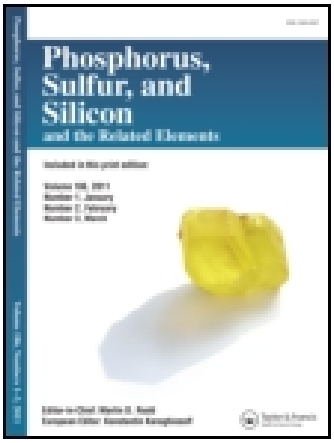

Phosphorus, Sulfur, and Silicon and the Related Elements

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Distribution and Elimination of ^{14}C-Ethion Insecticide in Chamomile Flowers and Oil

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DISTRIBUTION AND ELIMINATION OF ^{14}C-ETHION INSECTICIDE IN CHAMOMILE FLOWERS AND OIL

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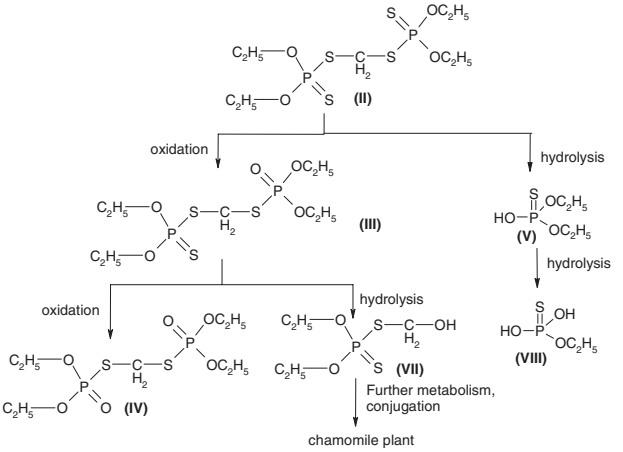
GRAPHICAL ABSTRACT



Abstract *The residual fate of ^{14}C-ethion labeled at the ethyl group in chamomile flowers and oil was studied. ^{14}C-ethion and some of its degradation products have been prepared for the present investigation. ^{14}C-residues in its flowers and oil were determined at different time intervals. Chromatographic analysis of chamomile oil extracts revealed the presence of the parent compound together with four metabolites, which were identified as ethion monooxon, ethion dioxon, O,O-diethyl phosphorothioate, and O-ethyl phosphorothioate. Effect of drying on ethion residues in chamomile flowers was investigated. Ethion residues were decreased by sun drying of chamomile flowers. The percentage of removal was 18%–47%. Effect of using low cost adsorbents for removal of ethion residues from chamomile oil was investigated. The removal percentage due to adsorption was 100% for CaO and saw dust adsorbers and for watermelon peels, bagass, and rice bran adsorbers were 36%, 41%, and 90%, respectively. Our results proved that the addition of CaO saw dust and rice bran reduced the pesticide residues, so we recommend the addition of these adsorbents during the extraction process to reduce the risk of pesticide residues on chamomile oil.*

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[Supplemental materials are available for this article. Go to the publisher's online edition of Phosphorus, Sulfur, and Silicon and the Related Elements *for the following free supplemental resource: Figure S1: ^{1}H NMR of Compound (IV)]*

Keywords Ethion; chamomile; removal; calcium oxide; rice bran

INTRODUCTION

Chamomile (*Matricaria chamomilla*) flower heads and extracts are used in the pharmaceutical industry as cosmetic repairs[1] for their antispasmodic, anti-inflammatory, and antimicrobial properties[2–4] and also due to natural hair dye and fragrance.[5] The organophosphorus pesticides most commonly used in the cultivation of essential oil plants travel through the vascular system of the plant and are absorbed at the cellular level.[6]

Ethion insecticide [*S*,*S*′-methylene bis (*O*,*O*′-diethyl phosphorodithioate)] is an organothiophosphate pesticide that was first registered for use in the United States in 1965.[7] This pesticide was first developed as a nonsystemic insecticide and acaricide for use on fruit trees, including citrus fruits, nut trees, cotton and seed, and forage crops as well as a wide variety of fruits and vegetables.[8] It is also used for control of aphids, spider mites, scale insects, thrips, lepidopterous larvae, leafhoppers, maggots, suckers, and soil-dwelling insects on a wide variety of food, fiber, and ornamental crops, including grapes, fruits, vegetables, and nuts.[9]

Information about the effect of drying on pesticide residues in herbs or other crops is limited in literature. Natural drying and hot air drying are still the most widely used methods to produce dried herb flakes because of their lower cost.[10]

Adsorption is one of the most efficient methods for removal of pollutants.[11] Activated carbon is a very efficient adsorbent for removing varieties of pesticides due to its high surface area and porosity.[12] However, because of the high cost of activated carbon, its use in the field is restricted on economical considerations.[13] Nowadays, low cost adsorbents have been investigated as an alternative to the high cost activated carbon, for example, fly ash,[13] carbon cloth,[14] porous polymeric adsorbents,[15] wheat residue black carbon,[16] bleaching earth,[17] lignin,[18] riverbed sand,[19] wood char coal,[20] and waste tire rubber granules.[21]

To the best of our knowledge, there are no studies on the fate and removal of ethion from chamomile oil. Therefore, this study attempted to investigate the residues of ethion on chamomile oil. In addition, a complete picture on the reduction of this compound in flowers and oil was also evaluated.

