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Determination of proposed maximum residue limit of cypermethrin in starfruit (*Averrhoa carambola*)

[Penentuan cadangan had maksimum residu cipermethrin dalam belimbing (*Averrhoa carambola*)]

C.K. Ngan*, A.M. Khairatul* and S. Mohammad Shahid*

Keywords: cypermethrin, starfruit, maximum residue limits

Abstract

Residue trials of cypermethrin in starfruit (*Averrhoa carambola*) were conducted to estimate the maximum residue limit (MRL). Five trials were conducted at two commercial growers' plots in Mantin, Negeri Sembilan. The study plots contained 120 mature starfruit trees which were selected and treated with cypermethrin using the manufacturer's recommended rate (0.023 kg a.i./ha). The insecticide was applied with a motorized sprayer at weekly intervals. Starfruit samples (2 kg) were randomly collected from the plots at 0, 1, 3, 4, 5 and/or 7 days after the last spray. Analytical procedure of cypermethrin extraction was validated prior to actual analysis. Satisfactory recoveries ranging from 92.6% to 106.7% were obtained for the fortified starfruit samples. Results showed low evels of cypermethrin residues in starfruit ranging from < 0.01 to 0.09 mg/kg. The determined value of proposed MRL was 0.2 mg/kg. The Dietary Intake Assessment based on Malaysian Food Consumption Pattern confirmed that the proposed MRL concentration will not cause health risks to consumers.

Introduction

Maximum Residue Limits (MRLs) is defined as the maximum levels of pesticide residues that can be detected in a foodstuff if a crop protection product (CPP) is applied according to critical good agricultural practice (FAO/WHO 1997). Critical good agricultural practice is a condition where maximum numbers of application and dosage coupled with minimum pre-harvest intervals are applied. In other words, a residue trial is conducted to give a 'worst case scenario' of residue or maximum level of residue that could be expected in the crop. In establishing MRLs, residue trials need to be conducted to reveal the decline pattern and the possible residue concentration at the recommended pre-harvest intervals (PHI). MRL is calculated by a statistical method and recommended after dietary intake assessment confirms the consumption of the relevant food does not result in dietary risk.

MRLs on agricultural commodities have been established in many countries in order to protect consumer health and to avoid trade barriers. MRL values vary between countries due to different agricultural practices and climatic conditions. The discrepancy in MRL values can result in trade dispute. This is where Codex MRLs, being recognized worldwide, play an important role. Codex MRLs are set by Joint FAO/WHO Meeting on

*Strategic Resources Research Centre, MARDI Headquarters, Serdang, P.O. Box 12301, 50774 Kuala Lumpur, Malaysia
Authors' full names: Ngan Chai Keong, Khairatul Azmah Mohamed and Mohammad Shahid Shahrun
E-mail: ckngan@mardi.gov.my

Pesticide Residue (JMPR) after evaluation of residue data submitted by companies or national governments. JMPR, comprising the World Health Organization (WHO) Core Assessment Group and the FAO panel of Experts on Pesticide Residues in Food and the Environment, is responsible for evaluating pesticide residue and toxicology data for estimation of MRLs and acceptable daily intakes (ADI). The World Trade Organization requires that all signatory countries comply with Sanitary and Phytosanitary (SPS) Agreement (Henson and Loader 2000), which applies to all sanitary and phytosanitary measures related to agricultural products and foodstuffs to protect the health of consumers, plants and animals. Under this agreement, Codex standards are recognized as international points of reference in case of a trade dispute. Products in compliance with the Codex standards are in compliance with the provisions of the SPS Agreement and can therefore move freely in trade. Without Codex MRL, Malaysian commodities will face difficulties in international trade. A violation in MRL by a single commodity in international trade could affect national reputation and a wide range of export commodities.

Starfruit (*Averrhoa carambola*), also known as carambola, is one of the important export commodities of Malaysia. Statistical data from Department of Agriculture Malaysia revealed that in 2010, carambola production was 11,280 metric tonnes valued at RM31.6 millions (Hii et al. 2011). The export will be dangerously threatened when WTO regulations are fully imposed. Sign of non-tariff trade barrier is emerging after a number of recent reported cases that the fruits were rejected due to pesticide residues. Thus, there is a need to determine the MRL of pesticide residues in starfruit.

