Pesticide Residues of MEP, MPP and PAP in Mulberry Stumps and Its Effect on the Silkworm (*Bombyx mori*, L.)

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Residual levels of pesticides, MEP, MPP and PAP, in parts of mulberry stumps were determined by means of *glc*. Ten days after application, a high concentration of all chemicals remained in the bark: MEP 66–790 ppm, MPP 52–120 ppm and PAP 58–347 ppm. Several ppm of these pesticides also remained in the wood. Only a small quantity, 0.14–1.08 ppm of MEP remained in the pith. Silkworms can be reared normally on mulberry leaves harvested from the pesticide-treated stumps, but a slightly reduced cocoon weight was noted case of MEP and MPP-treatment.

INTRODUCTION

In the field of sericulture, the use of MEP, MPP and PAP formulations for the protection of mulberry trees against damage by the yellow-spotted longicorn beetle (*Psacotha hilaris* PASCOE) has increased. The application of these pesticides is usually restricted to a short period immediately after the summer pruning of the mulberry tree, since experience has shown that the silkworm has been damaged when the mulberry leaves retain the contaminated residue of those pesticides. Larvae of the beetle which is most sensitive to these pesticides develop in September and/or October in the Kanto area.^{1,2)} Rearing of the silkworm is also carried out during this period by providing mulberry leaves. Therefore, it is essential to know the extent to which the pesticide residue remains on mulberry leaves after application. In this paper, we wish to report on the pesticide residue found in parts of a mulberry stump and the quality of the cocoons resulting from silkworms reared on mulberry leaves harvested from the pesticide-treated stump.

MATERIALS AND METHODS

1. Mulberry Tree

A "Takanegari" (semi-low cut training) of the Rosō strain, ten years old, was used for this study during the spring rearing season.

2. Silkworm

The fifth instar larvae of a hybrid from Nichi 134 and Shi 135 were used. The silkworm was reared on the leaves of the pesticide-treated mulberry stump.

3. Pesticides

MEP (*O,O*-dimethyl *O*-(3-methyl-4-nitrophenyl)thiophosphate, 50% emulsion) and MPP (*O,O*-dimethyl *O*-(3-methyl-4-methylthiophenyl)thiophosphate, 50% emulsion) with EDB (1,2-dibromoethane) added to 10% were diluted to 1/50, 1/100 and 1/200; each 200 ml was then sprayed on the mulberry tree. PAP (ethylacetate of *O,O*-dimethyldithiophosphorylphenyl, 10% emulsion) with EDB added to 10% was diluted to 1/10, 1/20 and 1/40, and each 200 ml of the diluted formulation was used for the test.

4. Analysis of Pesticide Residues

The bark, wood and pith of the pesticide-

treated mulberry stump (circumference, about 20 cm) were separated carefully at a definite day after the application of the pesticide. Ten g of the chipped samples were dipped in 100 ml of acetonitrile for a day and extracted twice with 50 ml of *n*-hexane. The hexane extract was concentrated and was subjected to chromatography on an alumina column containing water to 10%, and eluted with acetone. A certain volume of the concentrated acetone eluate was applied to gas liquid chromatography. The condition of *glc* was as follows: apparatus, Hewlett Packard 5710A; detector, N-P FID; column, 2% OV-101 chromosorb W, HP, 100/120 mesh, 1.2 m; detector temp., 300°C; column temp., 190°C; injection port temp., 250°C; N₂, 30 ml/min; air, 50 ml/min; H₂, 3 ml/min. The retention time of MEP, MPP and PAP was 3.3, 4.2 and 5.5 min, respectively. Quantitative assay of MEP and MPP was performed employing PAP as an internal standard. The standard curve of each pesticide was drawn with the height of peak.

5. Silkworm Rearing

Twenty female larvae of the fifth instar were reared from newly ecdysis to maturation on fresh mulberry leaves harvested from the mulberry stump which had been treated with pesticides. Cocoons were weighed on the seventh day after maturation of the larvae.

RESULTS AND DISCUSSION

Ten days after the application of the pesticides, a high concentration of MEP, MPP and PAP, remained in the bark of the mulberry stump (Table 1). The amount of pesticide residue was less in proportion to the magnification of the dilution applied. No residue was detected in pith in any case of MPP spraying. Residues of PAP were negligible in cases when 20 and 40 times dilutions were used. Moreover, the mulberry leaves of the treated stumps contained no detectable residues.

