

Household Activities Can Reduce Imidacloprid and Abamectin Residues in Greenhouse Crops

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ABSTRACT

Greenhouse products are subject to contamination by pesticides used to control pests and diseases. Toxicity on human and non-target animals, destruction of environment, and bioaccumulation are just some of the problems caused by indiscriminate use of pesticides. Imidacloprid and abamectin are two widely used pesticides to control greenhouse pests in Varamin region (Tehran Province, Iran). The aim of this study was to determine the effect of some household operations including refrigerated storage (48 hours), immersion in water (5 hours) and combination of these two treatments on reduction of pesticide residues in two most freshly consumed vegetables, cucumber and tomato. Sampling was performed in three randomly selected greenhouses of each crop in Varamin. Pesticides residue were extracted from samples according to QuEChERS method and determined by High Performance Liquid Chromatography (HPLC) system equipped with UV detection and analytical column C18 (250×4.6 mm). Results showed that crops were contaminated more than maximum residue level of these pesticides. Although in most cases, refrigerated storage treatment reduced pesticides residue more than immersion in water, their combination was the most effective treatment. Based on the results, this treatment caused imidacloprid residues reduction of 91.3 and 60.2% and abamectin residues reduction of 81.4 and 70.3% in cucumber and tomato, respectively. These findings showed that some easy, accessible, and domestic solutions can dramatically reduce residues of these two common pesticides.

Keywords: Cucumber, HPLC, QuEChERS method, Tomato.

INTRODUCTION

Fruits and vegetables are necessary for human diets, which contain a lot of materials with nutritional values. These agricultural products are subject to contamination by pesticides used to control pests and diseases. People are generally exposed to pesticides through ingestion of contaminated fruits and vegetables grown in contaminated field or directly treated with pesticides. Agricultural products could contain significant quantities of pesticides residue. By definition, pesticide residue refers to the pesticides or their break down products that remain on or in food

after they are applied to food crops (Wang *et al.*, 2009). Although many studies have focused on biological pest control, pesticides are still a common and reliable source to control pests in many regions of the world. Their indiscriminate use may lead to many adverse effects, such as toxicity of the human and non-target animals, destruction of environment, bioaccumulation as well as the pesticide residue problems. It has been estimated that over million tons of pesticides are being used in the world and this process is increasing with the passing of time (Tariq *et al.*, 2007). Therefore, post harvests safety period's regulations are created for

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pesticides on fresh fruits and vegetables in many countries in the world.

Face to face survey showed that imidacloprid and abamectin are much used to control greenhouse pests (e.g. whitefly, two-spotted spider mite, aphids etc.) in the research area (Varamin, Tehran Province) (personal communication with Dr. Shahriar Asgari from Greenhouse Cultivation Research Department, Tehran Agricultural and Natural Resource Research and Education Center, Varamin, Iran).

Imidacloprid [IUPAC name 1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine] is a systemic chloronicotinyl insecticide, belonging to a relatively new class of insecticides known as neonicotinoids that has high efficiency against sucking insects (El-Naggar and Zidan, 2013). This pesticide is the active ingredient in a wide variety of trade names, such as Gaucho, Alcador, and Confidor which were introduced by Bayer Agricultural Products for the first time (Daraghmeh et al., 2007). Imidacloprid consists of colorless crystals and has a broad spectrum activity and low mammalian toxicity along with excellent translaminar activity. The half-life of this neonicotinoid pesticide in soil is reported 29–190 days (Sarkar et al., 2001). This insecticide is quite water soluble even at the lowest solubility value reported (510 mg L^{-1}) and could leach to groundwater (Mulye, 1995). According to the codex Alimentarius, the maximum residue levels of this insecticide in cucumber and tomato are 1 and 0.5 mg kg^{-1} crop weight, respectively (FAO, 2017).

Abamectin belongs to the family of avermectins, a class of macrocyclic lactones produced by a soil actinomycete, *Streptomyces avermitilis* and is a mixture of two homologues containing at least 80% of avermectin B1a, and less than 20% of avermectin B1b (Sun et al., 2005). Abamectin is widely used to control insects and mites in vegetables and fruits such as cabbage, cucumber, tomato etc., due to its high toxicity to agricultural pests (Huang

and Casida, 1997; Wang et al., 2005). Although abamectin belongs to a bio-pesticide group, it may be toxic to mammals including human beings. According to the codex Alimentarius, the maximum residual levels of this insecticide in cucumber and tomato are 0.03 and 0.05 mg kg^{-1} crop weight, respectively (FAO, 2017).

