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Rotenone and Rotenoids in Cubè Resins, Formulations, and Residues on Olives

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Rotenone and rotenoids (deguelin, β-rotenolone (12aβ-hydroxyrotenone), tephrosin (12aβ-hydroxydeguelin), 12aα-hydroxyrotenone, and dehydrorotenone) were determined in cubè resins and formulations. Cubè resins from *Lonchocarpus* contain large quantities of deguelin (ca. 21.2%) and smaller quantities of tephrosin (ca. 3.5%) and β-rotenolone (ca. 3.0%). The composition of commercial formulations may present very different rotenoid contents depending on the extracts used to prepare them. Because these rotenoids also present insecticide activity, the efficacy of these formulations may be very different. The storage stability and photodegradation of some rotenone formulations were studied. Rotenone and rotenoids are very sensitive to solar radiation, which degrades them rapidly, with half-lives in the order of a few tens of minutes. Some formulations show greater disappearance rates than that of cubè resin, indicating that not much attention has been paid to protecting the active ingredients from photodegradation in the formulation. A study on the residues on olives was also carried out to assess not only the rotenone content, but also that of the main rotenoids. At harvest, the residues of deguelin, tephrosin, and β−rotenolone were 0.10, 0.06, and 0.10 mg/kg, respectively, very similar to rotenone (0.08 mg/kg), and though a few data indicate similar acute toxicity values for deguelin, only rotenone is taken into consideration in the legal determination of the residue.

KEYWORDS: Rotenone; deguelin; tephrosin; cubè resin; residues; olive

INTRODUCTION

Organic agriculture is practiced in about 111 countries in the world. Currently almost 23 million hectares are managed organically with 11.6 million hectares (mio ha) in Australia/Oceania, 5.1 mio ha in Europe, 4.7 mio ha in Latin America, 1.5 mio ha in North America, 0.6 mio ha in Asia, and 0.2 mio ha in Africa. Land area under organic management as a percentage of the total agricultural area is highest in Europe. In Italy, almost 8% (1.23 mio ha) of agricultural land is organic, while in Australia only 2.3% (11.5 mio ha) is organic. Organic agriculture is continuously on the increase, and an annual growth of 5−11% in Europe and of 15−20% in USA on the world markets for organic food and beverages is expected in 2003−2005 (*1*). In Europe, organic production is governed by EU regulation no. 2092/91. According to this regulation, only natural pesticides and not synthetic ones may be used in pest control. The list of allowed biopesticides is limited. It includes the botanical insecticides, which play a very important role. In Italy, the following extracts have been registered: *Azadirachta indica* (a.i., azadirachtin), *Derris elliptica, Lonchocarpus nicou, Tephrosia vogelii* (a.i., rotenone), and *Chrysanthemum cinerariaefolium* (a.i., pyrethrins). The use of these extracts has not always proved effective. The main causes of this erratic efficacy could be ascribed to formulation. The formulations are generally marketed by small firms that purchase extracts and formulate them in the same way as traditional pesticides but without taking into account that because these extracts are easily photodegradable they do not last long. These firms do not usually carry out stability studies on formulations, either because they do not have research facilities or for their cost. These issues are just starting to be investigated in the case of azadirachtin (*2*−*4*) and in particular in the study of compounds that can act as sun filters to reduce the degradative effect of solar radiation and increase their own persistence and efficacy (*5*−*9*). Studies on the photodegradative stability and shelf life of formulations based on rotenone are still scanty (*10*−*12*). Rotenone is extracted with trichloroethylene from the roots of some *Luguminosae* (*Derris. Tephrosia* and *Lonchocharpus*) to obtain cubé resin. This resin is used to prepare rotenone-based insecticide formulations. From the composition of cubè resin obtained from the roots of *Lonchocarpus utilis* and *urucu* from Peru, it has been shown that the major ingredients are four rotenoids: rotenone (44.0%), deguelin (22.0%), rotenolone (12aβ-hydroxyrotenone) (6.7%), and tephrosin (12aβ-hydroxydeguelin) (4.3%) (*13*). Another 25 minor rotenoids have been isolated and identified, but they were probably decomposition products of resin processing (*14*). Anticancer activity by cubè resin is reported in rats and mice, and most of it is probably due to the four major rotenoids (*13*).

