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Evaluation of pesticide residues in selected vegetables from Kuala Lumpur, Malaysia using modified QuEChERS and assessment of washing methods

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Abstract

Growing population in Malaysia has resulted in increased production of local vegetables as well as pesticide usage. This constitutes a health risk to human health. In the present work, the level of ten pesticide residues namely chlorpyrifos, profenofos, aldrin, endrin, cypermethrin, lambda-cyhalothrin, carbendazim, propamocarb, imidacloprid, and thiamethoxam in ten types of vegetables collected from six local markets were measured using modified QuEChERS (quick, easy, cheap, effective, rugged, and safe) coupled with gas chromatography-tandem mass spectrometry (GC-MS/MS) and ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). Results showed that 13.3% samples contained pesticide residues above the maximum residue limit (MRL) prescribed by the Malaysian Food Regulations 1985, 55.0% of samples contained pesticide residues below the MRL, and no pesticide residues were detected in 31.7% of samples. Carbendazim and chlorpyrifos were among the highest pesticides detected in the samples. For the type of vegetables, kale and spinach contained high concentrations of pesticide residues above the MRL. In order to produce safe vegetables, the efficiency of different washing methods (tap water, 10% sodium bicarbonate solution, and 10% acetic acid solution) in reducing carbendazim and chlorpyrifos residues in a kale model system was evaluated. Results showed that the levels of carbendazim and chlorpyrifos reduction for all three methods were significantly different ($p < 0.05$) with 10% acetic acid solution being the most effective followed by 10% sodium bicarbonate solution, and tap water. Washing kale with 10% acetic acid reduced 76.0 and 41.2% of carbendazim and chlorpyrifos, respectively. Therefore, it is recommended for consumers to practice 10% sodium bicarbonate washing method by soaking vegetables with an acidic solution followed by rinsing with tap water to reduce pesticide residues, and minimise the exposure to hazardous pesticides.

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Introduction

Vegetables are an important component in the human diet owing to their high nutrient and antioxidant contents. Vegetables comprise of essential micronutrients including vitamins and minerals, as well as phytochemicals like phenolics and flavonoids, which possess substantial anti-carcinogenic, anti-mutagenic, and anti-oxidative properties (Samtiya *et al.*, 2021). The examples of popular vegetables are leafy vegetables (spinach and lettuce), fruiting vegetables (tomato and chili), and root vegetables (carrot and radish).

In Malaysia, the total population has increased from 23.3 million in 2000 to approximately 32.7 million in 2021 (DOSM, 2021). As the population increases, so does vegetable production to support domestic consumption as well as for some export markets. Increased awareness among health-conscious consumers is believed to be among the

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driving factors leading to the surge in demand for vegetables. This trend has resulted in extensive vegetable production and also pesticide usage to minimise pest and maximise profit, which in some cases, without adhering to Good Agricultural Practices (GAP) (Wang *et al*., 2013).

Organophosphates (OP), synthetic pyrethroids (SP), and carbamates (CA) are some examples of pesticides being used in agriculture to combat pests such as insects, moulds, and weeds. Pesticides protect the crops by reducing the losses, thus enhancing the yield. The crops' quality, particularly their aesthetic appeal, is preserved well with the application of pesticides (Sharma *et al*., 2019).

In Malaysia, it is estimated that 97.6% of the farmers use chemical pesticides as a common practice or strategy to fight pests and diseases (Halimatunsadiah *et al*., 2016). This is mainly due to the low cost, ease of access, high efficiency, and simple application (Sharifzadeh *et al*., 2018; Kaur *et al*., 2022). Unfortunately, the widespread and malpractice of pesticides have negatively impacted food safety. Among the recorded malpractices including harvesting the vegetable products shortly after pesticide treatment (Halimatunsadiah *et al*., 2016).

