The Study of Biosurfactant as a Cleaning Agent For Insecticide Residue in Leafy Vegetables*

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Abstract

Pesticides are used as the main tool for agricultural pest control. Many pesticides are, however, toxic substances and persistent in character. Concern over the pesticide residues in fruits and vegetables have led to the development of many clean up and analysis methods.

Biosurfactant was used in this study to explore the possible potential for cleaning up cypermethrin residue. Lettuce was chosen as a representative for leafy veggy crop. Amounts of biosurfactant and the contact times needed to reduce cypermethrin residue in lettuce to below maximum residue limit of 2 ppm to make it safe for consumers were determined. Salt, vinegar and potassium permanganate are also tested for comparing the cypermethrin neutralizing effect on lettuce with biosurfactant. A simple method to determine cypermethrin residue is developed based on Ninhydrin test which is the reaction of Ninhydrin reagent with free nitrogen to form a color product which can be detected by spectrophotometer.

With the initial pesticide concentration of 100 ppm, the amount of biosurfactant that need to be used is 10 ppm of biosurfactant for 25 minutes, 15 ppm of biosurfactant for 15 minutes and 20 ppm of biosurfactant for 5 minutes. With the initial pesticide concentration of 10 ppm, the amount of biosurfactant that need to be used is 2 ppm for 3 minutes, 3 ppm for 3 minutes, and 4 ppm for 1 minutes minute. Adding KMnO₄ together with biosurfactant will cause the synergistic effect that will further enhance the efficiency of this cleaning method. From this study we concluded that biosurfactant can be used as an effective agent to clean up pesticide similar to the group of cypermethrin on leafy vegetables.

Keywords: Biosurfactant, cleaning agent, insecticide residue, leafy vegetables.

Introduction

The economic growth of Thailand largely depends on the agriculture-based industry. The agricultural sector is very important to Thailand as a source of export revenue and a part of a strategy to alleviate poverty through employment and subsistence.

In the past decades, vegetable production in Thailand has experienced considerable problems: increased competition for land and labor from rapid urbanization and industrialization has put pressure on traditional peri-urban vegetable production sites.

Agricultural chemicals, including pesticides have made significant contributions to the efficiency and productivity of Thai agriculture, making sure that the rise in Thai food production has kept ahead of its growing population. Responsible pesticide use delivers important benefits to agriculture and in turn society, such as: year round availability of agricultural produce; improved quality and variety; and reduced production costs which in turn results in lower prices for consumers.

Pesticides are used in Thailand as the main tool for pest control. According to the Agricultural Regulatory Division, Thailand imported 29,463 tons of pesticides in 1990. Of the total imports, herbicides constituted approximately 50%, insecticides 31.8%, whereas fungicides and miscellaneous pesticides constituted the rest (Chunyanuwat 2005). The intensity of pesticide application in vegetable and fruit production is higher than in other crops.

Many pesticides are, however, toxic substances and persistent in character. Pesticide residues in food and crops are a direct result of application of pesticides to crops. It causes serious health damage and even death. In 2003, the number of poisoning incidents was 2,342 cases or 3.72 per 100,000 capita (Bureau of Epidemiology, Department of Disease Control, Ministry of Public Health) (Chunyanuwat 2005). Survey on consumer perceptions of food safety indicated that pesticide residues are one of the most common food safety concerns.

The Pesticide Residues Research Sub-Division, Office of Agricultural Production Science Research and Development is responsible for pesticide residues research and monitoring in Thailand. In the year 2003, this Office analyzed 23,525 samples of food crop and found pesticide residue in 4,885 samples (20.76 percent). Out of this, residues exceeded the maximum residue limits (MRLs) in 1,007 samples (4.28 percent) and 3,878 samples (16.48 percent) did not exceed MRLs. The reason cypermethrin is chosen in this study is because pesticide residues most often found on leafy vegetable were cypermethrin with concentration of 0-8.48 ppm. (Chunyanuwat 2005).

According to Chia Thai Co. Ltd., cypermethrin is the pesticide which is mostly used in leafy veggy crop with the volume of 1,000 metric tons per year. Besides, the trend of reducing the use and banning of organophosphates pesticide in Thailand, pyrethroid in general (cypermethrin), specifically are becoming more popular and a more important class of insecticide. According to Codex Alimentarius annex by FAO/WHO, cypermethrin maximum residue limit for lettuce is 2 ppm. Acceptable Daily Intake (ADIs) and percentages of the ADI based on the highest daily intakes of Thais are published by Vongbuddhapitak et al. (2002) as shown in Table 1.

