ORIGINAL ARTICLE

Determination of plant species for the phytoremediation of carbofuran residue in rice field soils

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Abstract

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This study searched for plant species suitable for accumulating carbofuran residue in rice field soil. Three groups of plant, i.e. grass crops, upland crops, and vegetable crops, were grown in 8 inches diameter plastic pots filled with soil containing 5 mg/kg carbofuran. Parts of plants (stems and leaves, roots, fruits) were harvested at day 120 and analyzed for carbofuran residue using HPLC. The results indicated that *Helianthus annuus* L. (sunflower) was the most suitable species for phytoremediation of carbofuran residue in rice field soil because it highly accumulated carbofuran up to 93.4 μg/kg dry weight in its stems and leaves. In addition, *H. annuus* L. (sunflower) could tolerate carbofuran since it showed similar physical appearance (circumference and height) to control not receiving carbofuran.

Key words : carbofuran, phytoremediation, tolerate, accumulator

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บทคัดย่อ

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การคัดเลือกสายพันธุ์พืชเพื่อฟื้นฟูดินจากนาข้าวที่มีคาร์โบฟูรานตกค้าง

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การศึกษานี้ได้ค้นหาชนิดของพืชที่มีความสามารถในการฟื้นฟูดินจากนาข้าวที่มีการตกค้างของคาร์โบฟูราน โดยใช้พืช 3 กลุ่ม คือ พืชกลุ่มหญ้า พืชไร่ และพืชผัก โดยปลูกพืชแต่ละชนิดของทั้งสามกลุ่มในกระถางพลาสติกขนาดเส้นผ่าศูนย์กลาง 8 นิ้ว ในดินที่ผสมด้วยคาร์โบฟูรานในอัตรา 5 มก./กก. ดิน จากนั้นในวันที่ 120 เก็บเกี่ยวส่วนของลำต้นและใบ ราก ผล นำไปวิเคราะห์สารคาร์โบฟูรานที่ตกค้างด้วยเครื่องโครมาโตกราฟีแบบของเหลวแรงดันสูง ผลการศึกษาพบว่า ทานตะวัน (*Helianthus annuus* Linn.) ซึ่งเป็นพืชในกลุ่มพืชไร่มีความเหมาะสมสำหรับการฟื้นฟูดินจากนาข้าวที่มีการตกค้างของคาร์โบฟูราน เนื่องจากทานตะวันสามารถสะสมปริมาณสารคาร์โบฟูรานในส่วนที่เป็นใบและลำต้น ได้สูงที่สุดถึง 93.4 ไมโครกรัม/กก. น้ำหนักแห้ง จึงทำให้ทานตะวันเป็นพืชที่มีศักยภาพที่ดีในการสะสมคาร์โบฟูราน นอกจากนั้นทานตะวันยังมีความทนทานต่อคาร์โบฟูรานเนื่องจากมีลักษณะทางกายภาพ (ความสูงและเส้นรอบวง) ไม่แตกต่างจากพืชที่ไม่ได้ปลูกในดินที่ผสมคาร์โบฟูราน

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Phytoremediation is the method that uses plants to clean up or remediate pollutants from the environment such as soil (Visoottiviseth *et al*., 2002; Garbisu and Alkorta, 2001). It is used for treating many classes of contaminants including petroleum hydrocarbons (Plamroth *et al*., 2002), heavy metals (Schwartz *et al*., 2001; Brennan and Shelley, 1999; Brown *et al*., 1994), radionuclides (Entry *et al*., 1993; Demirel *et al*., 1994) and pesticides (White, 2002; Burken and Schnoor, 1997). Plants remediate organic contaminants via three mechanisms: (i) direct uptake of contaminants and subsequent accumulation of nonphytotoxic metabolites into plant tissue; (ii) release of exudates and enzymes that stimulate microbial activity and biochemical transformations; and (iii) enhancement of mineralization in the rhizosphere (the root-soil interface), which is attributable to mycorrhizal fungi and the microbial consortia (Schnoor *et al*., 1995). Plants that facilitate removal of pollutants by their ability to accumulate the compounds are called hyperaccumulators. There have been investigations of the ability of plants to accumulate pesticides such as soil-bound chlordane (Mattina *et al*., 2003) and diazinon - or parathion (Hsu and Bartha, 1979).

