonitrile that individually contained 0.5, 1.0, 1.5, 2.0 and 2.5 ppm. It was evident that following the injection into GC-ECD, diazinon, chlorpyrifos and dimethoate eluted at 3.21, 4.32 and 2.92 minutes, respectively (Figure 1). The peak area of the individual analyte versus the amount of analyte added was plotted and the obtained linearity curves were only accepted when (a) the

coefficient of determination (r^2) were greater than 0.995 and (b) the coefficient variation (CV %) was lesser than 20% [12, 13]. The calibration curves for diazinon, chlorpyrifos and dimethoate in cucumber (Figure 2) were prepared by spiking the extracted blank sample (organic cucumber) (500 µL) with the amounts of those standards detailed inTable 2. In accepting the calibration curve, the same criteria prescribed by Man *et al*. [13] and the National Poison Centre [12] were adhered.

Man *et al*. [13] indicate that Limit of Detection (LOD) is the lowest concentration of an analyte determined with signal to noise ratio of at least 3:1 by peak height while Limit of Quantification (LOQ) is the lowest concentration of an analyte in a calibration curve. In this present research, the LOD and LOQ for diazinon, chlorpyrifos and dimethoate were mathematically determined from the calibration curves (Table 2) utilizing the formulae described by the previous researchers [14, 15].

RESULTS

Detection and Quantification of Diazinon, Chlorpyrifos and Dimethoate Residues in Samples of Cucumber and Tomato

Our preliminary survey involving 47 permanent food outlets that were present within the Taman Universiti commercial area revealed that only 10 of them sold raw vegetables as complete dishes; cucumber (90%), tomato (80%), lettuce (60%) and *ulam raja* (50%) being the more common vegetables, justifying the choice of cucumber and tomato in this present research. Upon completion of the preliminary survey, four out of those 10 permanent food outlets were randomly chosen and results of the analysis of diazinon, chlorpyrifos and dimethoate residues in those samples of cucumber and tomato obtained are tabulated in Table 3. Although diazinon was potentially detected in one of the cucumber samples (Food Outlet No. 3) following analysis using GC-ECD, confirmatory analysis using GC-MS disproved its presence despite having the same retention time i.e. 3.21 minutes. Hence, this research revealed that the presence of diazinon, chlorpyrifos and dimethoate residues were not detected in all the cucumber and tomato samples obtained from the selected permanent food outlets within Taman Universiti. Additionally, result of this analysis revealed the possible presence of an unknown compound in a sample of tomato from the Food Outlet No. 1, eluted at 3.47 minutes in the chromatogram (Figure 3) generated by GC-ECD as well as two other unknown compounds eluted at 2.25 minutes and 3.58 minutes, respectively in both of the organic cucumber and tomato samples included in this research as controls (Figure 4).

DISCUSSION

Prevailing Knowledge on Issues Pertaining to the Use of Pesticides in Vegetable Farming

In general, organophosphorus pesticides have been regularly used for various vegetable and fruits crops mainly due to low level of persistency in the environment as well as high efficiency in controlling the infesting pests when compared with the other types of pesticides such as organochlorine and organonitrogen substances [16]. It has been reported that Cameron Highlands is accounted for 92% of the overall production of vegetables in the country with considerable proportions are exported mainly to the neighbouring country, Singapore [17]. Since the vegetable production in Cameron Highlands has been impeded by an over-reliance on the use of pesticide for controlling pests, long term environmental impacts such as local health hazards, build-up of resistance and contamination of the environment [17] resulted in public scrutiny by consumers in both Malaysia and Singapore since 1987 [18]. Additionally, the content of pesticide residues in fruits and vegetable crops are not entirely dependable on the amount of pesticide sprayed over them but also on the accumulation in soils and water during irrigation [16]. In this context, diazinon, chlorpyrifos and dimethoate have been regarded as amongst the more prevalent organophosphorus pesticides found in vegetable and fruits crops in many countries [9, 19] including Malaysia [20]. Due to such factors, continuous monitoring and stringent regulations of pesticides application on vegetable crops are considered needful [8, 9, 19]. In view of such importance, and the fact that no universal analytical methods are available, studies on developing robust, simple and multi-residues analytical methods, including that of conforming to the principles of green analytical chemistry, focusing on the different vegetable and fruit matrices have been acquiring considerable interest [5, 8, 21]. Hence, this present research aimed at investigating the presence of diazinon, chlorpyrifos and dimethoate in cucumber and tomato served as complete dishes, acquires public health importance.

