Australian Journal of Basic and Applied Sciences, 7(7): 190-195, 2013
ISSN 1991-8178

Carbamate Pesticides Residues in Different Imported Brands of Tomato Juice

[1]Asma Mohammed Shaderma, [3]Tawfiq Mustafa Al- Antary and [2]Maher Mahmoud Al-Dabbas

[1]Ministry of Agriculture, Jordan
[2]Faculty of Agriculture, Department of Nutrition and Food Processing, University of Jordan, Jordan
[3]Faculty of Agriculture, Department of Plant Protection, University of Jordan,Amman, Jordan

Abstract: This study was conducted to monitor methomyl, oxamyl and carbosulfan carbamate pesticides residues in different imported brands of tomato juice sold in the Jordanian local market during the period from June to September in 2012. Ninty one samples of imported tomato juice from different brands collected randmly from local market were analyzed for selected carbamate residues. Methomyl was found to be the most abundant carbamate pesticide. Fifty six percent of the collected tomato juice samples were found to contain methomyl residues, but less than the maximum residue limits according to EU-pesticides MRL.Oxamyl residues were not detected in any of tomato juice samples.In addition, carbosulfan residues were also not found in any of the collected tomato juice samples.

Key words: Monitoring, Residues, Carbamate Pesticides, Tomato Juice, Methomyl, Oxamyl, Carbosulfan.

INTRODUCTION

Tomato is one of the most common agricultural products in Jordan. It is the main component for several dishes in the Jordanian people diet. Around 129535.9 donums planted with tomatoes in open fields and plastic houses by the year of 2011 to produce 777820.4 tonnes of tomatoes for local use and exportation (Department of Statistics, 2011).

In 2010 the per capita consumption of tomato fruits in Jordan was about 56 kg/year. In addition, Jordan produced 2310 tonnes of tomato processed products like; tomato paste, tomato soup, tomato juice, and ketchup. The total produced and consumed amount in local market is 6181 tonnes/year. Furthermore, the per capita consumption of processed tomato products was about 1 kg/year (Department of Statistics, 2010).

Agricultural activities are closely associated with intensive use of pesticides to control plant pests and to increase the yield of cultivated areas. Tomatoes have been treated intensively with pesticides to control pests especially *Tuta absoluta* and white fly. However, many of these pesticides are used in large quantities. Carbamate was found to be an effective group of pesticides to control plant pests by contact or systemically. In addition, this group does not persist for a long time in the environment in comparison with organo-chlorinated pesticides (Worthing and Hance, 1991, Ahmed and Ismail (2006)). The total imported amount of carbamate pesticides in 2011 were 47799.9 kg and 28694.5 liter. In addition, 10081 kg were produced locally to be used as insecticides, acaricides, nematicides, and fungicides (Ministry of Agriculture, 2011).

Food and Agricultural Organization (FAO) database showed that in 2007 around 129.13, 1234, 549, 1 and 426 tonnes of carbamate pesticides were used in Germany, France, Japan, Bahrain and Saudi Arabia, respectively (FAO, 2007). Carbamate pesticides are classified by the Environmental Protection Agency (EPA) from moderate to highly toxic for humans. They affect the nervous system, which lead to acute toxicity, because they inhabit acetylcholinesterase enzyme (AChE), which regulates acetylcholine (ACh) neurotransmitter (Marrs and Ballantyne, 2004). The excess ACh accumulation causes different signs and symptoms; convulsions, paralysis, coma, nausea, vomiting, diarrhea, salivation, sweating, blurred vision, hypertension, twitching, skeletal muscle (Aldridge, 1971). On the other hand, some carbamate pesticides have a very high decomposition temperature up to 162 ^{o}C and 192 ^{o}C for oxamyl and methomyl, respectively (FAO, 2002 , 2008 ,Hsu, 2008 and Mansour et al 2008).

