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Monitoring of Residues of Oxydemeton-Methyl in Greenhouse Vegetables in Shahrekord, Iran

Mehraban Sadeghi[1], Abdolmajid Fadaei[*2], Ramazan sadeghi[3], Hassan Hashemi[4]

[1, 2]Department of Environmental Health Engineering, School of Public Health, Shahrekord University of Medical Sciences, Shahrekord, Iran

[3]Department of Pharmacy, Shahid Behesti University of Medical Sciences, Tehran, Iran

[4]Department of Environmental Health Engineering, School of Public Health, Isfahan University of Medical Sciences, Isfahan, Iran

[1]sadeghi1ir@yahoo.com; [*2]ali2fadae@yahoo.com; [3]ramezansadeghi@yahoo.com; [4]h2_hashemi62@yahoo.com

***Abstract*-In order to increase yield and quality of some fruits and vegetables, pesticides are repeatedly applied during the entire growth period. The aim of this study was to monitor Oxydemeton-methyl residuals (as a model pesticide) in cucumbers and tomatoes, as the most consumed vegetables and also grown in greenhouse farms of Shahrekord, Iran. The vegetable samples were collected in during a six-month period from two big greenhouse farms located in Shahrekord vicinity which use this pesticide. Sampling was accomplished after one week of any stage of pesticides spray application. Analyses were performed by Gas chromatography-Mass Spectrometry (GC/MS) method. The average values of pesticide residuals in unwashed, washed and peeled cucumber samples were 0.23± 0.17, 0.18± 0.1, 0.15± 0.6 0.08± 0.5(µg/kg) respectively. The average values of the residues in unwashed, washed and peeled tomato samples were 1.61± 0.8, 0.85± 0.09, 0.67± 0.04 (µg/kg) respectively. The results showed that the pesticide residuals in total cucumber and tomato samples were more than permissible limit. Peeling and washing were effective but didn't decrease the pesticide level lower than the permissible limit. Consumers are recommended to wash and to peel cucumbers and tomatoes before they consume in order to reduce daily intake of the toxicants through the diet.**

Keywords- Pesticide Residuals; Oxydemeton-methyl; Greenhouse Vegetables; Cucumber; Tomato

I. INTRODUCTION

Fresh vegetables, fruits and pulses are important parts of a healthy diet because of the presence of considerable amount of nutrients and minerals in them. They can also turn out to be a source of toxic substances such as pesticides [1]. The use of pesticides in agriculture for raising of crops and livestock has increased after World War II. Organophosphorus compounds consist of a group of 250 chemicals manufactured throughout the world. 140 of these compounds are pesticides, and others are mainly used in industries such as plasticizers and industrial hydraulic fluids and solvents. Organophosphorus Pesticides (OPPs) are the most widely used class of agricultural pesticides among the various pesticides that have used in recent years [2, 3, 4].

Oxydemeton-methyl is an organophosphate pesticide that **is** frequently used in agriculture. Lethal dose standard for oral exposure to Oxydemeton-methyl residual pesticide (LD_{50}) is 0.05 mg/kg [4]. In recent years, many of the studies have shown that OPPs to be mutagenic, carcinogenic, cytotoxic, genotoxic, teratogenic and immunotoxic [5]. In farming some fruits and vegetables, in order to increase quality and yield of the agricultural products, pesticides are repeatedly applied during the entire growth period and sometimes even at the production stage of the fruit. These are absorbed by the vegetables and turned out to be noxious when consumed by human [6]. In 1989, a possible case was reported of a worker developing a rash while picking cucumbers. The field was treated with chlorothalonil and oxydemeton-methyl. In 1985, another case was reported of a worker who developed rashes while harvesting cauliflower in a field that had been treated with chlorothalonil, oxydemeton-methyl, and mevinphos. Vegetables such as tomato, cucumber, cabbage, green beans, Pea, eggplant, spinach, leek, sweet pepper and smooth gourd were reported to have pesticide residues to be even more than Maximum Residue Level (MRL) values recommended by European Union (EU), World Health Organization (WHO) and Food and Agricultural Organization (FAO) [7, 8]. The content of pesticides in various fruits and crops does not only dependent on the sprayed amount over them but also on the content present in soil or water used for irrigation. Therefore, some studies have also been carried out to find out the pesticide content of soil and water directly [4, 9, 10]. The pesticides consumption in Chaharmahal and Bakhtiary Province 279000 kg in 2002 has achieved to 275000 kg in 2007 (1.5% decrease). During this time sub cultivated area reduced from 162644 hectares to 113081 hectares (about 40% decrease). This document has shown that pesticide application increased from 1.72 kg/ha to 2.43 kg/ha during the last five years (41 % increase). Increasing of greenhouse cultivated areas to 150% in recent decade, and also the conditions of greenhouse area are more susceptible to pest invasion than open cultivated areas, these places require more pesticide spray.

