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ORGANOPHOSPHATE AND CARBAMATE RESIDUAL LEVELS IN VEGETABLES OF TRANG MUNICIPALITY

Abstract:

□ This experimental research design aimed to study residual levels of organophosphate and carbamate in 360 samples of a vegetable widely consumed in Trang Municipality, located in southern province of Thailand. Three kinds of vegetables were most eaten vegetables, organic vegetables and locally grown vegetables. 190 samples of the most eaten vegetables included cilantro, kale, Chinese cabbage, cabbage, cauliflower, chili, spring onion, celery, yard long bean, cucumber, tomato, lettuce, egg plant, Thai egg plant, Chinese morning glory, white radish, devil's fig, asparagus, lemon. 50 samples of the organic vegetables included Chinese morning glory, Chinese cabbage, kale, cabbage and yard long bean. 120 samples of the locally grown vegetables included morning glory, curry leaf, Liang vegetable, star gooseberry, Thai basil, chili, Gotu kola, bird lettuce, bitter bean, fresh pod color (red yard long bean). The most eaten vegetables were collected from 3 wholesale markets and 200 grams for each vegetable was randomly collected from 3 areas (top, bottom and middle). The organic and locally grown vegetables were collected from 3 retail sale markets and 200 gram for each vegetable was randomly collected from 3 areas (top, bottom and middle). The residual level of organophosphate and carbamate was examined with GPO-M kit of Medical Science Department, Ministry of Public Health. The samples were collected during April - July 2015.

□ The results revealed that unsafe level (cholinesterase inhibitor level of 50-70%) of organophosphate and carbamate were found in most eat eaten vegetables 79 samples (41.58%) from 190 samples. Kinds of vegetables found pesticide residuals included cilantro, kale, Chinese cabbage, cabbage, cauliflower, chili, celery, spring onion, yard long bean, cucumber, tomato, Thai egg plant, white radish and lemon. All organic and locally grown vegetables were found safe residual level of organophosphate and carbamate. Based on the results of this study, public health authorities should encourage consumer to eat organic or locally grown vegetables and should properly wash vegetable before cooking. In addition, gardeners should be aware of the dangers of chemical pesticides for good quality and food safety of Thai vegetables. Meanwhile, authorities should work proactively to advise the use of chemical pesticides correctly and monitored continuously among gardeners throughout the country.

Keywords:

Organophosphate and Carbamate residues, most eaten vegetables, organic vegetables, locally grown vegetables

JEL Classification: I19

Introduction

Organophosphates and carbamate have been used as insecticides worldwide for more than 50 years. Organophosphate and carbamate are insecticides used against insects in all developmental forms, but mainly to kill mature insects. Organophosphate commonly used in agriculture is parathion, malathion, chlorpyrifos and dichlorvos. They are used to protect livestock, crops, homes and communities from the direct and indirect effects of insects and the diseases they carry (Clark, 2006, p. 1497–1512; Erdman, 2004, 1475 -1496). An estimated 3,000,000 people worldwide are exposed to organophosphate or carbamate agents each year, with up to 300,000 fatalities (Eddleston and Phillips, 2004, p. 42; Eyer, 2003, 165). The carbamate and organophosphorus pesticides have become increasingly important due to their broad spectrum of activity, their relatively low persistence, and their generally low mammalian toxicity when compared to organochlorine pesticides (Leibson and Lifshitz, 2008, p. 767-770; Eddleston et al., 2008, p. 597–607).

Organophosphates and carbamate are potent cholinesterase inhibitors capable of causing severe cholinergic. Clinical effects are manifested via activation of the autonomic and central nervous systems and at nicotinic receptors on skeletal muscle (Leibson and Lifshitz, 2008, p. 767-770). These pesticides combine with acetylcholinesterase at nerve endings in the brain and nervous system, and with other types of cholinesterase found in the blood. This allows acetylcholine to build up, while protective levels of the cholinesterase enzyme decrease. The more cholinesterase levels decrease, the more likely symptoms of poisoning from cholinesterase inhibiting pesticides are to show. Signs and symptoms of cholinesterase inhibition from exposure to organophosphate and carbamate include (Gupta, 2006, p. 735-763): (1) In mild cases (within 4 - 24 hours of contact): tiredness, weakness, dizziness, nausea and blurred vision; (2) In moderate cases (within 4 - 24 hours of contact): headache, sweating, tearing, drooling, vomiting, tunnel vision, and twitching; and (3) In severe cases (after continued daily absorption): abdominal cramps, urinating, diarrhea, muscular tremors, staggering gait, pinpoint pupils, hypotension (abnormally low blood pressure), slow heartbeat, breathing difficulty, and possibly death, if not promptly treated by a physician. However, some of the above symptoms can be confused with influenza (flu), heat prostration, alcohol intoxication, exhaustion, hypoglycemia (low blood sugar), asthma, gastroenteritis, pneumonia, and brain hemorrhage. This can cause problems if the symptoms of lowered cholinesterase

levels are either ignored or misdiagnosed as something more or less harmful than they really are.

