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Original Research Article

Evaluation of pesticide residues in vegetables from Mekong, Delta, Vietnam using LC-MS/MS

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Abstract

Purpose: *To assess the levels of pesticide residues in commonly used vegetables in Vietnam as a reference for future monitoring.*
Methods: *A total of 180 samples of six different fresh vegetables including watercress, mustard green, choy sum, daikon, okra, and yam were analyzed from Mekong Delta, Vietnam. Ten popular pesticides were evaluated using liquid chromatography-tandem mass spectrometry (LC-MS/MS) after extraction with a multi-residue method (QuEChERS method).*
Results: *The results were assessed according to the maximum residue limit (MRL) provided by Codex for each pesticide in each commodity. Pesticide residues above the MRL were detected in 107 samples (59 %) and 63 samples (47 %) contained residues below the MRL. Multiple residues were present in 38.3% of the samples with two to five pesticides, and 0.6 % of samples were contaminated with more than five pesticide residues. Of the pesticides investigated, eight pesticides were detected, of which abamectin, alpha-cypermethrin, acetamiprid, chlorpyrifos- ethyl, chlorantraniliprole, fenobucarb, fipronil, and trichlorfon exceeded their MRLs. Pesticide residues were detected above MRLs in samples of watercress (14 samples), mustard green (24 samples), choy sum (25 samples), daikon (26 samples), and yam (18 samples). Chlorpyrifos-ethyl, and fipronil were detected in most of the vegetable samples (100% and 89.44%, respectively).*
Conclusion: *The results indicate the occurrence of pesticide residues in commonly consumed vegetables in Vietnam. The need for the regular monitoring of pesticide residues and the sensitization of farmers to better pesticide safety practices, especially the need to adhere to recommended pre-harvest intervals is recommended.*

Keywords: Monitoring, Pesticide residues, Vegetables, LC-MS/MS

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INTRODUCTION

Chemical pesticides have continually proven their usefulness by enhancing worldwide agricultural output, lowering insect-borne and endemic diseases, and safeguarding plantations, forests, and harvested areas [1]. Pesticides are currently more valuable in developing nations, particularly those in tropical regions aiming to enter the global economy by supplying off-season fresh fruits and vegetables to nations with more moderate climates, such as Vietnam. Integrated Pest Management (IPM) programs were implemented by Food and Agriculture Organization (FAO) and other organizations in the 1990s [2]. In Vietnam, these IPM programs significantly improved the knowledge of local farmers about pesticide use, resulting in the reduction of pesticide application rates and a plunge in the total consumption of pesticides in this country [3].

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. The maximum residue limits (MRLs), expressed as the highest concentration of pesticide residues in vegetables and fruits (mg/kg), are used to determine the maximum intake legally permitted by regulations introduced by different countries and internationally by the Codex Alimentarius Commission [4]. The human intake of pesticide residues in vegetables can be much higher than the intake related to water consumption and air inhalation. It is very important to monitor such chemicals in vegetables and to assess their potential risks to human health [5]. Some indicators can estimate the number of chemicals in food, which is represented as an acceptable daily intake (ADI). The ADI can be used to predict the daily intake during a lifetime that would prevent health risks to the consumer [6]. The aim of this study was to assess the levels of pesticide residues in commonly used vegetables in Vietnam for use as a reference for future monitoring. The information is also critically important for developing measures that aim at reducing or preventing human health risks from harmful pesticide residues in primary and derived agricultural products. Ten kinds of pesticides included in this study, were selected on the basis of their wide use in vegetable production in Mekong Delta, Vietnam.

EXPERIMENTAL

Chemicals and reagents

All pesticide reference standards (purity ≥ 95%) were purchased from Dr. Ehrenstorfer (Augsburg, Germany). The internal standards (ISs), triphenyl phosphate (TPP) and D10-chlorpyrifos (CPR-d10), were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Acetonitrile (ACN), methanol (MeOH), and water (H_2O) were of HPLC grade. Acetic acid (CH_3COOH), anhydrous magnesium sulfate ($MgSO_4$), sodium acetate trihydrate ($CH_3COONa·3H_2O$), trisodium citrate dihydrate ($Na_3C_6H_5O_7·2H_2O$), disodium citrate sesquihydrate ($Na_2HC_6H_5O_7·1.5H_2O$), and sodium chloride were supplied by Merck (Darmstadt, Germany). Primary secondary amine (PSA), graphite carbon black (GCB), and octadecylsilane (C_{18}) sorbents were obtained from Agilent Technologies (Canada).

