Decay of Fenthion in Green Table Olives

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The period needed for fenthion, an insecticide frequently used in southern Italy against the olive fly, to decline to safety levels was assessed by developing a method for extraction and gas−liquid chromatography with nitrogen−phosphorus detection, and measuring fenthion persistence in green table olives after treatments in two periods of the year when the insect is particularly active. High overall recovery (90−100%), satisfactory linearity ($r^2 = 0.998$) and precision (4−5%) in the useful working range 0.03−3.962 ppm, and a low detection limit of the active ingredient (0.004 mg/kg) were obtained. The investigation on two olive cultivars showed swift degradation of fenthion in July, according to the first-order kinetic model with a half-life of about 1 day, while in September the decay seemed to be the result of two processes, the slower of which had a half-life ranging from 3 to 7 days. The method was used to evaluate the effect of sweetening olives with 2% alkaline solution on fenthion content, and a decrease in the pesticide concentration of 7−10% was found.

Keywords: *Fenthion; GLC; table olives; residue decay*

INTRODUCTION

The cultivation of table olives is of primary importance for the economy of southern Italy. It is mostly concentrated in the regions of Sicily, Apulia, and Calabria, which provide for 80% of the national output (Marsilio, 1993). The promising expectations for this fruit require very particular care toward its quality. Unlike oil olives, table olives must be uniform in size, homogeneous in color, and absolutely devoid of any parasitic injury (Russo et al., 1987). The fulfillment of these quality standards has made necessary chemical protection against several pests and diseases through appropriate use of pesticides. For consumer protection, though, levels of pesticide residue in olive fruit must not exceed those fixed by national (Italian Ministry Decree, 1990) and international institutions (European Economic Commission, 1976; FAO/WHO Codex Alimentarius Commission, 1993, etc.).

Investigation on the extent to which it is possible to parallel commercial validity of the product and appropriate use of pesticide is important. To this purpose, a GLC method optimized to the determination of some phosphorganic pesticides in olives was developed, and the degradation of fenthion during the ripening of green table olives was monitored. Fenthion is extensively used in Apulia on olive trees against insects and scales, especially the olive fly (*Bactrocera oleae*). The study was carried out on two varieties of olives, known as Bella di Cerignola (BC) (Ferrara et al., 1984) and S. Agostino (SA) (Ferrara et al., 1981), the most cultivated green table olives in Apulia (Marsilio, 1993).

Literature on insecticide residues in olives is very limited. Ferreira (1983) and Leone et al. (1990) investigated residues in oil products resulting from supervised field trials; Lentza-Rizos et al. (1991) and Lentza-Rizos (1994) screened several chemical contaminants in olives and olive oil; Lanza et al. (1986) performed tests on the decay of two insecticides in olives; Cabras et al. (1993) investigated the fate of fenthion mainly with regard to its metabolism in olives. In this paper, the decay of fenthion was investigated in olives collected after pesticide treatment, in two periods of the year according to the activity cycle of the olive fly, to tentatively evaluate the influence of climate, growth level of drupes, tissue composition, etc. on the degradation rate of the insecticide.

Finally, the effect of the alkaline treatment for the sweetening of table olives on the elimination of fenthion residue was studied. Experiments in this field have been carried out on some phosphorylated insecticides (Albi and Rejano, 1982; Albi and Navas, 1984, 1985; Russo et al., 1987), with no data on the persistence of fenthion residue in pickled green olives.

MATERIALS AND METHODS

Field Instrumentation and Sampling. The field trials were carried out in two olive groves near Foggia, Italy, in July and September of 1995, and were repeated in the same periods of 1996. Six olive trees for each cultivar were involved in the investigation; five trees randomly chosen in each block were treated with fenthion, and one tree was untreated and used as control. The treatments with fenthion were performed when weekly captures of adult individuals of *Bactrocera oleae* indicated the attainment of the intervention threshold (4 −5 insects per trap). For this purpose, about 8 L of fenthion mixture (50 g/hL of active ingredient) per olive tree was sprayed, until dripping, using a normal-volume back-pack spraying pump (GDM, Mary-10, Italy). The treatments were accomplished on July 13, 1995, September 9, 1995, July 8, 1996, and September 9, 1996, for the S. Agostino trees; and on July 8, 1995, September 10, 1995, July 9, 1996, and September 10, 1996, for Bella di Cerignola trees. The olive samples subjected to residue analysis were collected 24 h and 48 h, and 4, 8, 12, 16, 20, 24, 28, and 30 days after the treatment. Around 1000 g of olives was randomly picked from the trees, and stored in plastic bags at −25 °C.

