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Situation of Organophosphate and Carbamate Residue in Fresh Strawberry in Phitsanulok and Petchaboon Provinces, Thailand

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Abstract

Strawberries are a fruit that comes in a variety of flavors from sour taste to very sweet taste depends on the species grown. In Thailand garden strawberry are popular. Strawberry is rich in nutrient and substances that help fight against many types of free radicals. Strawberries are one of a very popular fruit nowadays. The strawberry is harvested in the winter in when is the New Year's season. Many people are travelling during the winter time. A long the road no. 12, it is a route from Phitsanulok to Lomsak, there are many popular tourist spots included Khao Kho and Phu Thap Buek , were also many strawberry fame s and stalls along each side of the road. Pesticide is used in growing strawberry, improperly used result in hazardous chemical residues in strawberry. The researcher is therefore interested in studying for pesticide residues of strawberry sold in this area. In this research, it is an examination of drugs, chemicals, pesticides, cholinesterase inhibitor groups by using the Colorimetric Cholinesterase Inhibitor Assay principle with the MJPK test kit of the Department of Medical Sciences. A simple random sampling method was used for sampling a total of 48 samples, 3 samples per store, from 16 different stores. All 48 samples were collected during December 25-31, 2022. Based on the results of the test kit, it was concluded that no chemical detected Organophosphate group and carbamate residues in all 48 strawberry samples.

Keywords: strawberry, pesticide residue, organophosphate, carbamate

Introduction

"Strawberry" is a subtropical fruit that belongs to the rose family and falls under the category of flowering plants. There are over 20 species of strawberries, each with many different hybrid varieties. Fresh strawberries have a diverse range of flavours, ranging from sour to intensely sweet. This variation depends on the specific variety being cultivated. Among the strawberries commonly grown in our country is the

garden strawberry. Garden strawberries are enriched with various antioxidants, including compounds such as quercetin, kaempferol, and anthocyanin, which contribute to their health benefits [(1)].

The aforementioned compounds play a role in inhibiting various cancer-causing agents. Additionally, strawberries contain a high amount of vitamin C. Approximately **100** grams of fresh strawberries can provide as much as **58** milligrams of vitamin C, which is significantly higher compared to other fruits. In terms of antioxidant capacity, strawberries have been found to contain more antioxidants than oranges (**1.5** times more), more than double the amount found in red grapes, more than triple that of kiwi, seven times more than tomatoes, seven times more than bananas, and up to fifteen times more than apples. Strawberries thrive in areas with an altitude above **800** meters above sea level, temperatures ranging from **10** to **25** degrees Celsius, and a cool climate throughout the year. They require fertile soil for cultivation. Planting usually begins in late August and continues until late October, with harvesting taking place from November to April of the following year [(2-3)]

During the period of strawberry harvesting, it aligns with Thailand's winter season and coincides with the extended New Year holiday. This is a time when many people prefer to travel and explore different provinces, resulting in a significant number of tourists. As a result, strawberry farms have become popular tourist attractions. Visitors are drawn to these farms not only for their picturesque photo opportunities but also for the chance to pick and purchase fresh strawberries for consumption. Apart from cultivating strawberries in dedicated farms, there is also a practice of planting strawberries along main roads, making it convenient for tourists to visit, take photos in the strawberry fields, and buy fresh strawberries to enjoy. This practice has gained immense popularity among travellers.

Based on previous studies, it has been found that residual chemical substances for plant pest control can be present in strawberries [(4-5)]. This is because strawberries can be vulnerable to plant pests that can cause damage to the yield[(6)]. In commercial strawberry cultivation, chemical substances are used to control production factors, such as weed and insect management, with the aim of producing beautiful, visually appealing strawberries that are free from wormholes or insect infestations.

There are four main groups of chemical substances commonly used by farmers for plant pest control: organochlorines, organophosphates, carbamates, and pyrethroids[(7)]. However, improper use of chemical substances for pest control can lead to residue build up in the produce, posing potential health risks for consumers in both the short and long term[(8)].

The health problems arising from the use of chemical substances for plant pest control are a significant and serious issue in Thai society. There is a lack of sufficient collective awareness and collaboration among relevant agencies and the Thai community, particularly concerning the impact on farmers and the general population. According to data from the year **1997 (2540** in the Thai calendar) by the Department of Occupational and Environmental Diseases, Ministry of Public Health, it was found that a considerable number of farmers had blood test results that were not within safe limits and were at risk of poisoning due to the use of chemical substances for plant pest control. This affected up to **16.35%** or **89**,**926** individuals out of a total of **563**,**353** farmers who underwent blood tests. This trend has continued to increase significantly, and by the year **2007 (2550** in the Thai calendar), random testing revealed that as many as **39%** of farmers were at risk of the aforementioned health issues [(9)]**.**

Furthermore, the issue of residual chemicals in vegetables and fruits remains a close and frequent threat in everyday life that may have implications for the health of individuals consuming agricultural produce. The health impacts resulting from the ingestion of chemical substances used for plant pest control can be divided into two categories: 1) Acute Toxic Effects: These effects occur immediately after exposure to chemical substances. They can lead to irritation of the respiratory system, gastrointestinal tract, nervous system, and muscles. Symptoms may include nausea, vomiting, headache, muscle pain, diarrhea, difficulty breathing, blurred vision, and more. And 2) Chronic Toxic Effects: These effects stem from the accumulation of toxins that lead to the development of diseases or other problems causing genetic changes. Examples include cancer, diabetes, autism, Parkinson's disease, various skin conditions,

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infertility, congenital disabilities, or sexual dysfunction [10-11].

