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Adsorption and Analysis of the Insecticides Thiamethoxam and Indoxacarb in Hawaiian Soils

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A method was developed for the simultaneous extraction and analysis of the insecticides indoxacarb and thiamethoxam from five Hawaiian soils. Using pressurized fluid extraction followed by liquid chromatography, optimized recoveries from the five soils were obtained ranging from 80% ± 5 to 101% ± 10 for thiamethoxam, and 83% ± 6 to 106% ± 7 for indoxacarb. Aging studies also showed strong binding of indoxacarb to all soils tested after 30 days, while thiamethoxam remained quite available for extraction during the length of the study (90 days). Freundlich constant (K_f) and empirical value (n) for thiamethoxam sorption on Lihue soil were 0.007391 mmol$^{(1-1/n)}$·$L^{1/n}$·g^{-1} and 1.1377, respectively; K_f and n were 0.007844 mmol$^{(1-1/n)}$·$L^{1/n}$·g^{-1} and 0.8473, respectively, on Wahiawa soil. The organic carbon adsorption constant (Koc) of thiamethoxam was 0.53 in Lihue soil and 0.23 in Wahiawa soil.

KEYWORDS: Insecticides; soils

1. INTRODUCTION

Thiamethoxam {3-[(2-chloro-5-thiazolyl)methyl]tetrahydro-5-methyl-*N*-nitro-4*H*-1,3,5-oxadiazin-4-imine} and indoxacarb {(*S*)-methyl 7-chloro-2,5-dihydro-2-[[(methoxycarbonyl)[4-(trifluoromethoxy)phenyl]amino]carbonyl]indeno[1,2-e][1,3,4]-oxadiazine-4a(3*H*)-carboxylate} are two new insecticides introduced to the American market in the past few years, indoxacarb being an oxadiazine developed by Du Pont and thiamethoxam a neonicotinoid by Sygenta (*1*, *2*). Thiamethoxam is approved for use as soil, foliar, and seed treatment for the control of aphids, whiteflies, and some beetles among others (*3*). Indoxacarb is used in liquid formulations for the control of lepidoptera larvae including beet armyworm and Egyptian cotton leafworm (*4*, *5*).

Thiamethoxam was shown to have some synergistic effect with nematodes against beetles (*6*), but to a lesser extent than imidacloprid. As a foliar spray, it is effective against sweet potato white fly (*7*) on melons, but its toxicity to nontarget honeybees was determined to be high with a LD_{50} value of 30 ng per bee (*8*). Indoxacarb is effective for the control of Lepidoptera (*9*) and found to have low toxicity to the beneficial parasitoid *Eretmocerus mundus* Mercet of the whitefly *Bemisia tabaci* (*10*). Also, when indoxacarb was mixed with a feeding stimulant, its action was shown to not be compromised (*11*). Indoxacarb was also studied for its toxicity toward nontarget anthropods (*12*), and it was found to be nontoxic to the mites *Amblyseius fallacis* and *Agistemus fleschneri*, but toxic to the mired *Hyaliodes vitripennis*. Field weathered residues of both indoxacarb and thiamethoxam were investigated for their toxicity to *A. iole* wasps, the biological control agent for the cotton pest *Lygus lineolaris*, and they both were found to be toxic to the wasps in varying degrees (*13*).

Due to these toxicity concerns to nontarget species, it is necessary to develop a reliable, fast, and accurate analytical method for the two insecticides. The method of choice for the analysis of the compounds has been high performance liquid chromatography (HPLC) coupled to ultraviolet detection (UV) (*2*, *14*) or electrospray ionization mass spectrometry (ESI-MS) (*2*, *15*, *16*). Sample extraction has been done using homogenization and solid-phase extraction clean up for larvae (*2*), and homogenization followed by liquid extraction for processed fruits and vegetable (*15*). This work focused on the simultaneous analysis of the two compounds in Hawaiian soils using pressurized fluid extraction (PFE), HPLC-UV and ESI-MS, and the determination of thiamethoxam adsorption constants on two Hawaiian soils.

2. MATERIALS AND METHODS

Chemicals. Extraction solvents were Optima grade acetonitrile and methanol (Fisher Scientific, Pittsburgh, PA). The analyte standards had purities of 99.4% for thiamethoxam synthesized in this laboratory (*17*) and 55.0% for indoxacarb (DPX-MP062) obtained from Du Pont Crop Protection (Wilmington, Delaware). Stock solutions and subsequent dilutions were made in acetonitrile and methanol for indoxacarb and thiamethoxam, respectively. All standard solutions were kept at 4 °C.

