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Variation of cypermethrin residue levels in selected tropical fruit samples within and among field trials

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Abstract
A comparison of pesticide residue data from field trials of three sampling methods was done to assess the variability of cypermethrin residue levels in terms of within the field trial and among between field trials. The fruit (mango/ papaya) samples were collected on individual tree basis (Study A: within field trial), on subplot basis (Study B: within field trial) and whole plot basis (Study C: among field trials). The field trials were conducted from 2006 – 2009. In Study A and B, replicate analysis yielded smaller measurement variation compared to sample replicates. Measurement of variation was highest (Relative Standard Deviations, RSD = 50.7%) among field trial samples (Study C) followed by samples from Study A (RSD = 28.9%) and Study B (RSD = 15.8%). The RSD of replicate samples were higher than the RSD of the analysis of replicates in Study A and B. The contributors of uncertainty to the measurement of cypermethrin residue in descending order, based on percentage variance were: sampling (82.2 – 95.6%) followed by sample processing (2.6 – 11.5%) and analysis (1.7 – 6.3%). The field factor was a significant contributor to the variation in pesticide residue measurement as compared to the laboratory factor.

Keywords: sampling, measurement uncertainty, pesticide residues

Introduction

Accuracy and reliability of pesticide residue measurements are important for decision making in terms of establishing the maximum residue limit (MRL), checking compliance to the MRL, risk assessment and food processing studies, as they ultimately would affect public safety and health. The ISO/IEC 17025 standard stipulates that accredited laboratories be used to determine all sources of uncertainty that could contribute to the total measurement of the analyte (ISO/IEC 2005). In the case of pesticide residue measurement, uncertainty in the method of sampling is known to contribute significantly compared to that of the laboratory that constitutes sample processing and analysis (Ambrus 2000; Ambrus and Soboleva 2004; Lyn et al. 2007). It is important to emphasise that pesticide residue measurements obtained have three major sources of uncertainty which are seen in the following equations:

$$u(C)^2 = u(C_S)^2 + u(C_{SP})^2 + u(C_A)^2 \quad \text{Equation 1}$$

where $u(C)$ = Uncertainty in the concentration of the analyte

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$u(C_S)$ = Uncertainty in the concentration of the analyt due to sampling
$u(C_{SP})$ = Uncertainty in the concentration of the analyte due to sample processing
$u(C_A)$ = Uncertainty in the concentration of the analyte due to analysis

In actuality, uncertainty due to sampling could also originate from non-sampling factors, such as biasness in pesticide application, causing non-uniformity in distribution of pesticide residues. Nevertheless for simple quantification of uncertainty sources towards the total measurement of uncertainty, often the non-sampling factors prior to the laboratory phase are grouped under 'sampling uncertainty'.

The contribution of sampling uncertainty to the total measurement of pesticide residue is largely due to the residue variation in the field. Factors affecting variation in residue levels are uniformity of application, distribution of sprayed crop in the field, wind conditions during application, weather conditions, sampling error, etc. Sampling design is very important in order that the actual distribution of the analytes of interest is well represented in the sample population. According to FAO (2009), regarding the submission of pesticide residue data for the MRL setting, a simple random sampling from an experimental plot is sufficient for collection of the field sample.

Variations in residue levels can be quantified by the variability factor or the uncertainty parameter. Nevertheless the ISO/IEC 17025 standard for laboratory accreditation emphasises the use of the uncertainty parameter. It is important to determine the variability of the residue level from the samples taken from within field and among field trials. Information on the variability factor is useful to the regulatory authority for determining the risk assessment and MRL setting.

The objective of the present study was to assess the variation of cypermethrin residue levels in mango/papaya fruits sampled within the field trial and among field trials. Discussion in the present paper combines data from the field trials conducted at the fruit farms from 2006 – 2009. Cypermethrin is a common insecticide used in mango and papaya farms.