Weed problems are projected to increase in Asian rice production. Weeds cause lower rice yields, reduced rice quality, increased insects and disease risk, restricted irrigation water flow, slower harvest operations, increased grain drying costs, and reduced land values (Smith *et al*., 1977; Smith and Hill, 1990). Carbofuran is one of the common pesticides used in rice fields. It is a broad-spectrum insecticide used to control rice water weevil (Getzin, 1973; Sieber *et al*., 1978). Carbofuran is moderately persistant in the environment (Mora *et al*., 1996) with a half-life of 26-110 days in soil depending on pH, soil type, temperature, moisture content and microbial populations. It has a very low ability to be adsorbed thus it easily leaches into groundwater. To remediate carbofuran residue in rice field soil, there is a need to determine plant species that are capable of accumulating carbofuran. This study was designed to search for carbofuran accumulating plants that have a short-life cycle which makes them suitable for planting after rice harvest.

Materials and Methods

Insecticide

Carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate), 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, Amphor 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 soil:water (1:2.5).

Plants

Three groups of plant species, i.e. (i) grass crops consisting of rice (*Oryza sativa* L.), cat tail (*Typha angustifolia* Linn.) and bulrush (*Cyperus difformis* Linn.), (ii) upland crops consisting of soybean (*Glycine max* Merr.), ground nut (*Arachis hypogaea* Linn.), mung bean (*Vigna radiate* (L.) Wilczek.), sunflower (*Helianthus annuus* Linn.) and corn (*Zea mays* L.), and (iii) vegetable crops consisting of water convolvulus (*Ipomoea aquatica*), tomato (*Lycopersicon esculentum* Mill), aubergin (*Solanum aculeatissimum* Jacq.), eggplant (*Solanum melongena* L.), chinese-kale (*Brassica alboglabra* Bailey.), chili (*Capsicum annuum* L.) and cabbage (*Brassica oleracea* var. capitata), were tested to determine their phytoremediation abilities. These 15 plants were chosen because of their short life cycles which made them suitable for planting after the rice harvest. It was hoped that some of 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-inch diameter plastic pots filled with rice field soil mixed with carbofuran at 5 mg/kg soil. All of the pots were watered every day during the experiment and were randomly assigned on the greenhouse bench and randomly relocated every two weeks to prevent light effect. Controls were plants receiving no carbofuran.

Extraction and analysis of carbofuran residues

After day 120, plant samples were harvested and analyzed for concentrations of carbofuran residue in different parts of the plants i.e., stems and leaves, roots and fruits. Each sample was chopped and blended before extraction. The blended samples were then put into an Accelerated Solvent Extractor (Dionex, ASE100) cell and extracted with acetonitrile. Conditions for the ASE were as follows: preheat 5 min, static 5 min, flush 60%, purge 100 s, 2 cycles, pressure 1500 psi, and temperature 80ºC. Sample extracts were cleaned up by C8 cartridges and analyzed for carbofuran concentration using a Shimadzu 10-A HPLC with UV detector. The Lunar C-18 5 µm (Phenomenex, USA) chromatography column (150 mm x 4.6 mm) was used. The percent recovery of the extraction method was greater than 92%.

Determination of carbofuran tolerance in plants

Circumference and height of plants were measured at days 0, 7, 14, 21, 35, 70, and 120 using ruler and vernier.

HPLC analysis/conditions

The HPLC system (Shimadzu, Japan) consisted of a Model LC-10AD solvent delivery system, and a Model SPD-10A UV visible-wave-

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
bulk density (g/cm^3)	1.3
Total N (%)	0.032
C/N ratio	7.84

length detector. The analyses were performed at 220 nm and the mobile phase was acetonitrile-water (50:50) at a flow-rate of 1 ml/min.

Statistical analysis

T-test was used to determine if statistically significant differences at the $p<0.05$ significance level were observed among the treatment means of the height and circumference of treated plants and control plants not receiving carbofuran.

Results

Soil physicochemical properties

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

Carbofuran residue in plants

To determine the potential carbofuran accumulating ability, phytoaccumulation, in selected plant species, we analyzed the carbofuran concentrations in different plant parts, i.e. roots, stems and leaves, and fruits, at day 120. The concentrations of carbofuran residue in various plant parts are shown in Figures 1, 2 and 3. Carbofuran was found mostly in stems and leaves in most plant species, indicating a phytoaccumulation mechanism. Grass crops and upland crops appeared to be suitable for phytoremediation of carbofuran

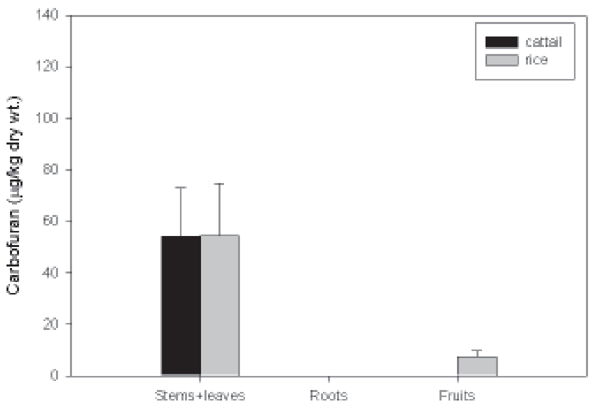

Figure 1. Amounts of carbofuran present in different plant parts (grass crops).

