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Vetiver Grass: a Natural Barrier to Protect Against Organophosphate Pesticides from Cabbage Fields

Maliwan Boonsaner, Thamnoon Kaewumput and Arthorn Boonsaner

ABSTRACT

The absorption of organophosphate pesticides by cabbage and vetiver grass (*Vetiveria zizanioides* Nash.) was investigated at Khunkong Watershed Research Station, Chiang Mai, between January and May 2003. The experimental plots with 12 cabbages per square meter and rows of vetiver grass about 0.5 m down slope below the plots were established on an area of about 30% slope. Two samples per plot of cabbage, vetiver grass and soil, were collected, twice a month at 15 days after application and at one day before next application, except the first collection, which was carried out right after the initial spray. Except methamidophos, the results showed that all the test compounds accumulated in cabbage, which was not found in any samples because of its low persistence. The investigation of test compounds in cabbage during the 139 day experiment showed that the average uptake rate was ethoprophos (0.78 μg/g lipid weight.day) < methyl-parathion (0.93 μg/g lipid weight.day) < methidathion (1.86 μg/g lipid weight.day) < EPN (7.7 μg/g lipid weight.day) and the concentration factor ($CF = C_B/C_S$, g organic carbon /g lipid weight) order was similar as ethoprophos (log CF = 0.54) was < methidathion (log CF = 0.98) < methyl-parathion (log CF = 1.18) < EPN (log CF = 2.17). The concentration factors of the test compounds were highly related to their hydrophobicity ($\log K_{ow}$) by $\log CF = 1.0696 \log K_{ow} - 2.2347$, $r^2 = 0.87$. In vetiver grass, only EPN was found in root samples with the absorbed concentration increasing with exposure time and in soil, EPN was the only compound which was found only in samples collected from the areas above vetiver grass rows. This result indicated that vetiver grass might be used to absorb organophosphate pesticides applied in agricultural areas.

Key words: organophosphate pesticides, bioacumulation, vetiver grass

INTRODUCTION

The northern part of Thailand is a mountainous area where deforestation is a problem. At present, some of this area is cleared for agricultural purposes which involves pesticide usage. Under government support, vetiver grass (*Vetiveria zizanioides* Nash.) has been introduced to this area in order to reduce soil erosion due to its long fibrous root system. Besides this important property, vetiver grass root is also known to have a high essential oil content which can be used to produce perfume (α-vetivone, β–vetivone, khisimone and khusitone) and soap (Bostid, 1993). The high oil content in the root may be used to absorb the organophosphate pesticides which are used in this area as well as to protect against soil erosion and conserve water quality.

Several previous studies have shown that vetiver grass can be used to absorb environmental contaminants. Pimpan *et al.* (1997) reported a substantial decrease of endosulfan residue after

Department of Environmental Science, Faculty of Science, Silpakorn University, Nakhon Pathom 73000, Thailand.

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passing through vetiver grass zones and Pinthong *et al.* (1999) also found the absorption of three pesticides: carbofuran, alachlor and monocrotophos, by vetiver grass. In China, Hamping (2000) used vetiver grass to control the effluent from a pig farm. However, the study of the translocation of pesticides to the upper part of vetiver grass is as important as the study of absorption via roots. Some species of vetiver grass are also used as an animal feedstock (Truong, 1999). Therefore, if translocation is found, vetiver grass may not be safe to be used as feed stock because it may lead to human exposure. In 2002, Boonsaner *et al*. reported the bioaccumulation of five organophosphate pesticides: methamidophos, ethoprophos, methidathion, methyl-parathion and EPN in vetiver grass root and stem. The results showed that the common sequence for the uptake rate of test compounds in root was methamidophos > ethoprophos > methidathion > methyl-parathion > EPN but the sequence was opposite in the stem. The accumulation of test compounds in both root and stem increased with their hydrophobicity. The linear relationship between log RCF (root concentration factor) and log K_{ow} as well as that between log SCF (stem concentration factor) and log K_{ow} can be represented by log RCF = 0.29 log K_{ow}– 1.05, r^2= 0.99 and log SCF = 0.59 log K_{ow}– 1.06, r^2 = 0.95, respectively. The results indicated that the hydrophobicity of test compounds may play an important role in governing the behaviour of organic compounds, especially organophosphate pesticides, in soil-root-stem partitioning.