Chemical residues from insecticides that linger on vegetables and fruits can exist in varying amounts, and their presence may not be visually detectable. These toxic substances can pose significant health risks when ingested, potentially leading to toxic effects and serious illnesses. Therefore, it's important to learn how to choose and purchase vegetables and fruits wisely, as well as adopt safe washing practices to minimize the potential risks associated with the residues of chemical substances used for plant pest control [12].

Highway Route **12** is a road that connects Phitsanulok to Lom Sak and is a well-known travel route with popular tourist destinations in the provinces of Phetchabun and Phitsanulok[13]. There are several highly regarded tourist attractions along this route, including Khao Kho and Phu Thap Boek, which have numerous strawberry farms. Each year, a large number of tourists visit these places. However, due to the lack of prior investigation and examination of fresh strawberries being sold along this route, the researchers became interested in conducting a study to find residual pesticide substances on the produce that is sold within this area. In this study, the researchers aimed to assess the presence and concentration of these chemical substances.

Study Objective

1. to study the levels of organophosphates and carbamates residues in fresh strawberries that are sold within the provinces of Phitsanulok and Phetchabun.

Instrument

This study constitutes a qualitative research that investigates the residual presence of organophosphate and carbamate pesticides in fresh strawberries sold along the roadside of Highway Route **12**, from Phitsanulok to Khao Kho. The study area spans a distance of **34.3** kilometers, commencing from the Ban Yaeng intersection in Nakhon Thai District, Phitsanulok Province, and concluding at the Camp Son intersection in Khao Kho District, Phetchabun Province.

Sampling

The study conducted a field survey in December **2022**, focusing on both sides of Highway Route **12** where fresh strawberries are sold. A total of **30** roadside vendors were surveyed. Using a simple random sampling method, a total of **48** samples were collected, with **3** samples taken from each of the **16** different vendors. Sampling was carried out from December **25**th to **31**st, **2022.**

Equipment

This research aims to examine the presence of cholinesterase inhibitor pesticides, specifically those that inhibit acetylcholinesterase, using the Colorimetric Cholinesterase Inhibitor Assay. The assay kit used in this study is the M.J.P. kit developed by the Department of Medical Sciences, Ministry of Public Health. The assay is designed to detect the minimum quantity of pesticide that inhibits enzyme activity by **15%**, which is considered to be a slightly toxic level. The test is conducted on vegetables and fruits to determine the presence of cholinesterase inhibitors from the group of pesticides known as acetylcholinesterase inhibitors. The results of the test can be obtained within **30** minutes.

Equipment and Chemicals for the Experiment

1. Plastic Bottles (Extraction Bottles) - **10** bottles
2. Glass Test Tubes - **10** tubes
3. Plastic Test Tubes - **10** tubes
4. **3** cc. Dropper Tubes - **4** tubes
5. Control Tubes - **1** tube
6. Gloves - **2** pairs
7. Extraction Solution - **1** bottle
8. Distilled Water - **1** bottle
9. Test Solution **1** - **1** bottle
10. Test Solution **2** - **1** bottle
11. Test Solution **3** - **1** bottle

The testing procedure

Cut the vegetables or fruits to be tested into small pieces and place them in the plastic bottles, filling about three-quarters of each bottle. Add **6** milliliters of the extraction solution to each bottle, close the lids tightly, and vigorously shake the bottles for approximately **2** minutes. Gradually open the bottle