RESULTS AND DISCUSSION

Effect of Drying

Table 1 shows the effect of drying on ethion residues on chamomile flowers after different time intervals of the last application. The concentration of ^{14}C-ethion activity decreased during experimental period and reached 2.42 ppm after 30 days; the percentage of removal because of drying was 18.2%. Cabras et al.[22,23] found that drying process could cause an important decrease in pesticide residues in apricot and raisin processed from grapes. Juraske et al.[24] noted that the degradation of pesticides is not only due to drying but also due to other processes such as metabolism and photodegradation.

Table 1 Effect of drying on the residues of ^{14}C-ethion in chamomile oil after the last application

Sample	Time of treatment*			
	(7 day) ppm	(15 day) ppm	(21 day) ppm	(30 day) ppm
Flowers before drying	4.21 ± 0.10	4.33 ± 0.08	3.75 ± 0.06	2.96 ± 0.08
Flowers after drying	2.21 ± 0.03	3.13 ± 0.10	2.94 ± 0.06	2.42 ± 0.04
% removal due to drying	47.44	26.48	21.59	18.18

*Results are expressed as mean ± SD for three determinations for each sample.

Distribution of ^{14}C-ethion Residues in Chamomile Oil

The topical application of ^{14}C-ethion on chamomile plant led to the appearance of ^{14}C-activity in the oil (Table 2). ^{14}C-ethion residue in hexane and methanol extracts increased during the first 2 weeks and then decreased rapidly at the end of experimental period. The nonextractable residues (bound residue) show the same trend. The maximum binding of ethion residues was 54.1% after 30 days from the last application, and the recovery percentage ranged from 94% to 98%. Half-life ($T_{1/2}$) for degradation of ethion on chamomile flowers was found to be 3.4 days at recommended dosage. A waiting period of 15 days is suggested for safe consumption of the chamomile flowers and oil. The data obtained are in agreement with those reported by Singh et al., on their studies, the persistence of ethion residues on cucumber.[25]

Identification and Characterization of Metabolites in Chamomile Oil

Table 3 shows the R_f values and the average concentration of ^{14}C-ethion (parent compound and metabolites) in chamomile oil extract after 2 and 4 weeks of the second application, in addition to the parent compound; ethion monooxon (III), ethion dioxon (IV), *O,O*-diethyl phosphorothioate (V), and *O*-ethyl phosphorothioate (VII) were found as free metabolites besides *O,O*-diethyl-*S*-hydroxymethyl phosphorodithioate (VII) as a conjugated metabolite. The concentration of ^{14}C-ethion in chamomile oil and its metabolites was decreased at the end of experimental periods. On the contrary, compound (VIII) which was increased at the same period due to all degradation products are hydrolysis to the compound (VIII) at the end of experiment period.

The metabolic fate of ^{14}C-ethion labeled at the ethyl group was studied in chamomile oil and flowers after double application on the leaves. At 15 days and 30 days after the second application, the parent compound and its metabolites were found in chamomile oil. Our results demonstrated that ethion was metabolized to ethion monooxon (III) and ethion dioxon (IV) by oxidation and to *O,O*-diethyl phosphorothioate (V) and *O*-ethyl phosphorothioate (VIII) by hydrolysis in the plant. Hydrolysis of ethion monooxon leads to the formation of *O,O*-diethyl-*S*-hydroxymethyl phosphorodithioate (VII), which may be conjugated with sugar in plants (Figure 1). While the basic features of ethion metabolism are known, detailed information is lacking. Like other organothiophosphate insecticides (chlorpyrifos, parathion), ethion is converted via desulfuration in the liver by cytochrome P-450 enzymes to its active oxygen analog, ethion monooxon.[26] It is not known whether ethion monooxon can then be desulfurated to ethion dioxon. Ethion monooxon is a potent inhibitor of cholinesterases and exerts toxicity by reacting with an inhibiting neural acetylcholinesterase.[27] The breakdown of ethion and ethion monooxon has not been characterized

Table 2 Distribution of radioactive ^{14}C-ethion in chamomile oil after the last application of ^{14}C-ethion

Sample	Time of treatment*			
	(7 day) ppm	(15 day) ppm	(21 day) ppm	(30 day) ppm
Hexane extract (containing oil)	0.650 ± 0.010 (29.4%)	0.900 ± 0.040 (28.7%)	0.780 ± 0.030 (26.5%)	0.620 ± 0.020 (25.6%)
Methanol extract	0.393 ± 0.003 (17.7%)	0.578 ± 0.007 (18.5%)	0.530 ± 0.014 (18%)	0.360 ± 0.014 (14.8%)
Bound residue (cake)	1.134 ± 0.003 (51.3%)	1.510 ± 0.021 (48.2%)	1.460 ± 0.042 (49.6%)	1.310 ± 0.035 (54.1%)
Recovery%	98.4	95.4	94.1	94.5

*Results are expressed as mean ± SD for three determinations for each sample.

