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# Phytotoxicity of heptachlor and endosulfan sulfate contaminants in soils to economic crops

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**Abstract**

The intensive use of organochlorine in the past decades has resulted in contamination of soil worldwide. The phytotoxicity of two organochlorine pesticide, endosulfan sulfate and heptachlor, on the early growth stage of sweet corn (*Zea mays*), waxy corn (*Zea mays*) cowpea *(Vigna sinensis*), cucumber *(Cucumis sativus*) and water morning glory (*Ipomoea aquatica*) were studied. In the range of concentration found in Thai agricultural soil, 0.4 – 40 mg kg$^{-1}$ of each pesticide, did not affect the percentage of seed germination. Heptachlor seemed to affect the shoot and root length of test plants more than endosulfan sulfate. The combined effect of both pesticides to corn seedling growth was tested. There was no significant effect on combined treatment of both pesticides to corn growth. The 0.4 – 40 mg kg$^{-1}$ concentration of endosulfan sulfate and heptachlor did not produced significant effect on early growth of plants.

**Key words**

Endosulfan, Heptachlor, Organochlorine, Phytotoxicity, Crop



## Introduction

Organochlorine pesticides including endosulfan and heptachlor have been widely used in the past to control insects and pests in agricultural area. However, both endosulfan and heptachlor have been banned worldwide during 1970's to 1980's due to their toxic effect on non-target organisms, low biodegradability and highly persistence in the environment (Singh and Mishra, 2009; Golfnopoulos *et al*., 2003). Due to their uncontrolled and intensive use in the last decades, organochlorine and their residues have been reported to contaminate in soils and sediments of many areas in Thailand (Fang *et al*., 2007). For example, Poolpak *et al*. (2008) reported the presence of 0.5-16.1 mg kg$^{-1}$ dry soil of all the isomer of endosulfan and 2.67-152.17 mg kg$^{-1}$ of heptachlor in agricultural area adjacent to the Mae Klong river in Central Thailand. The highest amounts of alpha-endosulfan in sediments along the eastern part of the Gulf of Thailand were 10-104 mg kg$^{-1}$ dry soil during drying season (Srivilas and Jaidee, 2006). Where as 11.91 mg kg$^{-1}$ dry soil of heptachlor epoxide were detected in agricultural area of the east part of Thailand (Thapinta and Hudak, 2000).

Although, various kinds of organochlorine still remained abundant in agricultural soils and product of organochlorine transformation, such as endosulfan sulfate and heptachlor epoxide, may be more persistent and more toxic than parent compounds (Cambpell *et al*., 2009). However, toxicity of these compounds on economic crops has rarely been reported compared to other contaminants. Although, several studies have reported abnormal seedling growth, reduced seed germination, shoot length, root length, and biomass in *Sorghum bicolor* L. Moench (variety JP-1-1) exposed to increased concentration of endosulfan (Vidyasager *et al*., 2009). In addition, heptachlor has also been found to effect cell density and carbon fixation of marine dinoflagellate *Exuviella baltica* (Magnani *et al*., 1978). In light of the above, the objective of the present study was to investigate the toxic effect of heptachlor and endosulfan insecticides on economically important crops of *Zea mays, Vigna sinensis*, *Cucumis sativus* and *Ipomoea aquatica*.

## Materials and Methods

**Spiking of soil:** Soil with no previous history of endosulfan-sulfate and heptachlor contamination was collected from Kookaew Temple, Kantharawichai District, Mahasarakham Province, Thailand. Soil used soil used for experiment was clayey in texture, with pH 8.1, 2.4% dry organic matter content, 109.5 $\mu$s cm$^{-1}$ electrical conductivity, 0.29% total nitrogen and 58.38 mg kg$^{-1}$ available phosphorus.

