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Residue distribution, safety evaluation, processing factor and dissipation kinetics of bifenthrin and ethion in date fruits

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RESEARCH ARTICLE

Abstract

Gas chromatography with ion trap mass spectrometry was used to simultaneously analyses bifenthrin and ethion residues in date fruits after acetonitrile extraction and dispersive solid phase extraction for clean-up. The method was validated in line with the SANTE/11813/2017 guideline with performance criteria including the linearity range, repeatability, intermediate reproducibility, matrix effect, recovery, and limit of quantitation. Attractive advances results include: (1) lower quantitation limit of 6.9 and 5.8 μg/kg; (2) the recoveries were between 91.61 and 98.84%, and the corresponding relative standard deviations (RSDs) were lower than 11.7%; (3) excellent repeatability RSD_r of lower than 10.3 μg/kg, and intermediate reproducibility RSD_R of lower than 15.6 μg/kg, Furthermore, the method is fast and simple. After validation, the developed method was successfully applied for the determination of the dissipation kinetics of the bifenthrin and ethion in date fruits over 45 days under field conditions of Saudi Arabia. The results showed that the dissipation kinetics of bifenthrin and ethion can be described by a first-order kinetics model with a half-life ($t_{0.5}$) of 1.92 and 2.43 days for bifenthrin and 2.2 and 2.59 days for ethion, at the low and high recommended application rates, respectively. For safe consumption of dates, waiting periods of 12 and 28 days for bifenthrin and 18 and 38 days for ethion are suggested. A total of 600 date samples were collected from the largest market in the world in Qassim Province, Buraydah City, in 2017-2018. A risk assessment of the residues was performed by calculating the risk quotients for children and adults in Saudi Arabia. The high consumption of dates by children may have hazards. The processing factors for washing with tap water, and washing plus boiling (date syrup production) showed that washing had little effect on residue reduction compared with washing plus boiling.

Keywords: risk assessment, method validation, QuEChERS, date fruits, ethion, bifenthrin

1. Introduction

Dates (*Phoenix dactylifera*) are currently produced in 40 countries with a total production of 8.5 million tons. Saudi Arabia is one of the most important producers and ranks third after Egypt and Iran according to FAO statistics. The quantity produced in Saudi Arabia reached about one million tons in 2016 (http://faostat.fao.org/). Buraydah in Qassim Province plays a major part in the production. Recently, interest in date products has increased among international consumers. Dates contain carbohydrates, minerals, and vitamins, as well as antimutagenic, anticarcinogenic, antiviral, and antioxidant properties (Baloch *et al.*, 2006).

Pesticide is used extensively for controlling agricultural pests and food storage. The consumption of crops contaminated with pesticide residues can lead to accumulation in the body, resulting in adverse effects on health (Ghani and Abdallah, 2016), such as cancer (Zhao *et al.*, 2012), diabetes (Son *et al.*, 2010), endocrine disruption (Mnif *et al.*, 2011), and infertility (Clementi *et al.*, 2008).

Many pesticides are registered for the application on date fruits, among them bifenthrin[(2-methyl-3-biphenylyl) methyl (1S,3S)-3-[(1Z)-2-chloro-3,3,3-trifluoro-1-propen-1-yl]-2,2 dimethylcyclopropanecarboxylate] (Figure 1A) is a synthetic pyrethroid with broad-spectrum activity. It acts as a non-systemic insecticide with contact and stomach poison and is effective against insects, mites, and foliar pests including Coleoptera, Diptera, Heteroptera, Lepidoptera, and Homoptera (Gupta *et al.*, 2009; Reddy and Rao, 2002), and ethion[O, O, O', O'-tetraethyl S, S'-methylene bis (phosphorodithioate)] (Figure 1B) is a non-systemic organophosphate insecticide and acaricide that is widely used on many crops for controlling pests such as caterpillars and aphids by inhibiting the cholinesterase enzyme (Abdel-Gawad *et al.*, 2014; MacNeil and Hikichi, 1976). Based on our preliminary survey of 193 date fruit samples collected from the markets during 2017, it was revealed that ethion and bifenthrin are the most frequently used pesticides, as the residues of these two pesticides occurred in real samples.

It is recommended that levels of pesticide residues be reduced in foods to reduce exposure and health risks (Gale and Buzby, 2009). Washing with water is one of the most important ways to reduce pesticide residues on fruit and vegetable surfaces. The efficiency of different removal processes depends on several factors: the physico-chemical properties of the washing solution and pesticide, morphological structure of the plant and exposed surface, the exposure period, the formulation type of the pesticide, and the application method (Al-Taher *et al.*, 2013).

Many reports investigate the dissipation of bifenthrin and ethion in several crops in open-field conditions with different chromatographic techniques. The main techniques are gas chromatography (GC) coupled to an electron capture detector, mass spectrometry (MS), and liquid chromatography coupled to a diode array detector and MS spectrometer (Ramarao and Goud, 2016; Reddy *et al.*, 2017; Seenivasan and Muraleedharan, 2015; Sharma and Parihar, 2013; Varghese *et al.*, 2011). It is challenging to prepare date samples for the quantitative determination of bifenthrin and ethion residues, using gas chromatography equipped with ion trap detector (GC-MS/IT) due to the high amounts of carbohydrates (44-88%), fats (0.2-0.4%), protein (2.3-5.6%), fibre (6.4-11.5%), minerals, and vitamins (Al-Farsi *et al.*, 2005). Thus, optimal sample preparation is one of the key conditions to achieve good performance.

