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REGULAR ARTICLE

Food safety in Thailand 1: it is safe to eat watermelon and durian in Thailand

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Abstract
Objectives The wide use of pesticides raises serious concerns regarding food safety and environmental impacts. There is increasing public concern about the potential health risks linked with exposure to pesticides. Regulation of maximum residue limits (MRL) of pesticide residues in food commodities has been established in many developed countries. For developing countries, like Thailand, this regulation often exists in law, but is not completely enforced in practice. Thus, pesticide residue levels in vegetables and fruits have not been thoroughly monitored. The present study aimed to examine potential health risks associated with pesticide exposure by determining the pesticide residues in two commonly consumed fruits, watermelon and durian.
Methods The fruit samples were purchased from markets in central provinces of Thailand and assayed for the content of 28 pesticides. Analysis of pesticides was performed by multiresidue extraction and followed by GC–MS/MS detection.
Results Of 28 pesticides investigated, 5 were detected in 90.7 % of the watermelon samples ($n = 75$) and 3 in 90 % of durian samples ($n = 30$). Carbofuran, chlorpyrifos, diazinon, dimethoate and metalaxyl were found in watermelons, whereas dichlorvos, dimethoate and metalaxyl were detected in durians. However, their levels were much lower than the recommended MRL values.
Conclusions These pesticide levels detected in the fruits are unlikely to harm the consumers; therefore it is safe to eat watermelon and durian in Thailand. While our results found negligible risk associated with pesticide exposure from consuming these common tropical fruits, special precautions should be considered to decrease total exposure to these harmful pesticides from various foods.

Keywords Pesticide residues · Fruits · Watermelon · Durian · Food safety

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Introduction

In the past few years, there has been increasing public concern for food safety. This is because of the extensive use of pesticides in agriculture. While pesticide use usually increases agricultural productivity, accumulated pesticide residues in treated plants present possible health risks to consumers [1]. Toxicity and human health risk associated with pesticide contamination in foods have made it necessary to limit pesticide residues in our foods [2]. Detection and quantification of pesticide residues in food samples are essential to verify whether these pesticides are within limits, the so-called "maximum residue limits (MRL)". This regulation was established by the European Commission and other regulatory authorities. Many developed countries have adopted this regulation to oversee and manage their food safety affair. However, in developing countries such as Thailand, good agricultural practices are not fully complied with.

Watermelon (*Citrullus vulgaris* Schrad.) is one of the most common types of melons. Watermelon is a sweet,

juicy, and very nutritious fruit that is rich in most of the important antioxidants in nature. Besides vitamins A and C, watermelon contains lycopene, an efficient free radical scavenger. These help to protect against chronic diseases such as cancers, cardiovascular diseases, and osteoarthritic inflammation [3, 4]. Watermelon is eaten as fresh fruit and is mainly used to make products such as juices, nectars, and fruit cocktails. Even the by-products, such as peel and seed, can be made into pickles, preserves, and other foods [5]. Thai people commonly use watermelon peel as a vegetable ingredient in their curry dishes (watermelon rind sour curry). Many kinds of pesticides have been applied to protect watermelon from pests and fungi [3, 6]. In Thailand, there is a common belief among people that watermelons are cultivated with excessive use of pesticide carbofuran and thus not safe to eat [7–11].

Durian (*Duriozibethinus,* family of Malvaceae) fruit is delicious, soft, succulent, and very popular for its unique characteristics. Durian is widely respected as the "King of Fruits"; it is a highly prized fruit in Southeast Asian countries. Its production and export are dominated by Thailand, followed by Malaysia and Indonesia [12, 13]. The tree is tropical and native to Brunei, Indonesia, Malaysia, and Thailand. The tree grows up to 50 m in height depending on the species. Durian is a seasonal fruit; its season lasts typically from June until August, which coincides with that of the other tropical-specific fruits such as mangosteen, jackfruit, and mango. The flesh or pulp can be eaten at various stages of ripeness, and it is used as a flavor base in a wide variety of culinary and sweet preparations in Southeast Asian cuisines. Its edible flesh emits a distinctive odor which can be described as strong and penetrating even when the husk is intact. This unusual stinky and intense odor of the fruit may have prompted many people to express diverse and peculiar opinions. Though it contains a relatively high amount of fats among fruits, it is free from saturated fats and cholesterol. Durian is abundant in dietary fiber; the fiber content helps to protect the colon mucous membrane by decreasing exposure time as well as by binding to cancer-causing chemicals in the colon [13]. The durian fruit is a good source of antioxidants and vitamin C and enriches with health-benefiting vitamin B groups. Fresh durian fruit is also a very rich source of potassium, an important electrolyte that helps control heart rate and blood pressure. Additionally, it contains high levels of essential amino acid, tryptophan (also known as "nature's sleeping pill") which in the body metabolizes into serotonin and melatonin. These neurochemicals have important functions like sleep induction and are used in the treatment of epilepsy.

Information on pesticide residues in watermelon and durian fruits does not exist in the country and is scarce worldwide. We could not retrieve any residue data for durian in the literature, and there is very limited data on watermelons. Therefore, this study aimed to determine possible pesticide contamination in these fruit samples by analyzing 28 pesticides and 2 metabolites using GC–MS/MS methods. Also, we aimed to clarify if it was safe to eat the two popular tropical fruits, watermelons and durians, sold in Thailand.