Starfruit is subjected to attack from a wide range of insects, pests and mites which reduce the quality thus necessitating the use of pesticides. One of the widely used pesticides in starfruit is cypermethrin ((RS)-α-Cyano-3-phenoxybenzyl(1RS)-cis,trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylate). This chemical, discovered by Elliott and co-workers (Elliott et al. 1975), is a synthetic pyrethroid insecticide used to control a wide range of insect pests, particularly Lepidopterous pests in cereals, citrus, cotton, fruit, rapeseed, tobacco and vegetables (Jin and Webster, 1998).

Setting of MRLs requires residue trial data. There are, however, limited residue trials conducted in Malaysia. The present study was conducted for the purpose of establishing MRL for a commonly used insecticide (cypermethrin) in starfruit.

Materials and methods

Study plot, treatment and sampling

Five supervised residue trials were conducted in 2004 at two commercial grower's plots in Mantin, Negeri Sembilan as listed in *Table 1*. The plots having 120 mature starfruit trees each were selected and treated with cypermethrin using the manufacturer's recommended rate (0.023 kg a.i./ha). The insecticide was applied with a motorized sprayer at weekly intervals up to a maximum of four applications. Starfruit samples (2 kg) were randomly collected from the plots at 0, 1, 3, 4, 5 and 7 days after the last spray. Overall the five trials represented five replicates of the residue decline pattern of applied cypermethrin at the recommended rate.

Analytical procedure

Extraction of cypermethrin residues from starfruit was carried out using a multi residue method modified from a published method (Anastassiades et al. 2003). Homogenized starfruit sample (30 g) was put into a 250 ml bottle, followed by $NaHCO_3$ (5 g), ethyl acetate (60 ml) and anhydrous Na_2SO_4 (30 g). The mixture was homogenized using a homogenizer (IKA UltraTurrax) for about 1 min. After the sample was shaken in an orbital shaker for about 2 h, 5 ml of the extracts was cleaned

Table 1. Residue data summary from supervised trials of cypermethrin in starfruit

Trial	Application rate per treatment (Manufacturer Recommended)			No. of treatment per trial	Residues (mg/kg)	Sampling interval (days after last treatment)
	kg a.i./ha	water litre/ha	kg a.i./ha water			
1	0.023	450	0.005	4	0.08, c<0.01	0^{++}
					0.09	3^{+}
					0.09	7
2	0.023	450	0.005	4	0.02, c<0.01	3^{+}
					<0.01	5
3	0.023	450	0.005	4	<0.01, c<0.01	0^{++}
					<0.01	1
					<0.01	3^{+}
					<0.01	5
					<0.01	7
4	0.023	450	0.005	3	0.02, c=0.01	0^{++}
					0.01	1
					0.03	3^{+}
					0.01	5
					0.02	7
5	0.023	450	0.005	4	0.01, c<0.01	0^{++}
					0.01	4
					<0.01	7

c = Untreated control
$^{+}$Recommended pre-harvest interval
$^{++}$Sampling at 2 hours after application

using the Primary-Secondary Amines (PSA) cartridge. The cleaned extract was analysed by gas chromatograph (Hewlett Packard 6890) equipped with micro-Electron Capture Detector (μECD). The column used was HP-5 capillary column (30 m length x 0.32 mm internal diameter and 0.25 μm film thickness) packed with 5% diphenyl and 95% dimethylpolysiloxane. Cypermethrin reference material of purity 95.6% purchased from Reidel de Haen, Germany, was used for preparation of calibration standard solution and spiking solution (recovery study).

Recovery study

Prior to the experiment, a recovery study was conducted to validate the analytical procedure for the extraction of cypermethrin from starfruit. Homogenized starfruits (30 g) in three replicates with no history of cypermethrin treatment were spiked with cypermethrin standard solution at 0.01 mg/kg and 0.5 mg/kg respectively. The spiked starfruits were left for half an hour for solvent evaporation, followed by analytical procedure described above. Recovery of cypermethrin was calculated using the following equation:

$$\text{Recovery of cypermethrin (\%)} = \frac{\text{Detected cypermethrin (mg/kg)}}{\text{Spiked cypermethirn (mg/kg)}} \times 100$$

Recovery between 70–120% indicates the method suitability in determining analyte quantitatively (Holland et al. 2000).

