

Original article (Orijinal araştırma)

Reduction of some insecticide residues from grapes with washing treatments

Üzümdeki bazı insektisit kalıntılarının yıkama işlemleriyle azaltılması

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Abstract

Insecticide application is the most common method of insect control in agriculture. Efficiency of washing treatments in reduction of insecticide (chlorpyrifos-methyl and lambda-cyhalothrin) residues from grapes were investigated in this study. The trial was established in a Sultana seedless vineyard in Sarıgöl District, Manisa Province, Turkey in 2020. Method verification was performed with the recovery, limit of quantification and precision. Pesticide-free grapes were spiked with 0.5, 1 and 5 times of MRL for pesticides. The recovery of chlorpyrifos-methyl and lambda-cyhalothrin were 102 and 101% respectively. QuEChERS method yielded an overall-recovery of 101%. These figures were within the SANTE recovery limits (60-140%) and the detection limits of the insecticides were below the MRLs. Grapes in a vineyard were sprayed with insecticides four times and harvested 0, 2, 4 and 7 d after the last spray. Washing (tap water, citric and acetic acid) and ultrasonic cleaning treatments were applied to harvested grapes. Washing treatments decreased residue levels and reductions increased with prolonged washing durations. Reductions also decreased with prolonged harvest durations from the last spray. The citric and acetic acid washing, and ultrasonic-cleaning methods provided more efficient reduction than washing with tap water.

Keywords: Chlorpyrifos-methyl, grape washing process, lambda-cyhalothrin, Manisa, pesticide residue reduction

Öz

Insektisit kullanımı tarımda zararlı kontrolü için en yaygın metottur. Bu çalışmada yıkama işlemlerinin üzüm üzerindeki insektisit kalıntılarının (chlorpyrifos-methyl ve lambda-cyhalothrin) azaltılmasına etkisi araştırılmıştır. Deneme Türkiye'de Manisa İli-Sarıgöl ilçesinde 2020 yılında Sultana çekirdeksiz üzüm bağında kurulmuştur. Metod doğrulama, geri kazanım, ölçüm limiti, tekrarlanabilirlik ve kesinlik ile gerçekleştirilmiştir. Insektisit içermeyen üzüm numuneleri her pestisit için 0.5, 1 ve 5 kat MRL seviyelerinde sabitlenmiştir. Chlorpyrifos-methyl ve lambda-cyhalothrin geri alımları, sırasıyla %102 ve %101 olarak bulunmuştur. Tüm QuEChERS yönteminin geri kazanımı %101 olarak bulunmuştur. Bu rakamlar SANTE geri kazanım limitleri (%60-140) arasındadır. Insektisitlerin tespit limitleri, MRL'nin altında bulunmuştur. Bağda üzümlere dört defa insektisit uygulanmıştır. Son insektisit uygulamasının 0., 2., 4. ve 7. günlerinde üzümler hasat edilmiş ve çeşme suyu, sitrik ve asetik asit ve ultrasonik yıkama işlemlerine tabi tutulmuştur. Yıkama işlemi kalıntıları azaltmış ve artan yıkama süresiyle kalıntının azalma oranları artmıştır. İlerleyen hasat zamanları ile kalıntının giderilmesi azalmıştır. Sitrik ve asetik asit yıkama ve ultrasonik yıkama, musluk suyu ile yıkamadan daha etkili bulunmuştur.

Anahtar sözcükler: Chlorpyrifos-methyl, üzüm yıkama işlemi, lambda-cyhalothrin, Manisa, pestisit kalıntı azaltılması

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Introduction

Grapes and their processed products are extensively consumed all around the world. The world grape production is about 79 Mt in 2018. With an annual production of 4 Mt, Turkey has the sixth largest producer. In Turkey, 38% (1.5 Mt) of this production is in Manisa Province (TÜİK, 2019). About 83% of grapes exported from Turkey are sent to EU countries. Grapes are either consumed fresh or processed into different products such as wine, vinegar, raisin and molasses. Turkey's exports of raisins were about 243 kt in 2019, about 30% of globally traded raisins (TÜİK, 2019). Grapes are rich in carbohydrates, thus highly prone to pest damage. Pests including *Lobesia botrana* (Denis & Schiffermüller, 1775) (Lepidoptera: Tortricidae), *Daktulosphaira vitifoliae* (Fitch, 1855) (Phylloxeridae: Hemiptera), *Otiorhynchus* spp. Germar, 1822 (Coleoptera: Curculionidae), *Tetranychus urticae* Koch, 1836 (Acari: Tetranychidae) create significant problems in viticulture and result in serious economic losses each year. *Lobesia botrana* causes 45-92% product loss every year (Önçağ, 1975). Insecticides are commonly used in vineyards against these pests. Chlorpyrifos-methyl is used against *L. botrana*, lambda-cyhalothrin is applied against *Otiorhynchus* spp. and *L. botrana*.