Materials and methods

Chemicals and standards

Anhydrous magnesium sulfate, sodium chloride, primary and secondary amine (PSA, particle size 40 μm), graphited carbon black (GCB), and C18 sorbent (particle size 40 μm) were purchased from Supelco (Sigma–Aldrich Corp., St. Louis, USA). HPLC-grade acetonitrile was obtained from Merck (Darmstadt, Germany). Twenty-eight pesticides and two metablolite standards including aldrin, atrazine, captan, carbaryl, carbofuran (and its two metabolites carbofuran-3-hydroxy and carbofuran-3-keto), carbosulfan, chlormefos, chlorpyrifos, chlorothalonil, cypermethrin, deltamethrin, diazinon, dichlorvos, dicofol, dimethoate, ethion, fenitrothion, fenvalerate, lambda-cyhalothrin, malathion, metalaxyl, methidathion, methomyl, paraoxon-methyl, phosalone, pirimicarb, pirimiphos-methyl, and profenofos were purchased from Dr. Ehrenstorfer (Augsburg, Germany). The purity of these pesticide standards was >98 %. Individual stock of standard solutions (1000 mg/L) was prepared in acetonitrile. Working surrogate spiking standard solutions were prepared by an appropriate dilution of the stock solutions with acetonitrile. These standard solutions were protected from light and kept frozen at −20 °C until assay.

Fruit samples

A total of 75 watermelon samples of whole fruit and 30 durian samples of peeled fruit were purchased randomly from markets in eight provinces, Bangkok, Ayutthaya, Nakhon Pathom, Nonthaburi, Pathumthani, Samutsakorn, Suphanburi, and Nakhon Ratchasima. These provinces are located surrounding Bangkok, Thailand, within a radial distance of 260 km. The samples were collected between April and September 2014. The watermelon fruits analyzed in this study have an edible peel and consumers may ingest surface residues. Therefore, the unpeeled watermelon samples were analyzed for pesticide residues. This is in accordance with protocols recommended by Codex Alimentarius Commission [14] for watermelon sample preparations. The whole watermelon was cut into four parts, and the two opposite angles were used for analysis. As the peel

of durian fruits is not edible, only the inner parts (flesh or pulp) were used for the analysis. The sample preparation for durian did not follow the protocols of Codex Alimentarius Commission [14], but the guidelines given by the Department of Food Safety, Ministry of Health, Labour and Welfare, Japan [15]. The durian samples consisted of flesh separated from peeled fruit. These samples were analyzed without seeds. The representative portion (150–200 g) of the fresh fruit was cut into small pieces, blended using a food processor, and mixed thoroughly. For durian samples, 10 % distilled water was added to the sample before homogenization. When calculations were made for durian samples, the correction for sample dilution was taken into account. The homogenized samples were then extracted and processed as described below.

Determination of pesticide levels in flesh and unpeeled watermelon samples

The same watermelon fruits were cut and divided into two portions, one without peel (i.e., flesh watermelon) and another with peel (unpeeled watermelon). The sample size employed for this experiment was 8 samples ($n = 8$ each). These samples were analyzed for pesticide residues in the same way as ordinary samples.

Sample preparation

To determine the concentrations of pesticide residues, the extraction procedure was performed following a modified acetate-buffered version of the quick easy cheap effective rugged and safe (QuEChERS) method as previously described [16–19]. In brief, the procedure for sample preparation was as follows: 15 g of homogenized fruit was extracted with 15 ml acetonitrile saturated with 6 g of magnesium sulfate and 1.5 g of sodium chloride. After the sample mixture was vigorously shaken and centrifuged, the sample was subjected to a cleaning up procedure. For watermelon samples, this was accomplished by transferring an aliquot of 1 mL of supernatant into a dispersive solid-phase extraction tube containing 50 mg of primary–secondary amine (PSA) and 150 mg magnesium sulfate. For durian samples, 1 mL of the supernatant was transferred into a dispersive solid-phase extraction tube containing 50 mg of primary–secondary amine (PSA), 50 mg C18 sorbent, and 150 mg magnesium sulfate. After shaking and centrifugation, the extract supernatant was then transferred to an autosampler vial for direct injection into the Bruker GC/MS/MS system.

GC–MS/MS analysis

Detection of pesticides was performed using a Bruker 456 gas chromatography (GC) coupled with Bruker Scion triple quadrupole mass spectrometer (GC–MS/MS). Details of the GC–MS/MS conditions were as in a previous report [20]. Multiple reaction monitoring (MRM) acquisition method and two-ion transition at the experimentally optimized collision energy (CE) were monitored for each pesticide analyte.

Recovery studies for method validation were made by adding appropriate volumes of working solution to blank samples. The method of validation with respect to recovery, reproducibility, calibration linear range, limit of detection (LOD), and limit of quantification (LOQ) was carried out for the fruit matrix as described previously [21, 22].

Calibration and quantification

Calibration curves of each pesticide of interest were run according to the established procedure [17, 18, 21]. These were conducted using the same procedure each time when a new unknown sample set was analyzed. The ions selected for quantification were *m/z*, which was the most abundant ions of each chemical pesticide, while the remaining ions were used for confirmation of the analyte identity. Aldrin was used as an internal standard in the analysis of watermelon samples. During the quantitative analysis of durian samples, fenitrothion was used as an internal standard. The ratio of the peak area of the pesticide standard to that of the internal standard was employed for quantification.