Monitoring of carbamate residues becomes a compulsory issue all over the world to protect consumer health, and to minimize the consumer exposure to the harmful and unnecessary intakes of pesticides. Many international organization established database for the maximum residue limits (MRL) based on allowable daily intake (ADI) in different commodities like, Codex Alimentarius Commission and European Union Committee to show the safe residual limits for pesticides. The objective of this study is to monitor methomyl, oxamyl and carbosulfan residues in imported tomato juices from different brands in the Jordanian local market.

MATERIALS AND METHODS

Corresponding Author: Asma Mohammed Shaderma, Ministry of Agriculture, Jordan

This study was conducted at Pesticides Residues Department in the Directorate of Plant Wealth-Ministry of Agriculture, Jordan.

Materials:

Chemicals and Reagents:

The following chemicals were used for extraction of methomyl, oxamyl and carbosulfan from tomato juices:

- Anhydrous magnesium sulphate ($MgSO_4$, assay 99%) (Sigma-Aldrich, USA), ashed at 500^oC for 5 hours was carried out before usage to eliminate phthalates and remained water.
- Acetonitrile (CH_3CN, HPLC-grade, assay of 99.8%) (LAB-SCAN analytical sciences, Ireland).
- Acetic acid (CH_3COOH, assay 99%) (J. T. Baker, USA).
- Acetone (C_3H_6O, GC-grade, assay 99.8%) (LabChem, USA).
- Sodium chloride (NaCl, assay 99.9%) (AVONCHEM, UK).
- Primary Secondary Amine (PSA) sorbent, with 40 – 60 µm particle size (Agela Technologies, USA).

2.1.2. Pesticides:

Standards of oxamyl ($C_7H_{13}N_3O_3S$) and Carbosulfan ($C_{20}H_{32}N_2O_3S$) pesticides were obtained from Dr. Ehrenstorfer- GmbH (Augsburg, Germany) with certified purity of 97% for both pesticides. Methomyl pesticide ($C_5H_{10}N_2O_2S$, assay 99.5%) was purchased from (Sigma-Aldrich, USA). Ditalimifos ($C_{12}H_{14}NO_4PS$, assay 97.5%) was used as internal standard.

2.1.3. Apparatus and Equipment:

The following apparatus and equipment were used for preparation and analysis of methomyl, oxamyl and carbosulfan.

- Teflon centrifuge tubes of 10 and 50 ml.
- Pipettes 0.5-5 ml for transferring the sample extract to the clean up centrifuge tube.
- Vials 2 ml capacity
- Containers: Weighing boats, volumetric flasks, graduated cylinders, and other containers in which to contain samples, extracts, solutions, standards, and reagents.
- Micro-syringes, 10-100 µL (SGE, Australia)
- Gas chromatography model (Agilent 6890N, USA) equipped with nitrogen phosphorus detector (NPD). The capillary column was HP-5 with composition of 5% Phenyl 95% methylpolysiloxane with dimensions 30 m x 0.32 mm, 0.50 µm (Agilent, USA).
- Balance (Mettler PM6400, USA) for weighing the samples.
- Balance (Bosch SAE200, Germany) of four digits with maximum weight 200g ± 0.0001 g for weighing reference materials.
- Muffle furnace (Carbolite EML, UK), used for ashing of anhydrous magnesium sulphate for 5 hours at 500^oC.
- Vortex mixer (Heidolph, Germany) used for shaking the samples with 1% acetic acid in acetonitrile solution.
- Freezer (Kelvinator, Italy) used for storing samples at temperature -18^oC before analysis.
- Centrifuge (Jouan, France) for holding 50 ml centrifuge tubes, with speed of 1500-3000 rpm.
- Centrifuge (Hettich, USA) for holding10 ml centrifuge tube for clean up, operated at ambient temperature with 3000 rpm speed.
- Food chopper (Fimar, Italy) used for sample homogenization before taking the required weight for extraction.
- Nitrogen gas cylinder to evaporate acetonitrile to replace it with acetone, since it is not compatible with NPD detector.
- Blender (Laboratory blender 8010D, USA) used for further homogenisation of subsamples

2.2. Sampling:

2.2.1. Imported tomato juice:

Ninety one imported tomato juice samples were collected from the local markets, representing the different brands and batches of tomato juice in Jordan market during the period between June and September, 2012. Thirteen samples from each brand were taken for analysis.