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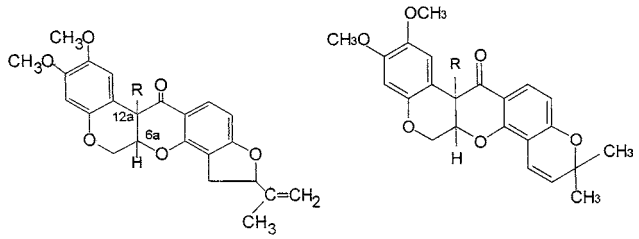

Rotenone (R=H)
α-rotenolone (R=OH)
β-rotenolone (R=OH)
dehydrorotenone (-R; -H; = between 6a and 12a)

deguelin (R=H)
tephrosin (R=OH)

Figure 1. Structure of rotenone and rotenoids.

Tephrosin has been shown to be active against tumors including skin cancer (*15*, *16*). Insecticide activity is also reported for deguelin, tephrosin, and rotenolone (*17*−*22*).

The acute toxicity of rotenoids to insects and mammals is attributable to the inhibition of NADH−ubiquinone oxidoreductase activity as the primary mechanism of activity. Rotenone and deguelin show similar activity and are more potent than their derivatives (*13*, *22*). Though the deguelin content in cubè resins is almost half that of rotenone, and though a few data indicate similar acute toxicity values for deguelin (*17*), only rotenone is taken into consideration in the legal determination of the residue.

This study is meant to be a contribution to the knowledge of the technical grade material, storage stability, and photodegradation of some rotenone formulations. We also carried out a study on the residues on olives to assess not only the rotenone content, but also that of the main rotenoids (deguelin, rotenolone, and tephrosin) and of another three minor rotenoids (12aα-hydroxyrotenone, 12 a*β*-hydroxyrotenone, and dehydrorotenone), which were possible to determine with the analytical method used (**Figure 1**).

MATERIALS AND METHODS

Chemicals. Rotenone was an analytical standard purchased from Sigma (purity 95−98%) (Chicago, IL). Metabolites 12aα-rotenolone (α-rotenolone) and dehydrorotenone were standards kindly donated by Prof. Casida (Berkeley University, Berkeley, CA). 12a*β*-Rotenolone (*β*-rotenolone), which is not available as a commercial standard, was obtained from rotenone by synthesis according to Crombie and Godin (*23*). Deguelin and tephrosin were isolated from cubè resin (*13*). Purity and control of the active ingredients were carried out in LC-MS.

Acetonitrile was HPLC grade, acetone and chloroform were solvents for analysis (Merck, Milan, Italy), and water was distilled and filtered through a Milli-Q apparatus (Millipore, Milan, Italy) before use. Na_2SO_4 was analytical grade. Stock standard solutions of the pesticides (ca. 500 mg/kg) were prepared in acetonitrile and stored in amber glassware to minimize photodecomposition and oxidative breakdown. Working standard solutions for HPLC determinations were prepared in amber glassware diluting with acetonitrile.

Preparation of Samples. Four cubè resins (named A, B, C, and D) were kindly supplied by Serbios (Italy) and four commercial formulations (named E, F, G, and H) were purchased for analysis. Their rotenone levels listed on the labels are given in **Table 1**. Cubè resins were accurately pulverized and homogenized using a mill (Malavasi, Bologna, Italy). The powder (10 mg) was solubilized in 100 mL of acetonitrile in an ultrasound bath. A 2-mL sample of this solution was diluted further to 10 mL with acetonitrile until complete solubilization of the resin. Because all the formulations were liquids (emulsifiable and emulsifiable concentrates), we prepared a solution by dilution with acetonitrile (about 500 mg/kg).

