J. Trop. Agric. and Fd. Sc. 48(2)(2020): 59 – 69

Maximum Residue Limit (MRL) setting of trifloxystrobin on curry leaves for application of Flint in curry leaf farms for control of *Ascochyta rabiei*

(Penetapan had maksimum residu trifloxystrobin pada daun kari untuk aplikasi *Flint* di ladang daun kari bagi kawalan *Ascochyta rabiei*)

Ngan, C.K.[1], Mohammad Nazrul Fahmi, A.R.[2], Khairatul, A.M.[1] and Mohammad Shahid, S.[1]

[1]Soil Science and Water Programme, Soil Science, Water and Fertiliser Research Centre, MARDI Headquarters, Persiaran MARDI-UPM, 43400 Serdang, Malaysia
[2]Pesticide and Fertiliser Control Division, Department of Agriculture, Kuala Lumpur, Malaysia

Abstract

Residue field trials for application of Flint (active ingredient: trifloxystrobin) on curry leaf plots were conducted in the states of Selangor (Serdang) and Johor (Ayer Hitam and Bukit Lawiang). In total, four residue field trials were conducted from the period of 2015 to 2016. In each residue trial, four applications of Flint were made (two-week intervals between applications). The number of applications in each trial was set at four within one growing season of curry leaves development from shoot development to mature leaves. The experimental plots were treated with foliar spray at the rate of 125 g/ha trifloxystrobin. Curry leaves samples were collected at 0, 1, 3, 7, 14, 21 and 28 Days After Last Application (DALA) of Flint. In all the four residue field trials, trifloxystrobin and trifloxystrobin acids were detected in the range of 0.089 – 21.182 mg/kg and <0.01 – 0.363 mg/kg respectively. Based on the residue data and selected Pre-Harvest Interval (PHI) of 14 days for Flint use on curry leaf farms, the Organization for Economic Co-operation and Development (OECD) MRL calculator proposed a Maximum Residue Limit (MRL) of trifloxystrobin at 3 mg/kg and in terms of dietary risk assessment, no acute or chronic risk to the general population resulting from the proposed MRL.

Keywords: residue field trial, Maximum Residue Limit (MRL), trifloxystrobin, curry leaves

Introduction

Curry leaves is one of the minor crops planted in Malaysia besides vegetables, fruits, nursery stock and ornamentals (Lamichhanea et al. 2015). Curry leaves are mostly used for flavouring local cuisines and are part of local diets especially among Indian and Malay communities. Curry leaves plants are often seen in home yards. Commercial planting of curry leaves is mainly for local markets although lately, there were some exports of curry leaf consignments to Singapore. Increase in acreage of curry leaves plants will inevitably lead to single monoculture species existing in large quantities, presenting opportunity for pests and diseases when conditions allowed. Field survey done by the extension officers of the Department of Agriculture (DOA) revealed that pesticide usage in

Article history
Received: 09.05.2019
Accepted: 28.09.2019

Authors' full names: Ngan Chai Keong, Mohammad Nazrul Fahmi Abdul Rahim, Khairatul Azmah Mohamed and Mohammad Shahid Shahrun
E-mail: ckngan@mardi.gov.my

commercial farms specialising in curry leaf production is unavoidable. Conventional chemicals will remain as important tools in pest management (Wheeler 2002). The reduction of current yield losses caused by pests is a major challenge to agricultural production (Popp et al. 2013). Based on interaction with the farmers, mites and black spots were among the common pests and disease problems encountered in commercial curry leaf farms. As such, chemical pesticides were used to control the pests and diseases. Fungicides were heavily used on a regular basis to prevent proliferation of disease on curry leaf plants. One of the major pathogens identified was *Ascochyta rabiei*. Trifloxystrobin (trade name Flint) was chosen as the fungicide for Miximum Residue Limit (MRL) development for curry leaves as it was already being used by some of the farmers and it has a relatively low toxicity in terms of acute reference dose.

At the beginning of the trials, there was no pesticide recommendation on curry leaf plants due to a small market demand causing pesticide manufacturers to lack interest and motivation to register their products for use on curry leaves. Cost was a major consideration for the pesticide manufacturers in pursuing registration (Ghidiu and Neary 1998). Registration of pesticides usage on new commodity requires significant investment on generation of pesticides efficacy and residue data, which to the pesticides manufacturers would not generate adequate returns of investment for minor crops such as curry leaves. Number of minor crops in Europe without pesticides solution has increased.