Detection and Quantification of Diazinon, Chlorpyrifos and Dimethoate Residues in Samples of Cucumber and Tomato

Results of this research indicated that diazinon, chlorpyrifos and dimethoate were not detected in any of the cucumber and tomato samples. However, it is pertinent to mention here that while Malaysia has been adhering to the prescribed MRL of not more than 0.50 ppm for diazinon in cucumber, considerably lower MRL for the same has been outlined by the European Commission (0.01 ppm), adopted by many agricultural countries such as Bangladesh [19]. Considering that diazinon would lead to numerous health implications [8, 10] and the fact that Malaysia is moving towards a developed nation of which health issue is an important factor, it is found pertinent for us to suggest the adoption of such a more stringent MRL for consideration. Results of this research that was generated using GC-ECD revealed the presence of an unknown peak with a considerable abundance in a chromatogram of a sample of tomato from the Food Outlet No. 1 eluted at 3.47 minutes was observed. Apart from that, two others unknown peaks (2.25 and 3.58 minutes) were observed in the chromatograms of both the organic cucumber and tomato samples considered as controls for analytical purposes. The fact that the organic vegetables are claimed to be free from the use of inorganic pesticides and fertilizers during farming and since an unknown peak was found in a tomato sample, the possible presence of pesticides/fertilizers or perhaps any other compounds in those samples cannot be excluded and this aspect merits further research, probably using a more sensitive analytical apparatus such as GC-FPD. Although the presence of diazinon, chlorpyrifos and dimethoate residues were not detected throughout the analysis, absolute absence of possible toxicants that may have adverse effects on human cannot be ruled out due to limited samples of vegetables and organophosphorus pesticides included in this research.

Table 1: Condition for GC-ECD Used in This Research

Parameters	**GC-ECD**
Temperature Gradient	Oven temperature was initially set at 200°C (held for 2 minutes) The temperature was increased at the rate of 20°C per minute until 290°C (no holding time)
Carrier Gas(Flow Rate)	Helium gas (1.1 mL min^{-1})
Make Up Gas(Flow Rate)	Nitrogen gas (32.4 mL min^{-1})
Column	HP-Ultra 2 (25 m × 0.2 mm i.d. × 0.33 μm film thickness)
Injector Temperature	260°C
Detector Temperature	350°C
Injection Mode	Splitless
Injection Volume	1 μL

Table 2: Range, Coefficient of Determination, Coefficient Variation, Limit of Detection and Limit of Quantification Obtained from the Calibration Curves for Dimethoate, Diazinon and Chlorpyrifos in Organic Cucumber

Organophosphorus Pesticides	**Range (ppm)**	**Coefficient of Determination, r^2**	**Coefficient Variation (%)**	**LOD (ppm)**	**LOQ (ppm)**
Dimethoate	0.05-2.50	0.996	9.67	0.09	0.30
Diazinon	0.30-3.00	0.997	19.35	0.16	0.54
Chlorpyrifos	0.01-2.50	0.996	11.75	0.08	0.28

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Table 3: Results of GC-ECD Analysis for Dimethoate,Diazinonand Chlorpyrifos Residues in Cucumber and Tomato Samples in Selected Food Outlets in Taman Universiti, Johor Bahru

Food Outlets/ Controls	Vegetable Samples	Organophosphorus Pesticide Residues			Observed retention time for unknown compound (minutes)
		Dimethoate (ppm)	Diazinon (ppm)	Chlorpyrifos (ppm)	
1	A	ND	ND	ND	ND
	B	ND	ND	ND	3.47
2	A	ND	ND	ND	ND
	B	ND	ND	ND	ND
3	A	ND	ND	ND	ND
	B	ND	ND	ND	ND
4	A	ND	ND	ND	ND
	B	ND	ND	ND	ND
Controls (Organic Samples)	A	ND	ND	ND	2.25; 3.58
	B	ND	ND	ND	2.25; 3.58

Legend: A = Cucumber; B = Tomato; ND = Not Detected

The retention times for dimethoate, diazinon and chlorpyrifos following injections in the GC-ECD were at 2.92, 3.21, and 4.32 minutes, respectively.

