The optimization of pyrethroid simultaneous analysis in tropical soil of Indonesian tea plantation: Preliminary study

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Abstract. The preliminary study for the simultaneous analysis of five synthetic pyrethroids (lambda-cyhalothrin, permethrin, cypermethrin, fenvalerate, and deltamethrin) in tropical soil of tea plantation has been performed. The objective of this study is to perform an optimum condition for simple method development, based on green analytical chemistry. The concentrations of 0.1 μg.g⁻¹ for all pyrethroids were evaluated using ultrasonic and microwave-assisted extraction. The quantification was performed using gas chromatography-electron capture detection (GC-ECD). Based on the experiment, the 0.5 mm soils size particles and 30 minutes sonication times were set as optimal condition for ultrasonic-assisted extraction with the best recovery yields of spiked soils were from 80 to 105 % for each pyrethroid and repeatability represent by the relative standard deviation (RSD, %) ranging of 1.1 – 4.8 %. Results were comparable with those found by microwave-assisted extraction. The result shows that the reproducibility, which is represented by recoveries obtained, ranged from 151 – 276 %, and were unacceptable because the value was beyond the specific range that was proposed by the Association of Analytical Communities (AOAC) International. The ultrasonic-assisted extraction was applicable for pyrethroids analysis in the real sample, with concentration found was 0.01 to 0.12 μg.g⁻¹.

Keywords: pyrethroids; soil tea plantation; ultrasonic-assisted extraction

1. Introduction
Tea is perennial plantation crop which also become main commodities besides horticulture, planted in upper Citarum watershed, with total area planted more than 15,000 Ha owned by private and government [1] Besides neonicotinoid, synthetic pyrethroid such as bifenthrin (Talstar(TM)), deltamethrin (Decis(TM)) and permethrin (Champion(TM)), also commonly used as insecticide in tea plantation. Synthetic pyrethroids are Persistent Organic Pollutants that were classified as group 3 “is not classifiable as to its carcinogenicity” based on the International Agency for Research on Cancer (IARC). Moreover, the World Health Organization (WHO) [2] classified several pyrethroids synthetic as moderately hazardous pesticides (Class II). In addition to that, the Indonesia government through the Ministry of Agriculture Regulation No 39, 2015 has already prohibit the utilization of pyrethroids synthetic, but still, the application of this compound was found. The utilization of these pesticides has
grown in recent years because they have advantages in comparison with any other pesticides, for instance, their selectivity, easy to degrade in the environment and low acute toxicity to mammals [3, 4, 5].

The occurrence of pyrethroid residues on agricultural soil not only may contaminate the water body through runoff but also has an adverse effect on human exposure by dermal contact or ingestion by crops cultivated in those soils. Long term and repeated applications of this compound will increase the residue accumulated in the soil and could enter the food chain through plants and water bodies [6, 7, 8]). Several studies have been carried out the occurrence and monitoring of pyrethroid in environment, among these are in China [9], United States [10] and India [11,12], and limited information can be found in the literature regarding the occurrence of pyrethroid pesticides at tea plantation in India [13, 14, 15].

Further studies regarding the occurrence of pyrethroids synthetic on tropical soil tea plantation especially in Indonesia is an important matter related to soil characteristics and climate differences. Soil matrices are very complex and their different characteristics such as soil and texture and organic matter can influence the occurrence and persistence of pyrethroid [16,17]. Despite it has short “half time” than any pesticide, however, due to its inherent nature in particulate and high affinity to organic matter and clay phases, pyrethroids are more persistent up to yearly in soil and sediment [18,19].

Several previous research show that pyrethroids were present at low concentrations in soils. Residues detected in soil samples were found in Mediterranean paddy fields range from 57 – 62.3 ppb [20]; soil samples with a concentration of 1.5 – 85 ppb for chestnut, walnut, and pinenut soils [8], even indicated not detected (below limit of detection) on tea plantation soil [14,15]. Moreover, a very sensitive and selective analytical method especially for tropical soil tea, based on green analytical chemistry with small number of samples, organic solvents, and less time-consuming is required.