In 2014, based on interaction with curry leaf farmers, a number of curry leaf consignments were rejected for import into Singapore due to detection of pesticide residues in some of the curry leaf consignments. At that time, there was no pesticide recommendation on curry leaf farms, resulting in non-existence of MRL of Pesticides in curry leaves. In international trades, agricultural crops detention by authorities of importing countries was sometimes due to the absence of MRL establishment on these crops (González 1999).

Realising that the trend of export crop rejections due to lack of MRL would result in economic loss to farmers and the national economy, the DOA allocated funds to establish pesticides recommendation and MRL on curry leaves with the collaboration of Malaysian Agricultural Research and Development Institute (MARDI) and Bayer CropScience. In this collaboration, Bayer CropScience conducted efficacy trials to derive proposed trifloxystrobin application rates for effective control of *A. rabiei*. MARDI was tasked to implement residue field trials and generation of residue data based on proposed trifloxystrobin application rates. Bayer CropScience then registered its product (Flint) on the new crop (curry leaf plants) as pesticide once sets of residue data were available.

Materials and methods

Residue field trials

A total of four supervised residue field trials were conducted on curry leaf plants between 2015 and 2016. The location of the trials is shown in *Table 1*. Trials 1 and 2 were conducted in the compounds of the Department of Agriculture (DOA) whereas trials 3 and 4 were conducted at the actual curry leaf tree farms. In each trial, two plots were established, namely, the control plot (15 trees) and the treated plots (35 – 50 trees). A distance separation between the two plots at a minimum distance of 20 m was established to minimise cross contamination factor due to drift during application.

Table 1. Location and period of supervised residue field trials

Trial no.	Location	Year
1	Ayer Hitam, Johor	2015
2	Serdang, Selangor	2015
3	Bukit Lawiang, Johor	2015 – 2016
4	Bukit Lawiang, Johor	2016

No application of Flint (active ingredient: trifloxystrobin) was applied on the control plot throughout the duration of residue field trials. As for the treated plot, four applications of Flint were applied using the knapsack sprayer and a hollow cone nozzle of size 5/64". The interval between each application was two weeks. Trifloxystrobin was applied at the rate of 125 g/ha based on the water rate of 1,000 L/ha. The application rate of 125 g trifloxystrobin/ha was found to be effective in controlling *A. rabiei* in an efficacy study conducted by Bayer CropScience. Curry leaves were collected at 0, 1, 3, 7, 14, 21 and 28 days after the last application of Flint. Samples weighing 500 g each were collected in single replicate and sent to the analytical laboratory located at the headquarters of the Malaysian Agricultural Research and Development Institute (MARDI) at Serdang, Selangor.

Water content determination in curry leaves

Few curry leaves samples were collected for residue determination and set aside for water content determination. Sampled curry leaves from the fields were immediately put in sealed sample bags which were subsequently placed in a cool box for sample transport to the laboratory. Determination of water content in curry leaf samples was performed using the drying method in MARDI. The percentage of water in the curry leaves is vital in order to derive concentration of residue in the dried form, as dried curry leaves in powder form is used as food condiments. Information of residue concentration in the dried form would provide additional information for regulatory authorities (Pesticide Board of Malaysia and Ministry of Health) on the extent of residue intake from consumption of the food.

Residue definition

Prior to analysis of samples in the analytical laboratory, it is important to establish residue definition for the analysis to ensure generated residue data is fit for purpose. In pesticide residue analysis, there are two types of residue definition, namely, MRL compliance and dietary risk assessment. *Table 2* shows two residue definitions of trifloxystrobin. [(E,E)-methoxyimino-{2-[1-(3-trifluoromethylphenyl) ethylideneaminooxymethyl]phenyl}acetic acid] or trifloxystrobin acid as it is known in some publications (Chen et al. 2014; Wang et al. 2014), is formed during plant metabolism via cleavage of the methyl ester group in the parent compound to form trifloxystrobin acid. From this text onwards, in order to avoid confusion with the residue definition of trifloxystrobin for dietary risk assessment, the parent compound is referred to as trifloxystrobin and its metabolite product is referred to as trifloxystrobin acid. In MRL setting, dietary risk assessment is part of the requirement in order to estimate risk posed to the general population following existence of residue at detected levels. Therefore as defined in *Table 2*, analysis of trifloxystrobin acids is required in addition to trifloxystrobin in dietary risk assessment.

