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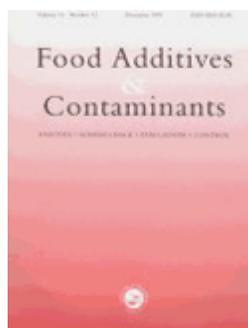
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Residues of rotenone, azadirachtin, pyrethrins, and copper used to control *Bactrocera oleae* (Gmel.) in organic olives and oil

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11 **Residues of rotenone, azadirachtin, pyrethrins, and copper**
12 **used to control *Bactrocera oleae* (Gmel.) in organic olives and**
13 **oil**
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Abstract

Rotenone, azadirachtin, pyrethrins and copper fungicide decay curves were determined in olives and olive oil samples after experimental trials consisting of one, two, and three applications of each active ingredient were carried out twice in the years 2005 and 2006. Rotenone, azadirachtin and pyrethrins were analysed by extraction with acetonitrile and determined by liquid chromatography; copper was extracted into aqueous HCl and determined by chemical stripping. Pyrethrins were always found but in amounts below the acceptable limits, whereas rotenone and copper residues always exceeded the limits, after the pre-harvest interval, the maximum threshold allowed for olives. As regards the residues in the olive oil, rotenone was found in concentrations higher than those detected on the olives. Copper tended to accumulate in the olives according to the number of applications, whereas in the oil the residue always proved to be lower than the maximum residue limit. The sensitivity of the method applied did not allow the detection of azadirachtin.

Keywords: *organic olive growing, rotenone, azadirachtin, pyrethrins, copper, residues, olives, oil.*

Introduction

Given the increasing concern about food safety, it is necessary to develop a control system of the production chain, which still displays some weaknesses, particularly for organic products. A knowledge of the post-treatment decay process for the active ingredients applied in organic agriculture on different substrates and in the various growing conditions to control pathogens is an important starting point to define proper application methods.

In the Mediterranean basin, olive growing and olive oil production play a major role in organic agriculture. However, the fungal infection by *Bactrocera oleae* (Gmelin) can seriously affect the quality of olives and therefore the quality of olive oil. Given the high incidence of infection, especially in some areas and on some varieties, appropriate control strategies are required, based also on the use of natural predators and/or products of plant origin (botanicals). The latter generally display a rapid degradation and a low toxicity risk for non-target organisms and mammals. However, their insecticidal action is sometimes of short duration (from a few hours to a few days), their cost is high and their availability on the market is limited. Furthermore, as it occurs with any plant protection product, they can entail risks for the operators and the environment. Among these natural substances, pyrethrins, rotenone, azadirachtin, quassine, nicotine ryanodine, sabadilla and some essential oils are the most used (Bagnoli and Petacchi 2004). As regards azadirachtin, pyrethrum and rotenone, little is known about their residual activity in olives and olive oil.

Azadirachtin A and its isomers act on the feeding behaviour or as growth regulators (Schmutterer 1990). Natural pyrethrins act, mainly by contact or ingestion, on the central and peripheral nervous system, thus causing immediately the insect paralysis (Casida and Quistad 1994). Pyrethrins are used against *B. oleae* as adulticide with a two-day pre-harvest interval.

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Rotenone is a broad-spectrum bio-insecticide (Copping 1998) which acts by contact and ingestion, inhibiting the respiratory processes. Recent studies have included rotenone among the causes of Parkinson's disease (Sherer et al. 2004).

Copper derivatives can be used in organic agriculture in accordance with the limitations set by Commission Regulation (EC) No 473/2002. The activity is exerted by the copper ion, which is mainly a fungicide, but also a repellent, an anti-oviposition elicitor and is active against the first larval stages of *B. oleae* (Girolami and Cavalloro 1972; Nuzzaci and Pizza 1995; Tzanakakis 1985).

In the present work, the post-treatment decay curves of pyrethrins, azadirachtin, rotenone and copper were studied as these substances were applied on olives in different control programmes against *B. oleae*, to assess their persistence and their transfer into the olive oil.

