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*Original article (Orijinal araştırma)*

# Insecticide residue analyses in cucumbers sampled from Çanakkale open markets[1]

Çanakkale açık pazarlarından örneklenen hıyarlarda insektisit kalıntı analizleri

**Hayriye ÇATAK[2]** **Osman TİRYAKİ[3*]**

## Abstract

The aim of this study was to investigate four insecticide residues in cucumbers with the aid of QuEChERS 2007.1 method. For method verification assessment, pesticide-free cucumber matrix was spiked with 0.1, 1 and 10 times of MRL for each pesticide. The QuEChERS-LC-MS/MS analytical method revealed that the detection limits of the insecticides were below the MRLs and the overall recovery of method was 97.7%. These figures were within the SANTE recovery limits (60-140%) and the values specified for the repeatability (≤20%). Cucumbers were collected from six different stands (A-F) at Çanakkale open markets for 6 weeks between 23 November and 28 December 2018. Residues of each sampling time and each stand were assessed. Acetamiprid residue of 257g and 236 µg/kg were detected in week 5 from stand B and in week 2 from stand E, respectively. These values are close to MRL (300 µg/kg). Formetanate hydrochloride residue of the week 3 from stand F (36.3 µg/kg) was more than MRL of 10 µg/kg. Pirimiphos methyl and chlorpyrifos residues were not detected in cucumbers. Theoretical maximum daily intake assessment showed that there was no chronic exposure risk for these four pesticides through cucumber consumption.

**Keywords:** Cucumber, insecticide residues, QuEChERS, risk assessment, toxicology

## Öz

Bu çalışma hıyarlarda dört insektisit kalıntısını QuEChERS 2007.1 yöntemi ile belirlemek amacıyla yapılmıştır. Metot doğrulama değerlendirmesi için pestisit içermeyen hıyar örneği MRL değerlerinin 0.1, 1 ve 10 katı seviyelerinde her pestisit ile zenginleştirilmiştir (fortifikasyon). QuEChERS-LC-MS/MS analiz yöntemi ile insektisitlerin dedeksiyon limitleri MRL'lerin altında ve tüm metodun geri alımı %97.7 olarak bulunmuştur. Bu değerler SANTE geri alım limiti (%60-140) ve belirlenen tekrarlanabilirlik değerleri (≤20%) arasındadır. Hıyarlar 6 hafta boyunca Çanakkale açık pazarlarından altı farklı tezgâhtan (A-F) 23 Kasım-28 Aralık 2018 tarihleri arasında toplanmıştır. Her bir örnekleme zamanı ve her bir tezgâha ait örneklerde kalıntılar araştırılmıştır. Acetamiprid kalıntısı, 5. hafta B tezgahında ve 2. hafta E tezgahında sırasıyla 257 µg/kg ve 236 µg/kg olarak tespit edilmiştir. Bu değerler MRL'ne (300 µg/kg) yakındır. Üçüncü hafta F tezgahında formetanate hidroklorür kalıntısı (36.3 µg/kg),10 µg/kg MRL değerinden daha fazla bulunmuştur. Hıyarlarda pirimiphos methyl ve chlorpyrifos kalıntısı bulunmamıştır. Teorik maksimum günlük alım değerlendirmesi hıyar tüketiminde bu 4 pestisitin kronik maruziyet riski oluşturmadığını göstermiştir.

**Anahtar sözcükler**: Hıyar, insektisit kalıntıları, QuEChERS, risk değerlendirmesi, toksikoloji

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[2] Çanakkale Onsekiz Mart University, Graduate School of Natural and Applied Sciences, Department of Plant Protection, 17100, Çanakkale, Turkey
[3] Çanakkale Onsekiz Mart University, Faculty of Agriculture, Department of Plant Protection, 17100, Çanakkale, Turkey
* Corresponding author (Sorumlu yazar) e-mail: osmantiryaki@yahoo.com
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## Introduction

Cucumber with an annual production of 1.9 Mt is the third placed vegetable produced Turkey after tomato and pepper. Of this production, 7.3 kt come from Çanakkale Province (TÜİK, 2019). About 83% of cucumber exported from Turkey is sent to EU (European Union) countries. Pests including *Bemisia tabaci* (Gennadius, 1889), *Trialeurodes vaporariorum* (Westwood, 1856) (Hemiptera: Aleyrodidae), *Aphis* spp. (Hemiptera: Aphididae) generate significant problems for cucumber production and result in serious economic losses each year. Insecticides are commonly used in cucumber production. Acetamiprid is used against the *B. tabaci* and *T. vaporariorum*, formetanate hydrochloride is applied against the *Frankliniella occidentalis* (Pergande, 1895) (Thysanoptera: Thripidae) and pirimiphos methyl is used against *Aphis* spp. Chlorpyrifos is used against *Aphis* spp. *Agrotis ipsilon* (Hufnagel, 1766) (Lepidoptera: Noctuidae), *Gryllotalpa gryllotalpa* (Mandal, 1982) (Orthoptera: Gryllotalpidae), *Agriotes* spp. (Coleoptera: Elateridae). However, chlorpyrifos was completely banned in Turkey in May 2020 and is not included in the BKÜ (Plant Protection Products) Database (BKÜ, 2020).