Sample collection

On the basis of information received during the survey, it was concluded that 10 pesticides were most widely used by the farmers (Table 1). As was expected, all the farmers interviewed in this study reported using various kinds of chemical pesticides. A total of 180 samples of 6 different kinds of vegetable samples including watercress, mustard green, choy sum, daikon, okra and yam was collected after harvesting from farmers' fields in Mekong delta for pesticide residue analysis. The sampling was performed in accordance with the general principles and methods of the European Commission (EC) directive 2002/63/EC for establishing MRLs in food commodities. Each representative vegetable was collected at preparing harvest for sale and avoiding the time of intense sun or rain. All the samples (1.5 – 2.1 kg each) were placed in sterile polythene bags, stored 4 °C, to avoid contamination and deterioration, labeled, and transported to the laboratory for processing and analyzed within 12 h.

Table 1: Commonly used pesticides and their classification

S/N	Pesticide	Chemical class	Toxicity class
1	Abamectin	Avermectin	II
2	Alpha- cypermethrin	Pyrethroid	II
3	Acetamipride	Neonicotinoid	II
4	Chlorpyrifos- ethyl	Organophosphorus	II
5	Chlorpyrifos- methyl	Organophosphorus	II
6	Chlorantraniliprole	Diamide	III
7	Fenobucarb	Carbamate	II
8	Fipronil	Phenylpyrazole	II
9	Thiamethoxam	Neonicotinoid	II
10	Trichlorfon	Organophosphorus	II

Preparation of standard solutions

The individual standard stock solutions (1 mg/mL) were dissolved in acetonitrile and stored at −18°C in the dark. The intermediate standard solutions in acetonitrile were prepared by mixing appropriate quantities of the individual standard stock solutions. A series of working standard solutions in the range of 0.2 – 8 μg/mL were prepared in acetonitrile by diluting the intermediate standard solutions in acetonitrile. The working standard solutions were used to prepare matrix-matched calibration and solvent calibration standards for the validation study. The ISs were prepared as described above. The working standard solutions and ISs were stored at −20 °C until they were used for the analysis.

Extraction procedure

A modified QuEChERS method was utilized to extract pesticides from the vegetable samples. After homogenization, a 10 g portion of each sample was placed in a 50 mL centrifuge tube. The ISs (TPP and CPR-d10) were added to the centrifuge tube, yielding a sample concentration of 20 μg/kg. Then, 10 mL of 1 % acetic acid in acetonitrile was added, and the samples were vortexed for 1 min. Then, 6.0 g of anhydrous $MgSO_4$ and 1.5 g of CH_3COONa were gradually added to the sample tubes. The centrifuge tubes were then tightly capped, vortexed for 1 min, and centrifuged at 6,000 rpm for 5 min. For the cleanup procedure, 6 mL of the supernatant was transferred to a 15 mL centrifuge tube containing 900 mg anhydrous $MgSO_4$, 150 mg PSA, and 45 mg GCB. The centrifuge tube was vortexed for 1 min and centrifuged at 12,000 rpm for 1 min. A 4 mL aliquot of the supernatant was dried under a nitrogen stream at 40 °C. The residue was then reconstituted with 1 mL of ACN:H_2O (40:60), and the extract was filtered through a 0.22 μm PTFE filter. Finally, the filtrate was transferred into a vial.