Materials and Methods

Trials in fields and preparation of samples

All the procedures applied to field studies were carried out under Good Laboratory Practice (GLP). The trials were carried out, in 2005 and in 2006, on two different organically cropped olive tree fields: in 2005, the field, cropped with cv. Nocellara del Belice was at the bio-farm "La Pizzica" in the area of Crispiano (Taranto, Italy); in 2006, the field cropped with cv. Coratina, was located inside the Mediterranean Agronomic Institute, in the area of Valenzano (Bari, Italy). Trees from the two cultivars were about 10 years old, and cropped, vase-trained, with a 6 x 6 (cv. Nocellara) or 8 x 8 (cv. Coratina) m planting distance. Twelve plots were identified per cultivar, each including 6 plants, which were submitted to one to three treatments with azadirachtin (Trial A), rotenone (Trial B), pyrethrum (Trial C) and copper (Trial D). Treatments were carried out with a knapsack sprayer, applying 6 or 10 L per tree, respectively in 2005 and in 2006. The product characteristics,

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4 dosage and pre-harvest interval are reported in Table I. The first sampling was
5 carried out before the applications and subsequent samplings after the
6 applications, at intervals reported in Table II.
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11 In order to evaluate the residues on the olives, each sample consisted of about
12 1 kg of olives collected from the whole canopy; in contrast, to assess the
13 residues in the oil, about 20 kg of olives, harvested following the post-harvest
14 interval for each active ingredient, were milled on the harvest day with an
15 electric grinder and the oil was extracted from the paste by centrifugation (15-
16 16% average oil content) at room temperature (12-16 °C).
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24 *Extraction of the chemical residues from olives and oil*

25 Protocols by Caboni et al. (2002) and Cabras et al. (2002) were applied to
26 extract azadirachtin, rotenone and pyrethrins from the olives: 25 g of olives
27 and 10 g of Na₂SO₄ were added to 25 mL of acetonitrile, stirred for 10 min,
28 and then sonicated for 15 min. The particulate was removed by filtering.
29 Extracts were kept at 4°C until testing. To detect the same substances in the
30 oil, the extraction protocol by Cabras et al. (2002) was applied. Acetonitrile
31 (10 mL) and 100 µL of oxalic acid (10% aqueous solution) were added to 1 g
32 of oil aliquots. The mix, stirred for 30 min, was then allowed to stand for 15
33 min for the phase separation; the organic fraction was filtered onto regenerated
34 cellulose microfilters, before being tested by HPLC.
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45 To determine copper on the olives, olive aliquots (20 g) were placed in 100
46 mL of a 10% HCl aqueous solution. After 30 min stirring, the aqueous phase
47 was recovered and then filtered prior to testing. To assess the presence of
48 copper in the oil, 50 mL of n-hexane and 15 mL of 37% HCL were added to
49 20 g of oil. The mix was brought to light boil for 30 min and then allowed to
50 cool to room temperature before the quantitative recovery of the lower
51 hydrochloric acid phase. The extraction flask was washed 3 times with 10 mL
52 of distilled water, making the mix boil lightly for 5 min; the aqueous phase
53 was recovered and added to the hydrochloric acid phase collected earlier. The
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4 solution, adjusted to 50 mL with distilled water, was eluted through a
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6 Supelclean NVI-CARB SP 500 mg column.
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10 At least three different extractions were carried out on each olive and oil
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12 sample.
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14 15 *HPLC analysis of chemical residues*