Concern over the pesticide residues in fruits and vegetables have led to the development of many clean up and analysis methods. Biosurfactants are surface-active substances synthesized by living cells. They have the properties of reducing surface tension, stabilizing emulsions, promoting foaming and are generally non-toxic and biodegradable. Interest in microbial surfactants has been steadily increasing in recent years due to their diversity, environmentally friendly nature, possibility of large-scale production, selectivity, performance under extreme conditions and potential applications in environmental protection (Banat et al. 2000; Rahman et al. 2002). Biosurfactants enhance the emulsification of hydrocarbons, have the potential to solubilise hydrocarbon contaminants and increase their availability for microbial degradation. Other applications include herbicides and pesticides formulations, detergents, health care and cosmetics, pulp and paper, coal, textiles, ceramic processing and food industries, uranium ore-processing and mechanical dewatering of peat (Banat et al. 2000; Rahman et al. 2002; Ron and Rozenberg 2001). Many of the known biosurfactant producers are also hydrocarbon-degrading (Rouse et al. 1994; Willumsen and Karlson 1997; Volkering et al. 1998). Several microorganisms are known to synthesise surface-active agents, most of them are bacteria and yeasts (Banat 1995; Kim et al. 2000).

When grown on hydrocarbon substrate as the carbon source, these microorganisms synthesise a wide range of chemicals with surface activity, such as glycolipid, phospholipid and others (Muriel et al. 1996; Desai and Banat 1997).

The objective of this study was to explore the possibility of using biosurfactant for cypermethrin residue to clean leafy vegetable and develop a simple method to analyze the amount of cypermethrin residue in solution. Lettuce was used as the representative of leafy vegetable in this study because of its popularity among the most regularly consumed veggies.
Table 1. Acceptable daily intake (ADIs) and percentages of the ADI based on the highest daily intakes of Thais (Vongbuddhapitak et al. 2002).

<table>
<thead>
<tr>
<th>Pesticides</th>
<th>ADI, mg/ kg/ body weight</th>
<th>ADI, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alorin &amp; dieldrin</td>
<td>0.0001</td>
<td>5</td>
</tr>
<tr>
<td>BHC</td>
<td>NA\textsuperscript{a}</td>
<td>-</td>
</tr>
<tr>
<td>Carbaryl</td>
<td>0.003</td>
<td>0.4</td>
</tr>
<tr>
<td>Carbofuran</td>
<td>0.002</td>
<td>0.25</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Cyhalothrin</td>
<td>0.002</td>
<td>0.2</td>
</tr>
<tr>
<td>Cypermethrin</td>
<td>\textbf{0.05}</td>
<td>\textbf{0.1}</td>
</tr>
<tr>
<td>DDT</td>
<td>0.02</td>
<td>0.21</td>
</tr>
<tr>
<td>Dicrotophos</td>
<td>NA\textsuperscript{a}</td>
<td>-</td>
</tr>
<tr>
<td>Dimethoate</td>
<td>0.004</td>
<td>1.05</td>
</tr>
<tr>
<td>Endosulfan</td>
<td>0.006</td>
<td>1.6</td>
</tr>
<tr>
<td>Fenvalerate</td>
<td>0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>Heptachlor</td>
<td>0.0001</td>
<td>7</td>
</tr>
<tr>
<td>Lindane</td>
<td>0.001</td>
<td>0.1</td>
</tr>
<tr>
<td>Malathion</td>
<td>0.3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Methamidophos</td>
<td>0.004</td>
<td>6.38</td>
</tr>
<tr>
<td>Methomyl</td>
<td>0.03</td>
<td>0.05</td>
</tr>
<tr>
<td>Mevinphos</td>
<td>0.0008</td>
<td>0.25</td>
</tr>
<tr>
<td>Monocrotophos</td>
<td>0.0006</td>
<td>2</td>
</tr>
<tr>
<td>Parathion</td>
<td>0.004</td>
<td>0.02</td>
</tr>
<tr>
<td>Parathion-methyl</td>
<td>0.003</td>
<td>0.43</td>
</tr>
<tr>
<td>Permethrin</td>
<td>0.05</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Profenofos</td>
<td>0.01</td>
<td>0.2</td>
</tr>
</tbody>
</table>

**Materials and Method**

**Materials and Reagents**

- Cypermethrin (Thai On Inter-Pack Ltd., Co.)
- Lettuce (Crisphead cultivar)
- Biosurfactant (Jeneil Biosurfactant Company JBR425)
- Ninhydrin
- KMnO\textsubscript{4}
- NaOH
- Salt
- Vinegar
- Spectrophotometer (Genesys 5, Milton Roy)

**Method**

**Preparation of Standard Curve**

Prepare 9 test tubes and
Label 0, 2, 5, 10, 15, 20, 50, 100, 150 ppm.

