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Effect of Planting Season on the Residue of Organophosphate in Chili (*Capsicum annuum* L.)

Megawati[1], Muhammad Ikhsan Sulaiman[2], Sabaruddin Zakaria[1]

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ABSTRACT

Background: Pesticide residues harm human health and are associated with hemopoietic disorders and many organ cancers. This study analyzes the pesticide residue content in chili cultivated by farmers in rainy and dry seasons, especially for organophosphates pesticides. The residual difference in chili was affected by planting seasons caused by farmers' adoption of crop cultivation technologies, including pesticide application.

Methods: The analysis was carried out using gas chromatography on 26 samples from 13 chili cultivation locations in Aceh province, Indonesia. Sixteen samples were harvested during the rainy season and the remaining ten were harvested during the dry season.

Result: The results showed that pesticide residues were found in two red chili samples planted in the rainy season, while no residue was found from the dry season. In the rainy season's harvested samples, the Organophosphate pesticide residues were chlorpyrifos 0.52 mg kg^{-1} and 0.16 mg kg^{-1} at Mutiara Tiga sub-district and Meurebo sub-district, respectively. From Meurebo sub-district profenofos 0.74 mg kg^{-1} was found. All detected pesticide residues samples are still below the maximum residue limit (MRL) set by the Government of Indonesia and CODEX Alimentarius.

Key words: Chili, Dry season, Organophosphate, Pesticide residues, Rainy season.

INTRODUCTION

Red chili (*Capsicum annuum* L.) is a vital horticulture commodity in Indonesia. Chili is widely used in daily culinary. The average consumption of fresh red chilies could reach 13.25 kg $person^{-1}$ $year^{-1}$. Indonesia needs around 68,000 tons of chili annually. According to Indonesia statistics (2019), chili production in Indonesia fluctuated from 53,800 tons in 2017, 68,153 tons in 2018 and 63,595 tons in 2019. Moreover, (FAOSTAT 2020) shows that China, Mexico, Turkey, Indonesia and Spain have the most significant production quantity and produce approximately 75% of the world's production of fresh chili.

The price of the chili varies depending on the power of supply and demand. The price ranged between US$ 0.75 and US$ 8.33 in the traditional market. Particularly during religious occasions, the demand for chili increased. Although chili could be grown all year round, the market is oversupplied in the dry season. The chili production is low during the rainy season, associated with a high risk of pests and diseases attack (Hussain and Abid 2011). The incidence of abnormal weather conditions has been more frequent and rampant recently, brought to the different yields of the chili (Tawang *et al.* 2001).

Many farmers, especially in Indonesia, try to gain more profit by growing chili in the wet season, although they should use high pesticide intensity.

The farmers rely on chemical pesticides because they are more effective and efficient than natural ones to control pests and diseases, such as caterpillars, leafhoppers, stink bugs and insects (Abedi-Lartey *et al.*, 2016; Sharifzadeh *et al.*, 2018; Zhang *et al.*, 2020). Usually, pesticides applied for vegetable crops belong to organo phosphates such as dimethoate, chlorpyrifos and cypermethrin. The pesticides are used in excessive doses and leave a residue above the permissible level (Latif *et al.,* 2011). The excessive use of pesticides might be due to the increasing attack of pests and diseases as an impact of increasing temperature due to climate change (Delcour *et al.*, 2015). Residual pesticides harm human health, which is estimated to increase the number of cancer cases in humans related to consuming fruits and vegetables containing pesticides residue (Valcke *et al.,* 2017).

Various studies have also shown that pesticide residues in food are associated with various diseases such as hemopoietic disorders or various types of human organ cancer (George and Shukla, 2011; Lozowicka, 2015). Very few studies have been carried out on its residues estimation; therefore, the present study was conducted to analyze pesticide residues in chili cultivated by farmers, primarily

[1]Department of Agrotechnology, Faculty of Agriculture, Universitas Syiah Kuala, Banda Aceh, 23111, Aceh, Indonesia.
[2]Department of Agricultural Product Technology, Faculty of Agriculture, Universitas Syiah Kuala, Banda Aceh 23111, Aceh, Indonesia.

Corresponding Author: Sabaruddin Zakaria, Department of Agrotechnology, Faculty of Agriculture, Universitas Syiah Kuala, Banda Aceh 23111, Aceh, Indonesia.
Email: zaksabar@unsyiah.ac.id

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due to differences in planting or harvesting seasons. Our studies aim to investigate the safety of fresh chili in Aceh Province, one of Indonesia's chili production areas and try to find out its relationship with the pesticide practice of local farmers.