Table 3 Amount and R_f values of ^{14}C-ethion and its active degradation products in chamomile oil

Metabolites	R_f values in various solvent systems			(15 day) ppm	(30 day) ppm
	1	2	3		
Ethion (**II**)	0.75	0.80	0.75	0.370	0.219
Ethion monooxon (**III**)	0.88	0.92	0.65	0.121	0.101
Ethion dioxon (**IV**)	0.69	0.56	0.55	0.194	0.162
O,O-diethyl phosphorothioate (**V**)	0.33	0.23	0.42	0.159	0.048
O-ethyl phosphorothioate (**VIII**)	0.24	0.50	0.71	0.056	0.090

System 1: Toluene: Xylene 20: 20 (v/v).
System 2: Dioxane: Xylene: Petroleum ether 10: 20: 20 (v/v/v).
System 3: *n*-Hexane: Ethyl acetate 98: 2 (v/v).

but is presumed to be by esterases in the blood and liver.[28,29] Samples were extracted with ethyl acetate; the aqueous and organic phases were analyzed by high-performance liquid chromatography (HPLC). More than 99% of the urine radioactivity was in the aqueous phase. Another sample was acidified (presumably to hydrolyze conjugates) and also extracted with ethyl acetate. Acidification converted about 30% of the radioactivity in the aqueous phase to an organosoluble form, which may indicate that some of the products

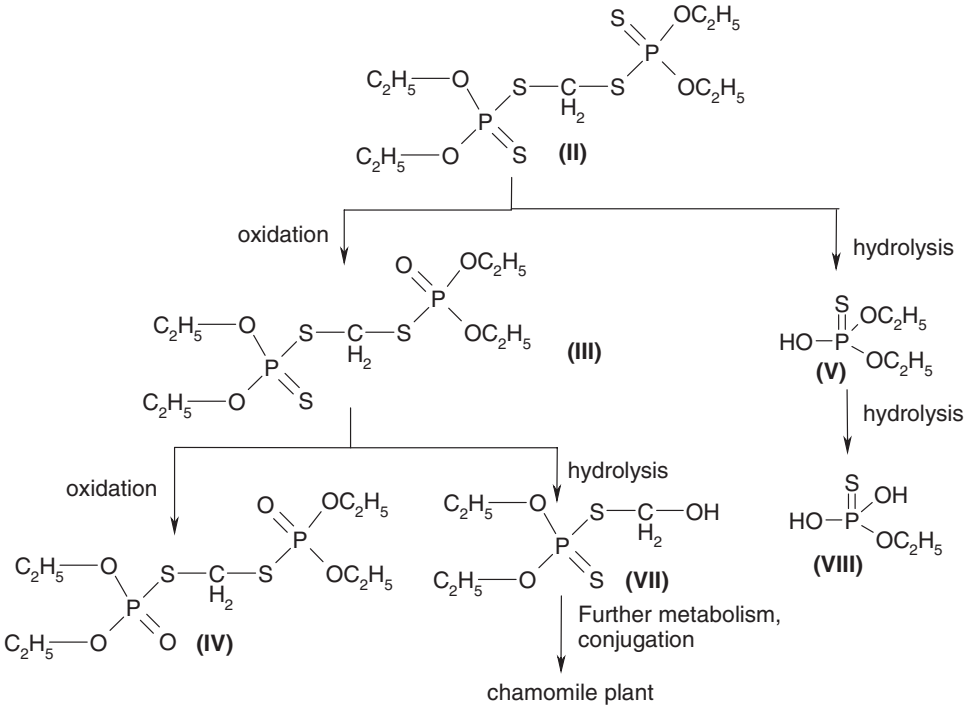

Figure 1 Metabolic pathway of ethion in chamomile oil.

Table 4 Removal of ^{14}C-activity from chamomile plant using foliar application of calcium chloride

	^{14}C-residues (ppm***)			
	Without $CaCl_2$		With 1.5% $CaCl_2$	
Sample	ppm	%*	ppm	%*
Flowers before drying	2.96 ± 0.08	0	1.74 ± 0.05	41
Flowers after drying	2.42 ± 0.04	0	1.55 ± 0.03	36
% removal**	18		11	
Oil	0.62 ± 0.02	0	0.36 ± 0.01	42

*% removal related to affect of adding $CaCl_2$.