Analytical method validation

Prior to analysis of actual residue trial samples, an analytical method was validated by conducting a recovery study. Validated

Table 2. Two residue definitions for trifloxystrobin in plant commodities according to Codex Alimentarius Comission

Residue definition	Compound
MRL compliance	Trifloxystrobin
Dietary risk assessment (for MRL development)	Sum of trifloxystrobin and [(E,E)-methoxyimino-{2-[1-(3-trifluoromethylphenyl)ethylideneaminooxymethyl]phenyl}acetic acid] (CGA 321113), expressed as trifloxystrobin

Source: JMPR (2004)

analytical method is an indication that the analytical method is accurate and precise in quantification of residue in the tested range of concentrations. The range of concentrations tested was 0.1 – 20 ng/mL. In a recovery study, a known quantity of analyte is fortified onto the homogenised sample that was sourced from the plot that was not treated with pesticide containing the same analyte. The fortified, homogenised sample was subjected to residue extraction procedure and residue analysis. Measured residue concentration from analytical method was then compared with the theoretical fortified concentration in order to obtain recovery parameter. The acceptable range of recovery was 70 – 120%. In validating the analytical method for quantification of trifloxystrobin and trifloxystrobin acid in curry leaves, 15 g untreated homogenised curry leaves were fortified with trifloxystrobin at concentrations of 0.01 and 0.1 mg/kg. Trifloxystrobin acid was fortified separately at the same concentrations. The lowest fortified concentration would be the Limit of Quantification (LOQ) of the analytical method. The spiked samples were analysed using the analytical methods that were shown in Residue Extraction and Residue Analysis sections. Recovery and relative standard deviation of recovery values were derived from the following equations:-

$$\text{Recovery} = \frac{\text{Concentration observed} - \text{concentration in blank sample}}{\text{Fortified concentration}} \times 100$$

$$\text{Relative standard deviation of recovery} = \frac{\text{Standard deviation of recovery}}{\text{Mean recovery}} \times 100$$

Residue extraction

Curry samples received in the form of curry leaves were homogenised using a food processor to obtain homogenised curry leaves and analysed. An analytical portion or sample size of 15 g was placed in a 250 mL bottle and 60 mL of 1% glacial acetic acid in acetonitrile was added to it, followed by 6 g of $MgSO_4$ and 1.5 g of sodium acetate. The content inside the bottle was homogenised by ultraturax at a high speed for 3 min and shaken in an orbital shaker at 150 rpm for 1 h. A volume of 10 mL was transferred into a 15 mL centrifuge tube and centrifuged at 4,000 rpm for 5 min. A volume of 5 mL was decanted into a 15 mL centrifuge tube that contained 1 g of activated Agilent Enhanced Matrix Removal (EMR). The tube was vortexed for 1 min and centrifuged at 5,000 rpm for 5 min. The contents were then transferred into another empty 15 mL centrifuge tube and 3 g of $MgSO_4$ added to it. The tube was vortexed for 1 min and centrifuged at 4,000 rpm for 5 min. The extract was diluted 5 times with 0.1% formic acid in acetonitrile and later the diluted extract was filtered using a syringe filter fitted with a 0.2 μm PTFE membrane filter. The filtered extract was transferred into a 2 mL vial for residue analysis using Liquid Chromatograph Mass Spectrometry (LCMS) equipped with a triple quadrupoles detector. Further dilution was made when the peak area of analyte was above the highest concentration of the calibration standard.

Residue analysis

Agilent 1290 Infinity UHPLC (Ultra High Pressure Liquid Chromatograph) with a Synergi 4μ Fusion-RP 80A column (4 μm pore size, 50 mm length, 2 mm outer diameter) and AB Sciex QTrap 5500 mass spectrometer were used in analysis of trifloxystrobin and trifloxystrobin acids. The temperature of the column was maintained at 30 °C and the injection volume was set at 5 μL. The mobile phases and gradient mode of the Liquid Chromatograph is shown in *Table 3*.

Results and discussion

A good linearity fit of trifloxystrobin and trifloxystrobin acid peaks in relation to its concentration range (0.1 – 20 ng/mL) has an R value of more than 0.99. Examples of trifloxystrobin and trifloxystrobin acid

Table 3. LCMS gradient setting for analysis of trifloxystrobin and trifloxystrobin acid

Time (min)	% A	% B	Flow (mL/min)
0.00	90.0	10.0	0.4
3.00	5.0	95.0	0.4
4.00	5.0	95.0	0.4
4.10	90.0	10.0	0.4
6.00	90.0	10.0	0.4

A: 0.1% formic acid in water
B: 0.1% formic acid in HPLC-grade methanol

calibration curves are shown in *Figure 1*. The Limit of Quantification (LOQ) of the analytical method for quantification of trifloxystrobin and trifloxystrobin acid was 0.01 mg/kg, given that recoveries for both analytes were within 70 – 120% and a precision of <15%. The Limit of Determination (LOD) was 0.004 mg/kg. The result of the recovery study was satisfactory (*Table 4*).