In 2017, about 54 t of pesticides were used in Turkey with nearly 5 t used in Manisa Province. Manisa Province ranks the second highest user of insecticides at 1 t/year after Antalya province (1.2 t/year) in Turkey (TÜİK, 2018). Despite the advantages of pesticide use in agricultural fields and vineyards in terms of pests and disease control, residues pose serious risks for human health. Insecticides used in vineyards may leave a problematic level of residue on grape berries. Since chlorpyrifos-methyl is neurotoxic and cholinesterase inhibitor and lambda-cyhalothrin is endocrine disruptor, eye irritant and skin irritant (PPDB, 2020), such residues may constitute a major concern for consumers. Residues also generate significant problems for international trade, they are even a criterion for import bans.

There are many studies on pesticide residues in grapes. These are generally monitoring studies conducted by sampling from markets and vineyards. There is limited information on the residues left after pesticide treatment. Some researchers have investigated pesticide residues in grapes.

Zengin & Karaca (2017) determined pesticide residues on grape berries directly sampled from a vineyard. These researchers applied GAP (good agricultural practice) in a vineyard located in Uşak Province, Turkey. About 45% of the samples had no residues, 55% had pesticide residues but none exceeded the maximum residue levels (MRL), with 13 pesticides detected. These researchers reported that lambda-cyhalothrin was the most commonly detected pesticide. They also emphasized the importance of GAP, especially in terms of pesticide application. Turgut et al. (2011) conducted a study in vineyards of Denizli, İzmir and Manisa Provinces and reported the most common pesticide residues were lambda-cyhalothrin (22 samples) and chlorpyrifos-methyl (15 samples). Gölge & Kabak (2018) investigated residues of pesticides in table grapes collected from local markets in four provinces of Turkey and reported residue of one or more pesticide in 60% of the samples. The residues were greater than MRL in 20% of the samples. Chlorpyrifos, azoxystrobin, boscalid and cyprodinil were identified as the most common residues encountered on grape samples. In another study conducted on fresh Sultana grapes, İçli & Tahmas Kahyaoğlu (2020) encountered lambda-cyhalothrin and iprodione residues in majority of the samples.

Researchers have mostly focused on efficient means of pesticide residue reduction from fruits and vegetables. Majority of them employed washing treatments with tap water and reported some positive outcomes with washing (Zhou et al., 2019; Corrias et al., 2020). Heshmati et al. (2020) worked on residues of some pesticides in unwashed and washed grapes and reported decreased residues with washing treatments. Tap water, acetic acid and sodium bicarbonate (NaHCO₃) were used in washing treatments and NaHCO₃ was reported as the most efficient means of pesticide residue reduction. Researchers reported that NaHCO₃ treatments yielded about 95% reduction for diazinon, 95% reduction for penconazole, 94% for hexaconazole, 72% for ethion and 63% for phosalone. However, Zhou et al. (2019) reported that

ultrasonic cleaning was the most efficient means of residue reduction in grape samples with reduction of 72-100%. These different degrees of reduction can be attributed to surface structure of the matrix.

Efficiency of washing treatments with different solutions in reduction of pesticide residues depends on the mode of action mode, solubility and physicochemical characteristics of the relevant pesticides (Hassan et al., 2019). Mode of action designates the behavior of the pesticides in the treated plant. Thus, mode of action (systemic or contact) is considered as an important factor for reduction of pesticide residues with washing treatments. It is harder to reduce systemic residues within fruits and vegetables than contact pesticides with remain on the fruit surface, whereas systemic pesticides absorbed by the leaves, fruits, shoots or stems, and penetrate deep into the plants and are transported to different locations within the plants (Lozowicka et al., 2013; Acoglu et al., 2018). In previous studies, it was found that it was easier to remove highly-soluble pesticides with small partition coefficients by washing (Kong et al., 2012; Lozowicka et al., 2016; Heshmati et al., 2020).

The PHI (preharvest interval), the time between the last spray and the harvest, is another factor to be taken into consideration in reduction of pesticide residues by washing. Özel & Tiryaki (2019) reported decreasing reduction with decreasing PHI. Solutions used for washing also significantly influence efficiency of the process. While some researchers used tap water, the others used acetic acid and ultrasonic cleaning processes for reduction of residues from fruits and vegetables (Khadre et al., 2001; Lozowicka et al., 2013; Polat & Tiryaki, 2020; Çatak et al., 2020). Previous research also focused on reduction of residue levels with different food processing techniques (Randhawa et al., 2104a; Lozowicka et al., 2016; Zhou et al., 2019; Heshmati et al., 2020).

Osman et al. (2014) reported that washing with acetic acid and citric acid (1-2%) was more efficient for reduction of insecticide (chlorpyrifos) residues than the other procedures, such as tap water washing and KMnO_4 and H_2O_2 . Randhawa et al. (2014a) studied the effects of various acid concentrations on residue reduction in capsicum and cucumber samples, and reported that greater acid concentration (9%) had higher reduction than the other concentrations (1.5, 3 and 6%). In another study, ultrasonic cleaning was found to be more efficient in reduction of pesticide residues from strawberries than the other methods (Lozowicka et al., 2016).