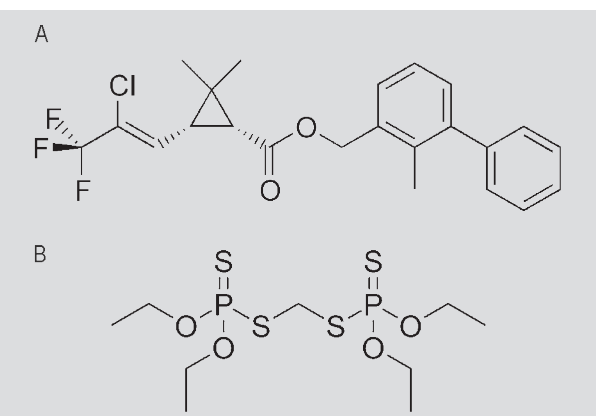

Figure 1. Chemical structure of bifenthrin (A) and ethion (B).

To our knowledge, very little report is available on residues of bifenthrin and ethion in dates (Ghani *et al.*, 2018). Therefore, an open-field study was carried out to investigate the residual behaviour, determine the half-life of both insecticides, and suggest suitable pre-harvest intervals (safe waiting periods) based on the maximum residue limits (MRLs) of 0.01 mg/kg (EC, 2005) for low and high recommended rates of application. This study also investigates the insecticides in date samples collected from Saudi Arabia in 2017-2018to gain insight into residue intake through the diet, as well as the effect of washing and the process of liquid sugar production on the reduction of residues. Validation criteria were applied to ensure the capability of the method for determining bifenthrin and ethion in dates using GC-MS/IT.

2. Materials and methods

Chemicals and reagents

Acetonitrile (pesticide residue grade), anhydrous magnesium sulphate, sodium hydroxide/and sodium chloride were purchased from Fisher Scientific (Loughborough, UK). Pure reference standards of bifenthrin (98.8% purity) and ethion (99.5% purity) were purchased from Sigma-Aldrich (St Louis, MO, USA). Bulk sorbent primary secondary amine (PSA) was purchased from Agilent (Wilmington, DE, USA). Commercial formulations of bifenthrin (8%, emulsifiable concentrate; EC) and ethion (40%, EC) were bought from a local market. A Milli-Q Integral system was used to produce ultra-pure water.

Preparation of standard solutions and reagents

Stock solutions of reference standards (1000 mg/l) were separately prepared in acetonitrile by weighing 0.1012 g and 0.1005 g of bifenthrin and ethion, respectively, and placing them in calibrated 100-ml volumetric glass flasks. The stock solutions were stored in dark volumetric glass flasks at -20 °C. Fortification levels, pure solvent calibration, and matrix-matched calibration solutions were prepared for gas chromatography-ion trap-mass spectrometric (GC-ITMS) analysis via further dilution with acetonitrile or spiking with appropriate volumes of standard to achieve concentrations ranging from 5 to 1000 μg/kg. A 0.1-N sodium hydroxide solution was prepared by dissolving 4 g of sodium hydroxide in 1 litre of deionised water.

Field trials

Field trials were carried out in Buraydah, Al-Qassim Governorate, Saudi Arabia, on May 15, 2018(two months before harvest). Fruit from date palms (*P. dactylifera* var. Sukkari) were sprayed with the commercial formulations of bifenthrin and ethion once at the manufacturer's low and high recommended rates of 75-125 ml/100 l water (equivalent to 60-100 mg a.i/l) and 100-200 ml per 100 l water (equivalent to 400-800 mg a.i/l), respectively. Each treatment involved five trees. Representative samples were collected at 0 (2 h after treatment), 1, 3, 7, 10, 15, 20, 25, 30, 35, and 40 days after the treatments. The collected sample weight was about 2 kg. Untreated (blank) samples were collected before the application of pesticides. Date kernels were separated, their weight was calculated, and they were divided into three replicates (0.5 kg each) and kept frozen (at -20 °C) until analysis. The weather conditions were as follows: the temperature ranged from 28 °C at night to 38 °C as during the day, and the relative humidity was 31-48%.

Monitoring of bifenthrin and ethion residues in real date samples

A total of 600 samples (2 kg each) of dates were collected in 2017-2018 from the world's largest market, which is held in the Buraydah city, Saudi Arabia, from July until October of each year. Millions of tons of dates are imported from all over Saudi Arabia during this period. Samples were collected according to published guidelines (EC, 2002) and then prepared and kept frozen (at -20 °C) until analysis.

Effect of home processing on reduction of pesticide residues

Real contaminated date fruit samples collected from the market were purchased in a large quantity from the same suppliers and re-analysed again to confirm the presence of bifenthrin and ethion residues separately in a concentration of 0.232 mg/kg for bifenthrin and 0.391 mg/kg for ethion, for further processing steps. About 18 kg of real contaminated date fruit samples was divided into three sets (each set has 6 replicates of 1 kg each). They were then subjected to the various treatments and analysed.

Effect of washing

Two sets of samples were washed under running tap water (pH=7.28 and total dissolved solids=560 mg/l) (Jenway® 3540, Bibby Scientific Ltd, Stone, Staffs, UK) with a flow rate of 2 l/min for 1 min. (set one) and 5 min. (set two) with gentle rubbing while avoiding damage to the outer morphological structure of the fruits. The samples were subjected to blowing air in the shade at room temperature for 2 h to remove the excess water from the surface. The third set was not processed.

Effect of washing plus boiling (liquid date syrup production)

Next, 100 g of the washed date samples were sliced into small pieces and put in an Erlenmeyer flask (1 l). Water was added at a ratio of 1:3 (w/v), and the samples were blended for 5 min. The pH was adjusted to 6.0±0.05 by an appropriate volume of 0.1 M sodium hydroxide solution. The samples were placed in a water bath at 70 °C for 2 h. After heating, the slurry was filtered through cheesecloth with a hand press to remove large impurities and insoluble matter and then centrifuged at 5,000 rpm for 10 min (El-Sharnouby *et al.*, 2014) The supernatant was decanted and cooled to 4 °C for pesticide residue analysis.

