LEACHING AND PERSISTENCE OF BENOMYL IN AN OIL PALM NURSERY

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ABSTRACT

A field trial on the leaching and persistence of benomyl in an oil palm nursery agro ecosystem was conducted at Labu, Negeri Sembilan, Malaysia. Benomyl was analysed as its degradation product carbendazims because benomyl is a very unstable compound and easily degrades in organic solvents and aqueous solutions. Therefore, the quantification of benomyl was expressed as carbendazim. Experimental plots in the nursery were sprayed with the fungicide benomyl, while the control plot was left untreated. The fungicide was sprayed using a knapsack sprayer at the recommended manufacturer's dosage as well as at double the recommended dosage. Composite soil samples were collected from each replicate plot at depths of 0-10 cm, 10-20 cm, 20-30 cm, 30-40 cm and 40-50 cm. Sampling of soils were taken at intervals of -1, 0, 1, 2, 3, 5, 7, 14, 30, 60 and 90 day/days after fungicide treatment. Analytical methods for the determination of carbendazim in soils were developed and good recoveries of over 80% with small standard deviations were obtained from soil samples. Carbendazim residues were detected until Day 1 after treatment. The carbendazim residues were found at a depth of 0-50 cm for both normal as well as double recommended dosages. The objective of this study was to evaluate the mobility and persistence of the fungicide benomyl in an oil palm nursery.

Keywords: leaching, persistence, nursery, depth, recovery.

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INTRODUCTION

Benomyl is a systemic benzimidazole fungicide that is selectively toxic to microorganisms and invertebrates, especially earthworms. It is used against a wide range of fungal diseases of field crops, fruits, nuts, ornamental plants, mushrooms and turf. Formulations of benomyl include wettable powder, dry flowable powder and dispersible granules. Benomyl is strongly bound to soil and is highly persistent. It completely degrades to carbendazim within several hours in acidic or neutral water. The half-life of carbendazim is two months (Howard, 1991). Benomyl is the common name for methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate, sold under the trade name of Benlate®. It is grouped under the benzimidazole fungicides. It has low solubility in water, about 2 mg litre^{-1} at 25°C. *Figure 1* shows the chemical structure of benomyl and its degradation product, carbendazim.

Benomyl is known to be a very unstable compound. Several analysts have studied the kinetics of benomyl degradation in organic solvents (Chiba and Doornbos, 1974; Singh *et al*., 1990) and aqueous solutions (Sims *et al*., 1969; Singh *et al*., 1990; Clemons and Sisler, 1969). The main degradation product of benomyl is carbendazim. For this reason, benomyl is generally expressed as carbendazim.

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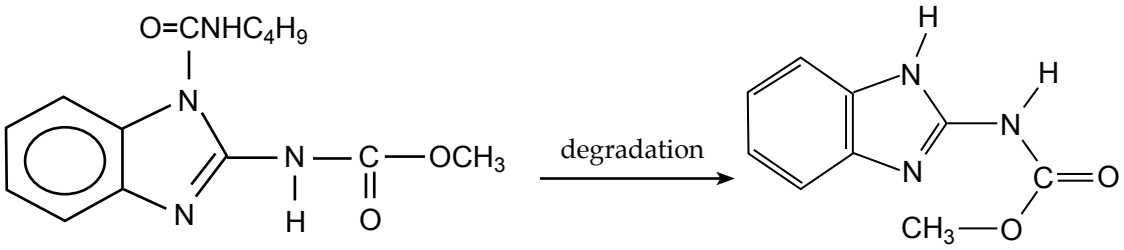

Figure 1. Chemical structure of benomyl and its degradation product, carbendazim.

The commonly used herbicides in oil palm plantations are glyphosate, metsulfuron-methyl, paraquat, 2,4-D, fluroxypyr, dicamba, triclopyr and glufosinate ammonium (Chung *et al*., 2000). In the case of insecticides cypermethrin, trichlorfon, chlorpyrifos and carbofuran (Chung *et al*., 1991; 2000; Sudharto *et al*., 1991; Wood, 1968; Wood and Ng, 1969) are often used. In oil palm nurseries, the major fungicides used are mancozeb, thiram and benomyl.