Table 1. Rotenone Concentration (%) in Cubè Resin and Commercial Formulations Given by the Supplier

sample	cubè resin	sample	formulation
A	46.9	E	8
B	46.8	F	6
C	38.9	G	4
D	38.8	H	2

Apparatus and Chromatography. *HPLC Determinations.* An Agilent Technologies (Waldbronn, Germany) model 1100 liquid chromatograph fitted with a diode array detector (DAD) model UV6000LP (Termo Quest, San Jose, CA) was used. The column was a Spherisorb S5 ODS2 (250 × 4.6 mm, 5 μm). The gradient profile for the separation of rotenone was as follows: initial mobile phase acetonitrile/water (50:50, v/v) reaching 85:15 (v/v) in 15 min. Before each injection, the LC system had to be stabilized for 10 min with an acetonitrile/water mobile phase (50:50, v/v). The injection volume was 20 μL, and the flow rate was 1 mL/min. The analysis was performed at a wavelength of 295 nm for rotenone and its derivates, at 270 nm for deguelin and tephrosin, according to the maximum absorbance in the spectra, and at 228 for pyrethrins.

LC-MS Analysis. An HPLC system (Shimadzu, Milan, Italy) equipped with an SPD M11 Avp DAD detector, an SIL 11 AD vp auto injector, and an LC 10 AD binary pump coupled on line with an MS 2011 mass spectrometer (Shimadzu, Milan, Italy) was used. UV and MS data were acquired and processed using Shimadzu "LCMS solution" software. The used column was a 150 × 2.1 mm i.d. 3.5 μm waters Symmetry C18. The injection volume was 20 μL, and the flow rate was 0.4 mL/min. UV detection was by absorbance at 295 nm. The MS conditions were as follows: APCI (±) source probe 400 °C, CDL 270 °C, block 300 °C, flow gas (N_2) at 2.5 L/min, probe voltage 4 kV.

Sunlight Photodegradation Experiments. Portions (to reach ca. 11 mg/kg), of cubé resin A and of the formulation solutions in acetonitrile of E, G, and H were poured into Petri dishes of a diameter of 5 cm and evaporated at ambient temperature. The dishes were exposed to direct sunlight and removed from sunlight at prefixed intervals (10, 20, and 30 min, 1 and 2 h). The samples were irradiated in April between 10 and 12 am. During this trial, the average daily solar radiation recorded with an AD-2 automatic weather station (Silimet, Modena, Italy) was 4118 W/m^2. A control was kept in the dark at room temperature (25 °C) in the laboratory. The residue contained in the dishes was dissolved with 2 mL of acetonitrile and injected for analysis. Each experiment was carried out in three replicates.

Field Trials. The trial was carried out in an olive grove at Uta, in the vicinity of Cagliari, Italy. The cultivar was *Tonda di Cagliari*. A random block design with four replications was used; each block contained three trees in a single row. Treatment was carried out on October 2, 2002, with an F-320 portable motorized sprayer (Fox Motori, Reggio Emilia, Italy). The commercial formulation H (2%) was used at the doses recommended by the manufacturers (700 mL/hL; 10 hL/ha). The weather conditions were continuously recorded with an SM 3800 automatic weather station (SIAP, Bologna, Italy). Rainfall was continuously recorded with an AD-2 automatic weather station (Silimet, Modena, Italy). After the last treatment, it did not rain during the entire experimental period. Maximum and minimum average temperatures were 24.5 and 18.1 °C, respectively. Olive samples (1 kg) were collected before and after the last treatment and subsequently at 1, 2, 6, and 9 days.

Extraction Procedure from Olives. Extraction of rotenone and rotenoids from olives was carried out with acetonitrile according to Cabras et al. (*24*). Four replicates of each harvest were analyzed.

RESULTS AND DISCUSSION

Analytical Method. The analytical determinations of rotenone and the major rotenoids in plant extracts and in the commercial formulation by GC (*19*) and HPLC (*25*−*27*) are reported in the literature. The method described by Draper et

Table 2. Rotenone and Rotenoids Concentrations (% ± SD) in Different Cubè Resins and Formulations

	cubè resin				
	A	B	C	D	average
rotenone	51.60 ± 1.84	43.58 ± 0.63	38.58 ± 1.74	36.87 ± 0.49	42.6 ± 4.9
deguelin	25.40 ± 1.45	19.78 ± 0.75	19.20 ± 1.23	20.46 ± 0.14	21.2 ± 2.1
tephrosin	3.17 ± 0.23	3.30 ± 0.16	4.58 ± 0.14	3.09 ± 0.02	3.5 ± 0.5
β-rotenolone	2.26 ± 0.37	2.44 ± 0.09	3.75 ± 0.12	3.66 ± 0.17	3.0 ± 0.7
α-rotenolone	0.38 ± 0.20	0.24 ± 0.05	0.26 ± 0.01	1.57 ± 0.02	0.6 ± 0.5
dehydrorotenone	0.26 ± 0.17	0.74 ± 0.00	0.43 ± 0.28	0.79 ± 0.00	0.6 ± 0.2