Soil. Five soils (**Table 1**) were collected from Oahu, Kauai, and Hawaii islands (*18*). The pH values of the soils were measured in a 1/1 ratio (w/v) of soil/water. All soil samples were air-dried for 24 h, sieved through a #10 metal sieve (Tyler equivalent 9 mesh, opening

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Table 1. Characteristics of Five Hawaiian Soils

soil series	soil classification[a]	pH	moisture, %	organic C, %
Nohili	fine, montmorillonitic (calcareous), isohyperthermic, mollisols	7.4	7.97	2.08
Helemano	clayey, kaolinitic, isohyperthermic, oxisols	5.2	15.71	0.83
Wahiawa	clayey, kaolinitic, isothermic, oxisols	5.3	4.21	3.37
Lihue	clayey, kaolinitic, isohyperthermic, oxisols	6.8	17.66	1.40
Lualualei	very fine, montmorillonitic, isohyperthermic, vertisols	7.6	7.73	0.43

[a] Information is cited from ref 19.

size 2.00 mm), and stored in clean glass jars prior to extraction. A 20-g portion of each sample was reserved for moisture content determination. Total organic carbon contents were measured with a Leco nitrogen autoanalyzer (Leco Corp., St. Joseph, MI) at the Agricultural Diagnostic Service Center, University of Hawaii at Manoa.

Extraction Procedure. A Dionex ASE 200 extractor (Salt Lake City, UT) was used for all extractions following a method described in detail elsewhere (*19*). Briefly, to reduce soil aggregation during extraction, all soil samples were mixed with clean Ottawa sand (Fisher Scientific) in a 2:1 w/w ratio prior to being loaded into the cells. The extraction solvent was a 1:1 mixture of acetonitrile and methanol, the pressure was kept constant at 1500 psi as pressure generally does not have a big impact on extraction efficiency (*20*, *21*), and the temperature varied from 25 to 100 °C to optimize extraction efficiency. The extraction time was 5 min. The extracts (approximately 30 mL) were concentrated with a gentle flow of nitrogen, brought to 2 mL, and filtered through a 0.45 μm membrane syringe filter (Gelman Laboratory) before LC-UV or LC-MS determination. All experimental samples were run in triplicates or quadruplets.

Fortification Procedure. The dried and sieved soil samples were used for fortification studies. A portion of the dried and sieved samples of each soil series was used to determine the background level of the analytes. It showed no presence of the target chemicals.

Freshly Spiked Sample. A soil sample (10 g of air-dried) was weighed, mixed well with about 5 g of clean Ottawa sand, and placed into an extraction cell. Aliquots of 250 μL of standard solutions of thiamethoxam and indoxacarb were placed on the top of the soil. The extraction cell was then closed and extracted immediately.

Aged Soil Sample. Five milliliters of thiamethoxam or indoxacarb standard stock solutions diluted into 150−200 mL of methanol−acetonitrile (1/1, v/v) was mixed with 200 g of each soil sample and let to stand at room temperature for 1 h with occasional swirling. The solvent was then evaporated with a vacuum rotary evaporator. This predried soil was then air-dried for 2−4 h under a fume-hood, placed in clean glass jars, and then stored in the dark at room temperature for 90 days for subsequent analysis of subsamples at days 1, 29 and 30, 59 and 60, and 90.

Water−Soil Phase Adsorption Isotherm. Isotherms of thiamethoxam (water solubility, 4.1 mg/mL) in Lihue and Wahiawa soils were determined. Four batches of water−soil adsorption isotherm samples were prepared by diluting the appropriate amount of thiamethoxam in water standard stock solution to 10 mL into 1.00 g of soil samples in 40-mL-Teflon centrifuge tubes. The tubes were then shaken in a Burrell Shaker for 16 h and centrifuged for 10 min at 3000 rpm (1100*g*) as preliminary experiments suggested that equilibrium was reached within 16 h for thiamethoxam. The top liquid layer was then filtered through a 0.45-μm nylon membrane syringe filter and diluted for HPLC analysis (*5*). The amount of thiamethoxam adsorbed was calculated from the amount in solution at the beginning of the experiment and at equilibrium. Isotherms of indoxacarb in the five soils were not examined in this study. Radioactive labeled indoxacarb would be required to determine the isotherms due to low solubility in water (0.2 μg/mL) and the method detection limit.