Besides sample preparation and clean-up process, extraction has play an important role in analytical methods, the failure of the extraction process will generate a low amount of extracted target compound. The extraction of pyrethroids from solid samples had been performed using Soxhlet extraction [3], microwave-assisted extraction [21,22] and continuing with pre-concentrated by solid-phase extraction (SPE) and liquid-liquid extraction. Above all methods mentioned, ultrasonic-assisted extraction is the most common conventional extraction method of pyrethroids from solid samples such as soils and sediment [3,20,23]. Apart from it, the utilization of microwave as a recent method for extraction was highly recognized through their fast and low solvent consuming. Through this research, the best extraction method which is in accordance with green analytical chemistry were determined through ultrasonic and microwave-assisted extraction. The objective of this research is to perform an optimum condition of simple analytical methods based on green analytical chemistry making them environmentally friendly for supporting pyrethroids monitoring. The result of this study is important as a reference to monitor the pyrethroids presence at the tropical tea plantation.

2. Material and Methods

2.1. Apparatus and Chemical and reagents (Analytical standards and working solution)

An Agilent 7890B gas chromatography coupled with a micro electron capture detector (GC-μECD) from Agilent was used for pyrethroid analysis. The optimum condition of GC-μECD was conducted, provided by previous research [24] with modification at detector temperature. Chemical analysis by GC-μECD was performed using the HP-5 Agilent column. Nitrogen was used as the make-up gas while Helium was acted as a carrier gas. The injector and detector temperature were set at 250°C and 300°C respectively. The column oven temperature was programmed from 200 °C (initial temperature, 1 min), to 280 °C at a rate of 20 °C per minute and hold for 8 minutes.

For the extraction of pyrethroids from the soils, Ultrasonic bath Branson-3510, and microwave digestion system (ETHOS EASY) with power setting 700 W [22] were employed. Both of the initial temperatures of the instruments above were set at room temperature (26 °C).
Pesticides standard: Fenvalerate (purity 99.5 %), Cypermethrin (purity 99.2 %), Permethrin (purity 99.6 %), Deltamethrin (99.3 %) and Lambda Cyhalothrin (99.5 %) were obtained from Chem Service. N-hexane and Anhydrous Sodium Sulphate from Merck. A stock solution of pyrethroid was prepared at 5 mg L-1 in n-hexane. Standard solutions were obtained by diluting the stock solution with n-hexane. All standard solution was stored in a freezer at -4ºC before use.

2.2. Preparation of spiked soil
The blank samples are topsoil samples (0-20 cm) that were collected from the area near the tea plantation which never been used for agricultural purposes. Whereas for real sample analysis, the topsoil (0-20 cm) were randomly collected from several tea plantation locations. All the samples were labelled properly and transported to the laboratory for immediate processed. The soils were air-dried in room temperature (less than 24 hours), grounded and sieved. The soil samples were stored in glass jars at a freezer with a range temperature -4 ºC – (-20 ºC) before analysis [25,26].

Blank soil samples for ultrasonic extraction were weighed (5 g, wet weight) into a centrifuge tube (50ml) and were spiked by adding 0.1 mL of 5 µg.mL⁻¹ standard mixtures of pyrethroids, whereas for microwave extraction the soil samples (1 g, wet weight) were spike by adding 0.02 mL 5 µg.mL⁻¹ pyrethroids standard mixtures, each spiked sample consist of one control and triplicate samples.

2.3. The Experiment of Ultrasonic extraction
In this study, several parameters that significantly affected the performance of ultrasonic-assisted extraction were determined based on an experiment. Before spiking with pyrethroids mixture standard as mentioned above, the soil samples were sieved with different particles size, each through 2-mm (Mesh No 10) and 0.5-mm (Mesh No 35). After the spike, the soils were vortex to homogenize the samples and stand with various time (1 hour and 24 hours) prior to extraction. After standing for each storage time, the 20 ml n-hexane was added to the samples and extracted using ultrasonic-assisted extraction methods. In this study, the optimum length of sonication time found out based on an experiment using various sonication times (15, 30 and 60 minutes). The extraction was performed twice, prior to clean up with a small portion of glass wool and anhydrous sodium sulphate.

