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DEGRADATION BEHAVIOUR OF CHLORPYRIFOS IN SPINACH *(SPINACIA OLERACEA)* AND SOIL

(Perlakuan Degradasi Chlorpyrifos di dalam Bayam *(Spinacia Oleracea)* dan Tanah

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Abstract

Chlorpyrifos, an organophosphorus insecticide that is widely used in pest control. This study focuses on the chlorpyrifos residue and degradation behaviour in spinach (Spinacia oleracea) and soil. The soil analyses include the determination of particle size, pH, percentage moisture and total organic carbon. The pH of soil was 4.95 with the content of 4.06 gkg^{-1} organic carbon, 15.55% moisture and 65.58% fine sand. A liquid-liquid extraction using acetone and water (70:30, v/v) followed by UV-Vis Spectrophotometer technique was performed to determine the residues of chlorpyrifos. The maximum absorbance (λ_{max}) of chlorpyrifos was observed at 229 nm. Exponential relations were found to fit the first-order rate equation. Results had also shown that chlorpyrifos degradation equation in spinach was $C=2.112^{e-0.09993t}$ with a half-life ($T_{0.5}$) of 6.93 days and R^2 = 0.99383. The rate equation for degradation of chlorpyrifos in the soil sample was $C=2.102^{e-0.01653t}$ with R^2 = 0.99383 and $T_{0.5}$= 42.09 days. When chlorpyrifos formulations were applied according to the recommended dose, the final residues in both samples exceeded the Codex Alimentarius Commission (CODEX) maximum residue limit of 1 $mgkg^{-1}$. Therefore, this work suggested that the harvest interval should be more than 14 days, which considered as safe to animal and humans.

Keywords: chlorpyrifos, degradation, rate, spinach, soil

Abstrak

Chlorpyrifos adalah racun serangga organofosforus yang digunakan secara meluas dalam kaedah kawalan perosak pertanian. Kajian ini memberi fokus pada analisis sisa dan pencemaran chlorpyrifos di dalam Spinacia oleracea (bayam) dan tanah. Analisa bagi sampel tanah adalah saiz zarah, pH, peratusan kelembapan dan jumlah kandungan karbon organik. pH tanah adalah 4.95 dengan 4.06 g kg^{-1} karbon organik, 15.55% kelembapan dan 65.58% pasir halus. Sisa chlorpyrifos diekstrak daripada bayam dan tanah dengan menggunakan aseton/air, membersihkan dengan pembahagian cecair/cecair dan kromatografi lajur, memekatkan kepada jumlah kecil dan dianalisis oleh UV-Vis. Keserapan tertinggi chlorpyrifos adalah pada 229 nm. Hubungan eksponen yang terhasil mematuhi persamaan kadar tertib pertama. Hasil kajian menunjukkan bahawa degradasi kepekatan chlorpyrifos dalam bayam bertepatan dengan $C= 2.112e^{-0.09993t}$ dengan separuh hayat chlorpyrifos dalam bayam adalah 6.93 hari dengan R^2= 0.99383. Degradasi chlorpyrifos dalam tanah dengan persamaan regresi dinamik dan separuh hayat chlorpyrifos dalam sampel tanah adalah $C= 2.102e^{-0.01653t}$, R^2= 0.99383 , $T_{0.5}$= 42.09 hari. Apabila rumusan chlorpyrifos telah digunakan mengikut dos yang disyorkan, sisa-sisa akhir dalam sampel bayam dan tanah adalah melebihi had maksimum CODEX dengan 1 mg kg^{-1}. Oleh itu, selang tuaian perlu lebih dari 14 hari, yang boleh dianggap sebagai selamat untuk haiwan dan manusia.