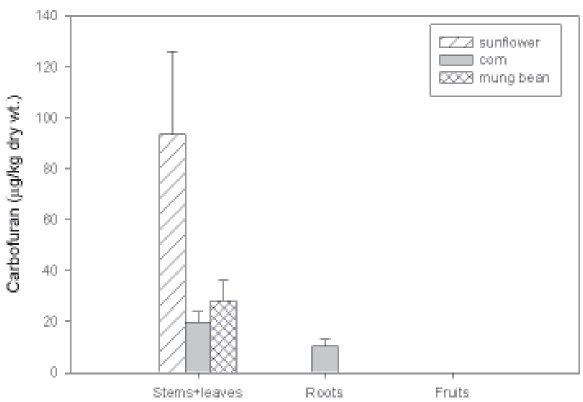

Figure 2. Amounts of carbofuran present in different plant parts (upland crops).

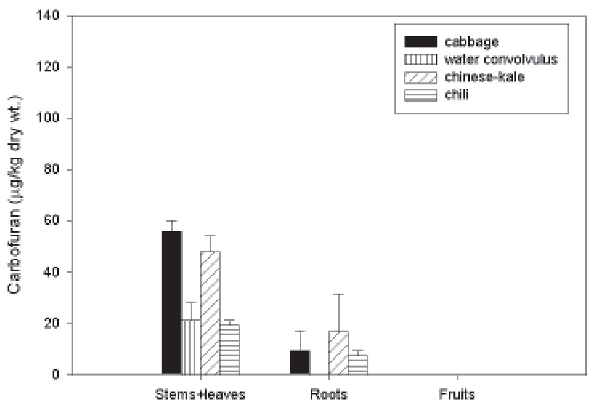

Figure 3. Amounts of carbofuran present in different plant parts (vegetable crops).

residue in rice field soil because they could tolerate carbofuran and highly accumulated carbofuran in stems and leaves (Figures 1 and 2). Carbofuran was highly accumulated in stems and leaves of sunflower, cabbage, rice, cattail, and chinese-kale with concentrations of 93.4, 55.6, 54.4, 54.1 and 47.8 µg/kg dry weight, respectively. Sunflower accumulated the highest concentration of carbofuran in its stems and leaves, 93.4 µg/kg dry weight, indicating it is a good potential accumulator for phytoremediation of carbofuran. Accumulation of carbofuran was highest in stems and leaves instead of seed which is the edible part, suggesting that sunflower is safe for people and for animals. Carbofuran residue was also found in the roots of some plants studied, but in a lower concentration than in stems and leaves. Differing carbofuran accumulations may be due to the varying mechanisms of plants to remove or uptake contaminants as they do with nutrients. In addition, since carbofuran is a plant systemic herbicide, which means that it has a high water solubility, it is easily uptaken into the roots or leaves (Ware, 1994). The direct uptake of a chemical through the roots depends on the plant's uptake efficiency and transpiration rate as well as the concentration of the chemical in soil water (Schnoor *et al.*, 1995). Caro *et al.* (1976) found that carbofuran was readily absorbed by roots and was transported via plant fluids to the areas of greatest transpiration, such as leaves. Burken and Schnoor (1997) reported transformation of atrazine occurred in roots, stems, and leaves.