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17 The analytical determination of azadirachtin, pyrethrins and rotenone was
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19 carried out by a Thermoquest SpectraSystem[®] Mod. 1000 (Thermo Finnigan,
20
21 USA) liquid chromatograph, provided with a UV-DAD detector, model
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23 UV6000LP (TSP UV 6000 diode array detector) and a Waters X-Terra RP18
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25 (250 x 4.6 mm, 5 μ m) column. The gradients for the pesticide separation were
26
27 80 H₂O:20 acetonitrile up to 18 min, 50 H₂O:50 acetonitrile up to 22 min, 40
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29 H₂O: 60 acetonitrile, up to 27 min, 20 H₂O: 80 acetonitrile until the end of the
30
31 run. Samples of 20 μ L were injected, elution set at 1 mL min⁻¹ flow rate and
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33 the column kept at 25°C.
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36 LC analysis for azadirachtin, rotenone and pyrethrins was carried out at 215,
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38 295 and 230 nm, respectively, according to the absorbance peak of the relative
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40 UV spectra. Calibration curves were plotted by the External Standard method,
41
42 reporting the concentrations of increasing standard solutions as a function of
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44 the area of the relative chromatographic peaks. High correlation coefficients
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46 (0.995-1.000) were obtained between 0.08 and 5 mg kg⁻¹ for azadirachtin,
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48 0.005 and 5.0 mg kg⁻¹ for rotenone, 0.02 and 5.0 mg kg⁻¹ for pyrethrin II and
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50 pyrethrin I. The limit of quantification (LOQ) was 0.08 mg kg⁻¹ for
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52 azadirachtin, 0.005 mg kg⁻¹ for rotenone and 0.02 mg kg⁻¹ for pyrethrins. The
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54 recovery of the active ingredient added to untreated matrices (samples were
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56 fortified with 0.5-5 mg kg⁻¹ azadirachtin, 0.02-2 mg kg⁻¹ rotenone, 0.1-2 mg
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58 kg⁻¹ pyrethrins) was between 93 and 102%. Retention times were: 18.6 min for
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60 azadirachtin, 24.6 min for rotenone, 25.7 min for pyrethrin II and 27.3 min for
pyrethrin I.

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Copper was determined by the standard addition method by chemical stripping by the equipment ION3 (Steroglass s.r.l, San Martino in Campo, Italy). Copper determination was carried out at a potential range of -800/-100 mV, setting the electro deposition potential at -950 mV and introducing 20 ml of 1 N HCl into the chamber, including 1 ml of Hg(II) and 1 g l⁻¹ of the extract to test. The integration interval was 370/-125 mV. For the quantitative analysis 100 µL of 5 mg kg⁻¹ standard copper were added twice and three addition measurement cycles were performed. The linearity interval was determined between 0 and 1000 µg L⁻¹ (R²>0.995). When olives or oil were fortified with 0.1-1.0 mg/kg copper, the recovery ranged between 90 and 100%.

Data management

Each evaluation of residue concentration is given as the average of the values coming from three different sample extractions ± standard deviation. Such values were converted into their natural logarithms and plotted as a function of time (in days) after applications, by first-order rate equations. Half-time decay values (DT₅₀) were obtained by these plots as ln2/k, where k represents the rate constant, and reported in the tables together with correlation coefficients (R²). .

Results

In both testing years and in the experimental conditions applied, azadirachtin was not detected in any olive or extracted olive oil, even when three applications were carried out and sampling was performed immediately after olive drying. Probably, the levels of azadirachtin residues on the olives were so low as not to be detected by HPLC analysis, as a result of the low sensitivity of the method (0.08 mg kg⁻¹) and the high decay rate of this chemical.

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Rotenone decay and half-life in olives given one to three treatments are indicated in Tables III-V. For olive samples collected for each trial 12 days after the last application, the residue proved to always be much higher than the admitted maximum residue level (MRL 0.05 mg kg⁻¹ according to SO G.U. 292, issued on Dec. 14 2004). The residue of rotenone found in the olives 12 days after a single treatment was 0.40 mg kg⁻¹ (2005) and 0.37 mg kg⁻¹ (2006), whereas, after three consecutive treatments, it reached 0.52 mg kg⁻¹ (2005) and 0.46 mg kg⁻¹ (2006). In the 2006 trial including three consecutive treatments, samples were collected up to 30 days after the last application; even at a such long time after application the residue concentration was still high (0.26 mg kg⁻¹). The analysis of the decay rates indicates that, within 5 days after the application, rotenone degrades quite rapidly, and then the decay process goes on very slowly. In our experimental conditions, rotenone half-life on the olives (DT₅₀= 4.5-5.5 days; R² = 0.9367- 0.9951) proved to be slightly higher than that found by Cabras et al. (2002). The rotenone concentration found in the oil samples was always higher than that estimated on the olives (Table VI). Generally, a proportionality was observed between the number of treatments and the residue amount in the oil; indeed, even 15 days after the last treatment, rotenone residues in the oil were 16 (0.82 mg kg⁻¹) to 54 (2.69 mg kg⁻¹) times higher than the MRL (0.05 mg kg⁻¹). Residues in the oil were higher than those on the olives by a concentration factor between 2.0 and 5.5.