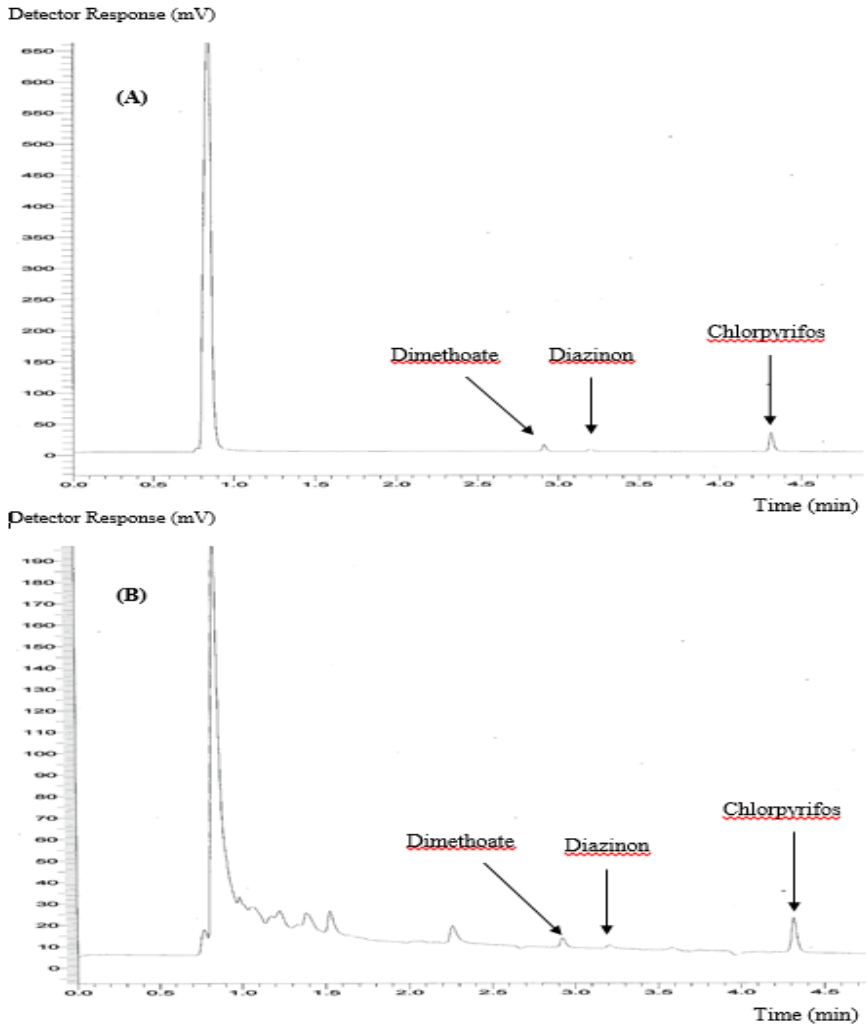

Figure 1: GC-ECD Chromatograms for (A) Standard Solutions of Dimethoate (2.92 min), Diazinon (3.21 min) and Chlorpyrifos (4.32 min) in Acetonitrile as well as in (B) Spiked Organic Cucumber Sample Matrix (1 ppm)

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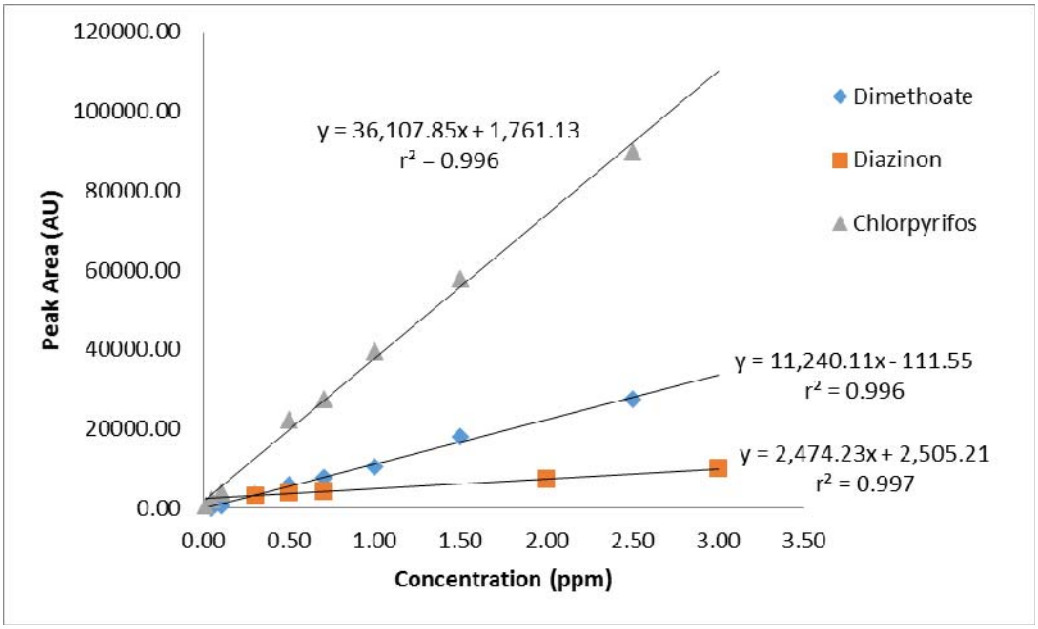