Despite the significant role in crop productivity and food security, chemical pesticides exert serious risks on human health and environment. Pesticide residues in agricultural products have negative effects on human health. Therefore, it is a major concern for consumers. Residues can constitute serious risks for human health (Council Directive 90/642/EEC, 1990). It is important to estimate pesticide exposure level from vegetables. Cucumber is consumed fresh and pickled. Pesticide exposure is very important, especially in fresh consumption. Farmers generally use pesticides for pest control in cucumber fields. However, some farmers use pesticides included in permissible lists but not recommended for cucumber. If integrated pest management (IPM) systems are not practiced, pesticides cause serious residue problems. Sometimes this topic is a barrier to international trade.

The QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method (Anastassiades et al. 2003) is largely employed in vegetable and fruit matrix safety analyses at well-equipped laboratories (Polat & Tiryaki, 2020). However, local laboratory conditions may require further verification for the method to be used reliably (Omeroglu et al., 2012).

Leili et al. (2016) used QuEChERS method to investigate pesticides residues in greenhouse cucumbers. The recovery of ethion and imidacloprid analyses ranged from 88 to 102%. Researchers have reported 35 and 31% reduction in ethion and imidacloprid levels, respectively, one day after pesticide application, 51 and 43% reduction with washing and 93 and 64% reductions with peeling. Wu & Hu (2014) conducted a study about method validation for fosthiazate residues in cucumber and soil. The recovery rates in cucumbers and soil varied between 91.2 and 99.0%. Researchers used QuEChERS and found that fosthiazate residues were lower than the MRL (maximum residue level) of 0.2 mg/kg.

Abdel-Ghany et al. (2016) worked on eight neonicotinoid insecticide residues in cucumbers and soil by LC/MS coupled with QuEChERS. Researchers set up a field trial in Qaluybiya, Egypt and sprayed pesticides on cucumbers. Cucumbers were sampled 1 h after application and after the 1, 2, 3, 5, 7, 14 and 21 d. The acetamiprid residue was found to be 938 ng/g in zero-time sample. However, 1, 2, 3, 5, 7, 14 and 21 d after pesticide application, residues were measured as 862 ng/g (8.1% reduction), 666 ng/g (28.9% reduction), 504 ng/g (46.3% reduction), 425 ng/g (54.7% reduction), 325 ng/g (64.9% reduction), 221 ng/g (76.4% reduction) and 87.5 ng/g (90.7% reduction), respectively.

In a study conducted by Hassanzadeh et al. (2012), imidacloprid was applied to greenhouse cucumbers at the recommended rate and twice that rate. The initial deposits were measured as 1.93 and 3.65 mg/kg in single and double doses, respectively, and recovery rates after 21 d were 94.5 and 99.2%, respectively. The residual imidacloprid level was lower than the MRL of 1 mg/kg after 3 d. Islam et al. (2015)

investigated pesticide residues on cucumbers sampled from the local markets and detected mancozeb residues in one out of three samples.

According to a study conducted in the Aegean Region of Turkey, the residues in 18 (26%) cucumber samples exceeded the MRL. Chlorpyrifos, dimethomorph and methomyl residues exceeded MRL in one, eight and four cucumber samples, respectively. Four or more pesticide residues were encountered in cucumber samples (Türköz-Bakırcı et al., 2014).

Nasiri et al. (2016) investigated residues of 12 pesticides on cucumber samples. The recovery of pesticides at five spiking levels using the QuEChERS method was in the range of 80.6-112%. The method was shown to be repeatable with RSD lower than 20%. Among 43 greenhouse cucumber samples, six samples contained chlorpyrifos residues at 97.1 µg/kg (range: 66.4-148 µg/kg) which was higher than EU MRL of 50 µg/kg.

Kaya & Tuna (2019) used QuEChERS to investigate pesticide residues in cucumbers collected from three open markets in İzmir Province and detected acetamiprid, chlorpyrifos, metalaxyl-M and thiamethoxam residues respectively as 0.01, 0.004, 0.033 and 0.025 mg/kg. The EU-MRL of them were 0.3, 0.05, 0.5 and 0.5 mg/kg, respectively.

Cara et al. (2011) worked on degradation of acetamiprid in greenhouse cucumber. When greenhouse indoor temperatures were between 29 and 35ºC, a rapid decline was seen in acetamiprid residues. The researchers demonstrated the importance of PHI (pre-harvest interval) for pesticide residues. In another study, consumer responses to pesticide residues in agricultural products were investigated. The study showed that about 40% of fruit and vegetable consumers had concerns about pesticide residues (Oraman, 2011).

Many studies have been conducted on the removal of pesticide residues by various product processing methods. In a study, chlorpyrifos residues (artificially spiked) on cucumber were reduced by 53.1, 59.2 and 62.9% with 5 ,10 and 20 min tap water washing, respectively (Liang et al., 2012). Indeed, mode of action of pesticide (systemic and/or contact) is important for reduction of residues by washing treatments (Polat &Tiryaki, 2020).