Endosulfan-sulfate (Dr. Ehrenstorfer GmbH Lot no. 81205, Germany, purity 98.5%) and heptachlor (Dr. Ehrenstorfer GmbH Lot no. 50511, Germany, purity 99.5%) were weighed separately and dissolved in acetone. Each pesticide solution was spiked to soil to final concentration of 0, 0.4, 4 and 40 mg kg$^{-1}$ dry soil. After mixing thoroughly, the soil was dried at room temperature (22-24°C) inside a fume hood to allow the solvent to evaporate. Soil was subdivided into 50 g dry soil portions in each 120 ml plastic planting containers. Control soil samples received only acetone.

**Phytotoxicity assay:** Phytotoxicity assay were performed according to Kirk *et al*. (2002). The seeds of sweet corn, waxy corn, cowpea, cucumber, and water morning glory seeds (commercial seeds of Chia Tai Group, Bangkok, Thailand) were immersed in distilled water for 3 hrs. Ten seeds were inoculated per replicate in plastic containers and kept at 30°C in a room which received natural sunlight. The soil was watered twice a day to maintain humidity and soil moisture. After 10 days, the number of seeds that germinated from each treatment was counted. Twenty plants were randomly removed to measure their fresh weight, dry weight, shoot length and root length. Dry weight/fresh weight ratio was calculated to estimate water accumulation.

The combine toxic effect of endosulfan-sulfate and heptachlor mixture was done by spiking soil with each chemical in 1:1 ratios to give final concentration of 0, 0.4, 4 and 40 mg kg$^{-1}$ dry soil. Sweet corn and waxy corn were selected as surrogate plant to test the combine toxic effect of heptachlor and endosulfan sulfate for comparing the different of toxicity on plant variety. It is possible that the same plant species but different variety were respond to pollutant in a different ways.

**Statistical analysis:** One-way ANOVA was used to test for statistically significant differences between treatments followed by Tukey's test.

## Results and Discussion

**Effect on seed germination:** In the present study, individual treatment of heptachlor and endosulfan did not significantly decrease seed germination of all the test plants. (Table 1 and 2). Although seed germination is reported as a critical stage of plant growth that are sensitive to environmental pollutants and can be used as an excellent bioindicator of pollutants (Kirk *et al.*, 2002; Malia and Cloete, 2002). However, both the organochlorines did not show toxicity to seed germination. Generally, seed germination may be affect by the presence of volatile, water soluble and low molecular weight compounds (Henner *et al*., 1999). Accordingly, total seed germination has been reported to be a non-sensitive variable for phytotoxicity testing of hexachlorocyclohexane (Calvelo Pereira *et al.*, 2010).

Pesticide usually effect seed germination by altering biochemical processes in seeds by depressing amylase activity in monocotyledon or suppressing seed respiration in dicotyledons (Calvelo Pereira *et al*., 2010). However, 0.2, 0.4 and 0.6% treatment of endosulfan has been reported to reduce percentage of seed germination in *Sorghum bicolor* (Vidyasager *et al*., 2009). Due to the fact that phytotoxicity of pollutants contaminated in soil depend on soil properties, the toxicity of endosulfan sulfate in this study was found to be less than that reported by Vidyasager *et al*. (2009). This difference may be due to adsorption of pesticides to soil organic matter rendering less bioavailability to crop plants. (Maliszewska-Kordybach and Smreczak, 2000).

**Effect on shoot and root length:** Shoot and root growth of early plant seedling is an important indication to assess phytotoxicity (Chouychai *et al*., 2007). In this study, shoot and root length of 5 plants were affected by heptachlor rather than endosulfan sulfate. Heptachlor did not affect to shoot length of sweet corn, water morning glory, cucumber and cow pea but the lowest concentration of heptachlor (0.4 mg kg$^{-1}$) significantly increased shoot length of waxy corn ($P<0.05$) and then decreased to the same level with control at highest concentration of heptachlor (40 mg kg$^{-1}$). Low concentration of heptachlor could stimulated shoot length of waxy corn with hormesis phenomenon. This is one of plant response to chemical stress by stimulate the growth of organ to increase capacity to uptake nutrient and enable plant to restoration of normal homeostasis (Wieczorek and Wieczorek, 2007). Calvelo Pereira *et al*. (2010) also reported that *Triticum* response to stress from lindane by increased root tissue. Shoot length of sweet corn, waxy corn, water morning glory, and cow pea was not different significantly in all concentration of endosulfan sulfate-contaminated soil (Table 2).