Blank samples of watermelon and durian employed for recovery experiments and for preparation of the matrix-matched multi-level calibration standards were those previously assayed. The blank samples of durian were demonstrated to be free of pesticides of interest. However, for watermelon, the blank sample was found to be free of other pesticides of interest except that it contained a very low concentration of metalaxyl. As metalaxyl is a common pesticide detected in every watermelon tested, modified calculations were adopted for the recovery studies and calibration curves for watermelon samples. Consequently, the calibration curves were constructed by subtracting the peak area of metalaxyl residue presented in the blank sample from each data point of the calibration curve, before subjecting data to regression analysis.

Excellent linearity and reproducibility of calibration curves were achieved as illustrated by the coefficient of determination (r^2) values of >0.92 and relative standard deviations (RSD) of <20 %. The signal-to-noise ratio of pesticides of interest measured at 0.01 ppb was well above 50 for all pesticides studied. Consequently, the detection limits were below 0.01 ppb using the sample preparation procedures described above. Determination of pesticides in an unknown fruit sample was done in duplicate unless otherwise stated. In each sample lot, a quality control

sample at a concentration of 0.05 ppm for each matrix was analyzed. This is to verify the reliability of the method with respect to detection of targeted analytes and precision of the assay. MRL values for each pesticide in the durians and watermelons were obtained from recommended MRL values established by the Thailand Ministry of Agriculture and Cooperation [23], and EU Pesticide Database [24].

Statistical analysis

All results are presented as mean ± standard deviation (SD). The mean differences of parameter between two sample groups were assessed by either unpaired Student's *t* test or the Mann–Whitney *U* test, depending on their normality of distribution. The statistical significance level was set to $p < 0.05$. All statistical analyses were conducted using the software SPSS statistical package for Windows version 18.0 (SPSS Inc., Chicago, IL, USA).

Results

Twenty-eight pesticides studied were selected on the basis of their wide use in agriculture in Thailand. The GC–MS/MS method employed in this study provided satisfactory separation with high sensitivity and selectivity for determination of all 28 pesticides of interest [20]. The absence of co-extracted interferences for all varieties of watermelons and durian fruits was demonstrated by blank extract analysis (Fig. 1a). In the GC–MS chromatogram of blank watermelon extract, there was no interfering peak co-eluted with analytes of interest (Fig. 1a). In the GC–MS/MS chromatograms of blank watermelon extracts, there was an abundant peak (peak 1) eluting with a retention time (RT) of 8.65 min (Fig. 1a). This peak almost co-eluted with the one of the pesticide studied, i.e., carbaryl was chromatographically eluted with RT of 8.82 min. Consequently, it was discovered from our previous study that this interfering peak (RT of 8.65 min) arose from the polypropylene plastic centrifuge tubes (50 mL, Jet Bio-Filtration Products Co. Ltd., Guangzhou, China) used in the extraction procedure [25]. This barrier, because of unknown interfering peak from the plastic tubes, had no influence on the quantification of carbaryl, as the interfering peak had different mass and mass ratios in MS analysis. For durian samples, the GC–MS chromatogram of blank extract also demonstrated that no interfering peak co-eluted with pesticides of interest (results are not shown). In addition, in all watermelon samples tested, there were no identifiable peaks detected with the same RT as aldrin (RT = 16.02 min) that was used as an internal standard in our watermelon GC–MS/MS assay. Similarly, in all durian samples studied, there were no identifiable peaks found with the same RT as fenitrothion (RT = 15.53 min) which was employed as an internal standard in the durian GC–MS/MS assay. This proves the validity of using aldrin and fenitrothion as the internal standards for the two different assays.

Representative chromatograms of watermelon and durian sample after extraction are illustrated in Fig. 1b, c, respectively. This particular watermelon sample had two pesticides, dimethoate (peak D) and metalaxyl (peak M), as shown in Fig. 1b. Also, there were possible peaks of cypermethrin (peak 10) eluted at 29.08 min (Fig. 1b) having matched RT and MS masses. Nevertheless, these complex peaks of cypermethrin were below the detection limit of the current assay. Two pesticides, dimethoate (peak D) and metalaxyl (peak M), were detected in the representative durian sample. Of note, under the extraction and GC–MS/MS analysis used in this current study, there were dissimilarities of endogenous peaks found in watermelon and durian samples. That is watermelons had more endogenous peaks (2, 4, 5, 6, 7, and 9) than those detected in durian samples (Fig. 1b). The endogenous peaks 4, 7, and 9 were not present in durian samples (Fig. 1c), while peaks 3 and 8 were found in durian but not in watermelon; they appeared to be unique endogenous compounds in durian fruit. These interpretations were based on evidence of retention times and GC–MS/MS mass identification. It is beyond the scope of this study to identify these unknown and possibly endogenous compounds in these two tropical fruits.

Of the 28 pesticides investigated, only five were detected in the watermelon samples. These were carbofuran, chlorpyrifos, diazinon, dimethoate, and metalaxyl. Seven watermelon samples were found to contain no pesticides; this represents a rate of free pesticides of 9.3 % (Fig. 2a). Pesticide residues were detected in 68 watermelon samples. This corresponds to a rate of pesticide detection of 90.7 %. Some samples contained only one pesticide, while others (41 %) had multiple pesticide residues. However, in all of the 68 watermelons in which the pesticides were found, the pesticide levels were considerably low, i.e., less than the recommended MRL values (Fig. 2a). Regarding the types of pesticides detected in watermelons (Fig. 2b), only one sample was found to contain carbofuran at a concentration of 0.01 ppb (μg/kg), which is much lower than its recommended MRL (0.1 ppm or 100 ppb). Two watermelon samples contained diazinon at concentrations of 0.02 and 0.04 ppb. Again, these levels found were well below the MRL suggested for diazinon in watermelons (0.01 ppm or 10 ppb). Chlorpyrifos was detected in one watermelon sample at a concentration of 1.3 ppb, which was below its MRL value. Of interest, dimethoate and metalaxyl were the most often found pesticides in the watermelon samples investigated (Fig. 2b). The overall occurrence rate of