Previous studies on the leaching of chlorpyrifos in an oil palm plantation, was reported by Halimah *et al.* (2010) and the results showed that chlorpyrifos residue was detected in the soil for up to five and seven days after treatment, when applied at the recommended and double recommended dosage. In another study, Badrul Hisyam (2012) proposed a method for the determination of λ-cyhalothrin and cypermethrin in soil using liquid-liquid extraction and identified these compounds using gas chromatography (GC) attached to a micro electron captured detector (μECD). This method was applied to real soil samples from an oil palm plantation in Malaysia and it was found that λ-cyhalothrin and cypermethrin residues were detected in soil at a very low level.

Halimah *et al*. (2011) has reported on leaching of chlorpyrifos in peat soil of an oil palm plantation in Malaysia. Results from this study showed that chlorpyrifos was detected at all levels of the soil profile (0-45 cm), and has very low persistence in the soil and therefore may have a low impact on the environment. There was a comprehensive study on the fate of fluroxypyr in oil palm agro environment. It was reported that fluroxypyr leached down to 50 cm depth. However, fluroxypyr persists for a short period in the soil and water ecosystems, but was not found in the oil palm leaf (Halimah *et al*., 2009; 2005a,b). Until today, there is no reported study on the fate and behaviour of fungicides in Malaysian oil palm agro environment. There is therefore a need for information on the environmental fate of fungicides under the Malaysian climatic conditions to ensure its appropriate use.

METHODOLOGY

Experimental Design

The study was conducted at Labu, Negeri Sembilan, Malaysia. The experimental plot was established on 0.5 ha flat land with nine sub-plots. These sub-plots were subjected to three different treatment conditions. Three sub-plots were treated with manufacturer's recomended dosage, another three sub-plots were treated with double recommended dosage and the last three sub-plots were control plots with water treatment. Each treatment was carried out in triplicate and each sub-plot was separated by a 2 m buffer zone. The sub-plots with the recommended and double the recommended dosages of benomyl were tagged to identify the sub-plots. The seedlings used for this study were eight months old.

Soil Sampling

For each plot, composite soil samples were taken randomly from the centre of the plot at five points using an auger at the following depths: 0-10, 10-20, 20-30, 30-40 and 40-50 cm. Soil samples collected from each replicate at same depth were combined before analysis. Soil sampling was carried out one day before treatment (-1), immediately after spraying (Day 0) and on 1,2,3,5,7,14,21,30,60 and 90 days after treatment (DAT). The collected soil samples were air-dried for three to five days in an air-conditioned room at 16ºC, sieved through a 2-mm sieve, and stored at -4ºC prior to analysis to inhibit microbial activity.

Reagents and Herbicide

All reagents and solvents used in this study were of analytical grade. Acetone was obtained from Merck. Standard carbendazim (99.4% purity) was purchased from Dr Ehrenstorfer Co., Germany. Solid phase extraction (SPE) cartridges, OASIS® HLB (polyvinyl dibenzene), containing 200 mg of sorbent were obtained from WATERS.

Apparatus

An Agilent 1100 HPLC Series fitted with ultra violet detector (UV) was used. The column used was Supelco Ascentis™ RP-Amide, 5 μm (250 mm X 4.6 mm I.D.). The vacuum N-Evap Model 1111, used to evaporate the solvent was purchased from Organomation Associates Inc., US. The Vortex mixture Type 37600 was purchased from Thermolyne Co., US. The pH meter used was a PHM 210 Radiometer from Copenhagen, Denmark. A vacuum manifold, IST VacMaster was from the International Sorbent Technology (IST), US. The aspirator connected to the vacuum manifold was a Buchi B-169 Vacuum N-Evap Model 1111 was from Organomation Associates Inc., US. Nylon filters of 0.45 μm were from WATERS.