The major differential diagnosis is carbamate poisoning, which is clinically indistinguishable (Leibson and Lifshitz, 2008, p. 767-770; Eddelston et al., 2008, p. 597-607). Carbamates are derivatives of carbamic acid. They have the same mechanism of action as organophosphate, but the chemical bond is completely reversible; therefore, when considering non-lethal doses, the assumed duration of the toxic effect is expected to be significantly shorter than that of organophosphate (Leibson and Lifshitz, 2008, p. 767-770). The types and severity of cholinesterase inhibition symptoms depend on (Gupta, 2006, p. 735-763): (1) the toxicity of the pesticide, (2) the amount of pesticide involved in the exposure, (3) the route of exposure and (4) the duration of exposure. Toxicity generally results from accidental or intentional ingestion or exposure to, agricultural pesticides (Eddelston and Phillips, 2004, p. 42; Watson et al., 2003, p.353). Other potential causes of organophosphate or carbamate toxicity include ingestion of contaminated fruit, flour, or cooking oil, and wearing contaminated clothing (Watson et al., 2003, p.353; Wu et al., 2001 p. 333).

The general population is exposed to pesticides on a daily basis via dietary ingestion of contaminated food products. Several studies have indicated that several foods contain higher levels of pesticide residue including fruits, juices, and vegetables (Curl et al., 2003, p. 377-382). Vegetables containing residue concentrations above the prescribed maximum residue level (MRL) may pose a health hazard to unwary consumers (Curl et al., 2003, p. 377-382; Dogheim et al., 1996 p. 949-952; Fillion et al., 2000, p. 698-713; Mukherjee and Gopal, 1996, p. 381-388; Sinha et al., 2012, 161-169. Fresh fruits and vegetables are important components of a healthy diet, as they are a significant source of vitamins and minerals. Different types of vegetables are consumed daily by locals in Trang Municipality of Thailand. Among them, there were three kinds of vegetables of the most common vegetables used in various local dishes of Trang province including most eaten vegetables, organic vegetables and locally grown vegetables. Thus, the current experimental research design aimed to study residual level of organophosphate and carbamate in three kinds of vegetables including most eaten vegetables, organic vegetables and locally grown vegetables that are both commonly consumed raw and cooked. The most eaten vegetables were cilantro, kale, Chinese cabbage, cabbage, cauliflower, chili, spring onion, celery, yard long bean, cucumber, tomato, lettuce, egg plant, Thai egg plant, Chinese morning glory, white radish, devil's fig, asparagus and lemon. The organic vegetables were Chinese morning

glory, Chinese cabbage, kale, cabbage and yard long bean. The locally grown vegetables were vegetable fern, sweet potato (vegetable shoots), aquatic morning glory, curry leaf, Liang vegetable, star gooseberry, Thai basil, chili, Gotu kola, bird lettuce, bitter bean, fresh pod color (red yard long bean).

Methods

A. Design

This study was experimental research design aimed to study residual levels of organophosphate and carbamate in samples of a vegetable widely consumed in Trang Municipality.

B. Collection and Preservation of Samples

To monitor the organophosphate and carbamate present in vegetable samples, most eaten vegetables including cilantro, kale, Chinese cabbage, cabbage, cauliflower, chili, spring onion, celery, yard long bean, cucumber, tomato, lettuce, egg plant, Thai egg plant, Chinese morning glory, white radish, devil's fig, asparagus, lemon were collected from 3 different retail and wholesale markets in Trang Municipally areas. The organic vegetables were Chinese morning glory, Chinese cabbage, kale, cabbage and yard long bean. The locally grown vegetables were vegetable fern, sweet potato (vegetable shoots), aquatic morning glory, curry leaf, Liang vegetable, star gooseberry, Thai basil, chili, Gotu kola, bird lettuce, bitter bean, fresh pod color (red yard long bean). The organic and locally grown vegetable samples that were used to investigate were collected from 3 retail sale markets in Trang Municipally areas. Several most eaten vegetables were selected because they are usually eaten raw. The locally grown vegetables and organic vegetables were selected because we belief that they are grown without using pesticide. 100 gram of each vegetable was collected from 3 different areas including top, middle and bottom. Only fresh, high-quality vegetables that were free from blemishes or rot were used. Following collection, the samples were collected in plastic bag and were refrigerated at $10 \pm 1^{\circ}\text{C}$ overnight and analyzed the next day. To reduce variability, all of the vegetable samples used in the study were collected within similar areas. The vegetable samples were collected during April – July 2015.