The size of each sample requested for pesticides residues analysis was (0.5 L) as recommended by Codex Alimentarius sampling guidelines (FAO/WHO, 1999).

2.3. Detection Limits and Recovery %:

To assess the efficiency of the extraction method, detection limits and recovery percentages were determined by spiking the blank samples with different standard concentrations solutions for each methomyl, oxamyl and carbosulfan. Tables (1) and (2) show the calculated detection limits and recovery percentages, respectively.

2.4. Analytical Method:

2.4.1. Sample Preparation and Extraction:

The method for extraction of pesticide residues in food called QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) based on the extraction by acetonitrile and partitioning with anhydrous magnesium sulphate was used for extraction of methomyl, oxamyl, and carbosulfan residues from tomato fruits and juice (Anastassiades *et al.*, 2003). Homogenized tomato juice samples were extracted as described for tomato fruits samples as follows:

a. Ten grams from each of homogenised subsample was transferred into 50 ml Teflon centrifuge tube.

b. Acetonitrile with 1% acetic acid solution was prepared on v/v basis (25 ml acetic acid in 2.5 L acetonitrile), solution A

c. Ten ml of solution A was added to the sample and was shaken by the Vortex mixer for 1 min at low speed.

d. Four grams of anhydrous $MgSO_4$ and 1 g of NaCl were added to the sample, then vortexed again for 1 min.

e. Ditalimifos of 0.5 ppm concentration was added as an internal standard.

f. Finally, sample was Vortexed for 30 s and centrifuged at 3000 rpm for 5 min. By this step the sample extract ready for clean-up.

2.4.2. Samples clean-up:

The same procedures for clean-up were followed for tomato fruits and juice samples, 1 ml of the upper acetonitrile layer was transferred into 10 ml centrifuge tube containing 25 mg Primary Secondary Amine (PSA) as sorbent and 150 mg anhydrous $MgSO_4$, then it was vortexed for 30 s, as a final step the centrifuge tube was centrifuged at 3000 rpm for 5 min. The extract was transferred into 2 ml GC vial.

Since acetonitrile is not compatible with NPD detector, it was replaced completely with acetone after flushing with nitrogen gas. After that samples were analysed with GC-NPD to determine the residues of methomyl, oxamyl, and carbosulfan.

2.5. Gas Chromatography With Nitrogen Phosphorus Detector Analysis:

2.5.1. Gas Chromatography Conditions For Determination Of Methomyl:

The chromatographic system consisted of a gas chromatography equipped with nitrogen phosphorous detector, splitless injector and the capillary column HP-5 model No.(19091J-413) with composition of 5% Phenyl and 95% methylpolysiloxane, dimensions are 30 m length, 0.32 mm nominal diameter, and 0.52 µm nominal film thickness. The carrier gas was helium. The operating conditions were: injection volume of 1 µl. The temperature program was: injector temperature 250^{o}C, and detector temperature 300 ^{o}C (Delgado *et al.*, 2001). The oven temperature program was modified to get the best response for methomyl as follows: initial temperature 60 ^{o}C for 1 min; 5 ^{o}C min^{-1} to 90 ^{o}C for 1 min; 20 ^{o}C min^{-1} to 150 ^{o}C for 1 min; 6 ^{o}C min^{-1} to 270 ^{o}C for 1 min (Delgado *et al.,* 2001). For data acquisition ChemStation software was used.

2.5.2. Gas Chromatography Conditions For Determination Of Oxamyl And Carbosulfan:

The same instrument used for determination of methomyl was used for determination of both oxamyl and carbosulfan but with different oven temperature program: initial temperature was 70 ^{o}C for 1 min; 12 ^{o}C min^{-1} to 280 ^{o}C for 15 min (Delgado *et al*., 2001). For data acquisition Chem-Station software was used.

