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Comparison of Soxhlet and Microwave-Assisted Extractions for the Determination of Fenitrothion Residues in Beans

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White and black "niebe" beans [*Vigna unguiculata* (L.) Walp] from Senegal were treated with fenitrothion (*O*,*O*-dimethyl *O*-4-nitro-*m*-tolyl phosphorothioate), and the residues were determined by high-performance liquid chromatography (HPLC) and electron capture gas chromatography (EC-GC). Fenitrothion residues from the beans were extracted by Soxhlet extraction (SE) and microwave-assisted extraction (MAE). A column cleanup procedure was used to remove the coextractives in the extract before HPLC and EC-GC analyses. The overall mean recoveries of fenitrothion residues in the 0.19−1.90 μg/kg fortification range determined from extracts obtained by SE and MAE were 88.4 and 89.8%, respectively, with respective relative standard deviations of <4%. The results show that MAE is a viable alternative to the commonly used SE for the determination of fenitrothion residues in beans.

KEYWORDS: Fenitrothion; "niebe" beans; Soxhlet extraction; microwave-assisted extraction

INTRODUCTION

Fenitrothion (*O*,*O*-dimethyl *O*-4-nitro-*m*-tolyl phosphorothioate) is a broad-spectrum organophosphorus insecticide and is used widely in agriculture to control insects on rice, fruits, cereals, soybeans, coffee, and tea (*1*). Fenitrothion has been also used for the control of stored product insect pests in a number of countries (*2*). In Senegal, fenitrothion has been used against insects affecting cereals and local white and black "niebe" beans [*Vigna unguiculata* (L.) Walp]. It is also used as a pre-emergent, soil-incorporated insecticide in cereal-growing regions. Beans are locally used for food because of their high nutrition values and high protein and low fat contents. Furthermore, they are also used as an export crop. The white and black beans are two varieties of the same species, and both colors appear in the crops. The white and black beans differ in their nutritional values and have different amino acid compositions. The annual production of beans in Senegal is ~80000 metric tones. The extensive use of fenitrothion in Senegal has raised concerns about its residues in white and black beans. Although no information is available on the maximum residue limits (MRLs) of fenitrothion for niebe beans, the value reported by the FAO/WHO Codex Committee for cocoa and soybeans is 0.1 mg/kg (*2*).

Soxhlet extraction (SE) followed by gas chromatography (GC) is reported in most published methods for the analysis of fenitrothion residues in crops. Möllhoff (*3*) determined fenitrothion residues in apple, lettuce, carrot, onion, tomato, and potato by electron capture gas chromatography (EC-GC) after Florisil column cleanup. GC determination of fenitrothion residues in vegetables and fruits using a selective NPD detector has been also reported (*4*, *5*). Funch (*6*) reported a high-performance liquid chromatography (HPLC) method for analyzing fenitrothion residues in apple. SE involves the use of a relatively large amount of solvent, and the extract often requires intensive cleanup steps to eliminate interferences before chromatographic analysis. Although SE is one of the oldest and most widely used techniques used for the routine extraction of pesticides from solid samples, an alternative method of extraction in detecting fenitrothion residues that is cost-effective, employs less solvent, and involves a short extraction time could prove to be useful. This would be especially useful in Senegal for the routine monitoring of fenitrothion residues in niebe beans used extensively for local consumption. Microwave energy to enhance the extraction of pesticide residues from solid matrices has been attempted by using conventional household microwave ovens (*7*, *8*). Microwave-assisted extraction (MAE) is becoming popular because of its rapidity, simplicity, and low cost of operation (*9*, *10*). Furthermore, it requires a very short time and uses less solvent than conventional SE. MAE has been applied for the extraction of pesticides from coffee beans (*11*), plants (*12*), and fruit matrices (*13*, *14*). A much smaller sample size and much less organic solvent are used in MAE (*9*, *14*). In this paper, we report on the feasibility of an MAE procedure that could be used in the routine monitoring programs. MAE was followed by HPLC and EC-GC for the analysis of fenitrothion residues in white and black beans from Senegal. The MAE

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method was compared with the conventional SE procedure for its usefulness in determining pesticide residues in beans.