	formulation			
	E	F	G	H
rotenone	8.41 ± 0.05	6.03 ± 0.31	3.99 ± 0.07	1.88 ± 0.01
deguelin	4.61 ± 0.03	4.02 ± 0.10	1.84 ± 0.03	0.95 ± 0.01
tephrosin	0.78 ± 0.05	0.56 ± 0.26	0.65 ± 0.01	0.22 ± 0.00
β-rotenolone	0.66 ± 0.04	0.44 ± 0.07	0.67 ± 0.01	0.32 ± 0.00
α-rotenolone	0.10 ± 0.03	0.04 ± 0.01	< 0.02	<0.02
dehydrorotenone	0.40 ± 0.02	0.18 ± 0.04	0.16 ± 0.00	0.06 ± 0.01

Table 3. Rate of Rotenoids versus Rotenone (%) in Cubè Resins and Formulations

	cubè resin					formulation				
	A	B	C	D	average	E	F	G	H	average
rotenone	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
deguelin	49.2	45.5	49.8	55.5	50.0 ± 2.7	54.8	66.7	46.1	50.5	54.5 ± 6.2
tephrosin	6.1	7.6	11.9	8.4	8.5 ± 1.7	9.3	9.3	16.3	6.4	10.3 ± 3.0
β-rotenolone	4.4	5.6	9.7	9.9	7.4 ± 2.4	7.8	7.3	16.8	17.0	12.2 ± 4.7

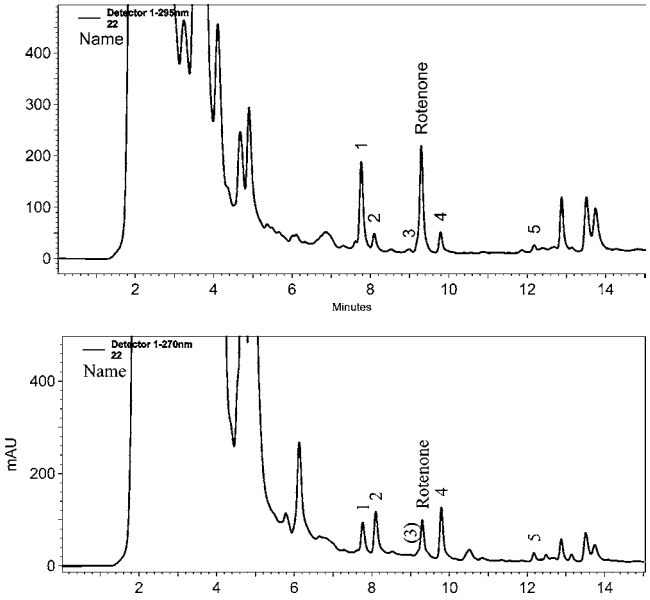

Figure 2. HPLC chromatograms of rotenone and rotenoids residue in olive at 295 and 270 nm: (1) β-rotenolone, (2) tephrosin, (3) α-rotenolone, (4) deguelin, and (5) dehydrorotenone.

al. (*28*) yielded a good resolution of the target compounds with a gradient of acetonitrile/0.025 M phosphoric acid. We used an acetonitrile/water gradient with an initial concentration of 50:50 (v/v) and a final concentration of 85:15 (v/v) in 15 min. Purification was not necessary because there were no interfering peaks in the plant extracts, formulations, and on the olive extracts for residue determination (**Figures 2** and **3**). With the DAD, it is possible to know the peak purity and confirm the a.i. by overlapping the sample spectra with those of the standards.

The standard calibration curves for rotenone and the rotenoids were constructed by plotting concentrations against peak areas.