MRL estimation

A European Union method was employed for the calculation of MRL values (Hyder et al. 2003). The estimation was based on the equation shown below:

MRL* = R + KS

R = Mean of HR** (Highest Residue after Pre-Harvest Interval, PHI)

K = One-sided tolerance factor for normal distributions with 95% confidence level

S = The standard deviation of HR after PHI

*Estimated MRL

**Highest residue after PHI from each of the field trial

Dietary intake assessment

The Malaysian Food Consumption pattern was acquired from the Ministry of Health Malaysia (2006). Acceptable Daily Intake (ADI) (derived from the toxicology study) of cypermethrin was extracted from Lu (1995). An assessment method by Global Environment Monitoring System – Food Contamination Monitoring and Assessment Program (GEMS/Food) and Codex Committee on Pesticide Residue was used (WHO 1989). In this assessment approach, National Theoretical Maximum Daily Intake (NTMDI) of pesticide concerned (including the proposed MRL of pesticide in new commodity) is estimated based on national dietary consumption pattern and the value of established MRLs and/or proposed MRL. The derived NTMDI should not exceed daily Maximum Permitted Intake (MPI = ADI of pesticide x standard body weight/ person) for safe conclusion that the proposed MRL will not cause health risk to the population.

Statistical analysis

A regression analysis was performed on the response of cypermethrin to the Gas Chromatograph- micro-Electron Capture Detector (GC-μECD). R^2 value of more than 0.995 indicates good linearity within the concentration range detected in the GC-μECD.

Results and discussion

Recoveries of cypermethrin

Recoveries ranging from 92.6% to 106.7% with a relative standard deviation (RSD) of <4.5% were obtained from overall recovery data of two levels of spiking (0.01 and 0.5 mg/kg), suggesting that the analytical method used was effective (*Table 2*). The limit of quantification (LOQ) of the analytical method for cypermethrin in starfruit is 0.01 mg/kg. The LOQ is the lowest level of spiking (0.01 mg/kg) that gives acceptable recovery (70–120%) and precision (relative standard deviation of recoveries <15%). One example of calibration curve (for quantification of detected residues) with good linearity (R^2 = 0.9974) within 0.02–0.1 μg/ml is shown in *Figure 1*. Chromatogram examples of cypermethrin peaks in standard solution and sample extract are shown in *Figures 2–3*.

Data on cypermethrin residue in starfruit from five supervised residue trials are presented in *Table 1*. Results from the residue trials indicated that cypermethrin applied at the manufacturer's recommended rate resulted in low residue in starfruit

Table 2. Percentage recoveries of cypermethrin from spiked starfruit

Spiked concentration (mg/kg)	Percentage recovered (%)					SD	%RSD
	R1	R2	R3	R4	Average		
0.01	100.0	103.3	106.7	97.6	101.9	4.0	3.9
0.5	101.1	96.1	92.6	102.2	98.0	4.4	4.5

SD = Standard deviation

RSD = Relative standard deviation

ranging from <0.01 mg/kg to 0.09 mg/kg. The highest recorded residue was 0.09 mg/kg during trial 1 at three days after the last treatment. For trial 3, the residue was found below the limit of quantification (<0.01 mg/kg) for the samples collected from all the sampling intervals. The observed low residue levels of cypermethrin could be due to low dosage used (0.023 kg a.i./ha) and the compound's rapid degradation. In addition, the trials were conducted between end of March and mid August, which was considered a relatively dry season. Rapid degradation of cypermethrin residue in crops has been reported by other researchers. Lettuce treated with 0.08 kg a.i./ha of cypermethrin had residues at 0.097 and 0.011 mg/kg by 7 and 14 days after the last treatment respectively (Braun et al. 1982). No cypermethrin residues were found in the grain samples at 1, 3, 6 and 27 days after application of cypermethrin to wheat at 0.028 kg a.i./ha (Westcot and Reichle 1987).