Moreover, highest concentration of heptachlor (40 mg kg$^{-1}$) only reduced root length of cucumber and sweet corn ($P<0.05$) but did not affect on root length of any plant used in this study (Table 1). Only highest concentration of heptachlor reduced shoot length of cucumber significantly ($P<0.05$). Also, 0.4-40 mg kg$^{-1}$ dry soil endosulfan sulfate did not decreased root length all of 5 tested plant (Table 2).

All of the above results confirmed that the test concentration of endosulfan sulfate was less toxic to the elongation of root and shoot than heptachlor. Moreover, endosulfan sulfate was reported to be toxic to plant cell division. Pérez *et al*. (2008) reported that 0.01–5 $\mu$l g$^{-1}$ of endosulfan reduced cell division in root meristem of *Bidens laevis* when exposed hydroponically. In the present study, there is the possibility of reduced cell division in root meristem of corn. Organochlorine pesticides, such as lindane, have been reported to decrease plant hormone level which control water balance, such as

**Table -1:** Growth of 5 plant seedlings growing in varying concentration of heptachlor contaminated soil for 10 days

| Concentration (mg kg$^{-1}$) | | % Seed germination | Shoot length (cm) | Root length (cm) | Whole plant | | |
|---|---|---|---|---|---|---|---|
| | | | | | Fresh weight (g) | Dry weight (g) | Dry weight/Fresh weight |
| Waxy corn | 0 | 96.70 ± 5.80a | 26.28 ± 2.51bc | 21.70 ± 3.40a | 1.04 ± 0.14a | 0.11 ± 0.01a | 0.11 |
| | 0.4 | 96.70 ± 5.80a | 28.75 ± 5.8a | 22.00 ± 4.30a | 1.12 ± 0.24a | 0.12 ± 0.03a | 0.11 |
| | 4 | 90.00 ± 10.00a | 27.15 ± 1.84ab | 21.30 ± 3.20a | 1.14 ± 0.21a | 0.11 ± 0.02a | 0.10 |
| | 40 | 96.70 ± 5.80a | 24.93 ± 3.014c | 19.70 ± 3.10a | 1.03 ± 0.18a | 0.12 ± 0.02a | 0.11 |
| Sweet corn | 0 | 70.0 ± 0.00a | 26.2 ± 4.0a | 15.1 ± 3.60a | 1.09 ± 0.19a | 0.13 ± 0.02a | 0.12 |
| | 0.4 | 70.0 ± 0.00a | 24.3 ± 3.8a | 14.90 ± 4.10a | 1.08 ± 0.23a | 0.12 ± 0.02a | 0.11 |
| | 4 | 76.7 ± 5.80a | 24.4 ± 3.6a | 14.60 ± 3.20a | 1.06 ± 0.21a | 0.12 ± 0.02a | 0.11 |
| | 40 | 63.3 ± 5.80a | 23.6 ± 4.2a | 8.90 ± 4.40b | 1.05 ± 0.23a | 0.12 ± 0.02a | 0.11 |
| Water morning glory | 0 | 76.70 ± 11.50a | 10.88 ± 1.77ab | 5.80 ± 1.20a | 0.29 ± 0.06a | 0.022 ± 0.00ab | 0.08 |
| | 0.4 | 66.70 ± 15.30a | 11.55 ± 1.52a | 5.40 ± 1.70a | 0.27 ± 0.04ab | 0.022 ± 0.01a | 0.08 |
| | 4 | 86.70 ± 5.80a | 9.60 ± 1.24b | 5.00 ± 1.60a | 0.24 ± 0.05ab | 0.017 ± 0.00b | 0.07 |
| | 40 | 66.70 ± 5.80a | 9.70 ± 1.90b | 4.80 ± 1.80a | 0.21 ± 0.07b | 0.018 ± 0.01ab | 0.08 |
| Cucumber | 0 | 90.00 ± 10.00a | 15.10 ± 1.91a | 7.70 ± 1.10a | 0.38 ± 0.09a | 0.02 ± 0.004a | 0.05 |
| | 0.4 | 96.70 ± 5.80a | 15.73 ± 1.19a | 7.40 ± 1.20a | 0.40 ± 0.08a | 0.02 ± 0.004a | 0.04 |
| | 4 | 80.00 ± 10.00a | 15.98 ± 1.51a | 7.20 ± 1.20a | 0.36 ± 0.10a | 0.02 ± 0.006a | 0.05 |
| | 40 | 93.30 ± 11.50a | 16.20 ± 1.29a | 5.60 ± 1.30b | 0.33 ± 0.08a | 0.02 ± 0.004a | 0.05 |
| Cow pea | 0 | 56.70 ± 5.80a | 15.79 ± 4.58ab | 6.00 ± 3.00a | 0.57 ± 0.07a | 0.05 ± 0.02ab | 0.09 |
| | 0.4 | 63.30 ± 15.30a | 16.18 ± 2.77ab | 7.20 ± 2.70a | 0.54 ± 0.06a | 0.05 ± 0.01a | 0.08 |
| | 4 | 76.70 ± 5.80a | 16.44 ± 2.12a | 7.10 ± 2.10a | 0.53 ± 0.06a | 0.05 ± 0.01ab | 0.08 |
| | 40 | 57.70 ± 15.30a | 13.26 ± 3.91b | 5.20 ± 2.90a | 0.41 ± 0.10b | 0.03 ± 0.01b | 0.07 |