Table 2. Effect of Temperature on Extraction Efficiencies of Thiamethoxam and Indoxacarb from Lihue Soil, $n = 3$

	recovery, % ± standard deviation	
temp (°C)	thiamethoxam	indoxacarb
25	70 ± 4	97 ± 9
40	69 ± 4	78 ± 9
100	98 ± 8	99 ± 9

Table 3. Recoveries of Thiamethoxam and Indoxacarb from Five Soils at 100 °C

		recovery, % ± standard deviation[a]	
soil series	moisture, %	thiamethoxam	indoxacarb
Helemano	15.7	82 ± 10	106 ± 7
Wahiawa	4.2	99 ± 7	83 ± 6
Kauai	17.7	98 ± 8	99 ± 0.5
Nohili	8.0	80 ± 5	101 ± 6
Lualualei	7.7	101 ± 10	90 ± 6

[a] PFE conditions: 1500 psi, 100 °C, 5 min cycle, acetonitrile and methanol (1:1). $n = 3$.

Soil pH Variation. The pH of Helemano series soil was modified for several batches with lime by alternating three wet−dry cycles of 1:1 w/v of water:soil. The resulting soil batches had pH values of 4.3, 6.0, and 6.8.

LC Analysis. LC-UV analysis was performed with a Hewlett-Packard (HP) HPLC Series 1090 equipped with an autosampler, photometric detector, and HP 3396 Series II integrator. The analytical column was an Agilent Eclipse XDB-C8 (4.6 × 150 mm i.d., 5 μm particle size) with a guard column. Gradient elution used deionized distilled water containing 1% of 2-propanol and methanol. The methanol percentage was set initially at 50% and held for 4 min, increased to 80% in 3 min, held at 80% for 6 min, decreased to 50% in 3 min, and held for 5 min for column equilibration before the next run. The total time per run was 21 min. The detection wavelength was set at 230 nm. The flow rate was 1 mL/min, and the injection volume was 10 μL. The retention times of thiamethoxam and indoxacarb were 2.2 and 10.8 min, respectively.

LC-MS analyses were performed with an Agilent 1100 LC-single quadrupole mass spectrometer. The chromatographic conditions were the same as for LC-UV analysis. The mass spectrometer parameters were optimized in electrospray positive mode for the best detection of indoxacarb (molecular ion 528^+) and thiamethoxam (ion 292^+).

3. RESULTS AND DISCUSSION

Extraction Optimization. Table 2 shows average recoveries of indoxacarb and thiamethoxam from Lihue soil at three different extraction temperatures. A quantitative recovery of indoxacarb and thiamethoxam was obtained at 100 °C.

The influence of soil pH was investigated for indoxacarb and thiamethoxam in Helemano soil. The recoveries of the two analytes did not significantly change when the pH varied from 4.3 to 6.8. Indoxacarb recoveries and standard deviations varied from 107% ± 6 to 110% ± 2, and thiamethoxam from 81% ± 9 to 83% ± 3.

In general, for all of the five soil series, the lowest optimized recovery for thiamethoxam was 80% ± 5 from Nohili series, and the highest 101% ± 10 from Lualualei soil. The lowest recovery of indoxacarb was 83% ± 6 from Wahiawa soil, and the highest was 106% ± 7 from Helemano soil (**Table 3**).

Soil Aging Effect on Recovery. Figure 1 shows the aging curves for thiamethoxam (**a**) and indoxacarb (**b**) in the five soils. Indoxacarb recoveries decreased for all soils from 3 to 30 days after incubation due to low water solubility and strong attraction