Efficiency of washing (tap water, citric acid and acetic acid) and ultrasonic cleaning in reduction of insecticide (chlorpyrifos-methyl and lambda-cyhalothrin) residues from Sultana grapes were investigated in this study with different PHI (days 0, 2, 4 and 7) and different washing durations (2 and 5 min). The QuEChERS-AOAC 2007.01 method coupled with UPLC-MS/MS detection was used for the pesticide residue analyses. Method reliability was verified based on international guidelines (EURACHEM, 2014; SANTE, 2019; TURKAK, 2019).

Materials and Methods

Field experiments and sampling

Field experiments were conducted in a vineyard located in Sarıgöl District, Manisa Province in 2020. Relevant cultural practices were conducted as needed throughout the growing season. Trials, sprays and sampling were conducted according to published standard trial methods for residue experiments with plant protection products (Anonymous, 2011). Lambda-cyhalothrin (Karate Zeon CS, 50 g/L, Syngenta) and chlorpyrifos-methyl (Reldan 22 EC, 227 g/L, Dow AgroSciences) insecticides were sprayed with at 25 and 200 mL/100 L water. Four sprays (15-d intervals) were applied throughout the growing season. Sultana seedless grapes were harvested 4 h (day 0) and 2, 4 and 7 days after the last spray. At each harvest, 5 kg grapes were collected (60 kg in total). Harvested samples were brought to laboratory in an icebox. Disposable polyethylene gloves were used to prevent contamination during the collection of samples.

Instruments

For washing treatments, about 1 kg of harvested grapes (EC, 2002) were submerged in tap water (5L, 20°C), acetic acid and citric acid solutions (5L, 9%, 20°C) for 2 and 5 min (Randhawa et al., 2014a). Samples were then dried for 30 min at room temperature before analysis. Analyses were conducted in four replicates. An ultrasonic cleaner (Medisson 12UT, Turkey) was used in ultrasonic cleaning treatments applied by immersing air-dried samples in 5 L tap water for 2 and 5 min (Figure 1). Unwashed samples were also analyzed for insecticide residues and resultant values were used to assess the efficacy of washing treatments.

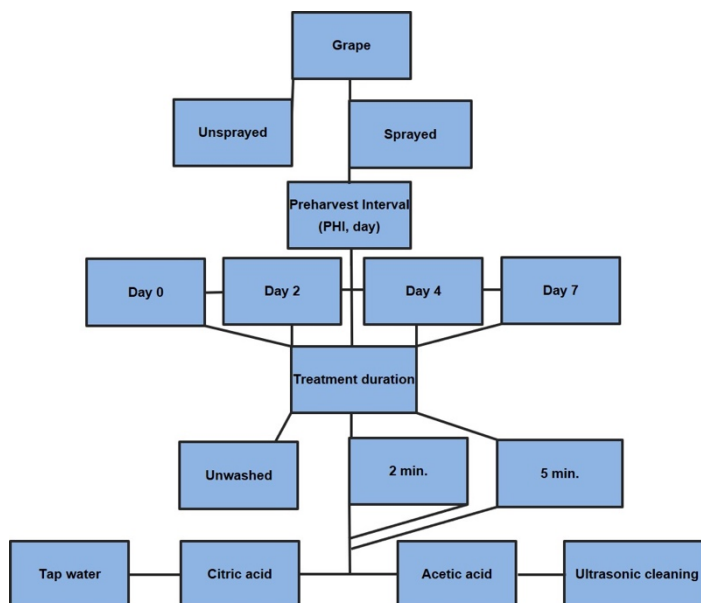


Figure 1. Steps of washing processes and sampling details of various washing treatments.

The processing factor (PF) for the washing treatments (mg/kg) (OECD, 2008) was calculated as the ratio of residue concentration in processed over unprocessed product. PF values of <1 indicate decreased concentrations with washing treatment (Dong et al., 2012). For assessing of pesticide residues in processed product, PFs is published by Turkish Ministry of Agriculture and Forest General Directorate of Food and Control (Anonymous, 2020).

Chemicals, reagents and solutions

Insecticide (chlorpyrifos-methyl and lambda-cyhalothrin) standards were purchased from a Dr. Ehrenstorfer Lab., GmbH (Wesel, Germany) at purity of 99.69 and 98.73%, respectively; and anhydrous MgSO₄, sodium acetate (NaAC), methanol (MeOH) and acetonitrile (MeCN) from Merck Co. (Darmstadt, Germany) at purities of 99.5, 99.0, 99.9 and 99.9%, respectively. PSA (primary-secondary amine 40 µm, 100 g) was sourced from Agilent (Santa Clara, CA, USA). Toxicological and physicochemical properties of insecticides are given in Table 1.

Stock solutions (400 µg/mL) were prepared adding 10 mg pesticide into 25 mL flasks and completing the final volume to 25 mL with acetonitrile. Working solutions (1.0 µg/mL) were prepared through dilution of the stock solutions. Calibration solutions were prepared over ranges of 20-4000 pg/µL and 10-2000 pg/µL. Spiking solutions equal to 0.5, 1 and 5 times of MRL were prepared. The standards and solutions were stored at 4°C in the dark. Representative apple matrix was used for matrix-matched calibrations and quantification, as indicated in Codex Alimentarius Commission Guidelines (CAC, 2003) and SANTE Guidelines (SANTE, 2019). Spiking with five times MRL was diluted to fit the calibration range.