Processing factors

Processing factors (PFs) were calculated as the ratio of the pesticide residue concentration (mg/kg) in the processed sample to the pesticide residue concentration (mg/kg) in the raw, non-processed sample. A PF less than 1indicates that the concentration of the residues in the processed samples are less than that in the raw samples and hence the processing step is effective, while a value higher than 1indicates that the processing step is not effective (Damalas and Eleftherohorinos, 2011).

Sample preparation

Sample hydration

A 0.5 kg sample of date fruit was weighed and mixed with 750 ml of cold deionised water (4 °C) (Abdallah *et al.*, 2018). The mixture was then well homogenised using a Stephan Universal (UM5) food processing machine (Stephan Machinery GmbH, Hameln, Germany) for 2 min at high speed.

Extraction and clean-up

A 10 g sample of re-hydrated date (corresponding to 4 g of raw date) or 10 g of date syrup was weighed in a 50 ml polytetrafluoroethylene (PTFE) centrifuge tube. Next, 10 ml of acetonitrile was added and vigorously shaken by hand for 1 min after adding a ceramic homogeniser. Then, 4 g of anhydrous magnesium sulphate and 1 g of sodium chloride were added and shaken by hand again for 1 min. The tube was centrifuged at 5,000 rpm for 5 min (Centurion Scientific Limited, K3 series, Chichester, UK), and 2 ml of the upper layer extract was cleaned up using 100 mg of PSA and 150 mg of $MgSO_4$. The tube was then vortexed for 30 sec., centrifuged for 5 min at 5,000 rpm, and filtered through a 0.22-μm PTFE syringe filter (Whatman, Maidstone, UK) for direct chromatographic analysis.

Gas chromatography-ion trap-mass spectrometry

Bifenthrin and ethion residues were analysed using GC (Varian® GC-450, Varian, Inc., Middelburg, the Netherlands) equipped with ion trap mass spectrometer detector (MS-220) (Varian, Inc., Walnut Creek, CA, USA) and operated in selective ion storage mode. A fused-silica HP-5MS ultra-inert capillary column was used (30 m, 0.25-mm i.d., 0.25-μm film thicknesses). For the oven temperature program, the initial temperature was 70 °C and held for 1 min, followed by an increase at 30 °C/min to 200 °C, holding for 5 min, a final increase to 280 °C at a rate of 10 °C/min, and holding for 6 min. The injector, manifold, ion trap, and transfer line were maintained at 250, 50, 230, and 290 °C, respectively. Helium gas (99.999%) was used as a carrier gas with a constant flow rate of 1.2 ml/min. The ion energy for electron impact was kept at 70 eV, and the injection volume was 1 μl. The retention times of bifenthrin was 14.19 min with major ions at 181, 182 and 165 m/z, whereas for ethion was 15.71 min with major ions of 230, 125 and 97 m/z (the first reported ion for each analyte was used for the quantification purposes).

Effect of clean-up on the matrix effect

The effect of PSA adsorbent to remove possible co-extract materials in the extract was studied in terms of the matrix effect. The study was conducted using spiking levels of 10 μg/l of bifenthrin and ethion in the mixture. Blank date extract was cleaned separately with 25, 50 and 100 mg of PSA in addition to 150 mg of $MgSO_4$ (mg/l ml of date extract). When PSA was not used, no bulk adsorbent or salts were added. The matrix effect was calculated by comparing the average of the chromatographic peak areas of each pesticide prepared in matrix extract X1 and that prepared in pure solvent X2 using the following equation:

$$\text{Matrix effect (\%)} = 100 \times \frac{x_1}{x_2} - 1 \qquad (1)$$

Data analysis

The residue concentration and half-life ($t_{0.5}$) of bifenthrin and ethion in dates were calculated by the equations $C_t = C_0\, e^{-kt}$ and $t_{0.5} = \ln2/k$, respectively, where C_t is the concentration (mg/kg) of pesticide residues at time t, C_0 is the initial concentration (0 days) after application, and k is the degradation rate constant (day^{-1}). The pesticide residues were plotted with respect to the interval times (Liang *et al.*, 2011). Significant differences between unprocessed dates and treated samples were determined by one-way analysis of variance (ANOVA). Significance was defined by $P<0.05$.

Safety evaluation of bifenthrin and ethion residues in date samples

The estimated chronic risk from the intake of contaminated dates was assessed using the reported determined concentrations (mean, median and 95^{th} percentile values) of bifenthrin and ethion residues during 2017-2018. Samples with undetected residues (< limit of quantification; LOQ), might not indicate the absence of pesticide residues, but only the sensitivity of the analytical method may not sufficient for detection of present lower levels (Claeys *et al.*, 2008). When the proportion of samples with undetected residue concentrations (<LOQ) is greater than 80%, two estimates of 0 and LOQ can be used to replace the results of <LOQ (WHO, 1995). The respective LOQ values were assumed to replace the undetected concentrations (<LOQ) in this paper as the worst case.

The assessment was done for populations of adults (60 kg body weight (BW)) and children (15 kg BW) based on the mean results of each pesticide. The analysis was based on the estimated average daily consumption of 0.0418 kg date/person/day in the Middle East (Abdallah *et al.*, 2018; WHO, 2003) and the respective acceptable daily intake (ADI) values. The following equations were used:

$$\text{EED (mg/kg, bw)} = \frac{\text{CRL (mg/kg)} \times \text{FI (kg)}}{\text{BW (kg)}} \qquad (2)$$

$$\text{RQ} = \frac{\text{EED (mg/kg, BW)}}{\text{ADI (mg/kg, BW)}} \qquad (3)$$

with EED = estimated exposure dose; CRL = calculated residue level; FI = food intake.

The ADIs are 0.015 mg/kg BW per day for bifenthrin(EFSA, 2011)and 0.002 mg/kg BW per day for ethion(FAO/WHO, 2008). An RQ value that is higher than one indicates that the risk of pesticide for humans is unacceptable.