In 2018, the Singaporean authorities issued a recall for Malaysian iceberg lettuce due to high level of fipronil residue. This could potentially tarnish Malaysia's reputation as an agriculture producer, as well as ruining other export opportunities, thus resulting in financial loss. As food safety is becoming a top priority among consumers, there is an increasing concern for pesticide residues in vegetables. Recently, various pesticide studies in lettuce, cauliflower, celery, tomato, chilli, and cabbage have been reported across the globe (Hu *et al*., 2020; Ramadan *et al*., 2020; Yi *et al*., 2020; Philippe *et al*., 2021; Toptanci *et al*., 2021). However, in Malaysia, due to the data limitation, researches are yet to be conducted particularly on those obtained from the Malaysian local market. In the present work, besides the evaluation of pesticide residues, the effectiveness of washing methods was also conducted to study the reduction of pesticide level in selected vegetable model. In the present work, pesticide residue analysis was performed by utilising modified QuEChERS (quick, easy, cheap, effective, rugged, and safe) extraction method coupled with gas chromatography-tandem mass spectrometry (GC-MS/MS) and ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) to determine ten pesticide residues from five major chemical groups.

The present work, therefore, aimed to evaluate ten pesticide residues (chlorpyrifos, profenofos, aldrin, endrin, cypermethrin, lambda-cyhalothrin, carbendazim, propamocarb, imidacloprid, and thiamethoxam) from five major chemical groups (OP, OC, SP, CA, and NEO) in selected vegetables. The concentrations detected were compared against the maximum residue limits (MRL) prescribed by the Malaysian Food Regulations 1985. Based on the findings, the most contaminated vegetable was selected as the model for the washing experiment to evaluate the effectiveness of the washing methods (tap water, 10% sodium bicarbonate, and 10% acetic acid solution) in reducing the selected pesticides.

Materials and methods

Sampling area

A total of 60 fresh vegetable samples were collected from six local markets (ten from each) in Kuala Lumpur. For each market, five leafy vegetables namely water spinach or *kangkung*, kale, mustard, spinach, and lettuce, and five fruiting vegetables namely tomato, chilli, cucumber, okra, and pumpkin were randomly collected. The sampling was performed by collecting a minimum of 1 kg sample according to Codex Alimentarius FAO-WHO methods of sampling for the determination of pesticide residues for compliance with MRLs (CAC/GL 33-1999). The samples were immediately transported to the Laboratory of Pesticide Residue, Department of Chemistry, Petaling Jaya, Malaysia for further analyses.

Chemicals and reagents

Certified reference standards (> 98% purity) of pesticide (chlorpyrifos, profenofos, aldrin, endrin, cypermethrin, lambda-cyhalothrin, carbendazim, propamocarb, imidacloprid, and thiamethoxam) and internal standard (IS) (triphenyl phosphate) were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Ultra-pure deionised water with 18 MΩ was obtained from a Millipore Milli-Q water purification system (Milford, MA, USA). Glacial acetic acid, anhydrous magnesium sulphate ($MgSO_4$), sodium acetate (NaOAc), and ammonium acetate were purchased from Merck (Darmstadt, Germany). Primary Secondary Amine (PSA) was purchased from Agilent (Santa Clara, CA, USA). All organic

solvents such as methanol and acetonitrile were liquid chromatography-mass spectrometry (LC-MS) grade, and purchased from Fisher Scientific (Leicestershire, UK).

Sample extraction and clean-up

Pesticide residues were extracted using the modified QuEChERS method according to Lehotay (2007). Briefly, homogenised sample (15 g) were weighed into a 50-mL polypropylene tube, into which 15 mL of 1% acetic acid in acetonitrile was added. The tube was manually shaken and subjected to a 1 min vortex. Next, IS was added, and the tube was manually shaken again. The tube was added with 6 g of $MgSO_4$ and 1.5 g of NaOAc, and shaken vigorously. The tube was then centrifuged (Eppendorf 5804 R, Hamburg, Germany) for 2 min at 4,000 rpm. Then, 5 mL of the supernatant was transferred to a 15-mL polypropylene tube containing 0.45 g of $MgSO_4$ and 0.15 g of PSA for clean-up. The extract was manually shaken and vortexed before being centrifuged again at 4,000 rpm for 2 min. Finally, 1 mL of extract was transferred directly to a vial for GC-MS/MS detection. As for UPLC-MS/MS, 0.1 mL of filtered extract was transferred to a vial containing 0.9 mL of ultrapure water for detection.