2.4. Microwave extraction
One gram of soil spikes with 0.5-mm particle size that was standing for 24 hours introduced to 100 ml PTFE high-pressure vessel. The 10 mL mixture n-hexane non polar-polar organic solvents were added and then extracted for 15 minutes [3,22,27], prior to clean up as similar as ultrasonic extraction.

3. Results and Discussions

3.1. Instrument performance
To evaluate the instrument feasibility, using the optimum condition of GC ECD mentioned above, the linearity of the calibration curve was performed at the concentration of synthetic pyrethroids standards at 1-50 µg.L⁻¹. The coefficient correlation obtained for each compound range from the lowest, 0.9961 for cypermethrin to the highest, 0.9996 for lambda-cyhalothrin (Table 1). This coefficient correlation shows that the performance of the instrument was accountable. The retention time obtained for each compound was 6.061 (Lambda-cyhalothrin), 6.630 and 6.721 (Permethrin), 7.425; 7.520 and 7.649 (Cypermethrin), 8.498 and 8.785 (Fenvalerate) and 9.583 (Deltamethrin).
Table 1. The instrument linearity.

| Pesticide standard      | Linear equation | Coefficient of correlation (r) |
|-------------------------|-----------------|-------------------------------|
| Lambda cyhalothrin      | $y = 19.324x + 11.405$ | 0.9996                        |
| Permethrin              | $y = 3.721x + 2.333$  | 0.9985                        |
| Cypermethrin            | $y = 14.289x + 18.515$ | 0.9961                        |
| Fenvalerate             | $y = 13.725x + 10.378$ | 0.9994                        |
| Deltamethrin            | $y = 12.379x + 2.149$  | 0.9990                        |

3.2. Ultrasonic parameters for pyrethroids extraction

In this study, ultrasonic-assisted extraction was proposed for the simultaneous extraction of several pyrethroids from the tropical soil tea plantation. This study indicates that the soil spikes that were standing up for 1 hour, gave the best percent recovery result (95-103 %) than the 24 hours storage time (82-104 %). The limitation of storage time up to 24 hours referred to previous research that indicated between 1, 7 and 14 days storage of soil spikes with carbofuran pesticides, the 1-day storage gave the best percent recovery up to 95 % incomparable with those found in 7 and 14-days storage [28]. Although both of this result were acceptable and has been fixed between 80% and 110 % based on AOAC regulation [29], in this study the 24 hours-spiked soils were employed to allow the spiked solution optimally penetrate to the soil matrices, inconsistent with the actual condition of real samples with the pyrethroid compounds that might be strongly attached yearly to the soils particles.

The particle size of the sample is the factor that was necessary to consider for extraction by ultrasonic. According to Capelo et al [30], the main focussed of ultrasonic is the smaller the particle size, the larger the total area exposed to the solvent. In this study, the selection of the 2-mm and 0.5-mm soils particles size as a variable were performed based on previous research [3,9,20,31,32]. This study indicated that with fixed sonication time, the best reproducibility and repeatability which indicated through percent recovery (91-104 %) and relative standard deviation (0.3 – 12.5 %) were obtained by the extraction using 0.5 mm particle size (see Table 2).

The smaller particle size also correlates with the presence of total organic carbon. Due to their high hydrophobicity, pyrethroids synthetic can strongly bind to soils in correlation with organic carbon matter and clay presence [16,17]. Previous research shows that the enrichment of pyrethroids has been found in fine particles with higher organic carbon and clay fractions [33]. In line with this, Nadeu et al [34] mentioned that soils with small size fraction seemed to contain silt and clay particles, which was correlated with the total organic carbon content.