Figure 2: Calibration Curves for Dimethoate, Diazinon and Chlorpyrifos

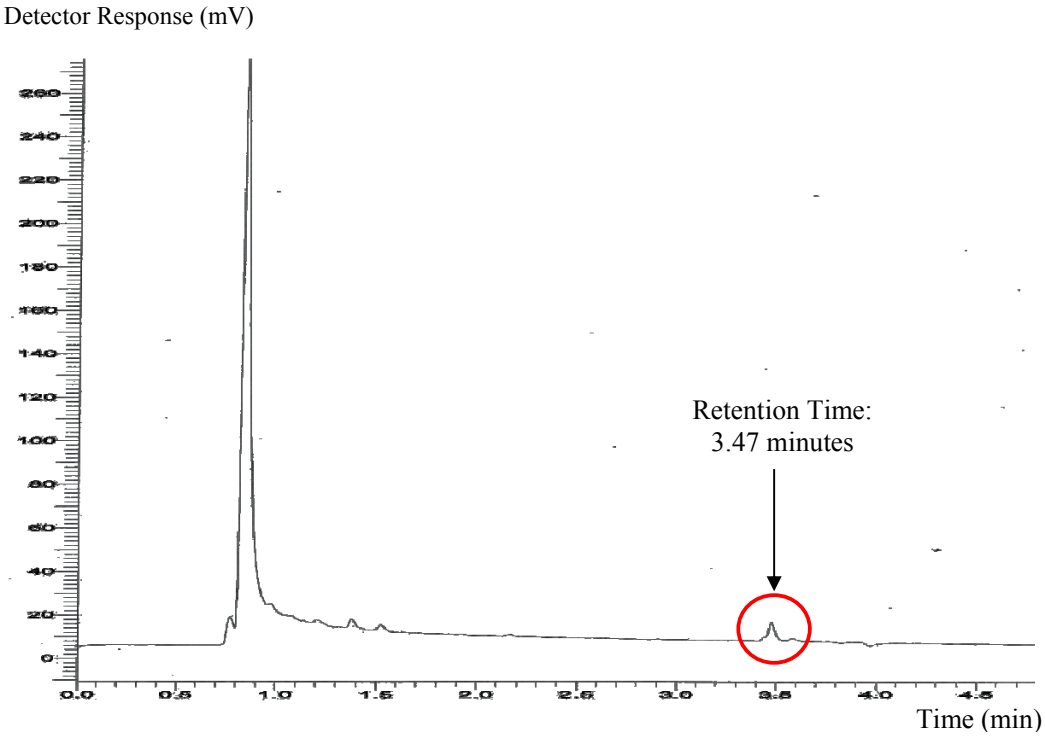

Figure 3: Chromatogram for Tomato Sample Obtained from Food Outlet No. 1

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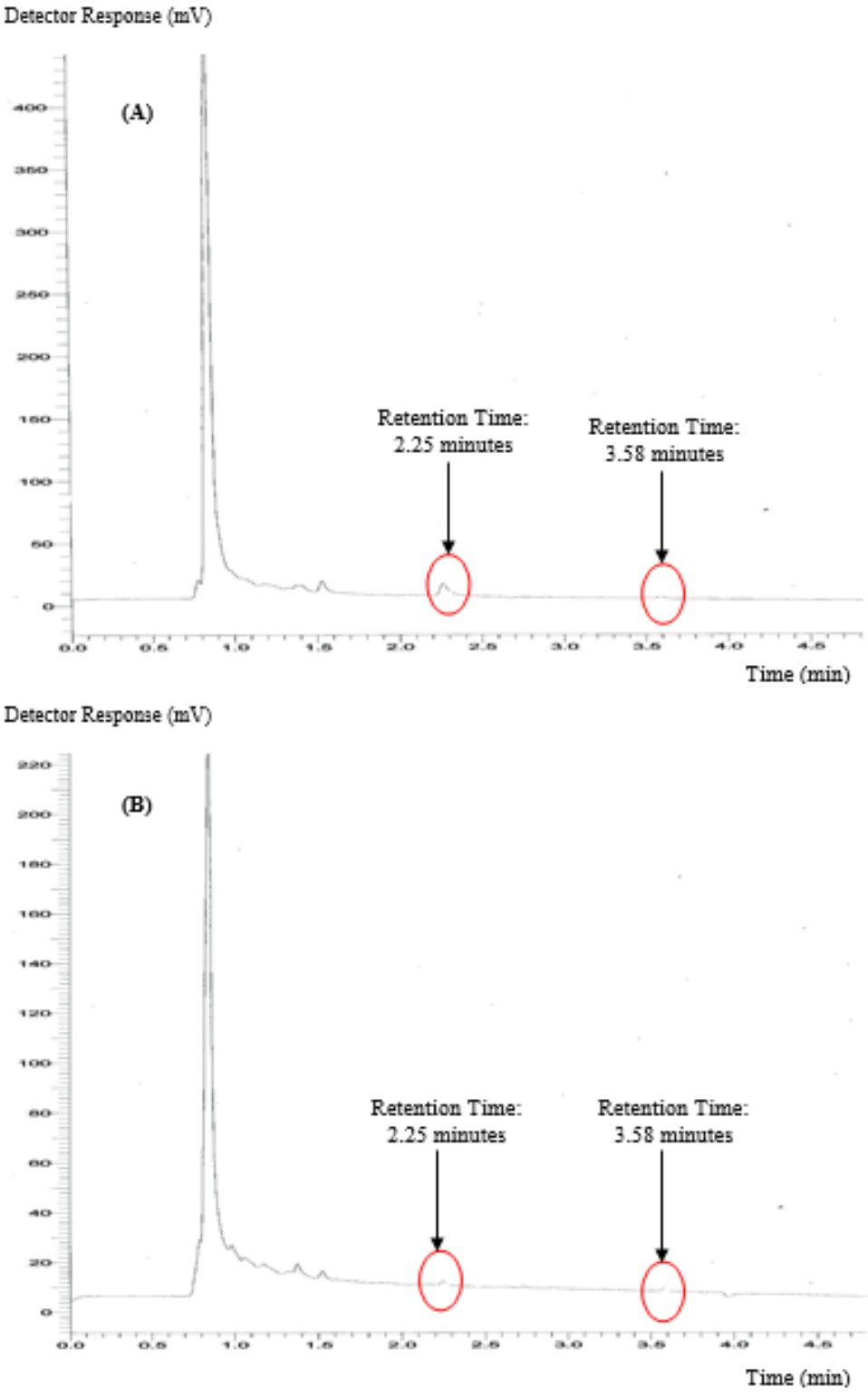

Figure 4: Chromatograms for Organic Cucumber (A) and Tomato (B) Samples Included as Controls for This Research

CONCLUSION

This research that utilized cucumber and tomato samples in the form of complete dishes obtained from several permanent food outlets within Taman Universiti, Johor Bahru was aimed at exploring the presence of three important and commonly found organophosphorus pesticides (diazinon, chlorpyrifos and dimethoate) in raw vegetables and its possible negative impacts on human health following consumption. In gist, all the cucumber and tomato samples did not contain any of the three organophosphorus pesticides at toxicity levels as recommended in the Sixteenth Schedule of the Food Act 1983 (Act 281) and Regulations (2013). Ironically, the MRL for diazinon in cucumber prescribed in our food act (0.50 ppm) is evidently higher than that of the MRL suggested by the European Commission adopted by a few of under-developed countries such as Bangladesh [19]. This would trigger an argument if the MRL for diazinon prescribed in our food act is adequate in ensuring the safe consumption of such vegetables for human, an avenue for further discussion. Since this research involved a small number of samples and it did not included a complete list of organophosphorus pesticides and other types of substances such as organochlorine and organonitrogen as well as pathogenic microorganisms too, an absolute exclusion of any possible negative impacts on human health cannot be concluded.

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