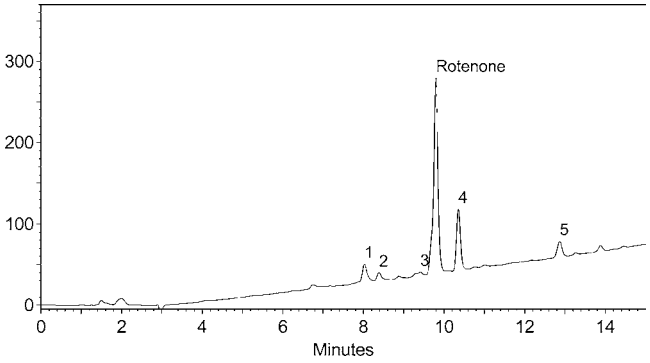

Figure 3. HPLC chromatograms at 295 nm of rotenone and rotenoids in cubè resin: (1) β-rotenolone, (2) tephrosin, (3) α-rotenolone, (4) deguelin, and (5) dehydrorotenone.

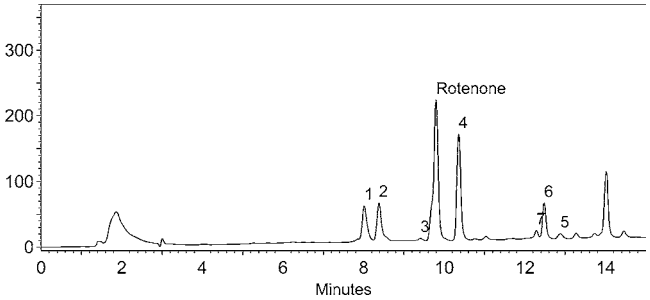

Figure 4. HPLC chromatograms at 228 nm of rotenone and rotenoidsand pyrethrins in formulation D: (1) β-rotenolone, (2) tephrosin, (3) α-rotenolone, (4) deguelin, (5) dehydrorotenone, (6) pyrethrin I, (7) pyrethrin II.

A good linearity was achieved for all active ingredients between 0.02 and 15.00 mg/kg with correlation coefficients ranging between 0.9995 and 0.9998. With this method, it is possible to determine also pyrethrin I and II, which were present in formulation D. In this case, it was necessary to use a wavelength

Table 4. Persistence of Rotenone and Rotenoids in Formulations during Storage

	concentration % ± SD							
	formulation E		formulation F		formulation G		formulation H	
	0 months	6 months	0 months	6 months	0 months	6 months	0 months	6 months
rotenone	8.41 ± 0.05	8.28 ± 0.04	6.03 ± 0.31	5.93 ± 0.15	3.99 ± 0.07	3.51 ± 0.04	1.88 ± 0.01	1.51 ± 0.05
deguelin	4.61 ± 0.03	3.24 ± 0.05	4.02 ± 0.10	3.08 ± 0.08	1.84 ± 0.03	1.32 ± 0.05	0.95 ± 0.01	0.65 ± 0.05
tephrosin	0.78 ± 0.05	1.26 ± 0.03	0.56 ± 0.26	1.40 ± 0.05	0.65 ± 0.01	1.39 ± 0.05	0.22 ± 0.00	0.55 ± 0.05
β-rotenolone	0.66 ± 0.04	0.31 ± 0.03	0.44 ± 0.07	0.35 ± 0.05	0.67 ± 0.01	0.45 ± 0.01	0.32 ± 0.00	0.17 ± 0.05
α-rotenolone	0.10 ± 0.03	0.03 ± 0.01	0.04 ± 0.01	0.08 ± 0.02	<0.02	0.12 ± 0.01	<0.02	0.05 ± 0.05
dehydrorotenone	0.40 ± 0.02	0.39 ± 0.02	0.18 ± 0.04	0.26 ± 0.03	0.16 ± 0.00	0.35 ± 0.02	0.06 ± 0.01	0.34 ± 0.05

