

Pesticide residue monitoring in green beans from Souss-massa (Morocco) and half-life times of dithiocarbamate fungicide on green beans after field treatments by Mancozeb and Mefenoxam

**L. BAZZI^{1,2}, M. ZOUGAGH^{3*}, R. SALGHI¹, A. HORMATALLAH⁴,
A. LEMERHYERATTE^{1,2}, M. MIHIT¹ and A. CHAKIR⁵**

¹Laboratoire d'Ingénieries des Procédés de l'Energie et de l'Environnement, Ecole Nationale des Sciences Appliquées, B.P 1136 Agadir (Maroc).

²Laboratoire Régionale de l'Etablissement Autonome de Contrôle et de Coordination des Exportations d'Agadir (Maroc).

³Department of Analytical Chemistry and Food Technology, Faculty of Chemistry, University of Castilla-La Mancha, Av. Camilo José Cela s/n, E-13004 Ciudad Real (Spain).

⁴Laboratoire des pesticides, Institut Agronomique et Vétérinaire Hassan II, Complexe Horticole d'Agadir (Maroc).

⁵GSMA, UMR CNRS 6089, Département de Chimie, laboratoire de Chimie-Physique, Faculté des Sciences, université de Reims, UMR 6089, Moulin de la housse, BP :1039, FR-51687 Reims Cedex 2 (France).

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ABSTRACT

Two hundred and fifteen green beans samples from an agricultural region of the Souss (Agadir, Morocco) were analyzed for endosulfan, dithiocarbamate, bifenthrin, chlorothalonil, iprodion, procymidon and deltamethrin. Of 189 samples that contained pesticide residues, dithiocarbamates (mancozeb and mefenoxam) were detected in 43 (20 %) in the concentration range of 0.045 to 2.651 mg Kg⁻¹. Four of them exceeded European Maximum Residue Limits (MRLs). Therefore, residue levels of mancozeb and mefenoxam were determined in green beans in an experimental greenhouse, during 17 days in which three consecutive treatments with both pesticides were applied. Sampling was carried out at 1 to 18 days after each multiple application, simulating the typical harvesting practices in greenhouse plantations. Residue levels of mancozeb and mefenoxam were determined by using a simple method consisting in a hot acid digestion of the whole sample to evolve carbon disulfide (CS₂), which is further quantified by spectrophotometry. During the study, residue levels in the plantation ranged between 1.99 and 0.0012 mg/kg for mancozeb and between 1.88 and 0.001 mg/kg for mefenoxam, with median values of 0.3425 and 0.3071 mg/kg respectively. The residual concentrations after the p.i. were high than legal limits in four samples. This finding indicates the need for careful control of the spraying doses of this fungicide in the green beans.

Key words: Pesticide residues, MRLs, green beans, persistence, dithiocarbamate.

INTRODUCTION

Introduced 40–70 years ago, dithiocarbamate fungicides (DTCs) still represent

an important class widely used in agriculture. They are characterized by a broad spectrum of activity against various plant pathogens, low acute mammal toxicity, and low production costs. In combination

with modern systemic fungicides, they are also used to manage resistances and to broaden the spectrum of activity. It therefore came as no surprise that the so-called "maneb group" (zineb, maneb, mancozeb, propineb, metiram) were some of the most frequently detected pesticides in the European Union, and that this group also had the highest frequency in exceeding maximum residue limits (MRLs)¹.

Mancozeb and mefenoxam (Figure 1), represent frequently used fungicides in the Morocco and also in EU countries. Mancozeb (ethylene[bis]dithiocarbamate), is a broad range contact fungicide used for control of wide variety of crops and seed treatment. It is applied especially for potato, green beans, vegetables and fruits protection. Mefenoxam [methyl N-(2,6-dimethylphenyl)-N-(methoxyacetyl)-D-alaninate], is the R-enantiomer of metalaxyl and has been on the market since 1996. It has activity against fungal pathogens of the order Peronosporales which cause late blight, downy mildew, damping off, and stem and fruit rots of many plants. Because of its broad spectrum activity, mefenoxam is registered for use on a wide range of crops and in several countries in temperate, subtropical and tropical regions.