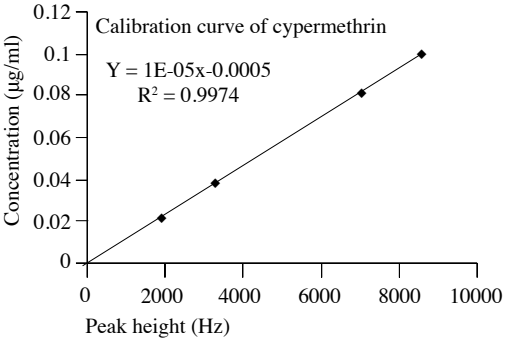

Figure 1. Calibration curve of cypermethrin

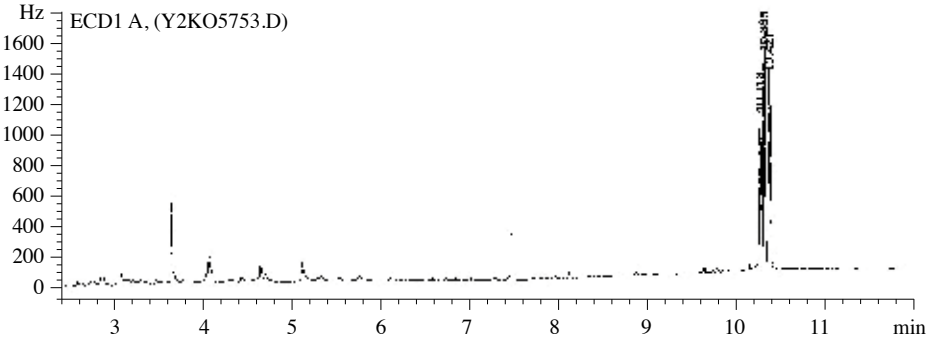

Figure 2. GC-µECD chromatogram of cypemethrin standard (0.04 µg/mL) depicting three peaks of cypermethrin isomers at retention time 10.318, 10.365 and 10.427 min respectively

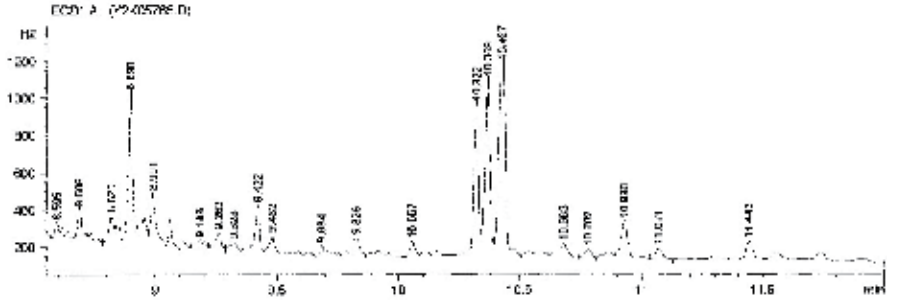

Figure 3. GC-µECD chromatogram of carambola extract sample (Trial 4, Day 0) depicting three peaks of cypermethrin isomers at retention time 10.322, 10.369 and 10.427 min respectively

MRL estimation

MRL for cypermethrin in starfruit is calculated using an European Union method (Hyder et al. 2003). This method requires a minimum of four residue trials for a minor crop which is defined as a crop which has a mean dietary intake of less than 7.5 g/person/day. Starfruit is considered as a minor crop even though the National Food Consumption Data by Ministry of Health Malaysia recorded its mean dietary intake for Malaysian at 10.48 g/person/day (Ministry of Health Malaysia 2006). For MRL calculation, the highest residue from each trial will be chosen as one data point; these data points are grouped and computed for relevant statistical values. For values that are below the limit of quantification (LOQ), they are assumed to be at the LOQ. The estimation was based on the equation shown below:

$$\begin{aligned} MRL^* &= R + KS \\ &= 0.032 \text{ mg/kg} + (4.202 \times 0.03) \\ &= 0.16 \text{ mg/kg} \end{aligned}$$

R is the mean of the highest residues (HR) of every trial after PHI (five trials with five highest residues in ascending order are 0.01, 0.01, 0.02, 0.03 and 0.09 mg/kg), S is the standard deviation and K is the one-sided tolerance factor for normal distributions with 95% confidence level. It should be noted that HR of each trial will be assumed to be at the LOQ when all residue results after the PHI are <LOQ. The estimated MRL value is 0.16 mg/kg. This value is rounded up to 0.2 mg/kg following the rules set by Codex in having the common classes of MRL values such as 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1.0, etc. Therefore, the proposed MRL of cypermethrin in starfruit based on residue data is 0.2 mg/kg.

