ORIGINAL ARTICLE

Phytoremediation of carbofuran residues in soil

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Abstract

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In this study, the ability of plants to clean up carbofuran residues in rice field soil was examined. Plants were grown in 8 inches diameter plastic pots filled with soils containing 5 mg/kg carbofuran. Phytoremediated samples were analyzed for carbofuran concentration. The results showed that carbofuran was rapidly degraded under planted soil and non-planted soil with half-lives ranging from 2-7 days. These facts suggest that phytoremediation could accelerate the degradation of carbofuran residues in soil and carbofuran was not persistent in the soil environment.

Key words : cabofuran, phytoremediation, plant

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การบำบัดสารคาร์โบฟูรานในดินโดยใช้พืช

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งานวิจัยนี้ได้ทำการศึกษาความสามารถของพืชในการบำบัดสารคาร์โบฟูรานที่ตกค้างในดินจากนาข้าว โดยได้ทำการปลูกพืชลงในดินจากนาข้าวที่ผสมสารคาร์โบฟูรานที่ความเข้มข้น 5 มก/กก ดิน ในกระถางที่มีขนาดเส้นผ่านศูนย์กลาง 8 นิ้ว จากนั้นนำตัวอย่างดินที่ผ่านการบำบัดด้วยพืชมาทำการวิเคราะห์หาความเข้มข้นของสารคาร์โบฟูรานที่ถูกย่อยสลายไป ผลการศึกษาพบว่า คาร์โบฟูรานสามารถถูกย่อยสลายได้อย่างรวดเร็วในดินที่มีการปลูกและไม่มีการปลูกพืช โดยมีค่าครึ่งชีวิตอยู่ในช่วง 2 ถึง 7 วัน ซึ่งแสดงให้เห็นได้ว่าพืชสามารถเร่งการย่อยสลายคาร์โบฟูรานที่ตกค้างในดินได้เร็วขึ้น นอกจากนั้นยังแสดงว่าสารคาร์โบฟูรานไม่มีความคงทนในสิ่งแวดล้อม

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Carbofuran (2,3-dihydro-2,2-dimethyl-benzofuranyl-N-methylcarbamate) is a phenyl-carbamate pesticide widely used in rice growing to prevent and eliminate pests such as *Nilaparvata lugens* Stal, mainly rice insects which cause wide-spread damage to rice culture (Rajagopal *et al.*, 1984). From the carbofuran characteristic report of WHO, 2003, carbofuran is a moderately persistent pesticide in the environment. Its half-life is between 26 and 110 days depending on pH, soil type, temperature, moisture content and microbial population. It has very low ability to be adsorbed, thus it can be easily leached into groundwater. In general, carbofuran can be degraded by the chemical hydrolysis and biological degradation process. The carbofuran degradation can take place more rapidly in alkaline condition.

A report showed that only 1% of insecticide sprayed would go to insect pest and 99% would be a residue in the environment (Kadsomboon, 2003). One of methods that can remediate pesticide residue in soil is phytoremediation. This method uses green plants to clean up contaminants in the environment (Ag-West Biotech Inc., 1999). Plants remediate organic contaminants via three mechanisms: direct uptake of contaminants and subsequent accumulation of nonphytotoxic metabolites into plant tissue, release of exudates and enzymes that stimulate microbial activity and biochemical transformations, and enhancement of mineralization in the rhizosphere (the root-soil interface), which is attributable to mycorrhizal fungi and the microbial consortia (Schnoor *et al.*, 1995). Phytoremediation is an aesthetically pleasing mechanism that can reduce remedial costs, restore habitat, and clean up contamination in place rather than entombing it in place or transporting the problem to another site (Zynda, 2001). Phytoremediation had been successfully used to clean up the radio nucleotides, pesticides and heavy metals (Anu *et al.*, 2001; Entry *et al.*, 1993; Gao *et al.*, 2000; Schnabel and White, 2000; Wilson *et al.*, 2000; Burken and Schnoor, 1996). Some pesticides which have been cleaned up by plants are DDT (Gao *et al.*, 2000) and simazine (Wilson *et al.*, 2000). However, there is no report on using plants to remediate carbofuran residue in rice field soil. Research on the selection of types of plants to degrade carbofuran residues in rice field soil deserves attention. Therefore this study was conducted to select the plants that possess ability to degrade carbofuran residues in rice field soil.

Materials and Methods

Carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranol methyl carbamate), 98% purity, was purchased from Sigma-Aldrich, USA.

Soil physicochemical properties

Soil samples were taken from 0-30 cm depth of rice field in Ban Non-Reung, A. Muang, Khon Kaen Province, Thailand. Physicochemical properties including organic matter, pH and soil texture were measured. Organic matter and soil texture were analyzed by the Soil and Plant Testing Laboratory, Faculty of Agriculture, Khon Kaen University. Soil pH was measured in water : soil (1:2.5).

