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The Determination of Profenofos Insecticide Residue Cabbage (*Brassica oleracea* L.) by Using Gas Chromatography

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ABSTRACT

The determination of profenofos insecticide residue in cabbage (Brassica oleracea L.) by using gas chromatography-flame photometric detector (FPD) had been investigated. The cabbage was collected from Koto Baru area, Tanah Datar district, West Sumatra. The results showed that the cabbage was contain profenofos residue. The unwashed cabbage, washed with water, and washed with detergent has an average residue 0.220, 0.075, and 0.068 (ppm), respectively. The residue levels from these sample do not exceed the Maximum Residue Limit (MRL) that established by Indonesian National Standard (SNI) and the Regulation of the Ministry of Agriculture number 88 in 2011 that is 1 (ppm).

Keywords: Pesticide residue, Profenofos, Organophosphate, *Brassica oleracea*

INTRODUCTION

One of food sources that plays big role in maintaining health is vegetables. In order to fulfill the needs of vegetables, peoples do many efforts in upgrading the production, but often this efforts constrained with pest attacks and diseases that caused crop loss or shrinkage of expected crops [1]. Studies showed that pest attack on vegetables plants is high enough, some of them is attacks on cabbage that caused crop loss until 100% [2]. To be able to overcome pests and diseases attack there is some alternative control method and most used methods is usage of pesticide.

Ministry of Agriculture Republic of Indonesian [3] gave recommendation and suggested usage of pesticide from organophospate group. Mostly farmers like pesticide from this group because it has strong and fast eradicate ability [4]. Organophospate is insecticide that classified as ester phosphate acid or thioposphate acid. Usually this used as insecticide because of the acute toxicity characteristic towards vertebrate like fishes, birds, lizards and mammals. Usage of organophospate become more encouraged in agriculture because this compound is easier to vanish in environment compared to dikloro difenil trikloroetan (DDT) [5]. Profenofos is one of organophospate insecticide with Maximum Residue Limit (MRL) that established by Indonesian National Standard (SNI) and the Regulation of the Minister of Agriculture number 88 in 2011 [6] that is 1 (ppm) on cabbage.

Result from Munarso *et al.* research (2006) [7] showed that residue from organophospate pesticide, carbamate and organochlorine group that found in cabbage from Malang and Cianjur, west Java. Residues found are chlorpirifos, metidation, malation (organophospate group), carbaril (carbamate group) and endosulfan (organochlorin group). Based on Alen *et al.* research (2013) [8], almost all kind of vegetables from vegetables center in Padang Luar, Agam District, West Sumatra market positive contained pesticide residue. On cabbage, found pesticide residue from organophospate group (terbufos), carbamate group (carbaril, thiobencarb, benthiocarb) and fungicide (triadimefon). Pesticide residue is specific substance that consist in agriculture crop for food or animal woof, either as direct effect or indirect effect from pesticide usages. This term also including pesticide derivate compound, like conversion-result compound, metabolite, reaction-result compound and pollutant compound that mostly toxic [9].

Pesticide residue in small amount can caused indirect effect towards the consumer, but in long period this can caused health disturbance some of them is nerve system and enzyme metabolism disturbance. Pesticide residue will transported along food and accumulated in body tissues that contain lipid. Accumulation of pesticide residue on human can corrupt liver function, nerve system, decreasing immune system, occurrence of inviable, allergy and cancer [7].

Because of that, in this research had been done profenofos insecticide residue checking of cabbage (*Brassica oleracea* L.) on Koto Baru, Tanah Datar district, West Sumatra, Indonesia. More than that, in order to overcome free market in 2015 through ASEAN Economic Society (MEA), consumer protecting issues become one part of ASEAN Economic Community with strengthen *consumer protection* in ASEAN through forming of *ASEAN Coordinating on Costumer Protection* (ACCP). This thing become challenge for monitoring and application of national consumer protecting area so monitoring and evaluation towards all the thing connected with consumer safety implied in pesticide residue found in vegetables eat by society. We need to be sure that vegetables that eaten has residue degree lower than BMR or even clean from pesticide residue so it can be advantages for consumer and also farmers to be defending free market next year.

MATERIALS AND METHODS

This research has been done from April-August 2014 at Laboratory of Pharmacy Chemistry Analysis and Central Laboratory of Faculty of Pharmacy, Andalas University along with Pesticide Laboratory Analyst Center for the Protection of Food Crops and Horticulture West Sumatra.