Effect of washing on pesticide residue reduction

The effect of washing on the reduction of pesticide residue was conducted according to Wu *et al.* (2019). Fresh organic kale samples were obtained from the supermarket, and analysed to confirm the absence of pesticide residue. This then served as blank sample. For spiked samples, the organic kale sample was immersed in 3 L of mixed carbendazim and chlorpyrifos solution at 10 mg/L for 5 min. The spiked samples were air-dried on the aluminium foil surface for 15 min at room temperature (30°C), and subjected to extraction and clean-up to determine the level of pesticide residues as described earlier. As for the washed samples, dry spiked kale samples were soaked with three types of washing solutions (tap water, 10% sodium bicarbonate, and 10% acetic acid) for 10 min, with the initial 15 s gentle rotation by hand. The sample was removed from the solutions, and rinsed with tap water for 15 s. The washed sample was placed on a surface with aluminium foil, air-dried for 15 min at room temperature (30°C), and further analysed for pesticide residue. Four replicates of blank, spiked, and washed kale samples were analysed in this experiment.

Analysis of pesticides using GC-MS/MS

The pesticide residues in the sample were detected according to Mezcua *et al.* (2009). Agilent 7890 gas chromatography (GC) equipped with Mass Spectrometer Agilent 7000 Triple Quadrupole MSMS were used for the determination of GC-amenable pesticides (chlorpyrifos, profenofos, aldrin, endrin, cypermethrin, and lambda-cyhalothrin). GC analysis was conducted using Agilent Technologies Ultra Inert 5% phenyl-methylpolysiloxane (HP-5MS) capillary column (30 m × 250 µm I.D. × 0.25 µm film thickness). Helium at 99.999% purity was used as the carrier gas at a flow rate of 0.9 mL/min. The oven temperature program was set at 60°C, and held for 1 min, then 40°C/min ramp to 170°C, followed by 10°C/min ramp to 310°C, and held for 5 min. The injector temperature was set at 250°C. The injection volume was set at 3 µL, utilising the splitless mode. The total run time was 25 min for each sample. The detection of the pesticide compounds was performed by multiple reaction monitoring (MRM) with a minimum of two mass transitions for each pesticide.

Analysis of pesticides using UPLC-MS/MS

The detection of LC-amenable pesticides (carbendazim, propamocarb, imidacloprid, and thiamethoxam) was conducted according to Grimalt *et al.* (2010) using Waters ultra-performance liquid chromatography (Waters ACQUITY Ultra Performance LC, USA) combined with a triple quadrupole detector MS system (Waters Micromass Quattro Micro, USA). Chromatography separation was performed by a reversed-phase Acquity UPLC BEH C_{18} LC column (2.1 × 100 mm, 1.7 µm). Chromatographic analyses were conducted using gradient elution. The mobile phases were 5 mM ammonium acetate (9:1; water:methanol) as eluent A; and 5 mM ammonium acetate (9:1; methanol:water) as eluent B. The total analysis run time was 13.5 min. The flow rate of 0.30 mL/min was used for the separation of analytes. The column temperature was set at 40°C, while the injection volume was 10 µL. An electrospray ionisation source (ESI) in positive modes was utilised. All the data analyses were acquired in Multiple Reaction Monitoring (MRM) mode with two transitions for each pesticide. The capillary temperature of the MS was set at 450°C, and the ion spray voltage was 3,500 eV. Collision-induced dissociation was performed using argon as the collision gas at a pressure of 3.8×10^{-3} mbar in the collision cell.

Method validation

Validation of the analytical method was performed through the determination of linearity, accuracy, limit of detection (LOD), and limit of quantification (LOQ) as suggested in analytical quality control and method validation procedures for pesticide residue analysis in food and feed; document SANTE/11945/2019 and Eurachem Guide: The Fitness for Purpose of Analytical Methods (Eurachem, 2014; EC, 2021). The calibration curves for ten pesticides were constructed from injections of matrix-matched internal calibration standard prepared using blank extract of mustard. The linearity and working range of the analytical procedure were 10 to 400 ng/mL for GC-MS/MS, and 1 to 100 ng/mL for UPLC-MS/MS.

