



The Investigation of Organophosphate and Carbamate Pesticide Residues Found in the Period 2022-2024 on Popular Vegetables in Bangkan County, Bangkok, Thailand

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Abstract

The experiment on organophosphate and carbamate has been conducted since 2022, therefore the yearly experiment has been done to observe the situation of pesticide residue in vegetables. Vegetables around Thailand have been used the organophosphate and carbamate as pesticides. Organophosphate and carbamate are inhibitors of acetylcholinesterase, an enzyme critical to the control of nerve impulse transmission from one cell to another. The LD_{50} of organophosphate and carbamate is 5 – 500 mg/kg. This investigation was performed on 10 kinds of vegetables found mostly in markets such as parsley, Chinese kale, cabbage, Chinese cabbage, water morning glory, carrot, lettuce, cowpea, tomato, and cucumber which were purchased in 2022, 2023 and 2024. They all were tested using the GT-Test Kit. The study found the combination of organophosphate and carbamate in morning glory was still over the maximum residue limits (MRLs) issued by the Thai Food and Drug Administration, issue number 288 (year 2005).

Keywords: Vegetables, Pesticide, Organophosphate, Carbamate, testkit

Introduction

The number of poisonings from pesticides in Thailand is increasing¹. A recent study has revealed that organophosphate and carbamate insecticides are the primary culprits behind poisoning cases in Thailand². Pesticide use in agriculture plays a crucial

role in boosting crop yields. However, the overuse of these chemicals can cause significant harm to the environment and human health. One major concern is the impact on the enzyme acetylcholinesterase (AChE), which is vital for breaking down the neurotransmitter acetylcholine in our nervous

system. The inhibition of AChE can lead to serious health issues, underscoring the need for careful management of pesticide applications³. Organophosphate and carbamate pesticides enter the body through ingestion, inhalation, and through the skin. Toxic will be in the same direction which is the toxicity depends on the rate of transformation of toxins in the body by liver hydrolysis. Both groups of pesticides were highly acutely toxic by inhibiting the activity of acetylcholinesterase. The enzyme AChE is responsible for the breakdown of the neurotransmitter acetylcholine. The function of controlling nerve impulses is choline and acetic acid, causing the accumulation of acetyl. Colin on nerve endings resulting in acute poisoning. This can cause severe nerve endings and death⁴. The initial symptoms found were Nausea, vomiting, golden streaks, lacrimation, sweating, iris constriction, urinary incontinence, urinary incontinence, bronchospasm muscle spasms and have a lot of phlegm⁴.

Organophosphate pesticides are classified as esters of phosphoric acid. Examples of substances in this group include monocrotophos, mevinphos, chlorpyrifos, dimethoate, dicrotophos, parathion-methyl and parathion⁵. Carbamates are a synthetic

group of organic compounds represented by the -O-CO-NH- functional group and the variations of carbamates are classified by the differences in substituent groups on the O terminal and N terminal. The variation of carbamates can significantly influence their biological activity and toxicity. Some common examples of carbamates include carbaryl, methomyl, and propoxur, each exhibiting different effects on target organisms and varying degrees of environmental persistence. Its application is used as an ingredient in various products for both domestic and industrial use and insecticides such as methomyl, carbofuran, carbaryl and methiocarb. for the decomposition of both pesticides, they have different period. Organophosphate pesticides decompose rapidly within a few weeks. Carbamate decomposes up to 50 weeks in the soil⁴. However, if pesticides are used more than needs and use in conditions where the soil nature is not balanced will result in a more stable group of pesticides and a higher half-life than used. These result in the excess formation of residues. Eliminate pests that exceed the acceptable Maximum Residue Limits (MRLs).

The determination of organophosphates and carbamate can be done using GT test

kits for quick, easy, and fast results. There are reports that the test kits had been used and the results showed that the test kits gave different results depending on the organophosphate and carbamate. However, no significant difference was found. But the sensitivity and accuracy of each test set were significantly different⁷. In Thailand, the detection of organophosphates and carbamates had been conducted using the GT test kit^{8,9}. The principle of this test is that inhibition of the enzyme cholinesterase (cholinesterase inhibition technique) by organophosphate and carbamates groups will inhibit the activity of the enzyme choline esterase. These prevents the enzyme from hydrolyzing acetylcholine. The remaining acetylcholine is indicated by the intensity of the resulting color. In the assay, when the sample contains large amounts of organophosphate and carbamate residues, the test will result in inhibition of the enzyme choline esterase as well, therefore, the residual amount of acetylcholine and the resulting color are dark accordingly¹⁰.