Materials and methods

Cypermethrin residue data from the field trial were divided into three types of sampling methodology, namely sampling on the per tree basis (Study A-within field trial samples), sampling on per sub-plot basis (Study B-within field trial samples) and sampling on per plot basis (Study C-between field trial samples). In Study B, the plot was divided into five subplots. Each subplot consisted of two rows of trees. Cypermethrin under the trade name of Kencis EC (5.5% cypermethrin) was applied using a motorised sprayer.

For the sampling process, each sample consisted of at least 2 kg of fruits. Although the samples were taken at the day after the last application (DALA), the DALA in Study B (14 DALA) was not the same as Study A and C (both were 0 DALA). This was due to different sampling design of each study. The purpose of the study was to assess the variation in residue levels at one sampling date. Information related to each study is shown in *Table 1*. The figurative representation of the sampling designs of Study A, B and C are shown in *Figure 1, 2* and *3* respectively.

Samples from all the field trials were sent to the Pesticide Laboratory, Strategic Resources Research Centre, MARDI Serdang for residue analysis. The laboratory test method (sample processing and analytical method) from Ma et al. (2005) was used for quantification of the pesticide residue. The detailed sample processing method followed that of Ngan et al. (2011). Recovery data of cypermethrin in mango and papaya using the analytical method are shown in *Table 2*. Inconsistency in the number of replicate analysis for Study A, B dan C was due to the fact that the field

Table 1. Information on samples from the three types of sampling designs

Study code	Active ingredient and purity	Crop	Plot location	No. of trees per trial	DALA	Sampling replicate	Analysis replicate
A	Cypermethrin 5.5%	Mango	Changlun, Kedah	20	0	20	3
B	Cypermethrin 5.5%	Mango	Changlun, Kedah	150	14	5	5
C	Cypermethrin 5.5%	Papaya	MARDI Serdang &		0	6	1
			MARDI Sintok	100		Replicate 1-Serdang	
				100		Replicate 2-Sintok	
				100		Replicate 3-Sintok	
				100		Replicate 4-Sintok	
				100		Replicate 5-Serdang	
				100		Replicate 6-Sintok	

A = Sampling per individual tree basis; B = Sampling per subplot basis;
C = Sampling per whole plot basis
DALA = Days after last application

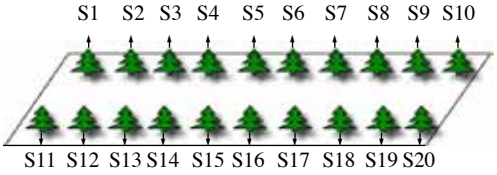

Figure 1. Figurative description of sampling design of Study A (within field trial) in which sampling was based on per tree basis (S refers to sample replicate; number of trees in the plot is more than what is shown in the figure)

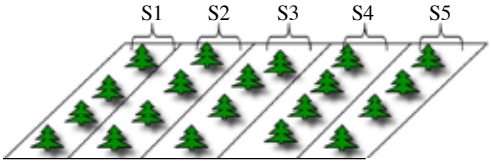

Figure 2. Figurative description of sampling design of Study B (within field trial) in which sampling was based on per subplot basis (S refers to sample replicate; number of trees in the plot is more than what is shown in the figure)

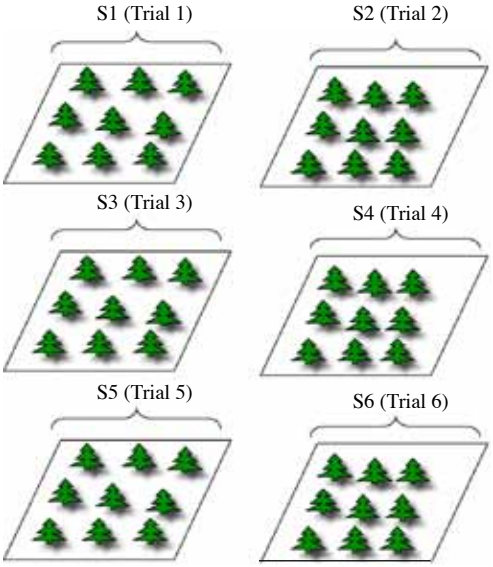

Figure 3. Figurative description of sampling design of Study C (among field trials) in which sampling was based on per plot basis (S refers to sample replicate; number of trees in the plot is more than what is shown in the figure)