Table 5. Sunlight Photodegradation of Rotenone and Rotenoids in Cubè Resin A

	concentration (mg/kg ± SD)					
time (min)	rotenone	deguelin	tephrosin	β-rotenolone	α-rotenolone	dehydrorotenone
0	12.18 ± 0.15	5.72 ± 0.11	1.13 ± 0.06	0.96 ± 0.06	0.20 ± 0.05	0.26 ± 0.00
10	8.70 ± 0.85	1.66 ± 0.37	0.71 ± 0.03	1.77 ± 0.44	1.25 ± 0.07	0.18 ± 0.07
20	5.71 ± 0.71	1.11 ± 0.12	0.63 ± 0.02	1.66 ± 0.22	0.79 ± 0.18	0.26 ± 0.03
30	3.93 ± 0.27	0.75 ± 0.04	0.56 ± 0.05	1.45 ± 0.14	0.41 ± 0.06	0.25 ± 0.01
60	1.53 ± 0.27	0.27 ± 0.04	0.34 ± 0.02	1.23 ± 0.10	0.15 ± 0.02	0.17 ± 0.02
120	0.32 ± 0.13	0.05 ± 0.04	0.11 ± 0.03	0.47 ± 0.09	0.02 ± 0.00	0.07 ± 0.00

Table 6. Sunlight Photodegradation of Rotenone and Rotenoids in Some Formulations

	concentration (mg/kg ± SD)					
time (min)	rotenone	deguelin	tephrosin	β-rotenolone	α-rotenolone	dehydrorotenone
			Formulation E			
0	13.42 ± 0.68	7.45 ± 0.41	1.30 ± 0.13	0.89 ± 0.10	0.15 ± 0.01	0.49 ± 0.05
10	8.21 ± 1.06	0.75 ± 0.20	0.22 ± 0.02	1.96 ± 0.12	0.58 ± 0.16	0.36 ± 0.07
20	7.19 ± 1.56	0.54 ± 0.23	0.18 ± 0.04	3.24 ± 0.39	0.32 ± 0.05	0.30 ± 0.08
30	4.00 ± 1.01	0.28 ± 0.01	0.14 ± 0.01	3.43 ± 0.90	0.21 ± 0.05	0.19 ± 0.04
60	2.35 ± 0.53	0.15 ± 0.04	0.02 ± 0.00	3.61 ± 0.42	0.09 ± 0.00	0.12 ± 0.01
120	0.52 ± 0.15	0.03±0.00	0.02 ± 0.00	2.58 ± 0.66	0.02 ± 0.00	0.07 ± 0.00
			Formulation G			
0	12.06 ± 0.25	6.33 ± 0.10	2.45 ± 0.05	2.48 ± 0.07	0.25 ± 0.01	0.48 ± 0.01
10	6.03 ± 0.71	1.17 ± 0.23	1.09 ± 0.09	4.03 ± 0.30	0.35 ± 0.17	0.18 ± 0.03
20	3.78 ± 0.35	0.76 ± 0.10	1.04 ± 0.10	4.62 ± 0.12	0.06 ± 0.04	0.12 ± 0.01
30	2.60 ± 0.16	0.51 ± 0.09	0.97 ± 0.11	4.86 ± 0.09	0.02 ± 0.00	0.11 ± 0.00
60	0.87 ± 0.12	0.12 ± 0.04	0.36 ± 0.08	4.50 ± 0.17	0.02 ± 0.00	0.07 ± 0.00
120	0.11 ± 0.05	0.03 ± 0.00	0.20 ± 0.08	2.07 ± 0.74	0.02 ± 0.00	0.07 ± 0.00
			Formulation H			
0	8.63 ± 0.26	4.56 ± 0.33	1.47 ± 0.04	2.41 ± 0.18	0.21 ± 0.06	0.16 ± 0.06
10	4.44 ± 0.53	1.58 ± 0.21	1.85 ± 0.05	3.67 ± 0.16	0.21 ± 0.03	0.16 ± 0.02
20	3.27 ± 0.69	1.08 ± 0.33	1.50 ± 0.20	3.45 ± 0.14	0.16 ± 0.02	0.19 ± 0.03
30	1.97 ± 0.58	0.53 ± 0.15	1.05 ± 0.09	3.53 ± 0.56	0.13 ± 0.03	0.14 ± 0.01
60	0.39 ± 0.18	0.14 ± 0.04	0.54 ± 0.13	3.49 ± 0.02	0.05 ± 0.03	0.09 ± 0.01
120	0.08 ± 0.00	0.07 ± 0.00	0.02 ± 0.00	2.29 ± 0.11	0.02 ± 0.00	0.07 ± 0.00

of 228 nm, depending on the maximum absorbance in the spectra of pyrethrins (**Figure 4**). Recovery assays of rotenone and rotenoids between 0.02 and 2.00 mg/kg ranged between 82 and 114%, with coefficients of variation between 3 and 14%. The limit of determination was 0.02 for all compounds.

