J. Trop. Agric. and Fd. Sc. 52 (1)(2024): 35 – 44

Generation of residue data from supervised residue trials for Maximum Residue Limit (MRL) setting of tebuconazole and trifloxystrobin on dragon fruits

Ngan, C. K.[1]*, Nor Hasimah, H.[2], Nurhafiza, H.[2] and Mohammad Nazrul Fahmi, A. R.[3]

[1]*Soil Science, Water and Fertiliser Research Centre, MARDI Headquarters, Serdang, 43400 Selangor, Malaysia*
[2]*Laboratory Services Division, Department of Agriculture, Kuala Lumpur, Malaysia*
[3]*Pesticides Control and Fertilisers Division, Department of Agriculture, Kuala Lumpur, Malaysia*

Abstract

Four residue field trials of fungicide application (trade name: Nativo, containing active ingredients of tebuconazole and trifloxystrobin) on dragon fruits were conducted in Peninsular Malaysia within 2015 – 2016 period. Three foliar applications of Nativo at were made at 240 g/ha of Nativo with retreatment period of 7 days. The first application was made 2 – 3 weeks before predicted harvest. Dragon fruit samples were collected at 0, 1, 3, 7 and 14 DALA (Days After Last Application) of Nativo. In analytical phase, tebuconazole, trifloxystrobin and trifloxystrobin acids were analysed in samples in accordance to residue definition in term of Maximum Residue Limit (MRL) compliance and dietary risk assessment. In all the four residue field trials, tebuconazole, trifloxystrobin and trifloxystrobin acids were detected in dragon fruit (whole fruit) in the range of <0.01 – 0.353 mg/kg, <0.04 – 0.327 mg/kg and <0.01 – 0.014 mg/kg, respectively. Based on the residue data and selected pre-harvest interval of 3 day for Nativo use on dragon fruit farms, Maximum Residue Limit (MRL) of tebuconazole and trifloxystrobin were proposed at 1 mg/kg and 0.6 mg/kg, respectively and in term of dietary risk assessment, no chronic risk to the general population resulting from the proposed MRLs.

Keywords: *residue field trial, Maximum Residue Limit (MRL), tebuconazole, trifloxystrobin, dragon fruit*

Introduction

In modern, commercial agriculture practice, pesticides are applied in most agricultural farms, crop processing facilities or warehouse for the purpose of controlling or reducing crop damage and/or yield loss (Hanford et al. 2015). Chemical pesticides will remain as an important component in pest management (Wheeler 2002). The continuous demand for pesticides based solutions to pest and disease problems and food security concerns, externalities problems would have to be addressed by authorities (Popp et al. 2022). Pesticides application on edible crops would inevitably leave pesticide residues on harvested crops. Public is very concern on remaining pesticide residues on food due to their concern on long-term effect toward health. Pesticide residues on edible crops resulting from pesticide use would have to be identified and quantified. Information on pesticide residue level in crops would be evaluated by the authorities in order for the approval of such use to be granted. Maximum Residue Limit (MRL) of pesticide residues would be established on pesticide use on edible crop after pesticide residue data is evaluated and later approved at administratively and legally by the relevant authorities. In other context, Maximum Residue Limits (MRLs) are also trading standards that represent the maximum residue that could be found if a crop protection product (CPP) is applied according to critical good agricultural practice (cGAP) (Hyder et al. 2003). The technical evaluation of pesticide residue data would also include assessment of pesticide residue levels after pesticide applications and long term impact to health consequences of ingesting such level of residue in food. Generation of pesticide residue data from the use of pesticide on particular crops is undertaken to fulfill mandatory requirement by the authority. *Figure 1* shows the basic work flow of MRL setting

Dragon fruits is one of the Malaysian favourite fruits. Production of dragon fruit in 2020 was 8562.64 mt with a value of about RM35 million. The acreage of dragon

Article history
Received: 18.9.23
Accepted: 8.11.23

Authors' full names: Ngan Chai Keong, Nor Hasimah Haron, Nurhafiza Hassan and Mohammad Nazrul Fahmi Abdul Rahim
Corresponding author: ckngan@mardi.gov.my

fruit farms in whole Malaysia as of 2020 was 670.54 ha according to the Department of Agriculture (2021). Pesticide use in dragon fruit farms in Malaysia is a norm due to pest and disease occurrences. Dragon fruit plants are susceptible to a number of diseases such as bacterial fruit soft rot, bipolaris black spot, sooty mold and anthracnose (Hieu and Van Hoa 2015). There are currently limited pesticide recommendations for use on dragon fruits. A check with an online, interactive database of pesticide use in Malaysia, called SISMARP (accessible through the following web address: www.portal.doa.gov.my/sismarp/) in March 2022, revealed that only two products were registered for use on dragon fruit crop. The two products are Antracol (active ingredient: propineb) and Ken-Mura 25 (active ingredient: difenoconazole) which were approved on 1st March 2018 and 1st May 2014, respectively. Difenoconazole and propineb are used to control *Colletotrichum gloeosporioides* and *Colletotrichum capsici*, respectively.

