Full Length Article

Phytotoxicity Assay of Crop Plants to Lindane and Alpha-endosulfan Contaminants in Alkaline Thai Soil

WARAPORN CHOUYCHAI[1] AND HUNG LEE†

Biology Program, Department of Science, Faculty of Science and Technology, Nakhonsawan Rajabhat University, Nakhonsawan, 60000, Thailand
†*School of Environmental Sciences, University of Guelph, Guelph, Ontario, N1G 2W1, Canada*
[1]Corresponding author's e-mail: chouychai@yahoo.com

ABSTRACT

Four plants (corn, pumpkin, sunflower & water morning glory) were tested for their ability to germinate and grow in an alkaline Thai soil contaminated with 0.2–20 mg/kg dry soil of either lindane or alpha-endosulfan, two organochlorine pesticides commonly found in agricultural soils in Thailand. Based on root length assessment, sunflower was the most tolerant to lindane contaminant, while corn was the most tolerant to alpha-endosulfan in the alkaline soil. Base on root dry weight assessment, corn was the most tolerant to both lindane and alpha-endosulfan. Corn was selected to further test its ability to tolerate a mixture of 0.2, 2.0, 20 mg/kg dry soil of lindane and alpha-endosulfan in the alkaline soil. The presence of both pesticides decreased root length of corn but did not affect its shoot and root dry weights. The results suggest that corn is suitable for use in the phytoremediation of alkaline soil contaminated with a mixture of lindane and alpha-endosulfan. © 2012 Friends Science Publishers

Key Words: Alkaline soil; Endosulfan; Lindane; Organochlorine; Phytotoxicity

INTRODUCTION

Organochlorine pesticides have been used widely in agriculture for many years. In recent years, the use of many organochlorine pesticides, in particular lindane and alpha-endosulfan, has been banned in many countries, including Thailand in 2001 for lindane (IPM Thailand, 2008). Despite the ban, there remains widespread contamination of lindane and alpha-endosulfan in some agricultural soils in Thailand. Poolpak *et al*. (2008) reported the presence of 0.34–24.17 mg/kg dry soil of lindane and 0.05–16.1 mg/kg dry soil of all the endosulfan isomers in the agricultural fields adjacent to the Mae Klong River in central Thailand. Thapina and Hudak (2000) reported that the average amounts of lindane and alpha-endosulfan in agricultural soils of Eastern Thailand were 0.37 and 8.82 μg/kg dry soil, respectively. Along the eastern part of the Gulf of Thailand, the highest amounts of lindane and alpha-endosulfan in sediments were reported to be 232 and 104 μg/kg dry soil, respectively, during the dry season. During the rainy season, the highest amounts increased to 508 μg/kg for lindane but decreased to 10 μg/kg for alpha-endosulfan (Srivilas & Jaidee, 2006).

Lindane and alpha-endosulfan are resistant to microbial degradation under aerobic conditions (Phillips *et al*., 2005) and this can limit the effectiveness of a bioremediation process based on the use of microorganisms alone. Phytoremediation, which involves the use of plants

in conjunction with competent microorganisms (Abedi-Koupai *et al*., 2007), may accelerate organochlorine contaminant degradation. Kidd *et al*. (2008) described a study in which 30-day-old seedlings of *Cytisus striatus* were planted in soil contaminated with 110 mg/kg dry soil technical grade hexachlorocyclohexane (HCH). After 180 days, the concentration of HCH decreased to 30 mg/kg dry soil in the planted soil, while in the unplanted soil, HCH concentration decreased to 40 mg/kg. Some plants have been shown to accumulate organochlorines in their tissues. Lindane concentration in the leaves of *Cytisus striatus* growing for 4 months in soil contaminated with 1,235 mg/kg lindane was about 60 mg/kg (Calvelo Pereira *et al*., 2006).