3. Results and discussion

Dispersive solid phase extraction clean-up and matrix effect

Co-eluting materials in the final extract may lead to changes in the chromatographic response, especially when using ion trap detector due to the change in the ionisation efficiency (suppression or enhancement). Consequently, the results obtained would be inaccurate. This phenomenon is known as the matrix effect (Lucini and Molinari, 2011). The matrix effect was evaluated for the pesticides by the relationship between the chromatographic peak areas of the analyte in the date extract and in the pure solvent at a concentration level of 10 μg/l (Equation 1).

The matrix effect of date extract enhanced the chromatographic peak area of bifenthrin by 31.3, 28.2, 26.1 and 22.3, whereas, the percentages for ethion were 88.56, 57.7, 40.6 and 12.3%, using a 0 (matrix w/out clean up), 25, 50, and 100 mg PSA/ml of extract, respectively, as comparing to the chromatographic peak area of each analyte in pure solvent. The use of 25 mg of PSA per 1 ml extract resulted in a non-significant reduction (P>0.05) of the magnitude of ionisation enhancement, whereas using 100 mg of PSA significantly reduced (P<0.05) the enhancement effect compared to the non-cleaned extract (Figure 2). Therefore, we recommend using 100 mg of PSA for clean-up of the date matrix. This finding is in agreement with other studies reporting that the d-SPE step of the QuEChERS method is effective for removing co-extracts. Once the optimal extraction and clean-up steps were set up, the matrix effect was finally evaluated at eight concentration levels ranging from 5 to 1000 μg/l of each analyte (Table 1). This was done by comparing the slopes of the established calibration standards dissolved in acetonitrile and matrix-matched calibration curves using Equation 4 (Ferrer *et al.*, 2011). Ionisation enhancement was confirmed to be the main matrix effect in GC-ITMS, which was calculated as 21.8 and 13.9 for bifenthrin and ethion, respectively.

$$\text{Matrix effect (\%)} = 100 \times \frac{\text{slope matrix}}{\text{slope solvent}} - 1 \qquad (4)$$

Method validation

Calibration curves based on the matrix-matched standard calibration method were used for quantification of bifenthrin and ethion residue in date samples. The linearity of the curves was examined in the range of 5-1000 μg/kg with eight calibration points of 5, 10, 25, 50, 100, 250, 500, and 1000 μg/kg in the solution. Three replicates were performed at each level. Linear calibration graphs of the concentrations versus the peak area were constructed by

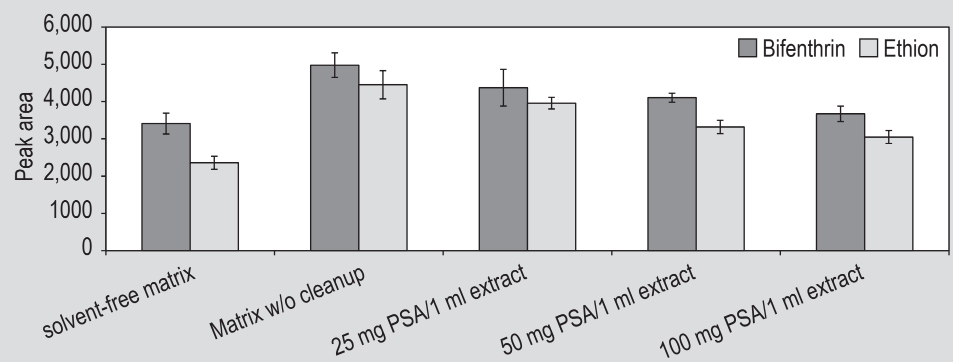

Figure 2. Effect of using primary secondary amine (PSA) on the matrix effect % (explained as peak area) of bifenthrin and ethion.

Table 1. Validation results.[1]

Parameters	Acetonitrile-free matrix		Date matrix extract	
	Bifenthrin	Ethion	Bifenthrin	Ethion
Calibration range	5-1000 (μg/l)	5-1000 (μg/l)	5-1000 (μg/kg)	5-1000 (μg/kg)
Linearity R^2	0.9891	0.9993	0.9931	0.9994
Intra-day repeatability (RSD_r, %)	–	–	10.3	8.8
Inter-day repeatability (RSD_R, %)	–	–	15.6	12.4
LOD(μg/kg)	–	–	2.1	1.5
LOQ (μg/kg)	–	–	6.9	5.8
Matrix effect %	–	–	21.8	13.9
Recovery %, (RSD%)	Spiked levels (μg/kg)			
	10		91.61 (11.7)	93.72 (10.9)
	50		94.15 (8.6)	96.33 (9.3)
	500		95.82 (7.4)	98.54 (6.7)

[1] LOD = limit of detection; LOQ = limit of quantification; RSD = relative standard deviations.

least-squares regression. The determination coefficients (R^2) of bifenthrin and ethion in the date matrix were 0.9931 and 0.9994, respectively.

The method efficiency was assessed by spiking blank date samples with corresponding volumes of bifenthrin and ethion working solutions at three different levels of 10, 50, and 500 µg/kg. Triplicate samples of each concentration were processed (n=3). The recovery ranged from 91.61 to 95.82% with a maximum RSD% of 11.7% for bifenthrin. For ethion, the recovery ranged from 93.72 to 98.84% with a maximum RSD% of 10.9%.

The intra-day precision (RSD_r) and inter-day precision (RSD_R) were also investigated. The intra-day precision was calculated by quantifying six spiked samples (n=6) at 10 mg/kg on the same day (RSDr) and on three different days (n=18) at seven-day intervals (RSD_R). The precision of the quantitation of bifenthrin and ethion ranged from 8.8 to 15.6% (Table 1). This finding is consistent with the SANTE guidelines (EU Reference Laboratories for Residues of Pesticides, 2017), which indicate that the precision of the method should be less than or equal to 20%. The values of recovery and precision indicated good method accuracy.