Add 0, 0.1, 0.25, 0.5, 0.75, 1, 2.5, 5, 7.5 μL of cypermethrin\textsuperscript{10% w/v}, which is equivalent to:

0, 0.01, 0.025, 0.05, 0.075, 0.1, 0.25, 0.5, 0.75 μg of cypermethrin

0, 2, 5, 10, 15, 20, 50, 100, 150 ppm

Transfer to cuvette and measure absorbance at 485 nm and 590 nm by spectrophotometer.

\textsuperscript{a}Not applicable.
**Study of Current Washing Method**

Prepare cypermethrin solution 100 ppm (200µl of cypermethrin 10% in 200 ml of water. Add 1 ml of NaOH and Ninhydrin reagent.

- Crisphead Lettuce
- Cypermethrin solution 100 ppm

Prepare washing solutions:
- Salt (dissolve 0.12g of salt in 200 ml of water);
- Vinegar (use 2 ml of 5 % distilled vinegar in 200 ml of water;
- KMnO₄ (0.01N, dissolve 0.951 g of KMnO₄ in 500 ml of water).

- Biosurfactant (10 ppm, 2 µl of biosurfactant in 200 ml of water)

Wash the contaminated lettuce in these solutions for 20 minutes.

Add few drops of Ninhydrin and heat.

Transfer to cuvette and measure absorbance at 485 nm and 590 nm by spectrophotometer.

**Study Effect of Concentration of Biosurfactant in Reducing Cypermethrin Residue**

**A. Initial Pesticide Concentration 100 ppm**

Prepare pesticide solution at 100 ppm by adding 200 µl of cypermethrin 10% in 200 ml of water. Add 1 ml of NaOH and Ninhydrin reagent.

- Crisphead lettuce
- Cypermethrin solution 100 ppm

- 1 µl 5 ppm
- 2 µl 10 ppm
- 3 µl 15 ppm
- 4 µl 20 ppm

Biosurfactant

200 ml of water
Prepare biosurfactant solution at 5, 10, 15, 20 ppm by adding 1, 2, 3 and 4 µl of biosurfactant in 200 ml of water.

Wash the contaminated lettuce in these solutions for 5 minutes.

Add few drops of Ninhydrin and heat.

Transfer to cuvette and measure concentration of pesticide residue by measure absorbance at 485 nm and 590 nm by spectrophotometer.

**B. Initial Pesticide Concentration 10 ppm**
Prepare pesticide solution at 10 ppm by adding 20 µl of cypermethrin 10% in 200 ml of water. Add 1 ml of NaOH and Ninhydrin reagent.

**Study the Effect of Concentration and Contact Time in Reducing Cypermethrin Residue**

**A. Initial Pesticide Solution 100 ppm**
Prepare pesticide solution at 100 ppm by adding 200 µl of cypermethrin 10% in 200 ml of water. Add 1 ml of NaOH and Ninhydrin reagent.
Take vegetable out at 5, 10, 15, 20 and 25 min.

Add few drops of cypermethin and heat.

Transfer to cuvette and measure concentration of pesticide residue by measure absorbance at 485 nm and 590 nm by spectrophotometer.

**B. Initial Pesticide Concentration 2 ppm**
Prepare pesticide solution at 10 ppm by adding 20 μl of cypermethrin 10% in 200 ml of water. Add 1 ml of NaOH and Ninhydrin reagent.

Study of Synergistic Effect of KMnO₄ and Biosurfactant
Prepare pesticide solution at 100 ppm by adding 200 μl of cypermethrin 10% in 200 ml of water. Add 1 ml of NaOH and Ninhydrin reagent. Prepare: biosurfactant 10 ppm (4 µl in 400 ml of water); KMnO₄ 0.01 N (0.951 g in 600 ml of water).
Wash for 20 minutes.

Add few drops of cypermethrin and heat.

Transfer to cuvette and measure concentration of pesticide residue by measure absorbance at 485 nm and 590 nm by spectrophotometer.

Results and Discussion

A standard method for pesticide residue analysis is chromatography followed by mass spectrometry. It is the process of separating the component in the mixture for analysis (Ștajnbaher and Župančič-Kralj 2008). The component of interest is dissolved in solvent (mobile phase). The mobile phase is then forced through an immobile, immiscible stationary phase in the column. The principle of separation is based on the difference in solubility (Pan et al. 2008). The standard method in detecting cypermethrin residue is gas chromatography (González-Rodríguez et al. 2008). In this study, we apply the method of detecting cypermethrin residue from the work of Drochioiu et al. (2004) using spectrophotometric to determine the trace element of cyanide with ninhydrin. A simple method to determine cypermethrin residue is developed based on Ninhydrin test (see Fig. 1) which is the reaction of Ninhydrin reagent with free amino groups to form a deep purple to blue color solution product which can be detected quantitatively by spectrophotometer (Drochioiu et al. 2004).