Table 1. Physicochemical and toxicological properties and mode of action of insecticides (WHO, 2009; EU, 2020; IRAC, 2020; PPDB, 2020)

Parameter		Chlorpyrifos-methyl	Lambda-cyhalothrin
Chemical formula		C ₇ H ₇ Cl ₃ NO ₃ PS	C ₂₃ H ₁₉ ClF ₃ NO ₃
Group		Organophosphates (1B)	Pyrethroids (3A)
Mode of action		Non-systemic acetylcholinesterase (AChE) inhibitors nerve action	Non-systemic sodium channel modulators nerve action
Physicochemical property	Log P (Octanol-water partition coefficient)	4.7	5.5
	Solubility in water at 20°C (mg/L)	2.74	0.005
	Degradation point (°C)	175	275
	Molecular mass (g/mol)	322.53	449.85
Toxicological property	EU MRL (µg/kg)	1000	80
	ADI (mg/kg/bw/day)	0.010	0.0025
	ARfD (mg/kg bw /day)	0.10	0.005
	Mammals -Acute LD ₅₀ (mg/kg)	5000	56
	Mammals-Inhalation LC ₅₀ (mg/L)	>0.67	0.06
	WHO classification *	III	II
	Health issues	Reproductive / Growth effects Neurotoxic, Cholinesterase inhibitor	Endocrine disruptor Eye irritant, Skin irritant

* II, moderately hazardous; III, less hazardous.

Sample preparation and analyses

The QuEChERS method was published in 2003 and the method has been widely used in residue analyses since. The QuEChERS-AOAC Official Method 2007.01 version has also been employed in residue analyses in agricultural commodities (Lehotay, 2007; Omeroglu et al., 2012; Polat & Tiryaki, 2019; Heshmati et al., 2020).

QuEChERS method should be verified under local conditions. Recovery assessment is the first step of method validation (SANTE, 2019). Therefore, fortification trials were performed. For this aim, 1 kg of blank (pesticide-free sample, no pesticide applied sample) grape sample was homogenized with a blender (Waring Commercial Blender). Then, 15 g homogenized sample (analytical portion) were placed into tubes supplemented then with 100 µL lambda-cyhalothrin and chlorpyrifos-methyl corresponding to 0.5, 1 and 5 x MRL spiking levels in four replicates (analytical portion) (Table 2). Resultant mixture was vortexed for 30 s and left for 15 min. After the salting step (MgSO₄ and NaAC), the extract was centrifuged (Hettich EBA 280) for 5 min at 5000 rpm. For further analyses, the steps in Figure 2 were followed. Analyses (extraction and cleanup) of all spiked and processed grapes were performed with the QuEChERS AOAC Method 2007.01 and LC-MS/MS (Lehotay et al., 2005). Both spiked and processed samples were analyzed in four replicates (analytical portion). About 1 mL of extract was sampled once for processed samples and three times for spiked samples into GC vials. The recovery was calculated as the percentage of measured over spiked concentration (Çatak & Tiryaki, 2020).

Table 2. Spiking pattern of grapes with chlorpyrifos-methyl and lambda-cyhalothrin

Spike	Code	Fortification (µg/kg)	
		Chlorpyrifos-methyl	Lambda-cyhalothrin
0.5 x MRL*	F1/1-4	500	40
1.0 x MRL	F2/1-4	1000	80
5.0 x MRL	F3/1-4	5000	400
Control	F0/1-3	none	none

* EU maximum residue limit, µg/kg.

Method precision and recovery rates were tested in accordance with SANTE European Guidelines (SANTE, 2019). Method linearity was checked for the ranges of 20-4000 pg/ μ L for chlorpyrifos-methyl and 10-2000 pg/ μ L for lambda-cyhalothrin.

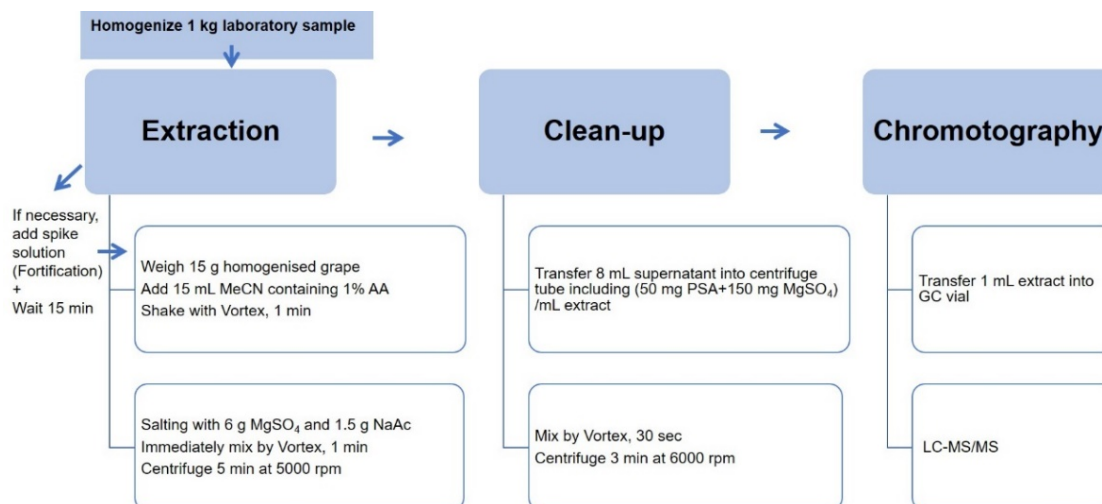


Figure 2. Analysis of chlorpyrifos-methyl and lambda-cyhalothrin in sultana grapes with the QuEChERS method.