The fact is that, about 60- 65% (in warm months) and 75-80% (in cold months) of the total consumed vegetables consumed in Iran are produced in greenhouse farms. No data are available on the presence or the level of pesticides residues in these vegetables. Also based on observation surveys, it's conceivable that spray application of the pesticides in these farms is the most common method to control pest invasion. The present study was undertaken for monitoring the residuals in the vegetables grown under greenhouse conditions located in Shahrekord greenhouse farms in order to provide background

information on the levels of the residuals in this region. Besides, the study also determined the current level of exposure by eating the products containing the pesticides on consumers.

II. MATERIALS AND METHODS

A. Study Area

The vegetable samples were collected during a six-months period from greenhouse farms located in Shahrekord vicinity. Sampling was accomplished after one week of any stage of pesticides treatment.

B. Sampling

About 1-2 kg samples of tomato and cucumber were collected at random from each treatment and the samples were delivered to the laboratory (Cellular and Molecular Researches Center) for analysis. The represent active samples of non washed, washed, peeled and stored for 7 days at 4°c (preservation condition in the refrigerator) were taken from each treatment Fruits that were cut in to small pieces and these sub samples (100 g of each) were weighed into polyethylene sacs and kept deep-frozen until extraction.

C. Reagent and Materials

Oxydemeton-methyl analytical grade with 97.0% purity was obtained from chemservice, inc. Methanol, acetone and ethyl acetate (GC/MS grades) were purchased from Dikma limited (Chine). The chemical structure and characteristics of oxydemeton-methyl are listed in table1 [1].

TABLE I PHYSICAL AND CHEMICAL PROPERTIES OF OXYDEMETON-METHYL [11]

Chemical formula	Molecular (g/mol)	Absorption coefficient	Solubility at 20 °C (g/L)	Hydrolsis half-life(d)	Class	WHO acute hazardous
$C_6H_{15}O_4PS_2$	246.3	30	-	40	Organophosphorous	Highly hazardous

D. Sample Extraction

During laboratory analysis, the samples were chopped and a subsample (l0 g) was weighed into 50 ml Teflon centrifuge tube and extracted with 20 ml acetone using a homogenizer at full speed for 2 minutes. The extract part was centrifuged at 3500 rpm for 5 min and the supernatant was transferred to a clean graduated measuring jar (25 ml) to measure its volume. The solid-phase extraction was carried out using SPE column according to Stajnbahar and Zupanics (2003) method [12]. The extraction columns were conditioned by passing 6 ml of ethyl acetate followed by 6 ml of methanol and then 8 ml of ultrapure de-ionized water. Then the extraction columns were fitted with detachable 70 ml polypropylene reservoirs to contain the diluted sample extract. Sample loading was performed under vacuum at flow rates of 5 ml min^{-1}. After the passage of the extract, the column was dried by vacuum aspiration under increased vacuum for duration time30 min. The pesticide was eluted with 3×2 ml aliquots of ethyl acetate – acetone at the ratio of 90:10 (v/v). The elute was evaporated to, less than 1 ml using gentle stream of nitrogen and then the solvent to low volume after each addition. The extract was quantitatively transferred to 2 ml clean vials and completed with acetone to 1 ml.

E. GC.MS Analysis

Analyses were performed by GC/MS. For identification, 1 ml samples were injected into the GC/MS (Varian CP-3800 GC with MS trap detector Varian Saturn 2200, run in EI mode). Injector temperature was 270 ^{0}C and analysis was done using a capillary column (Varian DB-5 column; 30 m 250 μm I.D., film thickness 0.25 μm). The method started at 150^{0}C, which was held for 2 min, then ramped to 120^{0}C at a rate of 25^{0}C / min, followed by an increase to 270^{0}C (held for 2 min) the method used a split, split ratio 1:10 Helium (99.999%) was used as carrier gas at 1 ml/min.

Recovery rate of oxydemeton–methyl was 80- 95% the minimum detection level (MDL) for oxydimeton – methyl was 0.002 mg/kg.

F. Statistical Analysis

Data were analyzed using non parametric test. Wilcoxon signed ranks and Fridman tests.

III. RESULTS

Tables 2 and 3 summarize the residues of oxydemeton–methyl for two concentrations of applied pesticide at different circumstances of vegetable prepared for consuming.

