Persistence of Insecticide Residues in Olives and Olive Oil

Paolo Cabras,*,† Alberto Angioni,† Vincenzo L. Garau,† Marinella Melis,† Filippo M. Pirisi,† Marlene Karim,‡ and Elizabeth V. Minelli§

Dipartimento di Tossicologia, Università di Cagliari, viale Diaz 182, 09126 Cagliari, Italy, Universidade Técnica de Lisboa, Lisboa, Portugal, and Departamento de Química Orgánica, Instituto de Química, UNESP, Araraquara, Brazil

The decay rate of six insecticides (azinphos methyl, diazinon, dimethoate, methidathion, parathion methyl, and quinalphos) used to control *Dacus oleae* was studied. Degradation of pesticides showed pseudo-first-order kinetics with correlation coefficients ranging between −0.936 and −0.998 and half-lives between 4.3 days for dimethoate and 10.5 days for methidathion. Residues in olive oil were greater than on olives, with a maximum concentration factor of 7. Dimethoate was the only pesticide with lower residues in the oil than on the fruits. Olive washing affects pesticide residues ranging from no reduction to a 45% decrease. During 8 months of storage of the olive oil, diazinon, dimethoate, parathion methyl, and quinalphos did not show any remarkable difference, while methidathion and azinphos methyl showed a moderate decrease.

Keywords: *Residues; insecticides; olives; olive oil; storage; washing*

INTRODUCTION

Olive oil is a typical food of the "Mediterranean diet". Thanks to its optimal composition in "essential" fatty acids and easy digestibility, it has a high biological and nutritional value (Turchetto, 1986). Moreover, because of its sensorial and flavor properties, it is more appetizing than other vegetable oils. Olive oil is obtained by simple pressure of the fruit, without any chemical intervention. The rediscovery of "natural" foods, such as olive oil, has contributed in recent years to the increase in its consumption, especially the extra-virgin type. This type is the best quality olive oil, and since it can only be obtained from mature sound drupes, parasite control is indispensable. There are several olive tree parasites, the most noxious being the olive fly, *Dacus oleae*, for whose control numerous insecticides are used. The use of pesticides leaves residues on the drupes, and the quantity depends mainly on the number of treatments, the degradation rate of the active ingredient, and the preharvest interval. In the processing of olive oil, residues present on the fruit are shared by the oil, the cake, and the vegetation water, depending on the physical and chemical characteristics of the active ingredient (De Pietri-Tonelli et al., 1965; Cabras et al., 1993). In general, since most pesticides are nonpolar, residues tend to be distributed mostly in the oil. Since on average about 5 kg of olives is needed to produce 1 L of oil, the residue concentration in the oil will consequently be greater than on the fruits. Recent reviews by Farris et al. (1992) and Lentza-Rizos and Avramides (1995) describe the results of research on olives and olive oil residues. These studies involved mainly the fate of pesticide residues from olives to oil during processing. No attention has been given to evaluate the effect of olive washing on residue levels. Washing is a preliminary procedure used commonly in olive oil production to remove foreign materials and leaves from olives. Another poorly studied issue is the persistence of pesticide residues during the storage of olive oil. To our knowledge, fenthion is the only pesticide studied in this respect (Lenza-Rizos et al., 1994).

In this paper we have studied the fate of six insecticides from olives to oil including washing and storage.

MATERIALS AND METHODS

Field Trials. The trial was carried out in an olive grove of the Consorzio interprovinciale di Frutticoltura at Villasor, near Cagliari, Italy. The grove was planted in 1982 with a tree spacing of 6 × 6 m; the cultivar was Yacouti. A random-block design with three replications was used, and each block contained three trees in a single row. Treatments were carried out on November 22, 1995, with an F-320 portable motorized sprayer (Fox Motori, Reggio Emilia, Italy). The commercial formulations Kition 30 (24% azinphos methyl), Basudin E (20% diazinon), Rogor L 40 (38% dimethoate), Supracaffaro (19% methidathion), Metox 20 (18% parathion methyl), and Ekalux (25% quinalphos) were used at the doses recommended by the manufacturers (respectively, 1.5, 2.5, 1.5, 3.0, 2.0, and 1.5 kg/ha). Sampling started 1 day after treatment and was repeated weekly. Random 3 kg fruit samples were collected from each block and immediately processed into olive oil. 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). During the experiments total rainfall was 102 mm, on November 23, 24, and 26 and December 5, 6, and 9, with 52.8, 2.6, 9.4, 8.4, 23.4, and 5.4 mm, respectively. Maximum and minimum average temperatures were 17.0 and 4.1 °C, respectively.