UPLC-MS/MS analysis

Chromatographic analyses were conducted with an LC-MS/MS (Waters I Class Plus UPLC + Xevo TQ-S micro MS Detector; ESI + mode) equipped with Acquity UPLC BEH C₁₈ column (1.7 μ m, 2.1 x 100 mm). Total run time was 15 min. Injection volume was 1 μ L (in MeCN) and flow rate was 0.35 mL/min. A gradient program including 10 mM NH₄CH₄CO₂, 95% MeOH (B) and 10 mM NH₄CH₄CO₂ pH 5, water (A) were used (Table 3). Selected ion groups, retention time and other parameters such as calibration ranges and limit of quantification of insecticides in LC-MS/MS analysis are shown in Table 4.

Table 3. Gradient solvent flow program (flow rate 0.35 mL/min)

	Time (min)	A%	B%
1		98.0	2.0
2	0.25	98.0	2.0
3	11.75	1.0	99.0
4	13.25	1.0	99.0
5	13.35	98.0	2.0
6	15.00	98.0	3.0

Table 4. Selected ion groups, retention time and other parameters insecticides in LC-MS/MS analysis

Parameter	Chlorpyrifos-methyl	Lambda-cyhalothrin
Quantification ion	321.80 > 124.93	467.22 > 225.04
Confirmation ion	323.99 > 124.93	467.22 > 141.06
Retention time (tr. min)	10.13	11.25
Calibration range, ppb, pg/ μ L	20-4000	10-2000
LOQ, ppb (μ g/kg)	20	10

Method verification

Analytical method was verified in accordance with the SANTE guidelines. Recovery (including relative standard deviation, RSD), linearity, precision, limit of quantification and accuracy assessments were performed for method verification (SANTE 2019).

Data analysis

SAS statistical software was used for statistical evaluation (SAS, 1999). Data were subjected to analysis of variance. Tukey's test was used to compare significant mean values.

Results and Discussion

Method performance verification

The calibration curves for standard solutions of chlorpyrifos-methyl and lambda-cyhalothrin were $y = 314.041x + 398.601$ and $y = 639.498x + 2277.72$, respectively. The curves were linear with the 20-4000 pg/ μ L range for chlorpyrifos-methyl and 10-2000 pg/ μ L range for lambda-cyhalothrin with $r^2 \geq 0.999$. Analytical functions were used as regression equations in matrix-matched calibrations and they were used for analyte (pesticide) quantification. The retention times for lambda-cyhalothrin and chlorpyrifos-methyl in matrix-matched solutions are presented in Table 4. Limit of quantification of chlorpyrifos-methyl and lambda-cyhalothrin for running method were 20 and 10 μ g/kg, respectively (Table 4). These values were substantially lower than the MRLs specified by EC. MRLs of chlorpyrifos-methyl and lambda-cyhalothrin for grape were 1000 and 80 μ g/kg, respectively (Table 2).

The recovery ratios varied between 84-127% ($n = 72$) (Table 5). Recovered chlorpyrifos-methyl from the grape matrix was 102% with the 36 samples ranging from 89-116%. For method precision, RSD was identified as 7.9% ($n = 36$). Recovery of lambda-cyhalothrin and RSD value were 100% and 10.5, respectively. Overall recovery was 101% with a standard deviation of 9.4% and RSD of 9.3% (Table 5). The recovery ratios and RSDs were within the SANTE recovery limits ($60\% \leq Q \leq 140\%$; $RSD \leq 20\%$) (SANTE, 2019; UGRL, 2020). Based on the accuracy values (Table 5), the QuEChERS-LC-MS/MS method yielded sufficient recovery ratios for lambda-cyhalothrin and chlorpyrifos-methyl insecticides in Sultana grapes.

Table 5. Method verification results (3 spiking level x 4 analytical portion x 3 times 1 mL for LC-MS/MS x 2 pesticide, $n = 72$)

Analyte	Concentration, μ g/kg		Recovery % (As a tool for trueness)	RSD % (As a tool for precision)
	Spiked	Measured		
Chlorpyrifos-methyl	500	465	93	4.6
	1000	1087	109	4.4
	5000	5208	104	4.7
	Mean recovery, $n=36$, Recovery range= 89-116%		102	7.9
Lambda-cyhalothrin	40	38	95	11.3
	80	78	98	4.1
	400	431	108	10.4
	Mean recovery, $n=36$, Recovery range =84-127%		100	10.5
Overall recovery of the QuEChERS (method accuracy):101% ($n=72$, $SD= 9.4$, $RSD=9.31$) Recovery range: 84-127%				

Analysis of unwashed grapes

Unwashed were also analyzed to compare the performance of washing methods through PF values. In day 0 samples, chlorpyrifos-methyl residues (1156 μ g/kg) slightly exceeded MRL at 1000 μ g/kg. However, lambda-cyhalothrin residues (325 μ g/kg) were about four times the MRL (80 μ g/kg). The residues of chlorpyrifos-methyl in day 2, 4 and 7 samples were 742, 124 and 42 μ g/kg, respectively. For lambda-cyhalothrin, these values were 276, 75 and 62 μ g/kg, respectively (Table 6). Chlorpyrifos-methyl and lambda-cyhalothrin residues decreased with increasing time after the last spray. This again highlights the importance of PHI.