High Performance Liquid Chromatography (HPLC) condition

An Agilent HPLC, 1100 series fitted with a variable wavelength UV detector set at 223 nm was used for quantification of carbendazim. The column used was a Supelco Ascentis™ RP-Amide, 5 μm (250 mm x 4.6 mm I.D.). The wavelengths were set at 280 nm. The mobile phase was methanol and acetate buffer (0.01% acetic acid in water) in the ratio of 20:80. Flow rate and injection volume were 0.8 ml min^{-1} and 100 μl, respectively.

Preparation of Standard Stock Solutions

Carbendazim standard in methanol for recovery study in soil. Standard carbendazim (0.05 g) was dissolved in 50 ml methanol to make up a stock solution of 1000 μg ml^{-1}. An intermediate stock solution of 100 μg ml^{-1} was prepared by dissolving 1 ml of the standard stock solution with methanol in a 10 ml volumetric flask. Working standard solutions containing 0.1 μg ml^{-1} to 8 μg ml^{-1} were prepared by appropriate dilution of the standard stock solution with methanol from the intermediate stock solution. All the standard solutions were stored at –20°C in glass bottles with Teflon-lined screw caps.

Carbendazim standard in acetonitrile:water (30:70) for calibration curve. Standard carbendazim (0.05 g) was dissolved in 50 ml in acetonitrile:water (30:70) to make up a stock solution of 1000 μg ml^{-1}. An intermediate stock solution of 100 μg ml^{-1} was prepared by dissolving 1 ml of the standard stock solution with acetonitrile:water in a 10 ml volumetric flask. Working standard solutions containing 0.1 μg ml^{-1} to 1 μg ml^{-1} were prepared by appropriate dilution of the standard stock solution with acetonitrile:water from the intermediate stock solution. All the standard solutions were stored at –20°C in glass bottles with Teflon-lined screw caps.

Determination of Benomyl

Since benomyl is known to be a very unstable compound and easily degrades in organic solvents and aqueous solutions to carbendazim, therefore the analysis of benomyl will be expressed as carbendazim.

Determination of Carbendazim in Soil

Thirty grammes of soil sample were added to a 100 ml conical flask. The soil was spiked with the standard carbendazim solution in methanol to obtain 0.01 to 0.3 μg gl^{-1} carbendazim in soil, and the contents were mixed using a vortex mixer. Subsequently, 40 ml of methanol:water (32:8) were added to the conical flask and the mixture was again mixed for 30 s on vortex mixture. The conical flask was placed in an ultrasonic bath for 45 min. The extract was then filtered using nylon filter and transferred into a 100 ml round bottomed flask. Twenty millilitres of the extract were then evaporated down to 2-5 ml using a rotavapour. The content was then mixed with 50 ml water and the solution was adjusted to pH 3.5 by addition of 10% hydrochloric acid. An SPE cartridge (OASIS® HLB), attached to a manifold, was pre-washed with 4 ml dichloromethane, 4 ml methanol and 5 ml distilled water and the washings discarded. One end of the SPE tubing was immersed into the mixture and the other end was connected to the OASIS® HLB cartridge before switching on the aspirator for the water to be sucked through the OASIS® HLB. The flow of water through the SPE cartridge was controlled by a pressure knob so as to get a drop wise elution of the water. A preliminary study on the effect of loading the water sample through the SPE cartridge had shown that the suitable flow rate was 8-10 ml s^{-1}. After the water was completely drawn through the SPE cartridge, the bottle was rinsed with 5 ml water. The rinse was also passed through the SPE cartridge and the cartridge was then vacuum dried. Carbendazim adsorbed in the cartridge was eluted with 5 ml methanol and 5 ml dichloromethane. The eluate was dried using nitrogen and redissolved in 2 ml acetonitrile:water (30:70) before injecting into the HPLC–UV. For each concentration, triplicate analysis was carried out, and a quantification of the analyte was made by comparison with the carbendazim standard solution.

