Residue Content of Chlorpyrifos Applied to Greenhouse Cucumbers and Its Reduction during Pre-Harvest Interval and Post-Harvest Household Processing

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ABSTRACT

Chlorpyrifos is widely used to control various insect pests on greenhouse cucumbers in Iran. In this study, the effects of the household processing such as washing, peeling, and refrigeration storage on the reduction of residue levels in cucumbers were investigated in different groups. Samples were collected at 1 hour to 14 days after application of chlorpyrifos and analyzed to determine the content of chlorpyrifos. Analysis was carried out by the QuEChERS method using GC-NPD. Residue levels in samples throughout a period of 14 days showed a gradual and significant (P< 0.05) decrease in contents of chlorpyrifos. The half-life (t1/2) of chlorpyrifos applied to cucumbers was 1.9 days. Chlorpyrifos was detected in concentration ranges of 0.02-4.73 mg kg⁻¹. Household processing, such as washing, peeling and refrigeration storage, was effective in reducing the residue levels. Peeling was the most effective way to reduce residues in the cucumbers. Obtained results showed that a waiting period after chlorpyrifos application and household processing are required for safe consumption of cucumber.

Keywords: Chlorpyrifos half-life, Food safety, Peeling, QuECHERS method.

INTRODUCTION

Pesticides are widely used in production of agricultural crops, which are susceptible to attacks by insects and diseases. Therefore, there has been a growing interest in detecting and quantifying pesticide residues in agricultural produce intended for human consumption (Hercegová et al., 2007; Yuan et al., 2014). The increasing public concern over the potential health risk associated with exposure to pesticides has led to the regulation of Maximum Residue Limits (MRLs) of pesticide in food commodities (Cengiz et al., 2007). Various pesticides are used in greenhouses to maintain high crop yields (Juraské et al., 2008; Juraské et al., 2009). Greenhouse cucumbers are among the major vegetable crops that are grown on a large scale in Iran, but they are attacked by several types of insects which require frequent use of insecticides. Chlorpyrifos is a non-systemic organophosphorus insecticide with broad spectrum insecticidal activity (Feo et al., 2010; Liang et al., 2012). This insecticide is registered for application on more than 40 different food commodities and is widely used to control various insect pests on greenhouse cucumbers in Iran (Hassanzadeh et al. 2012). As a result of

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their widespread application, the control and monitoring of residual levels of this insecticide in food is highly necessary, not only in order to meet regulatory requirements but especially to protect the consumers and the environment.

There are numerous studies in the literature which have examined the occurrence of chlorpyrifos in fruits and vegetables (Chavarri et al., 2004; Chavarri et al. 2005; Kurwadkar et al. 2015; Singh et al. 2007; Randhawa et al. 2007; Hernández-Borges et al. 2009). The emphasis has been on the safety periods for this organophosphorus insecticide in the tested vegetables (Singh et al., 2007).

Pesticide concentrations in foodstuffs have been shown to decrease by careful household processing. There is clear evidence that culinary treatment of the produce, including washing, peeling, and cooking can have a significant effect on the removal or degradation of pesticides (Chavarri et al., 2005).

The purpose of this study was to investigate the dissipation of chlorpyrifos residues in different time intervals and, furthermore, to determine half-life of chlorpyrifos. Another objective was to study effect of washing, peeling, and refrigeration storage for different periods on reducing the residues in cucumber samples treated with chlorpyrifos after household processing and post harvesting.

MATERIALS AND METHODS

Preparation of Chlorpyrifos Suspensions and Their Application in Greenhouses

The chlorpyrifos solution was prepared for spraying after dissolution in water according to the manufacturer's instructions. The commercial formulation of the chlorpyrifos-based product (Dursban 40 EC) was used for application. The spray solution of the pesticide was prepared by diluting 400 mL chlorpyrifos in 100 L water. In this way, a sufficient quantity of aqueous suspension was obtained for application in the greenhouse. The suspension was applied uniformly onto the cucumber plant using Aspee Hand Knapsack Sprayer. The recommended pre-harvest intervals were 14 days for chlorpyrifos as suggested by the manufacturer of this insecticide. Control samples were collected and analyzed immediately before spraying.

