Evaluation of Diazinon and Oxydemeton-methyl Residues by GC/NPD in Tomatoes Grown in Kerman Greenhouses

F. Ganjeizadeh Rohani¹, ²*, V. Mahdavi³, and M. M. Aminaee³

ABSTRACT

Pesticides are one of the major pollutants in the environment. The residue of pesticides has been reported to be at a critical level in agricultural crops, especially in greenhouse crops. Tomato is considered as an important vegetable in Iranian food basket and is produced in fields and greenhouses. So, the current study aimed to assess diazinon and oxydemeton-methyl levels in tomatoes sampled from five Kerman greenhouses. The extraction of these pesticides was carried out by mixture of dichloromethane and acetone. The extracts were cleaned up according to solid-phase extraction method. The pesticide residue was then determined by capillary gas chromatography and through nitrogen-phosphorus detection. The obtained results showed that the recovery level was estimated at 86.7 and 84.3% with RSD ≤ 16.0%, the limit of detection was estimated at 0.026 and 0.035 mg kg⁻¹, limits of quantification stood at 0.091 and 0.115 mg kg⁻¹, and linearity \( r^2 = 0.997 \) and \( r^2 = 0.989 \) for diazinon and oxydemeton-methyl, respectively, in tomato samples. The mean of diazinon residue was calculated at 0.276 mg kg⁻¹, which was 5.52 times the national Maximum Residue Limit (MRL= 0.05 mg kg⁻¹), and the mean of oxydemeton-methyl was 1.624 mg kg⁻¹, being 1.624 times the MRL (1 mg/kg).

Keywords: Maximum residue limit, Pesticide, Pollution, Solid Phase Extraction.

INTRODUCTION

Pesticides, as a risk to food safety, are one of the greatest environmental contaminants that are found in agricultural products. Different countries cultivate various types of crops every year that are exposed to damages by pests, diseases, and weeds. Since the global population is continually rising, increasing crops production and food is important for human. Greenhouse agriculture is one of the useful methods for raising crops production. Due to the seasonality of agricultural products and an increase in their consumption, especially consumption of fresh vegetables and fruits (for example, tomato in salads and sandwiches), farmers tend to plant tomatoes in greenhouses. Under greenhouse conditions, pesticides are the easiest way to control diseases and pests. Pesticide residues may cause many diseases such as tumors, cancer, nervous system disorder and brain damage, mental diseases and tumor in the central nervous system, respiratory system disorder (e.g. asthma), abortion, Parkinson, infertility, and dermatological diseases (IARC, 1991; Fleming et al., 1994; Gupta 2004).

Researchers studied pesticide residues in lettuce, green beans, artichokes, and...
tomatoes at Valencia University. They employed solid-phase cleanup by aqueous solutions for purification after diluting the extracts. Pesticide determination process was conducted by capillary Gas Chromatography (GC) and by electron capture detector. They analyzed 48 samples, of which ten samples contained pesticide residue at a level below the MRL (Maximum Residue Limit) in Spain. (Viana et al., 1996).

For 5 years, from 1991 to 1995, in Ontario-Canada, 802 fruit (apples and peaches) and 1536 vegetable (lettuce, potatoes, and tomatoes) samples that were important crops, were investigated. Generally, 31.5% of them had no detectable pesticide residue, whereas 68.5% of the samples contained one or more residues. Most of the residues were present at small amount; 48% of them were under 0.1 mg kg\(^{-1}\), and 86% were under 1 mg kg\(^{-1}\). Ninety tomato samples were analyzed, of which 48.9% contained one or more pesticide residues below MRL level. However, violations of MRL were observed in only 3.2% of vegetable samples, and in merely 3.1% of the fruit samples. This study indicated that Ontario fruits and vegetables contained only minimal amounts of pesticide residue and one third of the monitored commodities did not contain any residues. Furthermore, when residues were detected, they were typically at a level below the amount that is set as safe and allowable under the law (Ripley, 2000).

The relationship between the usage of pesticides and their residue in ripe greenhouse tomatoes in Poland was studied by researchers at the Institute of Plant Protection. They concluded that the average residue level of pesticide remaining in ripe tomatoes stands in a quantitative relation to its dose, which can be expressed by the equation \( R = 0.24 \text{ mg kg}^{-1} \). The quantitative dependence of residue level on dose enabled the evaluation of greenhouse tomato growers with respect to observing the rules of good agricultural practice and plant protection act, obligatory in Poland since 1996.