Olive Processing. The sample was processed into oil by a stainless steel laboratory unit, reproducing an industrial unit. The laboratory unit was made up of a hammer crusher, a breaking machine, and a centrifugal separator. After crushing, a sample of the fruit was collected and the paste was then broken for 45 min and centrifuged. The obtained must was centrifuged to separate the water from the oil. The percentage oil yield obtained with this unit was similar to that of an industrial unit.

Olive Washing. Olives used in washing experiments (October 1996) were of the cv. Koroneik, the fruits of which are very small. Treatments were carried out as described above. Two samples for each pesticide were collected 4, 11, and 18 days after treatment. Each sample (ca. 1 kg) was harvested from the same tree. The olive sample was divided into two

† Università di Cagliari.
‡ Universidade Técnica de Lisboa.
§ UNESP.

S0021-8561(96)00922-3 CCC: $14.00

equal parts. One was poured into a net and dipped for 1 min into a basin with running water. The washed sample and control were crushed and analyzed.

Olive Oil Storage. Olive oil samples at 0.5 and 2.0% acidity (expressed as oleic acid) without pesticide residues were fortified with hexane solutions of the insecticides. Each sample was divided in four subsamples and stored at room temperature. Samples were analyzed after 1, 45, 120, and 240 days.

Chemicals. Azinphos methyl, diazinon, dimethoate, methidathion, parathion methyl, and quinalphos were analytical standards purchased from Ehrenstorfer (Augsburg, Germany). Triphenyl phosphate (99%) was used as the internal standard (i.s.) and was of analytical grade (Janssen, Geel, Belgium). Chloroform was of HPLC grade, hexane was used as solvent for pesticides, and ethanol was of analytical grade (Carlo Erba, Milan, Italy). Stock standard solutions of the pesticide (ca. 500 ppm each) were prepared in ethanol. Olive and olive oil matrix standard solutions in hexane, containing i.s. at 0.6 ppm, were prepared by adding working standard solutions to untreated olive and olive oil extracts, evaporated to dryness under a nitrogen stream.

Pesticide Analysis. Pesticide analysis was carried out with the methods described by Cabras et al. (1993, 1997).

Apparatus and Chromatography. *GC Analyses.* An HRGC Mega 5160 gas chromatograph (Carlo Erba, Milan, Italy) fitted with an NPD-40 nitrogen−phosphorus detector, an AS 550 autosampler (Carlo Erba), a split−splitless injector,- and a Durabond fused silica column (30 m × 0.25 mm i.d.) (J&W Scientific, Folsom, CA) with DB-1701 (14% cyanopropylphenyl-methylpolysiloxane) liquid phase (film thickness 0.25 μm) was employed. The injector and detector were operated at 250 and 290 °C, respectively. The sample (2 μL) was injected in the splitless mode (60 s), and the oven temperature was programmed as follows: 110 °C for 1 min, raised to 280 °C (30 °C/min), and held for 18 min. Helium was the carrier and makeup gas at 100 and 130 kPa, respectively. The detector output was processed using a HP 3396-II reporting integrator (Hewlett-Packard, Avondale, PA). Calibration graphs for the insecticides were constructed with the i.s. method by measuring peak heights vs concentrations. Good linearities were achieved in the range 0−5.0 ppm, with correlation coefficients between 0.9993 and 0.9996.

Extraction Procedure. Five grams of crushed olives was weighed in a 30 mL screw-capped tube; 10 mL of chloroform containing 0.6 ppm of i.s. was added, and the tube was agitated in a rotatory shaker for 15 min. The organic layer was allowed to separate and was then poured into a 10 mL screw-capped tube containing 1 g of anhydrous sodium sulfate. Organic extract (0.4 mL) was evaporated to dryness under a nitrogen stream and then taken up with 1.6 mL of hexane containing the i.s. and injected for gas chromatographic analysis.