Sample Collection

Cucumber was cultivated in two commercial greenhouses located in Mazandaran, Iran. Samples were collected after a 1.5-month growth period. The row-spacing was 40 cm and the plant-to-plant spacing was 20 cm. Prior to the application of commercial pesticides, the absence of residual pesticides on samples was confirmed by residue analysis. Mature cucumber samples (marketable size fruits) were randomly collected (4.0 kg were sampled), at intervals of 0 (1 hour), 1, 3, 5, 7, 9, 11, 13 and 14 days after pesticide applications. The collected samples were placed in polyethylene bags and transferred to the laboratory after harvest and analyzed immediately.

Household Processing

In order to assess the effectiveness of household processing on reducing chlorpyrifos residues, the fruit samples collected in each time period were divided into four parts and processed separately. The first part was unprocessed; the second part was washed for one minute under running tap water without using detergent; the third part was peeled with a knife submerged previously in acetone for a short time; and the fourth part of samples was subjected to a storing procedure and kept in darkness at +4°C for 48 hours in the refrigerator in polyethylene bags.
Chemicals and Reagents

Pesticide analytical standard of chlorpyrifos and TriPhenyl Phosphate (TPP) were purchased with the purity certified by Dr. Ehrenstorfer Inc. (Augsburg, Germany). Chlorpyrifos stock solution (1000 µg mL⁻¹) and an internal standard solution Tri-Phenyl Phosphate (TPP) were prepared in pure acetonitrile (MeCN) and stored at −18°C. Calibration solutions were prepared with different concentrations just before use. HPLC grade MeCN and analytical grade anhydrous Magnesium Sulfate (MgSO₄) and sodium chloride (NaCl) were purchased from Merck (Darmstadt, Germany). Graphitized carbon black (GCB, 200 meshes) was obtained from Supelco. All glassware was rinsed with high purity acetone before use.

Apparatus

Gas Chromatography (GC) analyses were carried out using a Dani 1000 gas chromatograph (Monza, Italy) equipped with a Nitrogen–Phosphorus Detector (NPD). Chromatographic separation was achieved by using a HP-5 fused-silica capillary column (30×0.25 mm id, 0.25 µm film thickness, SGE). Helium (purity 99.999%) was used as carrier gas. All data were collected on Clarity software (DataApex, Prague, Czech Republic). Identification of chlorpyrifos residue was accomplished by Retention time (t_R) and compared with a known standard at the same conditions. The quantities were calculated by determining the area under the peak.

Operating conditions of the gas chromatograph were as follows: Injector and detector temperatures were 250 and 300°C, respectively; the column temperature was maintained at 100°C for 1 minute and then programmed at 10°C min⁻¹ to 200°C, then increased to 280°C at a rate of 5°C min⁻¹, and held for 10 minutes.

Extraction Procedure

The QuEChERS method was used for the extraction of chlorpyrifos from the cucumber samples. This method was carried out as described by other researchers (Nguyen et al., 2008). According to this method, the sample (1 kg of cucumber) was chopped and homogenized for 5 min at high speed in a laboratory homogenizer. An accurately weighed amount of 10.0 g of homogenized sample was placed into a 50 mL centrifuge tube with 10 mL of MeCN and 0.1 mg L⁻¹ TPP (Internal Standard). The screw cap was closed and tube shaken vigorously for 1 min by hand, ensuring that the solvent interacted well with the entire sample. Then, 4 g of anhydrous MgSO₄ and 1 g of NaCl were added repeating the shaking process again for 1 min to prevent coagulation of MgSO₄. After centrifuging, the upper layer was cleaned by dispersive solid-phase extraction with 0.5 g of graphite carbon black and 1.50 g of anhydrous MgSO₄. The mixture was then shaken for 1 min and centrifuged for 5 min at 4,000 rpm. The cleaned extract sample was concentrated to 1.0 mL with a gentle stream of nitrogen and then 1 µL of this solution was injected into GC-NPD.