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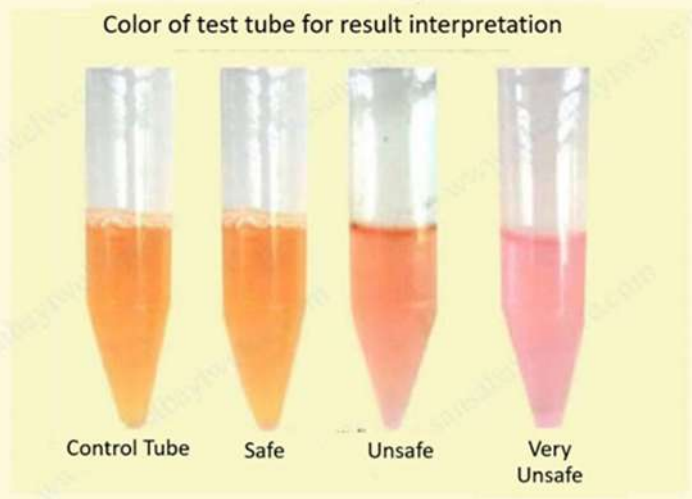
lids to release the extraction solution into the glass test tubes until empty. Submerge the test tubes in a water-filled beaker, with warm water filled about halfway. Gently shake the submerged test tubes to allow the extraction solution to evaporate, leaving approximately one drop in each tube.Mix the test solution **(1)** with **1** milliliter of distilled water in a dropper bottle, shake well to mix. Add the test solution **(2)** to a glass test tube, followed by **3** drops from the control tube, and shake well to mix. Add the prepared test solution **(1)** to another glass test tube, followed by **2** drops from the control tube, and shake well to mix. Rinse the test tube with distilled water and then mix the test solution **(3)** with **1.5** milliliters of distilled water.Add the test solution **(3)** to a sample test tube, followed by **2** drops from the control tube, and shake well to mix. Observe the immediate color change.Compare the color change rate between the sample and control tubes. If the sample tube changes color slower than the control tube, it indicates the presence of cholinesterase inhibitor chemicals. If the color of the dissolved solution turns dark orange similar to the control tube, it can be interpreted as safe. If the color of the dissolved solution has a pinkish tint, it can be interpreted as unsafe (inhibited by **15%).** Ensure quality control by including a control tube in each test round for color comparison. If the control tube color does not turn a deep orange, repeat the procedure. [14]

Data Analysis

The analysis of test results is based on the color change observed in the experimental glass test tubes. The rate of color change between the sample and control tubes is examined. If the color change in the sample tube is slower than that in the control tube, it indicates the presence of pesticide chemicals. If the color of the dissolved solution turns a deep orange similar to the control tube, it can be interpreted as safe. If the color of the dissolved solution has a pinkish tint, it can be interpreted as unsafe (inhibited by **15%).** Furthermore, if the color of the dissolved solution has a distinct pinkish hue, it can be interpreted as highly unsafe (Picture No. **1).**

Picture No. 1. Interpretation of Results from the Color of the Test Tubes.



Results

From the testing for residual chemicals of organophosphates and carbamates in fresh strawberries, with a total of 48 samples, no traces of organophosphate and carbamate chemicals were detected. This is evident in Figure 2, where the color of the control tube on the left is compared with the color of the experimental tube on the right. The orange color is the same in both tubes for all 48 samples, indicating that there are no residual organophosphate and carbamate chemicals present in the strawberry samples.

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Picture No. 2

Results of Residue Testing for Residual Organophosphate and Carbamate Pesticides in Fresh Strawberry Samples.

Table 1: Number and Percentage of Detected Organophosphate and Carbamate Residues in Strawberry Samples (n=48)

No. of sample	Results of Organophosphate and Carbamate Analysis		
	No residue detected	Dangerous Residue Absent	Highly Hazardous Residue Absent
48	48	0	0
Per cent	100	0	0

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Discussion

The investigation for residual pesticides, specifically organophosphate and carbamate pesticides, in a total of 48 samples of fresh strawberries revealed no detection of residual organophosphate and carbamate pesticides. This absence of pesticides was observed across all 48 strawberry samples. In commercial strawberry cultivation, a majority of farmers employ agricultural chemicals for crop protection due to the prevalence of various diseases, insects, and pests affecting strawberries[15-16]. Appropriate use of chemical pesticides, including their quantity and application duration, has an impact on residue levels in the harvested produce. The examination of residual organophosphate and carbamate pesticides in fresh strawberry samples did not yield any trace of these chemicals. This indicates that farmers are employing pesticides judiciously, both in terms of quantity and timing of application. For instance, adhering to practices such as ceasing pesticide use at least 7-14 days prior to harvesting allows for proper degradation of the chemicals before the strawberries are collected[20]. Consequently, there is no residual presence of these pesticides in the strawberry samples analyzed. [17]

This study's findings are consistent with the research conducted by Jarupong Prasopsuk and colleagues, which analyzed pesticide residues in vegetables and fruits in the upper northeastern region of Thailand in the year 2014. Their analysis did not detect any pesticide residues in strawberry samples [18]. Additionally, a study conducted by the Research Center and Food Safety Institute, in the year 2020, evaluated pesticide residues in fresh strawberries. A total of 58 types of insecticides were analyzed in 5 strawberry samples, comprising 2 imported and 3 domestic samples. The results indicated the absence of insecticide residues in all strawberry samples[19]. Furthermore, a subsequent analysis of 67 insecticide residues, belonging to 4 groups, in 5 samples of fresh strawberries, conducted in the year 2023, revealed the presence of diazinon and omethoate in only one strawberry sample out of the five analyzed[7].

However, it is noteworthy that traces of residual pesticides are still occasionally detected[7]. The presence of these residual pesticides, although in low amounts, raises health concerns. Therefore, before consuming strawberries, it is advisable to thoroughly wash and clean them to reduce the risk of ingesting lingering pesticide residues.

Limitation

The present study examined the presence of residual pesticides from the organophosphate and carbamate groups using a testing kit. It is important to note that the results obtained from this field testing may differ from laboratory analysis.

Conclusion

No residues of organophosphate and carbamate chemicals were detected in any of the 48 samples of fresh strawberries tested.

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