Nowadays, assessing pesticides residue and providing some strategies for their reduction in greenhouse crops is very important and many researches have been done in this area around the world (Andrade et al., 2015; Kong et al., 2012; Liang et al., 2012; Pirsaeheb et al., 2016; Vemuri et al., 2014). Although there are different methods to measure pesticides residue in vegetable, some studies have shown that imidacloprid and abamectin residues can well be measured by High-Performance Liquid Chromatography (HPLC) (Mandic et al., 2005; Roudaut, 1998; Valenzuela et al., 2001). The aim of this work was to determine imidacloprid and abamectin residues in greenhouse cucumber and tomato crops and evaluate some easy and accessible solutions to reduce these residues.

MATERIALS AND METHODS

Sampling and Application of Treatments

Three greenhouses growing cucumber (Soltan variety) and tomato (Nomarin variety) were randomly chosen from Varamin region ($35^{\circ} 19' 27'' \text{ N } 51^{\circ} 38' 45'' \text{ E}$, Tehran Province, Iran) in April 2016. In each greenhouse, ripe crops were randomly harvested, transferred to the laboratory and divided into four groups for application of treatments including: (i) Control, no clean-up activity applied, (ii) Refrigerated storage (4°C) for 48 hours, (iii) Immersion in water for 5 hours, and (iv) Refrigerated storage (4°C) for 48 hours + immersion in water for 5 hours.

Sample Preparation

After application of treatments, extraction and partitioning were carried out according to a modified version of the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method (Nguyen *et al.*, 2008; Paya *et al.*, 2007). According to this method, 1,000 g of the samples were homogenized with a blender, then, 10 g of each sample was accurately weighed and mixed with 10 mL acetonitrile (HPLC grade, Merck Company). Later, 1 g of sodium chloride and 4 g of anhydrous magnesium sulfate were added to the solution which was centrifuged (3,800 rpm) for 10 minutes at 4°C. Approximately 0.5 g of graphite carbon black was added to the materials to separate the pigments. After that, the supernatant was passed through filter paper (Sigma-Aldrich's Whatman®, Pore size= 25 micron) and concentrated to 1 mL with laminar flow of nitrogen gas. Each treatment was replicated three times.

Instrumental Analysis

The standards of pesticides with 99.5 to 99.9% purity were purchased from Sigma-Aldrich (USA). Chromatograms of five different concentrations ranging from 0.1 to 10 mg kg⁻¹, in acetonitrile as solvent, are shown in Figures 1 and 2. These standards were injected to HPLC system to draw the calibration curve of each pesticide (Figure 3). In both curves of Figure 3, the coefficients of determination were over 0.99 and represent the acceptable linear relationship between the concentration and the slope. The HPLC system (Knauer Company, Germany) equipped with UV detection and analytical column C18 (250×4.6 mm) was used in the study (at room temperature and 270 and 245 nm wavelength for imidacloprid and abamectin, respectively). The mobile phase was acetonitrile: water (80:20 v/v) at a flow rate of 1.4 mL min⁻¹ (Abdellseid and Rahman, 2014; Chauhan *et al.*, 2013). All samples were injected to HPLC system and

pesticides residue concentrations were estimated using calibration curve.

Data Analysis

All data were checked for normality using the Kolmogorov-Smirnov test and were found to be normally distributed. Data were subjected to one-way ANOVA followed by the Tukey's test ($\alpha = 0.05$) to separate means using IBM SPSS software (SPSS, 2011).

RESULTS

The imidacloprid residue in cucumber were measured and compared before and after clean-up operations (Table 1). These results showed that the greenhouses, except one, were contaminated more than the acceptable residues levels of this pesticide. Results showed that, generally, clean-up treatments significantly reduced the residue of this pesticide in cucumber. However, the average data showed that refrigerated storage for 48 hours reduced pesticide residue more than immersion in water, but statistical analysis indicated that there was no significant difference between these two treatments (P -value= 0.446). The combination of both treatments significantly reduced the amount of imidacloprid residue in cucumber. Noticeably, refrigerated storage for 48 hours + immersion in water for 5 hours treatment reduced residual amount of this neurotoxin pesticide to half of the acceptable maximum residue level.