RESULTS AND DISCUSSIONS

Standard Calibration Curve for HPLC-UV

To determine the reproducibility of the injection technique and linearity of the UV response, repeated injections of 0.8 - 8 μg ml^{-1} standard carbendazim solution were made into HPLC-UV system. The calibration curve, plotted using concentrations of standard carbendazim against the HPLC peak area, is as shown in *Figure 2*. The equation derived from the calibration data is Y = 617.19x + 37.084, where *y* is the peak area of standard carbendazim solution and *x* is the concentration in μg ml^{-1}. The r^2 of the calibration curve is 0.9993 at 99.9% confidence.

The method for analysis of carbendazim involved two steps namely, liquid-solid extraction followed by a clean-up step using SPE cartridges. *Figures 3a, 3b* and *3c* show the HPLC chromatograms corresponding to an untreated soil sample, carbendazim standard solution (1 μg ml^{-1}) and a spiked soil sample containing 0.3 μg g^{-1} of carbendazim, respectively. The retention time of carbendazim was 21.62 min. *Table 1* shows the recoveries and coefficients of variation of spiked soil samples containing 0.01 to 0.3 μg g^{-1} of carbendazim. It was found that the recovery ranged from 85.95%-97.89% and the coefficient of variation ranged from 1.7% to 8.2%.

Table 2 shows the physico-chemical properties of soil in the field trial. Soil samples used for the spiking study were collected from the oil palm nursery, air dried and sieved through a 2-mm sieve. The analysis showed that the soil contained clay (20.93%), sand (50.75%), silt (41.81%) with a total carbon content of 4.16%, pH 5.52, soil moisture 5.69% and cation exchange capacity (CEC) 13.35 Cmol (+)/kg soil. The soil was classified as sandy clay loam soil based on these soil characteristics.

Weather Conditions

Figure 4 shows the daily rainfall recorded in the months of July to October 2008 in Labu, Negeri

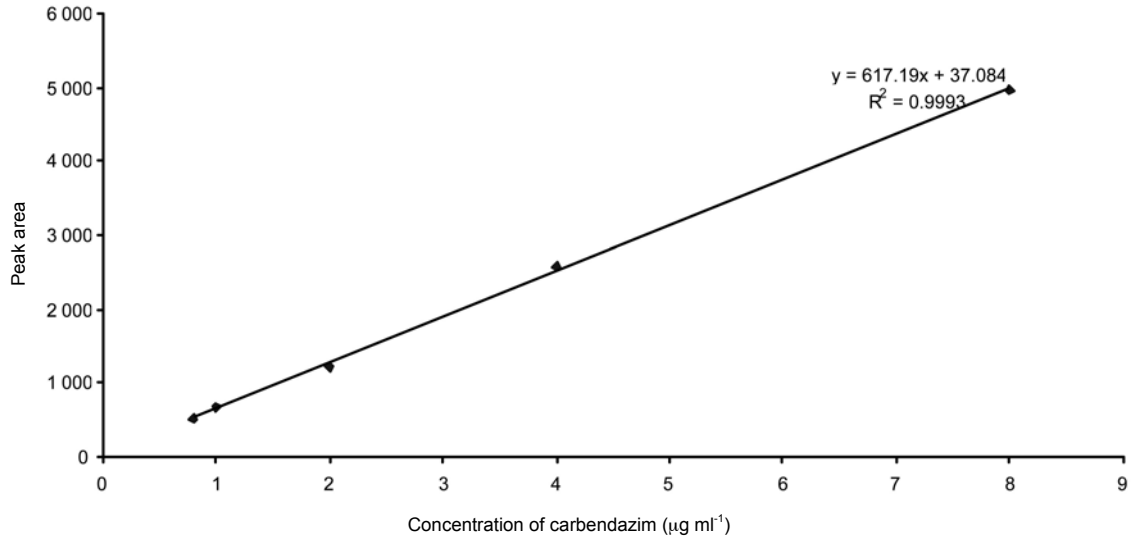

Figure 2. Calibration curve of carbendazim concentration against the high performance liquid chromatography (HPLC) peak area.