Table 2. Recoveries of cypermethrin in mango and papaya of analytical method

Sample	Spike concentration (mg/kg)	Mean (n = 5) recovery (%)	RSD (%)
Mango	0.1	97.2	2.4
	0.5	93.9	2.7
Papaya	0.1	81.7	2.3
	0.5	94.6	3.5

trial data was pooled from different field trial studies.

Since samples from the three studies were analysed under the same laboratory, contribution of sample processing uncertainty and analytical method uncertainty were confined to a single laboratory, thus contribution of sampling uncertainty can be discerned relatively. The uncertainty values were derived from unpublished works which were conducted in the author's laboratory. It should be noted that sample processing of sampling uncertainty can be derived from the equation 1 based on Relative Standard Deviation (RSD) of the measured residues given the values of sample processing and analytical method uncertainties. The total contribution of sampling, sample processing and analytical method uncertainties will be expressed as percentage of variance.

Results and discussion

Results of cypermethrin residue levels in the fruit samples are shown in *Figure 4* (Study A), *Figure 5* (Study B) and *Figure 6* (Study C). The RSD values of field samples in Study A, B and C were 28.9, 15.8 and 50.7% respectively. Among the field trials, the highest variation of residue levels occurred within the replicates. However for samples within the field trials (Study A and B), the RSD of field samples collected from individual trees exhibited higher RSD (28.9%) than samples collected from subplots (15.8%). This could be attributed to the larger pool of random samples from the subplot that could reduce variation.

As for the study A and B, where there were replicate analyses for each field sample replicate, the RSD values of the replicate analyses were lower than the RSD values of the sampling replicate. The range of RSD values for the replicate analysis for Study A and B were 1.5 – 21.0% and 9.1 – 13.4% respectively (*Table 3*). Although there were no replicate analysis for Study C, based on RSD of analysis replicate and sampling replicate in Study A and B (*Table 3*), it is believed that the replicate analysis if it was conducted, would be much lower than the reported RSD value of 50.7% for the sampling replicate. The lower RSD value of the replicate analysis compared to the sampling replicate suggests that additional factors, such as field factors contributed to the total variation of the pesticide residue levels.

The uncertainty and variance of the three error components (sampling, sample processing and analysis) in the cypermethrin residue levels in Study A, B and C are shown in *Table 4*. The sampling uncertainty is the largest source of uncertainty compared to the sample processing uncertainty and analysis uncertainty. Ambrus (2000) found that the difference in residue data can vary for as much as 80 – 100%. The variances in sampling data for the three studies seem to support the findings of Ambrus (2000).

The variability factor of as much as 19 has been reported for pesticide residues found in orchard fruits in a monitoring programme (Rawn et al. 2006). Based on the fact that sample processing uncertainty and analysis uncertainty are known from previous reports on measurement uncertainty, variation due to the field factor in the study by Rawn et al. (2006) was clearly a significant contributor of