Different letters in each column of crop represent significant differences with Tukey's test (*P<0.05*)

**Table- 2**: Growth of 5 plant seedlings growing in varying concentration of endosulfan sulfate contaminated soil for 10 days

| Concentration (mg kg$^{-1}$) | | % Seed germination | Shoot length (cm) | Root length (cm) | Whole plant | | |
|---|---|---|---|---|---|---|---|
| | | | | | Fresh weight (g) | Dry weight (g) | Dry weight/Fresh weight |
| Waxy corn | 0 | 83.30 ± 5.78a | 16.80 ± 5.21a | 16.00 ± 2.60a | 0.90 ± 0.16c | 0.42 ± 0.26c | 0.47 |
| | 0.4 | 93.30 ± 5.78a | 15.60 ± 3.42a | 16.20 ± 2.60a | 1.08 ± 0.15ab | 0.54 ± 0.11b | 0.51 |
| | 4 | 86.70 ± 5.78a | 17.10 ± 6.31a | 17.70 ± 2.80a | 1.16 ± 0.23a | 0.61 ± 0.18a | 0.53 |
| | 40 | 83.30 ± 5.78a | 15.70 ± 3.82a | 16.80 ± 2.70a | 0.99 ± 0.15bc | 0.31 ± 0.12c | 0.31 |
| Sweet corn | 0 | 50.00 ± 10.00a | 24.40 ± 4.80a | 13.70 ± 3.10a | 1.06 ± 0.28a | 0.12 ± 0.02a | 0.12 |
| | 0.4 | 43.30 ± 20.80a | 26.10 ± 4.40a | 14.20 ± 2.40a | 1.05 ± 0.24a | 0.13 ± 0.02a | 0.11 |
| | 4 | 43.30 ± 15.30a | 24.20 ± 4.60a | 14.00 ± 2.40a | 1.06 ± 0.23a | 0.13 ± 0.02a | 0.12 |
| | 40 | 50.00 ± 10.00a | 21.70 ± 6.20a | 12.80 ± 3.20a | 1.02 ± 0.18a | 0.13 ± 0.02a | 0.13 |
| Water morning glory | 0 | 70.00 ± 10.00a | 11.80 ± 1.51a | 3.60 ± 1.50a | 0.31 ± 0.06a | 0.02 ± 0.00a | 0.08 |
| | 0.4 | 76.67 ± 23.09a | 11.50 ± 1.61a | 3.20 ± 1.40a | 0.25 ± 0.06ab | 0.02 ± 0.00a | 0.09 |
| | 4 | 73.30 ± 5.78a | 11.80 ± 1.79a | 4.00 ± 1.00a | 0.25 ± 0.06ab | 0.02 ± 0.00a | 0.08 |
| | 40 | 90.00 ± 10.00a | 11.25 ± 1.51a | 3.40 ± 0.80a | 0.23 ± 0.06b | 0.02 ± 0.00a | 0.09 |
| Cucumber | 0 | 96.70 ± 5.78a | 17.18 ± 1.17a | 7.20 ± 1.40a | 0.37 ± 0.06a | 0.02 ± 0.002a | 0.06 |
| | 0.4 | 96.70 ± 5.78a | 16.90 ± 1.23a | 6.50 ± 0.90a | 0.34 ± 0.05a | 0.02 ± 0.003a | 0.07 |