Drochioiu et al. (2004) demonstrated that the reaction of cyanide and cyano group with ninhydrin initiate strong fluorescence product which can be measured at the wavelengths of 485 nm and 590 nm by spectrophotometer. The sensitivity of the test is increased with heat. The advantage of this method is that it is quick and simple. This method has many advantages such as high sample throughput, the use of smaller amounts of organic solvent and the use of no chlorinated solvents, Very little lab ware is used and therefore the safety for lab workers is increased (Nguyen et al. 2008).

Fig. 1. Ninhydrin test.

Comparing Current Methods for Cleaning Vegetables

Four different treatments: salt, vinegar, KMnO₄ and biosurfactant were tested for their effectiveness of reducing pesticide from lettuce. The sample was left in the 4 test compounds for 5 minutes. The result of 3 characters: color, texture and cypermethrin residue were observed and recorded as shown in Table 2. Color is observed using Munsell book of color. BG is hue and it is short for Blue-Green. Amounts of cypermethrin residue detected at OD485, OD590 and the average
between the 2 wavelengths were compared and plotted in Fig. 2. The results are synchronized well in all cases of plotting. Therefore, in the future experiment only the average value of wavelengths OD485 and OD590 will be used for graph plotting.

Table 2. Result of different washing treatments.

<table>
<thead>
<tr>
<th></th>
<th>Salt</th>
<th>Vinegar</th>
<th>KMnO₄</th>
<th>Biosurfactant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color before</td>
<td>5 BG8/4</td>
<td>5 BG8/4</td>
<td>5 BG8/4</td>
<td>5 BG8/4</td>
</tr>
<tr>
<td>Color after</td>
<td>2.5 BG8/4</td>
<td>2.5 BG8/6</td>
<td>2.5 BG8/8</td>
<td>2.5 BG8/8</td>
</tr>
<tr>
<td>Texture</td>
<td>Hard</td>
<td>Moderate</td>
<td>Soft</td>
<td>Soft</td>
</tr>
<tr>
<td>OD at 485 nm</td>
<td>0.055ᵃ</td>
<td>0.001ᵇ</td>
<td>0.019ᶜ</td>
<td>0.015ᵈ</td>
</tr>
<tr>
<td>Cypermethrin</td>
<td>5</td>
<td>1.4356</td>
<td>2.2356</td>
<td>2.0578</td>
</tr>
<tr>
<td>residue (ppm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OD at 590 nm</td>
<td>0.045ᵃ</td>
<td>0.000ᵇ</td>
<td>0.013ᶜ</td>
<td>0.010ᵈ</td>
</tr>
<tr>
<td>Cypermethrin</td>
<td>5.2098</td>
<td>1.2583</td>
<td>2.972</td>
<td>2.7622</td>
</tr>
<tr>
<td>residue (ppm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Salt is a very common ingredient in food processing. Salt preserves food by inhibiting microorganisms. However, it is not very effective in reducing cypermethrin residue (Fig. 2). The amount of cypermethrin residue after washing in salt solution is 5 ppm which is still much higher than the ADIs safety limit. Also, washing lettuce in salt solution will make the leave texture turned hard and the color is dull. Therefore salt is not recommended as a washing compound for leafy vegetable.

Vinegar is a mild acid. It is used in preparing salad and it is used in fermented vegetable products. In Fig. 2, vinegar shows a very effective reduction of cypermethrin residue. However, some people may not like the smell of vinegar. However, only 1.4356 ppm Cypermethrin residue left after washing in vinegar solution which is well below the maximum residue safety limit of 2 ppm. The result from Fig. 2 shows that KMnO₄ and biosurfactant are also effective in reducing cypermethrin residue. Biosurfactant is more effective than KMnO₄ and also provided a bright color on the lettuce after the treatment. This will benefit the fresh cut industry greatly (Fernández-Moreno et al. 2006). In this experiment, all the samples were washed in the treatment for 20 minutes.
Effect of Concentration of Biosurfactant

This experiment is conducted in two conditions: the first condition was carried on an industrial scale and the second condition was carried on a household scale. In the industrial scale, the vegetable is harvested directly from the field. The cypermethrin concentration that the farmers spray in the field is 100 ppm and we assume that the industrial scale has to manage with this high scale of cypermethrin residue. For the household scale, because there is a grace period between harvest to the market, and the market to the consumer, during this period of transformation the cypermethrin residue on the vegetable will be decomposed to a lower level. Therefore, we conduct the experiment for household scale with initial cypermethrin concentration at 10 ppm. In this experiment, we vary the concentration of biosurfactant used for the washing of industrial scale (Fig. 3) and household scale (Fig. 4) cypermethrin residue from 5 to 20 ppm. All the washing times were maintained at 5 minutes.

