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Residual pesticides monitoring of the horticulture soil in Lembang

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Abstract. The use of pesticides in agricultural systems is one of the most important factors which contributes to the massive increase of food production worldwide. In this study, spike technical approach was carried out to simulate the concentration of pesticide. The sample was taken from soil in Lembang region. The pesticide from the spiked soil was extracted with sonication technique and analyzed based on HPLC method. The study showed that 10 gram positive and 2 gram negative bacteria isolates were found from the Lembang soil. The inhibition concentration (IC_{50}) of pesticides on microbial growth was 50%.

1. Introduction

Pesticides have been used extensively since the compounds are beneficial to agriculture as well as its usage greatly increases from year to year [1]. The majority of such substances are applied directly to soil or sprayed over crop fields and hence released directly to the environment. Therefore, pesticides contaminate soil surface of farmland [2]. The slow degradation in the environment leads to environmental contamination and adverse effects to humans. Pesticides in soils are studied more than any other environmental contaminants. The monitoring of their residues in soils were reported [3].

Sample preparation prior to the determination of organic pesticides in soil usually consists of several steps due to the complexity of the matrix. The sample preparation step can be marked as the most critical. The isolation of pesticides from soil samples usually involves conventional solid–liquid extraction methods such as soxhlet extraction. However, these methods are time consuming, labor intensive, and demand large volumes of hazardous organic solvents with high cost of both purchase and disposal. The modern sample preparation procedures, such as supercritical fluid extraction (SFE), matrix solid-phase dispersion (MSPD), headspace solid-phase micro extraction (HS-SPME), ultrasonic-assisted extraction (UAE), and microwave-assisted extraction (MAE) have been developed to overcome the drawbacks of the traditional approaches. Various studies reported that ultrasonic extraction of organic analytes from soil and sediment are effective methods. However, the application of ultrasonication to extract the carbofuran from soil is rarely studied [2-6].

Pesticides help controlling pests for a good cropping and yield, but they also create several holistic anomalies in soil health as residual effects [7]. The soil contamination of pesticides may impact the soil microorganism by inhibit their growth as well as their activities [8,9]. Bacterial diversity in soil is altered by pesticide use [10] along with its population number [11]. In addition, decreasing in enzyme activity is also observed under pesticides use [12,13].

This research was aimed to monitor the concentration of carbofuran as pesticide residue in soil taken from agricultural area in Lembang, West Java. Bacteria isolated from Lembang soil based on type of

gram staining was characterized. The inhibition effect of carbofuran on the growth of indigenous microbes was also observed.

2. Experimental

2.1. Chemicals and reagents

Carbofuran was obtained from Hunan Research Institute of Chemical Industry-PR. China with 99.7% of purity. This pesticide was used for 100 mg/L stock standard solutions in methanol and stored at 4°C in the dark. Methanol and acetonitrile were HPLC grade from Merck. Deionized water was obtained from PT. Ikapharmindo Putramas. Membrane filter of 0.45 μm was used for filtration. Nutrient agar from Difco was used as growth medium for bacterial isolation. CH_3COONa, KH_2PO_4, $Na_2HPO_4.7H_2O$, and NH_4Cl used for toxicity test were purchased from Merck.

2.2. Apparatus

An ultrasonic water bath Branson-3510, centrifuge EBA-12 and vortex Genius-3 were used for extraction. An HPLC Hitachi-7000 series was used for the carbofuran analysis, which was performed on a μ-Bondapak C_{18} column with UV detector set at 275 nm. The mobile phase was acetonitrile and deionized water with the ratio of 1:1 (v/v). The flow rate of eluent was 0.8 mL/min with an isocratic mode. The analysis was performed at ambient temperature and the injection volume was 20 μL. The spectrophotometer Agilent Technologies Carry 60 UV/VIS was used for the optical density as microbial growth indicator was measured at 540 nm.

2.3. Source, collection and soil sample treatment

The soil samples were collected from Lembang farming area, West Java, Indonesia, from 0 - 20 cm under the vegetable plantations, then stored at 4°C. Soil samples were homogenized, sieved to obtain the same particle size and air-dried at room temperature before use. The control soil of which free from carbofuran was spiked with carbofuran to achieve its concentration to 5 mg/kg. The soils were then kept overnight at room temperature.

2.4. Extraction of carbofuran in soil sample

Extraction of carbofuran in soil was carried out by sonication technique. The 5 g of the spiked soil was extracted with 20 mL of acetonitrile for 1, 2, and 3 h of sonication. The soil samples were then centrifuged at 4000 rpm for 5 min. The extract then was filtered through 0.45 μm syringe filters and the filtered solutions were analyzed by HPLC. The blank samples were prepared with the same procedure but without carbofuran spiking.