Sweetening Procedure. Aliquots (300 g) of olives for each variety were fully immersed in a small glass basin containing a 2% (w/w) sodium hydroxide solution. The drupes were periodically cut crosswise and the removed sections subjected to a chromatic test with a 1% (v/v) alcoholic solution of phenolphthalein. When the pink color of the alkaline reaction showed soda piercing through two-thirds of the pulp thickness (after about 8−10 h), the soda solution was siphoned out, and the olives were repeatedly washed until a pH of about 7.5 in washing water was achieved and subjected to chemical analysis.

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Extraction Procedure. About 50 frozen olives for each replicate were thawed at room temperature. The olives collected after the treatment in July, having a tender stone, were finely chopped. The olives collected in September, instead, were destoned before chopping. Aliquots of about 25 g of chopped olives were carefully weighed and put into the glass of a homogenizer together with 50 mL of acetone and 1 mL of a solution of 5.301 μg/mL chlorpyriphos-methyl in acetone used as internal standard (IS). After a 15 min pause to allow diffusion of the IS into the vegetable matrix, the mixture was homogenized for 5 min at 8000 rpm, and then filtered in a Buckner funnel through Whatman no. 40 ashless filter paper. The solid fraction underwent a further extraction with 30 mL of acetone, following the same procedure.

Filtered solutions were combined and submitted to liquid−liquid partitioning in a separatory funnel with 50 mL of petroleum ether and 50 mL of methylene chloride. After separation, the aqueous layer was subjected to a second partitioning with 40 mL of methylene chloride, making use of 0.5 g of sodium chloride to improve fenthion extraction and prevent emulsion. Organic layers were combined and treated with 30 g of anhydrous sodium sulfate, and then filtered through Whatman no. 40 filter paper followed by rinsing the sodium sulfate twice with 20 mL of methylene chloride. The filtered solution was evaporated in a rotary evaporator under vacuum at 35 °C.

The residue was dissolved in 2 mL of hexane and 20 mL of a mixture of acetonitrile/1% sodium chloride aqueous solution (95:5, v/v), and then centrifuged for 3 min at 2000 rpm. After separation and recovery of the upper acetonitrile phase, the oily residue was subjected to the same procedure for a further extraction. The extracts were collected in a flask and evaporated in the rotary evaporator, under vacuum at about 35 °C.

Sample Cleanup. A disposable cartridge (Supelclean LC-Florisil; Supelco, Bellefonte, PA) covered with 1 g of anhydrous sodium sulfate and prewetted with 2 mL of acetonitrile/methylene chloride (1:3, v/v) (solution A) was used for sample cleanup. The sample residue was dissolved with 1 mL of solution A, transferred into the cartridge, and eluted twice with 2 mL of solution A. The eluate was collected in a flask, dried in the rotating evaporator, dissolved in 1 mL of isopropyl alcohol, and again dried to remove the acetonitrile traces and prevent interference in the subsequent pesticide detection with the NPD detector. Finally, the residue, redissolved in 1 mL of toluene and filtered, underwent chromatographic analysis.

Gas Chromatographic Analysis. A Fisons 8160 gas chromatograph (Italian Fisons, Milan, Italy) was employed, equipped with a split-splitless injector, a 25 m × 0.32 mm i.d. and 0.15 μm film thickness Mega SE-52 fused silica capillary column (Mega, Legnano, Italy), and a Fisons NPD 80FL nitrogen−phosphorus detector, and connected to a Fisons DP700 integrator. Helium at a flow rate of 1 mL/min was used as carrier gas; air at 315 mL/min, hydrogen at 5 mL/min, and nitrogen as makeup gas at 20 mL/min were used for operating the NPD detector. The operating temperatures of injector and NPD were 250 and 300 °C, respectively. The sample injection (1 μL) was splitless with a purge of 60 s. The oven temperature program was 105 °C for 1 min, raised to 280 °C at increments of 6.5 °C/min, and held for 20 min.

Chemicals. Petroleum ether, acetone, methylene dichloride, hexane, isopropyl alcohol, and acetonitrile were residue analysis quality (Prolabo, Fontenay, France). Sodium chloride and sodium sulfate were analytical grade reagents (Carlo Erba, Milan, Italy). Fenthion and chlorpyriphos-methyl were analytical standards purchased from Ehrenstorfer (Augsburg, Germany). Standard stock solutions were 8.130 mg/mL fenthion in acetone and 8.835 mg/mL chlorpyriphos-methyl in acetone. All the solutions were prepared using reagent grade water obtained by reverse osmosis using a Milli-RO plus module and a further purification with a Milli-Q plus unit (Millipore, USA).

