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Residues of Rotenone and Rotenolone on Lettuce and Tomato Fruit after Treatment in the Field with Rotenone Formulations

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Rotenone and 6aβ,12aβ-rotenolone levels were studied as a function of time on lettuce and tomato crops treated with dust or wettable powder formulations, according to label instructions. On both crops, the dust resulted in higher levels of residue. On lettuce, the half-life of rotenone applied as a dust or wettable powder was 2.9 or 3.6 days, respectively. The corresponding half-life of rotenolone was 4.5 or 5.0 days, respectively. Fourteen days after treatment 0.2–0.3 ppm of rotenone persisted, while 0.2 ppm of rotenolone was found after 9 days. The half-life of rotenone on tomato fruit was 2.7 days for the dust and 0.9 days for wettable powder formulations. Six days after treatment with dust, 0.2 ppm of rotenone was found on tomatoes, while 0.06 ppm of rotenolone was present after 2 days. Both rotenone and rotenolone were stable to boiling in tomato homogenate.

Rotenone [1,2,12,12a-tetrahydro-8,9-dimethoxy-2-(1-methylethenyl)[1]benzopyrano[3,4-*b*]furo[2,3-*h*][1]benzopyran-6(6*H*)-one] is a naturally occurring insecticide registered for use on a variety of food crops on a negligible residue basis. It is unstable in the presence of light and air, and when irradiated on leaf surfaces in the laboratory, it has been shown to degrade to a number of compounds (Cheng et al., 1972). Among the decomposition products, 6aβ,12aβ-rotenolone has been identified as the major compound formed after 4 h of exposure (Cheng et al., 1972).

In reviewing the literature of rotenone, Haley (1978) noted that rotenone has been found tumorigenic in rats but not in mice and that further studies are required to define the toxicity of rotenone and its decomposition products. Because of the potential hazard arising from agricultural use, the present study was initiated to provide residue data on rotenone and rotenolone on food crops obtained by liquid chromatographic methods. Tomato and lettuce crops were selected for study to represent two extremes with respect to surface area and thus amount of residue expected to be present. Further, two commercially available formulations were compared in terms of initial residue and persistence.

EXPERIMENTAL SECTION

Crops. Lettuce (Grand Rapids variety) and tomatoes (Ottawa 78 variety) were grown in field plots at the Ottawa Research Station, Agriculture Canada, during the summer of 1979. Lettuce was seeded in three plots, each containing two 25-ft rows. One plot served as a control, while the others were treated with dust or wettable powder formulations of rotenone. Similarly, tomatoes were grown in three plots (one control and two treatment plots), each consisting of two 30-ft rows containing 10 plants each.

Rotenone Application. The two formulations of rotenone studied were a commercially available dust containing 1% rotenone as active ingredient and a wettable powder containing 5% rotenone. Each formulation was applied at a rate as indicated by the label instructions. The dust was applied with a hand duster at a rate of 57 g/50 row ft. The wettable powder (7.8 g) was suspended in water (2 L) and applied with a Chapin no. 35 hand sprayer at a rate of 2 L/50 row ft. Both lettuce and tomatoes received three treatments of each formulation at intervals of 7 days.

Sampling. Each plot was divided into four areas and a sample was removed from each at various time intervals. Sampling was commenced as soon as the final spray treatment appeared dry on the plant. Upon receipt at the laboratory, lettuce plants were trimmed to remove roots and the leaves were rinsed with water to remove adhering soil. The leaves from several plants were homogenized in a Waring Blendor, and a sample of the homogenate was extracted immediately. Tomato fruit was not rinsed but was quartered, and representative quarters were combined and homogenized. Samples of homogenate were extracted immediately.

Analytical Method. *Materials.* Rotenone (97%) was obtained from Aldrich Chemical Co. (Canada) Ltd., Montreal, Quebec, and was used without further treatment.

Rotenolone was synthesized by aeration of an alkaline suspension of rotenone as described by Crombie and Godin

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(1961) for the preparation of isorotenolone from isorotenone. The crystals obtained by refrigeration of the reaction mixture were crystallized from methanol, and the recrystallized material was purified by column chromatography on silicic acid (50 g), using 1% isopropyl alcohol in dichloromethane as eluting solvent. The first 150 mL was discarded and the next 250 mL was collected and evaporated to dryness. The 6aβ,12aβ isomer was isolated by preparative high-pressure liquid chromatography on a 10 × 25 cm column of LiChrosorb Si 60, using 1% isopropyl alcohol plus 8% dichloromethane in hexane as mobile phase at a flow rate of 10 mL/min. This isomer (mp 89–90 °C) had a retention time identical with that of the isomer obtained on photodegradation of rotenone on glass plates by UV irradiation (Cheng et al., 1972).

Silicic acid for adsorption chromatography (Woelm, 100–200 μm) was purchased from ICN Pharmaceuticals Inc., Cleveland, OH, and was washed with dichloromethane and methanol. It was activated by heating overnight at 130 °C.

