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Assessment of four pesticide residues (diazinon, imidacloprid, primicarb and acetamiprid) in cucumber under greenhouse condition of Iran (Fars province)

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Abstract

The appearance of pesticides in agricultural products is a serious concern for consumers. The purpose of this study was to evaluate the amount of pesticides in cucumber in Fars province. The process of work was that 64 samples of fresh cucumber were analyzed for the presence of 4 pesticides (diazinon, imidacloprid, primicarb and acetamiprid) using the quick, easy, cheap, effective, rugged and safe (QuEChERS) multi-residue extraction, followed by high performance liquid Chromatography-Diode array detector (HPLC-DAD). The residual behavior of diazinon (60%EC), primicarb (50% WP) imidacloprid (35% SC) and acetamiprid (20% SP) in cucumber under the greenhouse condition was studied. The cucumbers were randomly sampled after 2 (initial), 5, 10 and 14 days period after pesticides application.

Both of acetamiprid and primicarb were found to be more persistent in cucumber compared with the other two tested pesticides; data also reported that the lowest residue (level 2.06 and 2.12 $mg.kg^{-1}$) in cucumber was detected 14 days after application of acetamiprid and primicarb, while the lowest residue of diazinon and imidacloprid was 0.24 and 1.16 $mg.kg^{-1}$ within 14 days. All tested residues dissipated 21 days after application in cucumber.

Keywords: Pesticide residues, QuEChERS, HPLC-DAD, Fars

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INTRODUCRION

Pesticides and their alternatives are an undeniable part of modern life, used to protect everything from flower gardens to agricultural crops from specific pests (Saravi & Shokrzadeh, 2011) Pesticides are considered a vital component of modern farming, playing a major role in maintaining high agricultural productivity. Consequently, in high-input intensive agricultural production systems, the widespread use of pesticides to manage pests has emerged as a dominant feature (Saravi & Shokrzadeh, 2011) Pesticides comprise a large number of substances that belong to many different chemical classes, they are applied to crops at various stages of cultivation to provide protection against pests and during post-harvest storage to preserve quality, to ensure the safety of food for consumers and regulate international trade and legislation. Pesticides are considered to be essential for agricultural development; some of them can cause serious ambient contamination, principally in food (Sitta et al., 2000; Gonzalez et al., 2003). This hazard, could be further increased in case of vegetable fruits which are usually consumed freshly e.g. consumed vegetable fruits freshly contaminated with pesticide residues, more than allowable tolerance (Romeh et al., 2000). Various human health related concerns are associated with pesticides, ranging from short-term impacts such as headaches and nausea, to chronic impacts, such as various cancers, birth defects, infertility and endocrine disruption (Romeh et al., 2000; Hiemstra & De Kok, 2007).

One of the major disadvantages of pesticides use is their residues that may remain on/ in food and feed with amounts above the maximum residue limits (MRLs) this could pose health hazards to consumers. The national monitoring program for pesticide residues are the key means of ensuring compliance with regulations and also to create a database to assess the levels of the greatest number of pesticide residues and the level of residues intake. Thorough monitoring of pesticide residues is crucial for proper risk assessment of human exposure through food and if it was within MRLs (Hiemstra & De Kok, 2007). Such information serves greatly to define human exposure to pesticide residues through dietary intake and also help in amending pesticide strategy in the country. Residue analysis provides a measure of the nature and level of any chemical contamination within the environment and of its persistence. The maximum residue levels (MRLs) limit and the types and amounts of residues that can be legally present on foods are set by regulatory bodies' worldwide (Subhash et al., 2014). Like other countries aiming to facilitate self-sufficiency in food production, Iran has rapidly increased its agricultural pesticide use, especially on vegetable crops (Pogačnik & Franko, 2003).

Determination of diazinon, imidacloprid, primicarb and acetamiprid has become increased in the recent years because of the widespread use of vegetables, which is due to their wide ranging biological activity and low persistence (Pogačnik & Franko, 2003). The present work the pesticides included in this study, were selected on the basis of their wide use in vegetable production in Fars province. This study was carried out to assess the residues of the recommended pesticides under controlled greenhouse condition following their applications including investigate the suitable residue determination procedures for selected pesticides, using analytical techniques such as HPLC-DAD.