| | 4 | 96.70 ± 5.78a | 17.00 ± 1.34a | 6.40 ± 1.30a | 0.33 ± 0.05a | 0.02 ± 0.003a | 0.06 |
| | 40 | 100.00 ± 0.00a | 15.60 ± 1.02b | 6.30 ± 1.30a | 0.31 ± 0.05a | 0.02 ± 0.003a | 0.06 |
| Cow pea | 0 | 70.00 ± 0.00a | 20.80 ± 5.41a | 7.90 ± 3.80a | 0.64 ± 0.23a | 0.05 ± 0.02a | 0.09 |
| | 0.4 | 80.00 ± 10.00a | 18.50 ± 4.76a | 10.20 ± 3.60a | 0.55 ± 0.19a | 0.05 ± 0.01a | 0.09 |
| | 4 | 86.70 ± 11.55a | 17.50 ± 4.78a | 10.20 ± 3.00a | 0.47± 0.14a | 0.06 ± 0.02a | 0.13 |
| | 40 | 76.70 ± 5.80a | 18.20 ± 4.12a | 8.50 ± 3.90a | 0.52 ± 0.16a | 0.05 ± 0.02a | 0.09 |

Different letters in each column of crop represent significant differences with Tukey's test (*P<0.05*)

**Table- 3**: Growth of corn seedlings growing in varying total concentration of heptachlor and endosulfan sulfate contaminated soil for 10 days

| Concentration ($mg\ kg^{-1}$) | | % Seed germination | Shoot length (cm) | Root length (cm) | Whole plant | | |
|---|---|---|---|---|---|---|---|
| | | | | | Fresh weight (g) | Dry weight (g) | Dry weight/Fresh weight |
| Waxy corn | 0 | 96.7 ± 5.8a | 20.2 ± 1.8a | 19.0 ± 3.8a | 1.4 ± 0.2a | 0.15 ± 0.02a | 0.11 |
| | 0.4 | 86.7 ± 15.3a | 21.5 ± 2.0a | 19.8 ± 2.6a | 1.5 ± 0.2a | 0.15 ± 0.03a | 0.09 |
| | 4 | 83.3 ± 15.3a | 21.0 ± 1.8a | 18.7 ± 3.6a | 1.5 ± 0.2a | 0.15 ± 0.02a | 0.10 |
| | 40 | 96.7 ± 5.8a | 20.2 ± 1.6a | 19.8 ± 2.9a | 1.4 ± 0.2a | 0.14 ± 0.02a | 0.10 |
| Sweet corn | 0 | 96.7 ± 5.8a | 20.1 ± 4.5a | 17.8 ± 3.2a | 1.4 ± 0.3a | 0.14 ± 0.03a | 0.12 |
| | 0.4 | 86.7 ± 15.3a | 20.3 ± 3.1a | 16.4 ± 4.6a | 1.3 ± 0.3a | 0.13 ± 0.02a | 0.12 |
| | 4 | 83.3 ± 15.3a | 20.2 ± 3.3a | 16.0 ± 5.0a | 1.3 ± 0.3a | 0.13 ± 0.02a | 0.11 |
| | 40 | 96.7 ± 5.8a | 19.2 ± 2.9a | 15.3 ± 4.5a | 1.3 ± 0.2a | 0.13 ± 0.03a | 0.11 |