In Morocco, mancozeb is commercially available through Basf under the trade name Dithane M45, whereas mefenoxam is commercially available from DuPont under the trade name Ridomil Gold 68 WG, and both pesticides are already widely used on different vegetable crops as effective substitutes for some traditional pesticides whose use in Morocco is being greatly restricted. Green beans production is widely diffuse in the Mediterranean area. Morocco is the major producer followed by Italy, Spain, Greece and France².

To the best of our knowledge, the study of mancozeb and mefenoxam residues in food particularly in green beans has not reported to date, but many papers describing analytical methods have been published³⁻¹². The most commonly used methods include carbon disulfide (CS₂) with spectrophotometry³⁻⁵ or gas chromatography⁶⁻⁸ and direct liquid chromatography using UV diode array (LC-UV)⁹, chemiluminescence¹⁰, electrochemical¹¹ or mass spectrometry (LC-MS)¹². However, these

direct methods are not in compliance with established regulatory rules relating to dithiocarbamate maximum residue levels (MRLs) in most countries, where the residue is defined as CS₂.

For the analysis of DTCs residues in foodstuffs, the official methods used are based on a hot acid digestion of the whole sample to evolve carbon disulfide (CS₂), which is further quantified by spectrophotometry or gas chromatography. These methods were early developed by many authors¹³⁻²⁴ following a method used in the rubber industry for the determination of vulcanization accelerators^{25,26} and consisting in CS₂ determination using a diethylamine/copper acetate reagent dissolved in a mixture of triethanolamine and ethanol, when yellow copper diethyldithiocarbamate is formed and measured at 430 nm. In 1974 Gullen²⁷ modified these methods and demonstrated that technical-grade triethanolamine contained high percentages of diethanolamine, which also entered into the color reaction forming a yellow copper chelate (λ_{max} 435 nm). He therefore simplified the reagent, just using diethanolamine and copper acetate dissolved in ethanol. But he pointed out that two chelate complexes (1:1 and 1:2) are formed with absorption maxima of 380 nm and 435 nm, depending upon the copper/CS₂ ratio. Two differently concentrated color reagents of 0.016 g/L and 0.048 g/L copper acetate were therefore suggested for the determination of 200–1000 μ g and <200 μ g CS₂, respectively. Cullen also substituted the lead-acetate scrubbing solution, formerly used, with zinc acetate, which facilitated cleaning the trap who recognized that technical-grade triethanolamine contained high percentages of diethanolamine, which also entered into the color reaction forming a yellow copper chelate (λ_{max} 435 nm). He therefore simplified the reagent, just using diethanolamine and copper acetate dissolved in ethanol. But he pointed out that two chelate complexes (1:1 and 1:2) are formed with absorption maxima of 380 nm and 435 nm, depending upon the copper/CS₂ ratio. Two differently concentrated color reagents of 0.016 g/L and 0.048 g/L copper acetate were therefore suggested for the determination of 200–1000 μ g and <200 μ g CS₂, respectively. Cullen also substituted the lead-acetate scrubbing solution, formerly used, with zinc acetate, which facilitated cleaning the trap.

This paper presents the results of pesticide residue monitoring in green beans from souss-massa (Morocco) and evaluation of the degradation behavior and residue levels of mancozeb and mefenoxam in green beans grown in a plastic greenhouse using the typical horticultural practices of this type of plantation (i.e., multiple pesticide applications and short preharvest intervals (p.i.). The residual concentrations after the p.i. were high than legal limits in four samples. This finding indicates the need for careful control of the spraying doses of this fungicide in the green beans.

MATERIAL AND METHODS

Chemical and reagents

All chemicals and solvents were of analytical and chromatographic grade. The organic solvents were obtained from Panreac (Barcelona, Spain). Analytical-grade reagents and deionized, double-distilled water were used throughout the experiment. All standard except dithiocarbamates were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Each standard was dissolved in acetone to obtain stock solutions of approximately 200 mg/L, which were stored light protected at 4 °C until further use. The freshly working standard solutions were obtained by dilutions with n-hexane. Florisil adsorbent (16–30 mesh) was obtained from Sigma–Aldrich (St. Louis, MO, USA).