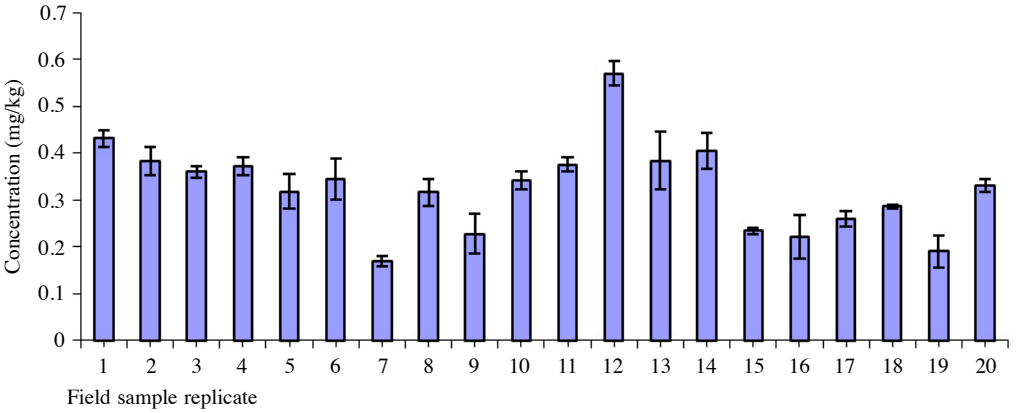

Figure 4. Cypermethrin concentration (mg/kg) in mango samples (20 replicates of field sample) at 0 day after last application in Study A (sampling from individual tree). Three replicates of analysis per replicate of field sample

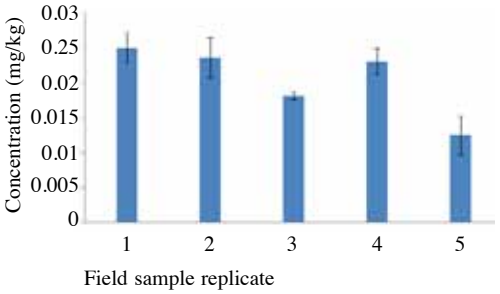

Figure 5. Cypermethrin concentration (mg/kg) in mango (five replicates of field sample) at 14 days after last application in study B (sampling from subplot). Five replicates of analysis per replicate of field sample

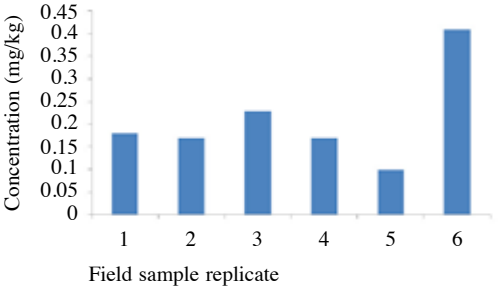

Figure 6. Cypermethrin concentration (mg/kg) in papaya (one field sample replicate per field trial) at 0 day after last application in Study C (sampling from whole plot). No RSD for replicate of analysis because one analysis replicate per replicate of field sample was performed in Study C

Table 3. Relative standard deviation of replicate analysis and replicate sampling in Studies A and B

Study	Analysis replicate RSD (%)	Sampling replicate RSD (%)
A	3.9, 7.7, 3.3, 5.1, 11.7, 12.7, 6.5, 9.3, 18.8, 5.8, 4.2, 4.6, 16.2, 9.3, 2.9, 21.0, 6.2, 1.5, 17.8, 3.9	28.9
B	11.5, 11.8, 9.1, 9.6, 13.4	15.8
C	No data	50.7

A = 3 analysis replicates per sample; 20 sampling replicates
B = 5 analysis replicates per sample; 5 sampling replicates
C = Only one analysis replicate was performed, thus analysis replicate RSD could not be derived; 6 sampling replicates from 6 field trials, respectively

Table 4. Relative standard deviation (uncertainty) and relative variance of measurement for three contributors of uncertainty (sampling, sample processing and analysis)

Study	Measurement (%)	Sampling component (%)	Sample processing component (%)	Analysis component (%)
A	28.9 (100)	28.2 (95.6)	4.7 (2.6)	3.8 (1.7)
B	15.8 (100)	24.3 (85.4)	4.7 (8.8)	3.8 (5.8)
C	50.7 (100)	50.0 (82.2)	17.2 (11.5)	12.7 (6.3)

Variance (%) value in bracket

uncertainty in residue measurement. The WHO/FAO JMPR (World Health Organization/Food and Agriculture Organization Joint Meeting on Pesticide Residue) uses the variability factor of 3 to reflect variation of residue levels that might be higher than composite samples in risk assessment of pesticide residues, where the calculation of the International Estimate on Short Term Intake (IESTI) in the case of medium sized crops (apple, orange, etc.) can be described by the following equation.