Equipment:

Equipments used in this research are blender, analytical scale, knife, Erlenmeyer 250 ml, Sonikator (Elma®), Beker glass 100 ml (Pyrex®), measuring glass 100 ml (Pyrex®), Volumetric flask 10 ml (Pyrex®), vial, filter paper (Toyo Filter Paper®), funnel, aluminium foil, spatel, pipette, volumetric pipette, micro pipette, parchment paper, oven, gas chromatography (GC-2010 AF Shimadzu®) flame photometric detector.

Materials

Materials used is unwashed cabbage, water-washed cabbage, vegetable detergent-washed cabbage, ethyl acetate, isooctana, natrium sulphate anhydrate *p.a*, before used heated until 200^0C for 3 hours, vegetable detergent (Mama lemon®), profenofos pesticide as standard.

Samples Collection

Samples are cabbage from farmers in Koto Baru, Tanah Datar District, West Sumatra. Cabbage plant used for this residue testing has been cared by farmers with pesticide spraying. Plants sprayed by farmers 3 times a week for 3 months. Kind of pesticide used by farmers are Profenofos (Curacron®) as insectisida, Dimetomorf (Acrobat®) as fungicide and Propineb (Antracol®) as fungicide. Pesticide that will assayed is profenofos from organophospate group. Samples were taken at five plots in one farming area. One cabbage taken in the central part of third plot. Then, from each of the plot is taken one cabbage, so 5 cabbages are collected.

Sample Preparation

Cabbage cleaned (not washed), then cut into one-quarter part, and leaf layers are removed. Cut the cabbage leaves and put into a container then make sure it homogenous. Samples divided into three group based on different treatments, i.e cabbage sample unwashed (cabbage A), cabbage washed with water (cabbage B), and cabbage washed with detergent (cabbage C). The washing process is done for 1 minute.

Samples Extraction

Samples that already cut weighed as much as 50 g, put into a blender then 50 ml water added, this mixture crushed for 3 minutes. Put the blender result into the Erlenmeyer (250 ml) and add 100 ml of ethyl acetate. Then, Erlenmeyer sealed and sonicated for 10 minutes. After that, pour sonication result into Erlenmeyer, add 20 gram anhydrous Na_2SO_4 and stir, then pour extraction result into Erlenmeyer.

Extraction result filtered with a funnel and filter paper. Result from filtering put into Erlenmeyer and ethyl acetate added until reach volume 100 ml. At this point samples ready to injected into gas chromatography instruments.

Extraction for Recovery

For recovery, cabbage samples that treated using pesticide other than profonefos by farmers are used. Pesticide used are insecticide Spinoteram (Endure®) and Sipermetrin (Capture®). Samples were cut and weighed for 50 g and put into Erlenmeyer (250 ml). Pipette 1 ml profenofos standard solution 10 ppm then it is added into Erlenmeyer containing the sample. Cover it tightly and let it stand for 2 hours. After that put in samples into blender and add 50

ml of water, crushed it for 3 minutes. The next process is same as the sample extraction.

Calculation % Recovery = $\frac{X}{k} \times 100\%$
Where,
x = earned value
k = known value

Preparation of Standard Solution
Pipette 1 ml of profenofos standard solution 10 ppm and put into volumetric flask (10 ml). Then add isooctane solvent into the flask until the volume reach 10 ml.

Residue Measurement by Gas Chromatography
Extraction result taken 1 µL, then injected with 3 times replication (triple) at the injecting gate using flame phototometric detector and observed the result on the chromatogram. Gas chromatography conditions are:
Detektor FPD (filter P),
Column Name : RTX-5
Column Lengh : 30 meter
Column Flow :1.65 ml/minute
Injection Temp : 250^0C
Carrier Gas : N_2/*Air*
Injection Pressure : 127.0 kPa
Detector Temp : 300^0C
H_2 *Flow* : 80 ml/minute
Air Flow : 120 ml/minute

Data Processing
From chromatogram data result can be calculated pesticide residue levels remaining. Levels of pesticides stated in µg/g or ppm for each sample and can be calculated using following equation:

$$R = \frac{\frac{a}{b} X\ c\ x \frac{d}{e} x\ f}{g}$$

Whereas:
A : Sample area (unit area)
b : Standard area (unit area)
c : Standard concentration (ηg/µl)
d : Standard volume injected (µl)
e : Extract volume injected (µl)
f : Final volume from extraction
g : Sample weight in gram (10)

RESULTS AND DISCUSSION

In this research, has been examined profonefos insecticide residues in cabbage (*Brassica oleracea* L.) in Koto Baru, Tanah Datar District, West Sumatra. This is done because the results from previous studies, it was found that almost all kinds of vegetables, including cabbage, located in vegetables center at Padang Luar market positive for pesticide residues [8] and this area is one of the producers for cabbage plant that distributed to various markets, included vegetables center at Padang Luar, Agam District, West Sumatra market.