The imidacloprid pesticide residue in tomato was measured and compared before and after clean-up operations (Table 2). The instrumental analysis showed that products of all selected greenhouses were notably contaminated with this pesticide. In greenhouse number 3, the contamination was even more than 20 times of the recommended allowance. In this crop, like cucumber, the refrigerated storage+immersion in water treatment reduced imidacloprid residue significantly,

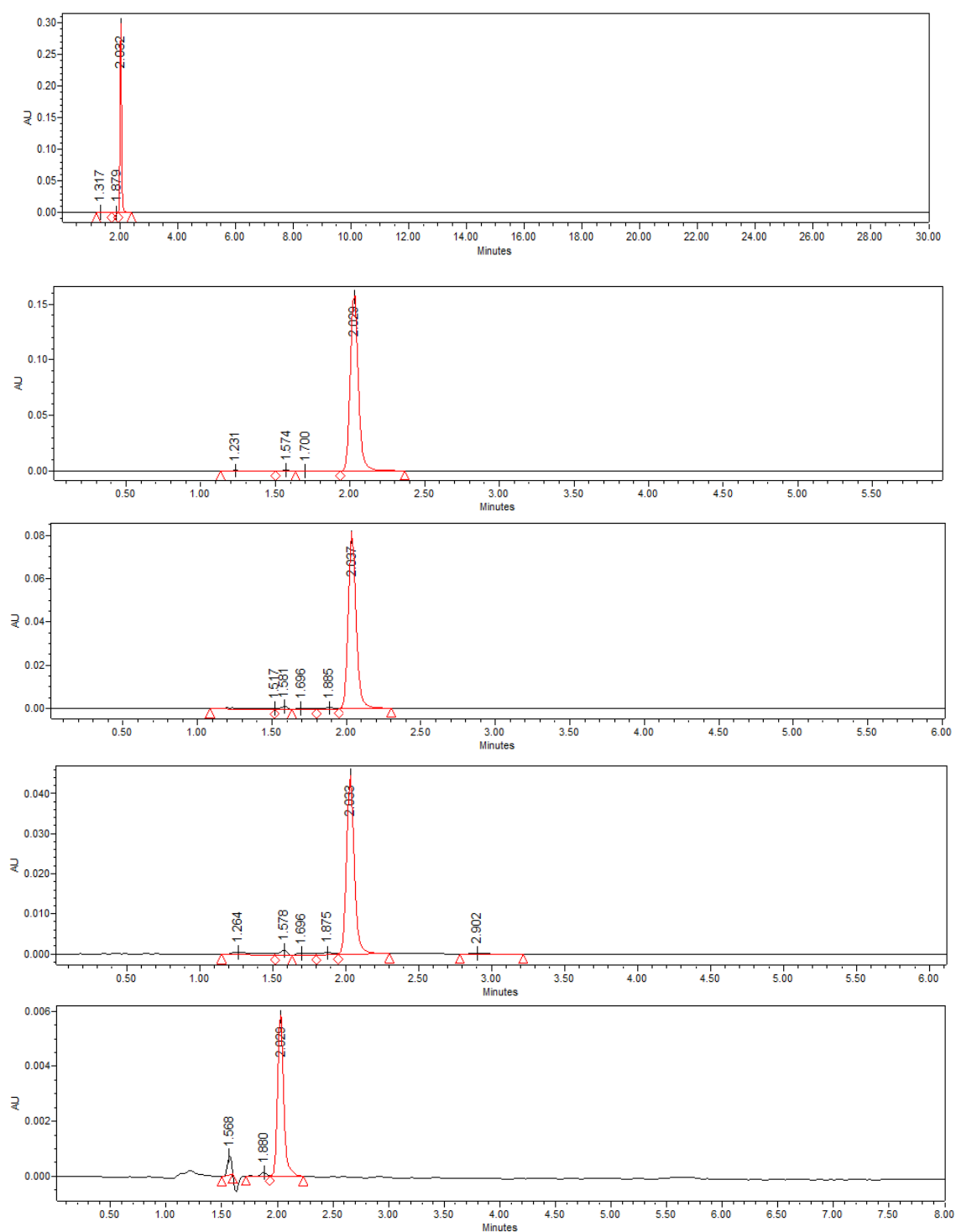


Figure 1. Chromatograms of imidacloprid standard at different concentrations.

