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DISAPPEARANCE OF DICHLOFLUANID, CHLOROTHALONIL, VINCLOZOLIN AND CARBENDAZIM ON GREENHOUSE TOMATOES

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Abstract. Disappearance of chlorothalonil, dichlofluanid, vinclozolin and carbendazim, as active ingredients of agrochemicals commonly used for the protection of greenhouse vegetables against fungal diseases, was studied comparatively. It was found that initial residues of chlorothalonil and dichlofluanid dropped by half within 4 and 2 weeks after treatment, respectively, while the deposits of vinclozolin and carbendazim were the most persistent and after 5 weeks still constituted 50% of their initial levels. Therefore, the obtained results indicated that iprodione, procymidone and vinclozolin should ensure the longest effective protection of greenhouse tomatoes while chlorothalonil, and especially dichlofluanid, for the shortest.

Key words: disappearance, chlorothalonil, dichlofluanid, vinclozolin, carbendazim, greenhouse tomatoes

I. INTRODUCTION

Greenhouse tomatoes are grown in Poland between February and November. During such a long season diseases caused by *Botrytis cinerea* Pers., *Phytophtora infestans* de Bary, *Oidium* spp. or *Septoria lycopersici* Speg. may occur. The fungicides most commonly used are those containing dicarboximides (vinclozolin, iprodione, procymidone and chlozolinate) and acylanilides (metalaxyl, furalaxyl and benalaxyl), as well as dichlofluanid, chlorothalonil, pyrazophos and thiophanate methyl. Thiophanate methyl produces carbendazim in plant tissue.

The biological activity of those four dicarboximidic fungicides and acylanilides has been studied by Cabras et al., Hedidar et al. and Valverde-Garcia et al. (1985; 1989; 1993). These authors showed persistence of the dicarboximidic products and reported a consequential accumulation effect of their residues. The same effect is also possible during frequent application of fungicides belonging to different chemical groups in circumstances of high infection pressure (Sadło 1994).

The objectives of this study were to confirm the results obtained so far for vinclozolin, to study disappearance of dichlofluanid, chlorothalonil and carbendazim by comparative method and, finally, to check the accumulation effect of fungicide residues after repeated sprayings.

II. MATERIALS AND METHODS

The trials were carried out inside a 1-ha commercial greenhouse, warmed and equipped with a drop irrigation system. A completely randomised block design was adopted with four replications. Each single plot consisted of two double rows of plants (plant density was 2.5 plants/m^2). Fungicides were applied with the portable mechanical sprayers, delivering

Table 1

Rate of fungicides used in trials I-VIII

No	Trademark of fungicides	Active ingredient	Concentration
I.	Bravo 500 SC + Rovral FLO 255 SC	chlorothalonil, iprodione	0.2 + 0.2%
II.	Bravo 500 SC + Rovral FLO 255 SC	chlorothalonil, iprodione	0.25 + 0.2%
III.	Euparen 50 WP + Sumilex 50 WP	dichlofluanid, procymidone	0.1 + 0.2%
IV.	Euparen 50 WP + Rovral FLO 255 SC	dichlofluanid, iprodione	0.2 + 0.2%
V.	Ronilan 50 WP + Rovral FLO 255 SC	vinclozolin, iprodione	0.1 + 0.2%
VI.	Ronilan 50 WP + Rovral FLO 255 SC	vinclozolin, iprodione	0.1 + 0.2%
VII.	Topsin M 70 WP + Sumilex 50 WP	thiophanate methyl, procymidone	0.1 + 0.1%
VIII.	Topsin M 70 WP + Sumilex 50 WP	thiophanate methyl, procymidone	0.1 + 0.1%

300 or 1,000 l of homogenous spray solution of two chemicals prepared according to recommendations of the Institute of Plant Protection (Tab. 1). Sampling started on the next day after treatment and was continued thereafter at weekly intervals.

Residue analysis. One hundred grams of each tomato sample was weighed and put into a blender jar, and homogenised with 150 ml of acetone. An aliquot of filtrate, equivalent of 20 g of the analytical portion, was placed in a separatory funnel. Fungicides were extracted with dichloromethane (Luke et al. 1975; Ambrus et al. 1981; Sadło 1998), and combined extracts were evaporated to dryness with a Waring apparatus (Rotavapor-R of Büchi) below 40°C. Residues were transferred quantitatively with n-hexane to a 25 ml flask and were analysed with a Gas Chromatograph equipped with a Electron Capture Detector (ECD) in the linear range of its indications (below 0.5 ng). Carbendazim residues were determined by Thin Layer Chromatography (Murawska 1980).