In a previous study^{8,9}, samples of 10 vegetables sold at large wet markets and department stores in 2014 and 2021 found that the contamination of organophosphate and carbamate pesticides in coriander, kale, and cucumber. The amount of contamination is at a safe level. In addition, it was found that

in the dry season, the amount of pesticide contaminants was higher than in other seasons⁹. In 2019, it was reported that roughly 41.3% of fresh fruits and vegetables available in supermarkets and markets contained pesticide residues exceeding permissible levels¹⁰. According to the information of the pesticide warning network, it was found that the results of the fruit and vegetable inspection for the year 2020 were from samples. Samples of 509 in total across the country found 58.7% contain pesticide residues beyond the standard and the types of vegetables found to have excess residues are small tomatoes, chili, celery, and kale¹¹. Based on the pesticide's contamination problem, the researchers realize the importance of monitoring the contamination of the disposal agent.

Leading to the experiments in this study that surveyed the residues of this group of pesticides from market Y in September of the years 2021-2024. The objective of this research was to update the determination of organophosphate and carbamate pesticides in vegetables commonly consumed from local store and to compared with previous studies using the test kits.

Materials and Methods



To monitor and compare contamination data of organophosphate and carbamate pesticides in popular vegetables, therefore, the same vegetables were randomly sampled as the previous experiment ⁸. Based on the research in 2014 ⁹, the results found that the contamination of organo-carbamate and organo-phosphate was mostly found in September of the year, so the samples were collected in September 2021 ⁸, September 2022 (this report), September 2023 (this report) and September 2024 (this report). They were from the same source at each market which is Market Y in Bangkan Province of Bangkok, Thailand. The 10 vegetables that were randomly sampled from market Y were cilantro, lettuce, morning glory, Chinese cabbage, kale, cucumber, tomato, carrot, cabbage, and long beans.

The samples were purchased randomly from market Y and divided into three samples of each vegetable. Each sample had been done in triplicate. Then, samples are extracted immediately in the laboratory by grinding. A random sample that has been crushed and weighs 5 g in each test gives the

total of each vegetable at 9 tests (total of n=90 tests/year). Then, vegetable samples were extracted with dichloromethane and 5% ethanol then the dichloromethane were evaporated using a nitrogen evaporator until approximately 1 mL of sample extract remained. The extracts were subsequently tested with the GT test kit. Perform the experiments as described in the test kit document with positive and negative control (Table 1, Figure 1). The temperature of the water bath has been monitored using a Fluke Thermocouple thermometer at 36 ± 2 °C. After adding all the test chemicals, read the results by comparing the color of the sample with the color of the control tubes. Colors more intense than or equal to the positive tube will interpret that residues were found at unsafe levels using the symbol (++) . If the color intensity is less than the positive control, but less than the negative control interpreted as the residue was found to be at a safe level using the symbol (+) . If the color intensity is less than or equal to the negative control means no organophosphate and carbamate residues were detected using the symbol (-) .

Table 1. Direction in adding each solution for the GT test kit.

| | Negative control | Positive control | sample | Post adding reagent |
|----------------|------------------|------------------|---------|---------------------------|
| 5% ethanol | 0.25 ml | 0.25 ml | - | - |
| Sample extract | - | - | 0.25 ml | - |
| GT-1 | 0.5 ml | 0.5 ml | 0.5 ml | In a water bath 5-10 mins |
| GT-2 | - | 0.25 ml | 0.25 ml | In a water bath 30 mins |
| GT-3 | 1 ml | 1 ml | 1 ml | Perform in a water bath |
| GT-4 | 0.5 ml | 0.5 ml | 0.5 ml | Perform in a water bath |
| GT-5 | 0.5 ml | 0.5 ml | 0.5 ml | Read the result |