Figure 3 shows a chromatogram of matrix-matched standards of bifenthrin and ethion at concentrations of 25 µg/kg. The LOQs were calculated based on the ratio between the standard deviation of the response (Sy) and the slope (S) of the matrix-matched calibration curve, assuming LOQ values at 10 Sy/S (Abdallah *et al.*, 2017). The LOQs were 6.9 and 5.8 µg/kg, respectively.

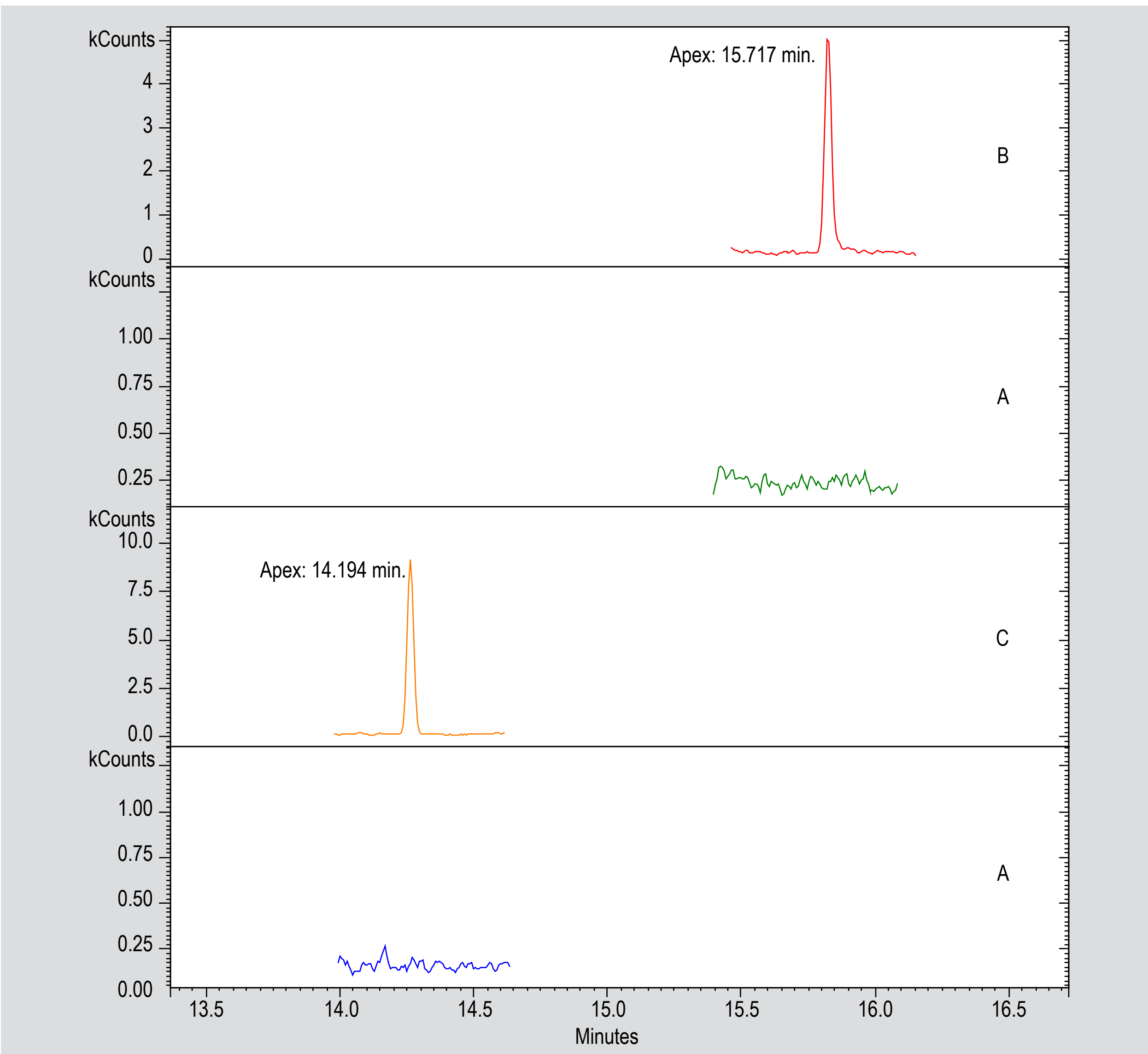

Figure 3. Chromatogram of blank (A), bifenthrin (B) and ethion (C) at a spiking level of 25 µg/kg.

Dissipation

The initial concentrations in date were 0.32and 0.549 for bifenthrin and 1.411 and 2.986 mg/kg for ethion at low and high doses, respectively. The residue of bifenthrin was found to be below the LOQ on the 15^{th} and 25^{th}days after spraying. The residue at the 45^{th} day after ethion spraying was below the LOQ and 0.005 mg/kg at low and high doses, respectively (Table 2) (Figure 4).

The residue dynamics of bifenthrin at low and high application rates can be described by the equations $C_t = 0.304e^{-0.316t}$ and $C_t = 0.488e^{-488t}$ with half-lives of 1.92 and 2.2 days, respectively. The residue dynamics of whereas ethion are described by the equations $C_t = 1.170\ e^{-143t}$ and $C_t = 2.417e^{-121t}$ with half-lives of 2.43 and 2.59 days at low and high doses, respectively. The results suggest that the dissipation rate of bifenthrin was faster than that of ethion in dates.

The reported half-lives of bifenthrin are 0.52 to 0.77 days and 1.20 to 1.32 days in tea (Tewary *et al.*, 2005), 4.85 to 5.22 and 4.38 to 6.66 days in apple fruits (Patyal *et al.*, 2010), 1.58 and 2.18 days in okra fruits (Kumari *et al.*, 2013), and 2.4 to 10.5 days in wheats (You *et al.*, 2013). Those of ethion are 1.27 days in okra fruits (Parmar *et al.*, 2012), 1.81 to 2.32 days in chilli (Sharma and Parihar, 2013), and 3.14 days in curry (Priyadarshini G, 2017). Our results revealed that the half-lives of bifenthrin were1.92 and 2.2 days, whereas those for ethion were 2.43 and 2.59 days at low and high rates of application, respectively.