C. Equipment

The residual levels of organophosphate and carbamate were examined with GPO-M kit of Medical Science Department, Ministry of Public Health (Fig. 1). The sensitivity of GPO-M kit is 98.2%, the specificity is

83.3% and accuracy is 93.6% (Shewonphisannukul, 2014, p 11). The GPO-M Kit is screening test kit for pesticides in vegetables, fruits and cereals (2 groups). The principal of GPO-M kit is colorimetric cholinesterase inhibitor assay.

Figure 1 GPO-M Kit:



Tool

- | | |
|----------------------------|---|
| 1. Chromatograph paper set | 1 |
| 2. Standard color tube | 3 |
| 3. Pin | 1 |

Reagent

- | | |
|----------------------|-----------------|
| 1. Solvent | 1 |
| 2. Reagent GPO-M 1 | 1 (Keep frozen) |
| 3. Reagent GPO-M 1.1 | 1 |
| 4. Reagent GPO M 2 | 1 |
| 5. Reagent GPO-M2.1 | 1 |
| 6. Reagent GPO-M 3 | 1 |
| 7. Reagent GPO-M 3.1 | 1 |
| 8. Reagent GPO-M A | 1 |

**Figure 2: Doper (a), chromatograph paper set (b),
Plastic Bottle (c), Small Silver Cup (d)**



a



b

c



d



Figure 3: Water Bath

D. Chemical Preparation

GPO-M chemicals were prepared as following (Shewonphisannukul, 2014, p 14-15):

1. GPO-M 1 was added with GPO-M 1.1 at room temperature and shake well (a). Then, the mixture was warmed in water bath at 37 degree Celsius before testing 15 minutes. If it can not be immediately examined, the mixture should be kept at 2-8 degree Celsius for 3 days.

2. GPO-M 2.1 was added with GPO-M 2 at room temperature and shakewell (b). If it can not be immediately examined, the mixture should be kept at 2-8 degree Celsius for 3 days.

3. GPO-M 3.1 was added with GPO-M 3 at room temperature and shake well (c). If it can not be immediately examined, the mixture should be kept at 2-8 degree Celsius for 3 days

E. Sample Extraction for Pesticide Analysis

Sample preparation was conducted by following the methods described by Medical Science Department (Shewonphisannukul, 2014, p 14-15).

1. An individual amount of vegetable sample (100 grams) was chopped (Figure 4).

2. A small amount (2-3 grams) was added in plastic bottle (4 level of plastic bottle) and was labeled. If it can not be immediately examined, the sample should be kept at -20 degree Celsius.

3. The vegetable sample in topic 2 was added with the extraction liquid for 5 ml and shake. Then, the mixture was leaved for 5 minutes.

4. One chromatography paper was dipped in small silver cup and was labeled.

5. The topped liquid of vegetable sample in topic 3 was sucked with doper for 5 ml, and then added it in small silver cup in topic 4. Then, the mixture was leaved in water bath at 50 degree Celsius (Don't leaved chromatography paper stick with small silver cup).

6. The dried chromatography paper in topic 5 was stabbed with pin and dipped it in test tube, and then leaved test tube in water bath (Figure 3) until experiment finished.

7. Then, added GPO-M 1 for 0.5 ml in test tube in topic 6, shake and leaved the experimental test tube for 15 minutes.

8. Then, added GPO-M 2 for 1 ml. in test tube in topic 7, shake and leaved the experimental test tube for 10 minutes.

9. Then, added GPO-M 3 for 3 drops in test tube in topic 8, shake, added GOP-M A for 2 drops and finally read the results.

Figure 4: Sample preparation & Sample Extraction



Source: Shewonphisannukul, 2014

F. Result Interpretations

Table 1 Chromatography paper color interpretation

Interpretation	Color of vegetable sample tube	Color of standard tube	Pesticide level	Cholinesterase inhibitor value
Negative	Dark purple	Tube 1 (Not found)	Not found & Safe level	< 50%
Positive	Lavender	Tube 2	Unsafe level	50-70%
	Gray	Tube 3	Toxicity level	> 70%

Results

Table 2 Residual levels of organophosphate and carbamate in most eaten vegetables.