Kata kunci: chlorpyrifos, degradasi, kadar, bayam, tanah

Introduction

Spinach (*Spinacia oleracea*) is best-known as one of the green leafy vegetables that have the richest contents of iron. The leaf part contains high antioxidants and vitamins such as vitamin A, vitamin E, vitamin C, vitamin K, and vitamin B, folate or folic acid. The leaves are sources of protein and many minerals such as calcium, magnesium, phosphorus, zinc, copper and potassium that can be part of healthy diet, maintain healthy skin and prevent oral cavity. Regular consumption of spinach can also prevent osteoporosis and protect the body from certain cancers. For a human's balanced healthy diet, the presence of nutrients and minerals in the vegetables is very important. However, some insects and diseases can be damaging those on the vegetables during the growing process [1]. In agricultural management practice, chemical were used to control the crops such as nematicides, herbicides, insecticides and fungicides. However, frequent exposure of the pesticides can cause vegetable to be contaminated thus lead the vegetables to become very toxic [2].

Organophosphorus insecticides such as chlorpyrifos are the most extensively used pesticides in agriculture purposes. The existence of pesticides residues in food can be shown in direct result of pesticides used on crops [3]. The residues of pesticides on fruits and vegetables constitute a possible risk hazard to consumers. Extreme exposure to chlorpyrifos can cause nausea, dizziness, abnormal facial sensation and immune system, birth defects, leukemia, anorexia and fatigue [4,5]. Moreover, chlorpyrifos can inhibit the cholinesterase and become toxic in human. The toxic effects are caused by the disrupting of the chemicals through the normal nervous system function. It occurs because of the accumulation of acetylcholine in the synapse excessively [4].

Early detection of pesticides contamination highly required as avoiding the adverse on health effect and interference on surrounding such as ecosystem besides to ensure the maximum quality of the vegetables before it can be consumed. The pests might be killed by the pesticides, but its residue is still absence on the plant and soil if the concentration used is not according to the advisable amount. The degradation methods for chlorpyrifos from plant were discovered by many researchers such as electro-enzymatic, ultrasonic treatment, microbial, soil type and surfactant [6 - 9]. The influence of duration in harvesting the plant on degradation of chlorpyrifos especially in spinach is still needed to be studied. Therefore, the aim of this research is to evaluate the degradation behavior of chlorpyrifos for different harvesting time in spinach plant and its soil.

Materials and Methods

Materials

The samples were analyzed using a UV-Vis Spectrophotometer (Shimadzu Co., Kyoto, Japan). The measured wavelength ranged between 200-400 nm. The method used was based on [10], where the chlorpyrifos standard was dissolved in acetonitrile at concentrations of 1, 2, 4, 6, and 10 mg kg^{-1}. Three samples were prepared for each concentration. Presence of pesticide residues in spinach and soil were detected through absorbance measurement of UV-Vis Spectrophotometer with 1 cm quartz cuvettes cell. The measurement was carried out at room temperature (25.0 ± 0.5 °C).

Field Study

Spinach plants were planted and grown in the experimental field at UiTM Jengka, Pahang. The experimental plot was applied on randomized complete block design (RCBD). The spinach was cultivated in open field condition. The recommended doses about 0.42kg of active ingredient per hectare was applied to the mature spinach plant while some plots were sprayed with water as a control [11]. The weather was in sunny conditions during the field experimental period.

Degradation Studies

The spinach and soil samples were collected randomly from each plot at day 3, day 5 and day 14 after the imposed of chlorpyrifos. Both control samples were not applied with chlorpyrifos throughout the 14 days. All of the samples were placed in a plastic bag and stored at -18 ^{o}C. The spinach samples (50 g) were cut into small pieces before the analyses were done. The samples extracted with 150 mL acetone by shaken for one hour on a mechanical shaker. The extracts were filtered and concentrated by using vacuum rotary evaporator until the volume reached 10 mL. The evaporated sample was extracted with 100 mL of 4% sodium chloride solution and followed by three portions of dichloromethane. The organic phase was combined and further concentrated to 2 mL.