Table 2. Recovery percentage of 0.1 μg.g-1 pyrethroid standards with different soil size samples

| Pyrethroids       | Average Recovery, t = 30 min (%) |
|-------------------|----------------------------------|
|                   | Size = 0.2 mm | Size = 0.5 mm |
| Lambda Cyhalothrin| 65±2.5        | 97±1.6        |
| Permethrin        | 78±15.7       | 104±7.5       |
| Cypermethrin      | 51±1.8        | 93±3.4        |
| Fenvalerate       | 66±0.1        | 91±12.5       |
| Deltamethrin      | 66±2.6        | 103±0.3       |
Besides particle size, according to Albaseer et al [3], sonication time is an important factor that must be considered to ensure that only the target compound was extracted. Three types (15; 30 and 60 minutes) of sonication time were performed in this research. The best sonication time was determined by the best recovery percentage according to the Association of Analytical Communities (AOAC) International [29]. This experiment indicated that, with fixed soils particle soil size, the 30 minutes sonication time gave the best result with average recovery range from 80-105 % with RSD which indicates repeatability for each compound were ranging from 1.1 – 4.8 %. The recoveries percentage obtained were acceptable and have been fixed between 80% and 110 % [29], compared to recovery percentage obtained at 15 and 60 minutes sonication times (see Table 3). Similar to this study, the previous research performed by Ali and Baugh [35] shows that the longer the sonication time, the larger the average recoveries percentage; however, the increases were not significant after 30 minutes. From Table 3, 60 minutes sonication had the lowest average recovery percentage (57-82 %) with any treatment. These phenomena indicated that the prolonged ultrasonic-assisted extraction times were open opportunities for re-adsorption of pyrethroids into the soil during extraction [3].

| Pyrethroids        | Average Recovery (%), Size = 0.5 mm |
|--------------------|-------------------------------------|
|                   | t = 15 min | t = 30 min | t = 60 min |
| Lambda Cyhalothrin| 83 ±6.5    | 90 ±1.4    | 76 ±2.6    |
| Permethrin         | 89±3.1     | 95 ±4.8    | 73±1.5     |
| Cypermethrin       | 65±10.8    | 80 ±1.6    | 57±0.8     |
| Fenvalerate        | 84±6.5     | 95 ±1.7    | 78±0.9     |
| Deltamethrin       | 89±7.7     | 105 ±1.1   | 82±1.1     |

3.3. Comparison between microwave and ultrasonic-assisted extraction

The chromatogram result by ultrasonic and microwave-assisted extraction was presented in Figure 1. The results indicated that the GC-ECD were applicable for pyrethroids analysis through ultrasonic as well as microwave-assisted extraction. The differences among them lie on the area generated, where ultrasonic-assisted extraction chromatograms had the largest area than chromatogram from microwave-assisted extraction.

Based on the experiment on ultrasonic-assisted extraction, the 24 hours spiking storage time, 0.5 mm particle size and 30 minutes sonication times had the highest recovery percentage, thus this method was selected to apply in microwave extraction except for extraction time that was diminished to 15 minutes. Different from ultrasonic-assisted extraction that was only used non-polar organic solvent, in microwave-assisted extraction the solvent used was not polar-polar organic solvent mixtures, with a small portion of organic solvent. According to Albaseer et al [3], the presence of polar organic solvent in the microwave serves as sensitizers that will absorb microwave radiation and pass it to the soil matrices. Acetone, methanol is polar organic solvent that was commonly used for sensitizers along with non-polar organic solvents such as hexane and toluene and can absorb more microwave energy [3,27,36]. Compare to ultrasonic-assisted extraction, the recovery percentage obtained by microwave-assisted extraction was 151 – 276 %. The recovery percentage obtained was unacceptable because it is beyond the specific range that proposed by AOAC International (80-110 %) (see Figure 2) attained with a value below 2/3 CVHorwitz (RSD, 11.3-17.8 %).
Figure 1. GC-ECD Chromatogram of spiked soil at 0.1 μg g⁻¹ concentration level extracted by ultrasonic-assisted extraction (top) and microwave-assisted extraction (bottom). (1) Lambda Cyhalothrin, (2) Permethrin, (3) Cypermethrin (3 isomer), (4) Fenvalerate, (5) Deltamethrin

Figure 2. The comparison between recovery percentages (0.1 μg g⁻¹ pyrethroid standards) extracted with microwave and ultrasonic-assisted extraction