The method accuracy was determined through a recovery experiment which was based on the fortification of known amounts of pesticides at 0.01, 0.05, and 0.10 mg/kg of the ten pesticides. Spiked and blank samples were analysed in five replicates (n = 5). The LOD of the method was obtained by multiplying three times the standard deviation of the lowest concentration in spiked samples (0.01 mg/kg), while the LOQ of the method was obtained by multiplying ten times the standard deviation of the lowest concentration in spiked samples (0.01 mg/kg).

Statistical analysis

Statistical analysis of all three washing methods was performed using a One-way analysis of variance (ANOVA) and Tukey's *post-hoc* test. The statistical analysis was done using the Minitab software (Version 19.0). A value of $p < 0.05$ was considered statistically significant. Values were expressed as means ± standard deviation (SD).

Results and discussion

Method validation

A good linearity response was obtained for all pesticides with six of them (chlorpyrifos, profenofos, cypermethrin, lambda-cyhalothrin, aldrin, and carbendazim) having correlation coefficient (R^2) values higher than 0.999, and the other four (endrin, propamocarb, imidacloprid, and thiamethoxam) having R^2 values higher than 0.990. Table 1 shows the results for LOD, LOQ, and recovery (%) obtained for the ten pesticides. Results showed that all pesticide analytes yielded recovery results within the range of 70 to 120%, thus considered acceptable for initial validation according to SANTE guidelines (Nimako *et al*., 2021). Figure 1 shows the chromatograms for chlorpyrifos and profenofos detected in kale sample as detected by GC-MS/MS.

Evaluation of pesticide residues

The concentrations (mg/kg) of five pesticide groups namely organophosphorus (chlorpyrifos and profenofos), organochlorine (aldrin and endrin), synthetic pyrethroid (cypermethrin and lambda-cyhalothrin), carbamate (carbendazim and propamocarb), and neonicotinoid (imidacloprid and thiamethoxam) were determined and shown in Table 2. The pesticide residue levels in the vegetable samples were calculated and compared against the MRL prescribed in the Regulation 41(3) of the Malaysian Food Regulations 1985.

Organophosphorus pesticide residues

Chlorpyrifos residue was detected above the MRL in two samples of kale and one sample of spinach, ranging between 0.024 to 0.149 mg/kg (Table 2). The use of chlorpyrifos is strictly regulated in Malaysia, and no permission is given on some crops such as kale and spinach. Perhaps this condition was violated due to the practice of using a registered pesticide on other crops cultivated on the same site (Farag *et al*., 2011). Other reasons could possibly be that farmers relied only on the pesticide retailers' and experienced farmers' recommendations which led them to wrong decision on pesticide usage (Farina *et al*., 2017).

Another MRL exceedance for chlorpyrifos was identified in one mustard sample at 3.172 mg/kg, which was three times higher than the MRL (1 mg/kg). This could be attributed to inappropriate pre-harvest interval (PHI) (Farag *et al*., 2011). This result was consistent with a previous work in Thailand (Wanwimolruk *et al*., 2015). Several studies have linked the toxicity of chlorpyrifos to human health including neurological dysfunction and neuro-development delay (Foong *et al*., 2020).

Banned profenofos residue was found in kale sample (0.321 mg/kg) which exceeded the MRL of less than 0.01 mg/kg (Table 2). Previous studies have also reported that vegetables from Sarawak, Malaysia were detected with profenofos between 0.10 to 1.80 mg/kg (Margaret and Chai, 2007). The presence of banned pesticides is a major GAP violation which

Table 1. Limit of detection (LOD), limit of quantification (LOQ), and recovery results for ten pesticides.