Two grams of olive oil was weighed in a 30 mL screw-capped tube; 2 mL of hexane was added and, after agitation, another 10 mL of acetonitrile. The tube was agitated in a rotatory shaker for 30 min. The acetonitrile layer was allowed to separate, and then 7.5 mL was poured into a 10 mL beaker and allowed to evaporate to dryness under a nitrogen stream. The residues were taken up with 1.5 mL of hexane containing the i.s. and injected for GC analysis.

RESULTS AND DISCUSSION

In Italy olives ripen between November and January (Vitagliano, 1982). Under favorable environmental conditions, treatments to control *D. oleae* may also be necessary in November (Leone et al., 1990), when our experiments were carried out.

Behavior of Pesticide Residues on Olives and in Olive Processing. The fruit weight did not increase during the experiment; therefore, the residue decline was not affected by growth dilution. The data relating to the residues in the fruit and oil, their ratio, and the yield in oil are reported in Table 1. The kinetic data calculated by pseudo-first-order kinetics are reported in Table 2.

Table 1. Insecticide Residues (Milligrams per Kilogram ± SD) on Olives after Treatment and in Olive Oil

pesticide	days after treatment	residues on olives	oil yield, %	residues in olive oil	residues in oil/ residues on olives
azinphos methyl	1	1.82 ± 0.63	15 ± 3	4.57 ± 0.88	2.5
	8	1.03 ± 0.13	16 ± 1	3.10 ± 0.578	3.0
	14	0.69 ± 0.18	16 ± 1	1.62 ± 0.32	2.3
diazinon	1	1.34 ± 0.11	17 ± 3	4.43 ± 1.26	3.3
	8	1.11 ± 0.47	16 ± 2	3.78 ± 0.32	3.4
	13	0.68 ± 0.36	14 ± 2	2.15 ± 0.46	3.2
	20	0.35 ± 0.10	14 ± 1	1.95 ± 0.80	5.6
dimethoate	1	1.60 ± 0.11	15 ± 3	0.53 ± 0.18	0.33
	8	1.08 ± 0.01	16 ± 1	0.24 ± 0.01	0.22
	14	0.17 ± 0.00	16 ± 1	nd	
methidathion	1	3.01 ± 0.60	15 ± 3	6.78 ± 2.83	2.3
	8	1.68 ± 0.79	16 ± 1	5.69 ± 1.78	3.4
	14	1.28 ± 0.43	16 ± 1	3.37 ± 0.33	2.6
parathion methyl	1	1.40 ± 0.12	17 ± 3	4.00 ± 1.02	2.9
	8	0.61± 0 .16	16 ± 2	2.91 ± 0.23	4.8
	13	0.35 ± 0.16	14 ± 2	1.77 ± 0.36	5.1
	20	0.19 ± 0.06	14 ± 1	1.33 ± 0.33	7.0
quinalphos	1	1.84 ± 0.10	17 ± 3	2.63 ± 0.60	1.4
	8	0.68 ± 0.15	1 6± 2	2.13 ± 0.22	3.1
	13	0.36 ± 0.14	14 ± 2	0.50 ± 0.40	1.4
	20	0.20 ± 0.04	14 ± 1	0.80 ± 0.14	4.0

[a] nd, not detectable (<0.01).

Table 2. Half-Lives (Days) and Correlation Coefficients (*r*) of Pesticide Residues on Olives

	azinphos methyl	diazinon	dimeth-oate	methida-thion	parathion methyl	quinal-phos
$t_{1/2}$	9.3	9.6	4.3	10.5	6.6	6.0
r	−0.998	−0.969	−0.936	−0.987	−0.997	−0.996

Azinphos Methyl. The decay rate of this insecticide shows a pseudo-first-order kinetics ($r = -0.998$) and a half-life ($t_{1/2}$) of 9.3 days. The residue of azinphos methyl in the oil was on average 2.6 times higher than on the olives. Because 6 kg of olives is needed to obtain 1 L of oil, it can be calculated that ca. 50% of azinphos methyl was transferred from the olives to the oil.

Diazinon. The degradative behavior of diazinon was similar to that of azinphos methyl ($t_{1/2} = 9.6$ days). The concentration factor of the residues on the olives that were processed into oil was on average 3.3 when the olive residues ranged between 0.68 and 1.34, while it was 5.6 when the residues were lower (0.35 ppm). Analogous results were obtained in Portugal (Ferreira and Tainha, 1983).