Plants

Morning glory (*Ipomoea aquatica*), cat tail (*Typha angustifolia* Linn.), bulrush (*Cyperus* spp.), soybean (*Glycine max* Merr.), groundnut (*Arachis hypogaea* L.), mung bean (*Phaseolus aureus* Roxb.), sunflower (*Helianthus annuus* L.), tomato (*Lycopersicon esculentum* Mill), aubergin (*Solanum xanthocarpum* Schrad.&Wendl.), egg-plant (*Solanum melongena* L.), Chinese-kale (*Brassica* spp.), and cabbage (*Brassica* spp.) were used to examine phytoremediation ability. These 12 plants were chosen because of their short life cycle which makes them suitable for planting after rice harvest. It was hoped that these plants could remediate carbofuran residues in rice soil and after the remediation was finished these plants would be edible.

Plants cultivation

Plants were grown in 8-inches diameter plastic pots filled with rice field soil containing carbofuran at a concentration of 5 mg/kg soil. All the pots were watered everyday during the experiment and were randomized on the greenhouse bench and re-randomized every two weeks. Control was non-planted pots containing soil with carbofuran at concentration at 5 mg/kg soil.

Degradation of carbofuran

Pots were sacrificed at day 0, 7, 14, 21, 35 and 70. Soils were extracted with methanol (1:1 wt/vol) and shaken at 200 rpm for one hour. After one hour, soils suspensions were filtered through Whatman Paper No. 1 using a vacuum pump. The filtrate was collected. This step was repeated three times. The filtrate were combined and analyzed for carbofuran concentration by Shimadzu 10-A HPLC with UV detector. The chromatography column (150 mm x 4.6 mm) Lunar C-18 um (Phenomenex, USA) was used. Percent recovery of the extraction method was greater than 95 %.

Carbofuran concentrations were fitted to a modified first-order kinetic model; $C = C_0e^{-kt} + Ya$, where C is the mean concentration of carbofuran as a function of time in days (mg/kg), Co is the initial Carbofuran concentration (mg/kg), k was the rate constant (/day, t was time (days), and Ya was an asymptotic estimate of the concentration of carbofuran that degrades very slowly over time (residual carbofuran) (mg/kg). Average initial carbofuran concentration (at Day 0) was 4.81 mg/kg. The mean concentrations used in the regression were weighted with inverse of the variance squared, S^{-2}. This method compensated for the non-constant variance and helped to improve estimation of parameters.

Sorption

Adsorption isotherm was determined by conducting a batch equilibrium experiment at carbofuran concentrations of 0.1, 1.0, 5.0, 10.0 and 20.0 µg/mL. All solutions were prepared in 0.01 M $CaCl_2$. A total of 3-g of air-dried soil was put into 15 ml glass tubes and mixed with 9 ml of 0.01 M $CaCl_2$ solution containing carbofuran. Tubes were shaken on a horizontal shaker for 48 hours at 90 cycles per minute. After centrifugation at 5,000 x g, supernatant was analyzed for carbofuran concentration using HPLC.

Results

Soil physicochemical properties

Physicochemical properties of soil samples are shown in Table 1. Soil was classified as loam. Carbofuran residues in soil before planting were under detectable limit (< 0.01 mg/L).

Degradation of carbofuran in soil

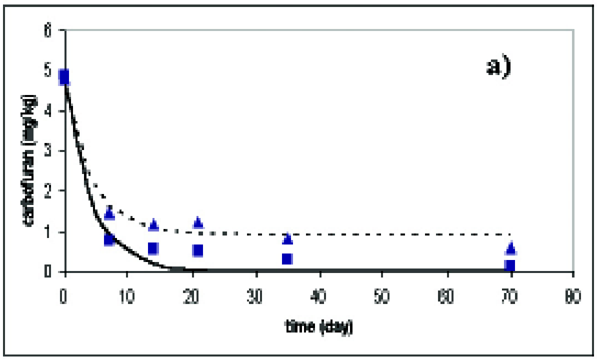
Degradation of carbofuran in planted and non-planted soils were described by a modified first-order kinetic model (Figure 1). The coefficients of determination, r^2, ranged between 0.89-0.99 and indicated good fit of the data to the first-order kinetic model (Table 2). Carbofuran was rapidly degraded under planted soil and non-planted soil (control) (Table 2) with the half-lives ranging from 2 days to 7 days suggesting carbofuran was not persistent in the environment and phytoremediation is a promising technique to degrade carbofuran in soil. Other researchers found a longer half-life of carbofuran, for example 7-259 days (WHO, 1996) and 30-120 days (Kamrin, 1997), than that found in this study. Variations of half-life depended on soil conditions i.e., pH, soil type, temperature, moisture content and microbial population (WHO, 2003). Carbofuran in soil was degraded by chemical hydrolysis and biodegradation. Previous studies demonstrated that the degradation of xenobiotics is greater in the presence of plant roots than in their absence (Hsu and Bartha, 1979; Sandmann and Loos, 1984; Walton and Anderson, 1990; Boyle and Shann, 1995). The rice field where soil samples were taken had a history of carbofuran use, therefore this may have been responsible for the rapid degradation of carbofuran in non-planted soil. Other researchers (Barriuso and Houot, 1996; Ostrofsky *et al.*, 1997; Jayachandran *et al.*, 1998) found evidence of microbial adaptation and growth in soil resulting from repeated pesticide (atrazine) exposure.