Kinds of sample	Total number of sample	Pesticide residues found					
		Not found & Safe level		Unsafe level		Toxicity level	
		Number	%	Number	%	Number	%
Cilantro	10	2	20	8	80	0	0
Kale	10	6	60	4	40	0	0
Chinese cabbage	10	5	50	5	50	0	0
Cabbage	10	3	30	7	70	0	0
Cauliflower	10	4	40	6	60	0	0
Chili	10	3	30	7	60	0	0
Spring onion	10	9	90	1	10	0	0
Celery	10	8	80	2	20	0	0
Yard long bean	10	1	10	9	90	0	0
Cucumber	10	5	50	5	50	0	0
Tomato	10	1	10	9	90	0	0
Lettuce	10	10	100	0	0	0	0
Egg plant	10	10	100	0	0	0	0
Thai egg plant	10	2	20	8	80	0	0
Chinese morning glory	10	10	100	0	0	0	0
White radish	10	6	60	4	40	0	0
Devil's fig	10	10	100	0	0	0	0
Asparagus	10	10	100	0	0	0	0
Lemon	10	6	60	4	40	0	0
Total	190	111	57.89	79	41.58	0	0

The results revealed that unsafe level (cholinesterase inhibitor level of 50-70%) of organophosphate and carbamate were found in most eat

eaten vegetables 79 samples (41.58%) from 190 samples. Kinds of vegetables found pesticide residuals included cilantro, kale, Chinese cabbage, cabbage, cauliflower, chili, celery, spring onion, yard long bean, cucumber, tomato, Thai egg plant, white radish and lemon. When considering each vegetable, 9 tomato and yard long bean samples (90%) were found unsafe level of organophosphate and carbamate from 10 samples. 8 cilantro and Thai eggplant samples (80%) was found unsafe level (cholinesterase inhibitor level of 50-70%) of organophosphate and carbamate from 10 samples. All organic and locally grown vegetables were found safe residual level of organophosphate and carbamate.

Discussions

The persistence of carbamate and organophosphate residues are a complex matter affected not only by the chemical and physical characteristics of the parent compound and their degradation products but also by the nature of the formulation applied, the adsorbents, and the type of solvents employed. Some types of vegetables have waxy surfaces that tend to trap sprayed pesticides, thereby making pesticides more resistant to removal, and they would be as true surface residues.

The present study, unsafe level (cholinesterase inhibitor level of 50-70%) organophosphate and carbamate were found in fifteen vegetables of 79 samples (41.5%) from 190 samples that are most eaten vegetables in Thailand. Kinds of vegetables found pesticide residuals included cilantro, kale, Chinese cabbage, cabbage, cauliflower, chili, celery, spring onion, yard long bean, cucumber, tomato, Thai egg plant, white radish and lemon. The results of this study were consisted with previous study (Prasopsuk et al., 2014, p. 430 - 439) to analysis of pesticide residues in vegetables and fruits for the certification of Good Agricultural Practice for Food Crop in upper Northeast Thailand (11 provinces) during 2011 – 2013. Pesticide residues were found in vegetables and fruits 173 samples (19.1%) and greater than the MRL 13 samples (1.4%) from 905 samples in 2011. Kinds of vegetables that found pesticide residue were cabbage, celery, yard long beans, Chinese cabbage, celery, chili, eggplant, and spring onion. In 2012, pesticide residues were found in vegetables and fruits 272 samples (26.5%) and greater than the MRL 18 samples (1.8%) in 1,027 samples. Kinds of vegetables that found pesticide residues were cauliflower, celery, cucumbers, chili, and spring onion. In 2013, pesticide residues were found in vegetables and fruits 332 samples (30.1%) and greater than the MRL 24 samples (2.2%) in 1,103 samples. Kinds of vegetables