Mancozeb and mefenoxam were obtained from Riedel-de Haen (Germany). Stock solution of Mancozeb and Mefenoxam was made by dissolving 10 mg in 100 mL of ethanol (Merck, Germany). The working standards were prepared by appropriate dilution of the stock solutions. Ethanol, potassium iodide, chloride acid, stannous chloride...ect, were purchased from Sigma (St. Louis, MO, USA).

Apparatus and Instruments

Green beans samples were ground using a food processor (Type, model blender). Analysis of the all pesticides except dithiocarbamates were carried out with a Hewlett–Packard 6890 gas chromatograph equipped with an ECD Detector, split/splitless injection port, and fused silica capillary column HP-5 column (5% diphenyl and 95% dimethylpolysiloxane, 25 m x 0.32 mm ID, 0.52 µm

film thickness); and temperature programming from 80°C to 250°C (15 °C/min); injector temperature 250 °C and detector temperature 300 °C. Carrier gas (helium) flow rate, 2.6 mL/min; makeup gas (nitrogen) flow rate, 60 mL/min; injection volume, 1 µl; and splitless time, 0.1 min. Data were acquired and the equipment controlled by using HP Chem-Station software, which was run under Microsoft Windows NT on an hp compatible personal computer. The identification of the a.i. peaks and the absence of interfering substances were assessed by comparing the sample chromatograms with those of a pesticide standard mixture and of an untreated sample. The internal standard method was used to quantify the residues by measuring peak areas vs. concentrations.

Analysis of dithiocarbamate fungicide residues in green beans were carried out with a shimadzu spectrophotometer equipped with an UV visible at 435 nm and 1-cm matched quartz cell.

Greenhouse plantation, treatments and sampling

The investigation was conducted in 2007-2008 in a plastic greenhouse, located in Massa (Agadir, Morocco). The plastic greenhouse size was 500 m², and the green beans planting density was 12050 plants/ha. Residue levels of dithiocarbamate were determined in green beans, during 18 days in which three consecutive treatments with Dithane M45 or Ridomil Gold 68 pesticide was applied to the different plantations (Table 1 and Table 2). The details of pesticides and doses used of treatment are summarized in Table 3. The chemical was sprayed onto the trees until run-off using a gun sprayer. Application was made with three replicates of 15 trees per line, and an equivalent number of trees was not treated and used as control.

In all cases, the greenhouse samples consisted of 30 pieces of green beans taken at random according to the method described by²⁸.

The daily maximum/minimum temperatures inside the greenhouse throughout the study were 12 and 24°C, whereas the daily medium relative humidity was 90 %. The irrigation flow was 40 m³/ha.

Sample preparation, processing and analysis

Immediately after picking, each sample consisting in 120 fruits were put into polyethylene bags, transported to the laboratory, and stored in the cold room at 4°C for further use. From the 120 fruits sample per grower, only a sub-sample of 30 fruits were chosen for analysis of pesticides residues.

For the extraction of pesticides except dithiocarbamates from green beans samples, the method was adapted from Charles and Raymond²⁹. For each 50 g of the sample ground using a food processor (Type, model blender), 150 ml of acetone was added and the mixture was homogenised for 2 minutes and after mixed during 2 hours. The mixture was filtered with glass wool. After filtration, the acetone residues were partitioned with saturated aqueous sodium chloride (30 ml) and dichloromethane (70 ml) in a separating funnel. The extraction was repeated with other 70 ml of dichloromethane and dried over anhydrous sodium sulphate. The dichloromethane fraction was collected and evaporated on a rotatory vacuum evaporator at 40°C and the residues were dissolved in an acetone-hexane (1:9) mixture (10 ml). For clean-up, 1 ml of the extract was passed through a florisil column previously conditioned with 5 ml of acetone/diethyl ether (6:4) and 5 ml of diethyl ether. The pesticide residues were eluted with acetone/diethyl ether (6:4) (4 ml). Samples were analysed by gas chromatography.