**% removal due to drying.

***Results are expressed as mean ± SD for three determinations for each sample.

of ethion metabolism are present in urine as conjugates. Four to six radiolabeled metabolites were detected by HPLC; none of them migrated with standards for ethion, ethion monooxon, or ethion dioxon. None of the metabolites were specifically identified.[30–32]

Removal of ^{14}C-Activity from Chamomile Plant and Oil

Foliar Application of Calcium Chloride on Chamomile Plant. The radioactivity in the flowers and oil after application of ^{14}C-ethion and calcium chloride solution (1.5%) was presented after 30 days (Table 4). ^{14}C-ethion residues decreased from 2.96 to 1.74 ppm due to foliar application of 1.5% $CaCl_2$ solution on the fresh flowers and decreased from 2.42 to 1.55 ppm on the dried flowers. The percentage of removal due to sun drying process was 11%. ^{14}C-ethion residues in oil extracts after sun drying process decreased from 0.62 to 0.36 ppm due to $CaCl_2$ addition.

By Chemical and Low Cost Adsorbent Derived From Agricultural and Industrial Wastes

Table 5 represents the removal of ^{14}C-activity from chamomile oil by using chemical and low cost adsorbents derived from agricultural wastes (rice bran, watermelon peels, saw dust, bagass). The effect of using low cost adsorbents for removal of ethion residues from chamomile oil was investigated. The percentage removal due to adsorption was 100% for

Table 5 Removal of ^{14}C-activity from chamomile oil using chemical and low cost adsorbent derived from agricultural wastes

	^{14}C-residues (ppm) (Flowers after treatment with 1.5% $CaCl_2$)*					
Sample	Without adsorber	With CaO	With rice bran	With bagass	With watermelon peels	With saw dust
Total ^{14}C-activity	0.360 ± 0.035	0	0.036 ± 0.003	0.210 ± 0.042	0.230 ± 0.028	0
% removal	—	100	90	41	36	100

*Results are expressed as mean ± SD for three determinations for each sample.

0.4% CaO and 0.5% saw dust adsorber and 36%, 41%, and 90% for 0.5% watermelon peels, 0.5% bagass, and 0.5% rice bran adsorber, respectively. The results obtained are in parallel with the data obtained by several authors[33–37] on their studies about the effect of low cost adsorbents on removal of pesticide residues.

CONCLUSION

The results demonstrate that the level of pesticide residues in chamomile oil is lowered, sometimes far below the recommended levels by drying or using adsorbents. The degradation products in chamomile oil were identified as ethion monooxon, ethion dioxon, *O*-ethyl phosphorothioate, and *O,O*-diethyl phosphorothioate. Among various methods used for removal of pesticide from chamomile, it is suggested that factories of essential oils should dry chamomile flowers carefully before extractions. Also, the addition of CaO, saw dust, and rice bran reduced the pesticide residues, so we recommend the addition of these adsorbents during the extraction process to reduce the risk of pesticide residues on chamomile oil.

EXPERIMENTAL

Spectral Analysis

The ^{1}H NMR spectra were acquired using a Jeol-EX (270 MHz) spectrometer; chemical shifts δ in ppm with tetramethylsilane (TMS) as the internal standard; coupling constants J in Hz for samples dissolved in $CDCl_3$. The EI-MS (70 eV) spectra were recorded with Jeol JMS-AX500 mass spectrometer. The IR spectra were recorded in potassium bromide rolled (KBr) on Nexus 670 FTIR spectrometer (Nicolet), at the National Research Centre. Elemental analytical data (in accord with the calculated values) were obtained from the Microanalytical Unit, Cairo University, Giza, Egypt.

Chemicals

^{14}C-ethion was synthesized by the reported methods with some modification as follows.[38–40]

Synthesis of ^{14}C-Ethion (Endo) *S,S′*-Methylene Bis (*O,O′*-Diethyl Phosphorodithioate) (II)

Synthesis of O,O-Diethyl Hydrogen Phosphorodithioate (I). Anhydrous benzene (40 mL) was stirred with P_2S_5 (10 g) and temperature of the mixture was maintained around 40 °C. ^{14}C-ethanol (12 mL, Sp. Act 37 MBq) was added dropwise and stirring was continued for 3 h. The crude oil was purified on silica gel column using hexane: acetone (98:2) for elution to leave colorless oil of 68% yield. Thin layer chromatography (TLC) on silica gel plate showed one spot of rate of flow (R_f) (0.68) in solvent system *n*-hexane: ethyl acetate 98:2. IR (KBr) ν_{max}/cm^{-1}, 3400 (SH), 2851, 1580 (C—H aliphatic), 1022 (POC) and 722 (P=S); ^{1}H NMR ($CDCl_3$, 270 MHz), $\delta = 1.24$ (t, $J = 8.0$ Hz, 3H, $\underline{CH_3}CH$), 2.80 (q, $J = 8.5$ Hz, 2H, $-\underline{CH_2}CH_3$), and 6.51 (s, 1H, SH); EI-MS (m/z), 186 (M+, 35.3%), 158 (68.7%), and 130 (91.5%); Anal. calcd. (%) for $C_4H_{11}O_2PS_2$ (186.23): C, 25.80; H, 5.95; P, 16.63; S, 34.44; Found (%): C, 25.75; H, 5.91; P, 15.98; S, 34.47 (Figure 2).