2.5. Isolation of bacteria in soil

To isolate the bacteria, several suspensions were made. Suspension 1 was made by mixing 0.5 g of soil sample and 10 mL of 0.9% salt solution mixed with 0.05% tween 80. Suspension 2 was made by mixing homogeneously 1 mL of suspension 1 and 9 mL of salt solution. Suspension 3 was made similarly, by mixing homogeneously 1 mL of suspension 2 and 9 mL of salt solution. The suspension 3 was then used to culture the bacteria in which 1 mL of the suspension was added into each sterile petri dish followed by pouring a nutrient agar media at 40-50°C. The petri dish was then closed tightly and incubated for 1-2 days at 37°C. Bacteria purification was done by tilting the petri dish and incubated for 6 days. Bacteria isolation was done through pour plate method.

2.6. Inhibition pesticides on microbial growth (IC_{50})

Inhibition concentration of pesticides on microbial growth by 50% was observed to determine the toxicity of the compound based on microbial assay. The toxicity of pesticides was carried out based on the method develop by Alsop *et al* [14]. The medium (150 mL) contained CH_3COONa, 1500 mg/L; KH_2PO_4, 850 mg/L; K_2HPO_4, 950 mg/L; Na_2HPO_4 $7H_2O$, 1500 mg/L, NH_4Cl; 850 mg/L and pesticides

(0-1000 mg/L). After inoculated with 1 mL microbial inoculum enriched from noncontaminated soil, the cultures were incubated at 30°C with orbital shaking (100 rpm) for 20 h. The optical density as growth indicator was then measured at 540 nm using a spectrophotometer.

3. Result and discussion

3.1. Qualitative analysis on spike soil samples

Extraction of carbofuran in soil reveals good results as shown in Figure 1. In the chromatogram, peak of carbofuran from the standard solution was appeared at the retention time (t_R) of 8.07 min (A), whereas carbofuran from the spiked soil sample at 8.04 min (C). The relative percent difference (RPD) both of the t_R was 0.37%, and the blank soil sample showed no peak of carbofuran (B).

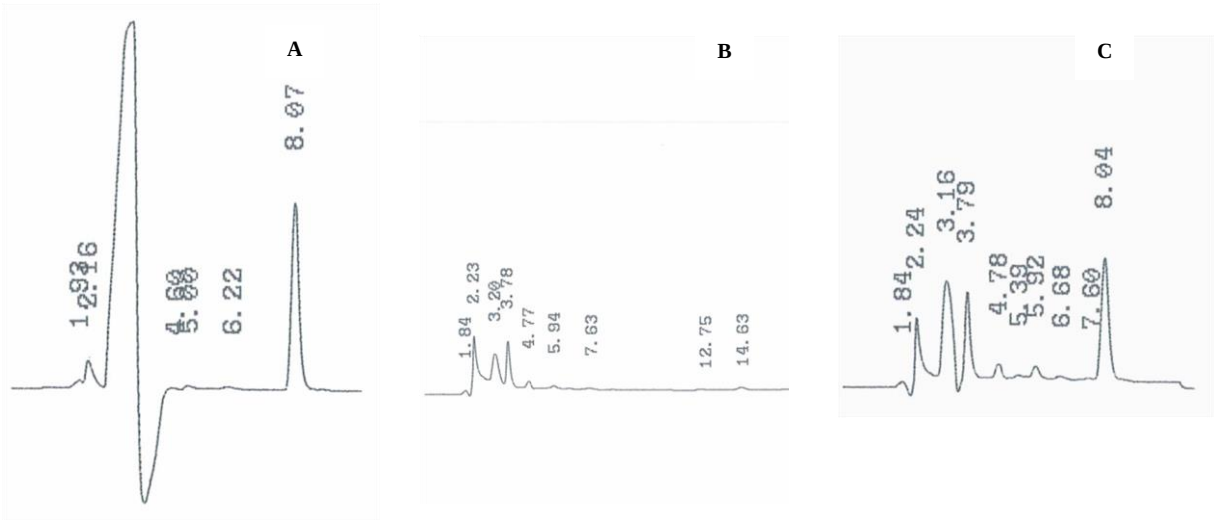

Figure 1. Chromatograms of (A) carbofuran standard solution, (B) blank soil sample, and (C) spiked soil sample.