Materials and methods

a) Pesticides Used

diazinon (60% EC) obtained from Golsam Corporation, iran, imidacloprid (35% SC) obtained from Kavosh Corporation, iran, primicarb (50% WP) and acetamiprid (20% SP) obtained from Mahan Corporation, iran.

b) Chemical and Reagents

Pesticide reference standards including diazinon, primicarb, imidacloprid, acetamiprid, were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany), with certified purity ranging from 97% to 99%. Acetonitrile and sodium chloride were obtained from Merck (Darmstadt, Germany) and anhydrous magnesium sulfate, Bondesil sorbents (primary and secondary amine; PSA particle size40µm) from Sigma-Aldrich (St. Louis, MO, USA). All the organic solvents used were higher performance liquid chromatography (HPLC) grade.

c) Sample Collection

The sample included cucumber is representative of commonly consumed commodity in Iran. The cucumbers were collected from farmer's greenhouses in Iran's major agricultural region (Fars province). Treated samples of cucumbers were randomly picked up for four pesticides (diazinon, imidacloprid, primicarb and acetamiprid) 2 (as initial), 5, 10, 14 days after pesticides spraying. Each sample was a composite of 10 subsamples of the same commodity collected through random sampling. All the samples (1–2 kg each) were placed in sterile polythene bags, in an ice chess box, to avoid contamination and deterioration, labeled, and transported to the laboratory for processing.

A representative portion (200 gr ± 0.01) of the samples was chopped into small pieces and blended using a food processor. The homogenized samples were analyzed immediately or stored in stainless steel jars at 4 ºC and analyzed within 24 h.

d) Sample Extraction and Clean-Up

we were taken for pesticide residues analysis using the QuEChERS method with slight modifications (Koesukwiwat et al., 2010; Hegazy & Nasr, 2003). Blended samples (10 gr ± 0.01) were mixed with (10 ml ± 0.01) acetonitrile and (4 gr ± 0.01) of anhydrous $MgSO_4$ in a polyethylene (PE) tube and shaken for 15 min at 150 rpm. The extract was centrifuged for 30 min at 4,000 rpm. Supernatant was collected and evaporated to dryness under a slow stream of nitrogen at 40°C. Dried extracts were reconstituted with 1ml of acetonitrile. A further 1ml of extract was cleaned with the mixture of (0.050 gr±0.01) primary secondary amine (PSA), (0.15 gr± 0.01) anhydrous $MgSO_4$. The extract was again shaken for 10 min at 50 rpm and centrifuged for 30 min at 4,000 rpm. After agitation and centrifugation, the aliquots of the extract were reconstituted to 3 ml with acetonitrile for clean supernatant was collected for HPLC analysis.

Cucumbers were sprayed after 45-60 days from sowing. The untreated control plots were sprayed with water only.

e) Solutions and Standards

Standard pesticide mix (1 $mg.ml^{-1}$) stock solutions were prepared in acetonitrile for HPLC-DAD analysis. Multi-residue working solutions containing pesticides analyzed by HPLC-DAD was prepared in acetonitrile at a concentration of 0.01, 0.05, 0.1, 0.5, 1, 2, 3,5, 7 and 10 $mg.ml^{-1}$for each pesticides.

f) High Performance Liquid Chromatography- Diode Array Detector (HPLC-DAD) Analysis

An Agilent Technology 1100 high performance liquid chromatography connected to diode array detector was employed. An instrument online workstation (Agilent) was utilized to control the system and for the acquisition and analysis of the data. An Agilent Zorbax XDB C_{18} column (250×4.6mm i.d., 5.0µm) was used for separations and column temperature keeps 25 ºC. The injection loop volume was 10.0 µL. The mobile phase was a mixture of acetonitrile: deionized water (65:35; v/v). The flow rate was 1.0 $ml.min^{-1}$. The DAD monitoring wavelength was chosen at 254 to 275 nm.