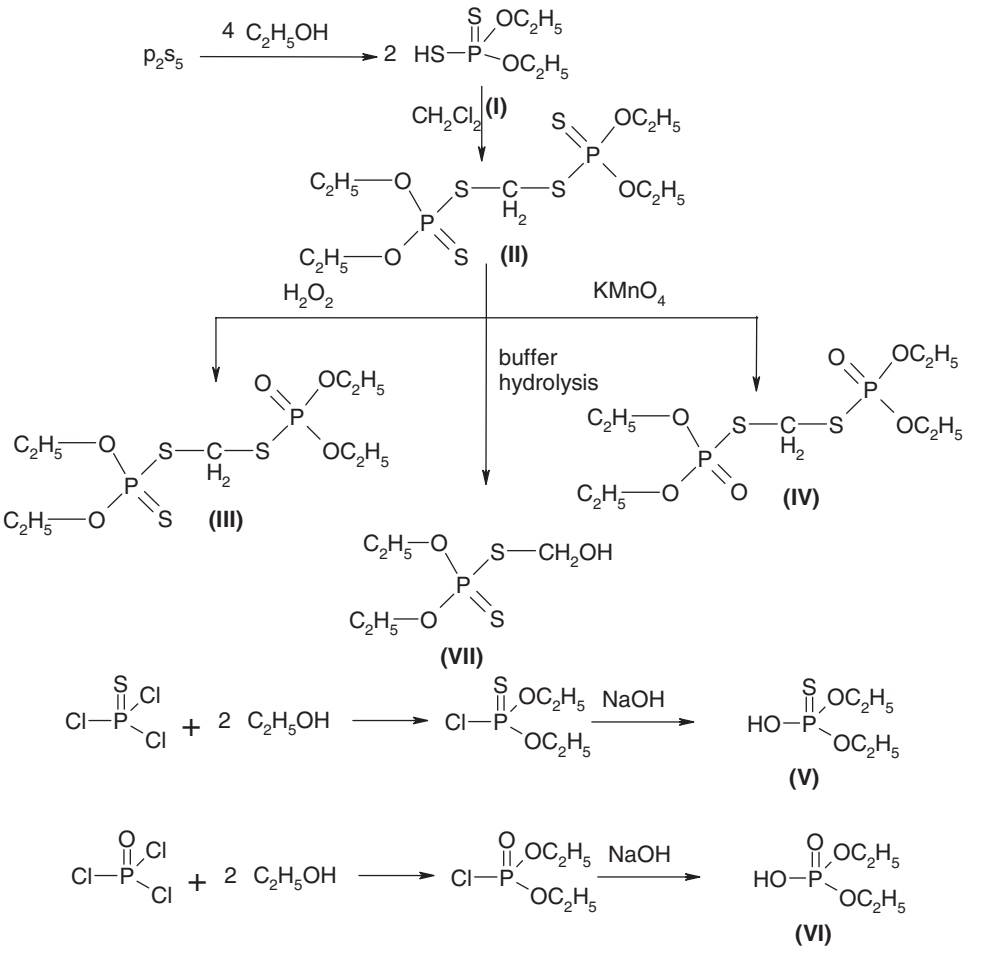

Figure 2 Preparation of ethion and its degradation products.

A solution of methylene chloride (0.05 moles, 3.5 mL) and triethylamine (0.1 mole, 13.5 mL) in dry benzene (20 mL) was added dropwise to (I). The reaction mixture was stirred at 25 °C for 20 min, and then refluxed for 3 h. It was filtered to remove triethylamine hydrochloride and evaporated under vacuum to remove the solvent. The crude oil (Sp. Act. 0.199 mci/g, 7.4 MBq/g, 68% yield) was purified on a silica gel column using hexane: acetone (98:2) for elution to leave colorless oil. TLC on silica gel plate showed one spot of R_f 0.75 in solvent system *n*-hexane: ethyl acetate 98:2. IR (KBr) ν_{max}/cm^{-1}, 2966, 1580 (C—H aliphatic), 1022 (POC), 720 (P=S) and 670 (C—S); ^{1}H NMR ($CDCl_3$, 270 MHz), $\delta = 1.24$ (t, $J = 8.0$ Hz, 3H, $\underline{CH_3}CH$), 2.80 (q, $J = 8.5$ Hz, 2H, $-\underline{CH_2}-CH_3$), and 7.30 (s, SCH_2S); EI-MS (m/z), 385 (M^+, 18.1%), 365 (9.1%), 231 (20.2%), and 153 (100%); Anal. calcd. (%) for $C_9H_{22}O_4P_2S_4$ (384.48): C, 28.12; H, 5.77; P, 16.11; S, 33.36; Found (%): C, 27.99; H, 5.90; P, 16.15; S, 33.41 (Figure 2).