Several research had been compare the performance of ultrasonic, microwave-assisted extraction and combination of both for various matrices, such as in sediments [27], soil [22], food [36,37], and plants [38], with various recoveries range obtained that were acceptable with AOAC International. Besides solvents, temperature, microwave power and the length of extraction were important factors that contribute to extraction performance by microwave [38]. According to Galesio et al. [39], the microwave power is created when the microwave has a contact with molecules, thus the increasing of microwave power is beneficial to diffusion and mass transfer during extraction. However, if the power setting is too high, this maybe result in the re-sorption or loss some of the samples through evaporation [36,38]. Similar with the microwave power, the temperature also has beneficial role to the extraction
performance, according to Zhou et al [40], the higher the temperature, the higher the desorption of compounds in the matrices, but if it exceed the optimum temperature, the decomposition of target compound might be take place [36]. The application of room temperature combined with high power setting (700 W) in this study, might be one reason why the recovery percentage were beyond the acceptable range (AOAC International). In this study, the recoveries value obtained by microwave-assisted extraction was too high. The higher recovery value result from assessment might be come from co-elution compounds which has similar characteristic to matrices thus eluting in the same retention time. Furthermore, the appropriate microwave-assisted extraction for pyrethroids analysis in tropical soil with appropriate analytical instrument besides GC-μECD should be proposed.

3.4. Real sample analysis
A total of five samples from each location sampling were analyzed for five pyrethroids consist of lambda-cyhalothrin, permethrin, cypermethrin, fenvalerate and deltamethrin. The result shows that only permethrin and deltamethrin were detected in all samples, with the highest value was permethrin (0.12 μg.g⁻¹) in sampling location no 4 (see Table 4). This result implies that the ultrasonic-assisted extraction method is appropriate for pyrethroids analysis at tropical soil tea, nonetheless this method still should be validated before it’s utilize for reliable monitoring pyrethroid at tropical soil tea plantation.

| Sampling location | Permethrin (μg.g⁻¹) | Deltamethrin (μg.g⁻¹) |
|------------------|---------------------|-----------------------|
| 1                | 0.06                | 0.01                  |
| 2                | 0.07                | 0.01                  |
| 3                | 0.05                | 0.01                  |
| 4                | 0.11                | 0.01                  |
| 5                | 0.12                | 0.01                  |

A large number of research had been compared the performance of ultrasonic, microwave-assisted extraction and combination of both for various matrices, such as in sediments [27], soil [22], food [36,37], and plants [38], with various recoveries, range obtained that were acceptable with AOAC International. Besides solvents, temperature, microwave power and the length of extraction were important factors that contribute to extraction performance by microwave [38]. According to Galesio et al. [39], the microwave power is created when the microwave has contact with molecules, thus the increase of microwave power is beneficial to diffusion and mass transfer during extraction. However, if the power setting is too high, this may result in the resorption or loss of some of the samples through evaporation [36,38]. Similar with the microwave power, the temperature also has beneficial role to the extraction performance, according to Zhou et al [40], the higher the temperature, the higher the desorption of compounds in the matrices, but if it exceeds the optimum temperature, the decomposition of target compound might be taking place [36].

4. Conclusion
The GC-μECD is eligible for simultaneous pyrethroids analysis either for ultrasonic or microwave-assisted extraction. The utilization of small amount of soil sample, low volume of n-hexane and less time-consuming of ultrasonic-assisted extraction with optimum sonication time and soils particles size were able to extract pyrethroids from spikes soil tea plantation sample with good recoveries and repeatability. Furthermore, the appropriate parameters extraction and analytical instruments for pyrethroids analysis through microwave-assisted extraction method still should be developed.
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Reference