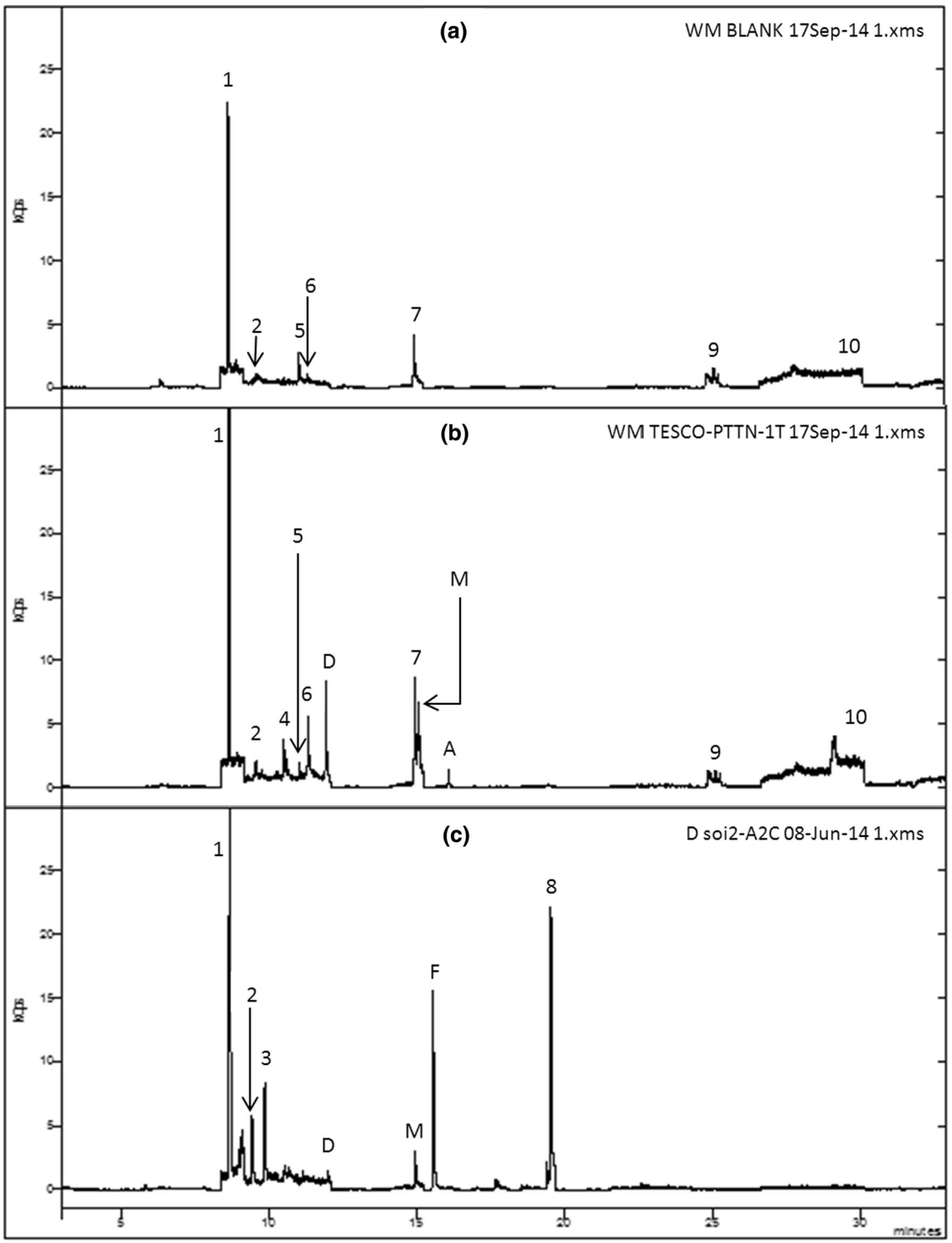

Fig. 1 Chromatograms of **a** blank extract of watermelon sample; **b** a representative chromatogram of watermelon sample extract containing dimethoate (D, 5.17 ppb) and metalaxyl (M, 23.5 ppb); and **c** a representative chromatogram of durian sample extract contained dimethoate (D, 1.44 ppb) and metalaxyl (M, 4.41 ppb) Peak identification: *1* unknown peak from plastic centrifuge tube, *2*, *4*, *5*, *6*, *7*, *8*, and *9* unknown endogenous peaks found in watermelon samples, *D* dimethoate, *M* metalaxyl, *A* aldrin used as the internal standard in watermelon assay, *F* fenitrothion, the internal standard used in durian assay, and *10* the second peak of cypermethrin