For determination of Mancozeb and mfenoxam, the extraction method used was that described previously by Gullen²⁷. It consists in two traps mounted in series. The first trap contains 15 mL of 10% NaOH solution, and the second contains 15 mL of color reagent. 100 g of grounded sample, 10 g of KI, 2 g of SnCl₂ and 200 mL of H₂O were put in the two-neck round-bottom boiling flask placed in a heating mantle and the acid decomposition of the dithiocarbamates take place with adding HCl. This flask is connected to air inlet tube and to a condenser, which is then connected to the proposed CS₂ reaction system.

After cooling at room temperature, 15 mL color reagent was added to CS₂ solution and diluted to 25 mL by adding ethanol and homogenized by

shaking. The absorbance of the solutions was measured at 435 nm against the reagent blanks prepared in the same way. The concentration of Mancozeb/mfenoxam was calculated using calibration graph, which was prepared by measuring absorbance with a series of standard solutions treated in the same way as sample solutions.

RESULTS AND DISCUSSION

Frequency, concentration ranges and identity of pesticides found in the samples analyzed are represented in Figure 2. Of the 215 samples analyzed, pesticides were detected in 189 (88 %) but the levels of pesticides were below the MRLs established by either, the Morocco Legislation or the European Union (EU) except in ten samples. The most commonly detected pesticide was endosulfan followed by dithiocarbamate, bifenthrin, chlorothalonil, iprodion, procymidon and deltamethrin. Folpet, fenhexamide, cypermethrine, tau-fluvalinate and cyhalothrine were detected in few samples.

Endosulfan was detected in 52 samples (24 %) at levels ranging from 0.011 to 0.124 mg kg⁻¹. Dithiocarbamate was found in 43 samples (20 %) in the concentration range of 0.045 to 2.651 mg kg⁻¹. Bifenthrin was present in 24 (11%) at concentrations from 0.005 to 0.367 mg kg⁻¹. Chlorothalonil was found in 24 (11 %) at levels ranging from 0.007 to 0.098 mg kg⁻¹. Iprodion was detected in 19 samples (9 %) at levels from 0.065 to 0.450 mg kg⁻¹. Procymidon was present in 17 (8 %) at concentrations from 0.012 to 0.336 mg kg⁻¹. Deltamethrin appeared in only 11 sample (5 %) at a concentration from 0.002 to 0.243 mg kg⁻¹. For folpet, fenhexamide, cypermethrine, tau-fluvalinate and cyhalothrine, the residues levels found in these green beans ranges from (0.01 to 0.032 mg kg⁻¹), (0.01 to-mg kg⁻¹), (0,005 to 0,521 mg kg⁻¹), (0.01 to 0.073 mg kg⁻¹) and (0.006 to 0.028 mg kg⁻¹) respectively.

If we consider MRLs of the pesticides established by UE regulations³⁰, it was found 10 samples with higher pesticide residues than EU established MRLs (4.7 % in total analysed samples). Four samples contaminated with dithiocarbamates, Three contaminated with endosulfan and three other

Table 1: Days of treatment and sampling of dithane M 45

Day	Treatment	Samples collected	Day	Treatment	Samples collected	Day	Treatment	Samples collected
0	I:	I+0	0	II :	II+0	0	III:	III+0
1	(07/11/07)	I+1	1	(14/12/07)	II+1	1	(12/01/08)	III+1
2	Dithane	I+2	2	Dithane	II+2	2	Dithane	III+2
3	M45	I+3	3	M45	II+3	3	M45	III+3
4		I+4	4		II+4	4		III+4
5		I+5	5		II+5	5		III+5
6		I+6	6		II+6	6		III+6
7		I+7	7		II+7	7		III+7
8		I+8	8		II+8	8		III+8
9		I+9	9		II+9	9		III+9
10		I+10	10		II+10	10		III+10
11		I+11	11		II+11	11		III+11
12		I+12	12		II+12	12		III+12
13		I+13	13		II+13	13		III+13
14		I+14	14		II+14	14		III+14
15		I+15	15		II+15	15		III+15
16		I+16	16		II+16	16		III+16
17		I+17	17		II+17	17		III+17
18		I+18	18		II+18	18		III+18