Quality Assurance and Recovery

Quantification was based on the peak area ratio divided by the internal standard (TPP) by comparison with calibration curves at various concentrations. Multi-level calibration curves were created for the quantification. Good linearity (R² > 0.998) was achieved for the tested intervals which included the whole concentration range found in the samples. Recovery tests were performed to check the efficiency of the residue analysis method for estimation of pesticide residues in the substrate of samples that were analyzed during the study. The method was optimized by recovery studies before...
the determination of pesticide residues in collected samples. Recovery experiments were carried out by spiking cucumber at different levels to establish the reliability and validity of analytical method adopted. Cucumber fruits from the control plots were spiked with this insecticide at levels of 0.1, 0.50 and 1.0 mg L\(^{-1}\) in triplicate. These standard solutions were added to the chopped cucumber sample in the blender jar before homogenization. The same extraction procedures and GC conditions were applied for both the sample analyses and recovery studies. In this way, the obtained recovery values were from 86.2-96%, with Relative Standard Deviation (RSD) values below 15%. Detection Limits (LOD) of 0.01 mg kg\(^{-1}\) was obtained for chlorpyrifos insecticide.

Statistical Analysis

All analyses performed on triplicate samples, and statistical analysis data presented as mean±SD, were subjected to Analysis Of Variance (ANOVA). The data were tested for homogeneity of variances at a significance level of \(P< 0.05\), and probability values of less than 0.05 were considered as statistically significant (one-way ANOVA). Significant means were subjected to analysis by Duncan’s multiple range test \((P< 0.05)\). When significant differences were found, the Least Significant Difference (LSD) test was used to determine the differences among means.

RESULTS AND DISCUSSION

Results of Chlorpyrifos Application

The residue concentrations in cucumbers obtained in the dissipation study of chlorpyrifos and the corresponding first-order decay fits is presented in Figure 1. The average residue concentrations of chlorpyrifos in cucumber fruits ranged from 4.73 mg kg\(^{-1}\) at 1 hour to 0.02 mg kg\(^{-1}\) 14 days after the spray application. The degradation kinetics of this insecticide deposits was well described by first-order decay equations, \([C(t) = 4.73 \times e^{-0.363 \times t}, R^2 = 0.94]\) (Juraske et al., 2008). According to experimental results, the half-life of

![Figure 1. Dissipation of chlorpyrifos from cucumber fruits.](image-url)
Chlorpyrifos Reduction in Cucumber

Chlorpyrifos is 1.9 days, if this insecticide is applied at the recommended dose on cucumber fruits. Residue levels in samples which were collected after the application of the pesticides throughout a period of 14 days showed a gradual and significant (P < 0.05) decrease in content of chlorpyrifos. The Maximum Residue Limit (MRL) of chlorpyrifos on cucumber has been prescribed as 0.05 mg kg\(^{-1}\) (EU MRL, 2016). The residues of chlorpyrifos on cucumber fruits were less than its MRL value 13 days after application at the recommended dosage. Therefore, the results of the present study indicate that residues of chlorpyrifos degrade below their EU MRL, 13 days after application, at the recommended dosage. Therefore it is suggested that a waiting period of 13 days should be kept before harvesting or consumption of cucumbers, in order to protect consumer's health.

In the samples collected from cucumber greenhouses, chlorpyrifos residues higher than their MRL values were observed in initial days. These results agree with other authors in that pesticide residue concentrations in different fruits and vegetables were higher than those recommended by MRL (Hernández-Borges et al., 2009; Singh et al., 2007). When the pre-harvest intervals between pesticide applications and harvest are not respected by the farmers, the risk of having higher pesticide levels is not negligible. In this case, the higher levels of pesticide residues can involve considerable consumer health risks and environmental pollution.

Table 1 indicates chlorpyrifos residue analysis and effects of household processing on the 14 days. The variance analysis was applied to these results. Also, Duncan's multiple range tests of significantly different means are presented in Table 1. According to the results of variance analysis, significant reductions in residue levels for chlorpyrifos were obtained through both the pre-harvest intervals and during the processes which were aimed at decreasing pesticide residues (P < 0.05). Significant interactions of these two parameters were observed on the reduction of residues (P < 0.05). According to the results of Duncan's multiple range test, when compared to the samples which were collected 1 h after the pesticide application, chlorpyrifos residues were significantly (P < 0.05) reduced in samples collected 7 or 14 days following the pesticide application because pesticide residues were rapidly lost from plant surfaces. These results agree with other authors who reported that chlorpyrifos residues were reduced by the pre-harvest time and other different processes (Randhawa et al., 2007). But, no significant effects were observed on the residue level by the washing and refrigeration storage processes.