Dimethoate. This insecticide degraded more rapidly than the others ($t_{1/2} = 4.3$ days). Results obtained in Italy (Lanza et al., 1986) and Portugal (Ferreira and Tainha, 1983) showed slower rates with half-times of 8.7 and 10.2 days, respectively, while in Spain (Albi et al., 1970) the rates were similar to ours. The residues of dimethoate in the oil were lower than those on the olives; this distinguishes dimethoate from other studied insecticides. When the residues in the olives decreased, the residue transferred from the olives to the oil also decreased, and when it was lower (0.17 ppm), there were no residues in the oil. This peculiar behavior is unanimously attributed to the high solubility of dimethoate in water (23.3 g/L), which determines a preferential split in the vegetable water.

Table 3. Effect of Washing on Olive Residues

pesticide	solubility in water (mg/L)		residues (mg/kg) 1	2	3	4	5	6
azinphos methyl	28	c[a]	3.02	2.73	2.15	2.12	1.01	0.72
		w[a]	1.85	2.49	1.40	1.28	0.92	0.79
diazinon	60	c	3.46	2.63	1.74	1.53	1.46	1.15
		w	2.29	1.72	1.73	0.91	1.53	1.27
dimethoate	23300	c	4.71	3.43	2.35	2.30	0.91	0.76
		w	4.02	2.47	1.70	1.98	0.85	0.82
methidathion	200	c	4.25	3.81	2.89	2.63	2.51	1.67
		w	3.59	2.88	2.51	2.36	2.55	1.74
parathion methyl	55	c	4.26	4.58	2.29	2.29	1.69	1.35
		w	3.03	4.67	1.51	2.36	1.71	1.40
quinalphos	18	c	3.56	1.90	1.75	1.46	0.88	1.06
		w	2.38	1.70	1.28	0.81	0.93	1.09

[a] c, control; w, washed sample.

Residues in olive oil were determined in Italy (Leone et al., 1990; Gambacorta et al., 1993), Spain (Albi and Navas, 1985), and Greece (Lentza-Rizos and Avramides 1995). In Portugal, Ferreira and Tainha (1983) did not find any residues in the olive oil, not even when it was obtained from olives with high residues (5.30 ppm).

Methidathion. The decay rate of this active ingredient (AI) was less rapid than that of the other pesticides ($t_{1/2}$ = 10.5 days). The residue concentration factor in the passage from olives to oil was similar to that of azinphos methyl (average 2.8). In trials carried out in Portugal (Ferreira and Tainha, 1983) the decay rate of methidathion on the fruit was similar ($t_{1/2}$ = 12.9 days), while the concentration factors were higher (average 4.8).

Parathion Methyl. The half-life of parathion methyl on the drupes, calculated as a pseudo-first-order kinetics (r = −0.997), was 9.3 days. In Portugal Ferreira and Tainha (1983) determined a slower decay rate ($t_{1/2}$ = 21.3 days) in olives. The Portuguese authors found residues in the oil with concentration factors of ca. 2.6, while in our experiments similar values were obtained only when the residues on the olives were higher. When the residues decrease on the olives, the percentage of residues transferred from the olives to the oil increases, and when the residues on the fruit are lower, the concentration factor in the oil is higher (Albi et al., 1970). In this case, since 7 kg of olives is needed to obtain 1 L of oil, 100% of the parathion methyl was transferred from the olives to the oil.

Quinalphos. Quinalphos degraded on the olives at the same rate as that of parathion methyl ($t_{1/2}$ = 6.0 days). The residues in the oil were 1.4 times higher than those on the olives in two of the samples and 3.5 times higher in another two samples.

Olive Washing. Table 3 shows pesticide residues of olives with and without washing and the solubility in H_2O values of pesticides. Of the studied pesticides only dimethoate is systemic and penetrates into the fruit. Very small olive sizes make a higher surface/weight ratio and, hence, higher residues than common olives.

After washing, olive residues decreased on average in the samples of all pesticides of the first (1 and 2) and second (3 and 4) harvest . The residue decreases ranged on average between 16 and 31%, with values from a minimum of 0 to a maximum of 45%. The samples at the last harvest (5 and 6) with and without washing did not show any remarkable difference. These were harvested after 2 days of intense rainfall.