No.	Type of pesticide	LOD (mg/kg)	LOQ (mg/kg)	Recovery (%)		
				0.01 mg/kg	0.05 mg/kg	0.10 mg/kg
1.	Chlorpyrifos	0.0009	0.0030	84.5	98.2	113.9
2.	Profenofos	0.0009	0.0030	81.9	102.4	111.2
3.	Cypermethrin	0.0016	0.0053	94.6	102.4	114.3
4.	Lambda-cyhalothrin	0.0009	0.0030	88.0	97.0	95.6
5.	Aldrin	0.0012	0.0040	75.9	103.8	112.4
6.	Endrin	0.0016	0.0053	88.9	104.7	73.3
7.	Carbendazim	0.0026	0.0086	103.6	83.6	91.0
8.	Propamocarb	0.0020	0.0067	75.7	85.6	88.9
9.	Imidacloprid	0.0025	0.0083	97.7	102.3	105.0
10.	Thiamethoxam	0.0027	0.0093	93.7	95.2	78.1

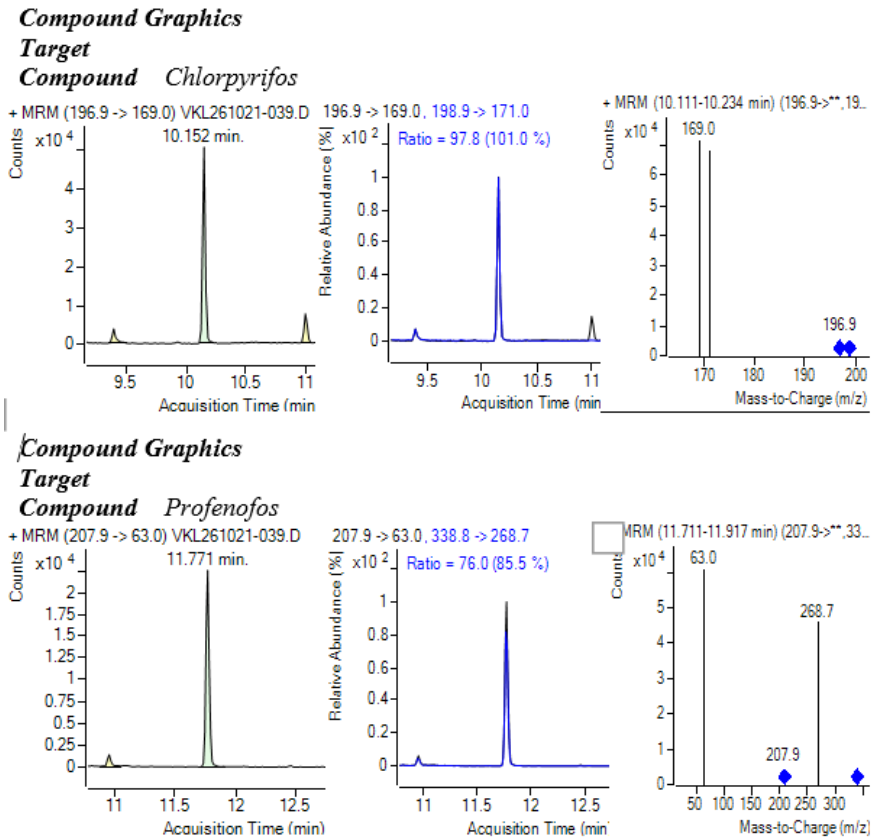

Figure 1. Chromatograms for chlorpyrifos and profenofos detected in kale sample by GC-MS/MS.

Table 2. Occurrence of pesticide residues in vegetable samples.