Results:

Detection limits (DL):

The results for the analysis of three blank tomato juice samples showed that the minimum detection limits were 0.0032, 0.0039 and 0.0063 ppm for methomyl, oxamyl and carbosulfan, respectively, as shown in Table (1).

Table 1: Detection limits (DL) of methomyl, oxamyl and carbosulfan pesticides, using GC-NPD.

Pesticide	Detection limits* ± SE (ppm)
Methomyl	0.0032 ± 0.0001
Oxamyl	0.0039 ± 0.0001
Carbosulfan	0.0063 ±0.0002

*Values calculated based on six replicates of the blank tomato fruits and juice samples.

3.2. Recovery test:

As shown in Table (2), the mean recoveries percentages from blank tomato juice samples of methomyl, oxamyl and carbosulfan were ranged from 96.4% to 98.2% for methomyl, 92.8% to 94.3% for oxamyl and 85.4% to 87.3% for carbosulfan.

Table 2: Recovery percentages of methomyl, oxamyl and carbosulfan pesticides from tomato juice samples spiked with different concentration levels.

Pesticide	Spiked amount (ppm)	Recovery %*± SE
Methomyl	0.1	96.7 ± 0.9
	0.5	97.2 ± 1.2
	1.0	96.4 ± 1.0
	5.0	96.9 ± 1.6
	10.0	98.2 ± 0.4
Oxamyl	0.1	92.8 ± 0.9
	0.5	93.3 ± 1.2
	1.0	93.6 ± 0.5
	5.0	94.2 ± 1.5
	10.0	94.3 ± 1.8
Carbosulfan	0.1	85.4 ± 0.4
	0.5	86.0 ± 0.7
	1.0	86.4 ± 0.6
	5.0	86.7 ± 0.6
	10.0	87.3 ± 0.7

* Values are means of three replicates.

3.3. Residues Of Methomyl, Oxamyl And Carbosulfan Inimported Tomato Juice Samples:

Table (3) shows the residues of 91 imported tomato juice samples of seven brands collected from the local markets from June to September, 2012; thirteen samples were collected from each brand.

Methomyl residues were detected in 51 samples, representing 56% of the total analysed samples. The concentrations of methomyl residues were ranged from 0.017 to 0.198 ppm. Methomyl residues found in all tested brands were lower than 0.2 ppm the EU harmonized maximum residue limits (EU, 2005). On the contrary, oxamyl and carbosulfan were not detected in any of the analysed tomato juice samples. Forty samples were found to be free of methomyl, oxamyl and carbosulfan residues, representing 44% of the analysed samples.

Table 3: Methomyl pesticide residues in different imported brands of tomato juice collected from local market from June to September, 2012, using GC-NPD.

Sample No.	Methomyl residues in seven brands* ± SE (ppm)						
	Brand 1	Brand 2	Brand 3	Brand 4	Brand 5	Brand 6	Brand 7
1	ND**	0.066±0.003	0.089±0.006	0.085±0.007	0.187±0.069	0.142±0.011	0.185±0.024
2	0.075±0.003	0.081±0.005	0.073±0.003	0.121±0.023	0.131±0.027	0.170±0.013	0.184±0.008
3	ND	0.075±0.006	0.063±0.006	ND	0.069±0.007	0.047±0.007	0.042±0.003
4	ND	ND	ND	0.149±0.026	0.065±0.006	ND	ND
5	ND	0.032±0.005	ND	0.084±0.005	ND	0.117±0.010	0.104±0.006
6	0.055±0.002	ND	0.085±0.008	0.101±0.018	ND	0.171±0.051	ND
7	0.095±0.001	0.087±0.006	ND	ND	ND	0.140±0.024	0.038±0.005
8	0.070±0.015	ND	0.175±0.035	ND	ND	0.060±0.007	ND
9	ND	ND	ND	ND	ND	0.024±0.004	ND
10	0.030±0.012	ND	ND	0.064±0.003	0.019±0.005	ND	0.060±0.006
11	ND	0.081±0.011	ND	0.158±0.017	0.033±0.015	ND	0.021±0.002
12	ND	0.185±0.015	0.017±0.005	0.026±0.008	0.192±0.027	0.028±0.004	0.198±0.007
13	0.039±0.007	0.138±0.013	ND	0.141±0.030	ND	0.080±0.017	ND