The present study was conducted to investigate residues of the four most widely used insecticide in cucumbers sampled from open markets of Çanakkale Province of Turkey. There are no previous studies investigating pesticide residues in cucumbers of Çanakkale Province. The selection of insecticides was made according to the RASFF (Rapid Alert System for Food and Feed) notification (there are RASFF notifications for three pesticides in cucumber for Turkey), residue data of EFSA (European Food Safety Authority) and registration (authorization) of them in Turkish - BKÜ Database. Of these, formetanate chloride is considered to be removed in Turkey and EU, due to environmental and toxicological risk (GKGM, 2020). The QuEChERS AOAC 2007.01 method (Lehotay et al., 2005) was used in the study. Verification of QuEChERS method was performed based on SANTE (EC Directorate-General for Health and Food Safety) guidelines (SANTE, 2019).

## Materials and Methods

### Reagents and chemicals

Standards of acetamiprid, chlorpyrifos, formetanate hydrochloride and pirimiphos methyl pesticides were supplied from a Dr. Ehrenstorfer Laboratories GmbH (Wesel,Germany) at purity of 98, 99, 99.2 and 97.6%, respectively. Some properties of insecticides are summarized in Table 1. $MgSO_4*7H_2O$, sodium acetate (NaAC), acetonitrile (ACN), toluene and methanol were supplied from Merck Company (Darmstadt, Germany) at purity of 99.0-100.5, 99.0, 99.9, 99.0 and 99.9%, respectively. Primary-secondary amine (PSA, 40 µM, 100 g) was sourced from Agilent (Santa Clara, CA, USA).

Table 1. Some properties of insecticides (WHO, 2009; EU, 2020; IRAC, 2020; PPDB, 2020)

| Parameter | | Acetamiprid | Chlorpyrifos | Formetanate hydrochloride | Pirimiphos methyl |
|---|---|---|---|---|---|
| Group | | Neonicotinoid | Organophosphate | Formamidine | Organophosphate |
| Formula | | $C_{10}H_{11}ClN_4$ | $C_9H_{11}Cl_3NO_3PS$ | $C_{11}H_{15}N_3O_3$ | $C_{11}H_{20}N_3O_3PS$ |
| Action mode | | Systemic, Nicotinic acetylcholine receptor (nAChR) | Non-systemic, acetylcholinesterase (AChE) inhibitors, nerve action | Stomach action and contact, acetylcholinesterase (AChE) inhibitors, nerve action | Contact and respiratory action, acetylcholinesterase (AChE) inhibitors, nerve action |
| Physicochemical parameters | Boiling | Degrades before boiling | Degrades before boiling | Degrades before boiling | Degrades before boiling |
| | Solubility in water (mg/L) | 2950 | 1.05 | 822000 | 11 |
| | logP | 0.8 | 4.7 | -0.0014 | 4.2 |
| | Degradation point (ºC) | 200 | 170 | 204 | 162 |
| | Molecular weight (g/mol) | 222.67 | 350.58 | 257.8 | 305.33 |
| Toxicological parameters | Acceptable daily intake, (mg/kg/bw/day) | 0.025 | 0.001 | 0.004 | 0.004 |
| | Acute reference dose (mg/kg/bw/day) | 0.025 | 0.005 | 0.005 | 0.1 |
| | Maximum permissible intake, (mg/person/day) | 1.5 | 0.06 | 0.24 | 0.24 |
| | Inhalation $LC_{50}$ (Mammals) (mg/L) | > 1.15 | 0.1 | 0.15 | > 4.7 |
| | Dermal $LD_{50}$ (Mammals) (mg/kg) | > 2000 | > 1250 | > 2000 | > 2000 |
| | Acute oral $LD_{50}$ (Mammals) (mg/kg) | 146 | 66 | 14.8 | 1414 |
| | WHO classification [a] | II | II | Ib | II |

[a] Ib, highly hazardous; II, moderately hazardous.

**Instruments**

Chromatographic analyses were performed with LC-MS/MS (Waters Acquity UPLC+Acquity TQD) equipped with BEH $C_{18}$ column (1.7 µm, 2.1 mm x 100 mm). Injection volume, flow rate and total run time were 20 µL, 0.3 mL/min and 15 min, respectively. Desolvation gas flow, cone gas flow and collision gas flow were 600, 50 and 0.19 mL/min, respectively. A gradient program of 5 mM ammonium acetate plus 95% MeOH (B) and 5 mM ammonium acetate plus 5% MeOH in water (A) were used. Quasimolecular ions were 222.1 m/z for formetanate hydrochloride, 223.1 m/z as [M+H]+ for acetamiprid, 306.15 m/z for pirimiphos methyl and 349.9 m/z for chlorpyrifos. For quantification, reactions of 222.1/165.1 m/z, 223.1/126.1 m/z, 306.15/164.11 m/z and 349.9/96.9 m/z were monitored through a multiple reaction monitoring mode for formetanate hydrochloride, acetamiprid, pirimiphos methyl and chlorpyrifos, respectively. Similar values for confirmation were 222.1/93.0 m/z, 223.1/90.0 m/z, 306.15/108.05 and 349.9/197.9 m/z for formetanate hydrochloride, acetamiprid, pirimiphos methyl and chlorpyrifos, respectively.