As regards pyrethrins, the results were expressed as the sum of pyrethrin I and II concentrations. Pyrethrins decay and half -life on olive samples are illustrated in Tables VII-IX. In general, a rapid decay (DT₅₀ = 1.0-1.5 days, R² = 0.94- 0.9971) was observed and, from the second day after each application, it resulted into a residue below the admitted MRL on the olives (1 mg kg⁻¹). No accumulation effect was reported on the olives and no residue was found in the oil.

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4 The extraction technique applied quantified copper on the olive surface,
5 regardless to the oxidation state of the metal. Results concerning copper on the
6 olives are reported in Tables X-XII. In any trial and number of treatments
7 tested, no decay of the copper ion was observed, even 50 days after
8 applications. The accumulation effect was proportional to the number of
9 applications. At the second application, the copper residue already exceeds its
10 MRL on olives (30 mg/kg). In contrast, in both 2006 and 2005 trials, copper
11 was detected in the oil at much lower concentrations than those detected on the
12 olives: concentration ranges in the oil were 0.09-0.13 mg kg⁻¹ in 2005 trial and
13 0.03-0.17 mg kg⁻¹ in 2006 trial (Table XIII).
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26 Discussion

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29 The study reported here has allowed monitoring of residue rates for pyrethrins,
30 rotenone and copper in olives and olive oil samples after different treatment
31 procedures. Although azadirachtin was one of the chemicals used in the
32 experiments, it was not possible to detect it on the treated olives, even when
33 sampling was performed within 24 hours after application, probably because
34 of the inadequate sensitivity of the method and the rapid decay of this
35 chemical. However, the actual concentrations of azadirachtin on the treated
36 olives must have been below the detection limit of the method (0.08 mg kg⁻¹)
37 and the MRL for this chemical is in fact 0.5 mg kg⁻¹ (Commission Regulation
38 (EC) No 149/2008). Thus, the quantities of azadirachtin used with the
39 described treatments applied to olive trees would result in residue levels of no
40 concern at any time after application. Also pyrethrins proved to be readily
41 degradable and the residue, regardless of the matrix, was always lower than
42 the accepted maximum limit (1 mg kg⁻¹). Therefore, the use of pyrethrins and
43 azadirachtin, within the tested conditions, may be feasible with respect to the
44 current needs of consumers and demands for environmental safety: given the
45 rapid decay observed, these substances can be applied also close to harvest, if
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Conversely, copper was the chemical which showed the slowest decay on the olives. Twenty days after the last application, as indicated in the label the residue level found was similar to that detected immediately after the application; moreover, the active ingredient increased on the olives according to the number of applications. Considering that copper-based products are generally applied to control plant pathogenic bacteria and fungi, and taking into account the strong accumulation effect on the olives, copper should only be used as an insecticide in the framework of an integrated management programme, especially when olives are to be marketed for fresh consumption. Indeed, in the oil, the metal was always found at concentrations lower than the accepted maximum limits. Since copper is concentrated in the olive aqueous fraction, it might be removed from the paste in the extracted water. It is necessary to validate this hypothesis above all for the appropriate management of vegetation water, which might contribute to copper ion accumulation when it is disposed in the soil.

Rotenone was found to be a very stable compound on olives. Its decay, was quite rapid in the early days after its application, decreased when the concentration on the olives reached 0.4–0.5 mg kg⁻¹ (values 10-fold over the limit) and continued slowly, without ever going below the maximum residue limit, not even 12 days after the treatment (two days more than the post-harvest interval). For rotenone, as well, a slight accumulation effect was found in the olives and olive oil, when two or three applications were consecutively performed. The highly lipophilic molecule (Cabras et al. 2002) markedly concentrated in the oil, where it reached an amount even five times higher than that detected on the olives (1.2–2.7 mg kg⁻¹). The effective biological activity of rotenone makes it a key active ingredient in the control of *Bactrocera oleae*, mainly in the case of heavy infection. However, a possible involvement of rotenone in Parkinson's disease was recently reported (Sherer et al. 2004). Thus, because of the possible hazard to human health, the slow degradation of rotenone on olives has caused concern about its use in the organic cropping of olive trees, as the residue concentration never falls below the legal limits, even