Method Assessment. Recovery assays and quantification of fenthion residues were accomplished by the internal standard method, measuring peak areas and using a response factor. The use of internal standards ensured that the between-day RSDs were not significantly different from the within-day RSDs obtained for the standards. The response factor was determined by adding 25 g of untreated homogenized olives to a mixture of a 1 mL standard solution of chlorpyrifos-methyl in acetone (5.301 μg/mL), and a 1 mL standard solution of fenthion in acetone (5.284 μg/mL), and subjecting the mixture to the extraction procedure of the two residues as already described under Materials and Methods.

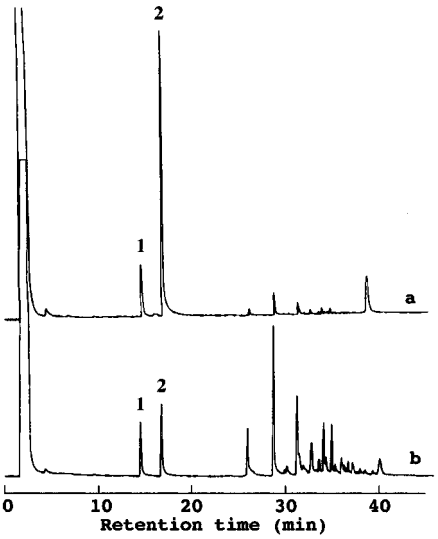

Figure 1. Gas chromatograms of S. Agostino olives picked 4 days after the pesticide treatments of September 1996 (a) and July 1996 (b). The chromatographic conditions are those reported under Materials and Methods. (1) Chlorpyriphos-methyl; (2) fenthion.

The linearity of the NPD response, y (peak area counts), versus fenthion concentration, c (ppm), was tested using five standard solutions, each in triplicate. The regression line was $y = (139 \pm 45) + (5.63 \pm 0.05)10^3c$. In this equation, the intercept was not significantly different for zero, at a 95% confidence level. The linearity of the response was satisfactory ($r^2 = 0.9979$) and was verified over the range 0.003−3.962 ppm. Under the reported chromatographic conditions, the minimum detectable amount of fenthion was 10.162 pg, at a signal-to-noise ratio of 3 and for five injections of the standard solution. The detection limit of fenthion residue in 25 g of untreated olives was 0.004 ppm.

The efficiency of the extraction and cleanup procedures was controlled by recovery assays on olives at various fortification levels. Samples were fortified by adding appropriate aliquots of fenthion standard solutions to each replicate portion of untreated olives, prior to the addition of the extracting solvents. The results showed fenthion losses of about 2%.

RESULTS AND DISCUSSION

Figure 1 shows typical chromatograms for SA olive samples under the optimal conditions reported in the experimental section. Table 1 reports the fenthion residues determined in the olives of both varieties collected during the field trials. The precision of the determinations ($n = 3$) ranged from 4−5% for most of the samples to 15−20% for levels of fenthion less than 0.03 ppm.

The measured persistence data of fenthion were fitted using the normal first-order decay model $y = c_0 \times \exp(-kt)$. A faster degradation rate of the pesticide in July resulted for both olive varieties, with mean half-life values of 0.96 day for SA and 1.01 days for BC; in September, the mean half-life values were 2.39 days for SA and 1.79 days for BC.

While the first-order decay model fitted well to the fenthion residue values of the July trials, it did not yield the same acceptable fits to the residue values deter-

Table 1. Residues of Fenthion, Parts per Millions (±SD), $n = 3$, in Two Varieties of Green Table Olives after Insecticide Treatment

	days after treatment									
sample[a]	1	2	4	8	12	16	20	24	28	30
SAJUL95	1.754 (0.042)	0.878 (0.030)	0.213 (0.016)	0.013 (0.004)	0.008 (0.003)	nd	nd	nd	nd	nd
SASEP95	2.191 (0.054)	1.482 (0.042)	0.788 (0.019)	0.401 (0.016)	0.269 (0.014)	0.184 (0.012)	0.118 (0.010)	0.076 (0.011)	0.041 (0.006)	0.034 (0.006)
SAJUL96	1.764 (0.047)	0.834 (0.034)	0.178 (0.015)	0.085 (0.011)	0.006 (0.003)	nd	nd	nd	nd	nd
SASEP96	2.576 (0.063)	1.745 (0.055)	0.868 (0.035)	0.456 (0.016)	0.246 (0.009)	0.156 (0.005)	0.075 (0.005)	0.031 (0.004)	0.007 (0.002)	0.005 (0.002)
BCJUL95	0.918 (0.035)	0.532 (0.017)	0.103 (0.007)	0.012 (0.004)	0.005 (0.002)	nd	nd	nd	nd	nd
BCSEP95	1.453 (0.051)	0.861 (0.031)	0.445 (0.015)	0.141 (0.010)	0.056 (0.008)	0.024 (0.004)	0.014 (0.003)	0.008 (0.003)	0.005 (0.001)	nd
BCJUL96	1.117 (0.042)	0.505 (0.020)	0.233 (0.011)	0.0071 (0.007)	nd	nd	nd	nd	nd	nd
BCSEP96	1.724 (0.044)	1.011 (0.034)	0.535 (0.021)	0.275 (0.013)	0.113 (0.012)	0.052 (0.007)	0.026 (0.003)	0.0120 (0.003)	0.009 (0.002)	0.006 (0.001)