Table 2: Days of treatment and sampling of dithane GW 68

Day	Treatment	Samples collected	Day	Treatment	Samples collected	Day	Treatment	Samples collected
0	I:	I+0	0	II :	II+0	0	III:	III+0
1	(07/11/07)	I+1	1	(14/12/07)	II+1	1	(12/01/08)	III+1
2	Ridomil	I+2	2	Ridomil	II+2	2	Ridomil	III+2
3	GW68	I+3	3	GW68	II+3	3	GW68	III+3
4		I+4	4		II+4	4		III+4
5		I+5	5		II+5	5		III+5
6		I+6	6		II+6	6		III+6
7		I+7	7		II+7	7		III+7
8		I+8	8		II+8	8		III+8
9		I+9	9		II+9	9		III+9
10		I+10	10		II+10	10		III+10
11		I+11	11		II+11	11		III+11
12		I+12	12		II+12	12		III+12
13		I+13	13		II+13	13		III+13
14		I+14	14		II+14	14		III+14
15		I+15	15		II+15	15		III+15
16		I+16	16		II+16	16		III+16
17		I+17	17		II+17	17		III+17
18		I+18	18		II+18	18		III+18

samples contaminated with each *L. cyhalothrine*, *deltamethrin* and *cypermethrin* respectively. Thus, to our knowledge, it is necessary to study a persistence of dithiocarbamate residues in green beans samples. The persistence of endosulfan was not studied in this work, because the treatment with this pesticide was prohibited now³⁰.

Dissipation study in green house

Figure 3 shows the residual values of mancozeb and mefenoxam studied in the experimental greenhouse samples of beans after the three applications. After three successive applications with 15 days interval between them, accumulative effects of these pesticides were observed in bean. On the basis of the linear fit carried out, the residue dissipation rate in beans was derived by fitting the experimental data to a pseudo-first-order kinetic function (31).

Table 3: Details of pesticides and doses used

Active ingredient	Commercial formulation	Dose (g/hL)	MLR (mg/kg)
Mancozeb	Dithane M45 (80%)	200	1
Mefenoxam & Mancozeb	Ridomil Gold 68 (4%-64%)	250	1

Table 4: Kinetic parameters of degradation of (a) Dithane M45 and (b) Ridomil WG68. $t_{1/2}$: half-Life time (days), k: kinetic constant

(a) Dithane M45									
Treatment 1	time (day)	3	4	6	9	11	12	14	Medium
	Ln (C0/C)	0,79	1,00	2,58	3,91	5,11	5,80	6,90	
	K (j ⁻¹)	0,86	0,70	0,73	0,63	0,63	0,63	0,62	0,69
	T _{1/2} (j)	2,89	3,56	3,41	3,92	3,96	3,92	4,00	3,66
Treatment 2	temps (j)	3	4	6	9	11	12	14	Medium
	Ln (C0/C)	0,79	0,90	2,45	3,80	5,33	5,72	6,84	
	K (j ⁻¹)	0,86	0,67	0,71	0,62	0,65	0,63	0,62	0,68
	T _{1/2} (j)	2,89	3,70	3,52	3,99	3,83	3,97	4,03	3,70
Treatment 3	temps (j)	3	4	6	9	11	12	14	Medium
	Ln (C0/C)	0,72	0,82	2,30	3,74	5,48	5,67	6,68	
	K (j ⁻¹)	0,84	0,65	0,68	0,61	0,66	0,62	0,60	0,67
	T _{1/2} (j)	2,97	3,80	3,64	4,04	3,76	3,99	4,10	3,76
(b) Ridomil WG 68									
Treatment 1	time (day)	3	4	6	9	11	12	14	Medium
	Ln (C0/C)	0,845	1,2	2,558	3,953	4,896	5,523	6,753	
	K (j ⁻¹)	0,88	0,75	0,72	0,64	0,61	0,61	0,61	0,69
	T _{1/2} (j)	2,83	3,32	3,43	3,89	4,08	4,07	4,07	3,67
Treatment 2	time (day)	3	4	6	9	11	12	14	Medium
	Ln (C0/C)	0,744	1,121	2,529	3,836	4,717	5,445	6,741	
	K (j ⁻¹)	0,84	0,73	0,72	0,62	0,59	0,60	0,61	0,67
	T _{1/2} (j)	2,94	3,41	3,45	3,97	4,20	4,12	4,07	3,74
Treatment 3	time (day)	3	4	6	9	11	12	14	Medium
	Ln (C0/C)	0,72	1,056	2,235	3,734	4,63	5,386	6,685	
	K (j ⁻¹)	0,84	0,71	0,67	0,61	0,58	0,60	0,61	0,66
	T _{1/2} (j)	2,97	3,49	3,70	4,04	4,25	4,15	4,10	3,81