Effects of washing treatments

The effects of different washing treatments on residue reduction are shown in Table 6. Increasing residue reduction were seen for both insecticides with longer washing. Processing factors of washing treatments are shown in Table 6. Since PF were all less than 1, the treatments were efficient in reduction of insecticide residues from Sultana grapes. Results of statistical analyses for insecticide residue levels are shown in Table 7.

Table 6. Pesticide residues of unwashed samples, reduction rates and their PFs achieved with the present applications (n = 4)

Application	PHI (d)	Time (min)	Chlorpyrifos-methyl			Lambda-cyhalothrin		
			Mean residue ($\mu\text{g}/\text{kg}$) ($\pm\text{SD}$)	PF	Reduction (%)	Mean residue ($\mu\text{g}/\text{kg}$) ($\pm\text{SD}$)	PF	Reduction (%)
Unwashed	0	-	1156 \pm 38.8			325 \pm 33.7		
	2	-	742 \pm 56.5			276 \pm 11.6		
	4	-	124 \pm 37.0			75 \pm 4.5		
	7	-	42 \pm 8.2			62 \pm 3.6		
Tap water	0	2	549 \pm 26.6	0.47	52.6	176 \pm 11.9	0.54	45.9
		5	430 \pm 35.0	0.37	62.8	142 \pm 16.6	0.44	56.1
	2	2	380 \pm 27.8	0.51	48.8	170 \pm 6.7	0.62	38.3
		5	307 \pm 20.4	0.41	58.7	164 \pm 10.5	0.59	40.5
	4	2	94 \pm 15.8	0.76	24.1	55 \pm 7.3	0.73	26.7
		5	88 \pm 8.0	0.72	28.4	50 \pm 16.3	0.67	33.0
	7	2	35 \pm 7.9	0.86	13.9	52 \pm 7	0.85	15.3
		5	33 \pm 3.5	0.79	20.7	44 \pm 2.8	0.71	29.2
Citric acid	0	2	485 \pm 41.2	0.42	58.1	124 \pm 9.5	0.38	61.7
		5	397 \pm 25.2	0.34	65.7	109 \pm 3.4	0.34	66.4
	2	2	339 \pm 21.5	0.46	54.4	132 \pm 3.1	0.48	52.3
		5	270 \pm 15.9	0.36	63.6	127 \pm 6	0.46	54.1
	4	2	83 \pm 10.3	0.68	32.5	50 \pm 4.2	0.67	33.5
		5	66 \pm 7.0	0.53	46.8	38 \pm 1.4	0.51	48.8
	7	2	34 \pm 4.6	0.81	19.5	41 \pm 8.5	0.67	33.2
		5	29 \pm 4.0	0.69	31.2	40 \pm 3.6	0.64	35.8
Acetic acid	0	2	516 \pm 13.8	0.45	55.3	143 \pm 7.4	0.44	56.1
		5	386 \pm 17.9	0.33	66.7	119 \pm 6.8	0.37	63.4
	2	2	351 \pm 23.3	0.47	52.8	154 \pm 15.7	0.56	44.3
		5	298 \pm 14.1	0.40	59.8	141 \pm 2	0.51	49.0
	4	2	82 \pm 12.2	0.67	33.5	49 \pm 5.5	0.65	34.8
		5	75 \pm 8.7	0.61	39.1	42 \pm 2.9	0.56	43.9
	7	2	30 \pm 2.2	0.72	28.0	40 \pm 4.5	0.66	34.4
		5	27 \pm 3.6	0.66	34.5	31 \pm 2.3	0.51	49.0
Ultrasonic cleaning	0	2	451 \pm 39.1	0.39	61.0	140 \pm 13.2	0.43	56.9
		5	334 \pm 55.5	0.29	71.1	104 \pm 15.0	0.32	68.0
	2	2	311 \pm 10.2	0.42	58.1	151 \pm 5.0	0.55	45.4
		5	274 \pm 16.6	0.37	63.1	132 \pm 4.3	0.48	52.2
	4	2	82 \pm 7.4	0.66	33.9	52 \pm 6.9	0.70	30.3
		5	69 \pm 8.2	0.55	44.5	48 \pm 4.4	0.64	35.5
	7	2	36 \pm 2.8	0.86	13.9	44 \pm 5.7	0.72	28.4
		5	30 \pm 3.7	0.71	28.8	41 \pm 3.8	0.67	32.8

Table 7. Significant differences in insecticide residue level of the washing applications