Pesticides were identified according to the retention times and quantification was based on peak area. Spiked blank samples were used as standards to counteract possible matrix effects and the samples were spiked before extraction. The applicability of the method to routine analysis was tested in real samples with good results and quality control systems applied demonstrated a good performance and stability in the time.

g) Quality Control

Calibration curves for each pesticide of interest was prepared in accordance with the European Commission guidelines (Hu et al., 2005). Matrix-matched calibration standards were prepared in cucumber blank acetonitrile extracts, using the multi-residue working solutions to reach a concentration ranging from 0.01 to 10 $mg.l^{-1}$ was added for HPLC-DAD determination. Areas under the peak versus concentrations were fitted using linear regression to obtain the equation for

the standard curves for the tested pesticides. Good linearity and reproducibility of calibration curves were achieved (r^2> 0.99).

The performance of the QuEChERS method was evaluated by performing recovery studies (Li et al., 2006; El-Sawi, 2007). The recovery and precision of the method (expressed as relative standard deviation (RSD), %)were measured by analyzing replicate pesticide-free samples of each cucumber, which were fortified at a concentration of 0.01 or 10 $mg.kg^{-1}$ for each pesticide (Figure 1). Sensitivity was evaluated by determining the limit of detection (LOD) and limit of quantification (LOQ), using the signal-to-noise ratio (S/N) of 3:1 and 10:1, respectively. The recovery values ranged from 89% to 93% (precision range, 3.34% to 6.8%) for the 0.1 to 2$mg.kg^{-1}$ concentration, 91% to 95% (precession range, 4.11% to 6.21%) for the 0.1 to 2 $mg.kg^{-1}$concentration,90% to 95% (precession range, 2.21% to 4.52%) for the 0.1 to 2 $mg.kg^{-1}$ concentration, 96% to 100% (precession range, 2.71% to 3.21%) for the 0.1 to 2 $mg.kg^{-1}$ concentration for diazinon, imidacloprid, primicarb and acetamiprid, respectively (Table 1). The LOD for the pesticides ranged from 0.01 to 0.3 $mg.kg^{-1}$and the LOQ from 0.03 to 0.9 $mg.kg^{-1}$ (Table 1). All the pesticides LOD and LOQ values were lower than the MRLs established by Codex (CCPR, 1997-2013) for the cucumber sampled.

RESULTS AND DISCUSSION

a) Residue of diazinon:

Results in Table (2) and Figure (2, a) showed that the concentration of initial deposits of diazinon in cucumber was 8.5 $mg.kg^{-1}$, then gradually decreased to 0.24 $mg.kg^{-1}$, 5 days after application revealing 14.11% loss. This value decline to 0.38 and 0.24 $mg.kg^{-1}$ recording the rate loss 55.29 and 71.76 % at 10 and 14 days, respectively, after 21 days diazinon was not detected. The data show that cucumber could be safely consumed after 21 days of application according to the recommended maximum residue limits (MRLs) for diazinon in cucumber 0.05 $mg.kg^{-1}$according to INSO 12581 (1394). These results are in agreement with those reported by several investigators (Reddy et al., 2007; Hu et al., 2005).

b) Residues of imidacloprid:

The data in Table (2), Fig (2,b) also showed the residues of imidacloprid in cucumber. The initial deposit of imidacloprid was 5.81 $mg.kg^{-1}$, then decreased to 3.62, 2.13 and 1.16 $mg.kg^{-1}$ indicating 37.69%, 63.33%, 80.03% loss after 5, 10 and 14 days respectively. The data indicated that cucumber could be consumed safely after 21 days after application, where (MRLs) of imidacloprid residue in cucumber was 1 $mg.kg^{-1}$ according to EU. Such results are in agreement with those reported by several investigators (Reddy et al., 2007; Iwata et al., 1981).

c) Residues of primicarb:

The residues of primicarb in cucumber in Table (2) and Figure (2,c) revealed that the initial deposit of primicarb was 8.14 $mg.kg^{-1}$, then decreased to 5.37, 3.62 and 2.12 detected after 15 days recording 34.02, 55.53 and 73.96% loss after 5, 10and 14 days, respectively. The data indicated that cucumber could be consumed safely after 21 days after application, where (MRLs) of primicarb residue in cucumber was 0.05 $mg.kg^{-1}$ according to INSO 12581 (1394). Such results are in agreement with those reported by several investigators (Reddy et al., 2007; Alamgir et al., 2014; Attalla, 2006; Al-Khalaf et al., 1995; Allshalaby, 2010).

d) Residue of acetamiprid:

The results given also in Table (2) and Figure (2, d) indicated the residues of acetamiprid in cucumber. The initial deposits found after one hour was 9.27 $mg.kg^{-1}$. The residue levels were decreased to 6.13, 3.18 and 2.06 $mg.kg^{-1}$ showing 24.70%, 65.70% and 77.78% loss after 5, 10, and 14 days, respectively. Maximum residue limits (MRLs) for acetamiprid in cucumber according to European Union was 2 $mg.kg^{-1}$. Data indicated that cucumber could be consumed safely after 21 days. These results were generally in agreement with a number of researchers (Hiemstra & De Kok, 2007; Reddy et al., 2007)

The present results indicated that: acetamiprid and primicarb were found to be more persistent in cucumber compared with the other two tested pesticides; data also reported that the lowest residue level 2.06 and 2.12 $mg.kg^{-1}$in cucumber was detected after 14 days of application for acetamiprid and primicarb, while the lowest residue of diazinon and imidacloprid was 0.24 and 1.16 $mg.kg^{-1}$ within 14 days.

All tested residues dissipated during 21days post treatment on/in cucumber vegetables.

Major priorities should be to create pesticide reduction strategies in agriculture by educating farmers on the use of pesticides and the safe use of clean and safe contaminants and promoting chemical pest control alternatives, such as biological control. Intervention strategies by regulatory agencies to strengthen the implementation of pesticide-control mechanisms at farm and retail levels are necessary to use pesticides. Adherence to pesticide label guidelines, especially before harvesting, must be ensured. It is also important to raise public awareness, which may be directly or indirectly exposed to pesticides, about the risk of these chemicals and how to reduce this risk. Consumers should be aware of practical measures to reduce pesticide contamination in fresh crops, especially fruits and vegetables that may be consumed raw. For example, washing, boiling, and especially peeling, has been shown to reduce pesticide residues in fruits and vegetables (Keikotlhaile et al., 2010; Shabeer et al., 2015). Consequently, a follow-up research is required to determine whether shelling of the skin, in particular, can reduce the pesticide residue in Iran. Finally, due to the increasing use of pesticides in Iran, routine monitoring of pesticide residues in crops is essential to ensure consumer safety.

Conclusion

It is important to respect the pre-harvest-interval (PHI) so that the maximum residue limits (MRLs) for a given crop is not exceeded. Residues found in excess of the MRLs on food would constitute a violation of the Regulations and could also pose a risk to consumers' health. In such situations, the harvested crop could be seized, destroyed or forbidden for export. Use pesticides only for the crops and pests listed on the product label and make sure to follow the application rates, number of applications and PHI stated on the label.

The survey showed that farmers lack knowledge of pests, diseases and their management and rely strongly on pesticides. Based on this information, researchers and extension workers in agriculture sector need to work with farmers in developing IPM strategies that will reduce their heavy reliance on pesticide usage.

