JOURNAL OF PLANT PROTECTION RESEARCH
Vol. 41, No. 3 2001

BEHAVIOUR OF PYRIMETHANIL RESIDUES ON TOMATO PLANTS

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Accepted: May 30, 2001

Abstract: The objective of this work was to estimate the disappearance of pyrimethanil the active ingredient of Mythos 300 SC. At present, Mythos 300 SC is commonly used for the protection of greenhouse vegetables against diseases of fungal origin. The behaviour of pyrimethanil deposits was studied on tomato plants grown in commercial greenhouses sprayed with homogenous 0.15% aqueous solution of this plant protection product. It was found that the value of pyrimethanil residues on ripening fruits decreased by half and reached zero levels in 5.7 and 13.7 days after Mythos 300 SC application, respectively. Pyrimethanil residues on tomato leaves decreased by half within 4.0 days and reached zero level in the first 10.5 days. Therefore, in conditions of high infection pressure, there is a need to repeat the fungicide application as early as after 3 or 4 days after previous application of Mythos 300 SC.

Key words: disappearance, pyrimethanil residues, tomato, leaves and fruits

I. INTRODUCTION

Pyrimethanil, the IUPAC name N-(4,6-dimethylpyrimidin-2-yl) aniline, is a colourless crystalline substance practically insoluble in water (121 mg/l), belonging to anilinopyrimidine class, for which Acceptable Daily Intake (ADI) was established at the level of 0.2 mg/kg of body weight/day. In the form of concentrates (tradenames: Mythos, Clarinet and Scala) it is used as a contact fungicide with protective and curative properties. In Poland, Mythos 300 SC was registered for application six years ago, though the behaviour of its active ingredient after treatment was poorly recognised and popularised (Cabras et al. 1997)

Pyrimethanil inhibits the secretion of fungal enzymes relevant for pathogenicity. It controls grey mould (*Botrytis cinerea* Pers.) on fruits, vegetables and ornamentals as well leaf scab (*Venturia inaequalis* or pirinal) on pome fruits. Compounds of the anilinopyrimidine class were not utilised so far. It means that there is the plant protection product with original mode of action available for the growers.

The objectives of this work were to determine disappearance parameters of pyrimethanil deposits on tomato plants grown in commercial greenhouses in order to optimise Mythos 300 SC application against *Botrytis cinerea* Pers. and *Alternaria solani* Sorauer, together with other fungicides of the same mode of action and to check an accumulation effect of fungicide residues, due to repeated applications of the same or different active ingredients. It is important to know these parameters, because in Poland the annual production of greenhouse tomatoes now exceeds 250,000 tons.

II. MATERIAL AND METHODS

1. Greenhouse experiments

Four experiments were carried out in 1-ha commercial greenhouses, air warmed and equipped with a drop irrigation system. Tomato plants (cv. Cunero F_1), receiving routine horticultural practices, were sprayed in the evening with Mythos 300 SC (active ingredient: 300 g of pyrimethanil per 1 l of the crop protection product) in the form of homogeneous 0.15% aqueous solution. A completely randomised plot design with four replications was used. Each single plot consisted of two double rows and contained 140 plants (2.5 plants per square meter).

Sampling was started the next day (about 12 hrs) after treatments and repeated in 1, 8, 15 and 22 days (Experiment 1), 1, 3, 5, 7 and 10 days (Experiments 2 and 4) and 1, 4, 6, 8 and 11 days (Experiment 3) after treatments. Each sample consisted of eight tomato fruits and leaves.

2. Extraction Procedure

One hundred grams of each fruit sample was put into a blender jar of Waring apparatus and homogenised for 2 min with 150 ml of acetone. Homogenate was filtered through a Büchner funnel and an aliquot of the filtrate, equivalent of 20 g of the analytical portion, was placed in a separate funnel. Pyrimethanil residues were extracted using the method published in the scientific journal of the Plant Protection Institute (Sadło 1998), based on the solvent system proposed by Luke et al. (1975) with its subsequent modification (Ambrus et al. 1981). The combined extracts were evaporated to dryness with a Rotavapor-R of Büchi below 40°C. Residues were transferred quantitatively with n-hexane to a 25-ml flask.