At the time when the trial began in 2015, there was only single pesticide recommendation (Ken-Mura) for dragon fruit crops, demonstrating the inadequacy of pesticide selection in pest and disease management of dragon fruit farms. Use of other pesticides than the approved product would constitute a violation of the Pesticide Act 1974. This poses a dilemma to the farmers in finding adequate plant protection products without law violation. In addition, some dragon fruit farms in Malaysia have been accredited according to *Skim Amalan Pertanian Baik Malaysia* (MyGAP) which forbids the use of pesticides that are not registered for use on particular crops. Certain desperate farmers would resort to use unauthorised pesticides in situation of overwhelming pest attack or disease occurrence and subsequently crops treated with unauthorised pesticides could violate MRL regulation if such crops were sampled and tested by the authority (Gonzales 1999). Affected farmers could be prosecuted or lose their farm certification scheme at worst. Recognising this problem, the Department of Agriculture sponsored a study to be undertaken by the Malaysian Agricultural Research and Development Institute to generate residue data from fungicide use in dragon fruit crops, with the ultimate aim of increasing pesticide recommendations for dragon fruit farmers.

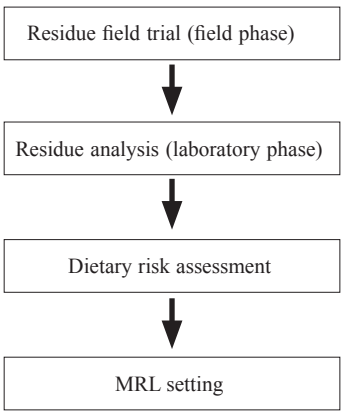

Figure 1. Work flow of setting of Maximum Residue Limit of pesticides

This paper describes a study to generate residue data from the use of Nativo (active ingredients: tebuconazole and trifloxystrobin) on dragon fruit farms so that MRL of tebuconazole and trifloxystrobin can be set by the authority for subsequent approval for such use on dragon fruit farms. The product is effective against *Colletotrichum gloeosporioides* or anthracnose disease in dragon fruit plants. Once absorbed, trifloxystrobin disrupts respiration by acting on target site of complex III: cytochrome bc1 (ubiquinol oxidase) Qo site (cyt b gene) and tebuconazole on the other hand, interrupted the sterol biosynthesis in membranes. The mode of action for trifloxystrobin and tebuconazole are classified as Code C and Code G under FRAC, respectively. There is currently registered use of difenoconazole (product trade name: Ken-Mura) for control of the same pest. Difenoconazole's mode of action is classified under Code G, which has the same mode of action with tebuconazole. Limited number of pesticides that are available for farmers could increase risk of disease resistance due to repeated use of the same pesticides (Ghidiu and Neary 1998; Lamichhane et al. 2015). Addition of tebuconazole and trifloxystrobin as approved fungicides could reduce risk of anthracnose resistance by alternating fungicide application with different mode of action, reducing anthracnose possibility to acquire resistance due to repeated exposure to fungicide of the same mode of action.

In MRL setting, the residue is defined according to MRL compliance and dietary risk assessment. Prior to the analysis of samples in analytical laboratory, it is prudent to determine targeted compound (s) during analysis so that residue data would be useful for the purpose. In some compounds, residue definitions of MRL compliance and dietary risk assessment are different. Residue definitions of tebuconazole and trifloxystrobin according to Joint Meeting on Pesticide Residues (JMPR) were used to determine targeted compounds during residue analysis. The residue definitions of tebuconazole and trifloxystrobin are shown in *Table 2*. [(E,E)-methoxyimino-{2-[1-(3-trifluoromethylphenyl)ethylideneaminooxymethyl] phenyl} acetic acid] or known as trifloxystrobin acid in some publications (Chen et al. 2014; Wang et al. 2014) is formed in plant metabolism via cleavage of the methyl ester group in parent compound to form trifloxystrobin acid. From this text onward, in order to avoid confusion with the residue definition of trifloxystrobin for dietary risk assessment, the parent compound is referred as trifloxystrobin (parent) and its metabolite product is referred to trifloxystrobin acid.

Materials and method

Residue field trials

A total of four supervised residue field trials were conducted on dragon fruit farms between 2015 – 2016. The locations of the residue field trials are shown in *Table 1*. In each trial, two plots were established, which were the control plot (30 trees) and the treated plots

(30 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. Nativo was not applied on the control plot throughout the duration of residue field trials. However, other fungicide (dithiocarbamate fungicide) was applied at both plots to ensure dragon fruit plants were not affected by anthracnose. As for the treated plot, three applications of Nativo were applied using motorised sprayer with a solid cone nozzle. The retreatment interval was one week. Nativo was applied at the rate of 240 g/ha based on the water rate of 1000 L/ha. Dragon fruits were collected at 0, 1, 3, 7 and 14 days after the last application of Nativo. Samples were collected in single replicate and consisted of 12 units of dragon fruits and at least 2 kg in total weight. Samples were sent to the analytical laboratory located at the headquarters of Malaysian Agricultural Research and Development Institutes (MARDI) at Serdang, Selangor.

Analytical method validation

Due to certain limitations encountered during the study, two analytical methods by the two different analytical laboratories were used to quantify the studied compounds. Tebuconazole and trifloxystrobin (parent) residues in dragon fruit samples were quantified by Central Services Laboratory, Department of Agriculture, Kuala Lumpur whereas trifloxystrobin acids residues in the same samples were quantified by Pesticide Laboratory, Soil Science, Water and Fertiliser Research Centre, Malaysian Agricultural Research and Development Institute (MARDI), MARDI Serdang, Selangor. Basically, the two analytical methods were consisted of residue extraction procedure and residue analysis by Liquid Chromatography Tandem Mass Spectrometry (LCMSMS). A residue extraction method on pitaya (dragon fruit) based on simple QuEChERS method followed by residue analysis using Gas Chromatography-Election Capture Detector (GC-ECD) was reported by Yao et al. (2017). The current residue extraction procedure used in the two laboratories was based on QuEChERS approach. Prior to the analysis of actual residue trial samples, the analytical method was validated by conducting a recovery study. A validated analytical method is an indication that the analytical method is accurate and precise in the quantification of residue in the tested range of concentrations. The concentrations tested were shown in *Table 4*. In recovery study, a known quantity of analyte is spiked onto homogenised sample that was sourced from plot that was not treated with tebuconazole or trifloxystrobin. The spiked sample was subjected to the analytical method that is shown in Residue Extraction and Residue Analysis section. In each spiked concentration, five or six replicates of spiked samples were prepared. Measured residue concentration from LCMSMS analysis was then compared with the theoretical spiked concentrations in order to obtain recovery parameter. The acceptable range of mean recovery is 70 – 120%. Relative standard deviation (RSD) was also derived to indicate analytical method precision. The analytical method is considered ideal if the RSD of recoveries is not more than 20%. Recovery and relative standard deviation of recovery value were derived from the following equations:

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

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

Fruit cutting and sample processing

In practice, for fruits other than small size fruits (e.g. grapes, longan, etc.), not all volume of the fruit units from a sample would be homogenised due to impracticality in term of time and resources to homogenise all units of whole fruit.

In the case of dragon fruits, each whole fruit unit of the 12 dragon fruits from each sample was cut into half longitudinally, the first half portion would be separated for analysis on a whole fruit basis in which peels and edible pulps would be homogenised. The second half portion was separated for additional analysis of edible pulps only, in which further separation of peel and edible pulps was performed. At the time of cutting each unit of fruits into half, the analyst would not know which part of the fruit surface would expose more to pesticide deposition, thus high number of fruit units per sample would reduce residue variation within the sample.

In term of dietary risk assessment, it is expected that only edible pulp of dragon fruit would be ingested leaving inedible peel of the fruit as waste. Thus, in reflecting the actual amount of residue taken by human, it is additionally required to further determine residue content in the edible pulp of dragon fruits.

Table 1. Residue definitions for tebuconazole and trifloxystrobin in plant commodities

Pesticide common name	Residue definition for MRL compliance	Residue definition for dietary risk assessment in plant commodities
Tebuconazole	Tebuconazole*	Tebuconazole*
Trifloxystrobin	Trifloxystrobin**	Sum of trifloxystrobin and [(E,E)-methoxyimino-{2-[1-(3-trifluoromethylphenyl)ethylideneaminooxymethyl]phenyl} acetic acid] (CGA 321113), expressed as trifloxystrobin**

* Source: JMPR 2011
** Source: JMPR 2004

Table 2. Location and period of supervised residue field trials

Trial no.	Location	Year
1	Sepang, Selangor	2015
2	Selandar, Melaka	2015
3	Renggam, Johor	2016
4	Sepang, Selangor	2016

The 12 units half-fruit units (half unit of whole fruits or edible pulps) would be further cut into smaller pieces and homogenised with dry ice in a Robot Coupe food processor in order to produce a homogenous matrix in terms of uniform residue distribution throughout the blended matrix. A well homogenous matrix would result in homogeneous distribution of residue throughout the volume of homogenised fruits so that if a certain mass of analytical portion is withdrawn from any part of homogeneous, the residue variation would be minimised. Cryogenic homogenisation with dry ice is known to promote good homogenisation and reduce residue degradation during high speed maceration of fruits by the blades.

Residue extraction at the DOA's laboratory [(extraction of tebuconazole and trifloxystrobin (parent)]

An amount of 15 g of homogenised sample was weighed in a 50-mL centrifuge tube, followed by the addition of 15 mL of acetonitrile (1% acetic acid v/v), 6 g of anhydrous Mg2SO4 and 1.5 g of sodium acetate. The tube was capped and shaken vigorously for one minute, followed by centrifugation at 2,500 rpm for 3 minutes. A volume of 3 mL of upper acetonitrile layer was transferred into a 15-mL centrifuge tube that has been added with 150 mg of PSA, 150 mg of C18 and 450 mg of anhydrous $MgSO_4$. The tube was shaken vigorously and centrifuged at 2500 rpm for 3 minutes. A volume of 1 mL of extract was transferred into a fresh tube. The extract was diluted with 1 mL of 0.1% formic acid in water. The extract was filtered using a 0.45µm filter into LC vials for further residue analysis in Liquid Chromatography Tandem Mass Spectrometry (LCMSMS) equipped with triple quadrupoles detector.

Residue extraction at the MARDI's laboratory (extraction of trifloxystrobin acid)

A mass of analytical portion would be withdrawn from the homogeneous matrix for residue extraction. The residue extraction procedure was described as followed. A 250-mL bottle containing 15 g of analytical portion (homogenised dragon fruits or homogenised pulps of dragon druits), 60 mL of 1% glacial acetic acid in acetonitrile was added, followed by the addition of 6 g of $MgSO_4$ and 1.5 g of sodium acetate. The content inside the bottle was homogenized using ultra-turrax at high speed for 3 minutes, then the bottle was shaken in orbital shaken at 150 rpm for 1 hour. A volume of 10 mL was transferred into a 15-mL centrifuge tube and centrifuged at 4000 rpm for 5 minutes. A volume of 5 mL was decanted into a 15 mL centrifuge tube that contain 1.5 g of Agilent Enhanced Matrix Removal (EMR). The tube was vortexed for 1 minute and centrifuged at 5000 rpm for 5 minutes. All the volume was transferred into another empty 15-mL centrifuge tube and 3 g of $MgSO_4$ was added. The tube was vortexed for 1 minute and centrifuged at 4000 rpm for 5 minutes. 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 2 mL vial for further residue analysis in Liquid Chromatography Tandem Mass Spectrometry (LCMSMS) equipped with triple quadrupole detector.