Quantitative dependence of residue level on dose may be a reliable base for registration of new agrochemicals (Sadlo, 2000).

In the central laboratories of Slovenia, pesticide residue, such as diazinon, of 115 samples of apple, carrot, cucumber, lettuce, pear, potato, spinach, and string bean were measured in 2005. Samples were analyzed for the presence of 66 different active compounds using three analytical methods. According to the results, 68.7% of the samples did not contain any residues, while only 2.6% of agricultural product samples exceeded MRL (Cesnik et al., 2006).

The level of pesticide residue in the tomato plants was examined in an open field. The obtained results suggested that the tomatoes sold as fresh products had a lower dose of benalaxyl (Gambacorta et al., 2005).

Researchers in Ghana evaluated the residue level of selected pesticide used on tomato crops. The results confirmed that pesticide residues, whose amount was quantified by further analysis, were indeed present in tomatoes. An analysis of organochlorine and organophosphorus residue levels in fruits indicated that chlorpyrifos, an active ingredient of pesticides being used on vegetables, had the greatest level of residue (10.76 mg kg\(^{-1}\)) in Ghana. The lowest residue level was 0.03 mg kg\(^{-1}\) for pirimiphos-methyl residue (Essumang et al., 2008).

Organophosphorus pesticide residues were investigated in vegetable by researchers in Thailand. They detected the highest diazinon levels in farm samples (13.6%), market samples (16.6%), and in supermarket samples (66.8%). Their finding showed that 16 samples from farms and 14 samples from markets contained pesticide residues at or above European Union MRL levels (Sapbamrer and Hongsibsong, 2014).

Human exposure to pesticide is thus a serious concern, but, farmers carry on using pesticides in developing countries (Monfared et al., 2015). Hence, monitoring programs are the first and the main strategy to decrease the risk of exposure for humans. Also, the total annual tomato production is
about 500,000 tons in Kerman that is about 10% of the total tomato production in Iran. Therefore, it is very important to investigate pesticide residue level in vegetables such as tomato that are widely consumed. The current study aimed to assess diazinon and oxydemeton-methyl levels in tomatoes sampled from five Kerman greenhouses.

MATERIALS AND METHODS

All the chemicals were used in analytical and pesticide grades. Diazinon (99%) and oxydemeton-methyl (95%) standards were obtained from Sigma-Aldrich®, USA. C_{18} (200 mg 3 mL⁻¹) tubes were purchased from capital HPLC Ltd for Solid Phase Extraction (SPE).

Samples

Tomato samples were obtained every 7 days from October through November, 2009, from five active greenhouses in north of Kerman Province. The cultivation area was more than 500 m² in all the selected greenhouses. Only one type of tomato seed (Lycopersicon Esculentum V.F. Jina) was used, and drip irrigation was employed for all samples. About 2 kg tomatoes were taken randomly from the rows in the greenhouses. They were carried in polyethylene bags to the laboratory. Also, the first sampling was done simultaneously with the first harvest for tomatoes market.

Extraction and Clean up

Samples were washed in distilled water (free pesticide residue) and were then dried at room temperature. Ten grams of homogenized fresh tomato sample was blended in a blender containing 100 mL of acetone, 75 mL of dichloromethane and 15 g of sodium chloride for 3 minutes. The homogenized mixture was allowed to separate into its organic and aqueous layers in a separator funnel. The organic phase was spilled into a flask and 3 g of sodium sulfate was added to remove the remaining water. At first, C_{18} column was washed by 10 mL of n-hexane for conditioning in SPE clean-up. Then, 2 mL of extract was transferred to the C_{18} SPE cartridge. Finally, it was washed with 10 mL of n-hexane (Kuet and Seng, 2004). The eluted substance was dried by nitrogen gas with high purity at room temperature and then solved in one mL of methanol for injection into gas chromatography (Kuet and Seng, 2004).

Determination of Insecticide Residue

Diazinon and oxydemeton-methyl residue in the samples were determined by a Shimadzu (2010 model) Gas Chromatography (GC) with a cb-5 capillary column (3 m×0.25 mm×0.25 mm) and nitrogen-phosphorus detection. In the gas chromatograph, the injector and detector were set at 280 and 300 C, respectively. The GC oven was programmed with 100 C for initial temperature and maintained for 2 minutes, then achieved 280 C at a rate of 20 °C min⁻¹ and maintained for 5 minutes.