UPLC–MS/MS parameters

The analysis was performed using an LC-MS/MS system consisting of an Acquity UPLC I-Class (Waters, USA) coupled with a Xevo TQ-S micro triple quadrupole mass spectrometer (Waters, USA) equipped with an electrospray ionization (ESI) source working simultaneously in the positive (ESI^+) mode for nine analytes and in the negative (ESI^-) mode for fipronil. The source settings were as follows: capillary voltage, 3.0 kV in the positive mode or −1.0 kV in the negative mode; source temperature, 150°C; desolvation temperature, 400°C; desolvation gas flow rate, 900 L/h. Argon was used as the collision gas. For the chromatographic conditions, a Waters Acquity BHE C_{18} column (1.7 μm; 50 × 2.1 mm) was used and maintained at 25°C. The flow rate and injection volumes were 0.25 mL/min and 10 μL, respectively. The mobile phases consisted of (A) 5 mM ammonium acetate/0.1 % formic acid in methanol and (B) 5 mM ammonium acetate/0.1% formic acid in water. The gradient elution program was as follows: 5 % A (0 – 1 min), 100 % A (2 – 3 min), 100% A (4 – 6 min), and 5 % A (until 7 min). The total chromatography run time was 7 min. The data acquisition in the multiple reaction monitoring (MRM) mode was optimized after direct infusion into the detector. Two ion transitions were selected for each compound; the quantifier and qualifier MRM and the analytical method were validated according to Association of Official Analytical Chemists (AOAC) guidelines [8].

RESULTS

Monitoring results

A total of 180 samples were analyzed for pesticide residues. Table 2 shows the seven chemical groups of pesticides analyzed in each commodity. Surprisingly, all of the samples were positive for at least one pesticide (≥LOD). Residual of OPs pesticide and pyrethroid pesticide was found in 6 kinds of vegetables with high ratio. All of the samples were pesticides detectable OPs in watercress, daikon and okra; whereas 100 and 96.7 % of the samples were detected pyrethroid pesticide in choy sum and mustard green, respectively. In contrast, no residual neonicotinoid was in the majority of samples. Obviously, seven chemical groups of

Table 2: Chemical group of pesticides detected in sample

Vegetable	No. of sample analyzed	Type of pesticides detected (n, %)						
		Avermectin	*Carbamate*	*Neonico-tinoid*	*OPs**	*Diamide*	*Pyrethroid*	*Pyrazole*
Watercress	30	15 (50%)	7 (23.3%)	0 (0%)	30(100%)	2 (6.67%)	14 (46.7%)	0 (0%)
Mustard green	30	10 (33.3%)	12 (40%)	0 (0%)	17 (56.7%)	11 (36.7%)	29 (96.7%)	21 (70%)
Choy sum	30	14 (46.7%)	16 (53.3%)	13 (43.3%)	19 (63.3%)	17 (56.7%)	30 (100%)	22 (73.3%)
Daikon	30	24 (80%)	23 (76.7%)	0 (0%)	30 (100%)	0 (0%)	23 (76.7%)	17 (56.67%)
Okra	30	0 (0%)	29 (96.7%)	1 (3.3%)	30 (100%)	30 (100%)	3 (10%)	27 (90%)
Yam	30	25 (83.3%)	0 (0%)	0 (0%)	10 (33.3%)	30 (100%)	23 (76.7%)	0 (0%)

**Organophosphorus (OPs) included 3 pesticides: Chlorpyrifos- ethyl; Chlorpyrifos - methyl; Trichlorfon*

Table 3: Frequency of vegetable samples without pesticide residue, and with residue below the MRL and above the MRL

Produce	Number of samples	With residue < MRL	With residue > MRL
Watercress	30	16 (53%)	14 (47%)
Mustard green	30	6 (20%)	24 (80%)
Choy sum	30	5 (17%)	25 (83%)
Daikon	30	4 (13%)	26 (87%)
Okra	30	30 (100%)	0 (0%)
Yam	30	12 (40%)	18 (60%)
Total	180	73 (41%)	107 (59%)

pesticide analyzed were found in most of choysum samples at the percentage of more than 40 %.

Moreover, six chemical groups of pesticides were also found in mustard green samples. Pyrethroid and pyrazole were especially chemical groups detected in mustard green at a high percentage (96.7 and 70 %, respectively). Yam samples had no detected carbamate, neonicotinoid and pyrazole groups. However, residual of diamide, abamectin and pyrethroid were detected in the high ratio in yam (100, 83.3 and 76.7 %) respectively.