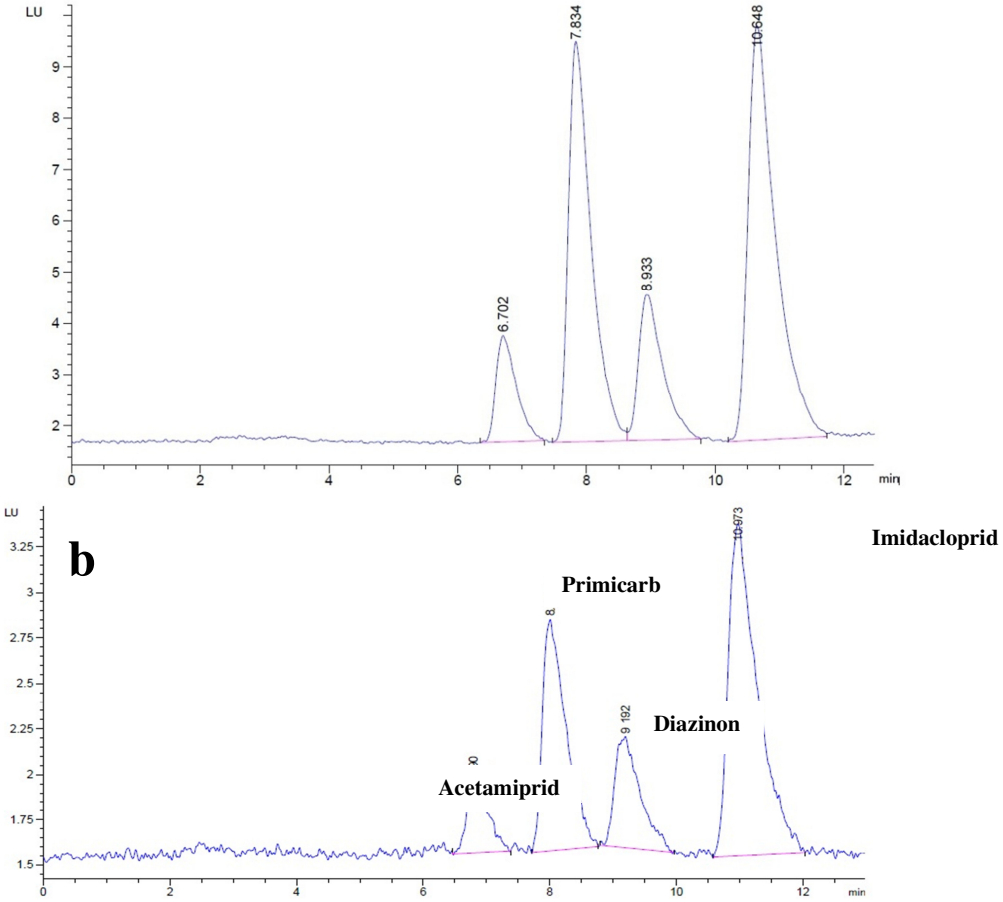

Fig (1). Chromatogram of four pesticides standards at 3 mg.kg^{-1} (a) and Chromatogram of four pesticides after 2 days of application (b)

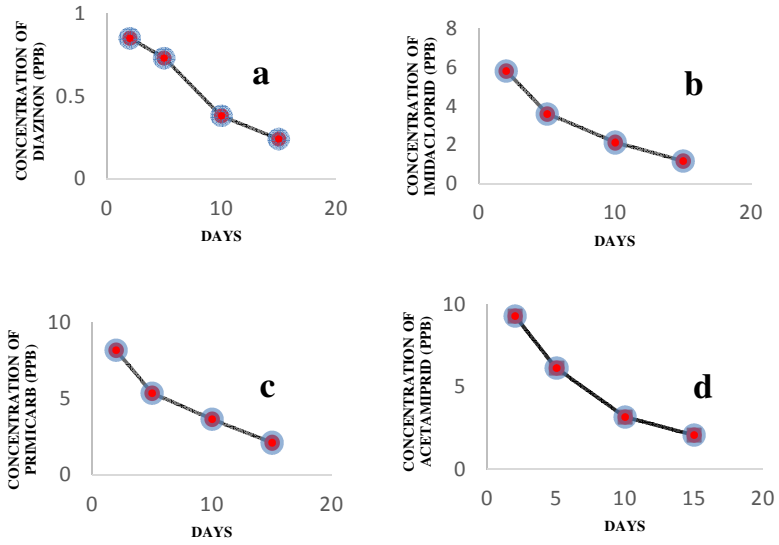

Fig (2) Behavior of diazinon, imidacloprid, primicarb and acetamiprid residues in cucumber.

Table 1. Determinations of pesticides in cucumber samples.