Plants intended for use in phytoremediation of soil contaminated with organochlorine pesticides should be tolerant of these pesticides. Little information is available in the literature on the phytotoxicity of lindane and alpha-endosulfan. In one study, 0.1-0.4 mg/kg lindane was reported to be non-toxic to the growth of germinated corn seeds (Benimeli *et al*., 2008). In another study, 15–50 mg/kg lindane was found to decrease the percent germination and vigor index of radish and green gram seedling using the filter paper method (Bidlan *et al*., 2004). Vidyasagar *et al*. (2009) reported that 2000–4000 mg/L endosulfan decreased the root length and root fresh weight of *Sorghum bicolor* seeds planted on filter paper.

The soils in Nakhonsawan province, such as the Takhli soil, are rich in limestone. The pH of the agricultural soil in this area can range between 7 to 9 (Department of Land Development, 2009). The potentially high alkalinity may pose a unique problem for phytoremediation of soils contaminated with organochlorine pesticides. High alkalinity in soil (pH 10.0) has been reported to inhibit PAH biodegradation by microorganisms. For example, Betancur-Galvis *et al*. (2006) examined the degradation of 1200 mg phenanthrene per kg dry soil at pH 6 and 10. At pH 6, the phenanthrene concentration decreased to around 600 mg/kg while at pH 10, the concentration only decreased to about 1000 mg/kg within 112 days. Also, high soil pH can induce iron and zinc deficiency (Clark *et al*., 1996) and this may in turn affect the response of plants to contaminants.

Phytotoxicity assays of seed germination and seedling growth can be a useful and effective screening tool to assess plant tolerance to pesticide found in soil and reduce the number of plants for pot or greenhouse study (Chouychai *et al*., 2007; Wibawa *et al.*, 2009). In this study, 4 easily grown plants native to Thailand, corn, water morning glory, sunflower and pumpkin were tested for their tolerance to lindane and alpha-endosulfan in alkaline agricultural soil in Thailand. The objective was to identify the plant(s) that may be most suitable for use in phytoremediation of alkaline Thai soils contaminated with these organochlorine pesticides.

MATERIALS AND METHODS

Agricultural soil: Alkaline soil with no previous history of organochlorine contamination was collected from Khaorad Agricultural Station, Faculty of Agricultural Technology and Industrial Technology, Nakhonsawan Rajabhat University, Nakhonsawan, Thailand. The soil was kept at room temperature (28-31°C) in black plastic bags. Before use, the soil was air-dried at 28-31^{0}C for at least 24 h to constant weight. A sample of the soil was sent to the Central Laboratory (Thailand) Co. Ltd., Bangkok, Thailand for chemical and physical characterization and measurement of background organochlorine contamination.

The soil used in this experiment was alkaline (pH 8.9), with low total phosphorus content (below 0.29 g/100 g soil). The soil contained (per 100 g dry soil): 0.21 g total nitrogen (N), 0.13 g total potassium (K), and 1.78 g organic matter. The soil was tested for a number of organochlorine compounds (benzene hexachloride, heptachlor & heptachlor epoxide, aldrin & dieldrin, dicofol, DDT, chlordane, endosulfan, endrin, DDE & DDD). None was detected.

Phytotoxicity testing: The procedure for the phytotoxicity assay followed that described by Chouychai *et al*. (2007). For each experiment, 50 g of dried soil were added to a glass Petri dish in triplicate. Lindane (Sigma-Aldrich, lot number 7038X, purity 99.8%) and alpha-endosulfan (Chem Service, Lot number 409-77A, purity 99.5%) were weighed separately and dissolved in acetone. Each pesticide solution was transferred to a glass sprayer and sprayed onto soil to final concentrations of 0.2, 2 and 20 mg/kg dry soil. As a control, acetone without any pesticides was sprayed onto soil. Soil in each dish was thoroughly mixed with a metal digger. The spiked soil was air-dried at 28-30°C for more than 24 h or until the smell of acetone had disappeared.