Type of pesticide	Level	Pesticide residue*									
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Chlorpyrifos	> MRL	0	2	1	1	0	0	0	0	0	0
	< MRL	1	1	2	2	0	1	1	1	1	0
	NR	5	3	3	3	6	5	5	5	5	6
Profenofos	> MRL	0	1	0	0	0	0	0	0	0	0
	< MRL	0	0	0	1	0	0	1	1	0	0
	NR	6	5	6	5	6	6	5	5	6	6
Aldrin	> MRL	0	0	0	0	0	0	0	0	0	0
	< MRL	0	0	0	0	0	0	0	0	0	0
	NR	6	6	6	6	6	6	6	6	6	6
Endrin	> MRL	0	0	0	0	0	0	0	0	0	0
	< MRL	0	0	0	0	0	0	0	0	0	0
	NR	6	6	6	6	6	6	6	6	6	6
Carbendazim	> MRL	1	2	2	0	0	0	0	0	0	0
	< MRL	0	0	0	3	4	1	1	0	1	0
	NR	5	4	4	3	2	5	5	6	5	6
Propamocarb	> MRL	0	0	0	0	0	0	0	0	0	0
	< MRL	0	2	0	1	1	3	0	2	0	2
	NR	6	4	6	5	5	3	6	4	6	4
Imidacloprid	> MRL	0	0	1	0	0	0	0	0	0	0
	< MRL	0	1	0	0	0	1	0	0	0	0
	NR	6	5	5	6	6	5	6	6	6	6
Thiamethoxam	> MRL	0	0	0	0	0	0	0	0	0	0
	< MRL	2	1	1	1	1	0	0	0	0	0
	NR	4	5	5	5	5	6	6	6	6	6

> MRL = above maximum residue limit; < MRL = below maximum residue limit; and NR = no residue. (*) 1 = *kangkung*; 2 = kale; 3 = spinach; 4 = mustard; 5 = lettuce; 6 = tomato; 7 = chilli; 8 = cucumber; 9 = okra; and 10 = pumpkin.

implies that some farmers are continuing the usage of illegal pesticide since they believe that the pesticides are highly effective in controlling pests.

Organochlorine pesticide residues

Aldrin and endrin were not detected in all the vegetable samples (Table 2). This indicated a good level of compliance among local farmers towards the banned usage of OC pesticides. It is possible that the farmers might have changed to less toxic pesticides such as OP in their farming practices. These results were comparable with a work reporting the absence of OC pesticides in 302 fruits and vegetables sourced from local markets in Selangor, Malaysia (Zawiyah *et al*., 2007).

Most OC pesticides are banned, and in some countries they are severely restricted. In Malaysia, OC pesticides such as DDT, aldrin, endrin, and dieldrin were totally banned since the late 1990s. This pesticide group was reported to increase the risk of chronic health hazards including hormone-related cancers such as breast, prostate, stomach, and lung

(Bolor *et al*., 2018). Although OC pesticides were not detected in all samples in the present work, constant monitoring of this pesticide group is nevertheless suggested due to its persistent nature and long half-lives.

Synthetic pyrethroid pesticide residues

The level of cypermethrin residue which was detected in kale, mustard, cucumber, *kangkung*, spinach, and chilli were all identified below the MRL (Table 2). This corresponded well with Jallow *et al.* (2017) who found cypermethrin as a frequently detected pesticide below the Codex MRL level. Cypermethrin is a non-persistent pesticide, and degrades easily in the presence of ultraviolet (UV) rays, thus resulting to most of its residue being below the MRL (Mahugija *et al*., 2017).

As for lambda-cyhalothrin, two spinach samples and one kale sample were found to exceed the limit, with levels ranging from 0.043 to 0.716 mg/kg (Table 2). Horská *et al.* (2020) opined that this could be due to the short PHI along with the high dosage application. These results well agreed with Mebdoua *et al.* (2017) who observed MRL exceedance in vegetables and fruits up to 0.260 mg/kg. The high detection amounts of the lambda-cyhalothrin insecticides in these non-permitted crops serves as necessary reason warranting the authority to continue pesticide residue monitoring programs in order to assure MRL compliance. Besides, education should be given for proper GAP application as this may help to reduce pesticide contamination risk.

Carbamate pesticide residues

Two samples of kale and spinach exceeded the MRL for carbendazim ranging between 0.096 to 0.747 mg/kg (Table 2). Meanwhile, one sample of *kangkung* exceeded the MRL by five times (0.054 mg/kg). MRL violations for carbendazim occurred as a result of illegal pesticide application on non-permitted crops which was consistent with present findings for chlorpyrifos and lambda-cyhalothrin. In the present work, carbendazim residues predominantly contaminated the leafy vegetables as compared to fruiting vegetables. This could have been due to the structure difference in leafy vegetables which have larger surface area and higher area-to-mass ratios that affect the residue deposit and dissipation (Ripley *et al*., 2003). Chronic exposure to carbendazim has been associated with endocrine disruption as well as embryotoxicity and teratogenic effects (Yan *et al*., 2011). Therefore, it is suggested for consumers to practice thorough washing procedures especially for leafy vegetables before consumption to reduce the pesticide residues to a safe level.