Synthesis of Ethion Monooxon (III). Ethion (3.8 mL, 0.01 mole) was dissolved in acetic acid (20 mL). A solution of sulfuric acid (2 mL) in hydrogen peroxide (5 mL) (3.5 mole of 30%) was added dropwise maintaining the temperature between 10 °C and 20

°C. The reaction mixture was left to stir at ambient temperature for 13 h, and chloroform (200 mL) was added to extract with distilled water (300 mL). Subsequently, the solution was washed with 5% aqueous sodium hydrogen carbonate until neutral; then, it was dried over anhydrous sodium sulfate and filtered, and the solvent was then removed under vacuum. The crude oil was purified on silica gel column using hexane: acetone (98:2) for elution to leave colorless oil of 73% yield. TLC on silica gel plate showed one spot of R_f 0.65 in solvent system *n*-hexane: ethyl acetate 98:2. IR (KBr) ν_{max}/cm^{-1}, 2966, 1580 (C—H aliphatic), 1220 (P = O), 1022 (POC), 720 (P=S), and 690 (C—S); ^{1}H NMR ($CDCl_3$, 270 MHz), δ = 1.24 (t, J = 8.0 Hz, 3H, $\underline{CH_3}CH$), 2.80 (q, J = 8.5 Hz, 2H, $-\underline{CH_2}-CH_3$), and 7.30 (s, SCH_2S); EI-MS (m/z), 369 (M^+, 19.1%), 349 (10.3%), 215 (100%), and 137 (69.2%); Anal. calcd. (%) for $C_9H_{22}O_5P_2S_3$ (368.41): C, 29.34; H, 6.02; P, 16.81; S, 26.11; Found (%): C, 29.45; H, 5.97; P, 16.75; S, 26.31 (Figure 2).

Synthesis of Ethion Dioxon (IV). Ethion (3.85 mL, 0.01 mole) was mixed with a solution of potassium permanganate (50 mL, 4.2 g, 0.01 mole) and conc. sulfuric acid (3 mL) was dissolved in water (50 mL). The solution was added dropwise to the stirred ethion mixture while maintaining the temperature between 15 °C and 20 °C. The reaction mixture was filtered and the filtrate was treated with sodium bisulfate to remove the excess potassium permanganate. The mixture was then saturated with sodium sulfate and extracted with dichloromethane (100 mL). The obtained organic extracts were then dried over anhydrous sodium sulfate and filtered. The solvent was then removed under vacuum. The crude oil was purified on silica gel column using hexane: acetone (98:2) for elution to leave colorless oil of 88% yield. TLC on silica gel plate showed one spot of R_f 0.55 in *n*-hexane: ethyl acetate 98:2. IR (KBr) ν_{max}/cm^{-1}, 2966, 1580 (C—H aliphatic), 1240 (P=O), 1022 (POC), and 670 (C-S); ^{1}H NMR (270 MHz, $CDCl_3$), δ = 1.24 (t, J = 8.0 Hz, 3H, $\underline{CH_3}CH$), 2.80 (q, J = 8.5 Hz, 2H, $-\underline{CH_2}-CH_3$), and 7.30 (s, SCH_2S); EI-MS (m/z), 353 (M^+, 8.1%), 333 (29.6%), 199 (100%), and 121 (79.3%); Anal. calcd. (%) for $C_9H_{22}O_6P_2S_2$ (352.34): C, 30.68; H, 6. 29; P, 17.58; S, 18.20; Found (%): C, 30.70; H, 6.33; P, 17.65; S, 18.35 (Figure 2). The ^{1}H NMR of compound IV is contained in the Supplemental Materials (Figure S1).

Synthesis of O,O-Diethyl Phosphorothioate (V). A mixture of absolute ethanol (0.01 mole, 0.6 mL) and triethylamine (0.01 mole, 1.4 mL) in dry benzene was added dropwise to a cooled (5 °C–10 °C) solution of thiophosphoryl chloride (0.005 mole, 0.507 mL) in dry benzene during 15 min. The reaction mixture was stirred at 25 °C for 18 h and filtered; most of benzene was evaporated using rotary evaporator. The residues were dissolved in sodium hydroxide solution (100 mL, 0.1 N) after extraction with diethyl ether (100 mL), and the aqueous phase was acidified to pH 1 with a few drops of concentrated HCl and extracted again with ether (100 mL). The ether extract was dried over anhydrous sodium sulfate and evaporated to leave colorless oil. The crude oil was purified on silica gel column using hexane: acetone (98:2) for elution to leave colorless oil of 68% yield. TLC on silica gel plate showed one spot of R_f (0.42) in *n*-hexane: ethyl acetate 98:2. IR (KBr) ν_{max}/cm^{-1}, 3440 (OH), 2851 (C—H aliphatic), 1022 (POC), and 720 (P=S); ^{1}H NMR (270 MHz, $CDCl_3$), δ = 1.24 (t, J = 8.0 Hz, 3H, $\underline{CH_3}CH$), 7.40 (s, 1H, OH), and 2.80 (q, J = 8.5 Hz, 2H, $-\underline{CH_2}CH_3$); EI-MS (m/z), 170 (M^+, 17.8%), 142 (100%), and 114(54.6%); Anal. calcd. (%) for $C_4H_{11}O_3PS$ (170.17): C, 28.23; H, 6. 52; P, 18.20; S, 18.84; Found (%): C, 28.30; H, 6.60; P, 18.35; S, 18.70 (Figure 2).