Results of residue analysis are shown in *Table 5*. No residues of trifloxystrobin and trifloxystrobin acid were detected at or above the LOQ (0.01 mg/kg) in all the untreated samples indicating that control (untreated) plot were not contaminated with studied compounds throughout the residue field trials. In treated samples, trifloxystrobin and trifloxystrobin acid were detected in the range of 0.089 – 27.182 mg/kg and <0.01 – 0.363 mg/kg respectively, throughout sampling intervals in the four residue field trials. Overall significant trends of residue decline were observed from 0 Days After Last Application (DALA) to 28 DALA in the four residue field trials.

Trifloxystrobin was detected as high as 21.182 mg/kg at 0 DALA in Trial no. 4 and at subsequent sampling intervals, concentrations were observed to decrease to 0.283 mg/kg at 28 DALA. In Trial no. 3 (same location but at different year and different trees), trifloxystrobin was detected at 17.931 mg/kg at 0 DALA and peaking at 1 DALA at 18.104 mg/kg. Highest residue was detected at 1 DALA instead of 0 DALA as a result of uncertainty of sampling

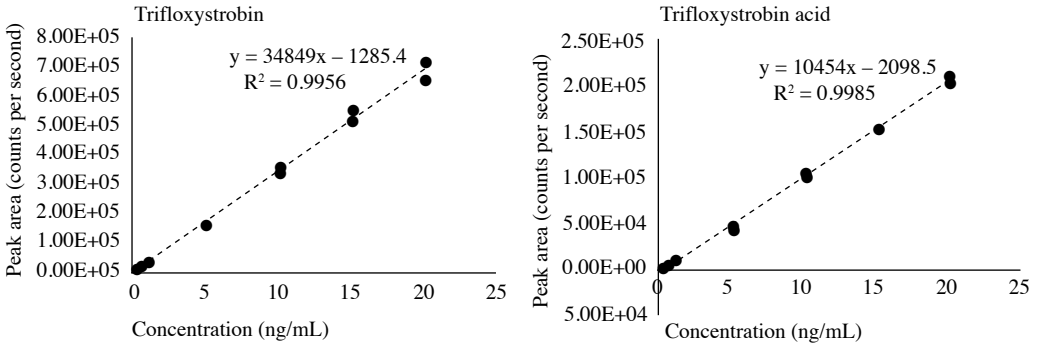

Figure 1. Calibration curves of trifloxystrobin and trifloxystrobin acid

Table 4. Recovery of trifloxystrobin and trifloxystrobin acid in curry leaves

Fortified concentration (mg/kg)	Replicate (n)	Mean recovery (%)	Relative standard deviation (%)
Trifloxystrobin			
0.01	5	82.1	3.4
10	5	99.8	1.0
Trifloxystrobin acid			
0.01	5	85.5	13.6
10	5	105.7	6.8

and very short intervals between the two sampling intervals. Residue variation in the experimental plot and the difference in one day intervals could be the reason that residue did not decline rapidly. The field factor was a significant contributor to the variation in pesticide residue measurement (Ngan et al. 2014). The concentration started to decline to 0.195 mg/kg at 28 DALA. In Trials no. 1 and 2, highest residue was detected at 1 DALA (8.319 mg/kg; no sample at 0 DALA) and 0 DALA (9.857 mg/kg) respectively. In both trials, residues declined to 0.090 mg/kg (Trial no. 1) and 0.089 (Trial no. 2).

Trifloxystrobin acid was detected at a very much lower concentration compared to trifloxystrobin. As with trifloxystrobin, a significant trend of trifloxystrobin acid decline was observed for the four residue field trials, indirectly indicating that the origin of trifloxystrobin acid was from applications of Flint during the trial phase. Highest residues were detected in 0 or 1 DALA irrespective of trial number and the decline trend in each trial was significantly noticeable to range from <0.01 – 0.016 mg/kg.

Based on the decline trends of residues from the four trials, Pre-Harvest Interval (PHI) of 14 days was selected. The highest data set at or after 14 DALA (italic values in *Table 5*) from each trial was chosen as input for MRL estimation and also derivatisation of Supervised Trial Median Residue (STMR) for further input in dietary risk assessment.

MRL estimation

In accordance with residue definition for MRL enforcement, only trifloxystrobin (parent compound) concentrations were selected for estimation of new MRL for curry leaves. The highest trifloxystrobin concentrations at or after 14 DALA in each of the four residue field trials (bold values in *Table 5*) were 0.085 mg/kg (Trial no. 1), 0.549 mg/kg (Trial no. 2), 0.807 mg/kg (Trial no. 3) and 1.039 mg/kg (Trial no. 4).