Thirty two discs were cut out from the sampled leaves using a leaf punch sampler (of inner diameter 0.5 cm), placed in a blender jar of Waring apparatus containing 100 ml of distilled water and homogenised for 2 min with 150 ml of acetone. An aliquot of filtrate, equivalent of one fifth of the analytical portion, was taken and further analysis followed as described above.

3. Recovery assays of pyrimethanil residues

Subsamples of untreated fruits and leaves were spiked with 2 ml of 10 μg/ml acetone standard solution. Samples were taken through the extraction procedure four times. Recoveries obtained with this method were satisfactory (Tab. 1).

4. Apparatus and Chromatography

A gas chromatograph, Hewlett Packard 5890, was employed, equipped with nitrogen-phosphorus detector (NPD) connected to HP 3396 series II integrator. The HP 17 wide-bore fused silica capillary column (10-m length, 0.53-mm i.d., and 2.0-μm film thickness) was used. The injector and the detector were operated at 240 and 260°C, respectively. Sample extracts (2 μl) were injected splitless and the oven temperature was programmed as follows: 150°C for 1 min, raised to 260°C (10°C/min), and held for 6 min. Good linearity was achieved in the range of 0–0.5 ng. Under these conditions, the detection limits for pyrimethanil were 0.01 μg/g (fruits) and 0.02 μg/cm^2 (leaves). Pyrimethanil residues on fruits and leaves were expressed in μg/g and μg/cm^2, respectively, and then their average levels and Variation Coefficients (Relative Standard Deviations; RSD) were calculated.

5. Chemicals

Acetone, dichloromethane and petroleum ether were of analytical grade. Pyrimethanil was purchased from Ehrenstorfer (Germany) and its stock standard solution (10 μg/ml) was prepared in acetone and stored at 4°C. Working standard solution (0.2 μg/ml) was obtained by diluting the stock solution with petroleum ether.

III. RESULTS AND DISCUSSION

1. Analysis

The average recoveries of pyrimethanil from spiked tomato fruit and leaves are given in Table 1. In all cases, their levels were higher than 80% with Variation Coefficients not exceeding 7%. These levels are generally considered satisfactory for determination of pesticide residues, and are comparable to those obtained for the other compounds insoluble in water (Sadło 1998). The limits of quantification of the method for pyrimethanil were calculated to be 0.01 μg/g and 0.02 μg/cm^2.

The residue data obtained in the disappearance studies of pyrimethanil deposits present on tomato plants after spraying carried out with Mythos 300 SC are summarized in Tables 2–5. Those data were subjected to mathematical analysis using Excel program and the obtained results were summarised in Table 6. Behaviour of the average pyrimethanil deposits was well described by linear ($Y = mX + b$) or logarithmic ($Y = cLn(X) + b$) equations, where Y is the variable we are trying to predict, X is the independent variable we are predicting from. In

Table 1

Pyrimethanil Recoveries and Detection Limits for Tomato Plants

Sample	Spiking level	Average ± RSD	Detection limit
Fruits	0.14 μg/g	99 ± 6.7 %	0.01 μg/g
Leaves	1.83 μg/cm^2	91 ± 4.5 %	0.02 μg/cm^2

the case of disappearance study expressed by linear regression, the dependent variable Y represents residue level (R) at a given moment (t) after treatment, while the b and m values express the initial residue of pesticide (R_0) and rate of its decrease with time. Finally, linear regression takes the form of $R = R_0 + mt$ while the logarithmic line $R = R_0 + cLn(t)$.

2. Disappearance of Pyrimethanil residues in tomato plants (Experiment 1)

The first laboratory samples were taken the next day (about 12 hrs) after treatment. Pyrimethanil residues in tomato fruits averaged at 0.33 μg/g, with Variation Coefficient amounting to 46% (Tab. 2) and decreased rapidly according to logarithmic line $R = 0.3178 - 0.1106Ln(t)$. The course of the line indicated that initial residues, $R_0 = 0.32$ μg/g, dropped by half ($t_{1/2}$) and reached the zero level ($t_{R=0}$) 4.2 and 17.7 days after treatment, respectively.