Residue analysis

Residues of trifloxystrobin and its metabolite, trifloxystrobin acid were analysed using HPLC-MS/MS in paddy straw, bran, brown rice and soil (Chen et al. 2014) and tomato (Wang et al. 2014). In this study, high performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS) was used as to analyse and quantify residues. Residues of tebuconazole and trifloxystrobin was analysed at Department of Agriculture laboratory. The sample extracts were re-analysed to determine trifloxystrobin acid residues at the Malaysian Agricultural Research and Development Institute laboratory, when it was realised that residue data of trifloxystrobin acid is required for dietary risk assessment. The parameters of both LCMSMS are shown in *Table 3*. All the samples were analysed within two years after sampling. Tebuconazole and trifloxystrobin residues were found to be stable up to two years in selected representative samples in a review by WHO/FAO JMPR (WHO/FAO Joint Meeting on Pesticide Residues). Hence, tebuconazole and triflosystrobin were deduced to be stable in dragon fruit matrix.

Results and discussion

The Limit of Quantification (LOQ) of the analytical method for quantification of tebuconazole or trifloxystrobin was 0.04 mg/kg, for the method that was validated at the Department of Agriculture laboratory. In the validated analytical method at the MARDI laboratory, the LOQ of trifloxystrobin acid was 0.01 mg/kg. Overall, mean recoveries were observed within 70 – 120% for tebuconazole, trifloxystrobin and trifloxystrobin acid. The results of recovery study (*Table 4*) were satisfactory in terms of recovery, however in terms of precision, higher than expected precision were reported for spiked tebuconazole (whole fruit and edible pulps) at spiked concentrations of 0.04 mg/kg and (whole fruit) 0.37 mg/kg. The higher RSD values indicated that the analytical method was less precise in determination of tebuconazole in the whole fruit matrix.

Table 3. LCMSMS setting for analysis of tebuconazole, trifloxystrobin and trifloxystrobin acid

LCMSMS parameter	DOA's laboratory			MARDI's laboratory			
Compound	Tebuconazole and trifloxystrobin			Trifloxystrobin acid			
LCMSMS	Agilent 1100 Series HPLC, AB SCIEX-3200			Agilent 1290 Infinity UHPLC (Ultra High Pressure Liquid Chromatograph) and AB Sciex QTrap 5500 mass spectrometer			
Column	Agilent Eclipse XDB-C18, 5µm, 4.6 x 150mm			Synergi 4µ Fusion-RP 80A (4 µm pore size, 50 mm length, 2 mm outer diameter)			
Injection volume (µL)	10			5			
Column temperature	35			30			
LCMSMS gradient setting	Time (min)	%A	%B	Flow (mL/min)	Time (min)	%A	%B	Flow (mL/min)
	0.00	90.0	10.0	0.4	0.00	90.0	10.0	0.4
	3.00	5.0	95.0	0.4	3.00	5.0	95.0	0.4
	4.00	5.0	95.0	0.4	4.00	5.0	95.0	0.4
	4.10	90.0	10.0	0.4	4.10	90.0	10.0	0.4
	6.00	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				A: 0.1% formic acid in water B: 0.1% formic acid in HPLC-grade methanol			

Results of residue analysis of field samples are shown in *Table 5* (tebuconazole) and *Table 6* (trifloxystrobin (parent) and trifloxystrobin acids). No residue of tebuconazole, trifloxystrobin (parent) and trifloxystrobin acid was detected at or above the Limit of Quantification in all the untreated samples indicating that the control (untreated) plot was not contaminated with studied compounds throughout the residue field trials. In treated whole fruit samples, tebuconazole, trifloxystrobin (parent) and trifloxystrobin acid were detected in ranges of <0.04 – 0.353 mg/kg, <0.04 – 0.327 mg/kg and <0.01 – 0.014 mg/kg, respectively, throughout sampling intervals in the four residue field trials. Overall significant trends of residue decline were observed from 0 DALA to 14 DALA in the four residue field trials. The observed residue concentrations were within the range of pesticide residue concentration detected by Wang et al. (2021). A total of 113 pesticide residues were detected in ranges of 0.01 – 1.21 mg/kg pitaya samples taken from markets in China (Wang et al. 2021).

Trifloxystrobin (summation of trifloxystrobin (parent) and trifloxystrobin acid) was detected at 0.337 mg/kg (One DALA) in Trial no. 1 (Sepang 2015) and at subsequent sampling intervals, its concentrations were observed to decreased to 0.073 mg/kg at 15 DALA. In the same trial, the highest tebuconazole residue was detected as high as 0.351 mg/kg at one DALA and decreased to 0.07 mg/kg at 14 DALA. In Trial no. 2 (Selandar 2015), tebuconazole was detected at 0.097 mg/kg (zero DALA) and decreased to <0.04 mg/kg (14 DALA). Trifloxystrobin residue was highest at 0.092 mg/kg (zero DALA) and decreased to <0.05 mg/kg by 14 DALA. In Trial no. 3 (Renggam 2015) tebuconazole and trifloxystrobin was detected below the limit of quantification in all sampling intervals mg/kg. In trial no. 4 (Sepang 2016), tebuconazole was detected at 0.119 mg/kg (0 DALA) and found to be in higher concentration at 0.178 mg/kg (seven DALA). Highest residue detected at seven DALA instead of zero DALA was a result in uncertainty of sampling and very short interval between the two sampling intervals. Residue variation in the experimental plot could be the reason that concentration at seven DALA was higher than zero DALA. The field factor (weather variation, sprayer variation, etc.) was a significant contributor to the variation in pesticide residue measurement (Ngan et al. 2014). Trifloxystrobin was detected at 0.063 mg/kg (zero DALA) and decreased to 0.059 mg/kg at seven DALA. In all the four residue trials, trifloxystrobin acid was detected at very much lower concentration as compared to trifloxystrobin (parent).