Fungicide residues, strongly influenced by uneven distribution of spray solution on fruit surfaces during treatment, were expressed in mg/kg and then their mean residue values and Standard Deviations (S.D.) were calculated. Their changes with time reflected the total effect of their real disappearance caused by degradation or evaporation and by dilution resulting from plant growth.

Disappearance study. The residue data obtained in the trials were subjected to mathematical calculations using the equation: $(RR)_t/(RR)_0 \times 100\%$, where $(RR)_t$ is the residue ratio of dichlofluanid, chlorothalonil, vinclozolin or carbendazim to iprodione or procymidone (assumed as stable compounds) at t weeks after application; $(RR)_0$ is the mean of residue ratios of the same compounds at t=0 (Sadło 1997). Thus, despite of plant growth and of uneven coverage of fruit surfaces with spray solutions during treatments, the percentages of initial amounts of dichlofluanid, chlorothalonil, vinclozolin and carbendazim present at the moment of sampling on tomatoes could be easily estimated.

III. RESULTS AND DISCUSSION

Chlorothalonil. Greenhouse trial I. Chlorothalonil residues on ripe tomatoes on the next day after treatment were in the range from 0.04 to 0.22 mg/kg and then their mean levels decreased irregularly with time (Tab. 2).

Table 2

Residues (mean, median and S.D.), residue ratios of chlorothalonil to iprodione (RR) and disappearance of chlorothalonil (as % of its initial amount)

Week after treatment	Iprodione			Chlorothalonil			RR	%
	mean*	median*	S.D.*	mean	median	S.D.		
0.	0.07	0.08	0.03	0.14	0.15	0.07	2.2	100
1.	0.06	0.06	0.01	0.05	0.06	0.02	0.9	40
2.	0.10	0.10	0.04	0.18	0.18	0.09	1.8	82
3.	0.07	0.07	0.02	0.08	0.08	0.04	1.1	50
4.	0.04	0.05	0.01	0.05	0.04	0.03	1.1	50
5.	0.04	0.04	0.00	0.03	0.03	0.02	0.7	31

* mean, median and S.D. (Standard Deviation) were expressed in mg/kg

Immediately after treatment, the mean residue ratio of chlorothalonil to iprodione $(RR)_0$ was at the level of 2.2 ± 0.9 (RR ± S.D.). The variation of RR, expressed by Standard Deviation, indicated imprecise mixing of the chemicals with water which could be caused by physical properties of the Bravo 500 SC product. The content of chlorothalonil decreased regularly and 3 weeks after its application date it averaged at approx. 50% of its initial quantity.

Greenhouse trial II. The residues of chlorothalonil in ripe tomatoes sampled on the second day after plant spraying were within 0.71 to 1.01 mg/kg and their mean level after one week did not change. Its value increased slightly after the second application and dropped considerably only one week after the second application and three weeks after the first application (Tab. 3).

The repeated application of Bravo 500 SC was enforced by the intensive pressure of pathogenic factor. It made the evaluation of real disappearance more difficult. Directly after the first application the residue ratio of chlorothalonil to iprodione was 2.3, stayed at the same level in the next week and then rose by 1.6 after the second application to return to its initial level two weeks later. Its variations show that the content of chlorothalonil after two

Table 3

Residues (mean, median and S.D.), residue ratios of chlorothalonil to iprodione (RR) and disappearance of chlorothalonil (as % of its initial amount)

Week after treatment	Iprodione			Chlorothalonil			RR	%
	mean*	median*	S.D.*	mean	median	S.D.		
0.	0.40	0.43	0.08	0.91	0.95	0.12	2.3	100
1.	0.40	0.43	0.05	0.95	0.96	0.04	2.4	103
2.**	0.29	0.26	0.08	1.11	0.99	0.26	3.9	166
3.	0.13	0.14	0.07	0.50	0.58	0.29	3.8	164
4.	0.11	0.12	0.07	0.26	0.23	0.17	2.4	102

* mean, median and S.D. (Standard Deviation)) were expressed in mg/kg

** repeated spraying of plants with Bravo SC 500

weeks decreased by approx. 50% of its initial amount. The results of both tests prove that the level of chlorothalonil residues decreases to the same degree through dilution caused by tomato mass growth and real disappearance caused by the process of its degradation or evaporation.