MATERIALS AND METHODS

Time of research and sampling procedure

This research was conducted from January to December 2019. Sampling was taken at 13 locations, including eight during the rainy season (October-March) and five during the dry season (April-September). These 13 locations are located within five districts of Aceh Province. Each location is represented by two samples so that there are 26 samples in total. These samples consist of 16 samples for the rainy season and ten samples for the dry season. Then the red chili samples from each location were composited to analyze pesticide residues. These five districts represented the eastern region (Aceh Besar and Pidie Districts), the central region (Aceh Tengah and Bener Meriah districts and the western region (Aceh Barat District). These three regions represented areas with low, medium and high rainfall types, as shown in Fig 1. Then the samples were immediately taken to the Food Safety Laboratory-Aceh Food Service.

Preparation for sample analysis

The analysis procedure tested samples against pesticides in the organophosphate group, consisting of diazinon, methidathion, dimethoate, chlorpyrifos and profenophos (AOAC 2007) method concerning Pesticide Residues in Food with Acetonitrile Extraction and Partitioning with Magnesium Sulfate Lehotay (2007). The collected samples were stored in a refrigerator with a temperature of 5°C. These samples were extracted and analyzed within 24 hours after sampling.

Extraction of sample

Fifty grams of the sample were chopped using a food chopper and fifteen grams of the homogeneous sample were weighed in a 50 ml tube, then the standard mixture was added (spiked). The sample was left to stand for 30 minutes, added with QuECHERS powder containing (6 g $MgSO_4$ and 1.5 g sodium acetate). Enter the Homogenizer ceramic and add 15 ml of solvent 1% acetic acid in acetonitrile. Then the sample was shaken for two minutes and centrifuged for five minutes at a speed of 4000 rpm. The extract was taken as much as 8 ml and carried out a clean-up process using absorbent (1200 mg MgSO4 + 400 mg PSA + 400 mg GCB) and centrifuged for two minutes at a speed of 4000 rpm. Finally, the extraction results were taken to be analyzed using gas chromatography (Anastassiades *et al.*, 2003; Yusuf *et al.*, 2021).

Analytical procedure

The instrument used to analyze pesticide residues was Gas Chromatography (Shimadzu-series 2010 plus model; Kyoto, Japan), equipped with a Flame Photometric Detector (FPD). The analysis was carried out simultaneously on pesticide residues in the organophosphate group (Dimethoate, Diazinon, Chlorpyrifos, Methidathion and Profenofos). Each residue has been validated on the linearity, precision, accuracy, reproducibility and detection limit values. A capillary column Rtx-5 (Crossband 5% diphenyl-95% dimethyl polysiloxane, Restek, USA) column size 30 m×0.25 mm, ID×0.25 µm df, mobile phase: Helium 99.9995% with a flow rate of 1.2 ml/min, detector temperature at 280°C, injector temperature at 230°C, splitless mode and injection volume of 1 µl. The processing of the raw chromatogram and data collection was performed using the GC-Solution program (Shimadzu Corporation). The pesticide residue test results were compared with the Maximum Residue Limit (MRL) of pesticides that have been used in Indonesia and the CODEX Alimentarius standard (Commission, 1992).

RESULTS AND DISCUSSION

Pesticide residues during the dry season

The GC analysis shows no pesticide residue was found from the samples of chilies harvested during the dry season.

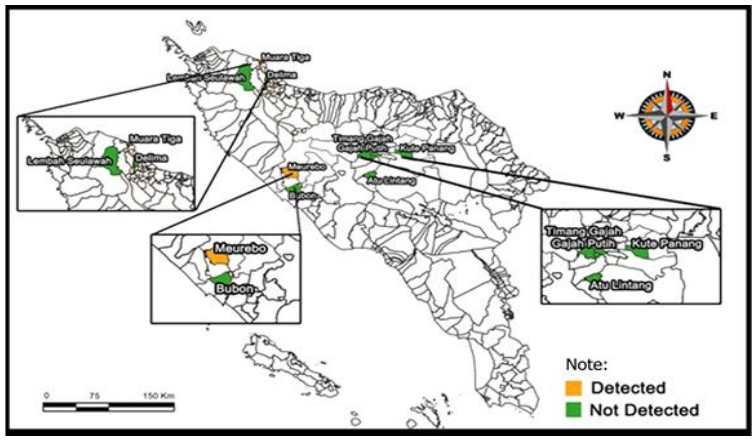

Fig 1: Distribution of sampling locations for analysis of residue pesticide belongs to organophosphate group in harvested chilies during the dry and rainy season in Aceh Province, Indonesia.

