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Determination of Profenofos Pesticide Residue in Tomato (*Solanum lycopersicum* L.) using Gas Chromatography Technique

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ABSTRACT

The determination of profenofos pesticide residue in tomato (Solanum lycopersicum L.) had been investigated by using Gas Chromatography technique with Flame Photometric Detector (FPD). The tomato was collected from Koto Baru, Tanah Datar, West Sumatera, Indonesia. The samples were divided into three groups: unwashed tomato, washed with tap water and washed with detergent. Samples were extracted once by ultrasonication for 10 minutes with 100 mL ethyl acetate. Results showed that the tomato contained profenofos pesticide residue. The unwashed tomato, washed with tap water and washed with detergent contains 0.159±0.0079; 0.070±0.0009 and 0.067 ±0.0016 ppm respectively. This data was obtained from the measurement as much as three times on each sample. The profenofos residue levels do not exceed the Maximum Residue Limit (MRL) that established by Indonesian National Standard (in ppm). Statistical tests with one-way ANOVA (SPSS 20.0) showed there was decrement in the levels of profenofos pesticide residues significantly between unwashed tomatoes, tomatoes washed with tap water and tomatoes washed with detergent ($P < 0.05$).

Keywords : residue, profenofos, gas chromatography, *tomato.*

INTRODUCTION

Tomatoes are one type of fruit that often consumed fresh, this fruit also traded a lot at supermarket and traditional market. Vitamin and mineral contents very beneficial in increase of nutrition and health. But, tomatoes production process often encounter pests and diseases attack. This condition can caused crop shrinkage or loss. The method control that most frequently performed by the farmers is usage of pesticide. The use of pesticide can leave any residues that could caused environmental pollution, human health disturbance and impede trade [1].

Pesticide usage could cause poisoning, both acute and chronic. Acute toxicity could resulted in sudden death. Acute toxicity measured based on lethal doses value. Chronic toxicity caused by low dose-exposure for long period or short period-exposure with chronic consequences. Chronic toxicity can be found in form of nerve and behavior disorder (neuro-toxic) or even formation of mutant [2,3].

Profenofos is one type of pesticide from organophospate group that mostly used for overcame pests attack on tomatoes plant. Research result of Munarso, Miskiyah and Wisnu (2009) note that profenofos residue was detected from all samples collection levels, i.e from farmer, seller and supermarket in Malang City, East Java, with highest concentration is 7.9 mg/kg [4]. Whereas, Purnama, Daud and Birawida (2013) research explained that profenofos residue concentration on tomatoes from Pasar Terong in Makassar, South Sulawesi, Indonesia is 0.015 ppm [5].

According to Alen et al. (2013) research, it can be concluded that tomato samples from Padang Luar vegetables centre, West Sumatra, Indonesia contained profonefos residues, triadimefon, jasmolin, prohydrojasmon and cinerin with profenofos residue concentration is 8.03 mg/kg [6]. High profenofos residue concentration and number of types pesticides residue were detected could caused by usage of some type of pesticide that mix with another type of pesticide. Another cause of this situation is doses of pesticide that is not appropriate with direction of use.

The pesticide usage on plants can be absorbed together with crop in form of residue that can be consumed by consumer. If pesticide residue consumed, it can endanger health. That is why a monitoring towards pesticide usage is needed. This situation can be obtained by fulfillment of pesticide Maximum Residue Limit (MRL), so it can assuring food security. Pesticide residue concentration control intended to prevent health disturbance caused by indigestion of food containing over-safe-limit dose pesticide.

Based on those issues above and result of previous research, then a research to determinate profenofos pesticide residue in unwashed, water-washed and detergent-washed tomato.

MATERIALS AND METHODS

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

Equipment's

Equipments were used in this study: blender, vial, aluminium foil, analytical scales, erlenmeyer (Pyrex®),beker glass (Pyrex®), measuring pipette, filter paper (Toyo filter paper®), measuring flask (Pyrex®),funnel, spatel, measuring glass (Pyrex®), Sonicator (Elma®), gas chromatography (Shimadzu® AF 2010).

Material

Unwashed tomato, water-washed tomato, vegetable detergent-washed tomato, ethyl acetate (Emsure®), natrium sulphate anhydrate (Emsure®), methanol (Emsure®), isooktana, standard solution of Profenofos 10 *ppm*.