A soil sample was taken from the surface layer of spinach soil (0 to 15 cm) and each of the samples was replicated to five samples using random sampling on site. The sample was dried and passed through 2.0 mm sieve and sub-sampled. A mass of 50 g of the soil sample weighed and placed in 250 mL conical flask with 200 mL of acetone/water (7:3, v/v). After an overnight, the samples were shaken for one hour and filtered with a filter paper. The extracts were evaporated to the final volume 20 mL by using vacuum rotary evaporator. The concentrated samples were extracted with 100 mL of 4% sodium chloride solution and continued with three portions of dichloromethane. The organic layer of dichloromethane was combined and concentrated to 2 mL.

Clean-up Procedure

A volume of 2 mL of the concentrated sample was dissolved in 20 mL of n-hexane and followed by a mixture of 2.0 g of anhydrous sodium sulphate and 2.0 g of activated carbon. The mixture was allowed to settle down until clear layer of slurry observed. Later, a clear solution was eluted into a chromatographic column that packed with silica gel and washed six times with 20 mL of n-hexane. The elute was combined and evaporated under vacuum to dryness and finally dissolved in methanol to reach 10 mL volumetric flask level [12].

Chlorpyrifos Analysis

The samples were analysed with a UV-Vis Spectrometer (Shimadzu Co., Kyoto, Japan). The measured wavelength ranged between 200-400 nm. The method used was based on [10]. Presence of pesticide residues in spinach and soil were detected through absorbance measurement of UV-Vis Spectrophotometer with 1 cm quartz cuvettes cell. The measurement was carried out at room temperature (25.0 ± 0.5 °C).

Soil analysis: pH Determination

20.0 g of air dried soil (< 2 mm) was weighed and placed into 50 ml beaker. The sample was added with 20 mL distilled water and stirred in interval 30 minutes. The pH values obtained were recorded [13].

Moisture Content

5.0 g of the sample (fine soil) was placed in a tin, and an initial mass was recorded (W_i gram) to 0.001 g accuracy. The sample was dried at 105 ^oC in the oven for overnight. Then, the tin was cooled to a room temperature and final mass (W_f gram) recorded (ISRIC FAO, 2002). The moisture content was determined by using Eq. (1).

$$\text{Moist (\% wt)} = \frac{W_i - W_f}{W_f} \times 100 \quad (1)$$

where, W_i = Pre-ignition weight, (g) and W_f= post-ignition weight, (g)

Soil Organic Matter (SOM) Analysis

The total organic carbon analysis was analysed using the loss of ignition method (LOI) that proposed by [14].The sample of soil was sieved to 2.0 mm size before used for analysis. The 5.0 g of sample placed into a ceramic crucible and was heated to 375 ºC for overnight. Then, the sample was cooled in desiccators and weighed. The percentage of organic matter was calculated using Eq. (2).

$$\text{\% OM} = \frac{W_i - W_f}{W_i} \times 100\% \quad (2)$$

where, W_i = Pre-ignition weight, (g) and W_f= post-ignition weight, (g)

Particle Size Distribution

Particle size is often used in soil science to evaluate the soil texture. The USDA classification scheme was used as a reference in particle size analysis. The dry method of sieve analysis was performed by weighing approximately 500 g of oven-dried soil sample and separated through three sizes of fractions (< 0.05 mm, 0.05-0.2 mm and 2.0 mm). The empty sieve and pan was weighed to 0.1 g before being analysed. The mass of each separation of soil size weighed, and the mass fraction was calculated.

Results and Discussion

Parameter Analysis

Spinach and soil samples were collected and analysed at day 3, day 5 and day 14 after the application of chlorpyrifos. The soil samples have been analyzed for four physical-chemical parameters which are particle size analysis, pH, percentage moisture and total organic carbon content. These physical-chemical parameters are important in degradation behaviour of pesticide in soil. The results from each parameter analysis of soil under study demonstrated in Table 1.