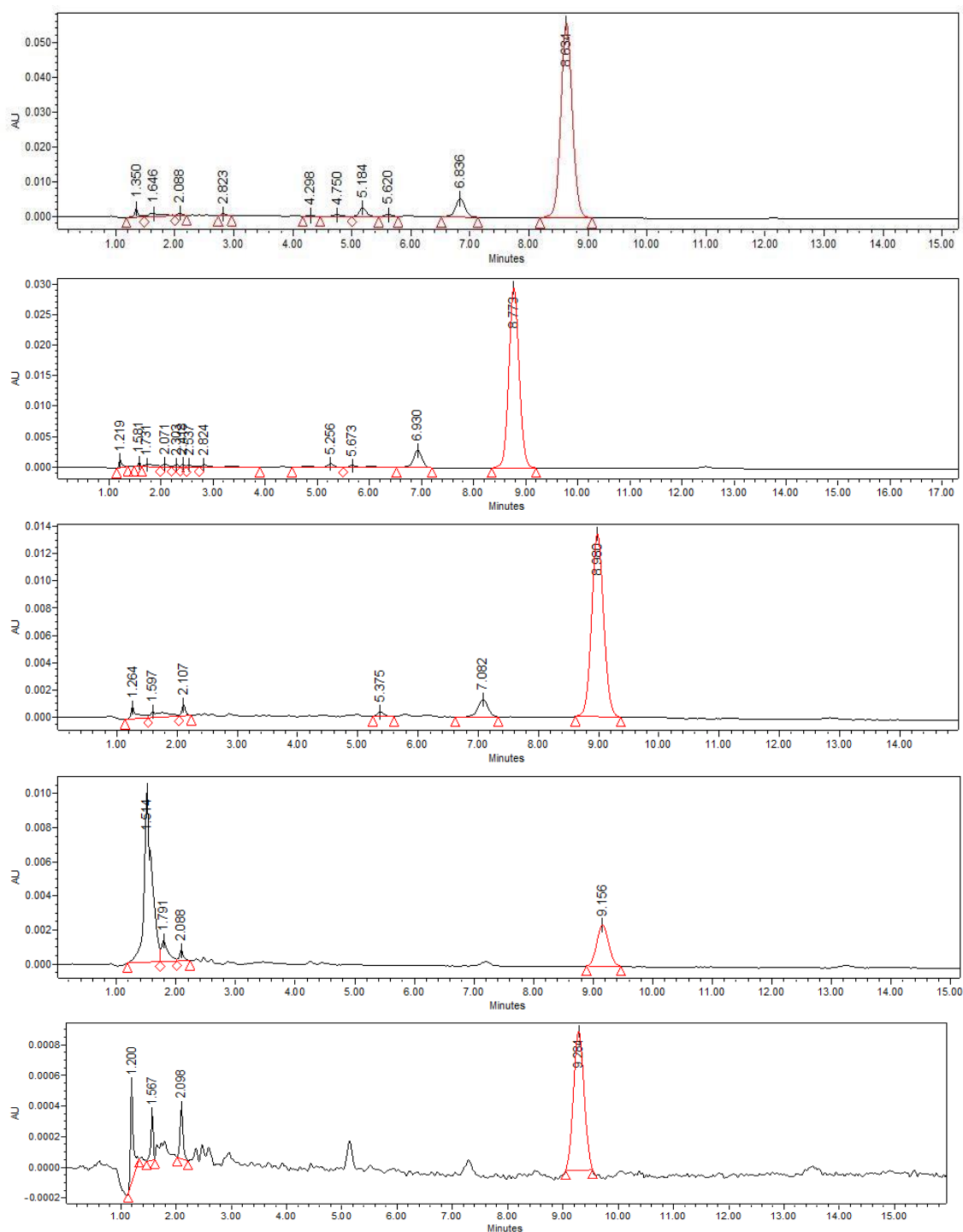


Figure 2. Chromatograms of abamectin standard at different concentrations.

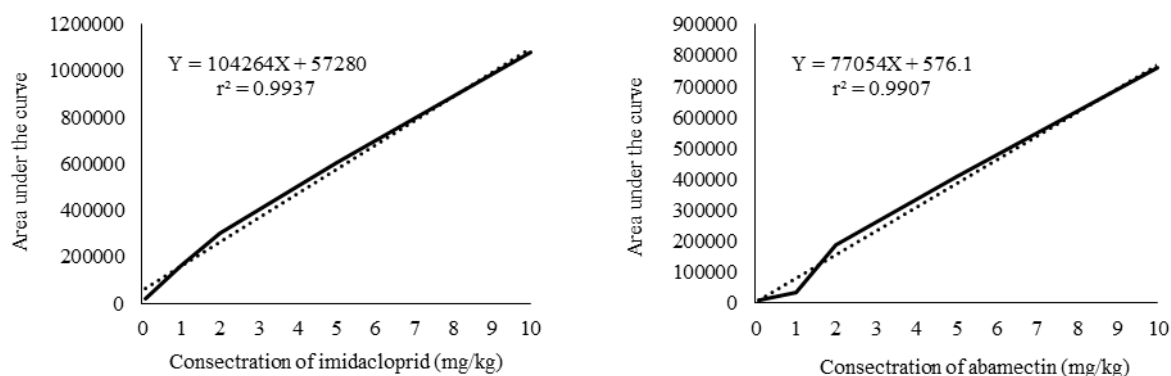


Figure 3. Standard calibration curves of imidacloprid and abamectin.