The different half-lives from other studies could be attributed to the different formulations, cultivated species, application doses, weather conditions, and intervals between application and harvest time. The lower persistence found in previous works could also be due to a dilution effect brought about by the rapid growth of the species studied. This apparent elimination due to a decrease of the residue (mg/kg) when the weight of vegetable material increases (Fenoll *et al.*, 2009) was unlikely to be observed in our study. In the present investigation, the pre-harvest intervals PHI's (safe waiting periods) for bifenthrin and ethion are suggested to be in the ranges of 12-18 days and 28-38 day at low and high application rates, respectively.

Monitoring

The permanent monitoring programs undertaken in recent years are of great importance for assessing the potential risk of insecticides for consumer health. A total of 600 date samples from the Al-Qassim market were analysed using the proposed method. The results of the occurrence of bifenthrin and ethion in dates are presented in Table 3.

Table 2. Dissipation kinetics of bifenthrin and ethion in date fruits.[1]

Days after application	Low dose Residue (mg/kg)[2] ± RSD (decline %)		High dose Residue (mg/kg) ± RSD (decline %)	
	Bifenthrin	Ethion	Bifenthrin	Ethion
0 (2 h)	0.320±0.13	1.411±0.32	0.549±0.24	2.986±0.56
1	0.192±0.08 (40.00)	0.924±0.41 (34.51)	0.362±0.11 (34.04)	2.289±0.41 (23.34)
3	0.121±0.05 (62.19)	0.641±0.27 (54.57)	0.227±0.07 (58.65)	1.198±0.36 (59.87)
7	0.041±0.03 (87.19)	0.421±0.16 (70.16)	0.151±0.04 (72.50)	0.860±0.28 (71.21)
10	0.011±0.01 (96.49)	0.308±0.18 (78.17)	0.053±0.023 (90.35)	0.565±0.21 (81.08)
15	BQL	0.117±0.06 (91.71)	0.023±0.007 (95.81)	0.368±0.16 (87.66)
20		0.078±0.02 (94.47)	0.008±0.003 (98.54)	0.210±0.09 (92.96)
25		0.039±0.017 (97.24)	BQL	0.172±0.04 (94.24)
30		0.016±0.006 (98.87)		0.113±0.03 (96.22)
35		0.007±0.004 (99.50)		0.049±0.01 (98.35)
40		BQL		0.021±0.003 (99.34)
45				0.005±0.004 (99.84)
Ct	$0.304e^{-0.316t}$	$1.170e^{-0.143t}$	$0.488\ e^{-0.205t}$	$2.417\ e^{-0.121t}$
r^{2c}	0.988	0.994	0.990	0.966
$t_{1/2}$ (days)	1.92	2.43	2.2	2.59
PHI	12	28	18	38

[1] BQL = below quantification limit; PHI = pre-harvest interval value (days); RSD = relative standard deviations; r^2 = determination coefficient.

[2] Average of three replicates.