Mixed N2/air was used as the carrier gas at a flow rate of 3.76 mL min⁻¹. Hydrogen was used as makeup gas at a flow rate of 27.5 mL min⁻¹. Figure 1 shows a GC/NP chromatogram of these pesticides in tomato samples. Calibration curves were created for each compound using five different concentration levels (0.5, 1.0, 1.5, 2.0, 2.5 mg kg⁻¹) with triplicate for each pesticide that is shown in Figure 2. The recoveries of diazinon and oxydemeton methyl from fortified organic tomato sample at 0.5 mg/kg with 5 replicates were studied. The SANCO Guidelines recommend recovery percentages of 70–120% and RSD≤ 20% (Pihlström et al., 2009). In this research, recoveries were obtained for diazinon and oxydemeton-methyl with 86.7 and 84.3% and with RSD≤ 16.0%, respectively.

The Limit Of Detection (LOD) and Limit Of Quantification (LOQ) were 0.026 and
RESULTS AND DISCUSSION

All samples were collected from five greenhouses in north of Kerman Province. The results of diazinon and oxydemeton-methyl residues are presented in Tables 1 and 2, respectively, which show the means for three replications.

The results show that, among various tomato samples, approximately 40% were contaminated with diazinon and 92% were polluted with oxydemeton-methyl, which are more than national MRL (0.05 and 1.0 mg kg\(^{-1}\), respectively). The mean of diazinon residue was calculated at 0.276 mg kg\(^{-1}\), which was 5.52 times the national Maximum Residue Limit (MRL= 0.05 mg kg\(^{-1}\)), and the mean of oxydemeton-methyl was 1.624 mg kg\(^{-1}\), being 1.624 times the MRL (1 mg kg\(^{-1}\)).

Diazinon residues detected in 1.7% of tomato samples was below MRL, while

0.091 mg kg\(^{-1}\) for diazinon and 0.035 and 0.115 mg kg\(^{-1}\) for oxydemeton-methyl, respectively. These effects considered in quantitative results by using tomato extract in standard solutions of the analytes and the calibration curves were prepared considering matrix effect.
Table 1. Diazinon residues in tomato samples in Kerman greenhouses (n=3).

<table>
<thead>
<tr>
<th>Period of sampling</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
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<tr>
<td>1st</td>
<td>&lt;0.026</td>
<td>0.456</td>
<td>&lt;0.026</td>
<td>1.863</td>
<td>0.928</td>
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<tr>
<td>2nd</td>
<td>&lt;0.026</td>
<td>0.573</td>
<td>&lt;0.026</td>
<td>&lt;0.026</td>
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<tr>
<td>3rd</td>
<td>0.889</td>
<td>0.422</td>
<td>&lt;0.026</td>
<td>1.752</td>
<td>&lt;0.026</td>
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<tr>
<td>4th</td>
<td>&lt;0.026</td>
<td>&lt;0.026</td>
<td>&lt;0.026</td>
<td>&lt;0.026</td>
<td>&lt;0.026</td>
</tr>
<tr>
<td>5th</td>
<td>&lt;0.026</td>
<td>0.866</td>
<td>0.895</td>
<td>1.098</td>
<td>1.091</td>
</tr>
<tr>
<td>6th</td>
<td>0.362</td>
<td>0.509</td>
<td>&lt;0.026</td>
<td>&lt;0.026</td>
<td>&lt;0.026</td>
</tr>
<tr>
<td>7th</td>
<td>&lt;0.026</td>
<td>&lt;0.026</td>
<td>0.783</td>
<td>&lt;0.026</td>
<td>0.161</td>
</tr>
<tr>
<td>8th</td>
<td>&lt;0.026</td>
<td>&lt;0.026</td>
<td>&lt;0.026</td>
<td>&lt;0.026</td>
<td>&lt;0.026</td>
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<tr>
<td>9th</td>
<td>&lt;0.026</td>
<td>&lt;0.026</td>
<td>&lt;0.026</td>
<td>&lt;0.026</td>
<td>&lt;0.026</td>
</tr>
<tr>
<td>10th</td>
<td>&lt;0.026</td>
<td>0.596</td>
<td>&lt;0.026</td>
<td>&lt;0.026</td>
<td>0.922</td>
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<tr>
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<td>&lt;0.026</td>
<td>0.228</td>
<td>0.121</td>
<td>0.321</td>
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<tr>
<td>12th</td>
<td>&lt;0.026</td>
<td>&lt;0.026</td>
<td>&lt;0.026</td>
<td>&lt;0.026</td>
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Table 2. Oxydemeton-methyl residues in tomato samples in Kerman greenhouses (n=3).