Pre-Harvest Interval (PHI) of three days was selected based on the decline trends of tebuconazole and trifloxystrobin residues after maximum applications of Nativo. In each trial, the highest concentration at or after three DALA (italic values in *Table 5*) was chosen as input for MRL estimation and also derivatisation of Supervised Trial Median Residue (STMR) for further input in dietary risk assessment.

Table 4. Recovery of tebuconazole trifloxystrobin and trifloxystrobin acid in dragon fruits (whole fruit and pulps)

Fortified concentration (mg/kg)	Replicate, n	Mean recovery (%)	Relative standard deviation (%)
Tebuconazole			
0.04	6 (6)	73.4 (75.7)	42.0 (18.5)
0.37	5 (6)	82.4 (93.4)	24.3 (11.4)
Trifloxystrobin			
0.04	6 (6)	102.3 (112.3)	13.2 (5.8)
0.37	6 (6)	104.1 (110.0)	11.6 (5.3)
Trifloxystrobin acid			
0.01	5 (5)	81.0 (80.5)	8.1 (3.5)
0.1	5 (5)	83.8 (87.0)	5.7 (5.4)

Note: Spike concentrations were similar for dragon fruit and dragon fruit pulp. Values in bracket represent relevant parameters related to dragon fruit pulps whereas values in non-bracket represent parameters related to dragon fruits.

MRL estimation

In accordance with residue definition for MRL enforcement, only tebuconazole and trifloxystrobin (parent) concentrations were selected for the estimation of new MRLs for dragon fruits. The highest tebuconazole concentrations at or after three days after application in each of the four residue field trials (bold values in *Table 5*) were 0.313 mg/kg (Trial 1), 0.094 mg/kg (Trial 2), <0.04 mg/kg (Trial 3) and 0.178 mg/kg (Trial 4). As for trifloxystrobin (parent), highest concentrations at or after three days after application in each of the four residue field trials (bold values in *Table 5*) were 0.258 mg/kg (Trial 1), 0.056 mg/kg (Trial 2), <0.04 mg/kg (Trial 3) and 0.049 mg/kg (Trial 4).

MRL calculator developed by OECD (Organization for Economic Co-operation and Development) was used to derive the estimated MRL values of 0.7 mg/kg and 0.6 mg/kg for tebuconazole and trifloxystrobin, respectively, based on input from selected highest concentrations from the available trials. The OECD MRL calculator is based on general equation (OECD 2014):

MRL = Mean + 4S

Where Mean = mean of the Highest Residues, HR from residue field trials

S = standard deviation of HR from residue field trials
Dietary risk assessment

Normally short-term (acute) and long-term (chronic) dietary risk assessment were performed after availability of residue data with input of selected Pre-Harvest Interval. Acute Reference Dose (ARfD) and Acceptable Daily Intake (ADI) shall be used in short-term (acute) and long-term (chronic) dietary risk assessment, respectively. In short-term intake risk assessment, The International Estimated for Short-Term Intake (IESTI) equations under case 2a (relevant to dragon fruit) was selected for estimation of tebuconazole acute risk. The equations are:

Table 5. Tebuconazole concentration in field trial samples

Days after last application	Concentration			
	Untreated		Treated	
	Whole fruits	Pulps	Whole fruits	Pulps
	Trial 1 (Sepang, Selangor/2015)			
0	<LOQ	<LOQ	0.197	0.192
1	NS	NS	0.351	0.032
3	NS	NS	***0.313***	***0.043***
5	NS	NS	*0.162*	*<0.04*
7	<LOQ	<LOQ	*0.149*	*<0.04*
10	NS	NS	*0.087*	*<0.04*
15	NS	NS	*0.070*	*<0.04*
	Trial 2 (Selandar, Melaka/2015)			
0	<0.04	<0.04	0.097	<0.04
3	<0.04	<0.04	*0.048*	*<0.04*
5	<0.04	<0.04	***0.094***	*<0.04*
7	<0.04	<0.04	*0.054*	*<0.04*
10	<0.04	<0.04	*<0.04*	*<0.04*
14	<0.04	<0.04	*<0.04*	*<0.04*
	Trial 3 (Renggam, Johor/2015)			
3	NS	NS	***<0.04***	*<LOQ*
5	NS	NS	*<0.04*	*<0.04*
7	<0.04	<0.04	*<0.04*	*<0.04*
	Trial 4 (Sepang, Selangor/2016)			
0	NS	NS	0.119	<0.04
1	NS	NS	<0.04	<0.04
3	NS	NS	***0.070***	*<0.04*
7	<0.04	<0.04	*0.178*	*<0.04*

DALA: Days after last application WF: Whole fruit P: Pulps NS: No sample

Note: Italic residue values at or after selected Pre-Harvest Interval (PHI) of 3 days. The highest residue in whole fruit (bold) from each trial was selected for MRL estimation. The highest residue in edible pulps (bold) among the four residue trials was used in dietary risk assessment (acute risk assessment).