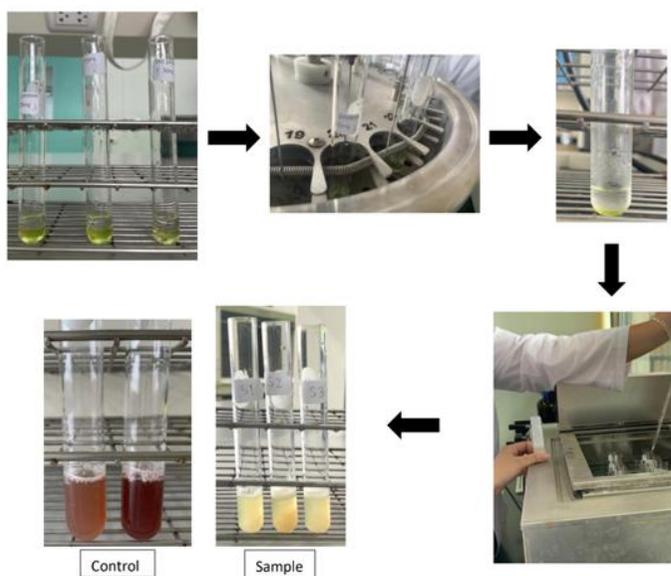


Figure 1. Method in determination using test kit from left to right, top to bottom, the sample were extracted using the solvent, the evaporation of solvent using nitrogen purge, the solution after evaporation, adding reagents as directed in Table 1 in each test, and the results after adding every reagent (GT1-GT5) both the indicators (positive and negative control), and sample tests.

Results and Discussion

The results from the test kit were shown as the example for the positive tests (Figure 2(b)) and the example that found the

residue under the limit (Figure 2(a)). For the negative results, the color of the sample turned to the same color as the negative control or even faded.

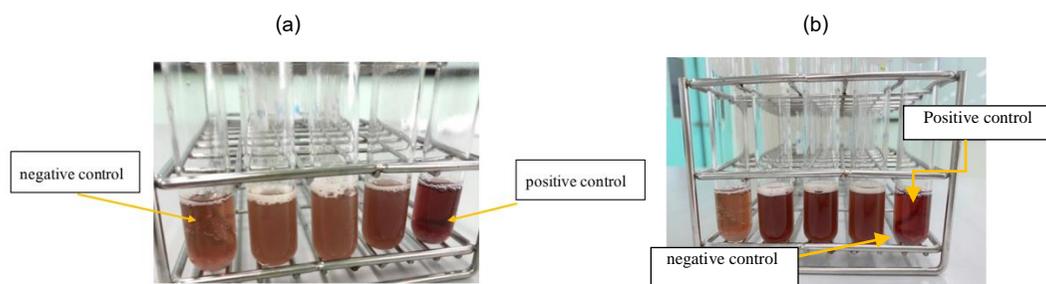


Figure 2. (a) results from the determining of morning glory as found in the safety level (+) (b) results from the determining of Chinese kale as found the unsafe level (++).

From the results of 10 vegetables that were randomly sampled from commercial sources that were tested using the insecticide “GT” test kit organophosphate and carbamate yielded the results shown in Table 2,3,4 and 5. From the experimental results, it was revealed that parsley from yearly sources where pesticide residues have been detected at unsafe levels. However, other vegetables mostly were found to have some residues but at safe levels. In some types of the vegetable, the carbamate and organophosphate residues were not found, for example, kale from the four markets had residues found at a safe level, and no residue was found in the samples from the department store.

The reason could be the regulation in the department store to promote the sale.

The results show parsley, Chinese kale, carrots, and cowpeas contaminated with carbamate and organophosphate over the limit by Thai regulations as shown the symbol (++) . The samples of Chinese cabbage, morning glory and tomato were found in the safety amount as shown in the symbol (+). However, cabbage and cucumber were not shown to be contaminated as shown in negative results (-). These were summarized in Table 2, Table 3, Table 4, and Table 5 for years 2021⁸, 2022, 2023 and 2024, respectively.

In 2022, the pesticide contamination in vegetables was compared to 2021. On the other hand, the pesticide residues are not detected in cucumber; this might because of

pesticide is leached into the soil by water from the rainy season. Residues are detected on tomatoes and lettuce which means that the pesticide is used in higher concentrations.

Table 2. Test of pesticide residues in samples of each vegetable from market Y in 2021⁸ (n=90)

| Vegetable | Number not detected (-)(%) | Number of detected | |
|-----------------|-------------------------------|--|---|
| | | Number detected in safe amount(+) (%) | Number detected in unsafe amount (++)(%) |
| | | Parsley | 3(33.33) |
| Chinese kale | 3(33.33) | 6(66.67) | 0(0.00) |
| Cabbage | 9(100.00) | 6(66.67) | 0(0.00) |
| Chinese cabbage | 3(33.33) | 6(66.67) | 0(0.00) |
| Morning glory | 6(66.67) | 3(33.33) | 0(0.00) |
| Carrot | 6(66.67) | 3(33.33) | 0(0.00) |
| Lettuce | 9(100.00) | 0(0.00) | 0(0.00) |
| Cowpea | 3(33.33) | 6(66.67) | 0(0.00) |
| Tomato | 9(100.00) | 0(0.00) | 0(0.00) |
| Cucumber | 6(66.67) | 3(33.33) | 0(0.00) |