<table>
<thead>
<tr>
<th>Period of sampling</th>
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<th>3</th>
<th>4</th>
<th>5</th>
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<tr>
<td>1st</td>
<td>0.494</td>
<td>2.076</td>
<td>0.955</td>
<td>5.099</td>
<td>3.715</td>
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<tr>
<td>2nd</td>
<td>0.681</td>
<td>2.338</td>
<td>0.905</td>
<td>0.626</td>
<td>2.441</td>
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<tr>
<td>3rd</td>
<td>4.012</td>
<td>1.499</td>
<td>0.707</td>
<td>3.944</td>
<td>0.944</td>
</tr>
<tr>
<td>4th</td>
<td>0.675</td>
<td>0.610</td>
<td>0.629</td>
<td>1.385</td>
<td>2.625</td>
</tr>
<tr>
<td>5th</td>
<td>0.245</td>
<td>3.545</td>
<td>3.246</td>
<td>&lt;0.035</td>
<td>2.778</td>
</tr>
<tr>
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<td>2.944</td>
<td>3.310</td>
<td>1.193</td>
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<td>0.565</td>
</tr>
<tr>
<td>7th</td>
<td>&lt;0.035</td>
<td>0.785</td>
<td>1.310</td>
<td>1.916</td>
<td>3.510</td>
</tr>
<tr>
<td>8th</td>
<td>0.337</td>
<td>0.658</td>
<td>0.361</td>
<td>3.192</td>
<td>1.739</td>
</tr>
<tr>
<td>9th</td>
<td>0.984</td>
<td>0.591</td>
<td>1.615</td>
<td>0.638</td>
<td>2.785</td>
</tr>
<tr>
<td>10th</td>
<td>&lt;0.035</td>
<td>3.773</td>
<td>1.035</td>
<td>1.457</td>
<td>4.689</td>
</tr>
<tr>
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<td>&lt;0.035</td>
<td>1.071</td>
<td>2.067</td>
<td>3.016</td>
<td>1.785</td>
</tr>
<tr>
<td>12th</td>
<td>&lt;0.035</td>
<td>1.021</td>
<td>1.991</td>
<td>2.018</td>
<td>1.221</td>
</tr>
</tbody>
</table>

38.8% of samples contained detectable amounts of diazinon residues above MRL, and diazinon was not detected in 60% of tomato samples. High residue levels of diazinon in some greenhouses in the sampling period were due to excessive application of pesticide and incorrect management in greenhouses (for example, in greenhouse 1 in the 3rd and 6th sampling, Table1). For oxydemeton-methyl, 31.7% of analyzed samples contained an amount of residue below MRL, 60% above MRL, and oxydemeton-methyl was not found in 8.3% of the tomato samples.

The means of these insecticide residues in tomatoes are shown in Table 3. This table shows that in greenhouse 1, the pesticide residue was lower than that in others. Health and safety regulations were observed in this greenhouse with greater precision and, as a result, less pests and diseases were found in samples taken from this greenhouse. According to reports and statistics, per capita consumption of fresh tomato in Iran was 25-50 kg in 2009-2011. Since it is a high level of consumption, production of healthy tomato crops is of vital significance. As per the obtained results, the mean of pesticide residue in
greenhouse tomato samples of Kerman stood higher than that of the national MRL. Overall, similar results were found in other research projects on pesticide residues in food and crops. Tomato samples as fresh vegetable were collected from Xiamen, and were analyzed in Chines Academy of Agricultural Science. They analyzed 231 samples 59.7% of which were without detectable pesticide residue, 29.9% of the samples contained residues bellow MRL, and 10.4% samples had pesticide residue above MRL (Chen et al., 2011). Also, Darko and Akoto (2008) analyzed 350 local fruits and vegetables that were purchased from markets in Kumasi in Ghana. The results showed that 37.5% of samples contained no detectable levels of the studied pesticide residue, 19.0% of them had residues above MRL, and 43.5% of the samples showed results below MRL. In their study, 30 tomato samples were analyzed and 67% of them contained pesticide residues. Quintero et al. (2008) determined the residue of organophosphorus pesticides in vegetables. They analyzed 96 samples; of which 46 samples were contaminated with pesticide residues. Methamidophos and chlorpyriphos were found in tomato samples and diazinon was not detected in samples (Quintero et al., 2008). In Colombia, researchers assessed the presence of pesticide residues in fresh tomatoes. At least one pesticide was detected in 70.5% of the samples. They concluded that monitoring programs must be in place to control the contamination of foods and crops such as tomatoes (Arias et al., 2014).