$$-d[C]/dt = k[C]$$

$$\ln[C] = \ln[C_0] + kt \text{ (general formulay = a + kt)}$$

where (C) is the mean residue level in beans at t days after treatment, (C_0) is the concentration initial of residue and (k) is the degradation rate constant.

The curve plotting $\ln(C_0 / C) = kt + Cte$ illustrating the kinetics of degradation of the two fungicides (Figure 4), allowed us to deduce the rate constants k. The values of correlation coefficients for the M45 Dithane and Ridomil WG68 show that the degradation of these fungicides in the first order, which is in good agreement with those found in previous works^{32,33}.

Table 4 lists the values of kinetic parameters on experimental data obtained from the experience of biological degradation of the two fungicides on the green beans.

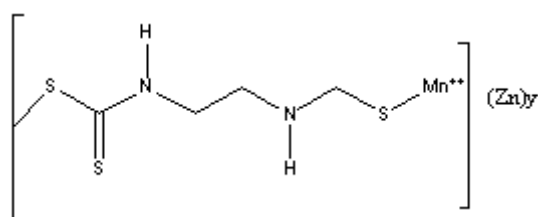
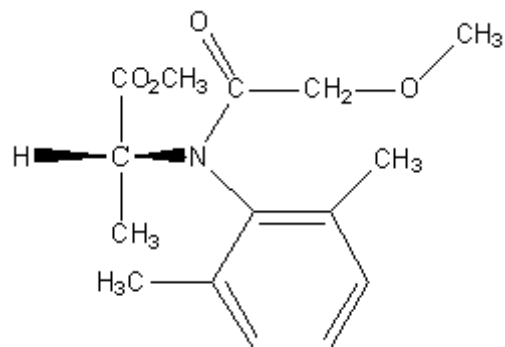


Fig. 1: Molecular structures of Mancozeb and Mefenoxam

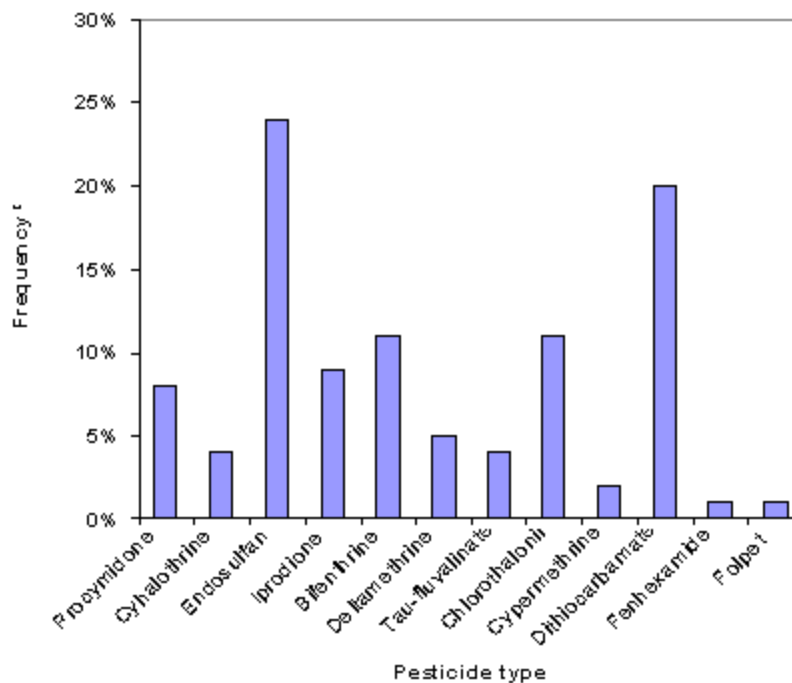


Fig. 2: Classification of chemical pesticides used by the Souss-Massa green beans growers in 2007-2008