Pesticide	% Recovery (RSD)[a] Fortification Levels ($mg.kg^{-1}$)			LOD[b] ($mg.kg^{-1}$)	LOQ[c] ($mg.kg^{-1}$)	MRLs[d] ($mg.kg^{-1}$)
	0.1	1	2			
Diazinon	90 (3.34)	93 (2.14)	89 (4.51)	0.01	0.03	0.05
Imidacloprid	95 (6.21)	91 (4.11)	92 (6.18)	0.1	0.3	1
Primicarb	90 (3.62)	95 (4.52)	93 (2.21)	0.3	0.9	0.05
Acetamiprid	99 (3.21)	96 (2.71)	100 (2.98)	0.3	0.9	2

[a] Numbers in parenthesis represent relative standard deviation (RSD); [b] LOD: Limit of detection; [c] LOQ: Limit of quantification; [d] MRLs: Maximum residue limits.

Table (2): Mean residues ($mg.kg^{-1}$) of the four tested pesticides in cucumbers during the experiments.

Name of pesticide	**diazinon**	**imidacloprid**	**primicarb**	**acetamiprid**
Times after Application	**Mean residue ($mg.kg^{-1}$)**	**Mean residue ($mg.kg^{-1}$)**	**Mean residue ($mg.kg^{-1}$)**	**Mean residue ($mg.kg^{-1}$)**
2 day	0.85	5.81	8.14	9.27
5 day	0.73	3.62	5.37	6.13
10 days	0.38	2.13	3.62	3.18
14 days	0.24	1.16	2.12	2.06

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Conflicts of Interest:

We don't announce any conflicts of concern. The founder sponsor didn't play a part in the design of the research. In gathering, analyzing, or interpreting data; in writing paper; and in deciding to publish results.

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ارزیابی باقیمانده سموم (دیازینون، ایمیداکلوپراید، پریمیکارب و استامی پراید) در خیار سبز گلخانه ای در منطقه فارس – ایران

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چکیده

وجود باقیمانده سموم بر روی محصولات کشاورزی می‌تواند نگرانی‌های جدی برای سلامت مصرف کنندگان به همراه داشته باشد. هدف از این مطالعه ارزیابی میزان باقیمانده آفت‌کش‌ها در خیار سبز گلخانه‌ای در استان فارس بود. روند کار به این صورت بود که ٦٤ نمونه خیار سبز گلخانه‌ای برای آزمون باقیمانده ٤ سم آفت‌کش (دیازینون، ایمیداکلوپراید، پریمیکارب و استامی پراید) با استفاده از روش استخراج سریع، آسان، ارزان، موثر، مفید و ایمن (کچرز) و دستگاه کروماتوگرافی مایع با کارایی بالا – آشکارساز فرابنفش و مرئی (HPLC-DAD) به‌کار گرفته شد. اثرات و رفتار باقیمانده سموم، پریمیکارب، دیازینون، استامی پراید و ایمیداکلوپراید، در خیار سبز گلخانه‌ای در استان فارس مورد مطالعه قرار گرفت. خیار سبز های سم‌پاشی شده در زمان‌های ۲ (ابتدایی)، ۵، ۱۰ و ١٤ روز پس از استفاده از آفت‌کش مورد نظر نمونه‌برداری گردید.

استامی پراید و پریمیکارب در مقایسه با دو سم دیگر میزان باقیمانده بیشتری داشتند. داده‌های آزمایشگاهی همچنین از میزان ۲/۰٦ و ۲/۱۲ میلی‌گرم در کیلوگرم سموم استامی پراید و پریمیکارب بعد از ١٤ روز حکایت دارد، در حالی‌که کمترین میزان سموم دیازینون و ایمیداکلوپراید بعد از ١٤ روز، ۰/۲٤ و ۱/۱٦ میلی‌گرم در کیلوگرم بود. تمامی بقایای سموم مذکور پس از ۲۱ روز از بین رفته است.

واژه‌های کلیدی: باقیمانده سموم، (HPLC-DAD) ، کچرز، فارس

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