Although the samples were analyzed for the five organo phosphate groups (Dimethoate, diazinon, chlor pyrifos, methidathion and profenophos), none of them were detected contained pesticide residue by GC analysis (Table 1). The absence of pesticide residues in chili samples harvested in the dry season during (May and June 2019) is affected by high temperatures after this time. According to Aceh Meteorology, Climate and Geophysical Agency, the highest temperature in Aceh Province was in May 34.1°C and June 33.6°C (Fig 3).

No pesticide residue was detected based on the analysis of pesticide residues in chili samples harvested in the dry season. Numerous climatic characteristics influenced pesticide loss, including precipitation and rainfall timing and extreme events concerning application date (Nolan *et al.*, 2008). Organophosphate pesticides are not resistant to high temperatures and the sunlight is primarily caused by the ultraviolet spectrum (Fest and Schmidt, 1973; Matsumura, 2012). The weather conditions such as this temperature make the pesticide evaporate (Damalas and Eleftherohorinos 2011). The degradation of plant surfaces is caused by photodegradation, evaporation, rainfall wash-off and growth dilution (Nasrin *et al.*, 2021; Zongmao and Haibin, 1997).

The loss of pesticide residues is due to several factors, including temperature, humidity and ultraviolet light (Kumar and Mukherji, 2017; Xu *et al.*, 2008). Organophosphate pesticides are pesticides that do not take a long time to experience degradation, pesticides that evaporate into the air will decompose due to the influence of temperature, humidity and sunlight, especially ultraviolet (Singh and

Table 1: GC analysis for organophosphate pesticide residues on chili harvested by farmers during the dry season of Aceh Province.

Location	Types of active pesticides belong to the organophosphate group.				
	Dimethoate	Diazinon	Chlorpyrifos	Methidathion	Profenofos
Aceh Besar district (Lembah Seulawah sub-district)	¬	¬	¬	¬	¬
Aceh Besar district (Lembah Seulawah sub-district)	¬	¬	¬	¬	¬
West Aceh district (Meurebo sub district)	¬	¬	¬	¬	¬
Pidie district (Delima sub-district)	¬	¬	¬	¬	¬
Pidie district (Mutiara Tiga sub-district)	¬	¬	¬	¬	¬

¬: Undetected.

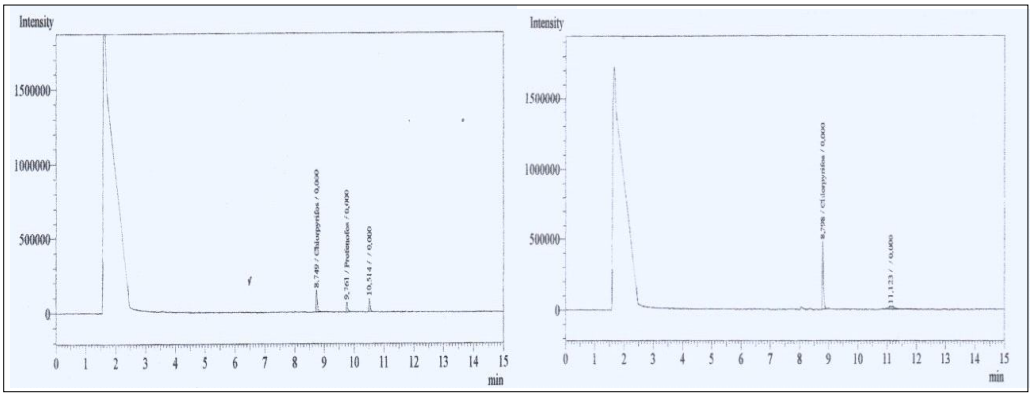

Fig 2: Chromatogram analysis results from blank sample chilies harvested in the rainy season (Meureubo /West Aceh district) (left) and MuaraTiga / Pidie district (right).