**Standard and fortification solutions**

Stock solutions (400 µg/mL) of experimental pesticides were prepared. Then, 1.0 µg/mL of working solutions and calibration solutions with the range of 2-50 pg/µL were prepared in ACN for all active ingredients. Spiking solutions corresponding to 0.1, 1 and 10 x MRL were prepared. The standards and solutions were stored at 4ºC in dark. Representative apple matrix was used for matrix-matched calibrations (MC) and quantification, as indicated in Codex Alimentarius Commission Guidelines (CAC, 2003) and SANTE Guidelines (SANTE, 2019). Spiking level of 10 times MRL was diluted to fit calibration range.

**Fortification trials and analyses**

Despite the widespread use of the QuEChERS method in sophisticated laboratories, there is still a need for validation/verification for local conditions of your own laboratories. Recovery assessment is the first step of method validation evaluation (SANTE, 2019). For this aim, 1 kg of blank (pesticide-free sample, no pesticide applied sample) cucumber sample was homogenized with a blender. Then, 15 g homogenized sample spiked with 100 µL ACN was mixed with acetamiprid, chlorpyrifos, formetanate hydrochloride solution and pirimiphos methyl at 0.1, 1 and 10 x MRL levels in three replicates (analytical portion) (Table 2). Resultant mixture was vortexed for 30 s and left for pesticide interaction for 15 min.

Table 2. Fortification parameters for four insecticides

| Fortification | Code | Level of fortification (µg/kg) | | | |
|---|---|---|---|---|---|
| | | Acetamiprid | Chlorpyrifos | Formetanate hydrochloride | Pirimiphos methyl |
| 0.1 x MRL* | F1/1-3 | 30 | 5 | 1 | 1 |
| 1x MRL | F2/1-3 | 300 | 50 | 10 | 10 |
| 10 x MRL | F3/1-3 | 3000 | 500 | 100 | 100 |
| Control | F0/1-3 | - | - | - | - |

* EU MRL(µg/kg).

Analyses of all spiked and market samples were performed with the QuEChERS AOAC Method 2007.01 and LC-MS/MS (Lehotay, 2005). Schematic diagram of the method is illustrated in Figure 1. Three 200 µL extracts of each analytical portion were subjected to chromatographic analysis. The recovery was calculated with the use of Equation1.

$$\text{Recovery } \% = \frac{\text{Measured concentration}}{\text{Spiked concentration}} \text{x}100 \quad (1)$$

Method precision and recovery rates were tested in accordance with SANCO European Guidelines (SANTE, 2019). Method linearity was checked for the range of 2-50 pg/mL.

**Collecting cucumber samples and analyses**

Cucumbers were collected from six different stands of Çanakkale open markets for 6 weeks (1 sampling per week) between 23 November and 28 December 2018. About 2 kg samples were taken in each sampling. Samples were immediately brought to laboratory for analysis. About 1 kg chopped cucumber sample was well homogenized and 15 g analytical portion was taken in three triplicates. Further analytical procedure of the QuEChERS-AOAC method are illustrated in Figure 1. In total 108 analyses (6 stands x 6 weeks x 3 analytical portions) were performed.

**Methodology for assessing dietary intake of insecticides**

WHO Guidelines were used to assess dietary intake of pesticides (WHO, 1997). Acceptable daily intake (ADI) (mg/kg/b.w/day) and maximum permissible intake (MPI) (mg/person/day) values of insecticides are provided in Table 1. Theoretical maximum daily intake (TMDI) values were calculated as percentage of ADI. In Turkey, annual cucumber consumption per person is 18.5 kg (i.e., 51 g of cucumber per day) (TÜİK, 2019). Mean national theoretical maximum daily intake (NTMDI) and ADI% were calculated with the use of Equations 2 and 3, respectively. According to WHO guidelines, chronic exposure levels of pesticides that have values not exceeding 100% of ADI are low (WHO,1997).

$$\text{Mean NTMDI, mg/kg} = \text{Daily cucumber consumption, mg/kgXMean residue, mg/kg} \quad (2)$$