Extraction. Samples of homogenate (5 g) were extracted with methanol (45 mL) containing oxalic acid (1 mg/mL). Oxalic acid was included to stabilize rotenone in the extract (Cahn et al., 1945). Extracts were filtered through Whatman No. 1 paper on a Buchner funnel, using gentle vacuum, and the filtrate was made to 50 mL in a volumetric flask. An aliquot (20 mL) of the extract was added to 0.25 N NaCl (50 mL) in a 125-mL separatory funnel. Rotenone and rotenolone were extracted by shaking first with 15 mL and then 10 mL of dichloromethane. The dichloromethane extracts were combined in a 50-mL round-bottomed flask and evaporated to dryness on a rotary evaporator. The residue was dissolved in dichloromethane (2 mL) and transferred to a silicic acid column for cleanup.

Column Chromatography. Cleanup was effected on a bed of silicic acid (2 g) prepared in dichloromethane in a 200 × 7 mm i.d. chromatographic tube (Chromaflex size 22, Kontes Glass Co., Vineland, NJ). After adsorption of the sample, the column was washed with dichloromethane (20 mL) and the eluate was discarded. Rotenone and approximately 50% of the rotenolone were eluted with 10% acetone in dichloromethane (10 mL). The remainder of the rotenolone was collected in a second fraction by elution with a second portion of 10% acetone in dichloromethane, followed by 30% acetone in dichloromethane (10 mL). Each fraction was evaporated to dryness on a rotary evaporator. The residue from each fraction was dissolved in methanol (0.5 mL) and water was added (0.5 mL). The resulting mixture which contained some insoluble pigment was filtered through a plug of glass wool in a Pasteur pipet and subjected to high pressure liquid chromatography.

High-Pressure Liquid Chromatography (LC). Samples were introduced with a Valco injector fitted with a 100-μL loop. Separations were performed at ambient temperature on a 3.2 × 250 mm column containing 10 μm of LiChrosorb C_{18}. Mobile phase (30% water in methanol) was supplied to the column by an Altex Model 110 pump at a flow rate of 0.8 mL/min. Under these conditions, the retention times of rotenolone and rotenone were 5.5 and 7 min, respectively. Sample components were detected with a Waters Model 440 absorbance detector containing a 280-nm filter and coupled to a 1-mV Honeywell recorder. Standards containing 0.2 μg/mL each of rotenone and rotenolone were run and used to quantitate the samples by comparison of peak heights. Samples were diluted to contain not more than 0.2 μg/mL before analysis.

Table I. Recovery[a] of Rotenone and Rotenolone from Fortified Lettuce and Tomato Homogenate

fortif level, ppm	recovery, %			
	rotenolone		rotenone	
	lettuce	tomato	lettuce	tomato
0.025		55		67
0.050		84		106
0.10	93	76	97	90
0.50	87	87	101	96
1.0	82	88	98	103
10.0	90		98	

[a] Recoveries are the means of duplicate determinations.

Table II. Residues[a] of Rotenone and Rotenolone on Lettuce at Various Intervals after Treatment with Rotenone Dust (DU) or Wettable Powder (WP) Formulations

time after treatment, days	residue found, ppm			
	rotenone		rotenolone	
	DU	WP	DU	WP
0	12.1 ± 1.1	4.2 ± 0.4	0.74 ± 0.06	0.45 ± 0.04
1	8.2 ± 0.7	3.5 ± 0.3	0.79 ± 0.07	0.48 ± 0.02
2	6.3 ± 1.4	3.1 ± 0.3	0.72 ± 0.17	0.40 ± 0.03
3	3.0 ± 0.6	2.2 ± 0.2	0.33 ± 0.06	0.29 ± 0.04
4	2.5 ± 0.4	1.7 ± 0.1	0.33 ± 0.04	0.25 ± 0.02
7	1.7 ± 0.2	1.1 ± 0.1	0.25 ± 0.02	0.13 ± 0.01
9	1.7 ± 0.3	1.3 ± 0.5	0.22 ± 0.04	0.18 ± 0.06
14	0.31 ± 0.07	0.21 ± 0.04	Tr[b]	Tr
control	0		Tr	

[a] Values are the means of determinations on four samples ± SE. [b] Trace.

RESULTS AND DISCUSSION

The approach used for the analysis of rotenone and rotenolone is similar to that described by Bowman et al. (1978) for the analysis of these compounds in animal chow and tissues. Methanol and dichloromethane were both examined as extracting solvents, but the latter was rejected because it resulted in interfering and late running peaks on the LC. The present method afforded acceptable recoveries, as shown by the data in Table I. For tomato, recoveries of both compounds at 0.05 ppm were greater than 80% but were reduced to 55–67% at 0.025 ppm. Thus, 0.05 ppm was considered to be the lower limit for quantitation. Measurable peaks below 0.05 ppm are indicated as being trace amounts. Values indicated as being zero are a result of there being no measurable peaks at the retention time of rotenone or rotenolone.