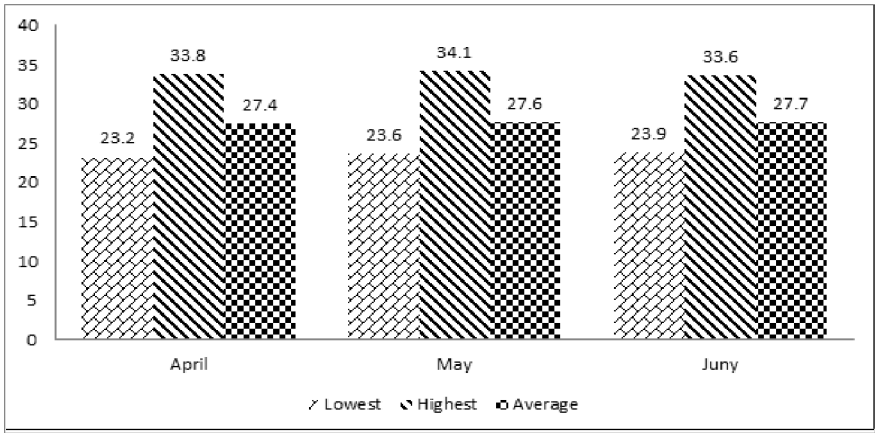

Fig 3: The lowest, highest and average temperatures of Aceh Province in April to June 2019.

Table 2: GC analysis for organophosphate pesticide residues on chili harvested by farmers during the rainy season of Aceh Province.

Location	Types of active pesticides belong to the organophosphate group (mg.kg^{-1})				
	Dimethoate	Diazinon	Chlorpyrifos	Methiadition	Profenoos
Pidie district (Muara Tiga sub-district)	¬	¬	0,52	¬	¬
Pidie district (Delima-sub-district)	¬	¬	¬	¬	¬
Bener Meriah district (Gajah Putih-sub district)	¬	¬	¬	¬	¬
Bener Meriah distric (Gajah Putih-sub district)	¬	¬	¬	¬	¬
Central Aceh district (Atu Lintang-sub-district)	¬	¬	¬	¬	¬
Central Aceh district (Kute Panang-sub-district)	¬	¬	¬	¬	¬
West Aceh distric (Meureubo sub-district)	¬	¬	0,16	¬	0.74
West Aceh district (Bubon sub-district)	¬	¬	¬	¬	¬

¬: Undetected

Table 3: Maximum residue limits on chili according to Indonesian minister of agriculture regulation No. 53 (2018) and CODEX Alimentarius for Organophospate groups.

Organophosphate group	(Maximum residue limit; MRL)	
	Indonesian Minister of Agriculture regulation No. 53 (mg.kg^{-1})	CODEX Alimentarius (mg.kg^{-1})
Dimethoate	0.5*	0.5*
Diazinon	0.05*	0.5*
Chlorpyrifos	2*	2*
Methiadition	0.1**	¬
Profenofos	3	3

* : MRL of chili; ** : MRL of tomato; ¬ : No data MRL.

Singh, 2013; Sule and Kumar, 2019). Moreover, the study also reported that organophosphate groups of insecticides are very toxic but can quickly decompose in nature (Lad *et al.* 2017).

The half-life (t½) period for each type of pesticide on chilies is 4.93, 8.85, 4.42, 3.20 days for chlorpyrifos, etion, quinalphos and spiromesifen, respectively (Sushil *et al.* 2018). There is a difference in the half-life between tropical climatic conditions and a non-tropical climate; tropical climates make the existing half-life shorter (Ngan *et al.* 2005). Farmers sprayed the last pesticide seven days before harvesting based on the questionnaire. It is estimated that the grace period between the last spraying and harvesting is the chili's evaporation (Table 4).

Pesticide residues during the rainy season

Four samples among 16 samples of chili have residues of organophosphate pesticides in this season (Table 2). The group chlorpyrifos is 0.52 mg kg^{-1} in each sample from the Muara Tiga sub-district Pidie district (Fig 2A). The samples from West Aceh District also had detected organophosphate residue chlorpyrifos 0.16 mg kg^{-1}andprofenofos 0.74 mg kg^{-1}, respectively (Fig 2B). These pesticide residues were found in the samples from the Meurebosub-district. Although residue of organophosphates was detected in this District, the amount of residue was still below the maximum residue limit, both based on regulation by the Indonesian Ministry of Agriculture no. 53/2018 and CODEX Alimentarius (Commission, 1992) (Table 3). The detection of pesticide residues such as chlorpyrifos and profenofos in chili was caused by the farmers using more insecticides containing these two active ingredients in their chili cultivation. The data shows that the most widely used pesticides are insecticides, followed by fungicides and bactericides. The insecticide trademark that was most widely used was Curacon, which this trademark used by 16 samples or more than 60% of farmers used this insecticide for controlling their chilly plant from pest attack. The active ingredient of this insecticide is profenofos belongs to the organophosphate group (Table 4). Besides, this fact also shows that farmers use more than one type of pesticide because it saves time and effort so that more than one active pesticide substance was detected in the same sample. Highly use of pesticides in chili cultivation is ultimately the poison for the human being because of its maximum uses beyond the immunity power of the human beings. It is to be why Indonesia Government does not recommend the application of insecticide through this method in plant cultivation.