$$\text{ADI}\% = \frac{\text{Mean NTMDI}}{\text{MPI}} \quad (3)$$

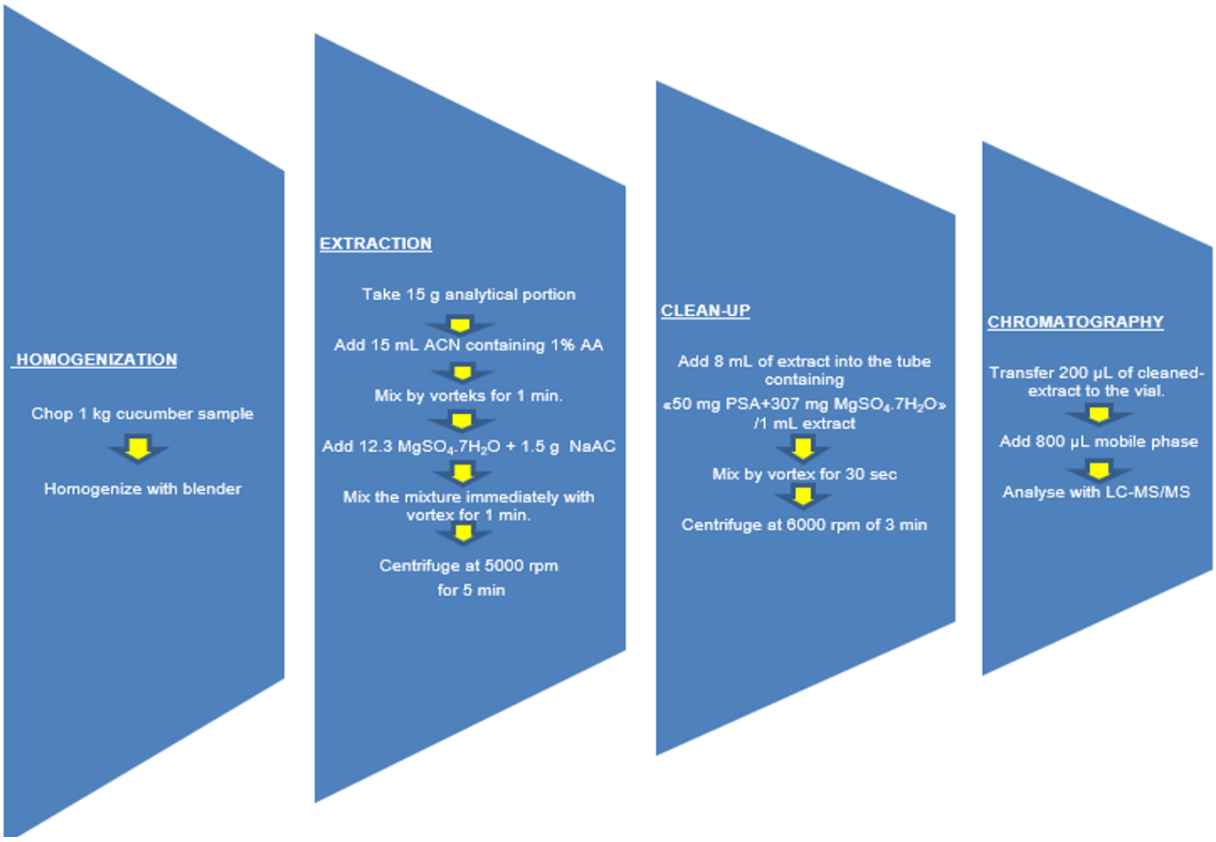


Figure 1. Schematic presentation of QuEChERS-AOAC method.

## Results and Discussion

### Method verification

### Linearity

Calibration curves of experimental pesticides are presented in Figure 2. Resultant curves were linear within the range of 2-50 pg/µL ($R^2 \geq 0.999$). Regression equations are used as the analytical function of MC. The regression equation, as the analytical function of MC, was used for analyte quantification.

### Repeatability of retention times

Retention time of pesticides (tR, min) should comply with the calibration standards with a ±0.1 min tolerance (SANTE, 2019). The repeatability of retention times for experimental pesticides was assessed through MC solutions of 2, 5, 10, 20 and 50 pg/µL. The retention time ranges were 10.18-10.19 min (with RSD of 0.05%), 2.85-2.86 min (with RSD of 0.19%) and 9.38-9.39 min (with RSD of 0.04%) for chlorpyrifos, formetanate hydrochloride and pirimiphos methyl, respectively. Acetamiprid tR was 4.91 min in all runs.

### Limit of Quantification

Limit of quantification (LOQ) was identified as 2 µg/kg (less than MRL of 300 µg/kg) for acetamiprid, 10 µg/kg (less than MRL of 50 µg/kg) for chlorpyrifos, 5 µg/kg (below than MRL of 10 µg/kg) for formetanate hydrochloride and 1 µg/kg (below than MRL of 10 µg/kg) for pirimiphos methyl.