The cypermethrin concentration used for the industrial scale was significantly reduced to the safe level below 2 ppm (1.84 ppm) when washed with biosurfactant concentration at 20 ppm as shown in Fig. 3.

The cypermethrin concentration used for the household scale was 10 ppm. The concentration of biosurfactant used for washing vegetable was varied from 1 to 5 ppm and the washing time was 1 minute. At 5 ppm of biosurfactant used, the pesticide residue significantly reduced to the safe level below 2 ppm (1.62 ppm) as shown in Fig. 4.

Fig. 3. Effect of concentration of biosurfactant at initial pesticide concentration of 100 ppm (5 minute washing time).

Fig. 4. Effect of concentration of biosurfactant at initial pesticide concentration of 10 ppm (1 minute washing time).
Interaction Effect of Concentration of Biosurfactant and Contact Time

This experiment is also conducted in two conditions like the previous experiment. However, the difference is that there are 2 factors of variation in this experiment: concentration and time instead of only variation in concentration as in the previous experiment.

Initial Concentration of Pesticide 100 ppm

There are three varying levels of concentrations in this experiment: 10 ppm, 15 ppm and 20 ppm. The contact times are varying at 5, 10, 15, 20, 20 and 25 minutes. Contact time is the time for which the vegetables are left in the washing solution. The results are shown in Fig. 5: concentration and contact time have a significant effect in reducing cypermethrin residue. Using 20 ppm of biosurfactant, the cypermethrin residue was reduced to safe level after 5 minutes, and the use of 15 ppm of biosurfactant resulted in 15 minutes, while 10 ppm of biosurfactant required 25 minutes, respectively.

![Fig. 5. Interaction effect of concentration of biosurfactant and contact time (initial 100 ppm cypermethrin).](image)

Initial Concentration of Pesticide 10 ppm

There are three varying levels of concentrations used in this experiment: 2 ppm, 3 ppm and 4 ppm. The contact times are varying at 1, 2, 3, 4, and 5 minutes. The results are shown in Fig. 6. Biosurfactant at 20 ppm cypermethrin residue level reaches the safety level after 5 minutes.

Using 4 ppm of biosurfactant, the cypermethrin residue was reduced to safe level after 1 minute, and using 3 ppm of biosurfactant resulted in 3 minutes, while 2 ppm of biosurfactant also required 3 minutes, respectively. Therefore, we can conclude that concentration and contact time have interaction effect on cypermethrin residue. Therefore, form the results of both previous and current experiments we conclude that concentration and contact time have interaction effect on the cypermethrin residue clean up process.
Study of Synergistic Effect of KMnO₄ and Biosurfactant

This experiment was set up to study the synergistic effect of biosurfactant and KMnO₄ which means that when biosurfactant and KMnO₄ combine together in a solution, they will work together in reducing the cypermethrin residue. Indeed, the result from Fig. 7 clearly shows that when combining biosurfactant and KMnO₄ together in the solution at the ratio of 1:1 (KMnO₄: biosurfactant), they work more effectively than either KMnO₄ or biosurfactant when being used alone.
Conclusion

From the results of this study, we can conclude that biosurfactant can be used as a reagent for the reduction of cypermethrin residue as effective as KMnO₄ and vinegar. But biosurfactant will have the benefit over vinegar and KMnO₄ in the aspect that it will not provide a bad smell and will result in a bright color on the lettuce after the treatment.

With the initial pesticide concentration of 100 ppm, the amount of biosurfactant that is needed during the treatment to reduce the cypermethrin residue to a safe level below 2 ppm is 10 ppm of biosurfactant for 25 minutes, 15 ppm of biosurfactant for 15 minutes and 20 ppm of biosurfactant for 5 minutes. With the initial pesticide concentration of 10 ppm, the amount of biosurfactant that need to be used is reduced to 2 ppm for 3 minutes, 3 ppm for 3 minutes, and 4 ppm for 1 minutes. Adding KMnO₄ together with biosurfactant will cause a synergistic effect that will further enhance the efficiency of this cleaning method. From this study, we concluded that biosurfactant can be used as an effective agent to clean up insecticide on leafy vegetable.

References


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