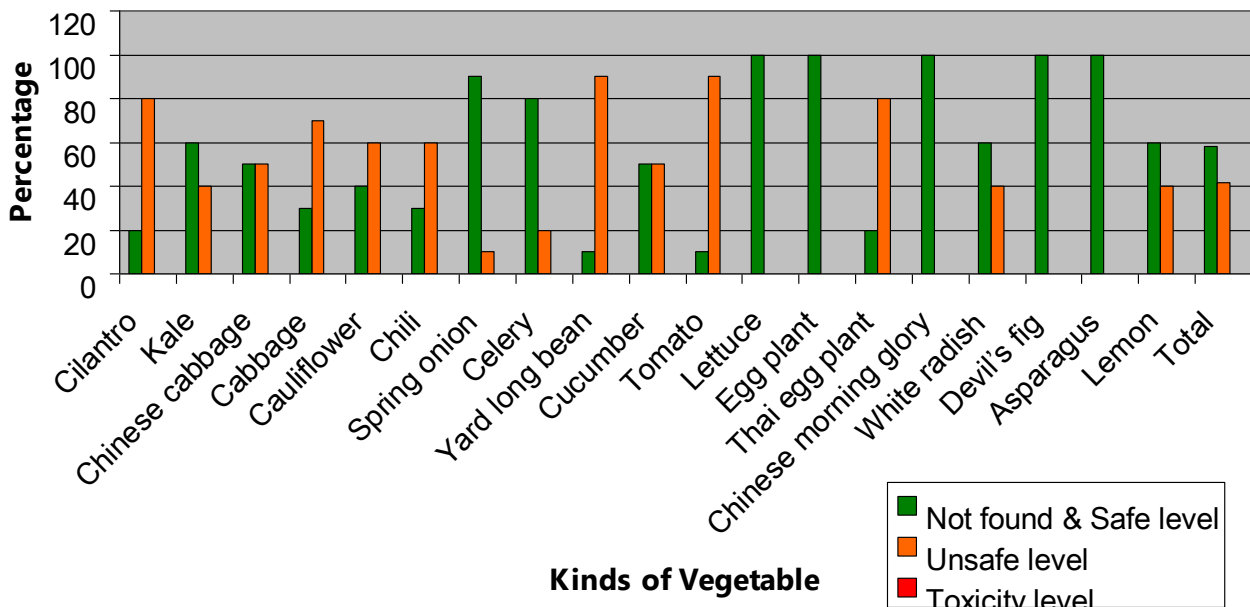
that found pesticide residue were cauliflower, celery, kale, coriander, Vietnamese coriander, lemon, yam bean, chili, mushrooms and spring onion. When comparing the results of previous study and current study, the results revealed that pesticide residues in vegetables were dramatically found higher.

Kinds of most eaten vegetables found pesticide residuals included cilantro, kale, Chinese cabbage, cabbage, cauliflower, chili, celery, spring onion, yard long bean, cucumber, tomato, Thai egg plant, white radish and lemon. These fifteen vegetables have waxy surfaces that tend to trap sprayed pesticides, thereby making pesticides more resistant to removal, and they would be as true surface residues. Although washing, peeling, and cooking remove a large amount of pesticides during food processing, some studies have indicated that they are inefficient in reducing pesticide residues below the MRL value. For example, (Kumari, 2002, p. 263–270) reported that organophosphate residues in cauliflower were reduced only to some extent by various home processing methods such as washing and cooking. It has been suggested that the inefficiency of home processes for decontaminating treated cabbage may be due to the strong adsorption properties of organophosphate (Jacobson, 1999, p. 783–789).

All organic and locally grown vegetables were found safe residual level of organophosphate and carbamate because they are grown without or less using pesticide.

Conclusions and recommendations

Only most eaten vegetables including cilantro, kale, Chinese cabbage, cabbage, cauliflower, chili, celery, spring onion, yard long bean, cucumber, tomato, Thai egg plant, white radish and lemon were found unsafe level (cholinesterase inhibitor level of 50-70%) of organophosphate and carbamate as Figure 5. All organic and locally grown vegetables were found safe residual level of organophosphate and carbamate. Based on the results of this study, public health authorities should encourage people to eat organic or locally grown vegetables. In addition, the only way to be sure that people are eating 100% natural products is by growing them their selves.

Figure 5: % of Most Eaten Vegetables found Pesticide Residuals

Consumer should properly wash vegetable before cooking and wash vegetable under running water rather than soaking or dunking it. In addition, people should use some chemical for example salt, potassium permanganate, calcium hypochlorite, vinegar and sodium bicarbonate for residuals of pesticide removal. Washing veggies is not enough, as it is very important to thoroughly dry them with disposable paper towels as well. This will remove the remaining pesticide residue and make the produce safer to eat. In addition, it is highly recommended to either throw away the outer layers of dark leafy greens and other vegetables, or to gently peel it.

However, for good quality and food safety of Thai vegetables, gardeners should be aware of the dangers of chemical pesticides. Meanwhile, authorities should work proactively to advise the use of chemical pesticides correctly and monitored continuously.

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