Synthesis of O,O-Diethyl Phosphoric Acid (VI). A mixture of absolute ethanol (0.01 mole, 0.6 mL) and triethylamine (0.01 mole, 1.4 mL) in dry benzene was added dropwise to a cooled (5 °C–10 °C) solution of phosphoryl chloride (0.005 mole, 0.7 mL) in

dry benzene during 15 min. The reaction mixture was stirred at 25 °C for 18 h and filtered; most of benzene was evaporated using rotary evaporator. The residues were dissolved in sodium hydroxide solution (100 mL, 0.1 N) after extraction with diethyl ether (100 mL), and the aqueous phase was acidified to pH 1 with a few drops of concentrated HCl and extracted again with ether (100 mL). The ether extract was dried over anhydrous sodium sulfate and evaporated to leave colorless oil. The crude oil was purified on a silica gel column using hexane: acetone (98: 2) for elution to leave colorless oil of 68% yield. TLC on silica gel plate showed one spot of R_f (0.81) in *n*-hexane: ethyl acetate 98:2. IR (KBr) ν_{max}/cm^{-1}, 3440 (OH), 2851 (CH aliphatic), 1220 (P═O), and 1022 (POC); ^{1}H NMR (270 MHz, $CDCl_3$), $\delta = 1.24$ (t, $J = 8.0$ Hz, 3H, $\underline{CH_3}CH_2$), 2.80 (q, $J = 8.5$ Hz, 2H, $-\underline{CH_2}CH_3$), and 7.10 (s, 1H, OH); EI-MS (m/z), 154 (M^+, 29.4%), 126 (79.1%), and 98 (100%); Anal. calcd. (%) for $C_4H_{11}O_4P$ (154.10): C, 31.18; H, 7. 19; P, 20.10; Found (%): C, 30.97; H, 7.30; P, 20.23 (Figure 2).

Synthesis of O,O-Diethyl S-Hydroxymethyl Phosphorodithioate (VII). A mixture of ethion insecticide (0.5 mL) and acetone (5 mL) was added to 10 mL of pH (5.5) buffer solution and placed in a covered circulating water bath at 30 °C for 3 h. The mixture was extracted with benzene-hexane (50:50 v/v) (50 mL). The extracted layer was dried over anhydrous sodium sulfate and evaporated to leave colorless oil. The crude oil was purified on silica gel column using hexane: acetone (98:2) for elution to leave colorless oil of 68% yield. TLC on silica gel plate showed one spot of R_f (0.85) in *n*-hexane: ethyl acetate 98:2. IR (KBr) ν_{max}/cm^{-1}, 3440 (OH), 2860 (CH aliphatic), 1022 (POC), and 722 (P═S); ^{1}H NMR (270 MHz, $CDCl_3$), $\delta = 1.24$ (t, $J = 8.0$ Hz, 3H, $\underline{CH_3}CH_2$), 2.40 (q, $J = 8.5$ Hz, 2H, $\underline{CH_2}CH_3$), 7.10 (s, 1H, SCH_2O), and 7.60 (s, 1H, OH); EI-MS (m/z), 216 (M^+, 18.6%), 188 (80.1%), 186 (65.7%), and 160 (88.3%); Anal. calcd. (%) for $C_5H_{13}O_3PS_2$ (216.26): C, 27.77; H, 6. 06; P, 14.32; S, 29.65; Found (%): C, 28.01; H, 6.08; P, 14.25; S, 29.73 (Figure 2).

Plant Material and Experimental Design

Chamomile plants were cultivated in an isolated and controlled area at the farm of the National Research Center located in Dokki, Cairo, Egypt. Plants were irrigated and fertilized as in practice; seeds were planted first in small containers, 1/8 inch deep. When the plants were big enough to handle, they were transplanted to soil including three replicates for each treatments. The flowers were harvested on a clear morning before the sun has drawn the valuable scent from the blossoms. Using scissors, opened heads were carefully picked and spread on paper in a cool, dry, airy place. After the heads became papery, they were stored in an airtight jar. At the time of blooming, the plants were divided into two parts; the first part was treated with insecticide and $CaCl_2$ solution (1.5%), while the second part was only treated with ^{14}C-ethion (4 mg/plant each time) on leaves of plants. ^{14}C-ethion was topically applied manually with a micropipette on healthy leaves of plants (twice, 15 days apart). Chamomile flowers were collected at, 7, 15, 21, and 30 days after the second spray of labeled ethion. The total radioactivity of flowers (before and after drying) was determined by digestion followed by liquid scintillation counting (LSC).