TABLE II AVERAGE VALUES OF OXYDEMETON–METHYL RESIDUES ON GREENHOUSE TOMATO AND CUCUMBER AT DIFFERENT CIRCUMSTANCES (C_O=1MGL^{-1})

Vegetables	Processing	Min.	Max	Mean ±SD
Cucumber	Non washed	0.03	0.21	0.1±0.05
	Washed	0.01	0.19	0.08±0. 04
	Peeled	0.01	0.16	0.05±0.03
	Storage for 7days	0.02	0.17	0.09±0.03
Tomato	Non washed	0.31	0.45	0.36±0.06
	Washed	0.11	0.39	0.21±0.10
	Storage for 7days	0.15	0.38	0.24±0.11

TABLE III AVERAGE VALUES OF OXYDIMETON–METHYL RESIDUES ON GREENHOUSE TOMATO AND CUCUMBER AT DIFFERENT CIRCUMSTANCES (C_O=2 MGL^{-1})

Vegetable	Processing	Min	Max	Mean ±SD
Cucumber	Non washed	0.08	0.73	0.23±0.11
	Washed	0.04	0.43	0.15±0.06
	Peeled	0.02	0.16	0.08±0.05
	Storage for 7days	0.06	0.25	0.18±0.03
Tomato	Non washed	0.99	1.95	1.61±0.08
	Washed	0.61	0.72	0.67±0.04
	Storage for 7days	0.59	0.92	0.85±0.09

Based on the presented results, processing methods like washing, peeling and storing which were applied for reducing the level of the residues before consumption, caused in a significant decrease ($P<0.05$) in oxydemeton–methyl residuals. No statistical differences were observed between the effects of peeling and washing processes on the reduction of the residue levels. In addition, the results show that peeling is the most effective process for the residue reduction of oxydemeton–methyl in cucumber. Washing and storage for 7 days at 4^0C decreased pesticide residues, but not as effectively as peeling. Tables 1 and 2 also show the residue of oxydemeton–methyl in tomato. According to the results of variance analysis, although no statistical difference was observed between the effects of washing and storage for 7 days at 4^0C on the reduction of oxydemeton–methyl residue levels. Significant reductions in the residue levels of pesticide were obtained through both the washing and storage which aimed at decreasing pesticide residue ($P<0.05$).

Figures 1 and 2 show percentages of detected average of the residues after different processes on the cucumber samples when initial concentration of spray application was 1 and 2 ppm, respectively. The results show the residue level of oxydemeton–methyl was decreased to 20% and 34.79% by the washing procedure, 50% and 65.22% by the peeling procedure and 10% and 21.74% by the storage procedure for 7 days at 4^0C, respectively. Figure 3 shows that the residue of pesticide on tomato was decreased to 58.30% by the washing procedure and 41.21% by the storage procedure for 7 days at 4^0C. Figure 4 shows that the residue of pesticide on tomato was decreased to 58.39% by the washing procedure and 47.21% by the storage procedure for 7 days at 4^0C. The results also show when higher concentration (2 ppm) of the pesticide was sprayed, the residue of pesticide was higher than significantly ($P<0.05$) than at low concentration(1ppm).

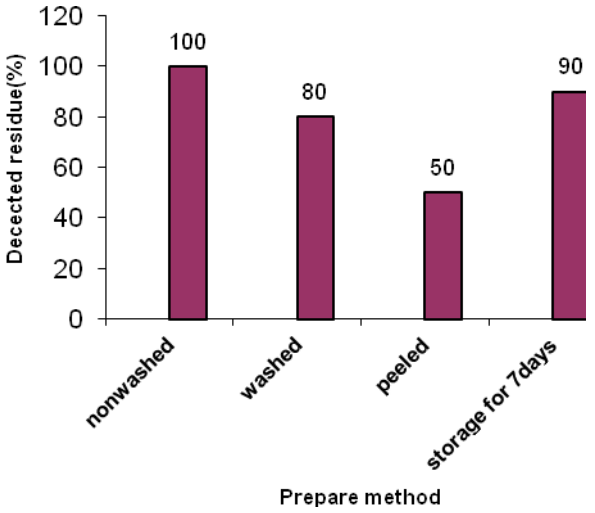

Fig. 1 Percentages of detected average residues after different procedures in the cucumber samples (C_0=1 ppm)