In determining the dosage of pesticides, farmers stated that they had used the recommended dosage. However, during the rainy season, farmers would increase the dose or increase the frequency of spraying, caused by the high intensity of pest attacks and diseases compared to the dry season. Even though there are no indications of pests and diseases, farmers believe that it is better to spray pesticides at the beginning of planting before pests and diseases attack the plants. Farmers also mix pesticides with adhesives that make the pesticides work more effectively.

Apart from the season factor, another factor that led to the identification of pesticide residues was probably because the chili fields were continuously used for chili cultivation, causing the pest population to increase and forcing farmers to use insecticides throughout the growing season continuously; according to Table 4, the respondent report that they had cultivated chili on the same land for ten consecutive years.

The average rainfall and rainy days in West Aceh district were 386.5 mm and 12 days, respectively, whereas for Pidie district were 124 mm and four days, respectively (Table 5). This data shows that the average rainfall and rainy days in

Table 4: Type of pesticide, trademark, active ingredient and cultivation system of chili by the farmers.

Pesticides Application and Cultivation System	Active Ingredient/Grup Pesticides	Sample	Total (%)
The type of pesticide used			
a. Organic Pesticides or biopesticides		0	0
b. Inorganic pesticides		26	100
The most widely used type of pesticide			
a. Insecticide		13	50
b. Fungicide		9	34.62
c. Bactericide		2	7.69
d. Herbicide		0	0
e. Growth regulator		2	7.69
The most widely used trademark of insecticides and their active ingredients			
a. Cuaron	Profenofos-Organophosphate	16	61.54
b. Curator	Karbofuran-Carbamat	3	11.54
c. Furadan	Furadan-Carbamat	3	11.54
d. Bamix	Abemektin Avermektin and Milbemi	2	7.69
	sin	2	7.69
e. Others		0	-
Cultivation system			
a. Continue chili cultivation for more than three years.		16	61.54
b. Apply plant rotation.		10	38.46
c. Chili cultivation with intercropping with other plants.		0	-
Last time pesticides application			
a. One week before harvest		22	84.62
b. Two weeks before harvest		4	15.38

Tabel 5: Total rainfall and rainy day at the time of chili collection at the location of the samples.

Sub district/District in Aceh Province	January 2019		March 2019	
	Rainfall (mm^3)	Rainy days (day)	Rainfall (mm^3)	Rainy day (day)
Pidie district (Muara Tiga sub-district)	-	-	124	4
Pidie district (Delima-sub-district)	-	-	87	9
Bener Meriah district (Gajah Putih-sub district)	257	18	-	-
Bener Meriah district (Gajah Putih-sub district)	257	18		
Central Aceh district (Atu Lintang sub-district)	176	18		
Central Aceh district (Kute Panang sub-district)	219	18		
West Aceh district (Meureubo sub-district)	-	-	386.5	12
West Aceh district (Bubon sub-district)	-	-	63	6

West Aceh district were higher than in the Pidie district. The difference in rainfall and rainy days is thought to be one of the factors that cause differences in the residual level of chlorpyrifos in chilies. It is suspected that the more rainfall before harvest, the washing tends to be high, thereby reducing pesticide residues on the surface of fresh chili skins.

Previous research on vegetables showed that pesticide residues concentration in the rainy season was lower pesticide compared to winter and summer (Bhanti and Taneja 2007). This study indicates that high rainfall in the West Aceh district caused the lower levels of insecticide residue contained in chilies.

CONCLUSION

The organophosphate pesticide residues were not found in chilies harvested during the dry season. However, in the rainy season's harvested samples, the organophosphate group of pesticide residues was obtained includes chlorpyrifos 0.52 mg $kg^{-1}$and 0.16 mg kg^{-1} at Mutiara Tiga sub-district and Meurebo sub-district, respectively. Moreover, at Meurebo sub-district was also found profenofos 0.74 mg kg^{-1}. All detected pesticide residues samples are still below the maximum residue limit (MRL) set by the Government of Indonesia and CODEX Alimentarius. Although the research results show the residue is still below the MRL limit, it is thought that continued consumption of these chili causes

chronic effects on humans in the long run. The government is obliged to play a role in extending and monitoring food safety to ensure food security through good agricultural practices (GAP).

Conflict of interest: None.

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