Dietary intake assessment and MRL determination

After MRL estimation, dietary intake assessment was conducted to ensure the MRL value does not give rise to dietary risk for the consumers. Dietary risk assessment was conducted by following the guideline from Global Environment Monitoring System – Food Contamination Monitoring and Assessment Program (GEMS/Food) and Codex Committee on Pesticide Residue (WHO 1989). Based on this guideline, National Theoretical Maximum Daily Intake (NTMDI) for each commodity was calculated by multiplying the established or proposed MRL by national average daily consumption and summed together. The calculated total NTMDI (*Table 3*) was

Table 3. Dietary intake assessment of cypermethrin

Commodity	MRL[a] mg/kg	National diet kg/person/day	NTMDI mg/person/day
Papaya	2.0	0.0061	0.0122
Cocoa beans	0.05	0.002	0.0001
Citrus fruits	2.0	0.0083	0.0166
Chilli	0.5	0.02	0.01
Maize	0.05	0.006	0.0003
Guava	2.0	0.0016	0.0032
Long Beans	0.5	0.0028	0.0014
Kale	1.0	0.0021	0.0021
Cabbage	1.0	0.0127	0.0127
Cauliflower	1.0	0.0019	0.0019
Okra	0.5	0.0037	0.00185
Mango	2.0	0.0024	0.0048
Palm Oil	0.5	0.065	0.0325
Lettuce	2.0	0.0006	0.0012
Mustards	2.0	0.016	0.032
Milk	0.05	0.27	0.0135
Tomato	0.5	0.0026	0.0013
Brinjal	0.2	0.0043	0.00086
Meat	0.05	0.13	0.0065
Starfruit	0.2*	0.01048	0.0003
		Total	0.15711
		% MPI**	13.1%

[[a]*Source: Sixteenth Schedule, Regulation 41, Malaysian Food Regulation 1984*]

*Proposed MRL for cypermethrin in starfruit

**Daily Maximum Permitted Intake
= Acceptable Daily Intake (ADI) x standard body weight/person
= 0.02 mg/kg body weight/day x 60 kg/person
= 1.2 mg/person/day

% MPI = (total NTMDI/MPI) x 100
= 0.15711/1.2 x 100 = 13.1%

0.157 mg/person/day which is 13.1% of the daily Maximum Permitted Intake (MPI), indicating there is no dietary risk. Therefore the proposed MRL is deemed acceptable.

The residue data was submitted to the Joint FAO/WHO Meeting on Pesticide Residue (JMPR) in 2008 and following procedure in the Codex MRL setting, the Codex MRL of cypermethrin in starfruit (carambola) was adopted in 2009 at the level of 0.2 mg/kg. Available Codex MRLs of cypermethrin for other fruits are 2.0 mg/kg for citrus fruits and peach, 0.5 mg/kg for berries and other small fruits (FAO 2004). The observed low residue levels of cypermethrin (<0.1 mg/kg) in the residue trials do not seem likely to result in MRL violation if the MRL is set at 0.2 mg/kg. However, if the MRL is not available, the starfruit exporters will face the risk of their exports being rejected by importing countries as they apply limit of determination (zero tolerance), usually below 0.05 mg/kg as rejection level.

Conclusion

Lack of MRLs for agricultural commodities may give rise to non-tariff trade barrier for the country bounded by WTO trade agreement. This study was a part of an effort by MARDI to establish MRLs of pesticides for export commodities. A proposed MRL of 0.2 mg/kg was determined for starfruit based on residue trials data. Dietary intake assessment confirmed the determined MRL was toxicologically acceptable.

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Abstrak

Kajian residu cypermethrin dalam belimbing segi telah dilaksanakan untuk menganggar nilai had maksimum residu. Lima kajian telah dijlankan di dua plot komersial di Mantin, Negeri Sembilan. Plot kajian yang mengandungi 120 batang pokok matang dirawat dengan cypermethrin pada kadar yang disyorkan oleh pengeluar racun tersebut (0.023 kg a.i./ha). Insektisid tersebut disembur menggunakan penyembur bermotor pada setiap minggu. Sampel belimbing segi (2 kg) dipetik secara rawak pada 0, 1, 3, 4, 5 dan 7 hari selepas semburan terakhir. Kaedah analisis mengekstrak cypermethrin disahkan sebelum analisis dijalankan. Perolehan semula yang memuaskan dalam julat 92.6% hingga 106.7 % diperoleh untuk sampel belimbing segi yang diperakukan. Keputusan analisis menunjukkan aras residu cypermethrin yang rendah dalam belimbing segi antara <0.01 hingga 0.09 mg/kg. Nilai cadangan had maksimum residu 0.2 mg/kg telah dianggarkan. Penilaian Pengambilan Diet berdasarkan Corak Pemakanan Rakyat Malaysia mengesahkan nilai cadangan had maksimum residu tersebut tidak akan menimbulkan risiko kesihatan kepada pengguna.

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