Propamocarb residue did not exceed its MRL (Table 2). A total MRL compliance for propamocarb showed that farmers were adherent to the approved pesticide application. It was noted that MRL specified on each pesticide for a particular crop did not indicate the safe level of pesticide residues to be exposed to humans. However, it can represent GAP that should be conducted by the farmers (Halimatunsadiah *et al*., 2016).

Neonicotinoid pesticide residues

Based on Table 2, a spinach sample was contaminated with imidacloprid residue (0.224 mg/kg), where the level was 22 times higher than its MRL (0.01 mg/kg). Khan *et al.* (2020) also detected high concentration of imidacloprid residues in spinach, mustard, and bitter gourd at 6.667, 1.9643, and 3.374 mg/kg, respectively. Imidacloprid, a systemic insecticide with LD_{50} of 380 - 500 mg/kg, has been linked to neurotoxicity, reproductive, and mutagenic health effects (Lu *et al*., 2006).

For thiamethoxam, at least one sample in each leafy vegetable was detected with this residue ranging between 0.043 and 1.081 mg/kg (Table 2) which was below the MRL level (3 mg/kg) for leafy vegetables. This suggested good compliance of this pesticide in the local farming activity. Other than that, results also showed higher occurrence of thiamethoxam residue in vegetable samples as compared to imidacloprid. This could have been contributed by the farmers' practices which replaced imidacloprid with thiamethoxam due to the increasing pest resistance towards imidacloprid (Jeschke *et al*., 2011).

Frequency of detection and exceedance of MRL

Out of 60 vegetable samples analysed, pesticide residue concentrations above the MRL prescribed by the Malaysian Food Regulations 1985 were detected in eight samples (13.3%) as shown in Table 3. Most of the MRL values were exceeded mainly in leafy vegetables. Kale (three samples) and spinach (three samples) recorded the highest sample number that exceeded the MRL. Kale was also found as the only vegetable detected with five different types of pesticide residues in a single sample. A possible explanation for this could be that kale has a

naturally occurring waxy layer on its surface which tends to trap the applied pesticides, thus making them more difficult to remove from the surface, which in turn led to higher concentration of pesticide residues detected as compared to the leafy vegetables without waxy layer (Chowdhury *et al*., 2014).

The present work also observed that 33 samples (55%) contained pesticide residues below the MRL, in which 17 samples were fruiting vegetables and 16 were leafy vegetables. Samples were not detected with any pesticide residue were 19; 13 samples of fruiting vegetables and six samples of leafy vegetables. Out of ten pesticide analytes analysed in the present work, eight pesticides were detected excluding aldrin and endrin. The most frequently detected pesticides were carbendazim (15 samples) and chlorpyrifos (15 samples).

The percentage of MRL violation (13.3%) observed in the present work was higher as compared to other studies from Korea (1.4%) and Poland (1.8%) (Yu *et al*., 2018; Yi *et al*., 2020). On the contrary, Asian countries such as Vietnam (59%) and Thailand (24%) reported MRL violations at higher rates than those reported in the present work (Sapbamrer and Hongsibsong, 2014; Nguyen *et al*., 2021).

Table 3. Frequency of detection and exceedance of MRL in vegetables.