MATERIALS AND METHODS

Chemicals. All solvents used were of pesticide grade (Burdick and Jackson, McGraw Park, IL) and used as received. Neat fenitrothion was obtained in high purity (98%) from DowElanco. A stock solution (10 mg/100 mL) was prepared initially in hexane. One milliliter of this stock solution was then diluted to 100 mL with hexane to obtain a working stock solution (1.0 μg/mL).

Bean Samples. Representative samples (~500 g) of white and black beans not previously treated with fenitrothion were obtained commercially in Senegal. About 250 g of beans was subsampled and ground using a mortar and pestle. The material was mixed thoroughly and retained for further study.

Sample Fortification. A precise amount (5−10 g) of ground bean was fortified in triplicate with an appropriate aliquot of the diluted working stock solution of fenitrothion to obtain concentrations of 0.19, 0.94, and 1.90 μg/g of beans. The solvent was allowed to evaporate for 24 h at ambient conditions, and then the sample was mixed thoroughly. The samples were fortified in triplicate. All recovery experiments were conducted in duplicate, and the overall means of the six analyses (per fortification level) are reported.

Soxhlet Extraction. SE (Soxhlet extraction apparatus, VWR Scientific Products, catalog no. 27611-049) was performed as described in EPA Method 3540. A 10 g sample portion of the fortified bean sample was extracted with 200 mL of dichloromethane (DCM) for 24 h at the boiling temperature of DCM. The extract was concentrated first to ~5 mL under reduced pressure on a rotary flash evaporator with the water bath temperature at 30 °C, quantitatively transferred to a 15 mL centrifuge tube, and evaporated to dryness with a gentle stream of dry nitrogen gas at room temperature; the residue was dissolved in ~0.5 mL of hexane and quantitatively transferred with hexane to a 5.0 mL (13 × 135 mm, Pyrex Brand) conical bottom glass centrifuge tube with 0.1 mL graduation, and the final volume was concentrated with a nitrogen stream to 1.0 mL.

Microwave-Assisted Extraction. A household microwave oven (Sharp model R-530CW) was used for extraction. The microwave oven operated with a 60 Hz single-phase output of 1100 W. A 2.5 g portion of the fortified bean sample was placed in a 25 mL glass bottle (20 mL vial, 1 $^1/_{16}$ × 2 $^1/_2$ in., borosilicate, VWR brand) followed by the addition of 10 mL of hexane/acetone (1:1). The bottle was tightly sealed using a Teflon-lined cap. The contents in the glass bottle were vigorously shaken by hand, and the bottle was subsequently placed in a plastic safety container (wide-mouth 500 mL capacity high-density polyethylene). The safety bottle was closed and then placed at the center of the microwave plate. Extraction was performed for 30 s at a power level of 80% (~900 W). After extraction, the glass bottle was kept closed, cooled in a refrigerator for 5 min, and vigorously shaken by hand; then the extraction was repeated for another 30 s as described above. The contents of the bottle were allowed to cool to room temperature before opening. The supernatant extract was carefully removed with a 9-in. glass pipet; the solid residue in the glass bottle was rinsed with hexane (3 × 10 mL) and combined with the extract. The combined extract was evaporated to dryness under reduced pressure on a rotary flash evaporator in a round-bottom flask. The dried residue in the flask was dissolved in 20.0 mL of hexane and allowed to stand for ~30 min; 10.0 mL of supernatant was carefully removed with a glass pipet into a graduated glass centrifuge tube and centrifuged to separate any fine particles from the extraction solvent. An aliquot of the supernatant (2.5 mL) was transferred to a 5.0 mL conical bottom glass centrifuge tube and evaporated to 0.5 mL with a gentle stream of dry nitrogen gas.

Column Cleanup. The extracts in hexane obtained from each of the extractions described above were subjected to a minicolumn chromatography cleanup at room temperature. Activated silica gel (silica gel 150, 60−200 mesh, Mallinckrodt Silica, preheated to 135 °C and cooled to room temperature) was transferred to a 9-in. glass pipet plugged with glass wool at the bottom tip and topped with a 0.5 cm layer of anhydrous sodium sulfate. The column was washed initially with 10 mL of hexane. An aliquot of the extract was transferred on the top of the column and eluted with 10 mL of hexane followed by 10 mL of DCM. In preliminary experiments it was observed that fenitrothion was present only in the DCM eluate from silica gel, whereas no fenitrothion was found in hexane eluate. The hexane eluate was discarded. The DCM eluate was evaporated under a gentle stream of dry nitrogen gas, solvent exchanged with 2.0 mL of acetonitrile, and concentrated to ~0.2 mL. An aliquot of the solution was analyzed using EC-GC and HPLC. The final solution was diluted or concentrated when necessary so that the injected volume contained an amount of fenitrothion in the linear range of the EC-GC and HPLC detectors' response.