Case 2a (if unit edible weight of raw commodity, U_e is less than large portion weight, LP)

$$IESTI = \frac{U_e \times HR \times v + (LP - U_e) \times (HR)}{bw}$$

Case 2b (if unit edible weight of raw commodity, U_e, exceeds large portion weight, LP),

$$IESTI = \frac{LP \times HR \times v}{bw}$$

Where
U_e = Edible weight of raw commodity (U_e)
HR = Highest residue (at or after PHI)
v = variability factor
LP = Large portion
bw = body weight

Table 7 shows result of acute risk assessment of ingestion of dragon fruit treated with tebuconazole. Highest Residue, HR of Tebuconazole residue in edible pulp (0.043 mg/kg) was used in IESTI to reflect realistic intake of tebuconazole. For two groups of population, namely children 3 – 6 years and general population (>3 years), the calculated IESTI for children 3 – 6 years and general population (>3 years) were only 0.51% and 0.19%, respectively of the Acute Reference Dose. This indicated that there is no acute risk to the both groups of population if tebuconazole concentration in edible pulp is as high as 0.043 mg/kg .

For chronic risk assessment, apart from tebuconazole residue in dragon fruit, its residue in other crops need to be taken into account due to long period of food intake that include various types of food. The statistical food intake was sourced from published food intake from national food intake survey in 2014 (IPH 2014). *Table 8* shows chronic dietary risk assessment of tebuconazole with latest addition of dragon fruit, with input of Acceptable Daily Intake of tebuconazole is 0.03 mg/kg body weight (JMPR 2011). The percentage of total tebuconazole intake based on MRLs in all the eleven crops was 3% from the daily maximum permitted intake based on Malaysian

Table 6. Trifoxystrobin and trifloxystrobin acid concentration in field trial samples

DALA	Concentration (mg/kg)											
	Trifloxystrobin (parent)				Trifloxystrobin acid				Trifloxystrobin (sum)			
	Untreated		Treated		Untreated		Treated		Untreated		Treated	
	WF	P	WF	P	WF	P	WF	P	WF	P	WF	P
Trial 1 (Sepang, Selangor/2015)												
0	<0.04	<0.04	0.201	0.183	<0.01	<0.01	0.012	<0.01	<0.05	<0.05	0.211	0.193
1	NS	NS	0.327	0.022	NS	NS	0.014	<0.01	NS	NS	0.337	0.032
3	NS	NS	***0.258***	0.028	NS	NS	0.012	<0.01	NS	NS	0.268	*0.038*
5	NS	NS	0.168	0.022	NS	NS	<0.01	<0.01	NS	NS	*0.178*	*0.032*
7	<0.04	<0.04	0.115	<0.04	<0.01	<0.01	<0.01	<0.01	<0.05	<0.05	*0.125*	*<0.05*
10	NS	NS	0.072	<0.04	NS	NS	0.012	<0.01	NS	NS	*0.082*	*<0.05*
15	NS	NS	0.063	<0.04	NS	NS	<0.01	<0.01	NS	NS	*0.073*	*<0.05*
Trial 2 (Selandar, Melaka/2015)												
0	<0.04	<0.04	0.082	<0.04	<0.01	<0.01	<0.01	<0.01	<0.05	<0.05	0.092	<0.05
3	<0.04	<0.04	<0.04	<0.04	<0.01	<0.01	<0.01	<0.01	<0.05	<0.05	*<0.05*	*<0.05*
5	<0.04	<0.04	***0.056***	<0.04	<0.01	<0.01	<0.01	<0.01	<0.05	<0.05	0.066	*<0.05*
7	<0.04	<0.04	<0.04	<0.04	<0.01	<0.01	<0.01	<0.01	<0.05	<0.05	*<0.05*	*<0.05*
10	<0.04	<0.04	<0.04	<0.04	<0.01	<0.01	<0.01	<0.01	<0.05	<0.05	*<0.05*	*<0.05*
14	<0.04	<0.04	<0.04	<0.04	<0.01	<0.01	<0.01	<0.01	<0.05	<0.05	*<0.05*	*<0.05*
Trial 3 (Renggam, Johor/2015)												
3	NS	<0.04	***<0.04***	<0.04	NS	<0.01	NS	<0.01	NS	NS	<0.05	*<0.05*
5	NS	<0.04	<0.04	<0.04	NS	<0.01	NS	<0.01	NS	NS	*<0.05*	*<0.05*
7	<0.04	<0.04	<0.04	<0.04	<0.01	<0.01	<0.01	<0.01	<0.05	<0.05	*<0.05*	*<0.05*
Trial 4 (Sepang, Selangor/2016)												
0	NS	<0.04	0.053	<0.04	NS	<0.01	NS	<0.01	NS	NS	0.063	<0.05
1	NS	<0.04	0.015	<0.04	NS	<0.01	NS	<0.01	NS	NS	0.025	<0.05
3	NS	<0.04	0.022	<0.04	NS	<0.01	NS	<0.01	NS	NS	*0.032*	*<0.05*
7	<0.04	<0.04	***0.049***	<0.04	<0.01	<0.01	<0.01	<0.01	<0.05	<0.05	0.059	*<0.05*

DALA: Days after last application WF: Whole fruit P: Pulp NS: No sample
Note: Italic residue values at or after selected Pre-Harvest Interval (PHI) of 3 days. The highest residue in whole fruit (bold) from each trial was selected for MRL estimation.