Seeds of sweet corn (*Zea may*) (commercial seeds of Kamlaithong Ltd., Bangkok, Thailand), water morning glory *(Ipomoea aquatica*) (commercial seeds of Chuayongseng Ltd., Bangkok, Thailand), sunflower (*Helianthus annuus*) (commercial seeds from a farm in Nakhonsawan province, Thailand), and pumpkin (*Cucurbita moschata*) (commercial seeds of Chuayongseng Ltd.) were used. Seeds were immersed in tap water for 3 h and then inoculated into pesticide-spiked soil at 6-7 seeds per Petri dish. The dishes were kept at 29^{0}C in a room which received natural sunlight. Each dish received 10 mL of water at daily intervals. After 10 days, the number of seeds germinated for each treatment was counted. Eight plants were randomly removed for measurement of their shoot length, root length, fresh weight, and dry weight.

In the agricultural field, each pesticide is typically found in combination with other organochlorine pesticides. Therefore, plants to be used for the phytoremediation of pesticide-contaminated soil should be tolerant to a mixture of pesticides. In this study, corn, the plant, which retained good root characteristics and germination rates in the presence of either lindane or alpha-endosulfan, was further tested for its tolerance to combinations of lindane and alpha-endosulfan. The toxic effect of lindane and alpha-endosulfan mixture was tested by spiking to final concentrations of 0.2, 2, and 20 mg/kg dry soil each of lindane plus alpha-endosulfan in different combinations. To each dish, seven seeds were added and the phytotoxicity test was done as described above.

Statistical analysis: ANOVA was used to test for statistically significant differences between treatments. One way ANOVA was used to examine the toxicity of individual pesticides. Two ways ANOVA was used to examine the toxicity of a lindane and alpha-endosulfan mixture on corn followed by Tukey's test.

RESULTS

Lindane phytotoxicity in alkaline soil: The presence of lindane in alkaline soil did not decrease seed germination for corn and sunflower (Table I). The percentages of seed germination declined significantly for pumpkin and water morning glory, even at 2.0 mg/kg dry soil lindane, but a higher lindane concentration of 20 mg/kg dry soil did not result in further decreases in percent seed germination (Table I).

The shoot lengths of all 4 plants decreased significantly in the lindane-contaminated alkaline soil. The shoot lengths of corn and pumpkin decreased significantly

even at the lowest lindane concentration tested (0.2 mg/kg dry soil). Shoot lengths of sunflower and water morning glory decreased significantly at 2.0 mg/kg dry soil lindane. In contrast to the shoot lengths, the dry shoot weights of all 4 plants did not decreased significantly, excepted pumpkin (Table I).

Increasing lindane concentrations in soil led to reduced root lengths of all the plants tested significantly. In contrast, the decreases in root dry weight at this lindane concentration were smaller than root length. Statistically significant decreases in the root dry weight were seen only in pumpkin (0.2, 2 & 20 mg/kg dry soil of lindane) and sunflower at 20 mg/kg of lindane only (Table I).

Alpha-endosulfan phytotoxicity in alkaline soil: The presence of alpha-endosulfan decreased seed germination of all 4 plants to similar extent as seen with lindane (Table II). Alpha-endosulfan exerted statistically non-significant effect on shoot lengths of the 4 plants, as their shoot lengths stayed mostly the same as the controls even at the highest concentration of alpha-endosulfan tested (Table II). Alpha-endosulfan also had no significant effect on the dry shoot weights of the 4 plants.

The root length of corn was the least sensitive to alpha-endosulfan. Corn root length exposure to any concentration of alpha-endosulfan was not significantly different from the controls (Table II). The root length of pumkin was reduced significantly when alpha-endosulfan concentration was increased to 2.0 mg/kg dry soil or higher when the root length of sunflower and water morning glory was reduced somewhat by alpha-endosulfan, with the greatest decrease seen at 0.2 mg/kg dry soil but lower decreases at higher concentrations (Table II). The root dry weight was less susceptible to the presence of alpha-endosulfan in the alkaline Thai soil. The root dry weight of pumpkin was the only one which decreased significantly at 20 mg/kg dry soil *(P<0.05).*

Table I: Shoot length, shoot dry weight, root length, root dry weight and seed germination of four plants grown in varying concentration of lindane-contaminated alkaline soil for 10 days. Values are the mean ± SD