Table 3. Test of pesticide residues in samples of each vegetable from market Y in 2022 (n=90)

| Vegetable | Number not detected (-)(%) | Number of detected | |
|-----------------|-------------------------------|--|---|
| | | Number detected in safe amount (+)(%) | Number detected in unsafe amount (++)(%) |
| | | Parsley | 0(0.00) |
| Chinese kale | 0(0.00) | 9(100.00) | 0(0.00) |
| Cabbage | 9(100.00) | 0(0.00) | 0(0.00) |
| Chinese cabbage | 0(0.00) | 9(100.00) | 0(0.00) |
| Morning glory | 3(33.33) | 6(66.67) | 0(0.00) |
| Carrot | 3(33.33) | 6(66.67) | 0(0.00) |
| Lettuce | 9(100.00) | 0(0.00) | 0(0.00) |
| Cowpea | 0(0.00) | 9(100.00) | 0(0.00) |
| Tomato | 9(100.00) | 0(0.00) | 0(0.00) |
| Cucumber | 3(33.33) | 6(66.67) | 0(0.00) |

The comparison percentages of pesticide residues found in tests of each vegetable from Market Y between the years 2014⁹, 2021⁸, 2022, 2023, and 2024 are shown in Figure 3 and Figure 4. It was found that carbamate and

organophosphate were not found in cabbage since 2021. The lettuce and cowpea are the vegetables in which the contaminate in some tests, but still in an amount that is not over the limit by Thai regulations.

Table 4. Test of pesticide residues in samples of each vegetable from market Y in 2023 (n=90)

| Vegetable | Number not detected | Number of detected | |
|-----------------|---------------------|-------------------------|---------------------------|
| | (-)(%) | Number detected in safe | Number detected in unsafe |
| | | amount (+)(%) | amount (++){%} |
| Parsley | 0(0.00) | 0(0.00) | 9(100.00) |
| Chinese kale | 0(0.00) | 0(0.00) | 9(100.00) |
| Cabbage | 9(100.00) | 0(0.00) | 0(0.00) |
| Chinese cabbage | 0(0.00) | 9(100.00) | 0(0.00) |
| Morning glory | 0(0.00) | 9(100.00) | 0(0.00) |
| Carrot | 0(0.00) | 0(0.00) | 9(100.00) |
| Lettuce | 0(0.00) | 9(100.00) | 0(0.00) |
| Cowpea | 0(0.00) | 9(100.00) | 0(0.00) |
| Tomato | 0(0.00) | 9(100.00) | 0(0.00) |
| Cucumber | 9(100.00) | 0(0.00) | 0(0.00) |

Table 5. Test of pesticide residues in samples of each vegetable from market Y in 2024 (n=90)

| Vegetable | Number not detected | Number of detected | |
|-----------------|---------------------|-------------------------|---------------------------|
| | (-)(%) | Number detected in safe | Number detected in unsafe |
| | | amount (+)(%) | amount (++){%} |
| Parsley | 0(0.00) | 0(0.00) | 9(100.00) |
| Chinese kale | 0(0.00) | 9(100.00) | 0(0.00) |
| Cabbage | 9(100.00) | 0(0.00) | 0(0.00) |
| Chinese cabbage | 0(0.00) | 9(100.00) | 0(0.00) |
| Morning glory | 0(0.00) | 9(100.00) | 0(0.00) |
| Carrot | 0(0.00) | 9(100.00) | 0(0.00) |
| Lettuce | 3(33.33) | 6(66.67) | 0(0.00) |
| Cowpea | 6(66.67) | 3(33.33) | 0(0.00) |
| Tomato | 3(33.33) | 6(66.67) | 0(0.00) |
| Cucumber | 9(100.00) | 0(0.00) | 0(0.00) |