**Table 1.** Effects of household processing on the removal of chlorpyrifos residues in periodic intervals following pesticide application (Mean±standard error of the mean, mg kg\(^{-1}\)).

<table>
<thead>
<tr>
<th>Time (Days)</th>
<th>Unprocessed</th>
<th>Washing</th>
<th>Peeling</th>
<th>Refrigeration storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>0(^{a})</td>
<td>4.73 ± 0.47(^{b})</td>
<td>3.03 ± 0.14(^{a})</td>
<td>2.23 ± 0.20(^{a})</td>
<td>3.87 ± 0.12(^{a})</td>
</tr>
<tr>
<td>1</td>
<td>3.87 ± 0.12(^{b})</td>
<td>2.83 ± 0.12(^{a})</td>
<td>1.33 ± 0.14(^{b})</td>
<td>3.17 ± 0.11(^{b})</td>
</tr>
<tr>
<td>3</td>
<td>2.63 ± 0.10(^{c})</td>
<td>1.03 ± 0.11(^{b})</td>
<td>0.77 ± 0.11(^{c})</td>
<td>1.83 ± 0.14(^{c})</td>
</tr>
<tr>
<td>5</td>
<td>1.47 ± 0.15(^{d})</td>
<td>0.80 ± 0.05(^{e})</td>
<td>0.50 ± 0.02(^{d})</td>
<td>0.87 ± 0.10(^{d})</td>
</tr>
<tr>
<td>7</td>
<td>0.93 ± 0.05(^{e})</td>
<td>0.47 ± 0.02(^{d})</td>
<td>0.23 ± 0.02(^{e})</td>
<td>0.50 ± 0.08(^{e})</td>
</tr>
<tr>
<td>9</td>
<td>0.56 ± 0.03(^{f})</td>
<td>0.13 ± 0.02(^{e})</td>
<td>0.09 ± 0.01(^{e})</td>
<td>0.13 ± 0.04(^{f})</td>
</tr>
<tr>
<td>11</td>
<td>0.13 ± 0.04(^{f})</td>
<td>0.05 ± 0.01(^{e})</td>
<td>0.02 ± 0.01(^{e})</td>
<td>0.07 ± 0.02(^{f})</td>
</tr>
<tr>
<td>13</td>
<td>0.06 ± 0.02(^{f})</td>
<td>ND(^{c})</td>
<td>ND</td>
<td>0.03 ± 0.02(^{f})</td>
</tr>
<tr>
<td>14</td>
<td>0.02 ± 0.01(^{f})</td>
<td>ND</td>
<td>ND</td>
<td>0.01 ± 0.01(^{f})</td>
</tr>
</tbody>
</table>

\(^{a}\) One hour after pesticide application. \(^{b}\) Values given are mean of three analyses. Values in the column followed by different letters are significantly (P < 0.05) different (Duncan's multiple range test). \(^{c}\) ND: Not Detected.
process (P< 0.05). No statistical differences were observed between the effects of washing and refrigeration storage (48 hours at 4°C) on the reduction of residue levels (P< 0.05). In addition, the results of the Duncan multiple range test shows that the most effective process for reduction of residues of chlorpyrifos was the peeling process.

The percentage (%) reduction of chlorpyrifos residues after three processes in cucumber samples collected following different pre-harvest intervals are presented in Figure 2, which shows a decrease in ranges of 27–100% by the washing procedure, 53-100% by the peeling procedure, and 18-76% by the storage procedure at +4°C for 2 days.

**Washing**

When the cucumber samples washed with tap water were compared with cucumbers without any treatment, the washed samples showed lower chlorpyrifos residues. The effect of washing was found to be dependent on the initial concentration of pesticide residue, more clearly in the last days of pre-harvest, while the least effect was found on initial days. Washing of cucumbers reduced the level of chlorpyrifos residues, which can be dissolved in water or physically dislodged from the surface of raw agricultural commodity. Chlorpyrifos is non-systemic organophosphorous insecticide and cannot penetrate into the plant. After application, they form a deposit on the surfaces of the leaves and fruit which facilitates their removal by washing and peeling. In a previous study, non-systemic residues were reduced by 9–40% on cucumber (Hassanzadeh et al. 2010). Polar water soluble pesticides are more readily removed than low polarity materials (Hassanzadeh et al. 2012).