High-Performance Liquid Chromatography. The HPLC (Hewlett-Packard model 1100 binary pump and model 1100 UV detector) was equipped with the variable UV detector set to 254 nm. A stainless steel analytical column (25 cm × 9.4 mm) packed with Partisil 10 ODS-1 particle size 10 μm (Whatman) stationary phase was used at ambient temperature. A stainless steel guard column containing pelicular C18 (Whatman Inc., Clifton, NJ) preceded the analytical column. The mobile phase consisted of an acetonitrile/double-distilled water (1:1) solvent system containing 0.1% acetic acid. The flow rate was 1.0 mL/min. Injection of 20 μL was performed in HPLC quantitative analysis. A four-point calibration curve was constructed from the peak area of the calibration run. The HPLC detector response was linear ($r^2 = 0.9$) for fenitrothion in the range of 0.1−2.0 μg/mL. The concentration of fenitrothion in the extract was determined by comparing the peak area with that of a reference standard via calibration curve. Under the experimental conditions described the HPLC retention time for fenitrothion was 11.7 min.

Gas Chromatography. The gas chromatograph was a Hewlett-Packard model 5890A fitted with a ^{63}Ni electron capture detector operated in splitless mode. Residues of fenitrothion (injection volume = 2.0 μL) were determined by using an HP-5 capillary column (30 m × 0.25 mm i.d., 0.25 μm thickness). The column temperature was programmed from 100 to 200 °C at a rate of 5 °C/min. The inlet temperature was 100 °C, detector temperature was 270 °C, and the flow rate of helium carrier gas through the column was 30 mL/min with the head pressure of 20 psi. The makeup gas used was a P-5 mixture (5% methane in argon) with a flow rate of 30 mL/min. From the peak heights of the reference standards a four-point calibration curve was obtained, which was linear in the concentration range of 0.2−4.0 ng. Under the GC conditions described fenitrothion had a retention time of 9.7 min.

Identification. The identity of the desired peak was confirmed by comparing its retention time with that of the reference standard and by cochromatography. In the latter case an aliquot of the sample showing a peak at the retention time of fenitrothion was injected together with an aliquot of the reference standard. The increase in the peak height at the retention time of fenitrothion confirmed the identity of the compound. The concentration of fenitrothion in the extracts was determined by comparing the peak areas or peak heights with those of the reference standard.

RESULTS AND DISCUSSION

Column cleanup was needed to remove coextractives from the Soxhlet extracts of beans prior to EC-GC and HPLC and from MAE extracts prior to EC-GC analysis. The silica gel elution system used in this study efficiently isolated fenitrothion from the coextractives. **Figure 1** illustrates the GC chromatograms obtained by injecting the extracts from the fortified (1.9 μg/g) black bean samples following the cleanup using silica gel chromatography. No residues of fenitrothion were detected in the hexane eluates from the column (curves b and c). The unknown peaks in the DCM eluates did not interfere with fenitrothion peaks (curves d and e).

Figure 2 illustrates the HPLC chromatograms of the extracts from the control and fortified (1.9 μg/g) black beans.The extracts obtained by MAE did not require column cleanup prior to HPLC analysis. A few unknown peaks appeared in the HPLC chro-

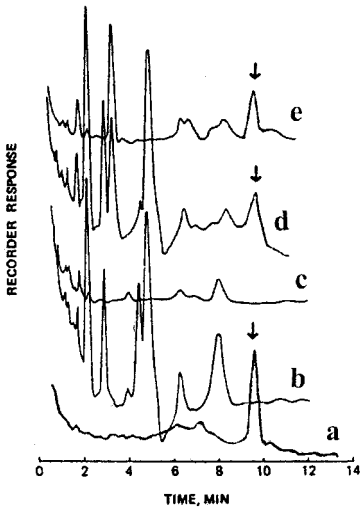

Figure 1. GC chromatograms of hexane and DCM eluates of silica gel chromatography following SE and MAE of fortified black beans: (a) fenitrothion; (b) SE of fortified beans, hexane eluate; (c) MAE of fortified beans, hexane eluate; (d) SE of fortified beans, DCM eluate; (e) MAE of fortified beans, DCM eluate.