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4 when a single treatment is carried out. Thus, from April 10, 2008, the EU
5 Commission recently removed rotenone from Annex I of the Directive 91-
6 414-CEE, although, its use is still permitted until October 10 2009. Therefore,
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8 finding alternatives to the use of rotenone is urgent for bio-farms producing
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10 olives for the fresh market and/or olive oil. The much more rapid decay
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12 observed with pyrethrins and that it was not possible to even detect
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14 azadirachtin residues provided to olive trees in this study, taking into account
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16 the positive data on the efficacy against the olive fly, suggest that these active
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18 ingredients may represent more ecologically-sound control strategies, which
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20 may be beneficial to the consumer, the environment, the operators and the
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22 host-pest system.
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Table I. Characteristics of the chemicals applied to the olive trees

Trial	Active Ingredient	Commercial product			Product dosage	Pre-harvest interval (days)
		Company	Name	a.i. (%)		
A	Azadirachtin	Serbios	Diractin®	3	150 ml/hl	3
B	Rotenone	Serbios	Rotena®	6	300 ml/hl	10
C	Pyrethrins	BioIntrachem Italia	BioPirenPlus®	2	150 ml/hl	2
D	Copper	Caffaro	Cuprocaffaro®	50	400 g/hl	20

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Table II. Treatments, sampling and harvesting times in 2005 and 2006 trials

Active ingredient	Trial	Treatment schedule ^a		Sampling after each treatment (days)		Harvesting time ^a
2005						
Azadirachtin	A-I	-	-	03/12	0, 1, 2, 5, 9	
	A-II	-	17/11	03/12	0, 1, 2, 5	07/12
	A-III	01/11	17/11	03/12	0, 1, 2	
Rotenone	B-I	-	-	28/11	0, 4, 8, 12, 16	
	B-II	-	12/11	28/11	0, 4, 8, 12, 16	12/12
	B-III	27/10	12/11	28/11	0, 4, 8, 12, 16	
Pyrethrins	C-I	-	-	03/12	0, 1, 2, 3, 9	
	C-II	-	17/11	03/12	0,1, 2, 3, 9	06/12
	C-III	01/11	17/11	03/12	0,1, 2, 3	
Copper	D-I	-	-	14/11	0, 5, 21	
	D-II	-	29/10	14/11	0, 5,10,15	06/12
	D-III	13/10	29/10	14/11	0, 10	
2006						
Azadirachtin	A-I	-	-	03/11	0,1,2,3	
	A-II	-	18/10	03/11	0,1, 2, 3	08/11
	A-III	02/10	18/10	03/11	0,1, 2, 3	
Rotenone	B-I	-	-	31/10	0, 4, 8, 12, 16, 30	
	B-II	-	15/10	31/10	0, 4, 8, 12, 16, 30	15/11-05/12
	B-III	29/09	15/10	31/10	0, 4, 8, 12, 16, 30	
Pyrethrins	C-I	-	-	03/11	0, 1, 2, 3	
	C-II	-	18/10	03/11	0, 1, 2, 3	08/11
	C-III	02/10	18/10	03/11	0, 1, 2, 3	
Copper	D-I	-	-	15/10	0, 5, 10, 15, 21	
	D-II	-	29/09	15/10	0, 5, 10, 15	07/11
	D-III	13/09	29/09	15/10	0, 5, 10, 15, 20, 29	

^adays/month

Table III. Rotenone decay on olives after one single application in 2006 and 2005 trials

Experimental Time (days)	2006 Trial	2005 Trial
0	2,2± 0,15 ^a	2,09± 0,37
4	1,07±0,12	1,04±0,25
8	0,76± 0,14	0,81± 0,2
12	0,37± 0,05	0,40± 0,26
16	0,29± 0,1	nd
DT ₅₀	5,4	5,3
R ²	0,9767	0,9713