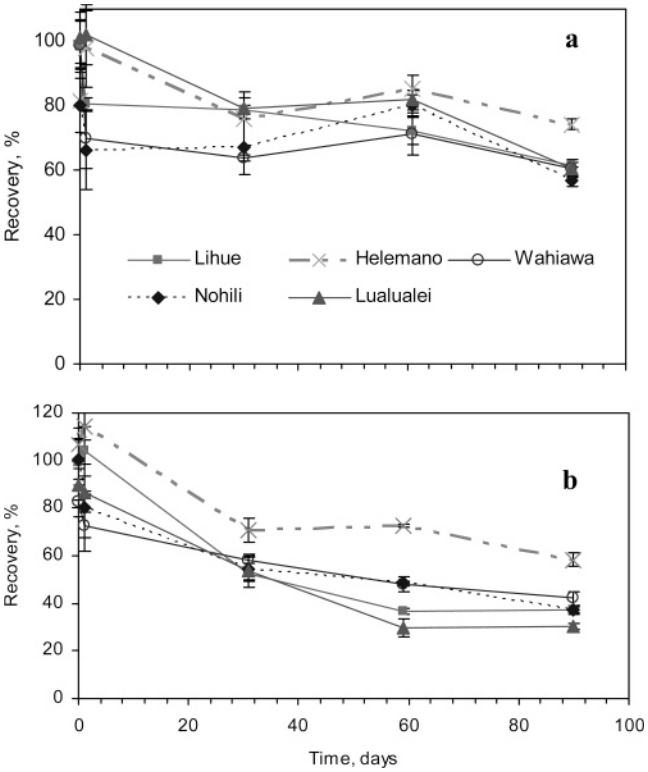

Figure 1. Aging of five spiked Hawaiian soils. (**a**) Soil spiked with thiamethoxam. (**b**) Soil spiked with indoxacarb. Error bars represent one standard deviation ($n = 3–4$).

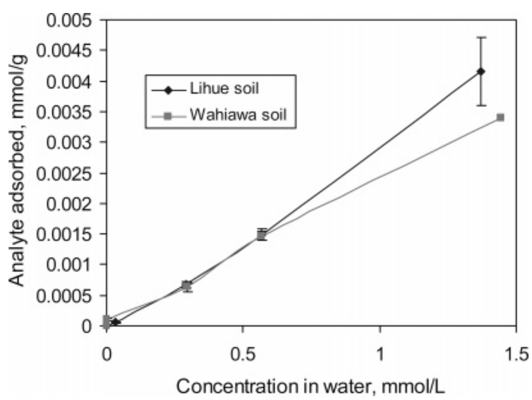

Figure 2. Sorption isotherms of thiamethoxam on Lihue and Wahiawa soils. Error bars represent one standard deviation ($n = 3–4$).

to the carbon fraction in the soils (*23*), followed by fairly constant recoveries from 60 to 90 days. The initial (1 day) recoveries of thiamethoxam were reversely proportional to the organic carbon content (oc) in the soils, that is, Nohili (oc 2.08) ≈ Wahiawa (oc 3.37), < Lihue (oc 1.4) < Helemano (oc 0.83) ≈ Lualualei (oc 0.43). Recoveries after 30 d were relatively constant. Previous work (*22*) showed that hydrolysis half-lives of thiamethoxam varied from several hundred days at pH 7 to a few days at pH 9; a half-life was 300 days in aerobic soils under laboratory conditions. Because the pH values of the soils were in a range of 5.2 and 7.6, hydrolysis of thiamethoxam is presumably negligible within the 90-day study period. The recovery decreases may be attributed to soil adsorption.

Soil Sorption. Figure 2 represents the adsorption isotherms of thiamethoxam on Lihue and Wahiawa series soils. From the Freundlich equation $S = K_f C^{1/n}$, where S is the sorbed thiamethoxam concentration, C the equilibrium concentration in water, K_f the distribution coefficient at equilibrium, and n the order of the reaction 24, K_f and n for each soil can be calculated (**Table 4**). Freundlich constant (K_f) and empirical value (n) for thiamethoxam sorption on Lihue soil were 0.007391 mmol$^{(1-1/n)}$•$L^{1/n}$•g^{-1} and 1.1377, respectively; K_f and n were 0.007844 mmol$^{(1-1/n)}$•$L^{1/n}$•g^{-1} and 0.8473, respectively, on Wahiawa soil. The correlation coefficients (R^2) were 0.9999 and 0.9892 for Lihue and Wahiawa soils, respectively. The organic carbon adsorption constant (Koc) of thiamethoxam was 0.53 in Lihue soil and 0.23 in Wahiawa soil.

Table 4. Freundlich Constants for Thiamethoxam Sorption on Lihue and Wahiawa Soils

soil series	K_f, mmol$^{(1-1/n)}$•$L^{1/n}$•g^{-1}	n
Lihue	0.007391	1.1377
Wahiawa	0.007844	0.8473

Conclusion. Thiamethoxam and indoxacarb were easily extracted with PFE from soil using methanol and acetonitrile mixture. The average recoveries of thiamethoxam ranged from 80% to 101% from the five Hawaiian soils and from 83% to 106% for indoxacarb. Variations of soil pH did not affect the recoveries. As compared to thiamethoxam, indoxacarb became quickly and tightly bound to the five soils tested within 90 days. Sorption coefficients (K_f and K_{oc}) were also determined for thiamethoxam.

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