[a] S. Agostino (SA) and Bella di Cerignola (BC) olives collected in July and September 1995 and 1996; nd = not detectable.

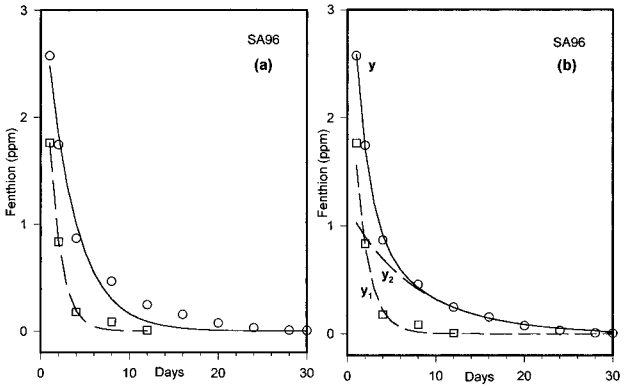

Figure 2. Fits of the fenthion residues in the S. Agostino olives collected in the field trials of July (□) and September (○) 1996. (a) Fit using the normal first-order decay model $y = c_0 \times \exp(-kt)$; (b) fit using the double exponential decay model $y = y_1 + y_2$, with $y_1 = c_0' \times \exp(-k_1 t)$ and $y_2 = c_0'' \times \exp(-k_2 t)$.

mined in the September trials, as can be seen in Figure 2a where only the fits to the persistence data of fenthion in S. Agostino olives collected in 1996 are shown for the sake of shortness.

The September fenthion data sets were more correctly fitted by a double exponential equation $y = y_1 + y_2$ with $y_1 = c_0' \times \exp(-k_1 t)$ and $y_2 = c_0'' \times \exp(-k_2 t)$. It was found that, regardless of the olive variety, the steeper component of this model, y_1 (see Figure 2b), fitted well also to the residue values of the July trials, and y_2 mainly described the persistence of fenthion in the olives collected in September from the 8th day after the treatment. From data analysis, we can infer that, for the examined green olive cultivars, and under the environmental conditions of the field trials carried out, the degradation of fenthion in July occurs according to the normal first-order decay law with a half-life of about 1 day; fenthion degradation in September occurs according to a bimodal decay process: at the beginning, it is as fast as in July, and in the course of late ripening its rate slows down to a half-life ranging from 3 to 7 days.

In order to assess the effect of sweetening, a treatment made for preparation of table green olives, on fenthion content, aliquots of olives collected after the September pesticide treatments underwent residue analysis with the method described here, before and after the sweetening treatment. A moderate decrease of the insecticide concentration ranging from 7% to 10% was determined, as reported in Table 2.

Table 2. Loss of Fenthion Residue in Green Table Olives after the Sweetening Treatment with 2% Alkaline Solution

sample[a]	residue before sweetening (ppm)	residue after sweetening (ppm)	loss (%)
SASEP96	2.576 ± 0.073	2.297 ± 0.045	10.83
SASEP96	1.745 ± 0.055	1.587 ± 0.015	9.05
SASEP96	0.868 ± 0.041	0.792 ± 0.019	8.76
BCSEP96	1.724 ± 0.094	1.583 ± 0.010	8.18
BCSEP96	1.011 ± 0.084	0.933 ± 0.008	7.72

[a] S. Agostino (SA) and Bella di Cerignola (BC) olives collected after the insecticide treatments of September 1996.

CONCLUSIONS

The developed method proved to be precise and accurate for the determination of fenthion residues in olives. The investigation on the decay of fenthion in the two examined olive cultivars highlighted different degradation laws of the pesticide; the degradation rate, fast in July, seemed to be influenced in September by a concurrent slow decay process with more importance in late ripening of the olive fruit.

Notwithstanding the slow decay rate of fenthion in September, this investigation confirmed the observance of the 28 day preharvest interval imposed by Italian law to be sufficient to keep fenthion residue below the legal maximum residue limit (0.8 ppm).

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