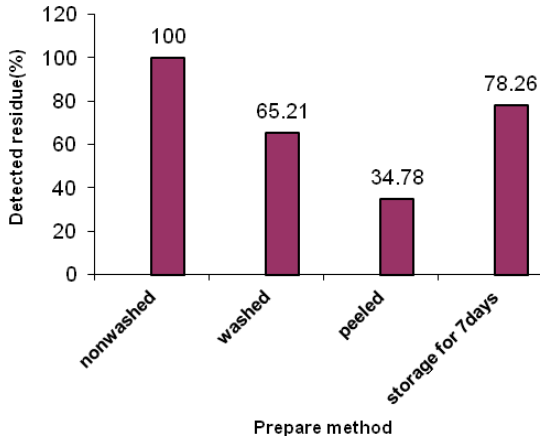

Fig. 2 Percentages of detected average residues after different procedures in cucumber samples (C_0=2 ppm)

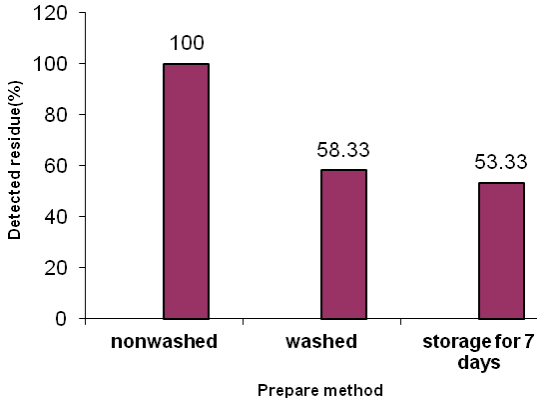

Fig. 3 Percentages of detected average residues after different procedures in tomato samples (C_0=1ppm)

The results showed the residues of oxydimeton–methyl in all the analyzed cucumber and tomato samples after carrying out procedures such as, peeling, washing and storage for 7 days at 4^0C and are higher than maximum residues limits MRL_s.

IV. DISCUSSION

Based on the presented results, processing methods like washing, peeling and storing which were applied for reducing the level of the residues before consumption, caused a significant decrease in oxydemeton–methyl residuals. These results agree with Cengiz et al (2006) and Kin and Huat (2009) [13-16]. It can be presumed that oxydemeton–methyl sustains due to some of its properties such as stability at low temperature, water solubility and vapor pressure. According to the results of variance analysis, significant reductions in the residue levels of pesticide were obtained through both washing and storage which aimed at decreasing pesticide residue. The results showed that the residues of oxydimeton–methyl in the all of the cucumber and tomato samples after peeling, washing and even after storage for 7 days at 4^0C were higher than maximum residues limits MRL_s (0.05 mg/kg) [4]. Similar results were reported by other authors also [17, 18, 19]. From the above results, it is clear that pesticides should be applied correctly according to good agricultural practice, using required amounts only.

Culinary processes are necessary to decrease the residues of pesticide. It can be concluded that setting controlled dose for spray application of this pesticide, controlled greenhouse treatment, peeling, washing and storage applications before consumption, have crucial role in the reduction of the residues of pesticide which pose a serious threat to human health and the environment. Overall, in this study peeling and washing were suggested as the most effective procedures for reduction of the residue of oxydemeton–methyl applied on cucumber and tomato plants.

V. CONCLUSION

The obtained experimental results led to the following conclusions:

Vegetables and fruits of greenhouse farms can be an important way for entrance of the pesticides to human body.

It was also experimentally found that the residues of oxydemeton–methyl in cucumber and tomato even after conventional procedures such as; peeling, washing and storage for 7 days at 4^0C are higher than maximum residues limits (MRL_s).

It was also experimentally found that peeling and washing are the most effect prepared methods for reducing the residue of the pesticide on cucumber and tomato, respectively.

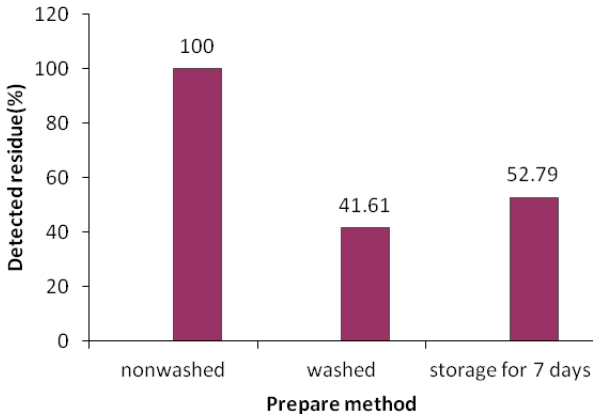

Fig. 4 Percentages of detected average residues after different procedures in tomato samples (C_0=2ppm)

REFERENCES

[1] Z. Kenzevic, M .Serdar, Screening of fresh fruit and vegetable for pesticide residues on croation market. Food Control. 20, pp. 419-422, 2009.