Pesticides residues in analyzed samples

After validation, the proposed method was applied to determine pesticide residue in 180 vegetable samples from farmers' fields in Mekong delta. Surprisingly, the result in Table 3 showed that all of the samples contained at least one detectable pesticide residue, whereas 107/180 samples (59%) contained pesticide residue above the MRLs established by Codex and those percentages were extremely higher than of Thailand's vegetables which had only 77.7 % detectable and 22.3 % exceeding samples [7]. Except for okra, all of the vegetables had high percentage of samples exceeding MRLs called exceeding samples (> 40 %). In 107 exceeding samples, the leaf vegetables (watercress, mustard green, choy sum) had higher percentages (59 %) than the root vegetables (daikon and yam). All of okra samples contained pesticide residue below MRLs, whereas daikon (87 %) had the highest number of detected samples.

MRL exceedances and detection frequencies of pesticides in analyzed samples

Figure 1 was illustrated that alpha-cypermethrin, fipronil and abamectin also had the highest number of exceeding samples with 82 (37.3%), 52 (23.6%) and 27 (12.3%) samples respectively. Whereas a residue of trichlorfon and chlorantraniliprole was found to exceed the MRLs in one choysum and watercress sample, respectively.

Residues of alpha-cypermethrin exceeding MRLs were primarily detected in leafy vegetables (56/90 samples). Moreover, mustard green had the highest numbers of samples exceeding MRL of alpha-cypermethrin (24/30 samples), whereas choy sum had 22/30 samples and watercress had 10/30 samples. In root vegetables, yam had 18/30 samples exceeding MRLs and was higher than daikon (8/30 samples). Residue of fipronil was detected in mustard green (19/30 samples), daikon (17/30 samples) and choy sum (16/30 samples) exceeded MRLs. Residue detected of Abamectin had the highest numbers in both daikon and choysum (11/30 samples), whereas mustard green only had 4/30 samples. Besides, the exceedance level of acetamiprid, chlorpyriphos ethyl and fenobucarb were detected in root vegetables with higher frequencies (39 samples) than leaf vegetables (17 samples). Fenobucarb was only detected in daikon samples (12/30 samples). Figure 1 shows that alpha-cypermethrin, fipronil and abamectin also had the highest number of exceeding samples with 82 (37.3 %), 52 (23.6 %) and 27 (12.3 %) samples respectively. Whereas a residue of trichlorfon and chlorantraniliprole was found to exceed the MRLs in one choysum and watercress sample, respectively.

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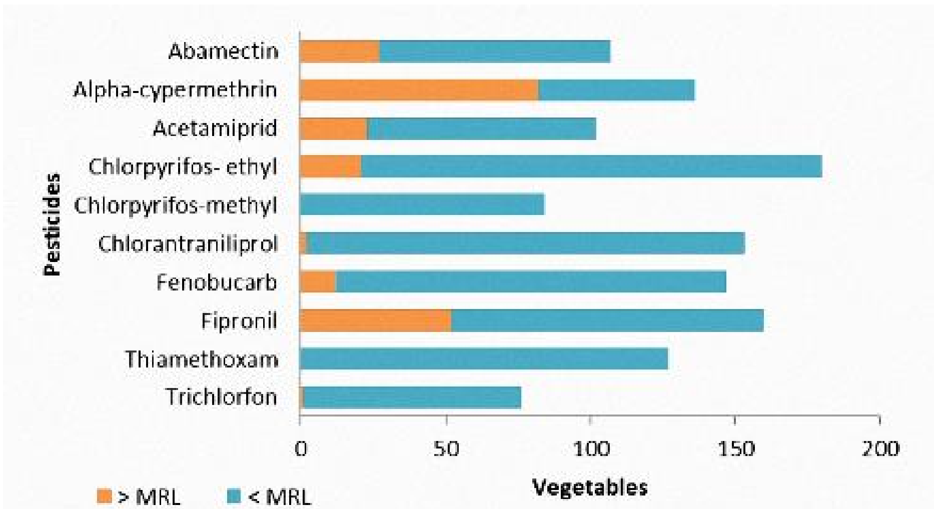