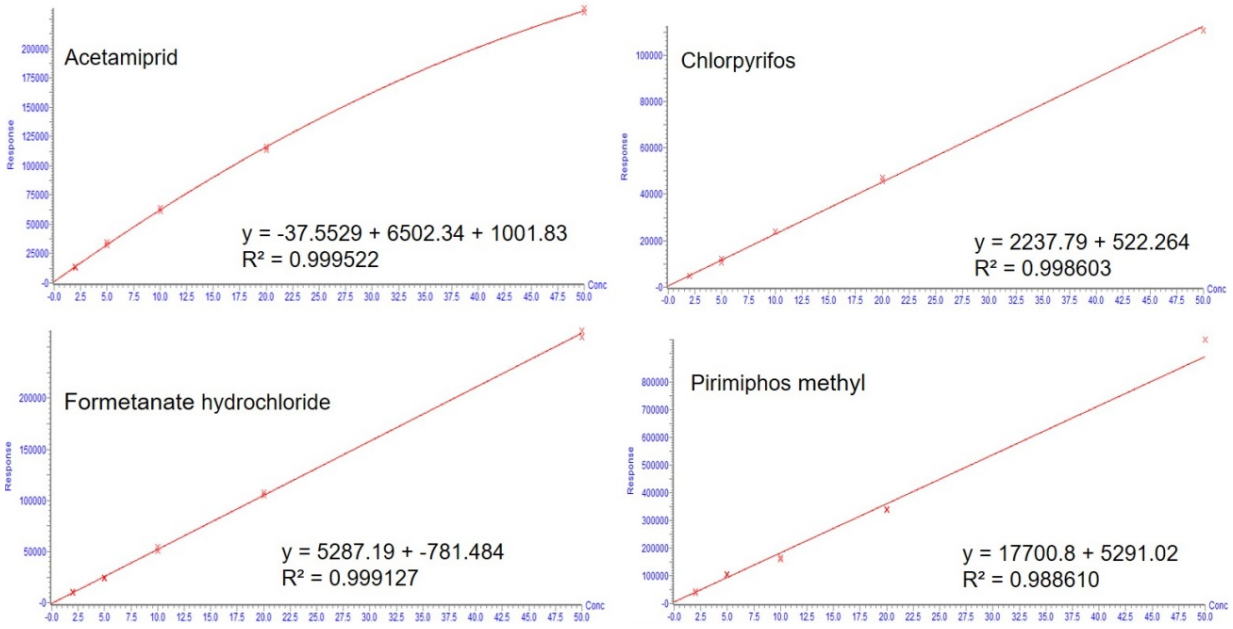


Figure 2. Calibration curves for four compounds in matrix-matched calibration.

**Precision and accuracy**

Method precision and trueness are generally assessed through repeatability (RSD%) and recovery (Q%) (SANTE, 2019; EURACHEM, 2014; TURKAK, 2019). Present recovery rates are provided in Table 3. Recovery rates of acetamiprid, chlorpyrifos, formetanate hydrochloride and pirimiphos methyl were 89.10% (RSD = 15.4%, n = 27), 84.1% (RSD = 15.8%, n = 18), 111% (RSD = 11.6%, n = 18) and 107% (RSD = 18.3%, n = 27), respectively. Mean recoveries varied between 84.1 and 111% (maximum RSD = 18.3%). The overall recovery rate was determined as 97.7% (RSD = 19.0%, n = 90). These figures were within the SANTE recovery limits (60% ≤ Q ≤ 140%) and the values specified for the repeatability (≤20%) for cucumber. The present findings on recovery rates also comply with the method verification parameters for pesticide residue analyses (SANTE, 2019; EURACHEM, 2014). In Hassanzadeh et al. (2012), mean recovery of imidacloprid in cucumbers was reported as 104%.

Accuracy is the closeness of the measured values to actual values (Tiryaki, 2016). Current accuracy values (as a tool for trueness) are provided in Table 3. Present findings revealed that QuEChERS yielded efficient recovery rates for experimental insecticides. Thus, it was thought that present analytical method may offer a rapid and accurate method for insecticide residue analysis in cucumbers.

**Residues of cucumber samples**

In this study, a total of 108 analytical portions, [36 samples (6-week x 6 stands designated as A to F) and three replicates] were analyzed. Evaluations were made for each insecticide on a weekly and stand basis. In cucumber samples, acetamiprid, chlorpyrifos, formetanate hydrochloride and pirimiphos methyl residues were detected. In addition, some traces of insecticide residues were encountered.

Table 3. QuEChERS-AOAC method verification

| Active ingredient | Concentration (µg/kg) | | Recovery % | RSD % |
|---|---|---|---|---|
| | Spiked | Measured[a] | (As a tool for trueness) | (As a tool for precision) |
| Acetamiprid | 30 | 30.67 | 102.24 | 9.73 |
| | 300 | 271.10 | 90.37 | 9.81 |
| | 3000 | 2241.07 | 74.70 | 3.90 |
| | Mean recovery, n=27 | | 89.10 | 15.44 |
| Chlorpyrifos | 5 | nd | - | - |
| | 50 | 38.72 | 77.44 | 11.10 |
| | 500 | 454.20 | 90.84 | 15.62 |
| | Mean recovery, n=18 | | 84.14 | 15.81 |
| Formetanate hydrochloride | 1 | nd[b] | - | - |
| | 10 | 10.86 | 108.68 | 15.62 |
| | 100 | 113.68 | 113.67 | 6.10 |
| | Mean recovery, n=18 | | 111.18 | 11.55 |
| Pirimiphos methyl | 1 | 0.83 | 83.77 | 9.28 |
| | 10 | 11.17 | 111.68 | 3.09 |
| | 100 | 126.54 | 126.54 | 2.27 |
| | Mean recovery, n=27 | | 107.33 | 18.30 |
| Whole recovery of the QuEChERS-AOAC (method accuracy): 97.71 % (n=90) RSD=19.01) | | | | |

[a] Mean of three analytical portions; [b] nd, not detected (below detection limit).