Two methods of MRL estimation were done using MRL estimation by Hyder et al. (2003) and OECD (Organization for Economic Co-operation and Development) MRL calculator. According to Hyder et al. (2003), their MRL estimation method could be used if the crop consumption is less than 7.5 g/person/day. Malaysian consumption of curry leaves is less than the limit mentioned. Using the Hyder et al. (2003) estimation method:

MRL = R + KS

Where R = mean of the Highest Residue (HR) from residue field trials
K = the one-sided tolerance factor for normal distributions with 95% confidence level
S = standard deviation of HR from residue field trials

With input of HR from the four residue field trials,

MRL = 0.620 mg/kg + (4.202*0.409)
= 2.338 mg/kg (MRL estimate after rounding up = 3 mg/kg)

For estimation of MRL, OECD MRL calculator was used. The OECD MRL calculator was initially introduced by OECD for harmonising estimation of MRL among OECD regulatory authorities (Hanford et al. 2015). The calculator is in the form of an excel spreadsheet in which selected HR at or after proposed PHI from each residue field trial was input into the spreadsheet. After input of HR from all the trials, the spreadsheet would recommend the MRL. The OECD MRL calculator was based on the general equation by OECD (2014):

MRL = Mean + 4S

Where Mean = mean of the HR from residue field trials
S = standard deviation of HR from residue field trials

With input of HR from the four residue field trials,

MRL = 0.620 mg/kg + (4 x 0.409)
= 2.256 mg/kg (MRL estimate after rounding up = 3 mg/kg)

Table 5. Residue data summary from supervised trials of trifloxystrobin in curry leaves

DALA	Concentration (mg/kg)			
	Ayer Hitam (2015)	Serdang (2015)	Bukit Lawiang (2015 – 2016)	Bukit Lawiang (2016)
Trifloxystrobin (for MRL estimation)				
0	9.857	17.931	21.182	ns
1	7.792	18.104	14.897	8.319
3	0.658	2.940	10.732	5.529
7	0.280	0.896	3.896	3.766
14	***0.085***	***0.549***	***0.807***	***1.039***
21	*0.093*	*0.296*	*0.614*	*0.258*
28	*0.089*	*0.195*	*0.283*	*0.090*
Trifloxystrobin acid				
0	0.039	0.097	0.363	ns
1	0.045	0.102	0.249	0.037
3	0.031	0.041	0.256	0.032
7	0.017	0.016	0.186	0.017
14	***<0.01***	***<0.01***	***0.064***	***0.012***
21	*<0.01*	*<0.01*	*0.027*	*<0.01*
28	*<0.01*	*<0.01*	*0.016*	*<0.01*
Sum of trifloxystrobin and trifloxystrobin acid (for dietary risk assessment)				
0	9.896	18.028	21.545	ns
1	7.838	18.206	15.146	8.356
3	0.689	2.980	10.988	5.561
7	0.298	0.912	4.082	3.783
14	0.095	0.559	0.871	1.051
21	*0.103*	*0.306*	*0.641*	*0.268*
28	*0.099*	*0.205*	*0.299*	*0.100*

ns: no sample

Note: Italic residue values at or after selected Pre-Harvest Interval (PHI). The highest residue (in bold) from each trial was selected for MRL estimation and dietary risk assessment. <0.01: Residue was not detected above the LOQ of analytical method

The MRL estimates of 2.338 mg/kg and 2.256 mg/kg by estimation method (Hyder et al. 2003) and OECD MRL calculator respectively, did not differ much. Both methods also recommended a MRL value of 3 mg/kg. Therefore, MRL for trifloxystrobin on curry leaves can be reliably proposed at 3 mg/kg. In *Figure 2,* it can been seen that in all the four residue field trials, trifloxystrobin residue decreased below the limit of 3 mg/kg by 14 DALA, thus showing that if the recommended dosage and PHI are followed, trifloxystrobin residue would not exceed the 3 mg/kg limit.