The Khunkong Watershed Research Station is located in Chiang Mai, Thailand, where cabbage is grown and organophosphate pesticides are used extensively. Vetiver grass has been employed as a plant model since it is widely grown to prevent soil erosion in this area. During January to May 2003, an investigation on the absorption of organophosphate pesticides by cabbage and vetiver grass was performed in order to study the capability of vetiver grass to absorb organophosphate pesticides, and hence act as the natural barrier to prevent organophosphate pesticide runoff from cabbage fields.

MATERIALS AND METHODS

Methamidophos, ethoprophos, methidathion, methyl-parathion and EPN, obtained from Novartis (Thailand) Co. Ltd., were used as the test compounds. Hexane (Nanograde), acetone (AR), acetonitrile (AR) and florisil were purchased from Mallinckrodt Chemical, Inc., Kentucky, USA. Methanol (AR), chloroform (AR), toluene (AR) and diethyl ether (AR) were obtained from BDH Laboratory Supplies, England. Glass wool was bought from Ajax Chemical Company, Australia, while anhydrous sodium sulfate (AR) was obtained from Merck KGgaA, Germany. Glass fibre filter paper (GF-C) was purchased from Whatman, England.

Anhydrous sodium sulfate and florisil were heated in a muffle furnace at 450°C and 650°C, respectively, for at least 4 h, then cooled in a dessicator, followed by deactivation with water (5% w/w) for florisil. Glass wool was prewashed with acetone and hexane, then air-dried before use. All glassware was soaked in detergent overnight, washed and rinsed with deionized water. Before use, all glassware was rinsed again with hexane.

The water content of soil and lipid content of vetiver grass were determined by standard methods of soil analysis (Page, 1982) and by soxhlet extraction (APHA, AWWA and WPCF, 1998), respectively. The extraction of test compounds from soil and the roots and stem of vetiver grass followed the method for analysis of pesticide residues as described by Moye (1981). A Perkin Elmer Autosystem XL Gas Chromatographs, fitted with FPD Detector and DB-5 column ($30 \times 0.32 \times 0.25$ μm) was employed for the analysis of test compounds. The condition of GC oven temperature program was hold at

120°C for 3 min, then increase to 200°C with ramp rate of 10°C/min hold for 5 min and increase to 250°C with 20°C/ min hold for 15 min. The injector and detector temperature were 220°C and 300°C, respectively. Helium (2 ml/ min) was used as carrier gas while hydrogen gas (75 ml/min) is fuel and air (90 ml/min) is the oxidant for the FPD. The detection limits were 200, 50, 100, 30 and 40°C µg/l for methamidophos, ethoprophos, methidathion, methyl-parathion and EPN, respectively.

The study on the bioaccumulation of five test organophosphate pesticides was conducted by preparing two experimental plots (5 m × 5 m each plot) with 12 cabbages per square meter and rows of vetiver grass located about 0.5 m below the plots down slope were established on an area of about 30% slope. The first cabbage plot was kept as a control. The second one was sprayed with the test mixture once a month. This mixture was prepared from five test compounds: 4 g of methamidophos, 4 g of ethopophos, 4 g of methyl-parathion, 4 g of methidathion and 4 of EPN in 20 l of water. The amount of each compound used was 1/5 of the required dose shown on each product directions. Test mixtures were sprayed on the cabbage once a month with care taken to avoid direct contamination of vetiver grass.

Two samples per plot of cabbage, vetiver grass and soil, were collected twice a month at 15 days after application and at one day before next application, except for the first collection inwhich samples were taken right after the spraying event. For soil samples, three collections were taken: one from the plots, another from between the cabbage plots and the vetiver grass rows and the last one from below the vetiver grass row. Regarding vetiver grass samples, the vetiver grass was separated into root and stem before performing the analysis. The concentration factor (CF) was calculated in terms of the concentrations of test compounds in plant lipid weight (either cabbage or vetiver lipid weight : C_b, mg/g lipid weight) divided by the concentrations of test compounds in soil organic carbon weight (C_s, mg/g organic carbon). The average uptake rate of test compound in plant was calculated from test concentration in plant (µg/g lipid weight) divided by duration of experiment (139 days).