^aconcentration expressed as mg Kg⁻¹ ± standard deviation
nd = not determined

Table IV. Rotenone decay on olives after two consecutive applications in 2006 and 2005 trials

Experimental Time (days)	First application 2006	First application 2005	Experimental Time (days)	Second application 2006	Second application 2005
0	2,42± 0,33 ^a	2,20± 0,4	16	2,73±0,45	2,5±0,4
4	1,09±0,22	0,98±0,13	20	1,02 ±0,23	1,01 ±0,22
8	0,734± 0,12	0,71± 0,14	24	nd	0,733±0,05
12	0,39± 0,09	0,42± 0,13	28	0,42±0,07	0,52±0,07
16	0,26± 0,08	0,24± 0,11	32	0,30±0,04	nd
DT ₅₀	5,0	5,2	DT ₅₀	5,2	4,8
R ²	0,9865	0,9852	R ²	0,9595	0,9636

^aconcentration expressed as mg Kg⁻¹ ± standard deviation

nd = not determined

Table V. Rotenone decay on olives after three consecutive applications in 2006 and 2005 trials

Experimental Time (days)	First application 2006	First application 2005	Experimental Time (days)	Second application 2006	Second application 2005	Experimental Time (days)	Third application 2006	Third application 2005
0	2,56± 0,32 ^a	2,05± 0,3	16	2,74±0,21	2,40±0,3	32	3,03±0,23	2,60±0,3
4	1,0± 0,25	nd	20	1,54±0,29	1,19±0,3	36	1,83±0,32	1,33±0,32
8	0,72± 0,17	0,71± 0,2	24	0,67±0,11	0,67±0,12	40	0,87±0,14	nd
12	0,35± 0,13	0,44± 0,12	28	0,42±0,18	nd	44	0,46±0,08	0,52±0,18
16	0,23± 0,15	0,21± 0,14	32	0,29±0,12	0,32±0,14	48	0,28±0,13	0,31±0,09
DT ₅₀	4,7	5,0	DT ₅₀	4,7	5,3	DT ₅₀	4,5	5,3
R ²	0,9773	0,9934	R ²	0,9815	0,9809	R ²	0,9952	0,9956

^a concentration expressed as mg Kg⁻¹ ± standard deviation

nd = not determined

Table VI. Rotenone residues on olives at harvest and in the extracted oil in 2006 and 2005 trials

Number of applications	2005 Trial			2006 Trial					
	Olives (12 dala)	Oil (15 dala)	CF	Olives (12 dala)	Oil (15 dala)	CF	Olives (30 dala)	Oil (30 dala)	CF
1	0,37±0,05 ^a	1,18±0,06	3,2	0,41±0,01	0,82±0,05	2,0	0,13±0,03	0,27±0,02	2,1
2	0,41±0,06	2,25±0,05	5,5	0,42±0,06	2,14±0,06	5,1	0,13±0,01	0,68±0,01	5,2
3	0,56±0,08	2,69±0,08	4,8	0,46±0,02	2,50±0,05	5,4	0,26±0,05	1,04±0,01	4,0

^aconcentration expressed as mg Kg⁻¹ ± standard deviation
dala = days after last application; CF = concentration factor

Table VII. Pyrethrin decay on olives after one single application in 2006 and 2005 trials

Experimental Time (days)	2006 Trial	2005 Trial
0	0,75± 0,09 ^a	0,77± 0,15
1	0,33±0,04	0,38±0,13
2	0,27± 0,1	0,22± 0,1
3	0,18± 0,1	0,17± 0,08
DT ₅₀	1,7	1,5
R ²	0,9479	0,9684

^aconcentration expressed as mg Kg⁻¹ ± standard deviation

Table VIII. Pyrethrin decay on olives after two consecutive applications in 2006 and 2005 trials