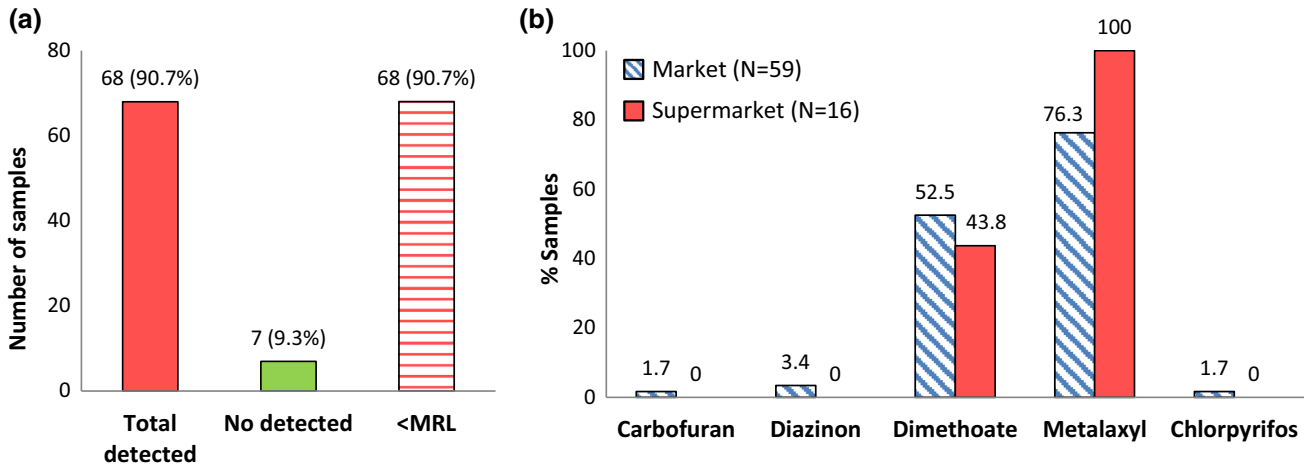

Fig. 2 **a** Overall incidence of detected pesticide residues in watermelon samples showing: total number of samples with detected pesticide residues, samples with no pesticide detected, and samples with pesticide residues detected at levels of <MRL. **b** Type of pesticides detected in watermelon samples purchased from local markets ($n = 59$) and supermarkets ($n = 16$). Rate of detection for each pesticide in watermelons was expressed as percentage

pesticide detected in the watermelon samples (including samples from both local markets and supermarkets) for dimethoate was 50.7 % (38/75), and for metalaxyl 81.3 % (61/75). The levels of dimethoate detected in the watermelons ranged from 0.1 to 5.5 ppb (mean ± SD: 0.7 ± 1.3 ppb), which were much lower than the recommended MRL for dimethoate (0.02 ppm or 20 ppb). Metalaxyl was detected in 61 samples and the levels ranged from 0.01 to 57.1 ppb (mean ± SD: 6.8 ± 14.8 ppb). These concentrations were well below the recommended MRL for metalaxyl (0.2 ppm or 200 ppb).

To distinguish where the fruit was purchased, the data were analyzed according to whether they were bought from local open-air markets or supermarkets. The results obtained are illustrated in Fig. 2b. Dimethoate and metalaxyl were found to be the most common pesticides detected in watermelon samples from both local markets and supermarkets. Samples bought from local open-air markets were found to contain carbofuran, diazinon, and chlorpyrifos, whereas these were not detected in those samples from supermarkets (Fig. 2b). Overall, the percentage of pesticides detected in watermelon samples purchased from the supermarkets (100 %) was higher than that observed in samples purchased from local markets (88.1 %). For dimethoate, 43.8 % of the watermelon samples from supermarkets were contaminated with this pesticide. Of the watermelon samples from local markets, 52.5 % contained dimethoate. The mean level of dimethoate in samples purchased from the supermarkets (1.41 ± 1.82 ppb) was not significantly different ($p > 0.2$) from that found in the watermelon samples obtained from local open-air markets (0.57 ± 1.18 ppb). For metalaxyl, 100 % of the watermelon samples from supermarkets were detected with this pesticide, while 76.3 % of the samples purchased from local markets contained metalaxyl (Fig. 2b). However, the metalaxyl levels in the watermelons purchased from the supermarkets (25.7 ± 18.8 ppb) were significantly greater than ($p < 0.001$) those from local markets (0.05 ± 0.04 ppb).

Table 1 Comparison of pesticide concentrations found in the flesh and the flesh with peel (rind) of watermelon samples

Pesticide	Concentration of pesticide (ppb)		p value
	Flesh	Flesh + rind	
Diazinon	0.019 ± 0.024	0.021 ± 0.025	0.92
Dimethoate	0.22 ± 0.20	1.95 ± 1.80	0.03*
Metalaxyl	0.024 ± 0.015	0.029 ± 0.015	0.42

The mean differences in parameter between the two groups were assessed by unpaired Student's t test

The results are expressed as mean ± SD ($n = 8$)

* Significant difference

The concentrations of pesticides in the flesh and whole watermelon (flesh with peels) samples were compared as shown in Table 1. The detected concentrations of pesticides in both the flesh and whole watermelon samples were very low with reference to their MRL values. The concentrations of diazinon and metalaxyl in flesh watermelon samples were similar to those found in the rind watermelon samples ($p > 0.4$), whereas the mean concentration of dimethoate in the flesh watermelon samples was significantly less than ($p < 0.05$) that detected in the whole watermelon samples (Table 1).