Figure 1: Frequencies of samples detected below or above MRL for each pesticide

Table 4: Concentration range of 10 pesticide residues in vegetable samples

Pesticide	Watercress	Mustard green	Choy sum	Daikon	Okra	Yam
			Concentration range (µg/kg)			
Abamectin	nd-9.05	nd-45.17	nd-54.72	nd-452.58	nd-0.44	nd-10.19
Alpha- cypermethrin	nd-369.78	nd-2774.76	0.6-3379.81	nd-791.77	nd-9.68	nd-83.89
Acetamiprid	6.91	391.92	403.71	2436.24	2.83	nd
Chlorpyrifos- ethyl	0.33- 12.81	nd-605.57	nd-26.33	nd-4155.65	0.81- 3.06	nd-19.69
Chlorpyrifos- methyl	nd-1.53	nd-5.14	nd-2.14	nd-1.83	nd-3.63	nd
Chlorantraniliprol	nd-83.67	nd-0.81	nd-1.22	nd	nd-7.14	3.65-8.07
Fenobucarb	nd-1.28	nd-1.61	nd-2.02	nd-1090.95	nd-0.39	nd
Fipronil	nd	nd-119.66	nd-102.18	nd-287.97	nd-1.05	nd
Thiamethoxam	nd	nd	nd	nd	2.86- 9.05	nd
Trichlorfon	6.19	5.59	14.71	2.33	3.60	1.34

**nd = pesticide residue not detected*

The highest concentrations of pesticide detected in samples were chlorpyriphos ethyl (4155.6 µg/kg) in daikon, alpha-cypermethrin in choysum (3379.8 µg/kg), acetamiprid in daikon (2436.2 µg/kg). The results, shown in Table 4, indicated that daikon had the highest numbers of high-level exceeding pesticides (> 200 µg/kg, approximately 20 times of MRLs), remarkable included acetamiprid (2436.2 µg/kg), chlorpyriphos ethyl (4155.6 µg/kg) and fenobucarb (1090.95 µg/kg). In contrast, okra and yam had low concentrations of pesticides, especially okra which had all of the samples were contaminated with lower MRLs. In leafy vegetables, the high-level exceeding MRLs pesticides were alpha-cypermethrin (2774.8 µg/kg in mustard green), acetamiprid (403.7 µg/kg in choysum) and chlorpyriphos ethyl (605.6 µg/kg in mustard green).

Samples with multiple residues

In 107 exceeding MRL samples, the results showed that the percentages of samples which contained more than one exceeding MRLs pesticide was 64.5 % (69/107 samples). In addition, the samples containing two exceeding pesticides had 35.51 % (38 samples), three exceeding pesticides had 17.76 % (19 samples), four and five exceeding pesticides had 10.28 % (11 samples) and 0.93 % (1 daikon sample), respectively. The most frequent combination of two pesticides was alpha-cypermethrin and fipronil in mustard green and choysum samples and alpha-cypermethrin and chlorpyriphos ethyl in daikon. Besides, the frequent combination of three pesticides was abamectin, fenobucarb and fipronil found in daikon (Figure 2).

Acceptable Daily Intake (ADI) results

Intake (ADI) is the estimate of the amount of a pesticide in food or drinking water that can be ingested daily over a lifetime without appreciable

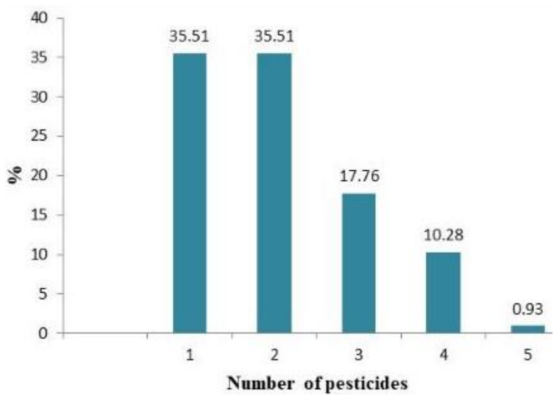

Figure 2: Vegetable samples with multiple exceeding MRLs pesticides

health risk to the consumer, on the basis of all known facts at the time of the evaluation. ADI in Figure 3 and Table 5 is calculated by using the sum of ADI of each pesticide detected sample followed the Ministry of Health and the Ministry of Agriculture regulation. The high ADI level (>200 µg/kg) accounted for 33.9 % of the total 180 samples, whereas, leafy vegetables had ADI >200 µg/kg higher than root vegetables. On average, ADI between the two groups of leafy vegetables and root vegetables had a statistically significant difference (95% significance level), in particular, leafy vegetables had an average ADI of 614.87 µg/kg higher than root vegetables 275.69 µg/kg (Figure 3).