Samples Collection and Preparation

Samples used in this research are ready-to-harvest tomato fruit from farmers at Koto Baru, Tanah Datar District, West Sumatra, Indonesia. During tomato planting, farmer was used some type of pesticide, i.e profenofos, simoxanil (fungicide) and isoprocarb. Concentration that will be determined is from profenofos pesticide (organophospate insecticide). Samples were taken at harvest time, when three days before that farmers sprayed plantation with pesticide. Samples were divided in several group based on different treatments, i.e unwashed, water-washed (at tap water for 30 seconds) and detergent-washed tomatoes (special detergent for vegetables).

Samples Extraction

As much as 300 g tomatoes from the preliminary treatment was cut and set to homogenous condition. After that, 50 g of the tomatoes were taken and blended for 3 minutes, and put into Erlenmeyer (volume 250 mL). Then, 100 mL of ethyl acetate were added into Erlenmeyer and sonicated for 10 minutes. The extract that is resulted from sonication then poured into other Erlenmeyer and 50 g natrium sulphate anhydrate added. Extract produced then poured into other Erlenmeyer and filtered with filter paper. The filter result put into Erlenmeyer and ethyl acetate were added until the solution reach 100 mL.

Extraction for Recovery

Fresh tomatoes weighed until 300 g, cut and set to homogenous condition. Then, it weighed as much as 50 g and put into Erlenmeyer (250 mL). As much as 1 mL profenofos standard solution 10 ppm then added into samples and it was covered with aluminium foil and incubated for 2 hours. Then, it put into blender and crushed for 3 minutes. Next processes are same as samples extraction. Calculation for recovery can be determined using this formula: [7]

$$\frac{\text{recovery concentration} - \text{samples concentration}}{\text{teoritical concentration}} \times 100\%$$

Standard Solution Preparation

As much as 1 mL profenofos standard solution 10 ppm were pipette and put into measuring flask 10 mL. Then isooctana solvent was added until reach 10 mL.

Extract Analysis with Gas Chromatography
The extraction result was measured for 1 μL using special syringe. Then it was injected into injecting gate using Flame Photometry Detector, the result can be observed on chromatogram. Gas chromatography condition were:

Detector	: FPD
Column name	: RTX-5
Column length	: 30 m
Column temperature	: 160^0 - 280^0 C
Injection port temperature	: 250^0 C
Carrier gas	: N_2/*air*
Injection port pressure	: 127.0 kPa
Detector temperature	: 300^0 C
H_2 flow	: 80.0 mL/min
Air flow	: 120.0 mL/min

Data Analysis
As much as 1 μL extract was injected on KG, which before arranged on maximum measuring condition for pesticide residue. Then, quantitative determination was done with comparing chromatogram area between standard solution and sample using formula below: [8]

$$R = \frac{\frac{Au}{Ab} \text{ x } Cb \text{ x } \frac{Vb}{Vu} \text{ x } Vakhir}{Wu}$$

where, R is pesticide residue concentration (ppm), Au is sample chromatogram area, Ab is standard chromatogram area, Cb is standard concentration (ŋg/ μL), Vb is volume of standard solution injected (μL), Vu is volume of sample solution injected (μL), Ve is volume of sample extract (μL) and Wu is sample weight (g).

RESULTS AND DISCUSSION

As shown in Table 1 and Table 2, profenofos pesticide residue was obtained on fresh tomatoes samples with average concentration as 0.159±0.0079 *ppm*. Whereas, in Munarso *et al.* 2009 [4] and Alen *et al* 2013 [6] research, profenofos residue level in tomatoes were higher, the value are 7.9 mg/kg and 8,03 mg/kg. Presumably this condition occurs because intense usage of profenofos pesticide by the farmers, samples collection time that closer to last spraying-time, and also different location of samples collection. Low profenofos pesticide residue level could be caused by samples time collection in wet season. Purnama *et al.* 2013 [5] notes that pesticide residue also influenced by several factors like persistent or non-persisten type of pesticide, pesticide application technique, climate and weather. Washery by rain could caused in decreasing of pesticide residue. Pesticide is more degradable by environment. Organophospate insecticide is highly toxic, but can degraded quickly in the environment. So, it can be said that organophospate group has effective effect to controlled pests.