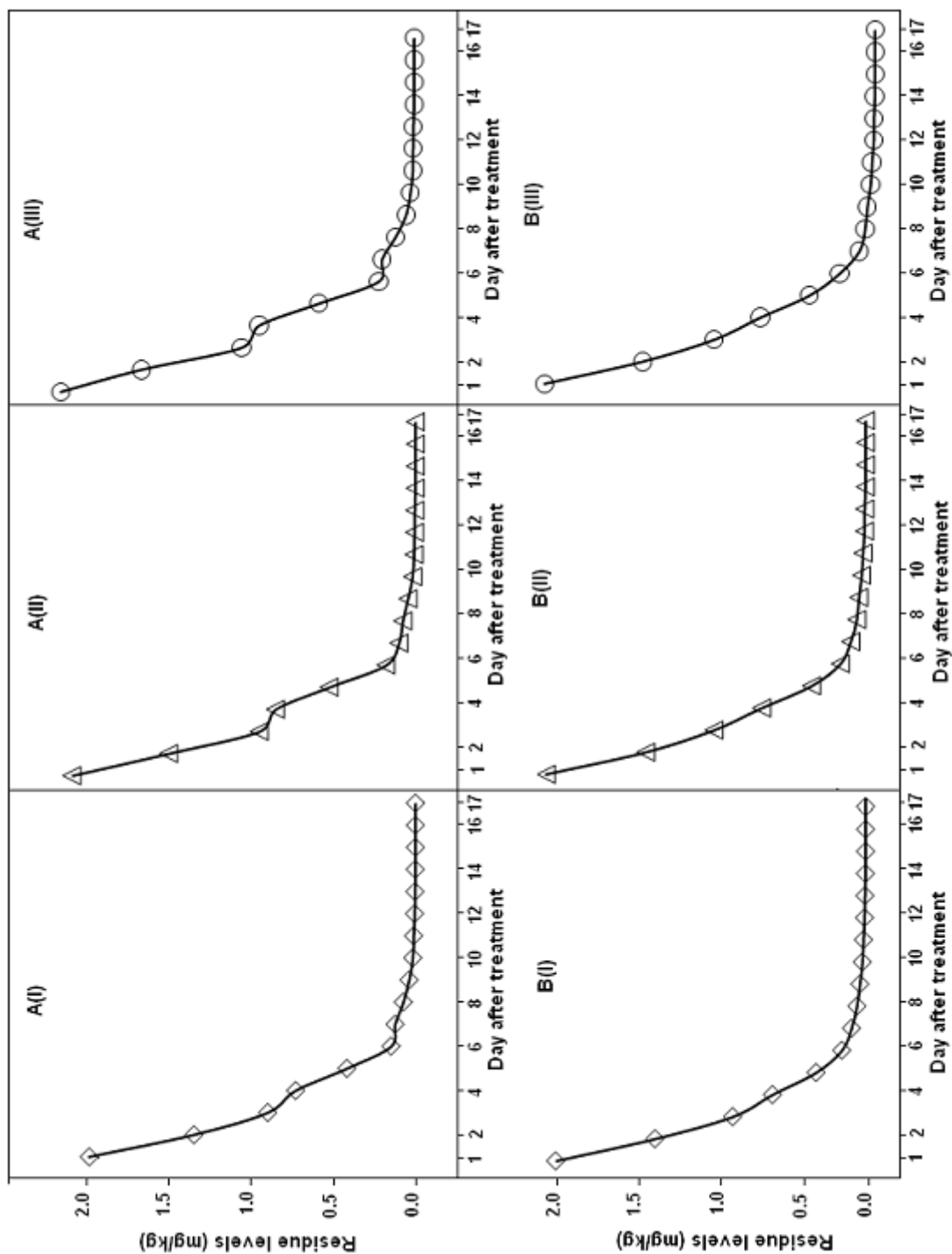


Fig. 3: Dissipation of Mancozeb (A) and Mefenoxam (B) residues in green house green beans after the first treatment (I), the second treatment (II) and the third treatment (III)