Thirty durian samples were evaluated for possible pesticide contamination. Of the 28 pesticides studied, only

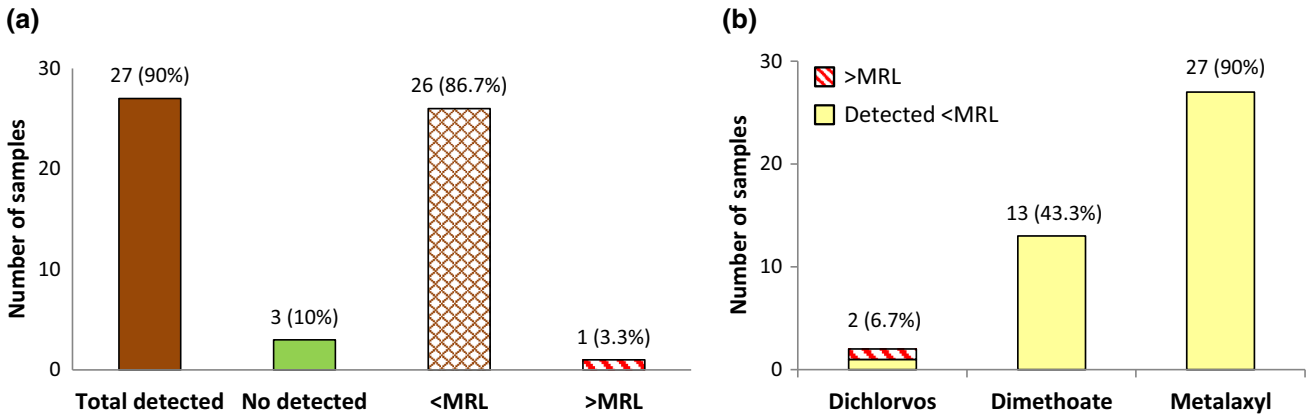

Fig. 3 Incidence of detected pesticide residues in durian samples ($n = 30$): with no pesticide detected; total number of samples with detected pesticide residues; detected pesticide residues with levels of <MRL; and detected pesticide residues with levels of >MRL

three pesticides were found in these durian samples. These were dichlorvos, dimethoate, and metalaxyl (Fig. 3). Overall, pesticides were detected in twenty-seven durian samples representing a rate (27/30) of pesticide detection of 90 % (Fig. 3a). Ten percent of durian samples were pesticide free. Even though these 27 durian samples contained pesticide residues, the level of pesticides was lower than the recommended MRLs. Of these durian samples contaminated with pesticide residues, 14 % contained one pesticide while 13 % had multiple (more than one) pesticide residues.

Dimethoate was detected in thirteen durian samples (Fig. 3b). This represents an occurrence rate of 43.3 %. The concentrations of dimethoate detected ranged from 1.4 to 5.9 ppb with a mean ± SD of 2.6 ± 1.3 ppb. These levels found in durian fruits were well below the recommended MRL for dimethoate in durians (20 ppb). Notably, metalaxyl was found in twenty-seven durian samples corresponding to 90 % (27/90) pesticide detection rate (Fig. 3b). Their levels ranged from 0.3 to 4.4 ppb (mean ± SD of 1.4 ± 1.0 ppb), which were much below the MRL (50 ppb). Two durian samples were found to contain dichlorvos at concentrations of 6.1 and 25.9 ppb (Fig. 3b). One of these samples had a dichorvos concentration which exceeded the recommended MRL for dichlorvos in durians (10 ppb). The incidence of pesticide detected exceeding MRL was 3.3 %.

Discussion

The GC–MS/MS analytical methods developed in our laboratory [25], including sample preparation and GC–MS/MS analysis, were validated. The sample preparation involved solvent extraction and the cleaning up process was executed using the QuEChERS procedure [16–18]. The methods were found to be suitable and applicable for determination of pesticide residues in two tropical fruits, watermelons and durians. This was supported by results of assay validation which have demonstrated good recovery, sensitivity, selectivity, linear calibration curves, good reproducibility, and accuracy. The uses of GC coupled with triple quadrupole MS technique not only eased the detection and quantitation of pesticides, but also provided great sensitivity for pesticide detection.

This study investigated the potential contamination of 28 pesticides in two tropical fruits, watermelons and durians. Five pesticides were detected in watermelons, namely carbofuran, chlorpyrifos, diazinon, dimethoate, and metalaxyl, while only three were found in durian samples, namely dichlorvos, dimethoate, and metalaxyl. Pesticide residues were detected in approximately 90 % of both the watermelon and durian samples tested. The results found in the present study indicate that pesticides are commonly used in the cultivation of watermelons and durians in Thailand. Dimethoate and metalaxyl appeared to be the most often used pesticides for these fruits' cultivation. Although five pesticides were detected in watermelons, the pesticide residue concentrations found were much less than their corresponding MRL values. For example, the mean level of dimethoate found in watermelons was 0.7 ppb, which was only 3.5 % of its recommended MRL (20 ppb). The mean level of metalaxyl detected in watermelon samples was 7.1 ppb, which was approximately only 3.5 % of the recommended MRL of metalaxyl (200 ppb). This finding implies extremely low levels of pesticide contamination in the watermelons sold in Thailand. Eating watermelons in Thailand, therefore, is expected to be safe. Surprisingly, the common belief of Thai people regarding the contamination of pesticide carbofuran in watermelons was not supported by our findings. It is evident from the present study that only one of 75 watermelon samples

monitored contained carbofuran, representing a rate of detection of 1.3 % (1/75). The level of carbofuran found in this particular watermelon was also very low (0.01 ppb). It is possible that this was not from direct pesticide applications used in the cultivation of watermelons. However, it rather came from other sources such as environmental contamination, contamination of irrigation water, and pesticide application affecting the adjoining crops [26, 27]. The findings of dimethoate residues in the watermelon samples were consistent with a previous report showing low levels of dimethoate in the edible parts of watermelons [27]. Few reports on watermelons were retrieved from literature. The analysis of 81 watermelon samples for 31 multi-class pesticide residues was conducted in Spain [28]. Their results have demonstrated that in no sample pesticides above the MRL were detected, representing a 0 % pesticide greater than MRL. In a relatively small sample size of 8 watermelons collected from Korean markets, none were found to be contaminated with pesticide residues [3]. These findings could be interpreted as a very rare or extremely low incidence of pesticides exceeded the MRL levels in watermelons, and is consistent with ours.

