Residues of Fungicides on Greenhouse Lettuce

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Lettuce cv. "Iceberg", cultivated in a greenhouse, was harvested at the time of its commercial maturation, and the residual concentrations of thiram, cymoxanil, benomyl, vinclozolin, and BMC were determined. The analyses were conducted with a reversed-phase high-performance liquid chromatographic method that possesses lowest detectable limits between 0.03 and 0.06 ppm; the recovery of pesticides from the lettuce, performed with a hexane–chloroform, 3:2 (v/v), mixture, varies from 52.0% to 110.9%. The results of analyses showed that residues were not found in the internal leaf samples at the lowest detectable levels for those pesticides; the residue concentrations, though, are very high in the external leaves and this leads to the supposition that, with treatments in the greenhouse, the safety time is not sufficient to guarantee lettuce with residue levels near the limits fixed by the laws.

Among the vegetable species cultivated in the greenhouse, lettuce tends to be increasing, since, due to its brief cultivation cycle (2 months), it can be easily interposed between other cultivations. It is well-known that the particular microclimate of the greenhouse, characterized by high temperature and humidity, favors the development of the fungal infections *Botrytis cinerea* and *Bremia lactucae*, diseases that are controlled by the use of various fungicides (Worthing, 1979). Those of most common usage in our environmental conditions are thiram (I), cymoxanil (II), benomyl (III), and vinclozolin (IV) (Figures 1 and 2). These fungicides are normally sprayed at fixed intervals.

With all other vegetables cultured in the greenhouse (tomatoes, eggplants, etc.), the commerical part represents a minimal portion of the whole plant; with lettuce, on the other hand, it coincides with the plant itself. Besides, lettuce is planted according to a plan that causes, in a short time, the complete coverage of the earth, due to the physical character of the lettuce itself. So, the pesticide sprayed deposits in an almost quantitative measure on the lettuce. Since the quantity of active ingredient (a.i.) used to control the pests in various vegetables, including lettuce, is the same, due to the characteristics of lettuce previously explained, the quantity of active ingredient that deposits per kilogram of edible part is much greater than both that of other vegetables and the maximal quantity fixed by laws (Italian Health Department Act, 1979). Evidently, this causes a potentially elevated toxicological risk, for which we wanted to study if, in the conditions described, in lettuce harvested at maturation, there exist high residual levels of fungicides (I–IV).

EXPERIMENTAL SECTION

Materials and Methods. The trial has been carried out inside a 500-m^2 greenhouse with structure of galvanized iron, air warmed and equipped with a drop irrigation system.

The lettuce employed was Iceberg hybrid Mesa 659 type, planted according to a plan of five rows 24 cm apart, resulting in a plant density of 17 m^{-2}.

The sampling procedure was performed at the time of commercial maturation and the analytical samples were prepared by cutting the lettuce in four slices.

The external leaves (first and second layers) were separated from the crop and analyzed separately. The pesticide applications were performed by spraying with portable mechanical sprayers solutions prepared from formulations according to the manufacturer's suggestions.

The extraction procedure was carried out as follows. Twenty grams of triturated leaves with 40 mL of a hexane–chloroform, 3:2 (v/v), mixture were shaken with a Multi-Wrist shaker (Lab-Line, Rome, Italy) for 10 min. After filtration through Millipore filters (0.45 μm), 2.0 mL of the organic extract was evaporated to dryness under reduced pressure ($T < 40$ °C). The residue was then recovered with 1.0 mL of a water–acetonitrile, 50:50, mixture.

Apparatus. A liquid chromatograph, Varian 5020, equipped with a UV–vis UV 50 detector, heater block, Hewlett-Packard 3390 A reporting integrator, and Valco AH 20 automatic injector (loop 50 μL) and a Spectra Phisics 8075 equipped with a 770 spectrophotometric detector, CDS 111 L data system, Hitachi 56 recorder, and Rheodyne 7105 injector (loop 50 μL) were used. Calculations on HP 3390 A and CDS 111 L were made by the external standard method.

Chromatography. Columns of Hibar LiChrosorb RP-18 (250 × 4.0 mm i.d., 10 μm, Merck, Darmstadt, West Germany) were employed with a 1:1 mixture of CH_3CN and a solution of H_2O + 1.2% (v/v) NH_4OH (32%) as the mobile phase at the flow of 1.5 mL/min. According to Farrow et al. (1977), the NH_4OH had to be used to allow the BMC elution. It would be noted, however, that the eluting mixture pH (≃12) leads to a relatively fast hydrolisis of the stationary phase, and the column loses its efficiency in about 3 days of continuous use.

The best wavelength for the simultaneous determination of the pesticides under study was found to be 220 nm, according to their UV spectra. Standard curves of each pesticide were constructed by plotting peak areas (external standard method) vs. concentrations. Good linearity was found in the range 0–10 ppm.