Also, hydrolysis is a major removal process for Organophosphate Pesticides (OPPs). Generally, hydrolysis involves the breakdown of one of the phosphoric or thio phosphoric esters and is strongly dependent on the pH and other solution (Liu et al., 2001). Washing with water resulted in a great reduction of chlorpyrifos residues in treated vegetable fruits and lead to residue levels lower than the MRL on several different days. It can be stated that washing process is effective in reducing chlorpyrifos residues. It has been indicated that these pesticides are hydrolyzed by water (Liang et al., 2012; Xu et al., 2012). Removal studies

![Figure 2. Percentage reduction of chlorpyrifos residues after different processes following different Pre-Harvest Intervals (PHIs).](image-url)
are reported in the literature by several researchers, who found that washing with water and/or other solutions resulted in a great reduction of various pesticide residues in treated vegetable fruits and lead to residue levels lower than MRLs (Randhawa et al., 2007; Baskaran et al., 1999).

Peeling

The effect of peeling was found to be the most pronounced in the last days of pre-harvest but least effective on initial days. However, chlorpyrifos residue levels in the unprocessed samples were much higher than in the peeled samples, and the small amounts of chlorpyrifos detected in the peeled samples could be in part the result of contamination during the peeling process. Also, the results indicate that the pesticide residues actually remain in the peel, as only small amounts were identified in the flesh, which may be, in part, the result of contamination during the process of peeling. This has already been reported for other non-systemic pesticide/fruit combinations (Fernández-Cruz et al., 2004). The data indicated that peeling has a significant effect on the declining of chlorpyrifos residues on different days. These results closely relate with the finding of 73% reduction in chlorpyrifos residues in asparagus after peeling (Chavarri et al., 2005). Also, the result of other research indicated that washing vegetables and fruits treated with non-systemic insecticides with tap water removed a magnitude of their residues (Keikothlaile et al., 2010). It is known that some of the pesticides penetrate from the surface into the deep layers of plant tissues. Usually the pesticide is first dissolved in the top waxy-like layers and then moves to the inside. Since some fruits are covered with a waxy layer and have a very thin skin, it is thought that the pesticides might easily penetrate the fruits and accumulate inside the pulp. This statement is mainly based on the fact that penetration depends on the stability of the insecticidal material in the lipoid-like or waxy-like layer which covers the cuticle. The thicker and oilier the skin, the more likely is the penetration (Kaushik et al., 2009; Keikothlaile et al., 2010). Because of their high lipophilicity, chlorpyrifos can be quickly absorbed and strongly retained by the waxes of the cucumber skin, making their elimination by peeling significant (Soliman, 2001).

Results from the flesh after peeling indicated that chlorpyrifos residues practically remain in the peel, as only small amounts were identified in the flesh. These results could be justified by the high Kow (octanol/water partition coefficient) values presented by this insecticide (Barcelo et al., 1997).

The data clearly indicated that the peeling process had a significant effect on the removal of chlorpyrifos residues in cucumber: because of a lower penetration power of the compound, its incorporation into the cuticle layer of the plant surface results in deposits removable by peeling (Liang et al., 2012).

Refrigeration Storage

Processing by refrigerated storage for 2 days at +4°C was the lesser effective way than peeling and washing procedures for reducing the chlorpyrifos residues of the cucumber samples. This can be probably attributed only to enzymatic degradation, since the process of evaporation and photo degradation under cold conditions and darkness are negligible (Liu et al., 2001) Samples kept under refrigerated storage at +4°C for 2 days had lower chlorpyrifos residue contents. This can be attributed to lower rates of physiological elimination reactions under refrigerated storage, but refrigeration assay for the refrigeration experiment depends on the specific Pre-harvest Time (PT) for each pesticide. These results of our study relate closely with those of other authors who found that pesticide residues in fruits already collected decrease
when they are stored for several days (Chavarri et al., 2005; Chavarri et al., 2004).