TABLE 1. RECOVERY OF CARBENDAZIM FROM SPIKED SOIL SAMPLES

Spiking level (μg g^{-1})	% Recovery	Relative standard deviation
0.01	94.55	8.2
0.03	97.89	7.2
0.1	87.08	4.6
0.2	85.95	1.7
0.3	90.44	2.1

TABLE 2. PHYSICO-CHEMICAL PROPERTIES OF THE SOIL AT LABU, NEGERI SEMBILAN, MALAYSIA

Soil type	Clay	Sand (%)	Silt (%)	Total carbon (%)	pH	CEC	Moisture content (%)
Sandy clay loam	20.93	50.75	28.32	4.16	5.52	13.35	5.69

Note: CEC - cation exchange capacity.

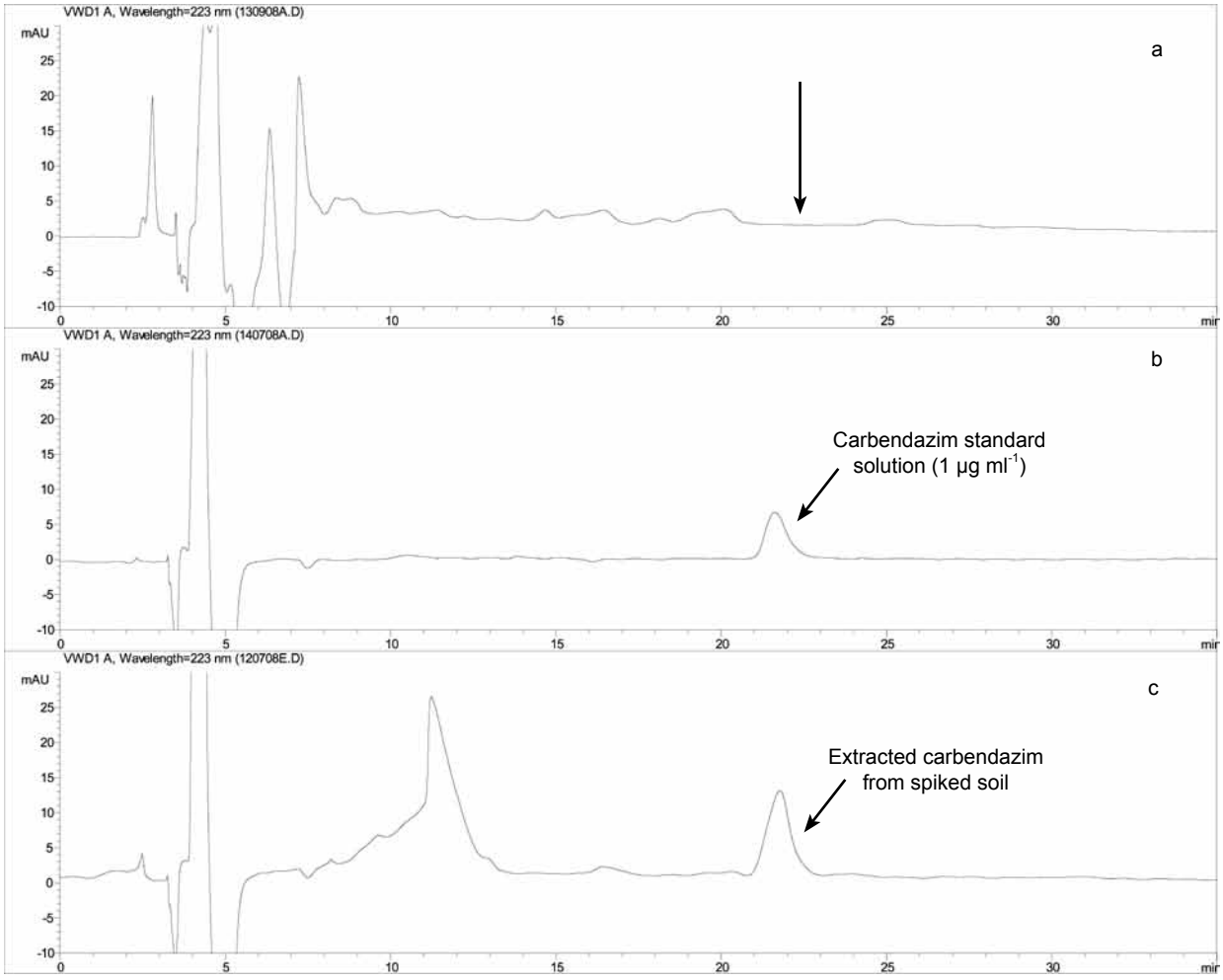

Figure 3. Chromatograms of a) blank soil sample, b) carbendazim standard solution (1 µg ml^{-1}) and c) spiked soil sample (0.3 µg g^{-1}).

Sembilan estate during the study period. The highest rainfall was in September and the lowest was in October 2008.

Figure 5 shows the carbendazim residue in soil samples at the recommended and double recommended dosage. Carbendazim residues were detected in soil samples collected at intervals of 0 and 1 DAT at both dosages. Residual amounts of carbendazim detected in the soil ranged from 3-11 µg kg^{-1} at sampling intervals of 0 and 1 DAT. Carbendazim residue was detected at the depth of 0-50 cm on day 0 (the sample was taken 6 hr after application). However, on 1 DAT carbendazim was only found at the depth of 0-30 cm. The highest concentration was found in the layer at a depth of 0-10 cm and the amount was 11 µg kg^{-1} on day 0. The amount of carbendazim reduced with increasing soil depth. The quantity of carbendazim at depth of 0-10 cm was reduced by 73% from 0 DAT to 1 DAT. Meanwhile, the amounts of carbendazim at depths 10-20 cm and 20-30 cm were reduced by 66% from 0 DAT to 1 DAT for the recommended dosage. However, carbendazim was not detected at all depths tested from 3 DAT onwards (0-50 cm).

Carbendazim residue was detected in the soil samples collected at intervals of 0 DAT and 1 DAT at