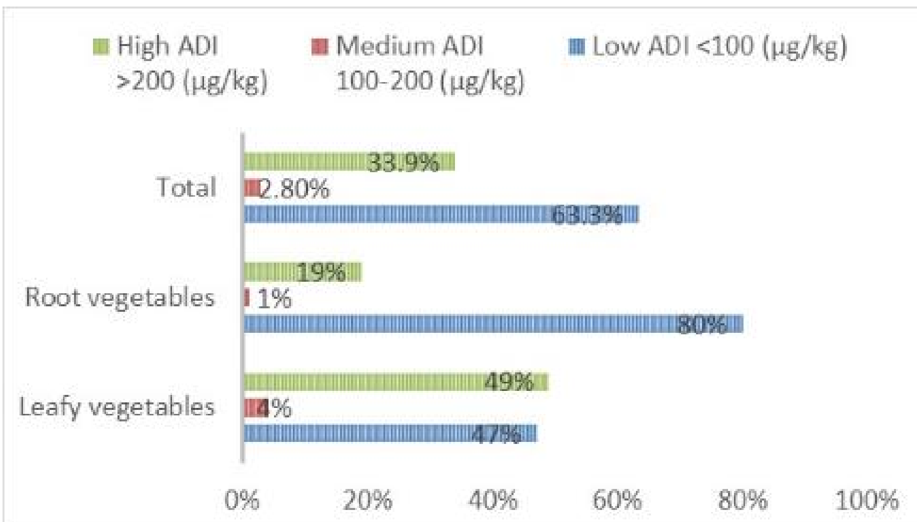

Figure 3: The difference of ADI level between leafy vegetables and root vegetables

Table 5: Acceptable daily intake (ADI) for each commodity

Vegetable	Maximum number of pesticides/ sample of vegetables	Total pesticide concentration found/ sample of vegetables (µg/kg)	ADI (µg/kg)
Mustard green	5	3094.87	740
Choy sum	6	3731.75	740
Watercress	3	373	20710
Okra	2	29.92	20
Daikon	6	5082	60
Yam	3	118.62	30

The maximum number of pesticides detected in choysum were 6 pesticides (Table 5) whereas average ADI of choysum 740 µg/kg. Watercress sample only detected 3/10 pesticides researched but average ADI was high remarkable (20710 µg/kg). This difference can be explained by the high residues of one pesticide on these watercress samples

DISCUSSION

This study shows evidence of the presence of pesticide residues in vegetables in Mekong Delta, Vietnam. All of the samples contained at least one detectable pesticide residue, 59 % (Table 3) contained pesticide residue above the MRLs. Results are significantly higher than the results of Jallow *et al* [9] in Kuwait with only 21 % contained pesticide residue above the MRLs and 79 % of the samples had no residues of the pesticides surveyed samples or contained residues below the MRL. In addition, Bempah *et al* [10] monitored pesticide residues in fruits and vegetables in Ghana. In that study, 37.5 % of the fruit and vegetable samples analyzed contained no detectable level of the monitored pesticides, 19.0 % of the samples gave results with levels of insecticide residues above the MRL, while 43.5 % of the samples showed results below the MRL [10].

The most common pesticides detected were chlorpyrifos-ethyl (180 samples) and fipronil (161 samples) that are classified as moderately hazardous. Chlorpyriphos-ethyl were detected in trace level (lower MRLs) more than 80 % of the samples. It is essentially a nerve agent, attacking chemical pathways and causing a breakdown in the ability of nerves to communicate. Whereas, Fipronil had the number of exceeding MRL with 52 samples (23.6 %). Clinical signs and symptoms reported after ingestion of fipronil by humans include sweating, nausea, vomiting, headache, abdominal pain. [11]. There are their extreme harmful effects on human health and their long-term persistence in the environment, therefore, chlorpyrifos-ethyl and fipronil pesticides are banned or restricted in many countries.