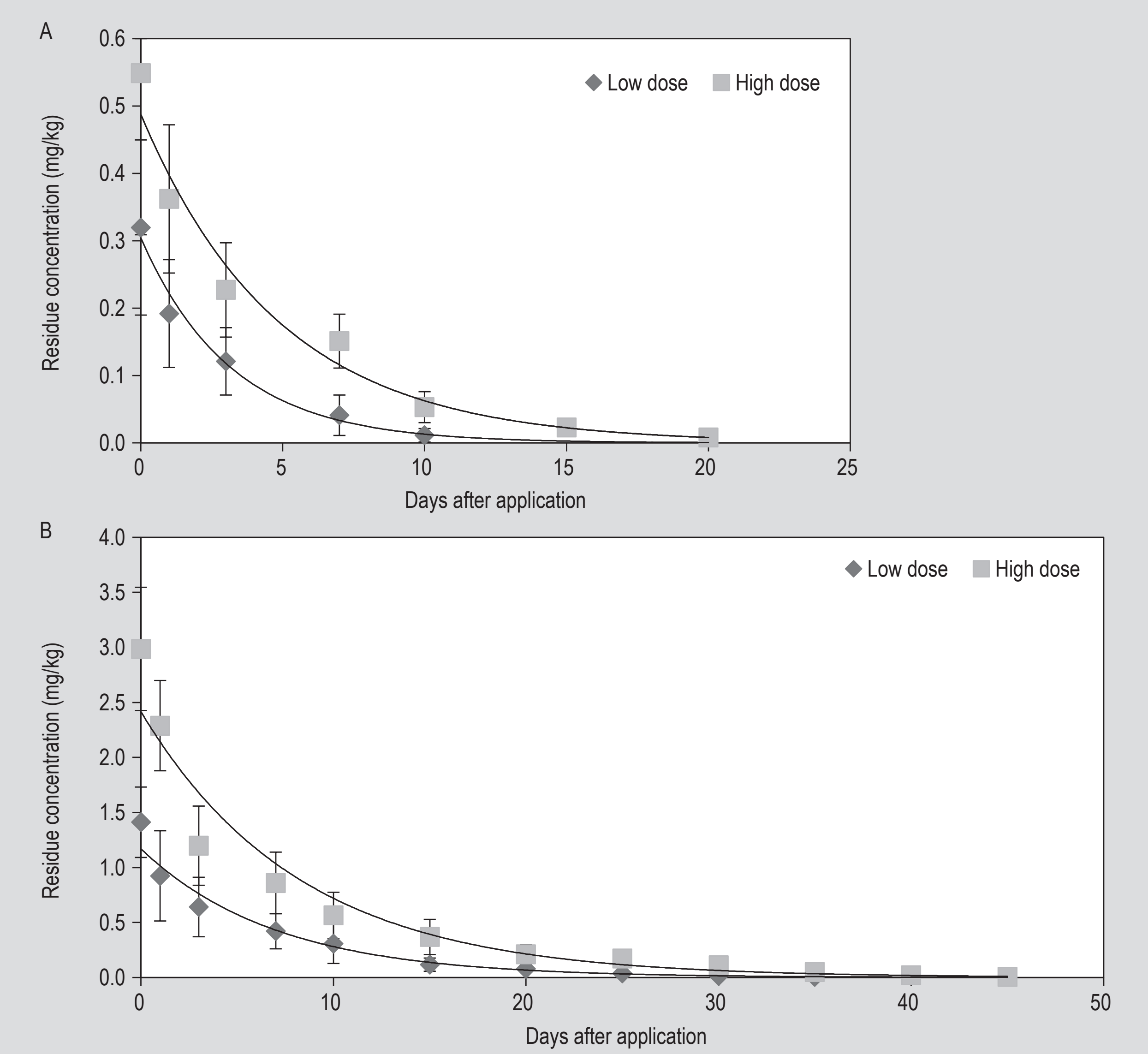

Figure 4. Dissipation dynamics of bifenthrin (A) and ethion (B) in date at the low and high rate of application.

Table 3. Residue levels (mg/kg) of bifenthrin and ethion in date fruit samples collected from Al-Qassim.[1]

Years	Number of samples	Number of contaminated samples, (%)	Detected pesticides	With residue >MRL	With residue <MRL	Min-max concentration (mg/kg)
2017	193	71, (36.79)	bifenthrin	23	2	0.005-0.239
			ethion	45	1	0.008-0.543
2018	407	112, (27.52)	bifenthrin	28	-	0.012-0.754
			ethion	82	2	0.007-0.621
2017-2018	600	183, (30.5)	bifenthrin	51	2	0.005-0.756
			ethion	127	3	0.007-0.624

[1] MRL = maximum residue limits.

In 2017, the concentration of bifenthrin residues in 25 samples (12.95%) out of 193 was from 0.005 to 0.239 mg/kg. Furthermore, 23 (11.92%) of the 193 samples were contaminated with concentrations above MRL value of 0.01 mg/kg (FAO/WHO, 2008), whereas only two samples (1%) were contaminated with concentrations lower than the MRL. There were 46 samples (23.83%) that were contaminated by ethion at levels ranging from 0.008 to 0.543 mg/kg. The concentration of ethion in 45 samples (23.32%) was higher than the MRL value of 0.01 mg/kg (EFSA, 2011), whereas one sample (0.5%) was contaminated with ethion at a concentration lower than the MRL.

In 2018, bifenthrin residues were detected in 28 (6.88%) out of 407 samples at levels ranging from 0.012 to 0.75 mg/kg. The detected bifenthrin concentration was above the MRL. There were 84 samples (20.64%) contaminated by ethion with concentration levels of 0.007 to 0.62 mg/kg. There were 82 samples (20.15%) with concentrations above the MRL, and only two samples (0.49%) had concentrations lower than the MRL.

Risk assessment

During the study period, 183 (30.5%) out of 600 samples were contaminated. Bifenthrin was found in 53 (8.83%) out of 600 samples with concentrations ranging from 0.005 to 0.75 mg/kg. Ethion residues were detected in 130 (21.67%) samples with concentrations ranging from 0.007 to 0.62 mg/kg (Table 3). The estimated chronic risk from date intake is indicated in Table 4. The estimated chronic exposure dose of the mean residues of bifenthrin and ethion was respectively 8.36×10^{-6} mg/kg bw/day and 3.34×10^{-5} mg/kg bw/day, and 1.81×10^{-5} mg/kg bw/day and 7.25×10^{-5} mg/kg bw/day corresponding to 0.06% and 0.22%, and 0.91% and 3.63% of ADI of 0.015 mg/kg bw/day and 0.002 mg/kg bw/day for children and adult. The calculated chronic exposure dose of the median (50th) residues of both pesticides for children and adult were nearly similar to that calculated from the mean intake with 0.03%, 0.21%, and 0.13% and 0.84%, of ADI, respectively.

At the 95th percentile, the estimated chronic exposure dose of bifenthrin and ethion was 2.16×10^{-5} (0.14% of ADI) mg/kg bw/day and 8.64×10^{-5} (0.58% of ADI) mg/kg bw/day, and 8.29×10^{-5} (4.45% of ADI) mg/kg bw/day and 3.32×10^{-4} (16.6% of ADI) mg/kg bw/day, for children and adult, respectively.

The health risk quotient (RQ) was investigated based on the mean, median and 95th percentile results. The RQs and the calculated estimated chronic exposure of bifenthrin and ethion (Table 4), were far below the acceptable intake exposure using the ADI and lower than unity. Thus, the residues of bifenthrin and ethion do not pose a health risk. However, Saudi Arabia has the highest global consumption of date fruit per capita of at least 100 g per day (equivalent to 10 kernel dates) (Abdallah *et al.*, 2018), so the risk of exposure to pesticides may be higher.

Effect of home processing on the reduction of bifenthrin and ethion residues

The un-processed samples of dates contained an average bifenthrin concentration of0.232 mg/kg and ethion concentration of 0.391 mg/kg. Washing dates in running tap water for 1 and 5 min removed 5.91-32.82 and 8.42-26.58% of the bifenthrin and ethion, respectively. The residues of bifenthrin in dates were reduced to below the LOQ (100%) when the washed samples were subjected to boiling for date syrup production that utilise in beverages, confectionery and ice cream as a sweetening and flavouring agent (Ganbi, 2012; Gohari-Ardebili, 2004), also a good substitute for sugar in making pan bread, cake, biscuit, toffee and candy

Table 4. Estimaton of exposure dose (chronic risk assessment) and risk quotient for children and adult population of Saudi Arabia due to date intake during years of 2017-2018.[1]