The insecticides in the present study, diazinon (Freeman et al., 2005; Guyton 2009) and oxydeneton-methyl(Pesticides, 2006) were classified as group 2A. The reported levels of pesticide residue indicate that the level of pesticides in greenhouse tomatoes in Kerman is high and critical. Also, results show that farmers use pesticides excessively and haphazardly. However, the information obtained from Kerman greenhouses seems inadequate. This shows that more samples is necessary for analyzing, and surveys need to be conducted over a more extended period of time to obtain data for various greenhouse conditions. Tables 1 and 2 present the results obtained for diazinon and oxydemeton-methyl residues, respectively.

In this study, the mean levels were above the maximum permitted level. The results pose a high risk to human health due to possible indirect exposure. Therefore, extensive and repeated monitoring is necessary. Due to the fact that pesticide residue is found worldwide in many tomato samples in greenhouses, the risk of cancer is significantly attributable to ingestion, and pesticide residue need further evaluation, which is hampered by limited availability of chronic toxicity data. More knowledge on people's diet and tomato consumption per capita such as processed, fresh, and varied forms of it in their diet is needed.

It should be noted that the level of these pesticide residues in contaminated samples had high degree. Also, sensitive methods with low detection limits are required for determining pesticide residues. The gas chromatography method is effective for achieving low detection limits, however, samples need to be cleaned up. GC is accurate, sensitive, and very reliable; hence, it is suitable for monitoring studies. Unfortunately, there is little information about the behavior of diazinon and oxydemethon methyl after tomato processing; therefore, more extensive investigations are necessary. It is better that the subsequent studies consider investigation of the factors affecting reduction or removal of pesticide residues.

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REFERENCES

بررسی باقیمانده دیازینون و اکسی دیمتون متیل در گلخانه‌های گوجه فرنگی قم

GC / NPD

ف. گنجه ای زاده روحانی، و. مهدوی، و. م. امینی

چکیده

آفت کش‌ها به عنوان یکی از مواد آلوده کننده محیط زیست به شماری آن‌ها، باقیمانده آفت کش‌ها در محصولات کشاورزی به تغییر محصولات گلخانه‌های از اهمیت خاصی برخوردار است. گوجه فرنگی به عنوان یکی از دسته‌های ایرانی آبزیان موجود است. هدف از این تحقیق ارزیابی میزان دیازینون و اکسی دیمتون متیل در گلخانه‌های گوجه فرنگی به کمک گونه‌گرایی گوجه فرنگی قم و ازمودن پذیرفته شده استخراج با استفاده از استخراج با فاز جامد به تغییر گرادیئن. سپس مقدار آن‌ها با کرومتوگرافی گازی مجزه به دکتر نیتروز-فسفر تعیین گردید. درصد اکسیژنی دو سم دیازینون و اکسی دیمتون متیل به ترتیب 7.8% و 0.25 mg kg⁻¹ با RSD ≥ 16% و 115 kg⁻¹ و درجه خطي 997 = 0.9893. حذ تشخیص 0.0242 و حد کمی 0.91 و mg kg⁻¹، MRL ملی (mg kg⁻¹) 0.00 = 0.08 mg kg⁻¹، حذ کمی (MRL) 0.08 mg kg⁻¹، حذ مکزیمم (MRL) 0.00 = 0.08 mg kg⁻¹، حذ مکزیمم (MRL) 0.08 mg kg⁻¹، حذ مکزیمم (MRL) 0.08 mg kg⁻¹، حذ مکزیمص (MRL) 0.08