Carbofuran residue was not detected in any parts of soybean, ground nut, tomato, eggplant, aubergin, or bulrush. We speculated that there should be trace amounts of carbofuran residue in these plants, but the detectable limit of the HPLC we used was not able to detect it (detection limit > 0.01 mg/kg). It is also possible that the carbofuran might be transformed into its metabolite, which we did not detect. Plant metabolites of pesticides can be classified into three broad groups: free compounds, conjugates and bound residues (Harvey, 1982). These bound pesticide residues in plants were reported to bind to components of the plant such as proteins, cell membranes and/or various other cellular structures (Still and Mansager, 1973). For aromatic and heteroaromatic rings from pesticide molecules, lignin appears to be the major binding site (Still *et al.*, 1981). There have been reports of carbofuran and metabolites found in plants. Knaak *et al.* (1970) revealed that the major metabolites of carbofuran in alfalfa were identified as the glycosides of 3-hydroxycarbofuran (37.5%), 2,3-dihydro-3, 7-dihydroxy-2, 2-dimethylbenzofuran (18.5%) and 2,3-dihydro-7-hydroxy-2, 2-dimethyl-3-oxobenzofuran (20%). Press and Saunders (1974) indicated that 3-hydroxycarbofuran and an unknown metabolite, tentatively identified as N-hydroxymethyl-carbofuran, were detected from the metabolism of carbofuran in mugho pines. IRRI (1977) indicated that the parent carbofuran metabolized to 3-hydroxycarbofuran and 3-ketocabofuran in rice plants.

Carbofuran tolerance in plants

One of the criteria to select plant species as being suitable for phytoremediation was the toleration of plants to the carbofuran. Therefore, we measured the circumference and height of the plants after they received the chemical (Table 2), and compared these results with the control (plants grown in no carbofuran-contaminated soil). The results indicated that most plants (except chili) showed a similar physical appearance, i.e. circumference and height, to the control, and also similar leaf color and size. Therefore, chili was not suitable to be used in remediating carbofuran residues in soil. The tolerance to toxicity of the chemical in plants involved different mechanisms. Salt *et al.* (1998) reported that organic compounds can be translocated to various plant tissues and subsequently volatilized, so they undergo partial or complete degradation or transformation to less toxic compounds, perhaps bound in the plant tissues. In addition, detoxification mechanisms may transform the parent pesticide to non-phytotoxic metabolites, including lignin, that are stored in various plant cells (Schnoor *et al.*, 1995).

Table 2. Height and circumference of tested plants and control plants not received carbofuran.

Species	Height (cm)[a]		Circumference (cm)[a]	
	Tested Plants	Control	Tested Plants	Control
Helianthus annuus Linn. (sunflower)[b]	30.2±1.0	32.3±8.1	0.8±0.0	0.8±0.0
Glycine max Merr. (soybean)[c]	64.4±2.4	67.5±9.2	0.9±0.1	1.0±0.0
Ipomoea aquatica (water convolvulus)	90.8±12.3	94.5±6.4	1.5±0.0	1.5±0.1
Lycopersicon esculentum Mill. (tomato)	48.7±11.2	65.0±19.8	1.4±0.3	1.6±0.3
Vigna radiata (L.) Wilczek. (mung bean)	27.5±1.1	28.7±0.9	1.1±0.1	1.2±0.1
Brassica alboglabra Bailey. (chinese-kale)	26.7±9.8	30.0±2.8	3.4±0.3	3.4±0.4
Capsicum annuum L. (chili)	14.7±3.0*	29.3±0.4	1.0±0.1*	1.4±0.2
Solanum melongena Linn. (eggplant)	32.7±2.6	33.0±7.1	3.1±0.4	3.1±0.9
Brassica oleracea var.capitata (cabbage)	16.3±0.4	17.5±0.7	3.1±0.4	3.1±0.9
Solanum aculeatissimum Jacq. (aubergin)	36.7±2.3*	52.0±0.0	2.9±0.7	3.1±0.8
Arachis hypogaea Linn. (ground nut)	31.8±2.8	36.0±11.3	1.3±0.0	1.3±0.0
Typha angustifolia Linn. (cattail)	137.5±21.7	144.5±24.8	3.9±0.5	4.1±0.9
Cyperus difformis Linn. (bulrush)	90.6±7.1	94.0±1.4	6.8±0.1	6.9±0.1
Oryza sativa L. (rice)	105.9±1.6	107.0±1.4	4.3±0.3	4.3±1.1
Zea mays L. (corn)[b]	116.3±4.0	121.0±1.4	4.0±0.2	4.1±0.0

***Significantly different when compared with control using t-test at 95% confidence level.**
[a]Data expressed as the mean and standard deviation of three replicate samples
[b]Harvested at day 100
[c]Harvested at day 70

Conclusion

In conclusion, our study found that sunflower was the most suitable plant species for phytoremediation of carbofuran residue in rice field soil. This was due to sunflower tolerating carbofuran, and highly accumulating carbofuran in its stems and leaves, indicating a phytoaccumulation mechanism. In addition, the half-life of carbofuran in soil planted with sunflower is only two days (Teerakun *et al.*, 2004) indicating a rapid degradation of carbofuran in this soil.

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