Table 1. The imidacloprid residues in greenhouse cucumber by applying different cleaning up treatments (mean \pm SE, mg kg⁻¹ product weight).^a

Treatment	Greenhouse 1	Greenhouse 2	Greenhouse 3	Overall
Without clean-up	-	5.2308 \pm 0.6656 a	5.4908 \pm 0.2062 a	5.3608 \pm 0.3170 a
Refrigerated storage for 48 hours	-	1.0956 \pm 0.3834 b	2.5075 \pm 1.1526 ab	1.8015 \pm 0.6283 bc
Immersion in water for 5 hours	-	2.3968 \pm 0.9381 b	3.2127 \pm 0.8623 ab	2.8047 \pm 0.5983 b
Refrigerated storage for 48 h and then immersion in water for 5 h	-	0.1530 \pm 0.0699 b	0.7776 \pm 0.0680	0.4653 \pm 0.1463 c
<i>F</i>	-	13.227	7.192	19.600
<i>df</i>	-	3, 11	3, 11	3, 23
<i>P</i> -value	-	0.002	0.012	< 0.001

^a In each column, the means with the same letter are not significantly different (one-way ANOVA, Tukey test, Alpha=0.05). In overall, different treatments in all greenhouses were compared. (-): Means without any imidacloprid residue.

Table 2. The imidacloprid residues in greenhouse tomato by applying different cleaning up treatments (mean \pm SE, mg kg⁻¹ product weight).^a

Treatment	Greenhouse 1	Greenhouse 2	Greenhouse 3	Overall
Without clean-up	8.8029 \pm 0.0900 a	4.6748 \pm 0.0155 a	10.2926 \pm 0.0661 a	7.9234 \pm 0.8408 a
Refrigerated storage for 48 hours	5.8964 \pm 0.0197 c	3.1160 \pm 0.0978 b	4.8275 \pm 0.0054 c	4.6133 \pm 0.4059 bc
Immersion in water for 5 hours	6.9293 \pm 0.1673 b	3.3621 \pm 0.1378 b	7.4685 \pm 0.0072 b	5.9200 \pm 0.6472 ab
Refrigerated storage for 48 h and then immersion in water for 5 h.	3.0229 \pm 0.0607 d	2.4111 \pm 0.0636 c	4.0144 \pm 0.0907 d	3.1495 \pm 0.2364 c
<i>F</i>	580.831	108.955	2547.714	12.202
<i>df</i>	3, 11	3, 11	3, 11	3, 35
<i>P</i> -value	< 0.001	< 0.001	< 0.001	< 0.001

^a In each column, the means with the same letter are not significantly different (one-way ANOVA, Tukey test, Alpha=0.05). In overall, different treatments in all greenhouses were compared.

unlike the immersion in water and refrigerated storage alone; but, even this treatment could not reduce the residue to the maximum acceptable level.

The percentage reduction of imidacloprid residues in cucumber and tomato by using various clean-up treatments in comparison with the without clean-up treatment is shown in Figure 4. This figure shows that clean-up treatments in cucumber are more effective than tomato crop. The refrigerated storage+immersion in water treatment reduced imidacloprid residue by approximately 90 and 60% in cucumber and tomato, respectively.

The abamectin residue in greenhouse cucumber was measured and compared before and after clean-up operations (Table 3). All selected greenhouses were contaminated with this pesticide. The contamination was to such an extent that only in greenhouse number 3 the refrigerated storage + immersion in water treatment was able to reduce pesticide residue to a level under the maximum recommended limit. In this pesticide, like imidacloprid, refrigerated storage treatment was more effective than immersion in water, however, combining these two treatments reduced the amount of residue effectively. Except greenhouse number 2, statistical analysis did not show any significant difference between the clean-up treatments. In overall, although

refrigerated storage + immersion in water treatment reduced the amount of residues more than 5 times in comparison with control treatment, the difference between cleaning treatments was not statistically significant. In general, using clean-up treatments can notably reduce the amount of abamectin residue in cucumber.