In this study, method modification has been done from Yulnefi research (2010) [10], including: extraction method used is sonication extraction. Sonication extraction method utilize ultrasonic wave with 42 kHz frequency that can accelerate contact time between sample and solvent even in room temperature. This caused mass movement process from bioactive compound from inside of plant cell into solvent became much faster. Samples sonicated for 10 minutes using solvent ethyl acetate, best extraction process is using solvent ethyl acetate and sonicated for 10 minutes.

Beside the method of extraction, addition time for Na_2SO_4 anhydrous also distinguished from the previous studies [10]. In this study, Na_2SO_4 anhydrous added into ethyl acetate extract, so Na_2SO_4 anhydrous can bind water particles contained in solvent. Whereas, in previous study addition of Na_2SO_4 anhydrous done at crushing stage with blender. This process can not give optimal result, because allegedly Na_2SO_4 anhydrous used can not work optimally if added when samples will be crushed in blender.

Method used in this study has some advantages, some of them are: the processes are easier and faster as well as the using of samples and solvent in small amount so this study can be done at a low cost.

The precision of the method used in this study can be seen from the price recovery of profenofos residue obtained. Accuracy is a measure of the degree of closeness of the result of the analysis with the actual analyte concentration. Prerequisites of this method is has a good precision when the percent recovery was in the range of 80-115%. Research result showed that profenofos residue percent recovery in cabbage is 112%.

This study has quite good analysis method accuracy, because according to Wonnacott that the maximum relative standard deviation (coefficient of variation) allowed is 20%. The smaller the relative standard deviation of the accuracy of the analysis method, the better. Data for standard deviation can be seen in Table 1.

Table 1. Calculation Result of Standard Deviation (SD) and % of Relative Standard Deviation (RSD) of cabbage

Sample	Concentration (ppm)	Average (x)	$(xi-x)^2$	SD	%RSD
Cabbage A	0,196	0,220	$5,76.10^{-4}$	0,0154	7,02%
	0,216		$1,6.10^{-5}$		
	0,249		$8,41.10^{-4}$		
Cabbage B	0,075	0,075	0	$5,77.10^{-4}$	0,77%
	0,076		1.10^{-6}		
	0,074		1.10^{-6}		
Cabbage C	0,064	0,068	$1,6.10^{-5}$	$3,05.10^{-3}$	4,49%
	0,074		$3,6.10^{-5}$		
	0,066		4.10^{-6}		
Average		0,121		$6,34.10^{-3}$	5,24%

Retention time and area of profenofos used as a standard for pesticide residue examination are 25.680 minutes and 3939874 unit area. Can be seen in Figure 1. Pesticide residue examination result showed that profenofos insecticide (organophospate group) positive found in cabbage samples. Research data can be seen in Table 2.

Table 2. Profenofos Insecticide Residue Concentration on Cabbage Data

Sample	Retention time (minute)	Wide area	Concentration (ppm)
Cabbage A (unwashed)	25.682	387517	0,196
	25.682	426789	0,216
	25.682	409692	0,249
	Concentration average		0,220
Cabbage B (water-washed)	25.682	147730	0,075
	25.683	149702	0,076
	25.682	146501	0,074
	Concentration average		0,075
Cabbage C (vegetables-cleansing detergent washed)	25.679	125993	0,064
	25.671	146413	0,074
	25.680	130683	0,066
	Concentration average		0,068
Recovery	25.676	430962	0,218
	25.676	435212	0,110
	25.676	459710	0,233
	Concentration average		0,187

Average pesticide residue in unwashed cabbage A is 0.220 ppm. Chromatogram result can be seen in Figure 2. Pesticide residue in cabbage not only found in Koto Baru area, Tanah Datar District, West Sumatra, research by Munarso *et al.* (2006) [7] also showed that organochlorine group positive found in cabbage from Malang and Cianjur but still under Maximum Residue Limit (MRL). As for the type of residues found, those are klorpirifos, metidation, malation (organophospate group), karbaril (carbamate group) and endosulfan (organochlorine group).