Table 7. Acute risk assessment of tebuconazole residues on dragon fruit

Population group	Edible weight of raw commodity (U_e)*	Large portion weight (g) per person*	Body weight (kg)	Case	IESTI (µg/kg bw)	% of Acute Reference Dose, ARfD
Children (3 – 6 year old)	280	202.5	17	2b	0.577	0.51%
General Population >3 years old	280	285.4	63	2a	1.537	0.19%

*The values are available from IESTI spreadsheet from JMPR.
ARfD tebuconazole = 0.3 mg/kg/bw, Highest Residue (HR) in edible pulps = 0.043 mg/kg, and variability factor (*v*) of 3 were used is IESTI estimation

Table 8. Chronic dietary risk assessment of tebuconazole

Crop	MRL (mg/kg)	Supervised Trial Median Residue, STMR	Food factor*	Theoretical Maximum Dietary Intake, TMDI**
Banana	0.05		0.02057	0.0010285
Rice	0.05		0.28968	0.014484
Mango	0.05		0.01048	0.000524
Long bean	0.02		0.00419	0.0000838
Chili	0.5		0.0087	0.00435
Tomato	0.2		0.0235	0.0047
Maize	0.05		0.00898	0.000449
Black pepper	1		0.0001	0.0001
Eggplant	0.1		0.0201	0.00201
Citrus	0.3		0.0364	0.01092
Dragon fruits	1		0.01048***	0.01048
Total Theoretical Maximum Dietary Intake, ∑TMDI 0.0491293				

* Food factors obtained from published national food intake survey (Institute for Public Health 2014)
**TMDI = MRL (or STMR) x food factor
***No data on national dragon fruit intake, therefore national mango intake was used
Acceptable Daily Intake (ADI) = 0.03 mg/kg body weight/day
Daily Maximum Permitted Intake (MPI) = ADI x average Asian weight^
= 0.03 mg/kg bw/day x 63 kg/person
= 1.89 mg/person/day
^ the value may differ from region to region
% intake from MPI = (∑TMDI/Daily MPI) x 100
= 0.0491293/1.89 mg/person/day
= 2.60% (<100% Daily MPI)
The daily total intake was 2.60% from daily MPI, indicating that no chronic risk from long term consumption

dietary data. Although OECD MRL calculator estimated MRL value of 0.7 mg/kg, value of 1 mg/kg tebuconazole was used in chronic risk assessment due to Pesticide Board decision to set tebuconazole MRL on dragon fruit at 1 mg/kg. Long-term intake of tebuconazole residue only constituted 2.6% of maximum permitted intake. With non-exceedance of maximum permitted intake, there is no chronic risk to population with residue concentration of 1 mg/kg in dragon fruit (MRL of tebuconazole in dragon fruit).

In the case of trifloxystrobin, due to its relatively low toxicity, parameter of Acute Reference Dose (ARfD) is not necessary (JMPR 2004), therefore acute dietary risk assessment is not necessary because such assessment require input of ARfD. In chronic dietary risk assessment, Acceptable Daily Intake (ADI) of trifloxystrobin is 0.04 mg/kg body weight (JMPR 2004).

The chronic dietary risk assessment of trifloxystrobin, following the inclusion of dragon fruit with residual trifloxystrobin for the Malaysian population, is presented in *Table 9*. Currently, there are ten registered uses of trifloxystrobin on ten different crops. As dragon fruit is the most recent crop to be registered, the total intake amount of trifloxystrobin residues from these crops must be estimated and added to the intake from dragon fruit. The percentage of total trifloxystrobin intake, based on MRLs, in all eleven crops was found to be 60% of the daily maximum permitted intake, according to Malaysian dietary data. Therefore, considering a Pre-Harvest Interval (PHI) of 3 days and a maximum level of 0.6 mg/kg of trifloxystrobin in dragon fruits, this level does not pose a long-term chronic risk to the population.

Table 9. Chronic dietary risk assessment of trifloxystrobin

Crop	MRL	Food factor*	Theoretical Maximum Dietary Intake, TMDI**
Rice	5	0.28968	1.4484
Mango	0.01	0.01048	0.000105
Cucumber	0.3	0.01385	0.004155
Chili	0.3	0.0087*	0.00261
Tomato	0.7	0.0235	0.01645
Eggplant	0.7	0.0201	0.01407
Long bean	0.1	0.00419	0.000419
Citrus	0.5	0.0364	0.0182
Black pepper	0.3	0.0001*	0.00003
Curry leaves	3	0.0001*	0.0003
Dragon fruits	0.6	0.01048***	0.006288
Total Theoretical Maximum Dietary Intake, ∑TMDI 1.511027			

* Food factors obtained from published national food intake survey (Institute for Public Health 2014)

**TMDI = MRL (or STMR) x food factor

***No data on national dragon fruit intake, therefore national mango intake was used

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

Daily Maximum Permitted Intake (MPI) = ADI x average Asian weight^
= 0.04 mg/kg bw/day x 63 kg/person
= 2.52 mg/person/day
^ the value may differ from region to region

% intake from MPI = (∑TMDI/Daily MPI) x 100
= 1.4916/2.52 mg/person/day
= 59.96% (<100% Daily MPI)

The daily total intake was 59.96% from daily MPI, indicating that no chronic risk from long term consumption

MRL establishment

Residue data were submitted to the Pesticide Board of Malaysia after the completion of the study. After evaluation of residue data and dietary risk assessment, The Pesticide Board of Malaysia would establish MRL and approve product use on the dragon fruits. As of now the Pesticide Board of Malaysia has established administrative MRL of 1 mg/kg and 0.6 mg/kg for tebuconazole and trifloxystrobin on dragon fruits. A higher than estimated tebuconazole MRL on dragon fruits was due to internal consideration by the Pesticide Board. Nativo could be used by dragon fruit farmers following establishment of administrative MRLs. Nevertheless, the new MRLs would be subjected to additional bureaucratic approvals under the Ministry of Health and Attorney General Chambers before final gazettement in the Food Act 1983 after approval by the Malaysian Parliament. Additionally, the same set of residue data was also considered by the Expert Working Group on Harmonisation of MRLs of pesticides among ASEAN Countries, and ASEAN MRL tebuconazole and trifloxystrobin were also established at the same level of MRLs as of national MRLs. Establishment of ASEAN MRLS for dragon fruits could widen the dragon fruit market in ASEAN Countries, especially in Singapore.