RESULTS AND DISCUSSIONS

The study of the bioaccumulation of 5 organophosphate pesticides in cabbage and vetiver grass was conducted for 139 days during January to May 2003. The experiment was set up at Kundkong Research Station where the slope of the growing area was about 28 - 30° and the organic carbon content of soil was between 2.8 and 5.0%. The temperature and relative humidity during the experimental period were 25 - 30°C and 40–90 %, respectively. Lipid contents in cabbage, vetiver stem and vetiver root were 0.13 - 0.19%, 0.17 - 1.09% and 1.98 - 3.94%, respectively.

Accumulation of test compounds in cabbage

Analysis for the five test compounds in cabbage showed that ethoprophos, methyl-parathion, methidathion and EPN had accumulated in cabbage while methamidophos was not found in all samples presumably because of its low persistence and high water solubility (Table 1). The hydrolysis of methamidophos can contribute to its low persistence (Ney, 1990). The study on the uptake of the remaining four test compounds showed detectable concentrations in cabbage 15 days after commencing the experiment with concentrations increasing with exposure time (Figure 1). The concentration factors (CF, g organic carbon/g lipid weight) in cabbage were in the order EPN > methyl-parathion > methidathion > ethoprophos (Table 1).

For this study, although the equilibrium of test compounds with cabbage may not be attained, the relationship between log CF and test compounds' physicochemical properties still

showed a high correlation which was represented by log CF = 1.0696 log K_{ow} – 2.2347, r^2 = 0.87 (Figure 2). This relationship is similar to the one obtained from Metcalf *et al.* (1975) and Veith and Kosian. (1983) which were log CF = 1.1587 log K_{ow} + 0.750, r^2 = 0.98 and log CF = 0.93 log K_{ow} – 0.40, r^2 = 0.93, respectively. The differences of the intercepts may be due to various factors such as lipid content of plant and organism, the soil organic carbon, test compounds, etc.

However, these results suggested that the accumulation of test compounds depends upon their hydrophobicity. This is also in accordance with Jorgensen *et al.* (1998) who noted that for any given compound, the higher log K_{ow} value of that compound, the higher the bioaccumulation factor.

Regarding the average uptake rate of each test compounds in cabbage during the 139 day experiment, the average uptake rate was EPN (7.7 µg/g lipid weight.day) > methidathion (1.86 µg/g lipid weight.day) > methyl-parathion (0.93 µg/g lipid weight.day) > ethoprophos (0.78 µg/g lipid weight.day). It was noticed that the uptake rate of test compounds which are bigger molecules with higher hydrophobicity tends to be faster than those with smaller molecular size and less hydrophobicity.

Absorption of test compounds in vetiver grass root and stem

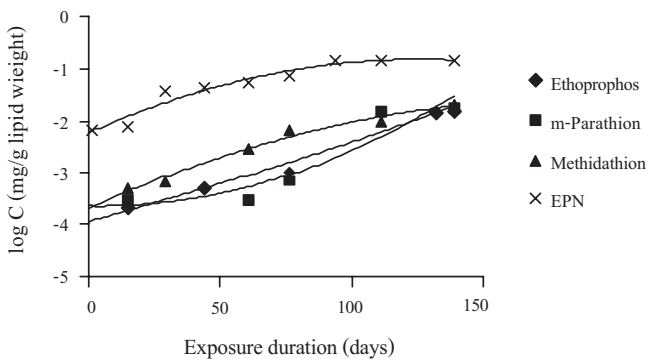

Figure 1 The accumulation of detectable test compounds in cabbage (mg /g lipid weight) with exposure time.

Table 1 Relevant physicochemical properties of test compound, the concentration factor and the average uptake rate in cabbage in 139 days experimental period.

Compound	MW	log K_{ow}	Persistence[3]	log CF (g organic carbon/ g lipid weight)	Average uptake rate (µg/g lipid content. day)
Methamidophos	141.3	0.08[1]	Low	ND	ND
Ethoprophos	242.3	2.57[1]	Low	0.54	0.78
M-parathion	263.2	3.52[1]	7 days	1.18	0.93
Methidathion	302.3	2.91[2]	2-3 weeks	0.98	1.86
EPN	323.3	3.91[3]	High	2.17	7.70

Note 1. From Isnard and Lambert (1989)
2. From Kamrin (1997)
3. From Worthing (1997)

From a previous study, the accumulation of the test compounds in vetiver grass root and stem increased with their hydrophobicity (Boonsaner *et al*., 2002). Although, the accumulation of test compounds in stem was comparatively small with very low concentrations, concentrations still consistently increased with time. In this study, all organophosphate pesticides except methamidophos were found, but not at every sampling time and when observed with very low concentration. Furthermore, the concentrations did not increase with the exposure time. This result suggested that the accumulation of test compounds in the stem might not arrive from the uptake of those pesticides via roots and subsequent translocation to the stem, but from the contamination of pesticide during application although precautions for direct contamination had been taken.