**CONCLUSIONS**

In this study, chlorpyrifos insecticide was reduced in cucumber samples 14 days after pesticides application compared to those 1 hour after the pesticide application. This indicates how pesticide residues are rapidly lost from plant surfaces not only by volatilization but also by other processes such as hydrolysis, photodegradation, oxidation etc., which play an important role here. The results showed that peeling was the most effective process for the reduction of residues of chlorpyrifos applied to cucumber plants. Refrigerated storage for 2 days at +4°C was the less effective way to reduce chlorpyrifos residues of the cucumber samples. Washing process is effective because chlorpyrifos is non-systemic organophosphorous insecticide and cannot penetrate into the plant. After application, they form a deposit on the surfaces of the leaves and fruit which facilitates their removal by washing (Elkins, 1989; Chavarri et al., 2005; Xu et al., 2012).

This probably reflects not only their higher solubility in the washing water, but also their reduced propensity to move into waxy layers. The extent to which pesticide residues are removed by commercial processing depends on a variety of factors, such as the chemical properties of the pesticide, the nature of the food commodity and the processing step, and the length of time the compound has been in contact with the food (Chavarri et al., 2005; Heshmati and Nazemi, 2017; Nazemi et al., 2016). Although water solubility plays an important role in removal of pesticides, the various results obtained were not necessarily dependent on this factor alone. In a different study, satisfactory relationships were found between water solubility, partition coefficient, and Henry’s law constant of the pesticides with the decrease of pesticides on fruits (Radwan et al., 2005).

When the peel was removed, a greater amount of the pesticide residue was also removed. The presence of pesticide residues in the skin of the tested vegetables indicates that a peeling process can help remove the pesticide residues from the commodity. Samples kept under refrigerated storage at +4°C for 2 days have low chlorpyrifos residue contents. But refrigeration storage was much less effective than peeling and washing procedures to reduce the chlorpyrifos residues of the cucumber samples. This can be probably attributed only to slow enzymatic degradation since process of evaporation and photo degradation under cold conditions and darkness are negligible (Juraske et al., 2008).

The results of the present study indicate that residues of chlorpyrifos, at the recommended dosage, degrade below its EU MRL after 13 days of its application. This is in accordance with the recommended pre-harvest interval for chlorpyrifos being 14 days for cucumbers. Pesticides should be applied correctly, according to good agricultural practice, using only the required amounts. Culinary applications are necessary to decrease the intake of pesticide residues. It can be concluded that processes such as controlled dose setting for the use of these pesticides, controlled greenhouse treatments, harvest and storage processes, and culinary applications before consumption have a crucial role in the reduction of residual pesticides which pose a serious threat to human health and the environment.

**ACKNOWLEDGEMENTS**

We sincerely acknowledge Prof. Amjad Shriam, from Chemistry Department of Taibah University, Ronnie Juraske from Department of Chemical Engineering (ETSEQ, Spain) and Prof. Mehmat Fatih Cengiz from Department of Food
Engineering (Akdeniz University, Turkey) for critically reading the manuscript and making a number of helpful suggestions, and Ellen Vuosalo Tavakoli (University of Mazandaran) for final editing of the English text.

REFERENCES

کروماتوگرافی گازی GC-NPD مورد شناسایی قرار گرفتند. نتایج نشان داد، دامنه غلظت باقیمانده کلرپیریفوس در دوره زمانی 14 روزه در خیار گلخانه‌ای به مقدار 4720/0 میلی گرم بر کیلو گرم متغیر است و مقادیر باقیمانده کلرپیریفوس در پیک دوره زمانی 14 روزه یک کاهش تدریجی و روند نزولی را نشان می‌دهد. نیمه عمر (1/2) کلرپیریفوس در این دوره 19 روز تعیین شد. تیمارهای خانگی از جمله شستن، پوست کنند و ذخيره‌سازی در بخارالان در کاهش مقدار باقیمانده کلرپیریفوس بسیار موثر بوده است و در بین روشهای مختلف، پوست کنند موثرترین روش برای کاهش مقادیر باقیمانده حشره کش کلرپیریفوس است. نتایج به دست آمده نشان می‌دهد که پیک دوره زمانی مناسب پس از سم‌پذیری کلرپیریفوس برای برداشت محصول و کاربرد روش‌های خانگی برای استفاده ایمن از خیار گلخانه‌ای جهت کاهش مقادیر باقیمانده سهم بسیار ضروری است.