Dietary risk assessment

For dietary risk assessment of plant commodity (applicable to curry leaves), residue definition of trifloxystrobin is the sum of trifloxystrobin and trifloxystrobin acids expressed together as trifloxystrobin. Thereby, from *Table 5* which shows values from summation of trifloxystrobin and trifloxystrobin acid, only the highest

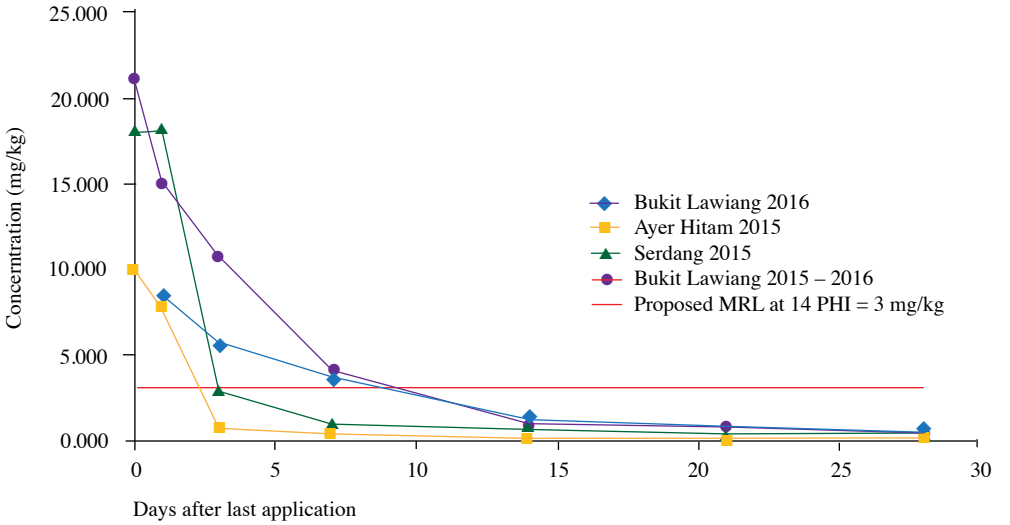

Figure 2. Decline of trifloxystrobin residue in curry leaves from four residue field trials

value in each residue field trial at or after 14 days application were selected, namely, 0.095 mg/kg (Trial 1), 0.559 mg/kg (Trial 2), 0.871 mg/kg (Trial 3) and 1.051 mg/kg (Trial 4). The Supervised Trial Median Residue (STMR) derived from these four highest values was 0.715 mg/kg and this value would be used in chronic dietary risk assessment.

Normally short-term (acute) and long-term (chronic) dietary risk assessment were performed following availability of residue data with input of chosen PHI. However in the case of trifloxystrobin, due to its relatively low toxicity, parameter of Acute Reference Dose (ARfD) was not necessary. Therefore, acute dietary risk assessment was not necessary because such assessment require input of ARfD. In chronic dietary risk assessment, Acceptable Daily Intake (ADI) of trifloxystrobin was 0.04 mg/kg body weight according to the Joint Meeting on Pesticide Residues (JMPR 2004).

For chronic risk assessment, HR and STMR from the four residue trials data were 1.051 mg/kg and 0.715 mg/kg respectively. The HR parameter would be used if the crop is consumed in fresh commodities whereas STMR parameter would be more applicable to processed food that involves thorough mixing of fresh commodities. Curry leaves are sometimes consumed in powder form as condiments or flavouring in cooking. In powder form, water content would be less than 1% of total weight, thus residue in powder form would be higher than those residue concentrations in the fresh leaves that were reported in this study. Pesticide residues of higher concentrations have been reported in dry pepper (Yap and Zender 2018) and dry chilli (Xavier et al. 2014) compared to fresh crops due to residues concentrated to higher concentrations during the drying process. The results of water content determination of curry leaves is shown in *Table 6*. Adjustment to HR concentrations based on water content was

Table 6. Moisture content (%) in curry leaf samples

Samples from trial site	Moisture content (%)
Trial No. 1	69.14%
Trial No. 1	61.66%
Trial No. 2*	63.58%
Trial No. 3	70.40%
Trial No. 3	63.26%
Trial No. 4*	74.20%
Average	64.07%

* Single replicate sample for moisture content test

made and only STMR values were adjusted because this parameter would be used as input in chronic risk assessment. Average water content from six measurements was 67.04%, therefore, adjusted STMR value was 1.07 mg/kg. This adjusted value was still lower than the worst case scenario of 3 mg/kg concentration that was assessed in *Table 5*, thus any form of increase of residue concentrations due to food processing would not pose significant risk to the population.

Chronic dietary risk assessment of trifloxystrobin following addition of curry leaves with residual trifloxystrobin for the Malaysian population is shown in *Table 7*. Currently there is registered use of trifloxystrobin on nine crops. As curry leaves would make the latest crops to be registered, total intake amount of trifloxystrobin residues from the nine crops must be estimated and compared to maximum permitted intake.

The percentage of total trifloxystrobin intake based on MRLs in all the ten crops was 55.46% from the daily maximum permitted intake based on Malaysian dietary data. Thus at the PHI of 14 days and maximum level of 3 mg/kg trifloxystrobin in curry leaves, the level does not provide long term chronic risk to the population.