Dichlofluanid. Greenhouse trial III. Dichlofluanid residues in ripe tomatoes sampled on the second day after plant spraying were within 0.23 to 0.41 mg/kg and then their mean level varied with time in a moderately regular manner (Tab. 4).

Table 4

Residues (mean, median and S.D.), residue ratios of dichlofluanid to procymidone (RR) and disappearance of dichlofluanid (as % of its initial amount)

Week after treatment	Procymidone			Dichlofluanid			RR	%
	mean*	median*	S.D.*	mean	median	S.D.		
0.	0.20	0.19	0.04	0.32	0.32	0.07	1.7	100
1.	0.33	0.31	0.09	0.40	0.35	0.18	1.2	70
2.	0.14	0.15	0.03	0.12	0.12	0.06	0.8	49
3.	0.16	0.16	0.07	0.08	0.06	0.03	0.5	32
4.	0.09	0.09	0.04	0.04	0.05	0.02	0.4	26
5.	0.07	0.06	0.04	0.03	0.02	0.02	0.3	21

* mean, median and S.D. (Standard Deviation) were expressed in mg/kg

The mean residue ratio of dichlofluanid to procymidone in ripe tomatoes sampled on the next day after plant spraying was 1.7 ± 0.3. Its variation indicated good/thorough mixing of chemicals with water. Contents of dichlofluanid underwent rapid variations and dropped to 50% of its initial quantity as early as after 2 weeks.

Greenhouse trial IV. Dichlofluanid residues in ripe tomatoes sampled on the second day after plant spraying were within 1.15 to 1.39 mg/kg and then decreased moderately regularly (Tab. 5).

Similarly as in the first test the mean residue ratio of dichlofluanid and iprodione in ripe tomatoes in the period of three weeks decreased from 1.7 to 0.5, so, after 3 weeks from application date, dichlofluanid deposits also constituted less than 50% of its initial amount.

Table 5

Residues (mean, median and S.D.), residue ratios of dichlofluanid to procymidone (RR) and disappearance of dichlofluanid (as % of its initial amount)

Week after treatment	Iprodione			Dichlofluanid			RR	%
	mean*	median*	S.D.*	mean	median	S.D.		
0.	0.81	0.91	0.21	1.28	1.29	0.09	1.7	100
1.	0.32	0.36	0.09	0.31	0.36	0.09	1.0	58
2.	0.30	0.17	0.26	0.34	0.19	0.31	1.1	63
3.	0.28	0.30	0.18	0.13	0.13	0.09	0.5	28

* mean, median and S.D. (Standard Deviation) were expressed in mg/kg

The results of both tests prove that initial residues of dichlofluanid decrease primarily through its real disappearance in tomatoes. Rapid rate of this process excludes the possibility of any significant summing of residues, thus indicating a need to repeat the treatment with fungicide as soon as next week.

Vinclozolin. Greenhouse trial V. Vinclozolin residues in ripe tomatoes sampled on the second day after plant spraying were within 0.17 to 0.51 mg/kg and then varied with time in a moderately regular manner (Tab. 6).

Table 6

Residues (mean, median and S.D.), residue ratios of vinclozolin to iprodione (RR) and disappearance of vinclozolin (as % of its initial amount)

Week after treatment	Iprodione			Vinclozolin			RR	%
	mean*	median*	S.D.*	mean	median	S.D.		
0.	0.35	0.34	0.12	0.33	0.32	0.12	0.9	100
1.	0.32	0.26	0.20	0.27	0.24	0.14	0.9	97
2.	0.18	0.18	0.02	0.13	0.13	0.03	0.7	77
3.	0.17	0.15	0.09	0.10	0.09	0.06	0.6	62
4.	0.19	0.20	0.07	0.08	0.08	0.03	0.4	45
5.	0.13	0.08	0.11	0.08	0.06	0.07	0.5	56

* mean, median and S.D. (standard deviation) were expressed in mg/kg

The mean residue ratio of vinclozolin to iprodione in tomatoes sampled on the next day after plant spraying was 0.9 ± 0.1. Its slight variation indicated good mixing of chemicals with water. Vinclozolin content underwent regular though slow variation but even approx. 5 weeks after application it remained at the level of 50% of its initial amount while its residues decreased in the same time period from 0.33 to 0.08 mg/kg.