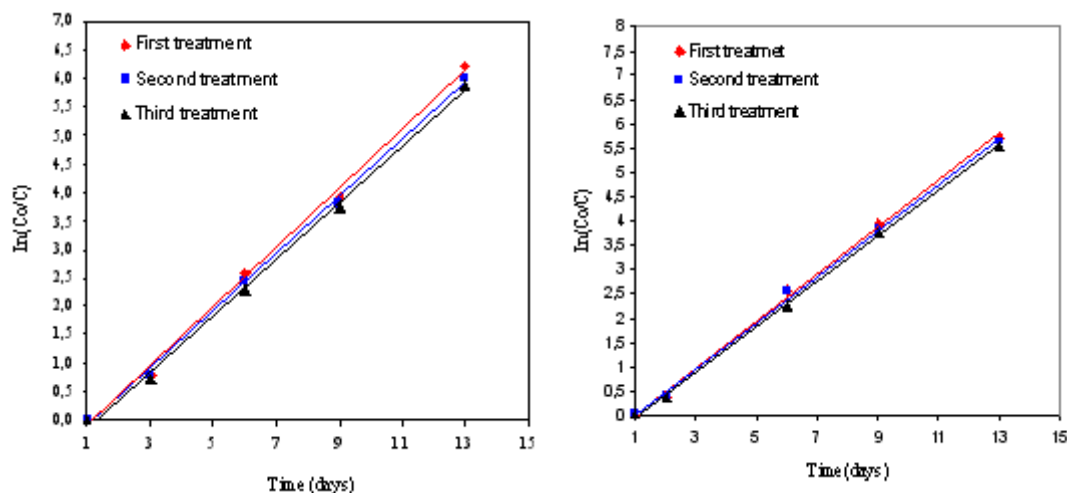


Fig. 4: Decrease of CS₂ in Dithane and Ridomil in green bean after treatment

Mancozeb

After the first application, residue of mancozeb ($1.9875 \text{ mg Kg}^{-1}$) was determined in beans (Figure 3 A(I)). The residue of this pesticide tended to decrease, showing pseudo-first-order kinetics with a half-life ($t_{1/2}$) of 3.66 days. Two weeks after the treatment, mancozeb residue was $0.0014 \text{ mg Kg}^{-1}$; therefore, at preharvest time (PT) (3 days) the residue was considerably below the MRL (1 mg Kg^{-1}). After the second and third treatments (Figure 3 (A(II)) and A(III)), the decrease rates were similar to that after the first one, with a half-life of 3.70 and 3.76 days respectively. Three and four days after the second and the third treatment the residue were also below MRL, while 17 days after these treatments, there were 0.0012 and $0.0015 \text{ mg Kg}^{-1}$ respectively. This pesticide showed the fastest decay rate in beans.

Mefenoxam

This insecticide had an initial residue of $1.8854 \text{ mg Kg}^{-1}$ that decrease with pseudo-first-order kinetics with a half-life ($t_{1/2}$) of 3.67 days. Our results show that 3 days after the first treatment, which correspond to the PT, mefenoxam residue was minor to the MRL (1 mg Kg^{-1}). Similar behaviours were obtained after the second and third treatments, with a initial residues of 1.9458 and $2.0096 \text{ mg Kg}^{-1}$ and a half-life ($t_{1/2}$) of 3.74 and 3.81 days respectively.

In the literature we have found no studies concerning mancozeb and mefenoxam dissipations in fruit or vegetables beans in the green house or in the field.

CONCLUSION

The mean residue levels of of mancozeb and mefenoxam during the course of the experiment after first, second and third treatment, were lower than the MRL. Mean residue levels of these pesticides application were lower than MRL 3 or 4 days after each application. The dissipation study of these pesticides after each application in green beans shows that the model that best fits the experimental data was the first Order model with PT below than 3.76 days. Finally, the Pre Harvest Intervals which have already been established by the EU competent authorities are safe enough for greenhouse green beans for both pesticides.

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