MRL setting

With no dietary risk concern at the proposed MRL (3 mg/kg) and PHI (14 days), the next step in MRL setting was for the Pesticide Board of Malaysia to establish

Table 7. Chronic dietary risk assessment of trifloxystrobin

Crop	MRL (mg/kg)	Food factor (kg/person/day)	Theoretical Maximum Dietary Intake (TMDI)* (mg/person/day)
Rice	5	0.27503[a]	1.37515
Mango	0.01	0.00723[a]	0.0000723
Cucumber	0.3	0.0106[b]	0.00318
Chili	0.3	0.00841[a]	0.002523
Tomato	0.7	0.00968[a]	0.006776
Eggplant	0.7	0.00292[a]	0.002044
Long bean	0.1	0.0107[c]	0.00107
Citrus	0.5	0.01029[d]	0.005145
Black pepper	0.3	0.00017[e]	0.000051
Curry leaves	3	0.00055[f]	0.00165
Total Theoretical Maximum Dietary Intake, ∑ TMDI			1.3976613

*TMDI = MRL (or STMR) x food factor
[a]Ministry of Health (MOH 2014)
[b]food factor under category of other fruiting vegetables (MOH 2014)
[c]food factor under category of other legume vegetables (MOH 2014)
[d]food factor under category of orange/mandarin (MOH 2014)
[e]food factor under category of pepper (black, white, pink, green), World Health Organization (WHO 2012)
[f]food factor under category of group of herbs, raw (including dried) (WHO 2012)

Acceptable Daily Intake (ADI) = 0.04 mg/kg body weight/day

Daily Maximum Permitted Intake (MPI) = ADI × average Asian weight**
= 0.04 mg/kg body weight/day × 63 kg/person
= 2.52 mg/person/day

** the value may differ from region to region

% intake from MPI = (∑ TMDI/Daily MPI) x 100
= 1.3976613/2.52 mg/person/day
= 55.46% (<100% Daily MPI)

The daily total intake was 55.46% from daily MPI, indicating no chronic risk from long term consumption

administrative MRL pending gazzetment of proposed MRL into Schedule 16 of The Food Act 1983. This would involve several steps before final addition into Schedule 16 after parliament gazettement. Following establishment of administrative MRL, the product in the study (Flint) was approved by the Pesticide Board for use on curry leaf farms and hence the recommendation was the first pesticide recommendation for curry leaf crops in Malaysia. Administrative MRL of trifloxystrobin on curry leaves was established at 3 mg/kg and the proposed MRL is currently at the Attorney General's Office before proceeding to parliament gazettement.

Conclusion

Based on the declining trends of trifloxystrobin and trifloxystrobin acids concentration from the four residue field trials, MRL of trifloxystrobin in curry leaves was estimated at 3 mg/kg with PHI set at 14 DALA with no significant acute or chronic risk to the general population in terms of dietary risk assessment.

Acknowledgements

This project was funded by the Department of Agriculture. The authors would like to express gratitude to Norhasimah Haron, Ismail Iberahim and Hayati Kumron from the Department of Agriculture. Special thanks are also due to Ho Mei Choo, Azmel Abdul Majid and Ooi Kuan Meng from Bayer CropScience for their support in terms of technical assistance, advice and supply of research materials (test items and pesticide analytical standards). Many thanks are also due to curry leaf farmer Lee Chan Loong and dedicated staff of the Department of Agriculture, Serdang and Ayer Hitam branches. We would also like to thank the staff and former staff, Lim Kim Peng, Nor Azhar Alias, Nor Ashikin Basiron, Siti Hajar Hadis@Hadas and Nurul Faizah Mohd Ridzuan of Pesticide Laboratory, Crop and Soil Science Research Centre.

References

Chen, X., Jun, X., Xingang, L., Yan, T., Xinglu, P., Yongquan, Z. and Fengshou, D. (2014). Simultaneous determination of trifloxystrobin and trifloxystrobin acid residues in rice and soil by a modified quick, easy, cheap, effective, rugged and safe method using ultra high performance liquid chromatography with tandem mass spectrometry. *J. Separation Sci.* 37(13): 1640 – 1647

Ghidiu, G.M. and Neary, P.E. (1998). An extension perspective of the minor use crops pesticides problem in vegetable production. *Journal of Extension* 32: 1

González, R.H. (1999). Pesticide residues in developing countries - A review of residues detected in food exports from the developing world. *Pesticide Chemistry and Bioscience* 386 – 401