There were considerable variations in the levels of pesticides detected in the watermelon samples tested in this study. For instance, the levels of dimethoate found in the watermelons varied over 50-fold (range 0.1–5.5 ppb), and for metalaxyl the levels varied dramatically over 5,000-fold (range 0.01–57.1 ppb). The large variation in the level of pesticides detected in the watermelons may be due to many factors affecting the residues remaining on the fruit at the time of harvest. These include dosage of pesticides applied, frequency of pesticides applied, and pre-harvest interval of crops [29, 30]. Proper education on pesticide use and the pre-harvest interval for crops is essential. This will help to reduce the amount of pesticides present in fruits and vegetables.

The quality of watermelons sold in Thailand markets was in general identified to be good with regard to very low levels of pesticide contamination. Such quality of watermelons marketed in Thailand seems to be similar, regardless of where the fruits were purchased from, i.e., from local open-air markets or supermarkets. The present study showed that there was similarity in the profiles of pesticides detected in the watermelon samples from these two sources. Of interest, dimethoate and metalaxyl were the most common pesticides detected in the watermelon samples purchased from both local markets and supermarkets. The average price of watermelons from supermarkets was 34 ± 13 Bahts/kg, (approximately US$1.1/kg), which was more expensive than those from local markets (26.1 ± 7.1 Bahts/kg, approximately US$0.84/kg). Despite this, the levels of pesticides, such as metalaxyl, were somehow significantly higher in the watermelons bought from supermarkets. This suggests that the quality of watermelons, with regard to low level of pesticide contamination, cannot be justified by the price of the produce. It may be true that fruits purchased from the supermarkets are fresher than those from local open-air markets.

Most people eat only the edible red juicy part (flesh) of watermelons, but watermelon peels or rinds are also edible. Watermelon rinds are used for making pickles, and sometimes used as a vegetable in various ethnic recipes including Chinese, Indian, and Thai [5, 31–33]. In this study, the possible risk of eating watermelon rinds was investigated by comparing the contents of pesticides in the flesh and the whole watermelon samples. The distribution studies have demonstrated that two pesticides, diazinon and metalaxyl, were able to diffuse into the flesh of watermelons as their concentrations were not significantly different in the flesh and the whole watermelon samples (Table 1). In contrast, the mean concentration of dimethoate in the flesh was significantly less than that found in the whole watermelon samples. This indicates that even though dimethoate is able to diffuse into the flesh of watermelon fruit, the transport of this pesticide is hindered by some kind of mechanisms. Such mechanism may provide protection from harmful substances. Little is known of the pesticide transport in plants. No direct evidence of transport mechanisms of dimethoate in watermelon but at least active transport system appeared to be one of important transport mechanisms for pesticides in some vegetables and fruits such as soybean, gourd, cucumber and zucchini [34–37]. In addition, a previous study has shown that the edible part (pulp) of vegetables including cucumber, pumpkin, yam, and sweet potato was less contaminated with an organochlorine pesticide, chlordecone, than the parts that are thrown away, i.e., peels or rinds [38]. This difference was accounted by the composition of lipids and fibers. Whatever the crop, Clostra et al. [38] found that the lipid and fiber contents were less in the pulp than in the peel. This may provide an alternative explanation for our finding that the concentration of dimethoate in the flesh (pulp) was significantly less than that in the whole watermelon samples with peel. It is naive to assume that the flesh part contains less lipid and fiber contents than the peel of watermelon fruit. Unfortunately, the lipid and fiber compositions of watermelon were not determined in this study. Pesticide contaminants may enter plant roots and leaves by passive diffusion and active transport. Then they move in the plant transpiration stream to other plant components [39]. Finally, the pesticides present an equilibrium in which the concentrations are presumed to be similar. The higher concentration of pesticide in some parts of plants could be caused by other factors [39] such as active transport system, pesticide deposition on the plant surface, and adsorption processes [40]. Foliar uptake of pesticides is a

complex process, depending on the characteristics of plant leaf surface, physicochemical properties and concentration of the active pesticide ingredients and additives, and environmental conditions [41]. It is possible that the pesticides may enter the watermelon fruit via the vine. Also, the concentrations of pesticides in the rind top side of the fruit might be different from that in the bottom side of the watermelon. However, these thoughts have not been verified.

Pesticides, at least dimethoate, were found at higher concentrations in the peel of watermelon. It would be advisable to wash the whole fruit before cutting it open with a knife. This is to avoid and reduce the contamination of pesticides into the edible flesh of watermelon. Washing the whole watermelon fruit before cutting it open is likely to reduce pesticide contamination. Even though the washing experiment was not performed to confirm this, previous studies have proven the advantage of washing in decreasing pesticide contamination. For instance, by washing tomato fruits, chlorpyrifos residues were reduced by 41–44 % [42]. Washing cucumbers under running tap water for 1 min appeared to remove carbaryl residues by 33 % [43]. Also after washing Chinese kale under running water for 2 min, approximately 55 % of profenofos residues were removed [25]. It is obvious that washing with water cannot remove the entire pesticide residues from vegetables and fruits, as some pesticides would have already been absorbed into plant components. Of note, most watermelons sold in the markets may have been cleaned and perhaps rubbed/shined up to improve their appearance. Unlike washing under running water method, the merchandise's preparation is unlikely to remove significant pesticide residues on the peel of watermelons. Therefore, our recommendation to properly wash the whole watermelon fruit before cutting it open is valid, because it will at least help to reduce human risk due to pesticide contamination far better than the superficial cleaning done by merchants.