Experimental Time (days)	First application 2006	First application 2005	Experimental Time (days)	Second application 2006	Second application 2005
0	1,2± 0,1 ^a	1,03± 0,11	16	0,88±0,11	0,8±0,11
1	0,62±0,1	0,57±0,09	17	0,54 ±0,05	0,65 ±0,05
2	0,46± 0,08	0,34± 0,08	18	0,36±0,03	0,29±0,03
3	0,22± 0,2	0,14± 0,02	19	0,16±0,1	0,16±0,08
DT ₅₀	1,25	1,06	DT ₅₀	1,26	1,07
R ²	0,9767	0,9854	R ²	0,9756	0,943

^aconcentration expressed as mg Kg⁻¹ ± standard deviation

Table IX. Pyrethrin decay on olives after three consecutive applications in 2006 and 2005 trials

Experimental Time (days)	First application 2006	First application 2005	Experimental Time (days)	Second application 2006	Second application 2005	Experimental Time (days)	Third application 2006	Third application 2005
0	1,2± 0,12 ^{aa}	1,3± 0,11	16	1,3±0,2	1,03±0,11	32	0,74±0,13	0,83±0,1
1	0,65± 0,13	0,63± 0,09	17	0,69±0,1	0,52±0,07	33	0,33±0,12	0,42±0,1
2	0,49± 0,11	0,45± 0,08	18	0,45±0,13	0,30±0,1	34	0,21±0,1	0,25±0,1
3	0,21± 0,11	0,26± 0,07	19	0,27±0,08	0,24±0,1	35	0,13±0,04	0,11±0,08
DT ₅₀	1,26	1,3	DT ₅₀	1,35	1,41	DT ₅₀	1,22	1,05
R ²	0,9658	0,9871	R ²	0,994	0,9586	R ²	0,9791	0,9928

^a concentration expressed as mg Kg⁻¹ ± standard deviation

Table X. Copper concentration on olives after three consecutive applications in 2006 and 2005 trials

Experimental Time (days)	First application 2006	First application 2005	Experimental Time (days)	Second application 2006	Second application 2005	Experimental Time (days)	Third application 2006	Third application 2005
0	18,4± 3,4 ^a	16,4± 3,7	15		32,3±3,4	30		51,0±3,5
5	18,5± 2,8	nd	20	31,9±3,1	30,9±3,1	35	54,9±2,3	50,7±5,7
10	18,6± 3,0	15,5± 2,4	25	30,4±2,1	32,5±4,5	40	nd	51,2±4,1
15	18,6± 2,2	nd	30	30,7±1,5	31,6±3,4	41	53,2±3,7	nd
20	18,0±2,8	nd	35	29,1±3,3	33,0±4,4	45	nd	52,6±3,4
						54	nd	49,3±2,2
						56	52,3±4,4	nd

^a concentration expressed as mg Kg⁻¹ ± standard deviation

nd = not determined

Table XI. Copper concentration on olives after two consecutive applications in 2006 and 2005 trials

Experimental Time (days)	First application 2006	First application 2005	Experimental Time (days)	Second application 2006	Second application 2005
0	17,9± 3,6 ^a	16,3± 3,2	15		33,2±2,6
5	17,4±4,3	14,9±2,4	20	34,1±4,1	33,1±4,0
10	16,6± 3,2	14,7± 3,2	25	nd	30,4±2,7
15	16,5± 2,8	14,3± 3,1	30	nd	30,9±2,1
			34	33,8±3,7	nd
			39	nd	29,7±3,0

^a concentration expressed as mg Kg⁻¹ ± standard deviation

nd = not determined

Table XII. Copper concentration on olives after one single application in 2006 and 2005 trials

Experimental Time (days)	2006 Trial	2005 Trial
0	14,6± 1,6 ^a	15,0±1,8
5	13,9±3,3	14,0±2,3
10	nd	14,4±1,1
15	nd	14,9±2,2
21	14,0± 2,5	14,4± 2,3

^aconcentration expressed as mg Kg⁻¹ ± standard deviation

nd = not determined

Table XIII. Copper concentration in the oil in 2005 and 2006 trials

Number of applications	2005 Trial	2006 Trial
3	0,075± 0,002 ^a	0,029±0,0025
2	0,029± 0,003	0,17±0,0045
1	0,011± 0,0013	0,077±0,0031

^a concentration expressed as mg Kg⁻¹ ± standard deviation