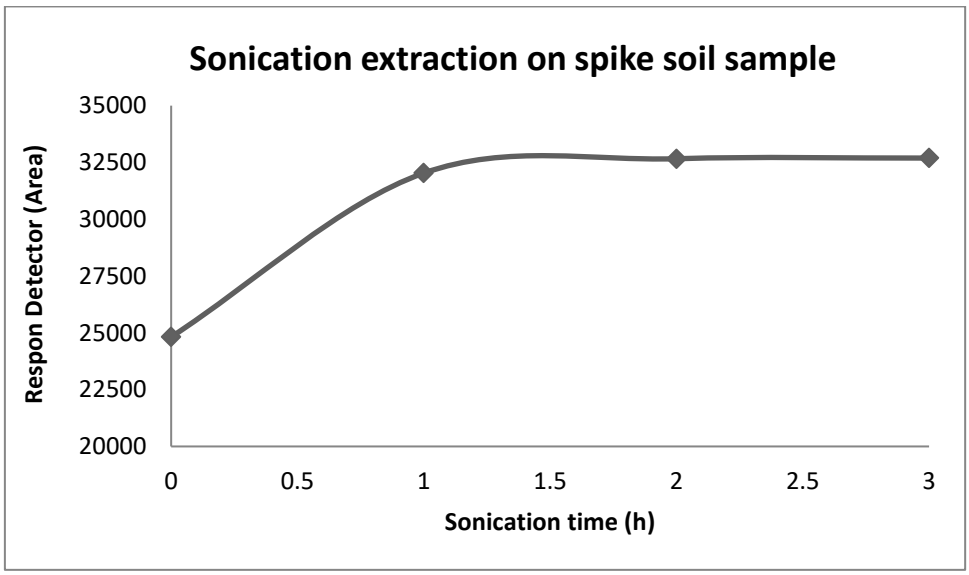

Figure 2. Sonication effect on spike soil sample extraction.

Parameter of the sonication efficiency such as time of the sonication was also investigated and the result can be seen in Figure 2. At the 5 mg/kg fortification levels of carbofuran in 5 g soil sample, the extraction was optimum at 1 h sonication with only 20 mL of acetonitrile as a solvent. The analytes in the extracts were analyzed directly by HPLC-UV without any further cleanup. The recovery of extraction was in the range of 109 to111%. Extraction by sonication technique was simple, time- and cost-effective. The concept of green analytical chemistry (GAC) has been done. The key goals to be achieved in greening analytical methods are elimination or reduction of reagents, waste, risk and hazard [15].

3.2. Bacterial isolation in soil samples

The Lembang horticultural soil analysis was carried out on moisture content and bacterial types. The moisture content was conducted by gravimetric method and its value was 31.5%. The bacterial isolate was shown in Figure 3. The composition of bacteria communities were 2 isolates of gram negative and 10 isolates of gram positive. The five carbamate insecticides *in vitro* and their average acute toxicity levels to the cyanobacteria were in a descending order: carbaryl > carbofuran, propoxur and metolcarb > carbosulfan [7].