Extraction of Chamomile Flowers

Chamomile oil was extracted from its dried flowers by using a Soxhlet apparatus for 12 h with *n*-hexane. After evaporation of hexane under reduced pressure, radioactivity

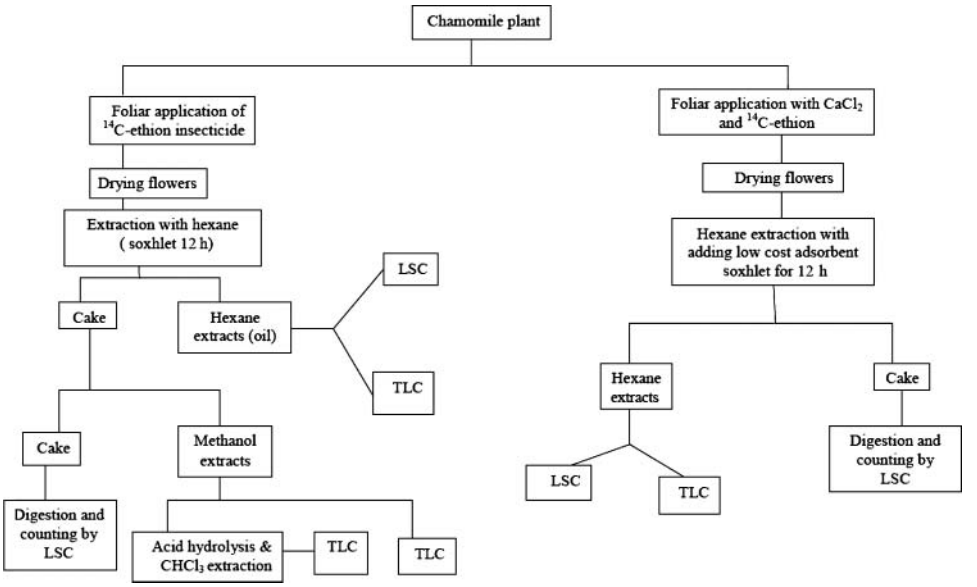

Figure 3 Diagram for removal of ^{14}C-ethion insecticide from chamomile plant and oil.

in the chamomile oil was measured. The residue remaining after extraction was further extracted with methanol (Figure 3). Aliquots of the hexane extract (oil) and the methanol extract were used for determination of radioactivity. The remaining cake was air dried and digested by using 1 mL Solusol (tissue and gel solubilizer), 1 mL 30% H_2O_2, and 70 μL glacial acetic at 40 °C–50 °C. The radioactivity was counted using LSC.

Adsorbent Development

Chamomile flowers were treated with low cost adsorbents, i.e., rice bran, sawdust, baggass, and watermelon peels. To improve the sorption capacity of materials, the chemical treatment was given by washing with deionized water to remove water-soluble compounds from the surface of materials and to develop its porous structure. The baggass was treated with hydrogen peroxide at 60 °C for 24 h to remove the adhering matter. Rice bran, rice husk, sawdust, and watermelon peels were then reacted with 0.1 M nitric acid for 1 h followed by soaking in methanol for 4 h to remove inorganic and organic matter from the surface of the materials and dried in an electric oven at 110 °C for 1 h, powdered, ground, and sieved to desired particle size before use. The treated materials were then stored in desiccators to be used as sorbents. The radioactivity in cakes after oil extraction was determined by digestion followed by LSC.

Isolation and Characterization of ^{14}C-Residues

An analysis of the radioactive compounds was achieved by TLC. Samples of crude oil after 2 and 4 weeks of the second application were partitioned between acetonitrile and hexane three times to remove the oil. The radioactive residues were distributed between the two layers. Analysis of oil extracts was achieved by TLC on silica gel plates (Merck-silica gel 60 F_{254}). The solvent systems used were:

- System 1: Toluene: Xylene 20:20 (v/v)
- System 2: Dioxane: Xylene: Petroleum ether 10:20:20 (v/v/v)
- System 3: *n*-Hexane: Ethyl acetate 98:2 (v/v)

For methanol extracts, the free metabolites were detected by TLC. The conjugated metabolites were liberated by heating the mixture with 2N HCl for 2 h at 100 °C. After cooling, the mixture was extracted with chloroform for three times, and the combined chloroform was dried over anhydrous sodium sulfate, filtered off, evaporated under vacuum, and identified by TLC analysis using the solvent systems used for identification of crude oil.

Authentic samples were run alongside as references and spots were detected by exposing to a UV source at 254 nm and made visible by subjecting the plates to I_2 vapor after a preliminary spray with $PdCl_2$ solution.

Radioactivity Measurements

The radioactivity in the oil and methanol extracts was measured directly by LSC (Packard Model TRI-CARB 2300 TR) in vials using a dioxane-based scintillation cocktail, which was composed of dioxane (1 L), naphthalene (100 g), PPO—2,5-diphenyloxazole (10 g), and POPOP—1,4-di[2-(5-phenyloxazolyl)]-benzene (0.25 g). Radioactivity in flowers or cakes (100 mg) was determined by digestion using H_2O_2 and Solusol followed by LSC. Silica gel plates were separately scraped (1-cm zones) and silica gel was eluted with methanol followed by LSC. The counts were corrected for background and quenching effect by the channel ratio method.

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