Table 1. Physico-chemical properties of the soil under study

Texture (%)			**pH**	**Moisture content (% wt)**	**Organic carbon (g kg^{-1})**
Coarse sand	**Fine sand**	**Clay and silt**			
19.54%	65.58%	14.77%	4.95	15.55	4.06

Soil texture can affect the permeability and porosity. Fine textured soil is having more pore space and holding more water than coarse textured soil. However, coarse textured soils tend to have large, well-connected pore spaces and hence high permeability. There is a correlation between the percentage of soil particle and the amount of carbon associated with the soil particle. High specific surface area of the soil that dominated by clays adsorbed more humic substances compared to low surface area. The small particles size of soil especially the clay and silt particles in the soil having high content of carbon than fine textured soil [15]. Organic matter is one of the major factors that influenced the extent of the adsorption of pesticide on the soil. Higher organic matter content will improve and increase the pesticide adsorption on the soil. Soil organic matter and clay texture of the soil are strongly associated because of their mutual attraction of molecular attraction and surface charges [16]. Most of the pesticides are effectively bound with soil organic matter which reduces the leach of pesticide through the soil.

High pH will enhance the organic carbon dissolution and dispersion of particle as well as lower the flow rate and increase the duration of a desorption process. Thus, it enhances the pesticide mobility in the soil. However, the result showed low pH number which is 4.95. The decreased in soil pH will reduce the solubility of soil organic matter and reduce the pesticide flow rate and movement [17]. In most of the cases, lowering in pH favors the bonding of organic to a clay particle. In this study, pH obtained was slightly acidic which are 4.95 and 4.06% of organic carbon content [15]. Soil acidity also affects the pesticide properties. The pesticides bind more with the clay in the soil when the pH decreases. Usually, the pesticides are less soluble in water with low pH values.

The moisture content can affect the pesticide leaching ability. Pesticides are broken down faster in moisture condition than drier soil. The result of these studies showed a low percentage of water content which was 15.55%. There are many possibilities affecting the moisture content value especially the role of weather. Climatic condition influences the water percolating system in the soil. Low percentage of moisture content obtained in these studies indicated the soil is quite drier; thus, lower the breakdown of chlorpyrifos in the soil.

Control Treatment

The control sample of spinach and soil where the sample is not applied to chlorpyrifos also showed respectively their concentrations which are 1.567 mg kg^{-1}and 1.481 mg kg^{-1}. The detected residue in control sample was probably due to the persistence retain of chlorpyrifos that was used previously and can be transported to plant so that positive detection of its residue in the control sample was identified.

Chlorpyrifos in Spinach Sample

Figure 1 demonstrates the residues of applied chlorpyrifos in spinach sample after spraying up to 14 days (336 h). The data was obtained through absorption of UV-Vis at 229 nm with 0.99383 of the correlation coefficient.

Chlorpyrifos showed the maximum initial deposit on spinach sample expressed as ppm or mg kg $^{-1}$. It was noticeable that the residue of unsprayed insecticide in sample is presence.