**Acetamiprid**

Acetamiprid (LOQ of 2 µg/kg) residues of 256.57 µg/kg and 235.93 µg/kg were detected in week 5 from stand B and week 2 from stand E, respectively. These two values were close to EU MRL of 300 µg/kg. Residues were 165 µg/kg in week 3 from stand E and 139 µg/kg in week 4 from stand D, which were well below the EU-MRL (Figure 3). The present samples all had acetamiprid residues below EU MRL of 300 µg/kg for cucumber. According to Türköz-Bakırcı et al. (2014), nine cucumber samples had acetamiprid residues below the LOQ. Kaya & Tuna (2019) found 10 µg/kg acetamiprid residues in cucumber samples.

**Chlorpyrifos**

LOQ and EU-MRL for chlorpyrifos were respectively identified as 10 and 50 µg/kg. Chlorpyrifos residues of all samples were below LOQ. Nasiri et al. (2016) found 97.13 µg/kg chlorpyrifos residues in six cucumber samples out of 43 greenhouse samples. In Türköz-Bakırcı et al. (2014), chlorpyrifos, residues exceeded MRL in one cucumber samples. In Kaya & Tuna (2019), chlorpyrifos residue in cucumber samples was identified as 33 µg/kg.

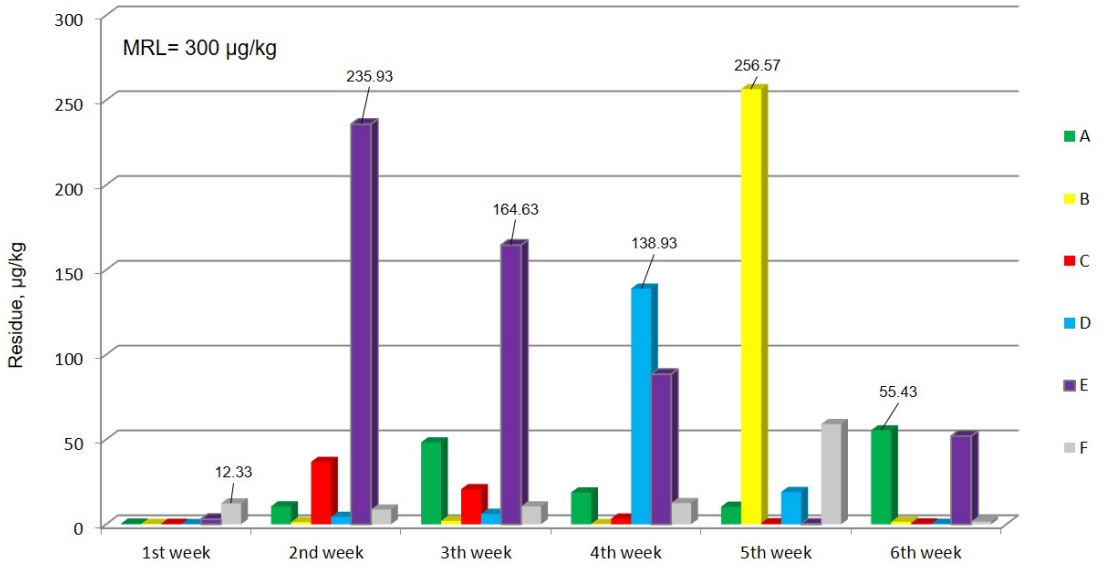


Figure 3. Acetamiprid residues in cucumbers based on week and stand.

**Formetanate hydrochloride**

LOQ and EU-MRL for formetanate hydrochloride were respectively identified as 5 and 10 µg/kg. In one sample (in week 3 from stand F), formetanate hydrochloride residue (36.3 µg/kg) was three times more than MRL. Residue of 11.5g, 11.4g, 10.7g and 10.1 µg/kg were found in week 3 from stand A, week 2 from stand C, week 4 from stand A and week 4 for from C, respectively. These values also slightly exceed the MRL (Figure 4). As shown in Figure 4, formetanate hydrochloride residues were not detected in week 5 and 6. This may indicate decreasing residues with increasing time after the harvest. Formetanate hydrochloride is considered to be banned insecticides in Turkey (GKGM, 2020). According to WHO classification, it is also highly hazardous (Class Ib) substance (WHO, 2009).