Greenhouse trial VI. Vinclozolin residues in ripe tomatoes sampled on the second day after plant spraying were within 0.17 to 0.46 mg/kg and then decreased regularly in the first two weeks after application (Tab. 7).

The mean residue ratio of vinclozolin to iprodione in tomatoes directly after application was 1.1 and then decreased slightly through a period of two weeks, thus indicating its persistence.

Table 7

Residues (mean, median and S.D.), residue ratios of vinclozolin to iprodione (RR) and disappearance of vinclozolin (as % of its initial amount)

Week after treatment	Iprodione			Vinclozolin			RR	%
	mean*	median*	S.D.*	mean	median	S.D.		
0.	0.38	0.45	0.16	0.36	0.41	0.11	1.1	100
1.	0.26	0.23	0.06	0.27	0.24	0.07	1.0	98
2.	0.20	0.20	0.00	0.20	0.19	0.02	1.0	96

* mean, median and S.D. (standard deviation) were expressed in mg/kg

Results of both tests prove that vinclozolin residues decreased almost exclusively through dilution caused by the tomato mass growth. Therefore Ronilan 50 WP should ensure effective protection of tomato fruit for a period of at least two weeks.

Carbendazim. Greenhouse trial VII. Carbendazim residues in ripe tomatoes sampled on the second day after plant spraying were within 0.06 to 0.22 mg/kg (Tab. 8).

Table 8

Residues (mean, median and S.D.), residue ratios of carbendazim to procymidone (RR) and disappearance of carbendazim (as % of its initial amount)

Week after treatment	Procymidone			Carbendazim			RR	%
	mean*	median*	S.D.*	mean	median	S.D.		
0.	0.41	0.26	0.27	0.10	0.07	0.07	0.3	100
1.	0.23	0.24	0.08	0.04	0.03	0.03	0.2	71
2.	0.21	0.19	0.09	0.04	0.04	0.02	0.2	77
3.	0.17	0.15	0.09	0.04	0.03	0.04	0.2	66

* mean, median and S.D. (standard deviation) were expressed in mg/kg

Residue ratio of carbendazim and procymidone remained near the same level throughout the period of this study, thus testifying to the persistence of carbendazim.

Greenhouse trial VIII. Carbendazim residues in tomatoes sampled on the next day after plant spraying was 0.04 to 0.30 mg/kg and then varied with time in a regular manner (Tab. 9).

Table 9

Residues (mean, median and S.D.), residue ratios of carbendazim to procymidone (RR) and disappearance of carbendazim (as % of its initial amount)

Week after treatment	Procymidone			Carbendazim			RR	%
	mean*	median*	S.D.*	mean	median	S.D.		
0.	0.42	0.41	0.19	0.16	0.15	0.10	0.3	100
1.	0.32	0.32	0.10	0.13	0.13	0.06	0.4	110
2.	0.25	0.19	0.13	0.12	0.09	0.07	0.5	138
3.	0.21	0.21	0.10	0.08	0.08	0.04	0.4	109
4.	0.24	0.24	0.07	0.11	0.12	0.03	0.5	131

* mean, median and S.D. (standard deviation) were expressed in mg/kg

Residue ratio of carbendazim and procymidone remained near the same level throughout the period of this study, thus testifying to the persistence of the former. Relatively low contents of carbendazim (compared to relation between concentrations of thiophanate methyl and procymidone in the working liquid) resulted from the fact that carbendazim comes from thiophanate methyl after the split of a fragment comprising nearly half of its particle.

The fact of significant persistence of carbendazim does not necessarily mean that we should expect long-time protection against diseases because due to systemic characteristics and relocation in plant tissue its concentration on fruit surface decreases rapidly.

IV. CONCLUSIONS

1. Residues of chlorothalonil underwent a slow but noticeable change and only after 4 weeks from application decreased by about 50% of their initial amount.
2. Among fungicides studied dichlofluanid residues disappeared the most quickly and reached the half of its initial amount as early as after 10-14 days.
3. Residues of vinclozolin and carbendazim practically remained at unchanged level throughout the period of study.
4. Vinclozolin, procymidone, iprodione, carbendazim and, in a shorter period chlorothalonil as well, are persistent compounds. Therefore, each chemical treatment performed a week after the application of Ronilan, Bravo, Sumilex, Rovral and Topsin shall cause a significant summing of combined residues.
5. Analysis of obtained results may lead to assumption that vinclozolin, procymidone, iprodione and chlorothalonil should ensure an effective protection of greenhouse tomatoes for a period of at least two weeks. Dichlofluanid is the compound which disappeared most rapidly and, consequently, one week application of Euparen 50 WP the treatment need to be repeated with the same product or with other one of similar effect.
6. Carbendazim is a persistent compound. However, due to its systemic characteristics, its residue (mg/kg) and concentration on the surface (mg/cm^2) undergoes variation caused by the fruit growth and migration of this compound to inside from tomato surface.