In vetiver grass roots, only EPN was found and its concentration increased with exposure time (Figure 3). For the other test compounds, their degradation may occur in soil before the leachate from the cabbage field could reach the downstream vetiver grass row. It should be noted that the other test compounds had a much lower persistence than EPN (Table 1).

Over the experimental period of 139 days, the concentrations of EPN in vetiver grass root increased with time (Figure 3). The concentration of EPN in vetiver grass root at day 139 was then compared with that obtained from a grass house experiment (Boonsaner *et al*., 2002). The comparison of log CF values of 3.8 from previous study and log CF = 1.36 from the present study indicated that vetiver root still has the capacity to absorb EPN or may be able to absorb more of other test compounds.

Residues of organophosphate pesticides in soil between cabbage field and vetiver grass row

Only EPN was found in soil between the cabbage field and vetiver grass row. Since the soil samples were collected 15 days after spray application and one day before next application,

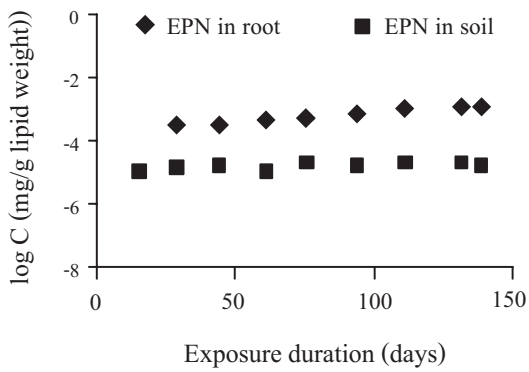

Figure 3 Accumulation of EPN in vetiver root and the soil between cabbage field and vetiver row.

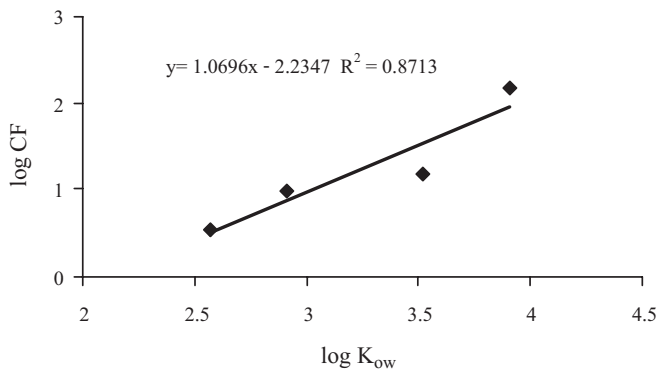

Figure 2 The relationship between log CF and log K_{ow}.

the other four pesticides might have degraded before collection. The concentrations of EPN did not increase with time. This is probably because although the soil accumulates the EPN runoff from the cabbage field, EPN undergoes degradation processes at the same time.

Vetiver grass as a natural barrier

Only EPN was found in soil samples collected from the areas above vetiver grass row. For those collected below the vetiver row, none of the test compounds were found. This result indicated that vetiver grass may be used to absorb organophosphate pesticides in an agricultural area.

CONCLUSION

The study on the bioaccumulation of organphosphate pesticides showed that the concentration factor (CF) in cabbage were in the order EPN > methyl-parathion > methidathion > ethoprophos. Methamidophos was not found because of its low persistence. The correlation log K_{ow} and log CF can be expressed by log K_B = 1.0696 log K_{ow} – 2.2347, r^2 = 0.87. However, the investigations of the accumulation of test compounds in vetiver grass root and stem had shown the very low concentration of all organophosphate pesticides except methamidophos which was not detected. For soil samples collected from area above vetiver grass row, only EPN was found. In comparison with those collected below the vetiver grass row, none of the test compounds were found. This result indicated that vetiver grass is capable of absorbing EPN.

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