Type	Commodity	Total sample	ND sample (%)	Sample with residue < MRL (%)	Sample with residue > MRL (%)
Leafy vegetable	*Kangkung*	6	1 (16.7)	4 (66.7)	1 (16.7)
	Kale	6	1 (16.7)	2 (33.3)	3 (50.0)
	Spinach	6	1 (16.7)	2 (33.3)	3 (50.0)
	Mustard	6	1 (16.7)	4 (66.7)	1 (16.7)
	Lettuce	6	2 (33.3)	4 (66.7)	0 (0)
	Total number	30			
	Total ND samples		6 (20.0)		
	Total samples < MRL			16 (53.3)	
	Total samples > MRL				8 (26.6)
Fruiting vegetable	Tomato	6	2 (33.3)	4 (66.7)	0 (0)
	Chili	6	2 (33.3)	4 (66.7)	0 (0)
	Cucumber	6	1 (16.7)	5 (83.3)	0 (0)
	Okra	6	4 (66.7)	2 (33.3)	0 (0)
	Pumpkin	6	4 (66.7)	2 (33.3)	0 (0)
	Total number	30			
	Total ND samples		13 (43.3)		
	Total samples < MRL			17 (56.7)	
	Total samples > MRL				0 (0)
Overall	Total number	60			
	Total ND samples		19 (31.7)		
	Total samples < MRL			33 (55.0)	
	Total samples > MRL				8 (13.3)

ND = not detectable; and MRL = maximum residue limit.

Washing effect on pesticide reduction

Table 4 shows the effectiveness of three different washing methods in reducing pesticide residues in kale. Analysis of variance (ANOVA) followed by a Tukey's *post-hoc* test showed significant differences among the washing solution treatments in terms of carbendazim and chlorpyrifos residue levels ($p < 0.05$). The results showed that washing kale with 10% acetic acid (W3) yielded the highest pesticide reduction for both carbendazim (76.0%) and chlorpyrifos (41.2%).

Acetic acid is considered a strong chelating agent (Amir *et al*., 2019). During the washing process, acetic acid chelates with metals such as nickel, chromium, and arsenic contained in the chlorpyrifos and carbendazim pesticide formulations, thus resulting in a greater amount of residues being washed away from the kale (Defarge *et al*., 2018). Zhang *et al*. (2007) reported that 79.8% of chlorpyrifos residues were removed by washing cabbage using 10% acetic acid solution. The amount of 10% acetic acid was also proven as the most efficient technique to reduce the residues of chlorpyrifos (94.21%), cypermethrin (89.99%), and deltamethrin (79.68%) in spinach samples (Amir *et al*., 2019).

The next best washing method determined in the present work was 10% sodium bicarbonate solution which yielded a reduction of 53.9% of carbendazim residues, and 31.9% of chlorpyrifos residues. On the other hand, the least effective method identified was tap water. Physicochemical properties of pesticides such as water solubility will affect the pesticide reduction from vegetables (Amir *et al*., 2019). Both carbendazim and chlorpyrifos have poor solubility in water, with carbendazim being soluble at 8 mg/L and chlorpyrifos at 2 mg/L. Therefore, this method only reduced carbendazim residues to 35.7% followed by 21.7% for chlorpyrifos.

Table 4. Effect of different washing methods on pesticide reduction in kale.

Washing treatment	Carbendazim		Chlorpyrifos	
	Residue (mg/kg ± SD)	Reduction (%)	Residue (mg/kg ± SD)	Reduction (%)
Untreated	2.063 ± 0.103^{d}		0.890 ± 0.024^{d}	
Tap water	1.326 ± 0.037^{a}	35.7	0.697 ± 0.031^{a}	21.7
10% sodium bicarbonate	0.952 ± 0.057^{b}	53.9	0.606 ± 0.024^{b}	31.9
10% acetic acid	0.495 ± 0.028^{c}	76.0	0.523 ± 0.031^{c}	41.2

Different lowercase superscripts in the same column indicate significant difference ($p < 0.05$).

Conclusion

The present work demonstrated the pesticide residue occurrence in vegetable samples collected from local markets in Kuala Lumpur, Malaysia. Some of the vegetable samples (13.3%) exceeded the permitted MRL with kale being the most contaminated. The present work also demonstrated that washing with acetic acid solution (10%) was the most effective method to reduce carbendazim (76%) and chlorpyrifos (41.2%) in kale. Therefore, it is recommended that authorities in Malaysia monitor the pesticide application, and educate farmers on the safety dosages, adherence to labels, and standard pre-harvest interval (PHI) as prescribed by the GAP. The usage of biopesticides as a substitute for synthetic hazardous pesticides should be widely promoted due to their safe and environmental friendly nature.

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