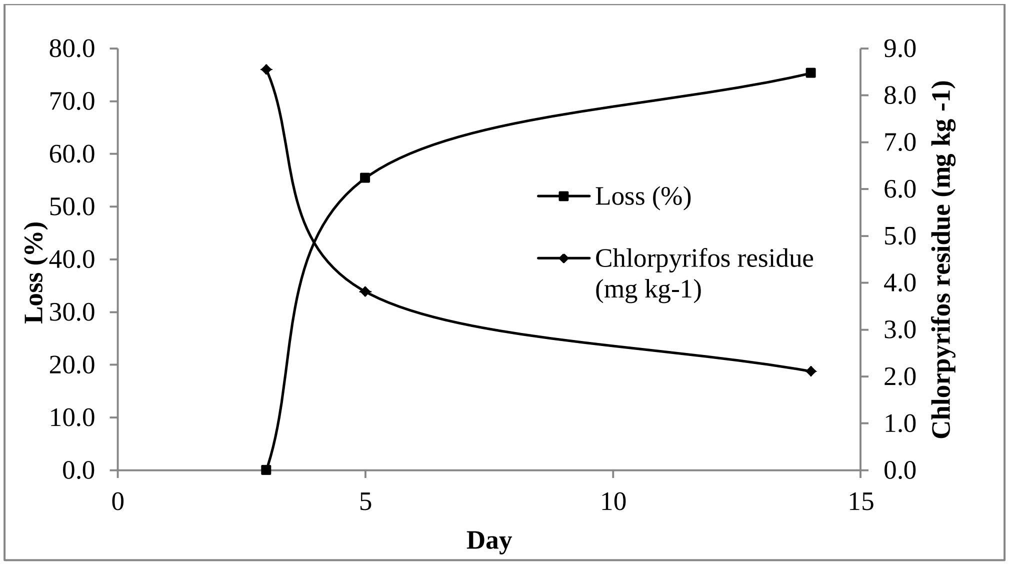

Figure 1. Persistence and degradation of chlorpyrifos in spinach plant

After three days of chlorpyrifos application, the detected residues were 8.556 mg kg^{-1}. The magnitude of loss was obtained to be after three days of application are 55.23%. As expected, a continuous and gradual deterioration of chlorpyrifos residues in treated plant was observed to seek the function of time after application. In 5 days after application, the chlorpyrifos residues detected were 3.830 mg kg^{-1}. Naturally, the decreasing of chlorpyrifos residues is due to the physical and chemical properties as well as the nature capability of the plant itself to breaking down the pesticide in the environment [18]. The residues of chlorpyrifos in spinach at day 14 showed 2.112 mg kg^{-1} and the magnitude of loss were obtained to be 75.32% after 14 days of chlorpyrifos applications. According to Codex Alimentarius Commission [19], the tolerance limits for chlorpyrifos in spinach is 1 mg kg^{-1}. In the result obtained in the present study, it is recommended to further prolong the experiment period up until 21 days before being harvested so that the minimum residue level attained are below the maximum residue limit (MRLs). The dynamic regression equation and the half-life of chlorpyrifos in spinach were as follows: $C=2.112^{e-0.09993t}$, R^2 = 0.99383, $T_{0.5}$= 6.93 days.

Chlorpyrifos in Soil Sample

Figure 2 shows the residues of chlorpyrifos insecticide and the percentage of loss in the soil sample after the application of chlorpyrifos up to 14 days. There was a detection of chlorpyrifos residue in control soil even though the soil is not applied with chlorpyrifos. Among the reasons of the presence of chlorpyrifos on soil are those agricultural soils were applied with chlorpyrifos previously and it had retained in the soil.

After application of chlorpyrifos, 2.647 mg kg^{-1} of residues was detected on day 3. Day 3 shows the highest value of chlorpyrifos that means more stability and penetration of those pesticides in the soil. A sharp declined of chlorpyrifos residues in a soil sample after application was observed. The concentration of chlorpyrifos residues at day 5 was decreased to 2.419 mg kg^{-1}. On the 14 day after application, the result showed 2.102 mg kg^{-1} of chlorpyrifos residues on the soil. In this respect, the magnitude of loss recorded was in about 8.614% and 20.59% respectively. According to US EPA's maximum residue limit (MRL) of chlorpyrifos in vegetable is 1 ppm but the concentration of the residues in spinach at 14 days was slightly higher than those MRL value. Therefore, it is suggested that the interval should be extended more a week at the recommended dosage which could be considered as safe for human and animal consuming ([12]. The dynamic regression equation and the half-life of chlorpyrifos in soil sample were as follows: $C=2.102^{e-0.01653t}$, R^2 = 0.99383, $T_{0.5}$= 42.09 days.