V. REFERENCES

1. Ambrus A., Lantos J., Visi E., Csatlos I., Sarvari L. 1981. General method for determination of pesticide residues of plant origin, soil, and water. I. Extraction and cleanup. J. Assoc. Off. Anal. Chem., 64: 733--742.
2. Cabras P., Meloni F.M., Pirisi F. M., Cabitza F. 1985. Behaviour of Acylanilide and Dicarboximidic Fungicide Residues on Greenhouse Tomatoes. J. Agric. Food Chem., 33: 86-89.
3. Hedidar S., Devolder F., Chebil A., Hullebroeck M. 1989. Etude des residus de quelques fongicides utilises dans la lutte contre la pourriture grise de la tomate sous serre en tunise. Med. Fac. Landbouww. Rijksuniv. Gent 54 (2a): 151-159.
4. Luke M.A., Froberg J.E., Masumoto H.T. 1975. Extraction and Cleanup of Organochlorine, Organophosphate, Organonitrogen, Hydrocarbon Pesticides in Produce for Determination by Gas-Liquid Chromatography. J. Assoc. Off. Anal. Chem., 58: 1020-1026.
5. Murawska M. 1980. Metoda bioautografii w zastosowaniu do oznaczania śladowych ilości fungicydów benzimidazolowych. Prace Nauk. Inst. Ochr. Roślin, 22 (1): 139-146.
6. Sadło S. 1994. Fungicide Residues on Tomatoes in Circumstances of High Infection Pressure of Potato Late Blight (*Phytophtora infestans*) in 1993. Prace Nauk. Inst. Ochr. Roślin, 35 (1/2): 120-128.
7. Sadło S. 1997. Adaptation of the internal standard method to a pesticide residues study in/on plants. J. Chromatography A 765: 115-119.
8. Sadło S. 1998. Partition coefficient - its determination and significance in estimation of pesticide residue losses in the course of extraction procedure. J. Plant Protection Res., 38 (2): 179-184.
9. Valverde-Garcia A., Gonzalez-Pradas E., Aguilera-Del Real A., Urena-Amate M.D. 1993. Determination and degradation study of chlorothalonil residues in cucumbers, peppers and cherry tomatoes. Analytica Chimica Acta 276: 15-23.

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ZANIKANIE DICHLOFLUANIDU, CHLOROTALONILU, WINKLOZOLINY I KARBENDAZYMU W POMIDORACH SZKLARNIOWYCH

STRESZCZENIE

Celem badań była ocena rzeczywistego zanikania chlorotalonilu, dichlofluanidu, winklozoliny i karbendazymu, stanowiących substancje aktywne preparatów chemicznych aktualnie najczęściej stosowanych do ochrony warzyw szklarniowych przed wystąpieniem chorób pochodzenia grzybowego. Przeprowadzono je w szklarni Państwowego Zakładu Ogrodniczego w Trzebownisku k. Rzeszowa. Rośliny opryskiwano wieczorem jednorodnymi mieszaninami wodnymi dwu preparatów chemicznych.

W wyniku przeprowadzonych analiz stwierdzono, że zdecydowanie najszybciej zanikały pozostałości dichlofluanidu, które już po upływie dwu tygodni stanowiły około 50% początkowej jego ilości.

Uwzględniając uzyskane efekty badań można przypuszczać, iż skuteczną ochronę pomidorów szklarniowych powinny najdłużej zapewniać iprodion, procymidon, chlorotalonil i karbendazym, a najkrócej dichlofluanid.

Powolne tempo rzeczywistego zanikania tych związków dowodzi również, że zabieg chemiczny wykonany tydzień po zastosowaniu Bravo 500 SC, Topsinu M 70 WP, Sumilexu 50 WP lub Rovralu FLO 255 SC spowoduje znaczące sumowanie się pozostałości.