Active ingredient	Application*	Residues at harvest ($\mu\text{g}/\text{kg}$)											
		Day 0		Day 2		Day 4		Day 7					
Chlorpyrifos-methyl	Raw	1156	A**	a***	742	A	b	124	A	c	42	A	d
	Tap water (2)	549	B	a	380	B	b	94	B	c	36	AB	d
	Tap water (5)	430	EF	a	307	DEF	b	88	BC	c	33	BC	d
	Citric acid (2)	485	CD	a	339	CD	b	83	BC	c	34	BC	d
	Citric acid (5)	397	F	a	270	F	b	66	C	c	29	BC	d
	Acetic acid (2)	516	BC	a	351	BC	b	82	BC	c	30	BC	d
	Acetic acid (5)	386	F	a	298	EF	b	75	BC	b	27	C	d
	Ultrasonic C (2)	451	DE	a	311	DE	b	82	BC	c	36	AB	d
	Ultrasonic C (5)	334	G	a	274	EF	b	69	C	c	30	BC	c
	Mean		523		a	364		b	85		c	33	
Lambda-cyhalothrin	Raw	325	A	a	276	A	b	75	A	c	62	A	c
	Tap water (2)	176	B	a	170	B	a	55	B	b	52	B	b
	Tap water (5)	142	C	b	164	BC	a	50	BC	c	44	C	c
	Citric acid (2)	124	CDE	a	132	FG	a	50	BC	b	41	C	b
	Citric acid (5)	109	E	b	127	G	a	38	D	c	40	C	c
	Acetic acid (2)	143	C	a	154	CD	a	49	BC	b	40	C	b
	Acetic acid (5)	119	DE	a	141	EF	a	42	CD	c	31	D	d
	Ultrasonic C (2)	140	CD	a	151	DE	a	52	B	b	44	C	b
	Ultrasonic C (5)	104	E	b	132	FG	a	48	BC	c	41	C	c
	Mean		154		a	161		b	51		c	44	

* Bracketed values are the time of application (min);

** Means followed by the same uppercase letters within the same column are not significantly different at $p \leq 0.01$;

*** Means followed by the same lowercase letters within the same row are not significantly different at $p \leq 0.01$.

Washing with tap water

Effects of tap water washing treatments on residue reduction are given in Table 6. Significant differences were observed in residue levels in unwashed grapes of chlorpyrifos-methyl in day 0, 2, 4 and 7 samples (Table 7). For lambda-cyhalothrin, significant differences were only observed in unwashed samples between the day 0 and 2 samples. With washing of 2 and 5 min, significant changes were only observed for chlorpyrifos-methyl between day 0 and 2 samples. However, this was valid for only in day 0 samples for lambda-cyhalothrin.

With 5-min washing, day 0, 2, 4 and 7 samples with tap water washing, respectively, gave 63, 59, 28 and 21% reductions for chlorpyrifos-methyl, respectively, with PF = 0.37, PF = 0.41, PF = 0.72 and PF = 0.79. The values for lambda-cyhalothrin were 56, 41, 33 and 29%, respectively, with PF = 0.44, PF = 0.59, PF = 0.67 and PF = 0.71.

Washing tap water significantly decreased insecticide residues on the grape samples. Increasing reductions were found with increasing washing duration corresponding to decreasing PF values. Similarly, decreasing reduction ratios were found with increasing PHI values corresponding to increasing PF values. Similarly, Lozowicka et al. (2016) also reported decreasing PF values for increasing washing duration.

Zhou et al. (2019) reported that reductions for five pesticides (difenoconazole, azoxystrobin, abamectin, thiamethoxam, tebuconazole) from grape samples were higher than 60%, except for abamectin, with tap water washing. Heshmati et al. (2020) reported the greatest efficiency in reductions of pesticide residues with tap water washing and the greatest for NaHCO_3 washing. Çelik et al. (1995) investigated insecticide (diazinon) reduction with tap water washing and reported 18% reduction in grapes, 10% in apples and 9% in tomatoes.

Holland et al. (1994) noted that water solubility and octanol-water partition coefficient (log P) are important for pesticide residue reduction. Previous researchers emphasized that highly-soluble pesticides with lower Log P could be removed more efficiently with the use of washing treatments (Kong et al., 2012; Randhawa et al., 2014b; Zhao et al., 2014; Lozowicka et al., 2016). Polat & Tiryaki (2020) investigated the efficiency of washing applications in reduction of pesticide residues. In day 0, 2 and 3 samples, they reported 64, 18 and 40% reduction for acetamiprid, respectively, with PF values of 0.36, 0.82 and 0.6, with tap water washing. Reductions were 38, 32% and 33% for chlorpyrifos and 76, 70 and 69% for formetanate hydrochloride, respectively.

In present study, chlorpyrifos-methyl gave greater reduction (63%) and lower PF (0.37) with lower log P (4.74) and high solubility value (2.74 mg/L) (Table 1). However, lambda-cyhalothrin (log P = 5.5, Sw = 0.005 mg/L) had lower reduction (56%) and higher PF (0.44) (Table 6). Such results indicated that log P and solubility were a significant factor in reduction of pesticides residues. Besides water solubility, mode of action of pesticide is also important in reduction of pesticide residues. In present study, both insecticides were non-systemic.