Plant	[Lindane] (mg/kg dry soil)	% seed germination	Shoot length (cm)	Shoot dry weight (mg)	Root length (cm)	Root dry weight (mg)
Corn	0	100 ± 0a	20.6 ± 1.5a[1]	54.0 ± 13.5a	14.6 ± 1.4a	54.0 ± 13.5a
	0.2	85 ± 5.8a	14.5 ± 1.5b	55.8 ± 20.5a	10.4 ± 1.7b	55.8 ± 20.5a
	2	80 ± 10a	14.0 ± 1.7b	53.0 ± 15.1a	9.2 ± 1.8bc	48.4 ± 14.0a
	20	75 ± 5a	13.0 ± 1.2b	43.5 ± 10.6a	7.0 ± 1.1c	41.0 ± 18.3a
Sunflower	0	100 ± 0a	8.4 ± 1.0a	28.5 ± 7.33a	7.9 ± 1.8a	16 ± 3.6a
	0.2	95 ± 5.8a	6.8 ± 1.4ab	26.4 ± 6.07a	4.3 ± 1.9b	20.6 ± 8.8a
	2	95 ± 5.8a	4.2 ± 0.9c	23.2 ± 4.33a	3.9 ± 1.7b	17.7 ± 4.2a
	20	85 ± 5.8a	5.6 ± 1.4bc	25.8 ± 7.28a	4.0 ± 1.3b	10.1 ± 5.5b
Water morning glory	0	85 ± 5.8a	7.7 ± 0.9a	18.4 ± 2.8a	7.3 ± 0.6a	7.0 ± 3.0a
	0.2	75 ± 5a	6.8 ± 0.7ab	16.8 ± 6.0a	4.9 ± 0.9b	6.9 ± 1.2a
	2	45 ± 15b	6.4 ± 0.9b	16.7 ± 8.4a	4.0 ± 0.7b	5.8 ± 2.1a
	20	65 ± 15a	5.6 ± 0.7b	14.3 ± 2.8a	3.6 ± 0.9b	5.2 ± 1.3a
Pumpkin	0	90 ± 10a	12.3±1.2a	63 ± 2.6a	6.5 ± 1.7a	15 ± 1.0a
	0.2	60 ± 10b	8.0 ± 1.3b	61.0 ± 1.7ab	1.8 ± 0.2b	10.3 ± 4.5a
	2	50± 10b	3.8 ± 1.8c	57.0 ± 3.0b	1.8 ± 0.2b	9.0 ± 2.6b
	20	55± 5b	4.4 ± 1.7c	54.7 ± 5.8b	1.8 ± 0.4b	9.0 ±3.6b

Table II: Shoot length, shoot dry weight, root length, root dry weight and, seed germination of four plants grown in varying concentration of alpha-endosulfan-contaminated alkaline soil for 10 days. Values are the mean ± SD