Average pesticide residue for cabbage B washed with water is 0.075 ppm. Chromatogram result can be seen in Figure 3. There is some decrement of profenofos residue concentration in cabbage B washed with water from unwashed cabbage A as much as 66%. This happened because pesticide remained at vegetables will dissolved when washed with water. Profenofos nature is soluble in water with ratio 1:20. According to Maruli *et al*. research (2012) [11], decrement of highest residue concentration from organophospate group found in cabbage washed with water and boiling which is equal 76.93%. Although decrement of residue is large enough, allegedly the nutrient contents in boiled cabbage can be reduced.

Detergent-washed cabbage also showed reduction of residue levels. Averages pesticide residues of cabbage C washed with vegetables-washing detergent is 0.068 ppm. Chromatogram result can be seen in Figure 4. There is some reduction of profenofos residue level in unwashed cabbage C compared to unwashed cabbage A by 69%.

Remained pesticide residue found in washed cabbage can caused by several things, those are use of some pesticide that mixed together in one time application, the use of uncontrolled pesticide dose, and unappropriated distribution time interval. It may be harmful for human health and environment.

Reduction of pesticide residue level happened in cabbage B washed with water and cabbage C washed with vegetables-washing detergent did not showed many differences, hence it is better to wash vegetables with water, because the usage of detergent can caused impact towards environment and health.

Reduction or degradation of pesticide residue can be caused by several factor i.e: Evaporation (Some kind of pesticide will reduced because it evaporated from plant surfaces), Mechanical and physical treatment (Pesticide reduced because it is dissolved in washing process), Chemical (Pesticide decreased/reduced/degradated because of chemical reaction/detergent washed).

Reduction of residue amount influenced by several factor, those are Solubility (pesticide residue can be dissolved in wash water. This is related with physical and chemical traits, that is solubility in water and pH of wash water), and Hydrolisis (pesticide residue can be hydrolyzed depend on amount of water available, pH and pesticide concentration).

Pesticide residue level found in this research did not exceed Maximum Residue Limit (MRL) that established by Indonesian National Standard (SNI) and the Regulation of the Minister of Agriculture of Republic of Ibndonesia, number 88 in 2011 that is 1 (ppm). Although the residue level still under the Maximum Residue Limit range, but it will be better if there is no pesticide residue in vegetables, especially in cabbage. So peoples can consume vegetables safely and do not threaten health. Maximum Residue Limit (MRL) is one of maximum concentration index from pesticide residue (set in mg/kg) that recommended legally in food and meat commodities. In addition to MRL, *Acceptable Daily Intake* (ADI) or the acceptable limit of body for a day also an international parameter need to be evaluated. Based on provisions by *Food Agricultural Organization* (FAO) and *World Health Organization* (WHO), ADI for profenofos is 0-0,03 mg/kg body weight [12].

General nature of organophospate pesticide is very readily degradable and has relatively short half-life so its residue found in plants just in small amount. Residue amount found is very related with its amount of usage in field, the fewer pesticides are used, residue in vegetables are also less. Pesticide residues in plants or vegetables are very depending on the time, manner and number of applications.

When organophospate insecticide exposed to someone, acethylcoline-esterase is blocked so there is accumulation of acethylcoline, acethylcoline dumped in the central nervous system will caused tremor, incoordination, and convulsions. In autonomous nervous system, this accumulation will caused diarrhea, uncontrollable urination, bronchial constriction and miosis [5]. Organophospate reaction and acetylacethylcoline-esterase. In this reaction, atom P will bind with atom O clusters of serine via phosphorylation reaction and created covalen bonding that will disturbed enzyme function.

Based on Regulation in Republic of Indonesia No.Per-03/Men/1986 article 2 paragraph 2a stated that to keep the undesirable effect, it is recommended that no more than 4 hours each day in a week respectively if used pesticide. Workers that managed pesticide must not have exposed more than 5 hours a day and 30 hours in a week. While WHO [13] established duration of a worker exposed to pesticide when they works for 5-6 hours per day and every week health examination must be held, including choline-esterase level in blood. In this condition, farmer's awareness and concerning about the dangers that they experienced also need to be done by doing intensive counseling, comprehensive and continuously.

CONCLUSION

Cabbage collected from Koto Baru market, Tanah Datar District, positive contains profenofos pesticide residue. Average concentration of insecticide residue on unwashed cabbage respectively 0.220; 0.075; and 0.069 ppm and this residue level not exceed Maximum Residue Limit (MRL) that established by Indonesian National Standard (SNI) and the Regulation of the Ministry of Agriculture number 88 in 2011 that is 1 (ppm).

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