Washing with acid solutions

In day 0 samples, significant differences were found between residues in 5-min acid washed and unwashed grapes for both pesticides (Table 7). The significant differences were found between 2- and 5-min citric acid washing for chlorpyrifos-methyl in day 0 and 2 samples. The differences between citric acid treatment duration for lambda-cyhalothrin in day 2 and 4 samples were significant and between acetic acid treatment durations of chlorpyrifos-methyl and lambda-cyhalothrin in day 0 and 2 samples (also day 7 samples for lambda-cyhalothrin).

The 5-min citric acid wash reduced chlorpyrifos-methyl by 66% (PF = 0.34) and lambda-cyhalothrin 66% (PF = 0.34) in day 0 samples. Acetic acid wash reduced chlorpyrifos-methyl by 67% (PF = 0.33) and lambda-cyhalothrin by 63% (PF = 0.37) (Table 6).

Since both insecticides were non-systemic, the reductions were not significantly different. These findings reveal that acid washing was more efficient in reduction of insecticide residues than the tap water. Randhawa et al. (2014a) conducted a similar study with acid washing treatments and reported a reduction 72% with citric acid washing and 69% with acetic acid. Polat & Tiryaki (2020) conducted a study with acetic acid washing and reported a maximum reduction of 78% for chlorpyrifos, followed by reduction rate of rate of 72% for formetanate hydrochloride and 34% for acetamiprid. Researchers reported the reduction rates of citric acid washing respectively as 78, 76 and 36%.

Washing with ultrasonic cleaner

Ultrasonic cleaning was effective in day 0 samples. The reductions with 5-min treatment were 71% for chlorpyrifos-methyl and 68% for lambda-cyhalothrin. In day 7 samples, the reductions were 29% and 33%, respectively (Table 6). Decreasing reduction rates were observed with increasing PHI. In day 4 samples, 5-min ultrasonic cleaning reduced chlorpyrifos-methyl by 45% (PF = 0.55) and lambda-cyhalothrin by 36% (PF = 0.64). Ultrasonic cleaning significantly reduced residues. In day 0 samples, the differences between unwashed grapes and 2 and 5-min treatments were significant (Table 7). Compared to tap water, ultrasonic cleaning gave greater reductions in residues of both insecticides. Polat & Tiryaki (2020) conducted a study with ultrasonic cleaning and reported a reduction of 30% for acetamiprid, 82% for chlorpyrifos and 74% for formetanate hydrochloride. In day 0 grape samples, such reduction rates were 77, 86 and 89%. Zhou et al. (2019) investigated the efficiency of different washing treatments in reduction of pesticide residues and found that ultrasonic washing more efficient than tap water treatment. Buakham et al. (2012) also reported greater reductions with ultrasonic washing.

Çatak et al. (2020) reported significant effects of different washing processes on reduction of pirimiphos-methyl residues. The order of reduction of pirimiphos-methyl residues (from highest to lowest) was ultrasonic cleaning > citric acid > acetic acid > tap water. The maximum reduction (87%) of pirimiphos-methyl was with ultrasonic cleaning. Lozowicka et al. (2016) reported reductions of 92% for ultrasonic cleaning, 20-68% for tap water and 36-75% for ozonated-water washing.

In the present study, citric acid washing and ultrasonic cleaning were more efficient in reduction of pesticide residues in grape samples than the acetic acid and tap water washing. The lowest residues (30 µg/kg for chlorpyrifos-methyl and 41 µg/kg for lambda-cyhalothrin) were achieved in day 7 samples with 5-min ultrasonic cleaning. In day 4 samples with 2-min washing, chlorpyrifos-methyl residues did not exceed the MRL (1000 µg/kg) but lambda-cyhalothrin residues (325 µg/kg) were about four times the MRL (80 µg/kg) in unwashed grapes. Residues of lambda-cyhalothrin were below the MRL with citric acid (50 µg/kg), acetic acid (49 µg/kg) and ultrasonic (52 µg/kg) washing.

Conclusions

These findings reveal increased residue reduction with longer washing. However, decreased residue reduction was observed with increasing PHI values. Greater reductions were achieved for the highly-soluble insecticide, chlorpyrifos-methyl, with smaller log P. Chlorpyrifos-methyl and lambda-cyhalothrin residues decreased with PHI, highlighting the importance of PHI. The residues of chlorpyrifos-methyl were below the MRL in day 2, 4 and 7 samples. With both 2- and 5-min washings, the residue of chlorpyrifos-methyl were reduced below MRL with all washing methods. For lambda-cyhalothrin, the residue levels were below the MRL in day 4 and 7 samples. The residues of lambda-cyhalothrin were above the MRL in day 0 and 2 samples with ultrasonic cleaning, citric and acetic acid and tap water washing treatments. It is concluded that as PHI increases the reduction of pesticide decreases with the various washing treatments and harvesting should be conducted according to the recommended PHI. Combined applications (for instance, ultrasonic cleaner plus acid solutions) are recommended for future studies.

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