The abamectin residues in tomato were measured and compared before and after clean-up operations (Table 4). Instrumental analysis showed that products of greenhouse number 2 were not contaminated with the pesticide. Product of greenhouse 3 was less contaminated than greenhouse 1, however, after applying treatment of refrigerated storage+immersion in water, abamectin residues in tomatoes were less than the maximum limit recommended. In overall, using refrigerated storage + immersion in water treatment reduced residues of this pesticide more than 3 times in comparison with the control i.e. without clean-up. Table 4 shows that there was no significant difference between cleaning treatments, except in greenhouse number 2. However, high levels of contamination in greenhouse products reinforce the hypothesis that the interval between spraying and harvesting was short.

Figure 5 shows the percentage reduction of abamectin residues in cucumber and tomato by using various clean-up treatments in comparison with the control treatment.

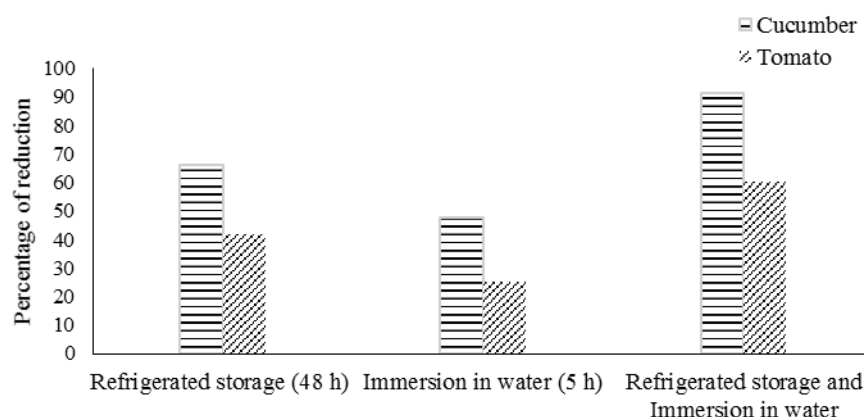


Figure 4. Percentage reduction of imidacloprid residues in greenhouse cucumber and tomato by applying different cleaning up treatments.

**Table 3.** The abamectin residues in greenhouse cucumber by applying different cleaning up treatments (mean \pm SE, mg kg⁻¹ product weight).^a

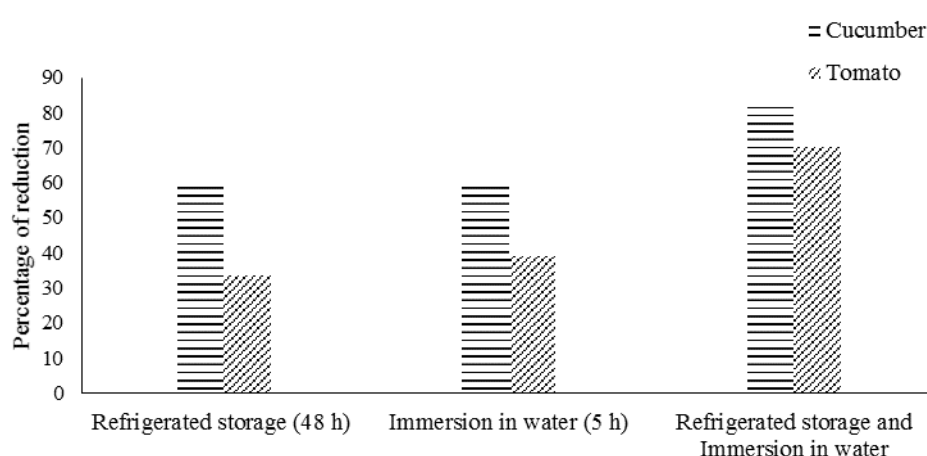
Treatment	Greenhouse 1	Greenhouse 2	Greenhouse 3	Overall
Without clean-up	0.1934 \pm 0.0520 a	0.1547 \pm 0.0067 a	0.1753 \pm 0.0169 a	0.1744 \pm 0.0169 a
Refrigerated storage for 48 h	0.0547 \pm 0.0342 ab	0.1159 \pm 0.0026 b	0.0355 \pm 0.0015 b	0.0687 \pm 0.0157 b
Immersion in water for 5 h	0.1079 \pm 0.0054 ab	0.0563 \pm 0.0015 c	0.0470 \pm 0.0041 b	0.0704 \pm 0.0097 b
Refrigerated storage for 48 h and then immersion in water for 5 h	0.0416 \pm 0.0035 b	0.0321 \pm 0.0021 d	0.0235 \pm 0.0018 b	0.0324 \pm 0.0029 b
<i>F</i>	4.845	211.914	64.801	23.711
<i>df</i>	3, 11	3, 11	3, 11	3, 35
<i>P</i> -value	0.033	< 0.001	< 0.001	< 0.001

^a In each column, the means with the same letter are not significantly different (one-way ANOVA, Tukey test, Alpha= 0.05). In overall, different treatments in all greenhouses were compared.

