Note

Further Evidence for the Presence of a Synthetic Insecticide in So-called Natural-Plant Extract-Formulations

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INTRODUCTION

The previous paper,¹⁾ studying the nature of active ingredient of so-called natural-plant extract-formulations (abbreviated as NPEF hereafter), reported the detection of cypermethrin in "Muso." The identification of the synthetic pyrethroid was based upon ¹H NMR spectra as well as chromatographic behaviors in silica gel TLC and HPLC using a chirally active column. Although concentration of the active ingredient was tentatively estimated to be in the range of 1 to 3% based upon the ¹H signals in the NMR spectra, accurate determination of the concentration was a subject for further studies. The HPLC analysis in the above paper also implicated the presence of cypermethrin in two other similar formulations.

The present study was undertaken to further confirm the presence and to determine the concentration of cypermethrin in several so-called NPEF used as organic agricultural materials.

MATERIALS AND METHODS

1. Chemicals

Formulations subjected to analysis in the present paper are "Muso"(A), "Muso"(B), "Hekiro," "Kensogen-Ten" and Agrothrin EC. The sources of these formulations were described previously.¹⁾ A technical grade (95.4%) of cypermethrin, (*R, S*)- α -cyano-3-phenoxybenzyl (1*RS*)-*cis, trans*-3-(2, 2-dichlorovinyl)-2, 2-dimethylcyclopropanecarboxylate, used as a standard was a gift from Sumitomo Chemical Industries, Ltd. All other chemicals used were of the highest grade available commercially.

2. GC-MS

Gas chromatography and mass spectrometry (GC-MS) of the insecticidal formulations were carried out using a Shimadzu Gas Chromatograph-Mass Spectrometer QP2000GF (Shimadzu Co.) under the following conditions—column:

Shimadzu CBI-S30-025 (30 m $\times$ i.d. 0.32 mm), oven temperature: 50 to 250°C with hold time of 1 min and ramp rate of 20 °C/min, injection temperature: 90 to 280°C with ramp rate of 30°C/min, carrier gas: He 0.8 kg/cm², ionization: electron impact (EI) at 250°C. One microliter of the NPEF solution, diluted with acetone to contain *ca.* 50 ppm cypermethrin, was injected without clean-up for analysis.

3. GC-ECD

A Hitachi Gas Chromatograph 163 (Hitachi Ltd.) equipped with an electron capture detector (ECD) was used to separate and quantitate cypermethrin under the following conditions—column: OV-17 (190 cm $\times$ i.d. 3 mm), oven temperature: 270°C, injection temperature: 300°C, carrier gas: N₂ 3.2 kg/cm². A calibration line was made by injecting seven doses between 0.1 and 1.0 ng of cypermethrin standard in acetone. The NPEF were diluted appropriately (see the dilution factors in Table 1) with acetone so that the peak areas fit within the areas of the calibration line. Diluted solutions were prepared in triplicate for each NPEF and 1 μ l was injected without clean-up for analysis.

RESULTS AND DISCUSSION

Figure 1 shows the GC-total ion chromatograms obtained in GC-MS analysis. Cypermethrin standard gave a single peak at retention time (*Rt*) 14.85 min (Fig. 1F). Agrothrin EC, a registered formulation containing 6% cypermethrin as the active ingredient, also gave a peak at the same *Rt* (Fig. 1E). Although the chromatograms of "Muso"(A), "Muso"(B), "Hekiro" and "Kensogen-Ten" (Fig. 1A-D, respectively) were more complicated than the former two and suggested the presence of numerous components possibly derived from plant extracts, a peak at *Rt* 14.85 min was clearly detected in all the so-called NPEF. Mass spectra of the peak at *Rt* 14.85 min are given in Fig. 2. With cypermethrin standard (Fig. 2F), major fragment ions detected were *m/z* 209, 181, 163, 127, 91, 77 and 51, but no molecular ion peak (*M.W.* = 416.3) was observed under the conditions. With all other formulations (Fig. 2A-E), the fragmentation patterns completely coincided with that of cypermethrin, confirming the identity of the common peak detected at *Rt* 14.85 min in GC-total ion chromatogram (Fig. 1) as cypermethrin. The result is an indubitable evidence that all the so-called NPEF examined are supplemented by cypermethrin, a synthetic pyrethroid.

Figure 3 shows the GC-ECD chromatograms of the six samples. Cypermethrin standard gave a single peak at *Rt* 4.80 min (Fig. 3F). All other formulations gave a corresponding peak at the same *Rt*, supporting again the above conclusion. The calibration line prepared by GC-ECD for cypermethrin gave a good linearity ($r^2=0.9926$) in this dose range.

$$Y = 0.1364X - 0.0765$$

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Fig. 1 GC-total ion chromatogram of various so-called NPEF, Agrothrin EC and cypermethrin standard.

A: "Muso"(A), B: "Muso"(B), C: "Hekiro," D: "Kensogen-Ten," E: Agrothrin EC, F: cypermethrin. The arrows (R_t 14.85 min) depict the fractions on which the mass spectra were examined.