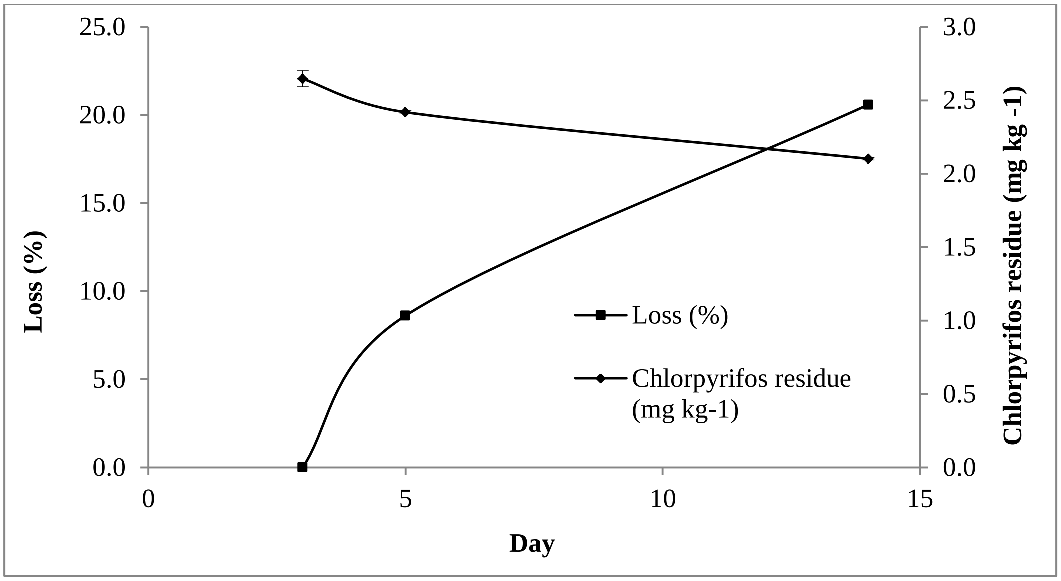

Figure 2. Persistence and degradation of chlorpyrifos in the soil sample

Degradations of chlorpyrifos in spinach and soil

Degradations of chlorpyrifos in samples are presented in Table 2. The chlorpyrifos disappearance on spinach was found to follow the first order kinetic model. The kinetic data for chlorpyrifos degradation on spinach for 3rd, 5th and 14th days were measured to be 0.507, 4.31 and 6.94 days after the treatment. Degradation of chlorpyrifos in the soil sample was longer than spinach sample. At day 3, the degradation rate was 0.39 day. However, significant increase of half-life of chlorpyrifos was measured at day 5 and day 14. In the soil, the half-life of chlorpyrifos in 5-day application was measured to be 38.4 days and 42.1 day at day 14. It showed that the chlorpyrifos in the soil sample was not easy to degrade. The degradation of chlorpyrifos was significantly influenced by the moisture content on the soil. [20] had obtained the similar results for another organophosphorus pesticide (OP). The degradation was slower due to the inactivation of the microbial activity under the low moisture content in the soil sample.

Table 2. Data on residue and degradation of chlorpyrifos in soil and spinach.

Day	**Residue (mg kg^{-1})**		**Half-life $t_{1/2}$ (day)**	
	Spinach	**Soil**	**Spinach**	**Soil**
3	8.556	2.647	0.50	0.39
5	3.830	2.419	4.31	38.4
14	2.112	2.102	6.93	42.1

Conclusion

Chlorpyrifos is an insecticide that is widely used in controlling flies, mites and other pests related to plant, vegetable and fruits. This study was designed to investigate the presence and the residues of chlorpyrifos in spinach (Spinacia Oleracea) and its soil. It has also determined the half-life between the intervals spraying and harvest required for the safe use of this spinach as well to prevent the health problem to the consumers. The results showed that soil texture, pH, soil moisture and organic matter are the critical variable affecting chlorpyrifos degradation. The results from this study implied that the chlorpyrifos degradation is slower in acidic soil, low moisture content with high fine sand texture. First-order kinetics could describe the degradation of chlorpyrifos in spinach and soil. In this study, the chlorpyrifos degradation was incomplete within 14 days of applications and it meant that the chlorpyrifos was not easy to degrade. Based on the limitation of recent work, it is suggested to further the extension of the interval period up to 21 days before being harvested, so the rate of degradation is complete. It would help in establishing the adequate monitoring of the residue and its wise pest management strategies in vegetable fields so it could be considered as safe for animal and human beings.

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