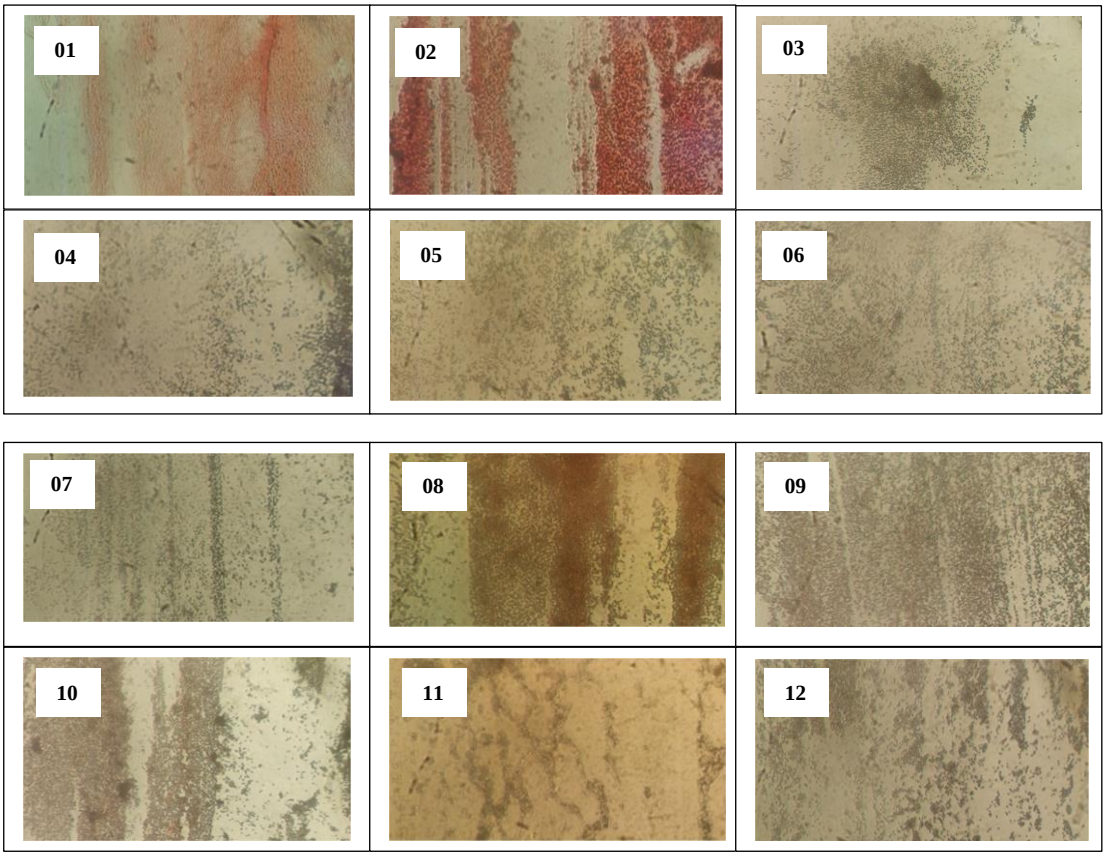

Figure 3. Bacterial types of Lembang soil: (01-02) gram negative and (03-12) gram positive.

3.3. Inhibition pesticides on microbial growth (IC_{50})

The results of the toxicity test are shown in Figure 4. The growth of microbes compared to the control was not significantly inhibited at pesticide concentration of 0.1 to 10 mg/L. This was shown by a high value of optical density of which was 87% at 10 mg/L of carbofuran. However, at 100 mg/L, the inhibition of the microbes observed with the optical density was 33% compared to the control.

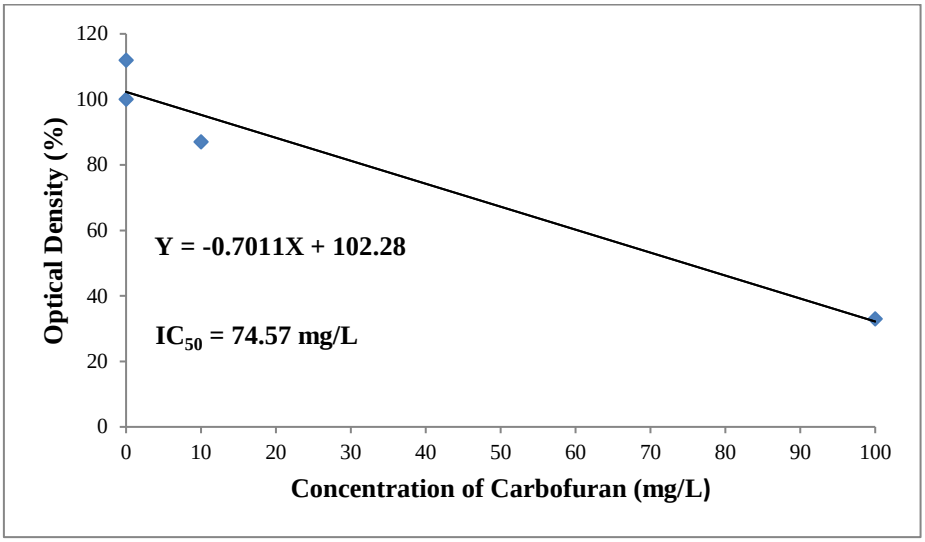

Figure 4. Toxicity test of carbofuran based on microbial growth.

The inhibition of microbial growth by 50% compared to the control was observed at the pesticide concentration between 10 and 100 mg/L. The IC_{50}, which is the concentration that inhibited the microbial growth by 50% of the control culture with no added pesticide, was 74.57 mg/L. Carbofuran is highly toxic after a single oral dose; the LD_{50} values in rats range from 6 to 18 mg/kg bw whereas and in other species such as mouse, guinea-pig, rabbit, cat and dog, ranges from 3 to 19 mg/kg bw [16]. Further study was recommended to observe the possible degradation of the carbofuran by microbial association.

4. Conclusion

From the current study, it is concluded that with the moisture content of was 31.5%, horticulture soil in Lembang contained 2 isolates of gram negative bacteria and 10 isolates of gram positive bacteria. The inhibition concentration of carbofuran on the microbial growth by 50% of the control culture without pesticide addition was 74.57 mg/L.

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