Active Ingredients in Cubè Resins and Formulations. Rotenone and the rotenoids were determined in four cubè resins (A, B, C, and D), and four formulations (E, F, G, and H). Four replicates were analyzed for each sample. The data reported in **Table 2** showed that the rotenone level determined in the cubè resins at purchase time were higher in sample A and lower in sample B and D compared to that declared by the supplier (**Table 1**). In the commercial formulations, only sample D showed a lower amount than that reported on the label. In cubè resins, the major rotenoids were rotenone and deguelin with an average amount of 42.6% and 21.2%, respectively, while β-rotenolone and tephrosin had an average amount of about 3% and α-rotenolone and dehydrorotenone of 0.6%. On calculating the percentage of the main rotenoids compared to rotenone (**Table 3**), it can be observed that the percentage of deguelin is about half that of rotenone. These data agree with the literature data for *Lonchocarpus* extracts (*11*, *19*), while for *Tephrosia* and *Derris* extracts, the reported rotenone/deguelin ratios are

Table 7. Half-Life ($t_{1/2}$) and Coefficient of Regression (r) of Rotenone, Deguelin, and Tephrosin in Cubè Resin A and Some Formulations after Exposure to Direct Sunlight

	rotenone		deguelin		tephrosin	
	$t_{1/2}$ (min)	r	$t_{1/2}$ (min)	r	$t_{1/2}$ (min)	r
cubè resin A	23	0.996	18	0.971	38	0.992
formulation E	27	0.992	10	0.908	11	0.956
formulation G	18	0.995	16	0.946	36	0.950
formulation H	18	0.987	20	0.932	19	0.965

Table 8. Degradation of Rotenone and Rotenoids Residues on Olive

	concentration (mg/kg ± SD)					
days after treatment	rotenone	deguelin	tephrosin	β-rotenolone	α-rotenolone	dehydrorotenone
0	0.99 ± 0.23	0.29 ± 0.08	0.29 ± 0.06	0.25 ± 0.06	0. 04 ± 0.00	0.05 ± 0.03
1	0.71 ± 0.13	0.24 ± 0.06	0.19 ± 0.03	0.47 ± 0.05	0.05 ± 0.00	0.05 ± 0.01
2	0.45 ± 0.08	0.22 ± 0.05	0.12 ± 0.02	0.39 ± 0.03	0.04 ± 0.00	0.04 ± 0.01
6	0.18 ± 0.03	0.18 ± 0.04	0.10 ± 0.05	0.20 ± 0.09	0.04 ± 0.00	0.02 ± 0.01
9	0.08 ± 0.02	0.10 ± 0.02	0.06 ± 0.02	0.10 ± 0.02	0.03 ± 0.00	0.02 ± 0.00
$t_{1/2}$ (days)	2.5	5.6	4.7			
r	0.996	0.955	0.925			

1.0/1.5 and 1.0/0.85 (*23*), respectively. Because the ratio between rotenone and the major rotenoids in the formulations is similar to that in *Lonchocarpus* extracts, it can be deduced that these formulations have been produced by extracts of these plants.

Formulation Stability. Formulations in normal storage (darkness and room temperature) conditions were analyzed at purchase time and after 6 months. Four replicates of each formulation were analyzed. The data are reported in **Table 4**. In the samples with a high rotenone level (A and B), the concentration of rotenone was the same after 6 months, while it was 12−20% lower in the sample with a low rotenone level (C and D). This shows that the lower the concentration, the greater the decrease in rotenone concentration in the formulations during storage. In all formulations, deguelin decreases by about 30%, while tephrosin increases. This indicates that during conservation, part of the deguelin is transformed into tephrosin by hydroxylation. β-Rotenolone decreases between 11 and 53%.