Conclusion

In the four residue field trials, residues were detected in the range of <0.04 – 0.353 mg/kg (tebuconazole), <0.04 – 0.327 mg/kg (trifloxystrobin) and <0.01 – 0.014 mg/kg (trifloxystrobin acid). Maximum Residue Limits of tebuconazole and trifloxystrobin in dragon fruits were estimated based on OECD MRL calculator at 1.0 mg/kg and 0.6 mg/kg, respectively with Pre-Harvest Interval set at 3 days after the last application with no significant acute or chronic risk to the general population in terms of dietary risk assessment. The national authority has set the administrative MRL of tebuconazole and trifloxystrobin on dragon fruits at 1 mg/kg and 0.6 mg/kg, respectively, based on the submitted study.

Acknowledgement

The project was funded by the Department of Agriculture. The authors would like to express gratitude to Mr. Ismail Bin Iberahim of Department of Agriculture and support staff of Laboratory Services Division, Department of Agriculture, Kuala Lumpur. Special thanks also to Ms. Ho Mei Choo from Bayer CropScience and Mr. Ooi Kuan Meng for their support in term of technical assistance, advice and supply of research materials (test item and pesticide analytical standard). Not forgotten also many

thanks to dragon fruit farmers and farm workers for their cooperation. Thanks also to the staff and former staff of the Pesticide Laboratory, Soil Science, Water and Fertiliser Research Centre, Mr. Mohd. Fadhilulrahman, Mr. Lim Kim Peng and Ms. Siti Hajar Binti Hadis@Hadas.

References

Chen, X., Jun, X., Xingang, L., Yan, T., Xinglu, P., Yongquan, Z. & Fengshou, D. 2014. Simultaneous determination of trifloxystrobin and trifloxystrobin acid residue 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., Neary, M. & Philip, E. 1998. An Extension Perspective of the Minor Use Crops Pesticide Problem in Vegetable Production. *Journal of Extension* 32: 1.

González, R. H. Pesticide Residues in Developing Countries – A Review of Residues Detected in Food Exports from the Developing World. 1999. *Pesticide Chemistry and Bioscience* 386–401.

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

Institute for Public Health (IPH). 2014. *National Health and Morbidity Survey 2014: Malaysia Adult. Nutrition Survey (MANS), Vol. III, Food Consumption Statistics of Malaysia* 2–9.

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

Jabatan Pertanian Malaysia. 2021. Statistik Tanaman Buah-Buahan Malaysia 2020, pp. 10.

JMPR (Joint Meeting on Pesticide Residues). 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. 2004. *Pesticide Residues in Food – 2004*. World Health Organization and Food and Agriculture Organization.

JMPR (Joint Meeting on Pesticide Residues). 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, 21–30 September 2010. 2011. *Pesticide Residues in Food – 2010*. World Health Organization and Food and Agriculture Organization.

Lamichhane, J. R., Arendse, W., Dachbrodt-Saaydeh, S., Kudsk, P., Roman, Johan C., van Bijsterveldt-Gels, José E.M., Wick, M. & Messéan, A. Challenges and opportunities for integrated pest management in Europe: A telling example of minor uses. 2015. *Crop Protection* (74): 42–47.

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

Nguyen, H.T. & Nguyen, H.V. Management strategies of major pitaya diseases in Vietnam. 2015 *Management*: 761.

Noegrohati, S., Sulasmi, S., Hernadi, E & Asviastuti, S. Dissipation pattern of azoxystrobin and difenoconazole in red dragon fruit (Hylocereus polyrhizus) cultivated in Indonesian highland (West Java) and coastal area (DI Jogyakarta) and its implication for dietary risk assessment. Food Quality and Safety. 2019 May;3(2): 99–106.

OECD (Organization for Economic Co-operation and Development). MRL Calculator: Users Guide and White Paper. 2014. Serie on Pesticides and Biocides, No. 56 Paris: OECD Publishing. https://doi.org/10.1787/9789264221567-en.

Popp, J., Pető, K. & Nagy, J. Pesticide productivity and food security. A review.

2013. *Agronomy for Sustainable Development* 33(1): 243–255.

Wang L., Li W., Li P., Li M., Chen S. & Han L. Residues and dissipation of trifloxystrobin and its metabolite in tomatoes and soil. 2014. Environmental Monitoring and Assessment 186(11): 7793–7799.

Wang P., Guo X., Duan Y., Li Q. & Yang X. Risk assessment of dietary intake of pesticide residues in pitaya. 2021. *Chinese Journal of Tropical Crops*. 42(7):2076.

Wheeler, W. B. Role of Research and Regulation in 50 Years of Pest Management in Agriculture Prepared for the 50th Anniversary of the Journal of Agricultural and Food Chemistry. 2002. J. Agric. Food Chem. 50(15): 4151–4155.

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

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

Yao D., Li X., Li Y., Chen N., Ma S., Deng G., Li J. & Liang J. The residue analysis of multiple pesticides in pitaya by the QuEChERS pretreatment method & gas chromatography. 2017. *Journal of Zhongkai University of Agriculture and Engineering*. 30(1):36–41.