Table 1 Cypermethrin concentration in so-called NPEF and Agrothrin EC determined by GC-ECD.

Formulation	Dilution factor	Peak area (Relative unit)	Cypermethrin concentration(%)
"Muso"(A)	$\times 50,000$	3.3892 ± 0.1354	1.9 ± 0.1
"Muso"(B)	$\times 50,000$	3.5133 ± 0.1961	2.0 ± 0.2
"Hekiro"	$\times 100,000$	3.0507 ± 0.1812	3.4 ± 0.3
"Kensogen-Ten"	$\times 50,000$	2.5981 ± 0.1333	1.4 ± 0.1
Agrothrin EC	$\times 200,000$	2.7477 ± 0.1419	6.0 ± 0.5

where Y is cypermethrin in $\text{ng}/\mu\text{l}$ and X is peak area in relative unit.

Cypermethrin concentrations in the so-called NPEF as well as in Agrothrin EC were determined using the calibration line (Table 1). In agreement with the previous estimation,¹⁾ "Muso"(A) and "Muso"(B) were found to contain 1.9 ± 0.1 and $2.0 \pm 0.2\%$, respectively, while "Hekiro" and "Kensogen-Ten" were found to contain 3.4 ± 0.3 and $1.4 \pm 0.1\%$ cypermethrin, respectively. The cypermethrin concentration of Agrothrin EC estimated in the present study, i.e. $6.0 \pm 0.5\%$, was in agreement with the concentration registered for the formulation and demonstrated the reliability of the present

analytical procedure.

The results described in the previous¹⁾ and present papers clearly reveal that the so-called NPEF which have been repeatedly advertised²⁻⁴⁾ as "natural" and therefore safe for both humans and the environment are deceptive and in reality contain a synthetic insecticide as the active ingredient. Since it is well known that cypermethrin has rather high fish toxicity (e.g. LC_{50} for brown trout at 96 hr: 0.0020 to $0.0028 \text{ mg}/\text{l}$,⁵⁾ it can cause a serious damage to fish populations if these formulations are sprayed carelessly near the aquatic environment. Although mammalian toxicity of cypermethrin is fairly low (e.g. acute oral LD_{50} for rats: 200 to $800 \text{ mg}/\text{kg}$),⁵⁾

the formulations can also be harmful to humans if used negligently as advertised.²⁻⁴⁾ Recent studies by several groups (unpublished data) are revealing that almost all the so-called NPEF examined contain synthetic pesticides—deltamethrin in “New Mushigie,” fenvalerate in “Mushigie,” diflubenzuron in “Sin-Muso,” cypermethrin in “Musikoro,” triadimefon in “Kensogen-Ku” and oxadiazon in “Kensogen-Chi.” The former three are insecticides, while

the latter two are a fungicide and a herbicide, respectively. These facts imply that the supplementation of synthetic pesticides to so-called NPEF is being widely practiced to deceive health conscious consumers who are willing to pay a higher price for agricultural produce labeled as “organic.” Regulation of these so-called NPEF is urgently due to prevent possible health hazard to users and damage to the environment. Consumers who purchase such produce are also

Fig. 2 Mass spectra of the fraction (*R_t* 14.85 min) separated from various so-called NPEF, Agrothrin EC and cypermethrin standard separated by GC.

A: “Muso”(A), B: “Muso”(B), C: “Hekiro,” D: “Kensogen-Ten,” E: Agrothrin EC, F: cypermethrin.

Fig. 3 GC-ECD chromatograms of various so-called NPEF, Agrothrin EC and cypermethrin standard.

A: “Muso”(A), B: “Muso”(B), C: “Hekiro,” D: “Kensogen-Ten,” E: Agrothrin EC, F: cypermethrin.

advised not to be misled by the label "organic."

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要 約

いわゆる天然・植物抽出液製剤に合成殺虫剤が混入されていることの追加証明

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有機農業で害虫防除に使用されるいわゆる天然・植物抽出液のなかには、合成ピレスロイド剤のサイパーメスリンが混入されているものがあることを明らかにした前報の結果をさらに確認するために、有効成分のガスクロマトグラフィー質量 (GC-MS) 分析を行なった。夢草 (A), 夢草 (B), 碧露, 健草源・天, および登録農薬であるアグロスリン乳剤のガスクロマトグラムには、標準のサイパーメスリンと一致する保持時間を示す共通のピークが検出された。このピークの質量スペクトルを比較したところ、分子イオンピークは観察されなかったものの、主要なフラグメントイオンとして m/z 209, 181, 163, 127, 91, 77, 51 が観察され、フラグメンテーションパターンはサイパーメスリンのそれと完全に一致した。したがって、供試した天然・植物抽出液にはすべてサイパーメスリンが混入されていることが確認された。GC-ECD で有効成分濃度の定量を行なったところ、夢草 (A), 夢草 (B), 碧露, および健草源・天のサイパーメスリン濃度は、それぞれ 1.9, 2.0, 3.4 および 1.4% であると推定された。