From the data presented in Table II, it is evident that the rotenone and rotenolone residues resulting from application of the dust are initially higher than those from the wettable powder but decline more rapidly and at 3 days are similar to the values for the wettable powder. For both treatments, measurable levels of rotenone are present 14 days after application. Rotenolone declined to trace levels by 14 days. The half-lives of rotenone, calculated from the first-order rate equation, were 2.9 and 3.6 days for dust and wettable powder formulations, respectively. The values for rotenolone were calculated to be 4.5 days for dust and 5.0 days for wettable powder. Stability studies on animal chow fortified with rotenone have shown rotenolone

Table III. Residues[a] of Rotenone and Rotenolone on Tomato Fruit at Various Intervals after Treatment with Rotenone Dust (DU) or Wettable Powder (WP) Formulations

time after treatment, days	residue found, ppm			
	rotenone		rotenolone	
	DU	WP	DU	WP
0	0.82 ± 0.05	0.53 ± 0.13	0.05 ± 0	0.05 ± 0.01
1	0.70 ± 0.03	0.25 ± 0.02	0.06 ± 0.01	Tr
2	0.79 ± 0.37	0.09 ± 0.02	0.06 ± 0.03	0
3	0.22 ± 0.04	0.06 ± 0.01	Tr	0
6	0.21 ± 0.03	Tr[b]	Tr	0
control	0		0	

[a] Values are the means of determinations on four samples ± SE. [b] Trace.

Table IV. Effect of Boiling on the Stability of 5.0 ppm Rotenone and 5.0 ppm Rotenolone in Tomato Homogenate

boiling time, min	residue found,[a] ppm	
	rotenone	rotenolone
5	4.3	4.6
10	3.7	3.7
20	3.9	3.9
40	4.0	4.0

[a] Values are the means of duplicate determinations.

to form and accumulate as a result of storage (Bowman et al., 1978). However, when exposed to light on glass plates in the laboratory, rotenolone (especially the trans isomers) appears to form and then degrade relatively rapidly (Cheng et al., 1972). The results of the present study in the field are consistent with the latter finding.

The data of Table III show that compared to lettuce, tomatoes contained much lower levels of both rotenone and rotenolone, as would be expected from the lower surface area. As with lettuce, the dust preparation resulted in higher residue levels. Significant levels of rotenone from the dust preparation remained after 6 days, while with the wettable powder only trace amounts remained at this time. The half-life of rotenone was calculated to be 2.7 days for dust and 0.9 days for wettable powder formulations.

The stability of rotenone and rotenolone to the effects of cooking was examined by boiling under reflux tomato homogenate fortified with 5.0 ppm of each for several time intervals. After each interval, the mixture was cooled, and samples (5 g) were taken for analysis. As shown by the data in Table IV, little if any decomposition of either compound occurred within 40 min.

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Precocene II Metabolism in Insects: Synthesis of Potential Metabolites and Identification of Initial in Vitro Biotransformation Products

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Syntheses are described for the following derivatives of the insect antijuvenile hormone precocene II (6,7-dimethoxy-2,2-dimethyl-2*H*-benzo[*b*]pyran), all of which are possible metabolites in insects: 6-hydroxy, 7-hydroxy, 3,4-dihydro-3-hydroxy, *cis*- and *trans*-3,4-dihydro-3,4-dihydroxy, 2-hydroxymethyl, 3,4-dihydro-4-hydroxy, and 3,4-epoxy. Of these, the first five are identified by cochromatography as metabolites of [^{14}C]precocene II formed in vitro on incubation with NADPH-dependent monooxygenases in fat body homogenates of cabbage looper (*Trichoplusia ni* Hübner) larvae. The 3,4-epoxide is inferred as a metabolic intermediate from the presence of the isomeric dihydrodiols, but could not be isolated directly. The extreme reactivity of the synthesized epoxide suggests this compound as a possible bioactivation product involved in the allatotoxic action of precocene II. One metabolite, which is apparently nonphenolic, has properties different from the synthesized standards and remains to be identified.

Precocene II (6,7-dimethoxy-2,2-dimethyl-2*H*-benzo-[*b*]pyran, 1) is selectively cytotoxic to the secretory cells of the insect corpus allatum (CA), thereby eliminating the production of juvenile hormone (Bowers and Martinez-Pardo, 1977; Unnithan et al., 1977; Pener et al., 1978; Schooneveld, 1979). There is considerable specificity in the action of 1 and related compounds. Thus, the interruption of normal nymphal development to produce precocious adults and the sterilization of adult females has been reported in several hemimetabolous species, whereas in holometabolous species only a few cases of sterilization, but no induction of precocious metamorphosis, have been reported (Bowers, 1976; Bowers et al., 1976; Pener et al., 1978). Even in generally sensitive groups, large differences

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