Sorption of carbofuran to soil.

Figure 2 shows the carbofuran sorption isotherm resulting from the Freundlich equation: $C_s = K_f C_{eq}^{1/n}$, where C_s is the carbofuran concentration in soil (mg/kg soil), C_{eq} is the equilibrium solution concentration of carbofuran (mg/L), K_f is

Table 1. Physicochemical Properties of Soil

Soil physicochemical properties	values
organic matter content (%)	0.30
pH	4.95
EC (mS/cm)	0.025
sand (%)	42.5
silt (%)	32.5
clay (%)	25.0



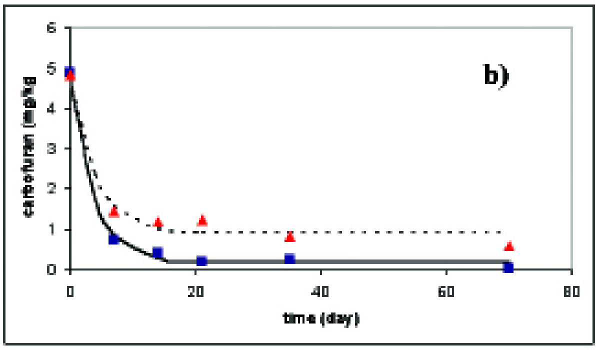


Figure 1. Dissipation of carbofuran in a) sunflower and b) soybean soils, (▲ = planted soil; ■ = non-planted soil; solid lines = carbofuran concentrations in planted soil fitted to the first order kinetic model; dashed line = carbofuran concentration in non-planted soil fitted to the first order kinetic model).

Table 2. Degradation rate constants (k) and half-lives ($t_{1/2}$) of carbofuran from soils under plants and non-plant.

Plants	k_1, day^{-1}	$t_{1/2}$, days	r^{2*}	Ya**	SSE***
Helianthus annuus L. (sunflower)	0.31	2	0.94	351.55	269.83
Glycine max Merr. (soybean)	0.27	3	0.95	182.89	251.94
Ipomoea aquatica (morning glory)	0.32	2	0.97	288.38	113.05
Lycopersicon esculentum Mill. (tomato)	0.17	4	0.92	862.59	294.29
Phaseolus aureus Roxb. (mung bean)	0.14	5	0.97	187.67	148.88
Brassica spp. (chinese-kale)	0.11	6	0.89	404.67	583.79
Solanum melongena L. (eggplant)	0.13	5	0.94	690.39	251.58
Brassica spp. (cabbage)	0.14	5	0.92	665.55	308.25
Solanum xanthocarpum Schrad.&Wendl. (aubergin)	0.11	6	0.93	408.52	381.82
Arachis hypogaea L. (ground nut)	0.10	7	0.96	245.51	220.08
Typha angustifolia Linn. (cattail)	0.41	2	0.96	269.53	189.30
Cyperus spp. (bulrush)	0.35	2	0.99	127.09	45.64
non-planted soil	0.24	3	0.92	907.46	254.15

*** Coefficients of determination for non-linear regressions** *****Sum of squares due to error**
**** Carbofuran concentration that degraded very slowly over time (mg/kg.).**

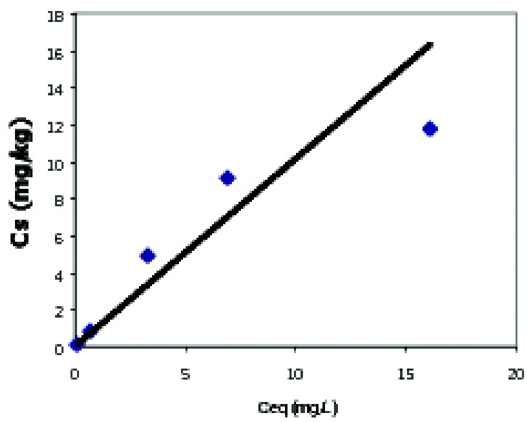


Figure 2. Sorption isotherms of Ban Non-Reung soil at 0-30 cm depth.

an index of the adsorption capacity, and 1/n is an empirical constant. K_f values for Ban Non-Reung soil is 1.0 L/kg, 1/n was 1.04 and r^2 of the adsorption isotherm was 0.98.

Conclusions

Soils from planted and non-planted (control) showed rapid degradation of carbofuran with half-lives ranging from 2 to 7 days. These facts suggest that phytoremediation could accelerate the degradation of carbofuran residues in soil and carbofuran was not persistent in soil environment.

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