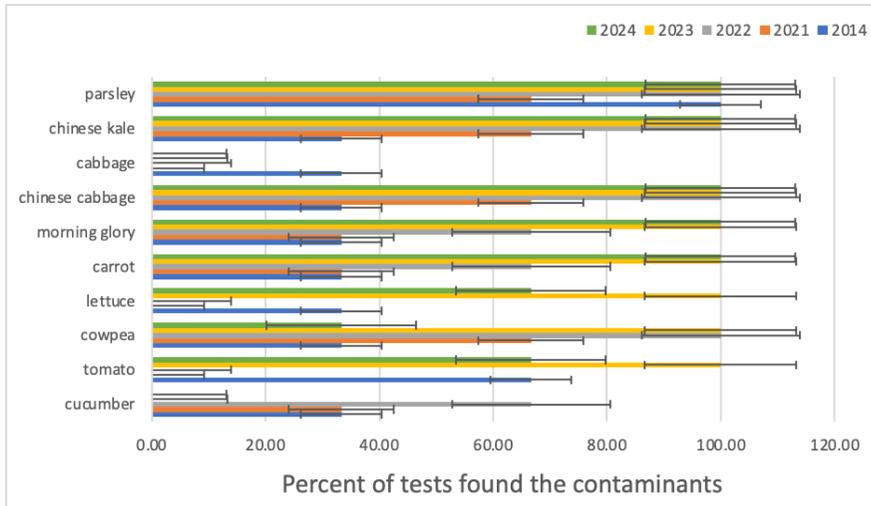


Figure 3. The comparison test of pesticide residues in samples found the contamination of organophosphate and carbamate on each vegetable from Market Y between 2014⁹, 2021⁸, 2022, 2023 and 2024

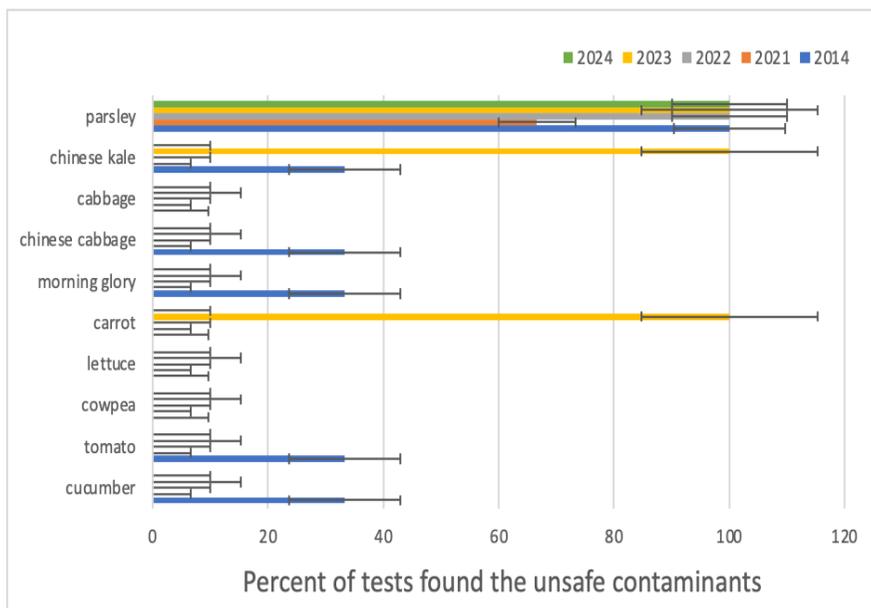


Figure 4. The comparison test of pesticide residues in samples found unsafe amount of the contaminated of organophosphate and carbamate on each vegetable from Market Y between 2014⁹, 2021⁸, 2022, 2023 and 2024

Conclusion

The problem of residues that are harmful to human health and safety. It has been studied about the health problems from poisoning of carbamate and organophosphate such as cardiac complication^{12,13}. Therefore, the consumer should be concerned about these contaminants or choose to buy organic vegetables that have agricultural product quality certification marks.

There are reports about the analytical process of carbamate and organophosphate in humans and the environment¹⁴. Those experiment need expertise and time consumed. However, the simplest way to test before consumption is such a test kit, which is important for screening those compounds contaminated in food¹⁵. The GT test kit has reported to be precise compared with standard methods¹⁶. The standard method found the contaminants 27.5% from 528 samples and GT test kit found the contaminants 33.7% from the same samples¹⁶. The test kit company has reported the sensitivity, the specificity, and accuracy as 92.3%, 85.1%, and 87.1%, respectively¹⁶.

The experiments showed the contamination of organophosphate and carbamate on various vegetables collected in the Market Y, Bangkan County, Bangkok,

Thailand. From Figure 3, it was found that the contaminated from organophosphate and carbamates was slightly lower in recent years. It should be noticed that the contaminated always found in parsley. From Figure 4, it was found that the contamination is reduced from a harmful amount to a non-harmful amount. However, the contamination in parsley is still found in harmful amounts in every experiment year. However, the research should be conducted in other areas of Thailand to be more precise and could lead to the main response by the government. As a results, consumers should consider how to protect themselves by rinsing with detergent such as baking soda or hydrogen peroxide⁸. The season has some factor on the amount of residue found since the not-safe number of residues was found in the late summer compared to yearly⁸.

Suggestion

This study examined vegetable samples collected from one area in Bangkok and studied only some types of vegetables. The results of this study may not be able to be used to conclude about organic contaminations in vegetables at the national level.



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