Silkworms can be reared on mulberry leaves of pesticide-treated stumps without any damage (Table 2). The larvae did not show any typical toxic symptoms. There were a few dead larvae due to flacherie, but not in any case because of the pesticidal effect of the

Table 1 Pesticide residues in parts of a stump 10 days after application of chemicals. (ppm)

Pesticide	Dilution	Bark	Wood	Pith
MEP	50	791.8	9.75	1.08
	100	305.0	2.03	0.37
	200	66.5	0.62	0.14
MPP	50	120.2	1.32	ND
	100	52.6	1.56	ND
	200	73.8	0.33	ND
PAP	10	347.7	5.9	0.58
	20	61.2	0.002	ND
	40	58.6	0.55	ND

Rainfall totalled 153 mm during this period.

The formulation of MEP and MPP used in the experiments was 50% emulsion and that of PAP was 10% emulsion.

Amount of pesticide residue (ppm) was indicated by an average value of two samples per series.

Table 2 Cocoons of silkworms reared on mulberry leaves of the pesticide-treated stump.

Pesticide	Dilution	Number of cocoons			
		Normal cocoon	Double cocoon	Dead worm cocoon	Larvae dead by flacherie
MEP	50	16		1	3
	100	17			3
	200	16		2	2
MPP	50	15		2	3
	100	16		1	3
	200	14	1	3	1
PAP	10	13	2		3
	20	17		1	2
	40	17		1	2
Control		16			4

residues. So-called cocoon qualities, i.e., cocoon weight, cocoon shell weight and percentage of shell weight, are presented in Table 3. Cocoon weight and cocoon shell weight proved to be less in those silkworms reared on mulberry leaves of the MEP-treated stump compared with the untreated stump. A significant difference in cocoon weight was noticed between silkworms reared on the MEP or MPP treated leaves and those reared on the control leaves. As mentioned above, pesticide residues on mulberry leaves were not detected. Satō *et al.*^{3,4)} have proposed safety levels for MEP and PAP of less than 0.02 ppm, and for MPP of less than 0.5 ppm.⁵⁾

Table 3 Cocoon quality of the silkworms reared on mulberry leaves of the pesticide-treated stump.

Pesticide	Dilution	Cocoon weight (g)	Cocoon shell weight (cg)	Percentage of shell weight (%)	Number of cocoons
MEP	50	2.030±0.102**	46.87±4.59	23.14±1.85	16
	100	2.186±0.124*	49.67±5.02	22.75±2.17	17
	200	2.219±0.116*	49.59±2.18	22.35±0.81	16
MPP	50	2.213±0.297**	51.10±5.55	23.08±2.01	15
	100	2.152±0.127*	49.12±3.76	22.79±0.70	16
	200	2.178±0.237	53.05±4.54	24.45±1.79	14
PAP	10	2.371±0.189	55.46±2.81	23.48±1.83	13
	20	2.445±0.147	57.97±4.46	23.67±0.98	17
	40	2.276±0.090	54.32±3.84	23.86±1.60	17
Control		2.651±0.230	58.59±7.43	22.01±1.53	16

The silkworms were reared on mulberry leaves harvested from stumps 20 days after application of pesticides.

** Significant difference p 0.01, * Significant difference p 0.05.

Double cocoon was eliminated from the calculation and f -test.

The cause of the deterioration of quality of cocoons from silkworms reared on mulberry leaves of the MEP- and MPP-treated stumps is not clear at the present time.

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要 約

桑株中の MEP, MPP および PAP の残留とカイコに及ぼす影響

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桑株に各薬剤を散布して 10 日後, その残留量を *glc* で測定した。樹皮中においては各農薬は高濃度に残留していた。MEP は 66~790, MPP は 52~120, PAP は 58~347 ppm であった。木部にはそれぞれ数 ppm が残留した。髓では MEP のみが 0.13~1.08 残留していた。薬剤を処理した桑株から収穫した桑葉からの農薬は検出されなかった。この桑葉でカイコを飼育した結果, カイコは正常に発育し営繭した。しかしながら MEP と MPP 処理株の桑葉で飼育したカイコの繭は繭重において若干軽かった。