Table 4. The abamectin residues in greenhouse tomato by applying different cleaning up treatments (mean \pm SE, mg kg⁻¹ product weight).^a

Treatment	Greenhouse 1	Greenhouse 2	Greenhouse 3	Overall
Without clean-up	0.1323 \pm 0.0224 a	-	0.0464 \pm 0.0018 a	0.0894 \pm 0.0217 a
Refrigerated storage for 48 h	0.0911 \pm 0.0069 ab	-	0.0276 \pm 0.0023 b	0.0593 \pm 0.0146 b
Immersion in water for 5 h	0.0660 \pm 0.0098 b	-	0.0430 \pm 0.0022 a	0.0545 \pm 0.0068 b
Refrigerated storage for 48 h and then immersion in water for 5 h	0.0338 \pm 0.0025 b	-	0.0192 \pm 0.0005 c	0.0265 \pm 0.0035 b
<i>F</i>	10.603	-	50.509	3.581
<i>df</i>	3, 11	-	3, 11	3, 23
<i>P</i> -value	0.004	-	<0.001	0.032

^a In each column, the means with the same letter are not significantly different (one-way ANOVA, Tukey test, Alpha= 0.05). In overall, different treatments in all greenhouses were compared. (-): Means without any abamectin residue.

**Figure 5.** Percentage reduction of abamectin residues in greenhouse cucumber and tomato by applying different cleaning up treatments.

Like Figure 4, this figure also shows that the cleaning treatments were more effective on cucumber than tomato. However, Figure 5 indicates that both refrigerated storage and immersion in water treatments had almost the same result in cucumber, although immersion in water was more effective in reducing the abamectin residues in tomato. Generally, the most effective treatment in reducing pesticide residues has been the combined treatment of refrigerated storage and immersion in water. Reduction of approximately 70 to 80% contamination in this treatment is very noticeable.

DISCUSSION

The current study revealed that imidacloprid and abamectin residues in selected crops were more than the permissible Maximum Residue Level (MRL) of these pesticides. For instance, tomatoes of greenhouse number 3 were contaminated with imidacloprid residue more 20 times than MRL (Table 2). In addition, all harvested cucumbers showed illegal abamectin residue at least 5 times more than its maximum residue level (0.03 mg kg^{-1} crop weight) (Table 3). Imidacloprid residue has been measured in fruits, vegetables, and water samples (Daraghmeh *et al.*, 2007). Their results showed that crops such as eggplant, potato, maize, peach and watermelon were severely contaminated by this pesticide. Latest researchers have also proven that 100% of sampled apple, eggplant, and potato were contaminated by imidacloprid in both years (1998 and 1999). These findings enhance need of providing some easy, accessible, and domestic solutions to reduce residues of these two widely used pesticides, especially in highly consumed vegetables.

There are several studies on the effect of different treatments on reducing pesticides residue (Andrade *et al.*, 2015; Kong *et al.*, 2012; Liang *et al.*, 2012; Pirsheh *et al.*, 2016; Vemuri *et al.*, 2014). Comparing the effects of washing with water using different

cleaning products and storage at different temperatures on the residue of organic phosphorus pesticides in cucumbers showed that washing with detergent was more effective (Liang *et al.*, 2012). The detergent sodium bicarbonate caused the greatest reduction in dichlorvos, fenitrothion and chlorpyrifos. This study also indicated that storage at 4°C for 48 hours reduced pesticide residues by 60.9–90.2% (Liang *et al.*, 2012). Another study revealed that washing and peeling process reduced 99% of difenoconazole residue in tomato (Kong *et al.*, 2012). In case of fruits, Ryad and Mahmoud (2016) showed that combination of washing and pickling treatments can reduce residues of some organophosphate pesticides in olive fruits by 82%. Furthermore, washing for three minutes, refrigerated storage at 4°C for 48 hours, and peeling caused reduction of 17, 61 and 80% of diazinon and chlorpyrifos in apple (Pirsheh *et al.*, 2016). All of these results are in accordance with the results of the current study. To clarify, refrigerated storage was more effective in reduction of pesticides residue than immersion in water in both crops, but combination of these two treatments caused reduction in pesticides residue ranging from 60% to 91%. Interestingly, all clean-up treatments in both pesticides had better performance on cucumber in comparison with tomato (Figures 4 and 5). This may be due to the physiological nature of crops texture or their morphological characteristics (surface area to volume ratio, etc.). The immersion in water treatment was more effective in reduction of abamectin (more than 39 and 59% in tomato and cucumber, respectively) than imidacloprid (about 25 and 47% in tomato and cucumber, respectively) residue. It is clear that immersion in water treatment mainly reduces pesticides in surface of fruit not in the fruit tissue, so, this result is expected due to systemic mode of action of imidacloprid.