* Values are means of three replicates.
** ND: Not Detected.

Discussion:

The present study showed that the detection limits for methomyl, oxamyl and carbosulfan were 0.0032, 0.0039 and 0.0063 ppm, respectively, using QuEChERS method for extraction and GC-NPD for determination. Delgado (2001) found that the detection limits of methomyl and carbofuran were 0.006 and 0.003 ppm, respectively, using same method of extraction and determination of these carbamate pesticides from powdered potatoes. In addition, similar results were reported by Berger *et al*. (1999), who showed that carbamates were directly and selectively detected using gas chromatography equipped with NPD detector with detection limits ranging from 0.003 to 0.06 ppm.

Methomyl, oxamyl and carbosulfan recoveries were found in the range of 96.4% to 98.2% for methomyl, 92.8% to 94.3% for oxamyl and 85.4% to 87.3% for carbosulfan using GC-NPD and QuEChERS method for extraction. In agreement with these results, Lehotay *et al.* (2005) found that the recoveries for methomyl and oxamyl were > 90% for different commodities. Moreover, Glauner (2012) achieved 98.9% and 103.3% recoveries for methomyl and oxamyl, respectively, from tomato fruits samples in a study performed to validate QuEChERS method for extraction of 313 compounds in different commodities.

Oxamyl and carbosulfan were not detected in any of the collected tomato juice samples from local market in Jordan. On the contrary, methomyl residues were found in 56% of tomato juice samples from different brands. All of the detected methomyl residues were less than 0.2 ppm, which is below the maximum residues limits established by the European Union countries (EU, 2005); e.g. UK (0.2 ppm), France (0.5 ppm) and Spain (0.5 ppm). Our results were in agreement with Rwan *et al*. (2006) results, whom found that methomyl and carbaryl residues were present in juices, cereals, fruits, vegetables and meat in the Canadian retail market at concentrations below the MRL established for these compounds in Canada (0.1 to 10 mg/kg). Lin and Hay (1990) found that the processing of tomatoes could not eliminate carbamate oximes, since they found oxamyl residues in washed tomato, whole canned, wet pomace, juice, paste, ketchup, puree and dry pomace, in concentrations of 0.2, 0.11, 0.06, 0.18, 0.54, 0.36, 0.24 and 0.02 ppm, respectively. The existence of these residues in tasted tomato juice could be explained according to methomyl physio-chemical properties (FAO, 2002). The decomposition temperature of methomyl was 192 ± 3.1°C, which is very high temperature to be reached, while processing of tomato juice to maintain the sensory properties and nutritional value of the tomato juice. Hsu (2008) recommended the use of high pressure (500 MPa) for processing tomato juice at ambient temperature to produce good quality instead of traditional tomato juice processing operation including hot-break at 92 ^{o}C for 2 min, or cold- break 60 ^{o}C for 2 min, since these processing induced significant changes in color, viscosity, and nutritional quality.

The results of carbamate pesticides residues monitoring study in tomato juice indicated the following conclusions:

1- QuEChERS method for extraction of pesticides residues was an efficient method to extract methomyl, oxamyl and carbosulfan from tomato juice with high recoveries.

2- Carbosulfan and oxamyl residues were not detected in any of the collected samples.

3.Methomyl residues were found in 56% of the collected samples of different brands. However, these juice samples were found to contain methomyl residues less than the European Union committee maximum residues limits.