Immediately after treatment the average initial deposit of pyrimethanil on tomato leaves was at the level of 1.0 μg/cm^2 and dropped by half within 3.4 days reaching the zero level after 11.7 days. The obtained results indicated that pyrimethanil deposits disappeared rapidly. Thus, proper sampling procedure changes were introduced in methodology before starting the next three experiments.

3. Disappearance of Pyrimethanil residues in tomato plants (Experiment 2, 3 and 4)

Pyrimethanil residues in ripe tomato fruits taken the next day after treatments were, on average, 0.16, 0.30 and 0.34 μg/g and were in good correlation with its application rate

Table 2

Average Residues of Pyrimethanil (μg/g, cm^2 ± SD) on Tomato Plants (Experiment 1)

Days after treatment	Fruits			Leaves	
	weight, g	residue	% of initial level	residue	% of initial level
1	119 ± 5	0.33 ± 0.15	100	1.00 ± 0.45	100
8	125 ± 2	0.05 ± 0.03	16	0.02 ± 0.00	2
15	115 ± 7	0.02 ± 0.01	5	0.00 ± 0.00	0
22	121 ± 9	0.00 ± 0.00	0	0.00 ± 0.00	0

Table 3

Average Residues of Pyrimethanil (μg/g, cm^2 ± SD) on Tomato Plants (Experiment 2)

Days after treatment	Fruits			Leaves	
	weight, g	residue	% of initial level	residue	% of initial level
1	130 ± 15	0.16 ± 0.07	100	0.78 ± 0.48	100
3	131 ± 4	0.20 ± 0.08	124	0.80 ± 0.22	103
5	134 ± 9	0.07 ± 0.03	43	0.64 ± 0.09	82
7	128 ± 4	0.05 ± 0.03	34	0.45 ± 0.09	58
10	127 ± 3	0.06 ± 0.06	38	0.22 ± 0.05	28

(Sadło 2000). The average residue levels dropped by half within 5.3–7.2 days after treatment by the route of real disappearance (lack of dilution effect caused by fruit growth) (Tab. 6). Pyrimetanil residues reached the zero value within two weeks. Acceptable Daily Intake

Table 4

Average Residues of Pyrimethanil (µg/g, cm^2 ± SD) on Tomato Plants (Experiment 3)

Days after treatment	Fruits			Leaves	
	weight, g	residue	% of initial level	residue	% of initial level
1	142 ± 4	0.30 ± 0.14	100	1.40 ± 0.11	100
4	115 ± 8	0.28 ± 0.13	95	0.31 ± 0.09	22
6	123 ± 1	0.21 ± 0.15	70	0.15 ± 0.05	11
8	117 ±14	0.12 ± 0.07	41	0.00 ± 0.00	0
11	120 ±19	0.09 ± 0.05	30	0.00 ± 0.00	0

Table 5

Average Residues of Pyrimethanil (µg/g, cm^2 ± SD) on Tomato Plants (Experiment 4)

Days after treatment	Fruits			Leaves	
	weight, g	residue	% of initial level	residue	% of initial level
1	127 ± 7	0.34 ± 0.19	100	2.22 ± 0.91	100
3	121 ± 4	0.27 ± 0.06	79	0.90 ± 0.15	41
5	121 ± 6	0.23 ± 0.20	69	0.56 ± 0.08	25
7	121 ± 9	0.05 ± 0.04	15	0.25 ± 0.09	11
10	131 ± 9	0.05 ± 0.06	15	0.00 ± 0.00	0

Table 6

Statistical Parameters Corresponding to the Disappearance Trends of Pyrimethanil Residues on Greenhouse Tomato Plants (Experiments 1–4)