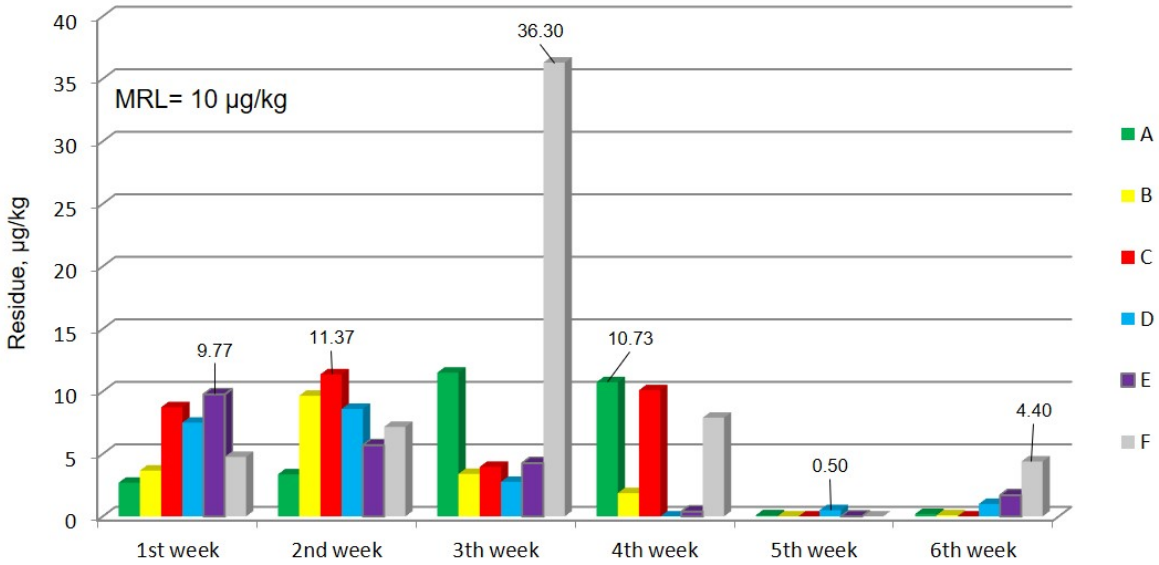


Figure 4. Formetanate hydrochloride residues in cucumbers based on week and stand.

**Pirimiphos methyl**

LOQ and EU-MRL for pirimiphos methyl were 1 and 10 µg/kg, respectively. Residues of pirimiphos methyl were neither exceeding MRL nor close to LOQ.

In addition to these four insecticides, 9 µg/kg of oxadixyl residues (LOQ = µg/kg) were detected in week 1 from stand F. This value is close to the MRL of 10 µg/kg.

Hassanzade et al. (2012) reported recovery rates for imidacloprid in 21 d respectively as 94.5% and 99.2% at single and double doses. Residue levels decreased below MRL of 1 mg/kg in 3 d. In a residue monitoring project conducted between 1996 and 2000 in Turkey, about 1000 vegetable and fruit samples were studied. Insecticide residue levels were below MRL in 45 greenhouse cucumber samples (Anonymous, 2002). Islam et al. (2015) investigated pesticide residues on cucumber samples from local markets and detected Mancozeb residue (about 50 ppm) in one out of three samples. Kaya & Tuna (2019) investigated pesticide residues in cucumbers and detected thiamethoxam residues as 0.025 mg/kg. The EU-MRL of the pesticide was 0.5 mg/kg.

**Risk assessment for dietary intake of insecticides**

**Acetamiprid**

Acetamiprid was the most abundant residue in present cucumber samples. Acetamiprid residue levels varied between 2.03 and 257 µg/kg. Overall mean residue of acetamiprid was 53.3 µg/kg. Risk assessments were made over 24 residues. Mean NTMDI was calculated as 2.7 µg/day (Equation 2). Average NTMDI, as a percentage of ADI, was calculated as 0.18% (Equation 3). Gölge & Kabak (2015) reported daily acetamiprid intake with tomato as 0.04 µg/kg/b.w. Chronic exposure level of this insecticide is low, since acetamiprid has a value not exceeding 100% of the ADI (WHO, 1997).

**Chlorpyrifos**

Risk assessment was not made for chlorpyrifos since no residue (more than LOQ) was detected in any of the cucumber samples.

**Formetanate hydrochloride**

Formetanate hydrochloride levels varied between 4.4 and 36.3 µg/kg. Overall mean residue of formetanate hydrochloride was 0.01115 mg/kg. Risk assessments were made over 13 residue data. Mean NTMDI was calculated as 0.00056 mg/day. Average NTMDI, as a percentage of ADI, was calculated as 0.233. Chronic exposure level of this insecticide is low, since formetanate HCl has a value less than 100% of the ADI (WHO, 1997).

**Pirimiphos methyl**

Since neither exceeding MRL nor close to LOQ, residues were not detected in any cucumber samples, therefore, risk assessments were not made for pirimiphos methyl.

## Conclusion

Agrochemicals have a significant role in improving agricultural production and reducing labor inputs for pest control. Pesticides may prevent yield losses to some extent, but exert serious risks on human health and environment. The current work was conducted to investigate some insecticide residues in cucumbers, sampled from open markets of Çanakkale Province of Turkey. In the study, the required method validation criteria were met. The QuEChERS method was successfully used in acetamiprid, chlorpyrifos, formetanate hydrochloride and pirimiphos methyl residue analyses in cucumbers. None of the cucumbers sampled from Çanakkale open markets contained residues of acetamiprid, chlorpyrifos and

pirimiphos methyl exceeding their MRLs. It was concluded based on present findings that consumption of cucumbers in Çanakkale Province did not pose a risk to human health, except formetanate hydrochloride. In one sample, formetanate hydrochloride was 3 times greater than the MRL. It can also be concluded that the absence of formetanate hydrochloride residue in any samples of week 5 and 6 emphasized the importance of PHI. Cucumber should be sampled at different PHI from the same field. It was concluded based on present data that there was no risk for cucumber consumption in terms of four insecticides. However, it is important to work with large data in order to evaluate risk of exposure in such studies.

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