Table 4. Persistence of Insecticide Residues (Milligrams per Kilogram) in Olive Oil at Different Acidities during Storage

pesticide	storage, days	acidity 2%	0.5%
azinphos methyl	1	5.06 ± 0.15	4.90 ± 0.29
	45	4.86 ± 0.19	4.65 ± 0.33
	120	4.68 ± 0.29	4.38 ± 0.46
	240	4.07 ± 0.03	4.53 ± 0.21
diazinon	1	4.40 ± 0.15	4.26 ± 0.05
	45	4.16 ± 0.21	4.03 ± 0.17
	120	4.07 ± 0.35	4.07 ± 0.23
	240	3.94 ± 0.15	3.78 ± 0.03
dimethoate	1	2.49 ± 0.07	2.49 ± 0.07
	45	2.41 ± 0.08	2.38 ± 0.15
	120	2.52 ± 0.06	2.42 ± 0.12
	240	2.58 ± 0.07	2.45 ± 0.01
methidathion	1	7.78 ± 0.32	7.70 ± 0.38
	45	7.35 ± 0.42	7.29 ± 0.34
	120	6.95 ± 0.25	6.41 ± 0.41
	240	5.83 ± 0.11	5.55 ± 0.10
parathion methyl	1	5.25 ± 0.19	5.21 ± 0.22
	45	5.14 ± 0.35	5.04 ± 0.33
	120	5.32 ± 0.21	4.76 ± 0.34
	240	5.43 ± 0.16	4.55 ± 0.04
quinalphos	1	2.48 ± 0.21	2.32 ± 0.09
	45	2.43 ± 0.09	2.28 ± 0.06
	120	2.58 ± 0.21	2.19 ± 0.07
	240	2.62 ± 0.05	2.40 ± 0.12

The residue decrease after washing cannot attributed to pesticide solubilization in H_2O, as many washed samples showed no reduction in residues. Moreover, the residue decreases were not correlated to pesticide solubility in H_2O. At treatment time, dust could be on the fruit; therefore, pesticides would settle both on the wax of the fruit surface and on the dust. During washing the dust is removed from the fruit together with the surface residues. Therefore, if at the treatment time or at harvest, there is not dust on the fruit because it has been washed away by the rain, the washing will not decrease the residue. This would account for the absence of residue decrease in many samples. By adsorbing the pesticides, the wax on the fruit surface (Riederer and Schreiber, 1995) would not allow their solubilization in H_2O. This hypothesis was confirmed in washing experiments in which dipping prewashed olives in water for 10 min did not cause the residue to decrease.

Olive Oil Storage. Table 4 shows pesticide residues in olive oil at 0.5 and 2.0% acidity during the storage. Diazinon, dimethoate, parathion methyl, and quinalphos remained unchanged during all storage times (8 months). In olive oil at 0.5% of acidity, diazinon and parathion methyl showed a little decrease (ca. 10%), which could be ascribed to analytical variability. Azinphos methyl was stable in olive oil at 0.5% acidity, while in a more acidic sample after 8 months, it showed a decrease of ca. 20%.

Methidathion showed the same moderate decrease in both oils (ca. 25%).

Conclusions. Azinphos methyl and quinalphos are two insecticides for which no olive residue data have been published in the literature. The decay rate of the studied pesticides has been properly described by pseudo-first-order kinetics. The use of parathion methyl and quinalphos could lead to residues in the olives above the Italian maximum residue levels (MRL). The residues in the olive oil were always higher than those on

the olives with the exception of dimethoate. Residues of the latter in the olive oil were lower than on the olives. Olive washing affects pesticide residues ranging from no reduction to a 45% decrease. During 8 months of storage of the olive oil, diazinon, dimethoate, parathion methyl, and quinalphos did not show any remarkable difference, while methidathion and azinphos methyl showed a moderate decrease.

ACKNOWLEDGMENT

E. V. Minelli is grateful to the CNPq—Brazil for a fellowship.

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Received for review December 5, 1996. Revised manuscript received March 3, 1997. Accepted March 11, 1997.[⊗]

JF960922E

[⊗] Abstract published in *Advance ACS Abstracts,* May 1, 1997.