Experiment		Equation	R_0	r	R^2	$t_{1/2}$	$t_{R=0}$
1	Fruits	$R = 0.3178 - 0.1106Ln(t)$	*0.32*	-0.9848	0.9699	4.2	17.7
	Leaves	$R = 0.9700 - 0.3948Ln(t)$	*0.97*	-0.9788	0.9581	3.4	11.7
2	Fruits	$R = 0.1877 - 0.0153t$	*0.19*	-0.7920	0.6272	6.1	12.2
	Leaves	R = 0.9294 - 0.0676t	*0.93*	-0.9667	0.9346	6.9	13.8
3	Fruits	$R = 0.3417 - 0.0236t$	*0.34*	-0.9616	0.9246	7.2	14.5
	Leaves	$R = 1.3665 - 0.6859Ln(t)$	*1.37*	-0.9934	0.9868	2.7	7.3
4	Fruits	$R = 0.3732 - 0.0356t$	*0.37*	-0.9425	0.8883	5.3	10.4
	Leaves	$R = 2.1271 - 0.9639Ln(t)$	*2.13*	-0.9924	0.9849	3.1	9.1
Mean	Fruits	–	*0.28*	–	–	5.7	13.7
	Leaves	–	*1.35*	–	–	4.0	10.5

Equation – the best approximations; R_0 – initial residue/deposit; r – correlation coefficient; R^2 – coefficient of determination; $t_{1/2}$ – half-life period

(ADI), established for pyrimethanil at the level of 0.2 mg/kg body weight/day (Tomlin 1994), shows that a 60-kg person could eat even 36 kg/day of ripe tomato fruits harvested immediately after treatment containing the average residue about 0.30 mg/kg.

The average initial pyrimethanil deposits on fully developed tomato leaves were, on average, 0.78, 1.41 and 2.22 μg/cm^2 and then dropped by half within 2.7-6.9 days after treatments (Tab. 6). Fast disappearance rate of pyrimethanil deposits on leaves strongly suggests that Mythos 300 protects tomato plants only within 4-day period of time. In order to assure effective protection the next treatment should be carried out.

IV. CONCLUSIONS

Reported research showed that residues of pyrimetanil in tomato fruits were reduced by half within 6 days after spraying of tomato plants and reached zero level after two weeks. As tomatoes are harvested twice a week (every 3 or 4 days) and chemical treatments are performed immediately after harvest, it should be pointed out that significant summation of residues in ripening tomatoes may take place only in conditions of high infection pressure. In case of a subsequent treatment, performed on the seventh day after former application of Mythos, the ripe tomatoes shall contain only the residues of the last applied plant protection product.

Within 4 days after the treatment, the pyrimetanil deposits on leaves were reduced by half and might not ensure effective protection for tomato crops. Therefore, in conditions of high infection pressure, there is a need to repeat the fungicide application as early as after 3 or 4 days after previous application of Mythos 300 SC.

The studies of fungicide behaviour on crops constitute an important step towards rationalisation of crop protection against diseases of fungal origin and their results shall be included in instructions of use (or label) of specific crop protection agent.

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ZACHOWANIE SIĘ POZOSTAŁOŚCI PIRYMETANILU NA POMIDORZE SZKLARNIOWYM

STRESZCZENIE

Tematem niniejszej pracy było oszacowanie przebiegu zanikania pirymetanilu, substancji biologicznie czynnej fungicydu o nazwie Mythos 300 SC, powszechnie stosowanego do ochrony warzyw szklarniowych przed chorobami pochodzenia grzybowego. Przebadano zachowanie się pirymetanilu na roślinach pomidora szklarniowego opryskiwanych jednorodną zawiesiną preparatu o stężeniu 0,15%. Stwierdzono, iż pozostałości pirymetanilu na dojrzewających owocach spadły o połowę już w okresie 5, 7 dni, osiągając poziom zerowy w trzynastym dniu po aplikacji Mythosu 300 SC. Początkowe depozyty pirymetanilu na liściach spadły o połowę po czterech dniach, osiągając poziom zerowy w jedenastym dniu po aplikacji. W warunkach zatem dużej presji czynnika chorobotwórczego istniała potrzeba powtórnej aplikacji fungicydu już po 3–4 dniach.