In a case where the unit edible weight of raw commodity is less than the large portion of the weight:

$$IESTI = \frac{U \times (HR \text{ or } HR_p) \times v + (LP\text{-}U) \times (HR \text{ or } HR_p)}{bw} \quad \text{Equation 2a}$$

In a case where the unit edible weight of the raw commodity exceeds a large portion of the weight:

$$IESTI = \frac{LP \times (HR \text{ or } HR_p) \times v}{bw} \quad \text{Equation 2b}$$

Where LP = Highest large portion reported (97.5th percentile of eaters), kg food/day
HR = Highest residue in composite sample found in residue trials
HR_p = Highest residue in processed commodity
bw = Mean body weight of population
U = Unit weight of edible portion
v = Variability factor

Based on the results from the three studies, the variability factor exceeding 1 was not observed, indicating that the variation level of cypermethrin residue was less than that stipulated by the JMPR. This result indirectly supports the JMPR assumption of allocating the variability factor to be as large as 3 for the worst case scenario in risk assessment.

The three types of field trials in terms of sampling design only checked the residue variation from whole plot to the individual tree but did not consider all that parts of the individual trees. Future studies could incorporate sampling of different tree parts. A study by Xu et al. (2006) found residue level variation for as much as 49% based on sampling of fruits from lower, middle, upper, inner and outer zones of the trees. Again this showed that the variation of residue levels at the different tree zones could be as large as residue variation for the whole plot. Thus any measurement of pesticide residue levels must be understood in the perspective that the uncertainty parameter can be large because of variation due to field factors. This observational fact has not been fully understood by a large sector of society in terms of interpreting the test measurements of pesticide residues.

Conclusion

Measurement variation was highest among field trial samples (Study C) followed by samples from individual trees (Study A) and subplots (Study B). The Relative Standard Deviations (RSD) of replicate sampling was higher than the RSD of analysis replicates in Study A and B. The contributors of uncertainty to the measurement of cypermethrin residue in descending order

based on percentage variance are: sampling followed by sample processing and analysis. The field factor is a significant contributor to variation in pesticide residue levels compared to the laboratory factor.

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Abstrak

Satu perbandingan data residu pestisid daripada kajian lapangan yang berdasarkan tiga reka bentuk pensampelan dijalankan untuk menilai variabiliti aras residu cypermethrin dalam aspek kajian dalam lapangan dan kajian antara lapangan. Sampel buah (mangga dan betik) dikutip mengikut pensampelan pokok individu (Kajian A: sampel kajian dalam lapangan), pensampelan subplot (Kajian B: sampel kajian dalam lapangan) dan pensampelan dalam keseluruhan plot (Kajian C: sampel kajian antara lapangan). Kajian lapangan tersebut dilaksanakan dalam tempoh 2006 – 2009. Dalam Kajian A dan B, analisis replikat menghasilkan variasi pengukuran yang lebih kecil berbanding dengan replikat pensampelan. Variasi pengukuran yang tertinggi (Relative Standard Deviation, RSD = 50.7%) untuk sampel kajian antara lapangan (Kajian C) diikuti dengan sampel daripada Kajian A (RSD = 28.9%) dan Kajian B (RSD = 15.8%). RSD bagi replikat pensampelan adalah lebih tinggi daripada RSD bagi replikat analisis dalam Kajian A dan B. Penyumbang kepada pengukuran ketakpastian residu cypermethrin dalam turutan menurun berdasarkan peratus varian ialah pensampelan (82.2 – 95.6%) diikuti oleh pemprosesan sampel (2.6 – 11.5%) dan analisis (1.7 – 6.3%). Faktor lapangan merupakan penyumbang yang signifikan kepada variasi pengukuran residu pestisid berbanding dengan faktor makmal.