Chemicals. Water was distilled twice and filtered through a Millipore apparatus before use; acetonitrile, hexane, and chloroform were HPLC grade (Carlo Erba, Milan, Italy); the NH_4OH solution (32%) was RP grade (Carlo Erba, Milan, Italy).

Analytical standards of benomyl (≥99.0%) and cymoxanil (≥98.8%) were furnished by du Pont de Nemours Italia S.p.A. (Milan, Italy) and vinclozolin (≥99.0%) by BASF Agritalia (Milan, Italy). BMC (≥99.0%) and thiram (≥99.0%) were purchased from Hoechst (Pestanal, Milan, Italy).

RESULTS AND DISCUSSION

Table I shows the percent recovery obtained by extraction from lettuce fortified with known amounts of the pesticides I–V. With regard to this it is noteworthy that benomyl tends to degrade during the operations of ex-

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Figure 1. HPLC chromatogram of lettuce blank (b, dotted line) and of a standard mixture of pesticides (s). Peaks: (1) cymoxanil (1.0 ppm); (2) BMC (1.0 ppm); (3) benomyl (1.0 ppm); (4) thiram (3.0 ppm); (5) vinclozolin (1.5 ppm). Chromatographic conditions: column RP-18, mobile phase H_2O–CH_3CN, 50:50, plus 0.6% NH_3; flow rate 1.5 mL/min; sample size 50 μL; UV detector at 220 nm; 0.032 AUFS; temperature 30 °C.

Table I. Percent Recovery from Fortified Lettuce[a]

fungicide	fortification level, ppm	% recovery ± SD
thiram	0.50	75.3 ± 7.6
	1.00	85.2 ± 11.0
	2.00	108.7 ± 18.3
cymoxanil	0.50	62.3 ± 7.6
	1.00	52.0 ± 12.9
	2.00	62.0 ± 6.1
benomyl	0.50	*b*
	1.00	88.4 ± 4.0[c]
	2.00	*b*
vinclozolin	0.75	104.9 ± 5.5
	1.50	104.9 ± 9.1
	3.00	92.7 ± 7.2
BMC	0.50	99.8 ± 11.7
	1.00	110.9 ± 12.9
	2.00	109.7 ± 10.8

[a] Results are the means of four replications. [b] Not determined. [c] As BMC.

traction from the lettuce, for which it was always determined as BMC (V). Such behavior appears to conform with that already known in the literature (Chiba and Cherniak, 1978).

Fortification was made at three levels; to each 20-g sample 10, 20, and 40 μL of a 1000 ppm of solution of each pesticide were added, so that the concentrations of the pesticides in those samples were equivalent to 0.5, 1.0, and 2.0 ppm, respectively (vinclozolin does not follow this guideline).

The recovery obtained appears satisfactory for all pesticides, except cymoxanil. Nevertheless, we also used this system for I because the extraction conducted with other solvents (petroleum ether, benzene) and different mixtures (hexane–chloroform) furnished extracts full of interfering materials (benzene and hexane–chloroform mixtures) or reduced recoveries (petroleum ether) for all the pesticides examined. With the above reported method we obtained the minimal detectable values (MDV) varying betweetn 0.03 and 0.06 ppm (see Table II). MDV were considered those concentrations giving a signal 2 times higher than

Table II. Residues of Fungicides on Lettuce

fungicide	sprayed amount, g/ha	spraying date	sampling date	days after spraying	safety times, days	lettuce prodn, kg/m²	MTDA,[a] mg/kg	residues in leaves,[b] ppm: external leaves	residues in leaves,[b] ppm: edible part	legal limits, ppm	MDV, ppm
cymoxanil	890	2/2/82					12				
	890	2/8/82					12				
	890	2/15/82	3/2/82	15	10	7.6	12	1.20	⩽0.06	0.1	0.06
thiram	14280	1/28/82	3/2/82	33	10	7.6	186	11.62	⩽0.03	3.0	0.03
benomyl	890	12/17/81					12				
	890	1/15/82					12				
	890	1/26/82	3/2/82	35	15	7.6	12	3.60[c]	⩽0.06[c]	1.0[c]	0.06[c]
vinclozolin	2680	12/31/81					35				
	2680	1/7/82					35				
	2680	1/20/82	3/2/82	41	21	7.6	35	1.85	⩽0.05	1.5	0.05

[a] See the text. [b] Results are the means of four replicate determinations performed on 20 samples. [c] As BMC.