Different letters in each column of crop represent significant differences with Tukey's test (*P<0.05*)

Indol-3-ylacetic acid (IAA) (Mansfield and McAlinsh, 1995; Sharada *et al*., 1999). It is possible that endosulfan sulfate could disrupt plant hormone level however, further research is needed. However, toxicity of heptachlor on plant was rarely reported and detailed of toxicity mechanism should be also study.

**Effect on fresh weight and dry weight:** Plant fresh and dry weight of sweet corn, waxy corn and cucumber did not affect with increasing concentration of heptachlor. Forty mg $kg^{-1}$ dry soil of heptachlor reduced fresh weight of water morning glory and cow pea significantly ($P<0.05$). Heptachlor in the range of 4- 40 mg $kg^{-1}$ did not show significant effect on dry weight of test plants (Table 1). Endosulfan sulfate had no significant effect on fresh and dry weight of sweet corn, cucumber and cowpea. However, the presence of endosulfan sulfate in the range of 0.4 and 4.0 mg $kg^{-1}$ dry soil tended to increase fresh and dry weight of waxy corn significantly ($P<0.05$). On the other hand, highest concentration of endosulfan sulfate decreased fresh weight of water morning glory but showed no effect on the dry weight of water morning glory (Table 2).

Plants belonging to family Cucurbitaceae have been reported to be effective in phytoremediation of organochlorine. Campbell *et al*. (2009) reported that *Lagenaria siceraria* (Cucurbitaceae) could accumulate heptachlor with bioaccumulation factor ranging from 1-5.2. Similarly *Cucurmis sativus* and *Cucurbita pepo* have been found to accumulate dieldrin and DDT in their biomass (Otani and Seike, 2007; Åslund *et al*., 2010).

**Effect of endosulfan sulfate and heptachlor mixture:** Because the pesticide contaminated soil in agricultural site often found the combination of organochlorine pesticides. The presence of combined treatment endosulfan sulfate and heptachlor in soil did not produced significant effect on the root and shoot elongation and fresh and dry weight. The result revealed that concentration of endosulfan sulfate and heptachlor mixture of these organochlorine compounds in the environment did not produced toxic effects on crops (Table 3). However, the combined effect of pesticides in soil to plant growth have been reported. Lin *et al*. (2008) reported that pyrene at the initial concentration of pyrene at 50, 100, and 500 mg $kg^{-1}$ tended to alleviate the toxic effect of copper to corn. Shoot dry weight of corn tended to increase in the presence of pyrene . In addition, *Festuca arundinaceae* (Poaceae) have been reported to tolerate phenanthrene, pyrene, and benzo[*a*]pyrene mixtures. Germination of *F. arundinaceae* was not affect by any concentration of these hydrocarbon mixtures. *F. arundinacea* could survive more than a half of initial concentration even though at 5,000 mg $kg^{-1}$ of hydrocarbon mixtures (Reynoso-Cuevas *et al*., 2008). Mixture of phenanthrene and pyrene in acidic soil was shown synergistic toxicity to groundnut growth but not for corn (Chouychai *et al*., 2007). However, the antagonistic effect of endosulfan sulfate and heptachlor in this study was found with root length of sweet corn only. The contaminated level of endosulfan sulfate and heptachlor at contaminated sites in Thailand did not exert toxic effect on seedling growth of selected crops in Thailand. Heptachlor seem to be more toxic to these plants than endosulfan sulfate.

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