Plant	[Alpha-endosulfan] (mg/kg dry soil)	% seed germination	Shoot length (cm)	Shoot dry weight (mg)	Root length (cm)	Root dry weight (mg)
Corn	0	100 ± 0a	20.6 ± 1.5a[1]	44.6 ± 9.3a	14.6 ± 1.4a	54.0 ± 13.5a
	0.2	85 ± 5.8a	17.3 ± 1.7a	49.3 ± 15.9a	15.2 ± 1.9a	50.3 ± 15.6a
	2	85 ± 5.8a	17.4 ± 1.6a	51.9 ± 14.5a	15.1 ± 0.9a	57.3 ± 19.0a
	20	90 ± 10a	17.0 ± 1.4a	50.8 ± 11.4a	12.3 ± 1.9a	66.1 ± 24.2a
Sunflower	0	100 ± 0a	8.4 ± 1.0a	28.5 ± 7.3a	7.9 ± 1.8a	16 ± 3.6a
	0.2	100 ± 0a	8.5± 1.6a	23.9 ± 5.5a	4.8 ± 2.8b	12.9 ± 3.6a
	2	95 ± 5.8a	9.3 ± 1.6a	22.9 ± 5.1a	5.3 ± 1.6b	13.6 ± 7.9a
	20	80 ± 5.8a	9.2 ± 0.8a	23.8 ± 4.6a	5.6 ± 1.6b	15.5 ± 5.0a
Water morning glory	0	85 ± 15a	7.7 ± 0.9a	17.8 ± 2.2a	7.3 ± 0.6a	7.8 ± 2.2a
	0.2	70 ± 10a	6.2 ± 3.4a	15.9 ± 3.5a	3.8 ± 1.1b	7.7 ± 2.2a
	2	40 ± 20b	6.0 ± 2.6a	16.2 ± 4.1a	3.7 ± 1.0b	7.7 ± 2.5a
	20	80 ± 10a	5.7 ± 1.4a	14.0 ± 2.1a	2.6 ± 1.8b	4.8 ± 6.1a
Pumpkin	0	90 ± 10a	12.3±1.2a	63.0 ± 7.9a	6.5 ± 0.5a	15.0 ± 1.0a
	0.2	60 ± 10b	9.2 ± 1.8b	62.7 ± 6.4a	6.5 ± 0.5a	15.7 ± 1.2a
	2	50± 10b	11.8 ± 1.7a	62.3±5.9a	4.3 ± 0.6b	15.0 ± 1.0a
	20	55± 5b	12.1 ± 1.0a	60.7 ± 2.3a	1.3 ± 0.6c	9.0 ± 1.7b

[1]Values followed by different letters are statistically different (P< 0.05)

Corn, which retained good germination rate, as well as exhibiting the longest root length and highest root dry weight in the presence of either lindane or alpha-endosulfan in the alkaline Thai soil, was selected for subsequent mixed contaminant testing.

Phytotoxicity of a mixture of lindane and alpha-endosulfan in alkaline soil: Organochlorine-contaminated soils in Thailand typically contain more than one organochlorine compounds (Thapina & Hudak, 2000; Srivilas & Jaidee, 2006; Poolpak *et al.*, 2008). Since our goal was to select plants suitable for use in the phytoremediation of organochlorine-contaminated alkaline soil in Thailand, it was important to directly test the ability of the plant selected, corn, to tolerate a mixture of lindane and alpha-endosulfan at varying concentrations, each ranging from 0 to 20 mg/kg dry soil, with a focus on assessing the seed germination and root health.

The root length of corn seedlings grown in soil contaminated with both lindane and alpha-endosulfan was reduced significantly when there were 20 mg/kg lindane+0.2 – 20 mg/kg alpha-endosulfan and 2 mg/l lindane + 20 mg/kg alpha-endosulfan compared with corn grown in non-contaminated soil (Fig. 1A). The decreases in the root length at the highest concentrations of the 2 organochlorine contaminants were about 50% from that of the control plant. The root length of corn plants also decreased with increasing of lindane concentrations irrespective of the amount of alpha-endosulfan present (Fig. 1a). The primary effect of the contaminants appeared to be reduction in the thin and fibrous features of the roots, while the thicker root structure remained. As a result, there was no statistically significant difference in the root dry weights of corn plants grown in any of the combinations of lindane and alpha-endosulfan concentrations tested (Fig. 1b).

DISCUSSION

Some authors have reported on the phytoxocity of some organochlorine compounds. For examples, endosulfan was shown to affect division of root meristem cells of *Bidens laevis*, a wetland macrophyte in the Asteraceae family, when grown hydroponically in the presence of 0.01– 5 µL/g endosulfan solution (Pérez *et al.*, 2008). Endosulfan was also found to induce enzymes such as catalase and glutathione reductase as well as the production of H_2O_2 involved in oxidative stress response of the aquatic microphyte *Myriophyllum quitense* (Menone *et al.*, 2008). Lindane was reported to disrupt the membranes of leaf cells of *Elodea densa*, resulting in reduced selectivity of sodium/potassium ions (Schefczik & Simonis, 1980) and decreased levels of the plant hormone, indoleacetic acid, in rice seedlings (Sharada *et al.*, 1999).