[2] Y. Bai, L. Zhou, J Wang, Organophosphrous pesticide reduces in market foods in Shaanxi area, China. Food Chem. 98, pp.240-242,2006.

[3] V.D. Toan, V.D. Thao, J. Walder, H.R. Schmutz, C.T. Ha, Contamination by selected organochlorine pesticides (OCPs) in surface soil in Hanio Vietnam. Bull. Environ Contam Toxicol. 78, pp. 195-200, 2007.

[4] L. Wang, Y. Liang, X. Jiang, Monitoring of organophosphorus pesticide residues in vegetable of agricultural area in Venezuela. Bull Environ ContamToxicol. 81. pp, 393-396, 2008.

[5] D. Sharma, A. Nagpal, Y.B. Pakade, J.K. Katnoria, Analytical Methods for estimation of organophosphorus pesticide residues in fruits and vegetables. Talanta. 82, pp. 1077-1089, 2010.

[6] B. Kumari, V.K. Madan, J. Singh, T.S. kathpal, Monitoring of Pesticidal contamination of farmagte vegetable from Hisar. Environ Monit Assess. 90, pp. 65-71, 2004.

[7] S. Cakir, R. Sarikaya, Genotoxicity testing of some organophosphate insecticide in the drosophila wing spot test. Food Chem Toxicol. 43, pp.443-450, 2005.

[8] G. Gerais, S. Brosillon, A. Laplanche, C. Helen, Ultra – pressure liquid chromatography – elector spray tandem mass spectrometry for multiresidue determination of pesticides in water. Chermotography A.1202, pp.163-172, 2008.

[9] C. Lopez-Balanco, S. Gomez – Alvarez, M. Rey-Garrote, B. Cancho-Grande, J. Simal Gandara, Determination of pesticides by solid phases extraction followed by gas chromatography with nitrogen – phosphorous detection in natural water and comparison with solvent drop microextraction. Anal Bioanl Chem.384, pp.1002-1006, 2006.

[10] R.M. Gonzalez –Rodriguez, R. Rial- Otero, B. Goncho – Grande, C. Gonzalez-Barreiro, J. Simal- Gandara, A review on the fate of pesticides during the processes with the food – production chain. Food Sci and Nutr. 51, pp. 99-114 ,2011.

[11] US.EPA, Pesticide product information system viewed on December 19, 2010.

[12] D. Stajnbahar, J.L. Zupancic – kralt, Multiresidue method for determination of 90 pesticides in fresh fruits and vegetable using solid – phase extreation and gas chromatography – mass spectomety. Chromatogy A. 1015, pp. 185-198, 2003.

[13] M.F. Cengiz, M. Certel, B. Karakas, H. Gocmen, Residue contents of DDVP (Dichlorvos) and diazinon applied on cucumbers grown in green house and their reduction by duration of a pre-harvest interval and post harvest culinary application. Food Chem, 98, pp. 127-135, 2006.

[14] C.M. Kin, T.G. Huat ,Comparsion of HS- SDME with SPME and SPE for the determination of eight organochlorine and organophosphorus pesticide residues in food matrices. Chromatogr Sci .44, pp. 694-699, 2009.

[15] M.F. Cengiz, M. Certel, B. karakas, H. Gocmen, Residue contents of captan and procymidone applied on tomato grown in greenhouses and their reduction by duration of a pre-harvest interval and post-harvest culinary applications. Food Chem. 100, pp. 1611-1619, 2007.

[16] C. Lentza-Rizos , A. Balokas, Residue levels of chlorpropam individual tubers and composite samples of post- harvest – treated potatoes. Agric and Food Chem. 49, pp. 710-714, 2001.

[17] I. Al- Saleh, I. Al – Doush, A. Echerdo, Residues of pesticides in grains locally grown in Saudi Arabia. Bull Environ Contam Toxicol. 63, pp. 451-459, 1999.

[18] A. Quintero, M.J. Caselles, G. Ettiene, N.G. Decolmenares, T. Ramirez, D. Medina Monoitoring of organophosphorus pesticide residues in vegetable of agricultural area in Venezuela. Bull Environ Contam Toxicol. 81, pp. 393-396, 2008.

[19] B. Kumari, V. Mandan, T. Kathpal Monitoring of pesticide residues in fruits. Environ Monit Assess. 123, pp. 401-412, 2006.