Dimethoate and metalaxyl emerged as the most commonly used pesticides in the cultivation of durians. However, the levels of pesticides detected in durian samples were well below their recommended MRL values. For example, the mean level of metalaxyl found in durian samples was 1.4 ppb corresponding to approximately 3 % of the suggested MRL for metalaxyl in durians (50 ppb). Dimethoate was detected in 13 samples with a mean concentration of 2.6 ± 1.3 ppb, corresponding to about 13 % of the recommended MRL for dimethoate in durians (20 ppb). An exception was one durian sample with dichlorvos at a concentration of 25.9 ppb exceeding its suggested MRL (10 ppb). This makes the rate of pesticides detected greater than MRL of 3.3 % (1/30), which is in an acceptable range when compared with that found in developed countries with respect to the incidence found in other fruits. The monitoring program for pesticide residues in the European Union and Norway carried out in 1996 and 1997 reported low incidence of pesticides exceeding their MRL [44]. For example, in mandarins, the proportion of samples tested with chlorpyrifos exceeding the MRL was 1 %, and 2 % of grape samples monitored contained the benomyl pesticide residues exceeding the MRL. A screening study of berries harvested from Poland [45] showed that 4 % of blackcurrant samples had pesticide levels exceeding the MRL. In apples from Poland, only 1.9 % of samples tested were found to have pesticide residues exceeding the MRL levels recommended [46]. Unfortunately, data on durian in the literature is very limited, so comparison of our results on durians to those in previous reports is not possible. Considering that only a few pesticides were detected in fresh durians sold in Thailand, and very low levels of these pesticides were found, i.e., lower than their MRL values, we conclude that consuming durian will not pose a danger to health of consumers.

Many of the pesticides of interest are known to undergo natural degradation processes such as photolysis and thermal degradation. For instances, in the environment photolysis is the main degradation path of pyrethroids (cypermethrin and cyhalothrin), and mutagenic compounds were generated during photolysis of an organophosphorus pesticide fenitrothion [47–49]. Similar to humans, plants develop a detoxification mechanism to avoid the deleterious effects of harmful pesticides [50]. Some pesticides can be metabolized by various xenobiotic metabolizing enzymes found in plants. These include phase I metabolizing enzymes such as cytochrome P450 (CYP) enzymes, esterases, and peroxidase, and phase II detoxificating enzymes, e.g., glutathione *S*-transferase, and UDP-glucuronyltransferase [50–52]. CYP enzymes are well known as important enzymes in phase I metabolism of numerous xenobiotics and have been implicated in the detoxification of pesticides. For example, thiocarbamates such as molinate and thiobencarb are initially metabolized in plants through thiol sulfur oxidation to the corresponding inactive sulfoxide metabolites [51]. On the other hand, some pesticides including organophosphate pesticides parathion, diazinon, and chlorpyrifos are metabolized by CYP enzymes to form toxic intermediates oxons causing neurotoxicity in humans [53, 54]. The oxon metabolites are recognized to have acute toxicity, due to their ability to bind to and inhibit acetylcholinesterase in the nervous system and at neuromuscular junctions. The scientific community has expressed a great concern for consumer health about the possible adverse effects that the residues of these pesticides in water, vegetables, and fruits may have. The possible chronic effects of these pesticides are

suspected to be linked with a wide spectrum of medical problems such as cancer, neurotoxic effects, reproductive health concerns, and endocrine disruption [50, 55]. Toxicity of pesticides is dependent upon the amount of pesticide intake from foods and exposure duration, toxic potency of pesticide, and individual susceptibility due to variability of pesticide-metabolizing enzymes [50]. In addition, many of these pesticides may also serve as inhibitors or inducers of human drug-metabolizing enzymes. For example, DDT and fenvalerate are known to induce several human CYP enzymes [50]. An endocrine disrupter pesticide endosulfan has been shown to reversibly inhibit human CYP3A4 enzyme [56]. Dimethoate pretreatment in rats caused an increase in the activities of glutathione peroxidase and glutathione reductase, as compared to the control animals [57]. A recent study has also shown that a new fungicide propiconazole inhibited P-glycoprotein (P-gp) transporter protein with an inhibition potency similar to erythromycin [35]. These further raise additional consequence for human risks of possible pesticide–drug interactions that may occur between pesticides and conventional medicines. Clinical implication of pesticide–drug interactions remains to be confirmed.

In summary, even though residues of a few pesticides including carbofuran, diazinon, dichlorvos, dimethoate, and metalaxyl were detected in some watermelon and durian samples tested, their levels were well below the recommended MRL values. These levels are unlikely to harm the consumers; thus eating watermelon and durian sold in Thailand is considerably safe. Despite that our findings discovered negligible risk associated with intake of pesticide residues in these tropical fruits, consumers may be exposed to many of the same pesticides from a variety of other foods. The diet, in general, must be taken into consideration to assess the true risk associated with pesticide residue exposure. In addition, the results derived from this study would be helpful for the Thai government to establish MRL of pesticides in watermelons and durians and to provide guidance on the safe and proper use of the pesticides.

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Conflict of interest The authors declare they have no conflict of interest.

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