Sunlight Photodegradation. Photodegradation was studied on solutions of cubè resins and formulations at about 11 mg/kg. The rotenone and rotenoids decay rate were calculated as a pseudo-first-order kinetics, and the data are reported in **Table 7**. Because the compositions of the four cubè resin analyzed were identical, as it is reported above, the experiment was carried out only on the cubè resin A. In cubè resin, sunlight exposure of rotenone, deguelin, and tephrosin produced a decrease in photo degradation, with half times of 23, 18, and 38 min (**Table 6**), respectively, while β-rotenolone and α-rotenolone increase initially and subsequently decrease progressively. This is because they are photodegradation products of rotenone (*10*, *11*). In addition, in the formulations, the photodegradative trend is qualitatively the same with rotenone, deguelin, and tephrosin, which decrease progressively in time, while α and β-rotenolone increase first and subsequently decrease progressively. The formulations present different disappearance rates compared to those of cubè resin for rotenone, deguelin, and tephrosin. As a matter of fact, in formulation A, we have a lower disappearance rate for rotenone (27 vs 23 min) and a greater one for deguelin and tephrosin (10 and 11 vs 18 and 38 min, respectively). Formulation C presents similar photodegradative behavior to that of cubè resin, while in formulation D, the disappearance rate of deguelin (20 min) is similar to that of cubè resin, and rotenone and tephrosin degrade faster (18 and 19 vs 23 and 39 min, respectively).

Olive Residues. In Italy, rotenone is registered on many crops, including olives, with a maximum residue limit (MRL) of 0.04 mg/kg and a pre-harvest time of 10 days (D. M. January 22 1998). After treatment, the rotenone residue was 0.99 mg/kg (**Table 8**). The residue decreased progressively to 0.08 mg/kg in 9 days, which is higher than the legal limit. The decay rate, calculated as a pseudo first-order kinetics ($r = 0.996$), shows a half-life ($t_{1/2}$) of 2.5 days. Because the average weight of the olives was constant during the experiment, no dilution effect occurred. These data were similar to those reported in the literature (*24*), with the residues at preharvest interval higher than the MRL. In Italy, PHI and MRL are the same for all registered crops. This shows that the PHIs have not been correctly determined. After treatment, deguelin and tephrosin showed the same residue (0.29 mg/kg). The disappearance rates of deguelin and tephrosin were similar but slower than that of rotenone ($t_{1/2} = 5.6$ and 4.7 days, respectively). Nine days after treatment, deguelin and tephrosin residues were, on average, 0.10 and 0.06, respectively. The high β-rotenolone content (0.25 mg/kg) after treatment indicates that before rotenone penetrated the fruit cuticle the photodegradative process had already transformed part of it into β-rotenolone. The residues of α-rotenolone and dehydrorotenone were negligible.

Besides rotenone, cubè resins from *Lonchocarpus* contain large quantities of deguelin and smaller quantities of tephrosin and β-rotenolone. Extracts of *Tephrosia* and *Derris* can contain equal or greater quantities of deguelin compared to rotenone. Therefore, the composition of commercial formulations may present very different contents of rotenoid, depending on the extracts used to prepare them. Because these rotenoids present insecticide activity, the efficacy of these formulations at the same concentration of rotenone may also be very different.

Rotenone is the only active ingredient considered both for efficacy and legal limit for residues. It should be remembered that with some formulations, the rotenone titer may decrease in time, and their use may, therefore, prove less effective because a smaller real dose is given. Rotenone and rotenoids are very sensitive to solar radiation, which degrades them rapidly with half-lives in the order of a few tens of minutes. Some studied formulations (C and D) showed greater disappearance rates than that of cubè resin, indicating that not much attention has been paid to protect the active ingredient from photodegradation in the formulation. Besides this aspect, formulators should take particular care to use an adjuvant that should increase to a maximum the penetration rate of active ingredients in the cuticle (*29*). As long as active ingredients are on the surface of the fruit, they will be liable to photodegradation, which is very fast, and they will only be protected when the cuticle is penetrated. The pre-harvest interval of rotenone formulations should be considered better, taking into account that after 9 days the residue is over the legal limit and that we should not only consider the limits for rotenone but also those for deguelin, tephrosin, and β-rotenolone, especially those for deguelin, whose toxicity is similar to that of rotenone.

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