Table 1. Profenofos pesticide residue concentration data on tomatoes

Sample	Retention time (minutes)	Area width	Concentration (ppm)	Average
Unwashed tomatoes	25.678	326455	0.1657	0.159 ± 0.00796
	25.684	320291	0.1625	
	25.678	296667	0.1506	
Water-washed tomatoes	25.676	138002	0.0700	0.0700 ± 0.00092
	25.696	136299	0.0691	
	25,681	139844	0.0709	
Vegetables-detergent-washed tomatoes	25.681	135295	0.0687	0.067 ± 0.00163
	25.696	130191	0.0661	
	25.688	129430	0.0657	

Washed-water tomatoes samples has average concentration as 0.070±0.0009 *ppm*. Profenofos pesticide residue level was decreased for 56% from profenofos residue level at unwashed tomatoes. Decrement of residue level was influenced by several factors, those are: (1) solubility. Pesticide residue could dissolved in water for washing. This condition was related to physical and chemical characteristic, i.e solubility in water and washing-water pH. (2) Hydrolysis. Pesticide residue can be hydrolyzed depend on amount of available water, pH, pesticide concentration. Reduction of chlorpirifos pesticide residue because tap water washed is 76.93% and with clean-water-bath is 24.64%. Usage of tap water for washing fruits and vegetables is more effective to reduces pesticide residue than usage of clean-water-bath.

The detector used in gas chromatography was Flame Photometry Detector. This kind of detector is very suitable for organophospate pesticide analysis because it is equipped with P filter that could detects phosphor-contained compound [9]. First, in this research addition of ethyl acetate solvent done after samples blended. Ethyl acetate was used as solvent because it can dissolve profenofos completely [10]. Then, based on Susilowati, Primaharinastiti and Soerjono (2013) research, ethyl acetate also gave better recovery value than acetonitril and acetone [11]. Beside, ethyl acetate is more economical compared to acetonitril. Second, extraction had done with sonicator instrument. Sonication extraction can accelerates contact time between sample and solvent that resulted in faster movement of bioactive compound mass from the inside of plant cell to the solvent. Sonication extraction for 10 minutes will gave the best result. Based on those reasons, this research was used sonication for 10 minutes. Third, addition of Na_2SO_4 anhidrat was done to filtrate of ethyl acetate extract. The purpose of addition Na_2SO_4 anhydrate is to binds water particles from the extract [12]. Water particles inside the extract can dissolves semi polar substance and also influenced the polar characteristic, that influenced process of analysis from pesticide residue. Method modification done was good enough, it can be seen from high recovery result. Beside, this method characteristic is fast, cheap and easy.

The method accuracy can be seen from percentage of profenofos residue recovery in matrix of tomatoes samples. This method has good accuracy if recovery percentage between 80% - 115% range [7]. From this research was obtained profenofos recovery percentage in tomatoes samples for 113.4%. Calculation examples can be seen in Table 2. Recovery percentage value already qualified for the accuracy

Standard Deviation (SD) calculation in unwashed, water-washed and detergent-washed tomatoes are 0.00796; 0.00092; 0.00163 respectively. Relative Standard Deviation (RSD) in each treatment are 4.98%, 1.31% and 2.44% respectively. This value were obtained from three times level measurement from each sample. Mean value of Standard Deviation is 0.0035 and Relative Standard Deviation is 3.5%. The calculation for SD and RSD can be seen on Table 3. Based on relative standard deviation were obtained, it can be said that analysis method was used had enough accuracy category, because it is qualified for the test criteria i.e 2.5% [13].