On February 12, 2019, the Deputy Minister of Agriculture and Rural Development signed Decision No. 501/QD-BNN-BVTV on the removal of pesticides containing chlorpyrifos ethyl and fipronil chemicals from the list of real protection drugs objects allowed to be used in Vietnam, together with 228 commercial drugs containing ethyl chlorpyrifos and 152 commercial drugs containing Fipronil active ingredients [12]. However, these pesticides are still being used clandestinely in some developing countries. Previous studies have detected chlorpyrifos-ethyl and fipronil pesticide residues in different commodities, including vegetables from Ghana [13], Thailand [7] but most residues were below the MRL allowed. Nevertheless, continuous consumption of food products even with moderate pesticide contamination may have negative consequences on human health in the long term [14]. Pesticides can accumulate in the tissues of organisms as they are not easily soluble [15].

The detection of multiple pesticides in this study is a cause of concern. In 180 samples, the results showed that the percentages of samples that contained more than one exceeding MRLs pesticide were 38.34 % (69 samples). Multiple residues may be expected in some crops because the application strategy is usually to alternate pesticide classes to prevent the buildup of pest resistance. According to a survey on Vietnamese Farmers' Knowledge of Nguyen *et al* [16], a high number of farmworkers received training on integrated approaches to pest management but still relied heavily on pesticides. Perhaps the farmers believe that using different types of pesticides is the most effective solution to control pests and diseases [17].

Most farmers apply chemicals at high frequency, resulting in use in excess of label specifications. Excessive application of pesticides may lead to high residue levels on plants, which may be dangerous to farmers and to vegetable consumers [16]. Almost farmers mix pesticides inappropriately and do not consider that this could reduce pesticide effectiveness and cause damage to their health or the environment. Farmers explained that mixing chemicals saves time and labor cost and that they anticipated higher pest control efficacy. However, in addition to health and environmental concerns, inappropriate mixtures of various pesticides can increase pest resistance and reduce effectiveness [18].

Greater priorities must be given to develop strategies for pesticide reduction in agriculture through farmer training pesticide use and promote alternatives to chemical pest control. Training farmers should be mandatory, yearly, and include assessment of knowledge and understanding gained as well as intent to adopt improved practices. Current training events reach the majority of farmers, but there is no assessment of the knowledge gained or level of understanding of the impact of their actions. Moreover, intervention strategies by regulatory agencies to strengthen the enforcement mechanisms of current pesticide laws at the farm and retail level are a necessity in promoting safe pesticide use.

Pesticide residues should be monitored on agricultural products and soil and water resources. A baseline of food residues and soil and water pesticide levels is the first step in implementing a monitoring program [16]. It is also critical to raise awareness among the general public who may be directly or indirectly exposed to pesticides, about the risk of these chemicals and how to reduce this risk. Consumers should be aware of practical measures to reduce the contamination of pesticides in fresh agricultural produce, especially vegetables that may be consumed raw. Finally, due to the increasing trend in pesticide use in Vietnam, routine monitoring of pesticide residues in agricultural produce is a necessity to ensure the safety of consumers.

CONCLUSION

The findings of this study indicate that the majority of vegetable samples obtained from Mekong Delta, Vietnam are contaminated with pesticide residues, with concentrations above the MRL. From a public health perspective, the observed levels of pesticide residues pose a potential health risk to consumers. On the basis of these findings, there is a need for continuous survey and monitoring programs for pesticides in all food commodities in order to protect the user from a high level of exposure to pesticides. Furthermore, scientific data on residues and other aspects of chemical use must be open to the public to satisfy the right-to-know of citizens.

DECLARATIONS

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. This study was conceived and designed by Van Ngoc Thi Nguyen. The data were collected by Van Ngoc Thi Nguyen and Lien To Thi Pham. The data analysis was done by Van Ngoc Thi Nguyen and Ngan Kim Huynh Nguyen. The article was written by Ngan Tuyet Duong and Ngan Kim Huynh Nguyen with reviews and corrections from Van Ngoc Thi Nguyen, Van De Tran and Kyeong Ho Kim

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