Pesticides	ADI (mg/kg bw)	Concentration levels mg/kg[2]		Estimated exposure dose (mg/kg bw/day)		Risk quotient	
				Children	Adult	Children	Adult
Bifenthrin	0.015	mean	0.012	8.36×10^{-6}	3.34×10^{-5}	5.57×10^{-4}	2.23×10^{-3}
		median (50th)	0.007	4.87×10^{-6}	1.95×10^{-5}	3.25×10^{-4}	1.3×10^{-3}
		95th	0.031	2.16×10^{-5}	8.64×10^{-5}	1.44×10^{-3}	5.76×10^{-3}
Ethion	0.002	mean	0.026	1.81×10^{-5}	7.25×10^{-5}	9.06×10^{-3}	3.62×10^{-2}
		median (50th)	0.006	4.16×10^{-6}	1.67×10^{-5}	2.09×10^{-3}	8.36×10^{-3}
		95th	0.119	8.29×10^{-5}	3.32×10^{-4}	4.45×10^{-2}	0.165

[1] ADI = average daily intake; LOQ = limit of quantification.
[2] A default value of LOQ value were used for samples with values below the LOQ.

(Ashraf and Hamidi-Esfahani, 2011; Sidhu *et al.*, 2003). The ethion residues were reduced by 96.47 and 97.32%washing for 1 and 5 min. and boiling, respectively. This indicates the great role of heat in the reduction of bifenthrin and ethion residues (Table 5). The water solubility of bifenthrin and ethion is <0.001 mg/l and 2 mg/l (at 20 °C), and the values of log K_{ow} are 6.6 and 5.02, respectively. K_{ow} is the ratio of the concentration of a chemical in n-octanol and water at equilibrium at a specified temperature (Tomlin, 2009). The results indicate that bifenthrin and ethion are more likely to be associated with the solid organic date matrix.

The skin of dates may adhere to the mesocarp and be wrinkled, or it may be separated by air filling the empty space between it and the shrunken pulpy mesocarp layers. Like most biological structures, the skin has visco-elastic properties, and when the surface area increases, the skin may extend irreversibly. Therefore, pesticide residues can be translocated internally, and washing would not reduce the pesticide residues very easily. Our findings are consistent with previous studies (Yang *et al.*, 2012) which reported that the water solubility of pesticide and K_{ow} are not important factors for removing pesticide residues.

Processing factors

Table 5 presents the effect of PFs on bifenthrin and ethion in washed samples and washed and boiled processed samples. The highest value was found when washing for 1 min, and the lowest value was found for the washing plus boiling process. When the residue level in the processed samples was below the LOQ, it was considered as not detected, and the numerical value used to calculate the PF was zero. The results showed that the PFs were less than 1, indicating that the whole process could reduce the residue of bifenthrin and ethion in dates.

The rinsability of a pesticide depends on many factors, such as the physicochemical properties of the pesticide, the crop skin, the water solubility of the pesticide, the location of the residues, the age of the residue, the temperature, and the type of washing solutions. Washing for 5 min was more efficient than washing for 1 min, and washing plus boiling was more effective than washing alone. The results indicate the important role of washing and washing plus boiling in the removal of pesticide residues from contaminated dates.

4. Conclusions

An effective method was developed to analyse bifenthrin and ethion in dates by GC-IT/MS. The satisfactory accuracy and precision revealed the usefulness of the approach and highlighted the practical benefits in the monitoring of bifenthrin and ethion residues. The dissipation of bifenthrin and ethion from dates followed first-order rate kinetics. The half-life of bifenthrin and ethion was in the range of 1.92 to 2.59 days, which indicated slower dissipation on dates compared to other crops. This may be attributed to the slow-growing nature of dates. Since dates are consumed at various growth stages, it is essential to determine PHIs to allow the insecticide levels to reduce to their respective MRLs. The results suggest that low-dose treatments of bifenthrin (8% EC) and ethion (40% EC) require PHIs of 12 and 18 days, whereas high-dose treatments required 28 and 38 days, respectively.

This study also investigated the levels of bifenthrin and ethion in dates marketed in the Qassim region in 2017-2018. The exposure assessment even when using the more conservative assumptions (95th percentile values) for each pesticide showed that the contribution of date fruits to the dietary intake represents only a small portion of the respective ADIs. The dietary intake of bifenthrin and ethion residues detected through date fruits consumption for the Saudi consumers does not pose a relevant risk. The results indicated that from a public health perspective, bifenthrin and ethion levels may pose potential health risks to children due to the high consumption of dates. Washing the dates with tap water for 1 and 5 min did not reduce the residues of bifenthrin and ethion to safe levels.

Table 5. Effect of processing on bifenthrin and ethion residues in date fruits.[1]

	Bifenthrin			Ethion		
	Concentration, (mg/kg) ± SD (n=6)	% reduction	Processing factor	Concentration, (mg/kg) ± SD (n=6)	% reduction	Processing factor
Un processed	0.232±0.011	-	-	0.391±0.021	-	-
Washing 1 min	0.218±0.008	5.91	0.94	0.358±0.015	8.42	0.92
Washing 5 min	0.156±0.007	32.82	0.67	0.287±0.006	26.58	0.73
Washing 1 min plus boiling	BQL	100	0.00	0.014±0.007	96.47	0.04
Washing 5 min plus boiling	BQL	100	0.00	0.010±0.003	97.32	0.03

[1] BQL = below quantification limit.

Washing plus boiling reduced the residues of bifenthrin to below the LOQ, and ethion was reduced to below the MRL of 0.01 μg/kg. This study could provide data for the Saudi government to establish the MRLs of bifenthrin and ethion in dates to provide guidance on the proper and safe use of these insecticides. We recommend that regular monitoring studies of pesticide residues be continued to include other vegetables fruits grown in various areas of Saudi Arabia to determine the actual state of pollution and to enhance consumer confidence in food quality as well as food quality control.

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Conflict of interest

The authors declare that they have no conflict of interest.

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