Hanford, C.E., Elliott, C.T. and Campbell, K. (2015). A review of the global pesticide legislation and the scale of challenge in reaching the global harmonisation of food safety standards. *Integrated Environmental Management and Assessment* 11(4): 525 – 536

Hyder, K., Travis, K.Z., Welsh, Z.K. and Pate, I. (2003). Maximum Residue Level: Fact or fiction? *Human and Ecological Risk Assessment* 9(3): 721 – 740

JMPR (2004). Report of the joint meeting of the FAO panel of experts on pesticide residues in food and the environment and the WHO Core Assessment Group on pesticide residues, Rome, Italy, 20 – 29 September 2004. *Pesticide Residues in Food – 2004*. World Health Organization and Food and Agriculture Organization

Lamichhanea, J.R., Arendseb, W., Dachbrodt-Saaydehc, S., Kudskd, P., Romanb, J.C., van Bijsterveldt-Gelsb, José E.M., Wickc, M. and Messéana, A. (2015). Challenges and opportunities for integrated pest management in Europe: A telling example of minor uses. *Crop Protection* 74: 42 – 47

MOH (2014). Food consumption statistics of Malaysia – For adult population aged 18 to 59 years. Volume 3, Ministry of Health

Ngan, C.K., Khairatul, A.M. and Mohammad Shahid, S. (2014). Variation of cypermethrin residue levels in selected tropical fruit samples within and among field trials. *J. Trop. Agric. Food Sc.* 42(2): 191 – 198

OECD (2014). MRL Calculator: Users Guide and White Paper. Series on Pesticides and Biocides, No. 56 Paris: OECD Publishing. Retrieved from https://doi.org/10.1787/9789264221567-en

Popp, J., Pető, K. and Nagy, J. (2013). Pesticide productivity and food security. A review. *Agronomy for Sustainable Development* 33(1): 243 – 255

Wang, L., Wenming, L., Puyu, L., Minghui, L., Shuo, C. and Lijun, H. (2014). Residues and dissipation of trifloxystrobin and its metabolite in tomatoes and soil. *Environmental Monitoring and Assessment* 186(11): 7793 – 7799

Wheeler, W.B. (2002). Role of research and regulation in 50 years of pest management in agriculture. Prepared for the 50th Anniversary of the *Journal of Agriculture and Food Chemistry. J. Agric. Food Chem.* 50(15): 4151 – 4155

WHO (2012). Template for evaluation of chronic exposure. Retrieved from https://www.who.int/foodsafety/areas_work/chemical-risks/gems-food/en/ on 9 June 2020

Xavier, G., Chandran, M., Thomas, G., Beevi, S.N., Mathew, T.B., Paul, A., Arimboor, R., Sree, V., Pradeepkumar, G.T. and Rajith, R. (2014). Persistence and effect of processing on reduction of fipronil and its metabolites in chilli pepper (*Capsicum annum L.*) fruits. *Environment Monitoring Assessment* 186(9): 5429 – 5437

Yap, C.A. and Zender, J. (2018). Residue levels and dissipation behaviours of chlorpyrifos in black pepper berries and soil. *Food Research* 2(6): 587 – 593

Abstrak

Kajian residu lapangan *Flint* (bahan aktif: trifloxystrobin) pada plot tanaman daun kari dijalankan di Serdang, Selangor, Ayer Hitam dan Bukit Lawiang Johor. Keseluruhannya empat kajian residu lapangan dijalankan dalam tempoh 2015 – 2016. Dalam setiap kajian residu lapangan, empat aplikasi *Flint* dibuat (selang dua minggu di antara dua aplikasi). Bilangan aplikasi dalam setiap kajian lapangan ditetapkan pada empat aplikasi dalam satu musim pertumbuhan daun kari iaitu dari pembentukan pucuk daun hingga daun matang. Plot eksperimen disembur secara foliar pada kadar 125 g/ha trifloxystrobin. Sampel daun kari diambil pada 0, 1, 3, 7, 14, 21 dan 28 hari selepas semburan terakhir *Flint*. Dalam kesemua empat kajian residu lapangan, trifloxystrobin dan asid trifloxystrobin dikesan dalam julat 0.089 – 21.182 mg/kg dan <0.01 – 0.363 mg/kg masing-masing. Berdasarkan data residu dan tempoh 14 hari dilarang mengutip hasil yang terpilih untuk penggunaan Flint pada tanaman daun kari, kalkulator OECD (*Organization for Economic Co-operation and Development*) mencadangkan had maksimum residu trifloxystrobin pada 3 mg/kg dan dalam aspek penilaian risiko diet, tiada risiko akut atau kronik kepada populasi awam berikutan daripada cadangan had maksimum residu.