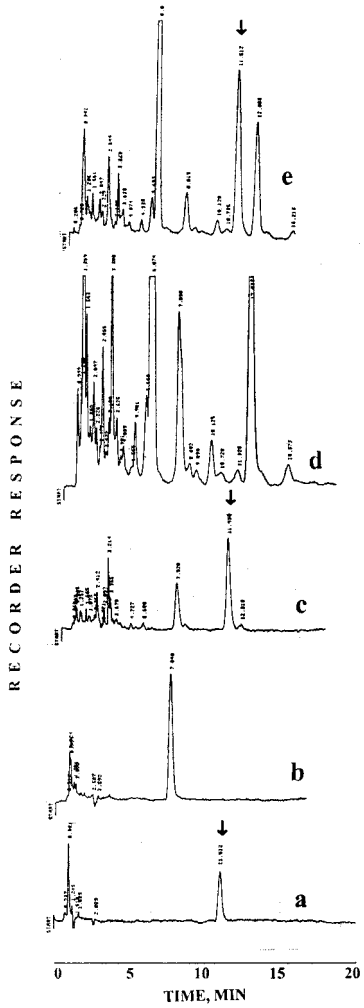

Figure 2. HPLC chromatograms of extracts from black beans following SE and MAE: (a) fenitrothion; (b) MAE of control beans; (c) MAE of fortified beans; (d) SE of fortified beans, hexane eluate; (e) SE of fortified beans, DCM eluate.

matograms due to coextractives, but they did not interfere with the fenitrothion peak (curves b and c). However, column cleanup was necessary for SE extracts obtained from black beans (curves d and e). The GC and HPLC chromatograms obtained from the SE and MAE extracts of white beans were very similar to those obtained for black beans as shown in **Figures 1** and **2**.

Table 1. Mean Recoveries (Percent) and Relative Standard Deviations (RSDs) of Fenitrothion from Fortified Beans after Soxhlet Extraction

fortification level (μg/g)	method of analysis			
	EC-GC		HPLC	
	white beans	black beans	white beans	black beans
0.19	91.0 (2.2)	84.3 (2.2)	91.3 (3.2)	88.2 (1.8)
0.94	91.0 (1.8)	82.4 (1.9)	91.1 (1.3)	88.1 (3.4)
1.90	89.5 (1.3)	86.7 (3.2)	93.9 (3.8)	83.6 (0.7)
overall mean	90.5 (1.8)	84.5 (2.5)	92.1 (2.8)	86.6 (2.0)

Table 2. Mean Recoveries (Percent) and Relative Standard Deviations (RSDs) of Fenitrothion from Fortified Beans after Microwave-Assisted Extraction

fortification level (μg/g)	method of analysis			
	EC-GC		HPLC	
	white beans	black beans	white beans	black beans
0.19	90.2 (1.8)	90.5 (3.3)	90.8 (2.0)	88.0 (0.9)
0.94	89.6 (2.1)	82.6 (1.5)	92.5 (2.6)	91.9 (2.3)
1.90	93.1 (0.2)	89.5 (2.5)	88.2 (1.8)	90.1 (1.4)
overall mean	91.0 (1.3)	87.5 (2.4)	90.5 (2.2)	90.0 (1.4)

Tables 1 and **2** summarize the recovery of fenitrothion from fortified beans by the two extraction techniques used under the experimental conditions described in this study. The average recoveries were >84% for the two methods of analysis employed, EC-GC and HPLC. The percent recoveries from the white beans were somewhat better than those from the black beans. It was observed that SE removed more coextractives from the beans (**Figures 1** and **2**). The results shown in **Tables 1** and **2** illustrate the effectiveness of MAE in obtaining recoveries of fenitrothion equivalent to the most commonly used SE method. When compared to SE, MAE of fenitrothion from niebe beans appears to be more convenient and faster and to use less solvent. From the results obtained in this study, MAE seems to be a viable alternative to SE of fenitrothionin from the black and white beans. It is obvious that microwave energy can cause selective migration of the target pesticide from the material to the surrounding solvent at a more rapid rate and with similar recoveries compared with conventional SE. The main advantages in using MAE, particularly in developing countries, are the low cost of the household microwave unit, short extraction time, and low use of solvent.

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