ACKNOWLEDGMENTS

The authors would like to thank the Ministry of Agriculture of Jordan and the University of Jordan for supporting this study.

REFERENCES

Ahmed, M. and S. Ismail, 2006. Residues of Methomyl in Strawberries, Tomatoes and Cucumbers. Pesticide Science, 44(2): 197-199.

Aldridge, W., 1971. The Nature of the Reaction of Organophosphrous Compounds and Carbamates with Esterases. Bulletin of the World Health Organization, 44: 25-30.

Anastassiades, M., S. Lehotay, D. Stajnbaher and F. Schench, 2003. Fast and Easy Multi-residue Method Employing Acetonitrile Extraction/Partitioning and Dispersive Solid-Phase Extraction for Determination of Pesticide Residues in Produce. Journal of AOAC International, 86(2): 412-431.

Berger, T., W. Wilson, T. Berger and W. Wilson, 1999. Recommended Method of Sampling for Determination of Pesticide Residues for Compliance with MRLS, CAC/ GL 33-1999, 2A, Part 1, (2nd ed.), Rome.

Delgado, M., S. Barroso, G. Tostado and L. Diez, 2001. Stability Studies of Carbamate Pesticides and Analysis by Gas Chromatography with Flame Ionization and Nitrogenphosphorus Detection. Journal of Chromatography A, 921: 287-296.

Department of Statistics, 2010. Annual Book, Amman, Jordan.

Department of Statistics, 2011. Annual Book, Amman, Jordan.

EU, 2005. EU-Pesticides Database, Regulation (EC) No 396/2005. Retrieved from http:// www.ec.europa.eu/sanco-pesticides

FAO, 2002. FAO Specifications and Evaluations for Plant Protection Products; Methomyl. Retrieved from http:// www.Fao.org/ag

FAO, 2007. Food and Agriculture Organization Statistics. Retrieved from
http:// www.Faostat.org

FAO, 2008. FAO Specifications and Evaluations for Plant Protection Products; Oxamyl. Retrieved from http:// www.Fao.org/ag

FAO/ WHO Food Standard Program, 1991. Recommended Method of Sampling for Determination of Pesticide Residues for Compliance with MRLS, CAC/ GL 33-1999, 2A, Part 1, (2nd ed.), Rome.

Glauner, T., 2012. New Tools for Unmatched Sensitivity and Unequivocal Confirmation of Pesticides in Food. Agilent Technologies Publications, USA.

Hsu, K., 2008. Evaluation of Processing Qualities of Tomato Juice Included by Thermal and Pressure Processing. LWT-Food Science and Technology, 41(3): 450-459.

Lehotay, S., A. Dekok, M. Hiemetra and P. Van-Bodegraven, 2005. Validation of a Fast Easy Method for Determination of Residues from 229 Pesticides in Fruits and Vegetables Using Gas and Liquid Chromatography and Mass Spectrometric Detection. Journal of AOAC International, 88(2): 595-614.

Lin, W. and R. Hay, 1990. Magnitude of Residues of Oxamyl Insecticide in Tomatoes. DuPont de Nemours Company Report No. 1039.

Mansour, R., I. Kalak and J. Hajar, 2008. Study of Oxamyl Pesticide Residues in Tomato Plant. Journal of Damascus for Agricultural Science, 24(2): 365-377.

Marrs, T. and B. Ballantyne, 2004. Pesticide Toxicology and International Regulation, (1st ed), London: John Wiley and Sons Ltd.

Ministry of Agriculture, 2011. Annual Report, Amman, Jordan.

Rawan, D., V. Roscoe, R. Trelka, C. Hanson, T. Kalovich and R. Dabeka, 2006. N-methyl Carbamate Pesticide Residues in Conventional and Organic Infant Food Available on the Canadian Retail Market. Food Additives and Contaminants, 23(7): 651-659.

Worthing, C. and R. Hance, 1991. The Pesticide Manual, (9thed.), England: British Crop Protection Council.