Based on company information, the recommended pre-harvest interval for imidacloprid and abamectin are



approximately 21 and 14 days, respectively. Based on our results, both crops were heavily contaminated with pesticides residue, indicating that, probably, the farmers did not adhere to recommended pre-harvest interval or dosage. On the other hand, results showed that cucumber greenhouse number 1 had not any imidacloprid residue (Table 1), while it was the most contaminated greenhouse in term of abamectin (Table 3). This means that pesticides were used interchangeably. Besides, the weather is warm in the spring and summer in Varamin and farmers are forced to harvest greenhouse cucumber and tomato about every other day. All of these indicate that there is no choice except using Integrated Pest Management (IPM) for the greenhouse pests. Biological control is inseparable part of any IPM plan. Fortunately, there are a lot of study on biological control agents of greenhouse pests including mites, whitefly, aphids, tomato leaf miner, etc. (Hassanpour *et al.*, 2016; Khanamani *et al.*, 2017; Riahi *et al.*, 2016; Tazerouni *et al.*, 2016). Clearly, appropriate and timely application of these agents can dramatically reduce the needs of chemical pesticides. In addition, teaching economic injury level about pests and food safety concepts to the farmers may be effective in reduction of pesticides residue in crops.

Finally, although using biological control in pest management and some household activities can reduce pesticides residue in crops, applying some strict rules on use of pesticides in agricultural crops (such as forcing farmers to use other pest control methods and producing organic products, increasing pesticides costs, disposal of products with high pesticides residue etc.) can effectively reduce use of pesticides. In addition, it is necessary to study some chemical solutions which can reduce pesticides residue in vegetables and fruits.

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اقدامات خانگی می توانند باقیمانده آفت کش های ایمیداکلوپراید و آبامکتین را در محصولات گلخانه ای کاهش دهند

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چکیده

محصولات گلخانه ای همواره در معرض آلودگی توسط آفت کش های شیمیایی استفاده شده علیه آفات و بیماری ها هستند. مسموم شدن انسان و موجودات غیر هدف، تخریب محیط زیست و تجمع در محیط زیست تنها بخشی از اثرات زیان بار استفاده بیش از حد از آفت کش های شیمیایی هستند. ایمیداکلوپراید و آبامکتین از جمله آفت کش های بسیار پر مصرف برای کنترل آفات گلخانه ای در ورامین استان تهران هستند. هدف از این مطالعه بررسی تاثیر برخی فعالیت های خانگی نظیر نگهداری در یخچال (به مدت 48 ساعت)، غوطه وری در آب (5 ساعت) و ترکیب این دو تیمار بر کاهش میزان باقیمانده آفت کش ها در دو سبزی پر مصرف خیار و گوجه فرنگی بود. نمونه برداری از سه گلخانه از هر محصول و به صورت تصادفی در شهرستان ورامین استان تهران انجام گرفت. باقیمانده آفت کش ها با استفاده از روش کپرز (QuEChERS) استخراج و با استفاده از دستگاه کروماتوگرافی مایع با کارایی بالا (HPLC) مجهز به آشکارساز فرابنفش و ستون C18 (250 × 4.6 mm) اندازه گیری شدند. نتایج نشان داد که محصولات بیش از میزان حداکثر باقیمانده مجاز این آفت کش ها آلودگی داشتند. اگرچه در بیشتر موارد تیمار نگهداری در یخچال بیش از تیمار غوطه وری در آب باعث کاهش باقیمانده آفت کش ها شد، ولی ترکیب این دو تیمار پاک سازی بیشترین تاثیر را در کاهش میزان آلودگی محصولات به آفت کش ها داشت. بر اساس نتایج، تیمار نگهداری در یخچال و غوطه وری در آب میزان باقیمانده آفت کش ایمیداکلوپراید را حدود 91/3 و 60/2 درصد در خیار و گوجه فرنگی کاهش داد. همچنین این تیمار میزان باقیمانده آفت کش آبامکتین را در خیار و گوجه فرنگی حدود 81/4 و 70/3 درصد کاهش داد. این یافته ها نشان داد که برخی راه حل های ساده، قابل دسترس و خانگی می توانند به طور چشمگیری در کاهش باقیمانده این دو آفت کش پر مصرف موثر واقع شوند.