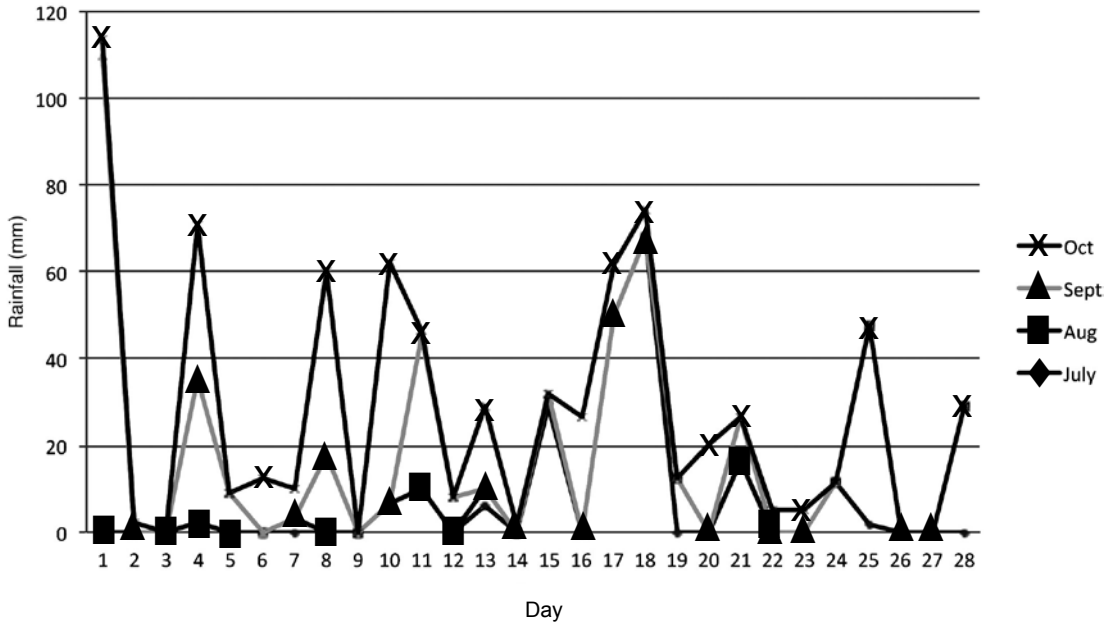

Figure 4. Daily rainfall at Labu from July to October 2008.

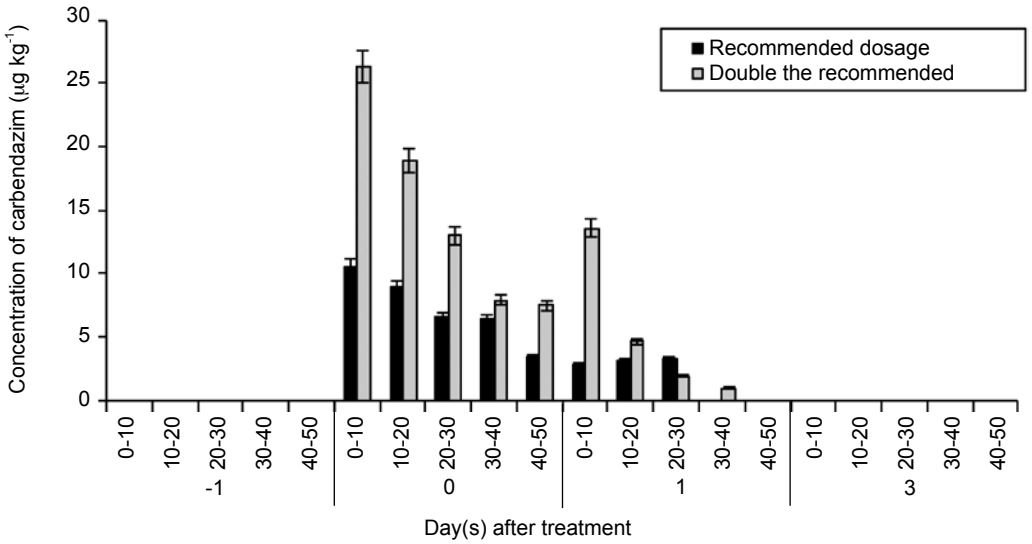

Figure 5. Carbendazim residue in soil samples at the recommended and double recommended dosage.

the double recommended dosage (*Figure 5*). Residual amounts of carbendazim detected in the soil, ranged from 1- 26.3 µg kg^{-1} at sampling intervals of 0 and 1 DAT. Carbendazim residue was detected at the depth of 0-50 cm on Day 0 (the sample was taken 6 hr after application) and up to 1 DAT. The highest concentration was found in the layer at a depth of 0-10 cm and the amount was 26.3 µg kg^{-1} on Day 0. The amount of carbendazim at depth of 0-10 cm was reduced to 52% from 0 DAT to 1 DAT and 25% at depth of 10-20 cm from DAT 0 to DAT 1. Meanwhile, it was also observed that carbendazim was not detected at all depths tested from 3 DAT onwards (0-50 cm).

CONCLUSION

Carbendazim leaches easily into the lower layer of the soil profiles (0-50 cm), probably because of the soil characteristics. Carbendazim residue in the soil was detected up to 1 DAT for both the recommended and double recommended dosages. As expected, the amount of carbendazim was less when applied at

the recommended dosage as compared to the double recommended dosage. The results suggested that carbendazim persists for a short period in the soil.

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