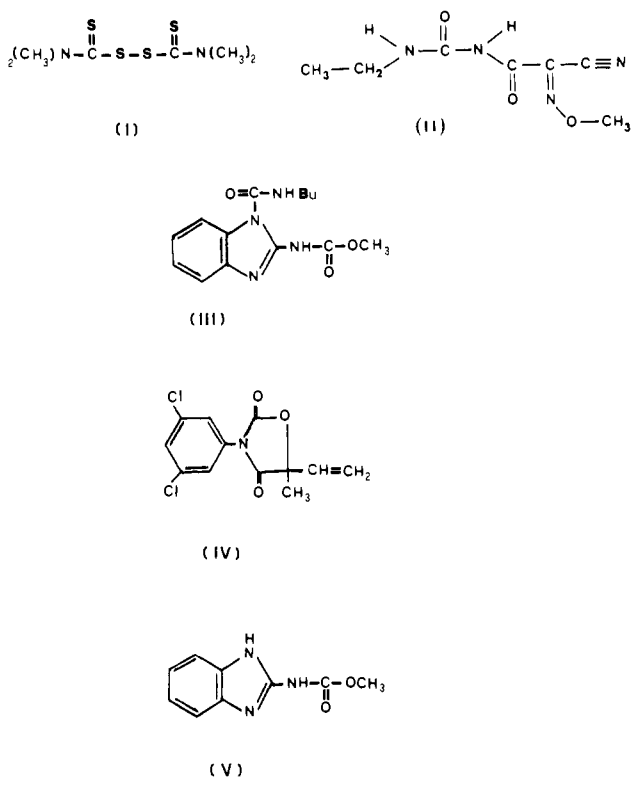

Figure 2. Pesticide formulas: (I) thiram, (II) cymoxanil, (III) benomyl, (IV) vinclozolin, and (V) BMC.

the blank signal, at the studied pesticides retention times when 50 μL of extract were injected.

The results are shown in Table II. Also shown is the MTDA, empirical quantity calculated considering the amount of active ingredient sprayed per square meter, the number of lettuces per square meter, and their average weight determined at harvest. This quantity represents the maximum theoretically detectable amount of pesticide contained in the lettuce.

It is of note that the values of the MTDA vary from 186 mg kg^{-1} for thiram to 12 mg kg^{-1} for benomyl and cymoxanil; such quantities are much higher (12–120 times) than the legal limits allowed in Italy for lettuce.

The harvesting of the lettuce was always carried out at maturation and also after the expiration of the safety period.

These lettuces were prepared by separating the external leaves from the internal ones, which represent the edible part of the plant.

On the internal leaves residues in concentrations exceeding the lowest detectable limits of the method were not encountered, while on the external leaves the residues were quite elevated.

This difference between external and internal leaves can be explained by the physical formation of the cultivar of the lettuce studied. In fact, the external leaves completely cover the internal ones, forming a true shell that protects the internal leaves.

The average values on the external leaves are slightly above the legal limit in the case of vinclozolin; with thiram and benomyl these values are 3.6 times higher and with cymoxanil 12 times higher than the limit. With regard to benomyl, it was very likely that a fairly high percentage of intact benomyl was still present as residues on the plant (Baude et al., 1973; Chiba and Veres, 1981), but its determination was made as BMC because we wanted to use a common method for all the pesticides tested.

The values found in the external leaves demonstrate that, in the condition within the greenhouse, elevated residual levels can be encountered even when the safety times have been accurately respected (e.g., cymoxanil 16 days vs. 10 days expected).

The above reported data appear to be in accordance with that of other authors (Van Wambeke et al., 1980; Dejonckheere et al., 1982); therefore, we are led to believe the following: (1) The lettuce cultivated in greenhouses should undergo frequent controls to ascertain whether or not they contain elevated, and toxicologically dangerous, residues. In fact, even though the external leaves are usually eliminated and not placed on sale, not all the cultivar of lettuce possess the form of Icerberg and, of consequence, dangerous levels of residues could be found on the internal leaves (Van Wambeke et al., 1980). (2) Safety times, as also noted by other authors (Dejonckheere et al., 1982), for treatments in greenhouses do not appear to guarantee the finding of residues below the legal limits, for which it would be opportune to revise and prolong them to obtain an increased toxicological security.

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LITERATURE CITED

Baude, F. J.; Gardiner, J. A.; Han, J. C. Y. *J. Agric. Food Chem.* **1973**, *21*, 1084.
Chiba, M.; Cherniak, E. A. *J. Agric. Food Chem.* **1978**, *26*, 573.
Chiba, M.; Veres, D. F. *J. Agric. Food Chem.* **1981**, *29*, 588.
Dejonchkheere, M.; Verstraeten, R.; Steurbaut, W.; Melkebeke, G.; Kips, R. H. *Pestic. Sci.* **1982**, *13*, 351.
Farrow, J. E.; Hoodless, R. A.; Sargent, M.; Sidwell, J. A. *Analyst (London)* **1977**, *102*, 752.
Italian Health Department Act (Jan 6, 1979) *Gazz. Uffic. Repub. Ital.* **1979**, *Feb 8*, 1282.
Van Wambeke, E.; Vanachter, A.; De Wit, A. *Parasitica* **1980**, *36*, 117.
Worthing, C. R., Ed. "The Pesticide Manual", 6th ed.; British Crop Protection Council: Croydon, England, 1979.

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