In this study, both lindane and alpha-endosulfan were found to be toxic to plants, as shown by diminished seed germination, plant elongation and plant dry weight. However, the responses of the plants to each pesticide differed.

Fig. 1: Root length (A) and root dry weight (B) of corn grown in varying concentrations of lindane plus alpha-endosulfan-contaminated alkaline soil for 10 days. Symbols; ☐ 0 mg/kg alpha-endosulfan, ▨ 0.2 mg/kg alpha-endosulfan, ■ 2 mg/kg alpha-endosulfan, ▥ 20 mg/kg alpha-endosulfan, * significant difference from non-contaminated soil *(P<0.05)*
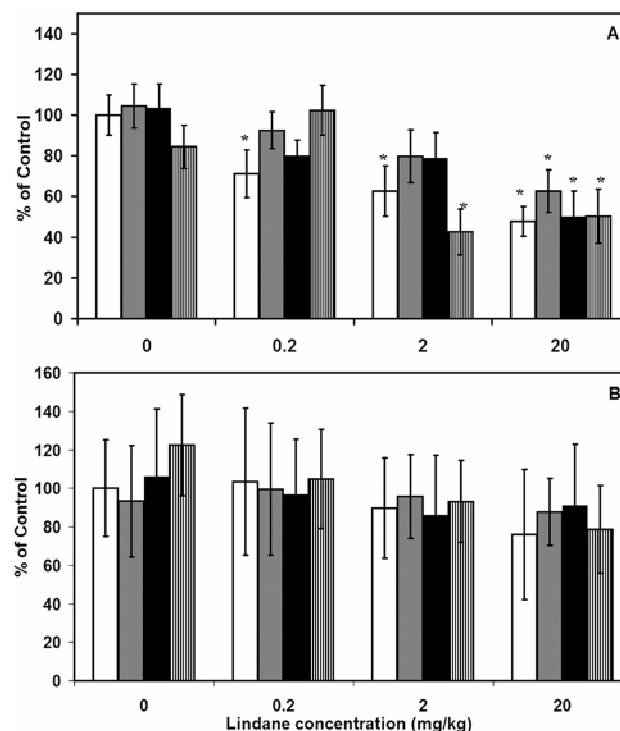
Corn tends to tolerate alpha-endosulfan better than lindane, while sunflower and water morning glory tolerated lindane better than alpha-endosulfan. Pumpkin was susceptible to both pesticides.

The phytotoxicity of lindane observed in this study differed from the result of Benimeli *et al.* (2008) who reported that lindane was not toxic to corn. The difference may be due in part to lower lindane concentrations (0.1 – 0.4 mg/kg) used in the study of Benimeli *et al.* (2008) compared to our study. Another reason may be that Benimeli *et al.* (2008) started with germinated seedlings while we started with seeds in this study. In another study using acidic soil (pH 4.8), technical grade HCH (300 – 12500 mg/kg; containing all the isomers of HCH including 5% of the gamma-isomer or lindane) was shown to reduce the shoot and root lengths, as well as the dry weight of various plant species (Calvelo Pereira *et al.*, 2010). In that study, the tolerant plant species for HCH–contaminated soil were *Hordeum vulgare*, *Brassica* sp., and *Phaseoulus vulgaris*. In our study, the presence of lindane alone decreased the shoot and root lengths of all the plants tested. The presence of alpha-endosulfan decreased the root length of sunflower, water morning glory and pumpkin.

Plants to be used for phytoremediation of organochlorine-contaminated soils should germinate well and have healthy root systems that distribute extensively in soil (Chouychai *et al.*, 2007). Based on this criterion, root length and weight were used to select the organochlorine-tolerant plants. Among the four plants tested, corn appeared to be the most suitable for phytoremediation of organochlorine-contaminated alkaline soil, as its seeds had good germination rate. In addition, the corn plant had the longest root length and highest root dry weight of the 4 plants tested. Further studies are needed to assess if the corn plant can indeed enhance the degradation of these organochlorine compounds by competent microorganisms in alkaline Thai soil.

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