Table 2. Profenofos recovery data

Sample	Recovery concentration	Teoritical concentration	Recovery percentage
50 g	0,362	0,2	101,2
50 g	0,393	0,2	116,7
50 g	0,404	0,2	122,2
Average			113,4

At detergent-washed tomatoes samples, the profenofos residue average concentration is 0.067±0.0016 *ppm* with degradation level as 58%. Difference in degradation percentage of profenofos residue level was insignificant between water-washed and detergent-washed samples. This condition caused by profenofos characteristic which is highly dissolvable in water. Profenofos resolvability in water is 1: 20 [10]. Then, according to Atmawidjaja *et al.* 2004 [9] decrement or degradation of pesticide residue could caused by several factors, i.e: (1) evaporation, partly of pesticide will reduces because it had evaporated from plant surface. (2) Mechanic and physic treatment, pesticide was decreased because it dissolved in washery process. (3) Chemical, residue level was reduced or degraded because chemical event, e.g detergent washing. But, detergent usage could be dangerous if detergent residue remained because of uncompleted rinsing. Beside soap usage, there is natural chemical that recommended for pesticide residue reduction puposes, i.e salt (NaCl), natrium bicarbonat ($NaHCO_3$), and acetic acid (CH_3COOH) [18].

Table 3. Standard Deviation (SD) and Standard Deviation Relative (RSD) Data

Sample	Concentration ppm (xi)	Average (x)	$(xi-x)^2$	SD	% RSD
Unwashed tomatoes	0.1506	0.1596	8.1×10^{-5}	0.00796	4.98 %
	0.1625		8.41×10^{-6}		
	0.1657		3.72×10^{-5}		
Water-washed tomatoes	0.0691	0.0700	7.92×10^{-7}	0.00092	1.31 %
	0.0701		4×10^{-8}		
	0.0709		8.46×10^{-7}		
Vegetables-detergent-washed tomatoes	0.0657	0.0668	1.21×10^{-6}	0.00163	2.44 %
	0.0661		4.9×10^{-7}		
	0.0687		3.61×10^{-6}		
Average		0.0988		0.0035	3.5%

Addition of the natural chemical compound could expands reduction of residue levels, this because degradation level of pesticide residue at salt, sodium bicarbonate and acetic acid solution significantly higher than branch water [14]. Solution from natural compound also non-threatening for health compared to synthetic fruit-washer solution.

Based on statistical examination result with One-way ANOVA (SPSS 20.0) profenofos residue level in unwashed samples was significant compared to running-water-washed and detergent-washed samples (p<0,05). Whereas, running-water-washed tomatoes samples and detergent washed samples did not had profenofos residue level significant difference (p>0,05).

Profenofos pesticide residue level on unwashed, tap water-washed and detergent-washed samples do not exceed the Maximum Residue Limit (MRL) that established by Indonesian National Standard (SNI). The pesticide contained profenofos BMR which is allowed by *Food and Agriculture Organization* (FAO) dan *World Health Organization* (WHO) (2013) and Indonesian National Standard (SNI 7313: 2008) is 2 mg/kg (*ppm*). For all that, pesticide residue expected to has very small amount or even none, because it could be accumulated in human body. This is appropriated with Kusnoputranto (1996) that noted pesticide in organisms body can not excreted perfectly, but accumulation toxic compound will happened and resulted in variety of health disturbance. Codex commitee, FAO and WHO (2013) was established *Acceptable Daily Intake* (ADI) for profenofos as 0-0.03 mg/kg body weight. As some compound became toxic or dangerous, ADI allowed will has smaller value.

Vegetables that positive contains pesticide will be dangerous if consumed on and on. The residues will accumulated in the body and influenced nerve formation, specially for nerve membrane and it will collected in fat. Residue that had saved in fat cannot excreted by urine and it will be accumulated endlessly and caused tissues damage and cancer [15]. Exposure towards pesticide from organophospate group for long duration and high relative amount will inhibit function of acetylcoline esterase, this can caused salivation, dizziness, bradichardia and even a comma. Inhibition of enzyme works happened because organophospate pesticide was done enzyme phosporilation in stabil component form. This can be obtained with consumed healthy food that will make healthy life [16,17].

CONCLUSION

Tomatoes collected from farmers at Koto Baru, X Koto, Tanah Datar District, West Sumatra, Indonesia are positive contained profenofos pesticide, but the level do not exceed the Maximum Residue Limit (MRL) that established by Indonesian National Standard (SNI) and the codex committee. Profenofos residue level for unwashed samples as 0.159±0.0079 ppm, water-washed 0.070±0.0009 ppm, and detergent-washed is 0.067±0.0016 ppm. Statistic examination result with one-way Anova (SPSS 20.0). Difference of profenofos residue level from unwashed samples is significant compared to water-washed and detergent-washed samples (p<0,05).

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