[1] Central Bureau of Statistics 2018 Bandung Regency in Figures. Statistics of Bandung Regency
[2] World Health Organization (WHO) 2005 Safety of Pyrethroids for Public Health Use. Communicable Disease Control, Prevention and Eradication WHO Pesticide Evaluation Scheme (WHOPES) and Protection of the Human Environment Programme on Chemical Safety (PCS
[3] Albaseer SS, Rao RN, Swamy YV, Mukkanti K 2010 J. Chromatogr. A 1217 5537 – 54
[4] Bronshtein A, Chuang JC, Van Emon JM, Altstein M 2012 J Agric Food Chem 60 4235- 42
[5] Yoo M, Lim YH, Kim T, Lee D, Hong YC 2016 Ann Occup Environ Med 28 2
[6] Akoto O, Andoh H, Darko G, Eshun K, Osei-Fosu P 2013 Chemosphere 92 67-73
[7] Ortiz-Hernández ML, Sánchez-Salinas E, Dantán-González E, Castrejón-Godínez ML 2013 Pesticide Biodegradation: Mechanisms, Genetics and Strategies to Enhance the Process. In: Chamy R (ed) Biodegradation - Life of Science. InTech.
[8] Han Y, Mo R, Yuan X, Zhong D, Tang F, Ye C et al. 2017 Chemosphere 180 42-7
[9] Liu Y, Li S, Ni Z, Qu M, Zhong D, Ye C, Tang F 2016 Sci.Total Environ 542 620-28
[10] Riederer AM, Hunter RE, Hayden SW, Ryan PB. 2010 Environ. Sci. Technol 44 483-90
[11] Kumari B, Madan VK, Kathpal TS 2008 Environ Monit Assess 136 239-44
[12] Murugan AV, Swarnam TP, Gnanasambandan S 2013 Environ Monit Assess: 185 8135 - 45
[13] Gurusubramanian G, Rahman A, Sarmah M, Ray S, Bora S 2008 J. Environ. Biol. 29 : 813 – 26
[14] Bishnu A, Chakraborti K, Chakraborty A, Saha T 2008 Environ Monit Assess. 149 457 – 64
[15] Paramasivam M, Chandrasekaran S 2014 Chemosphere 111 291-95
[16] Palmquist K, Salatas J, Fairbrother A 2012 Pyrethroid Insecticides: Use, Environmental Fate, and Ecotoxicology, Insecticides - Advances in Integrated Pest Management, Dr. Farzana Perveen (Ed.), InTech.
[17] Cycon M, Piotrowska-Seget Z 2016 Front Microbiol. 7- 1463 1-26
[18] Oros DR, Werner, I 2005 Pyrethroid insecticides: an analysis of use patterns, distributions, potential toxicity and fate in the Sacramento-San Joaquin Delta and Central Valley. In White Paper for the Interagency Ecological Program, vol. SFEI Contribution 415. Oakland, CA: San Francisco Estuary Institute.
[19] Amweg EL, Weston DP, Ureda NM 2005 Environ. Toxicol. Chem. 24 (4) 966 -72
[20] Aznar R, Moreno-Ramon H, Albero B, Sanchez – Brunete, C, Tadeo JL 2016 J. Soil Sediments.
[21] Ericsson, M, Colmso A 2000 J. Chromatogr. A 877 141-51
[22] Esteve-Turrillas FA, Aman CS, Pastor A, de la Guardia M 2004 Anal Chim Acta 522 73-78
[23] Gu XZ, Zhang GY, Chen L, Dai RL, Yu YC 2008 Environ Geochem Health 30 67-77
[24] You J, Weston DP, Lydy MJ 2004 Arch. Environ. Contam. Toxicol. 47 141-147
[33] Gan J, Lee SJ, Liu WP, Haver DL, Kabashima JN 2005 *J. Environ. Qual.* **34** 836 – 41
[34] Nadeu E, De Vente J , Martinez-Mena M , Boix-Fayos C 2011 *J. Soils Sediments* **11** 667 – 78
[35] Ali M A, Baugh P J 2003 *Int. J. Environ. Anal. Chem.* **83** 909
[36] Wu L, Song Y, Xu X, Li N, Shao M, Zhang H, Yu A, Yu C, Ma Q, Lu C, Wang Z 2014 *Food Chem* **162** 253-260
[37] Wang K, Xie X, Zhang Y, Huang Y, Zhou S, Zhang W, Lin Y, Fan H 2017 *Food Chem* doi: http://dx.doi.org/10.1016/j.foodchem.2017.08.061
[38] Zheng S, Wu H, Li Z, Wang J, Qian M 2015 *J. Sep. Sci* **38** 121- 127
[39] Galesio M, Mazzarino M, de la Torre X, Botre F, Capelo JL 2011 *Anal Bioanal Chem* **399** 861-875
[40] Zhou T, Xiao X, Li G 2012 *Anal Chem.* **84** 420-427