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Investigation of Phosalone Residues in the Early and Late Varieties of Peach Products of Orchards Saman City, Chaharmahal and Bakhtiari Province in 2019

Reza Faraji; MSc[1], Mehraban Sadeghi; PhD[1], Ramezan Sadeghi; PhD*[1], Gashtasb Mardani; PhD[2], Morteza Sedehi; PhD[3] & Farshid Shabani Borujeni; MSc[4]

[1] Department of Environmental Health Engineering, School of Health, Shahrekord University of Medical Sciences, Shahrekord, Iran; [2] Medical Plants Research Center, Shahrekord University of Medical Sciences, Sharhekord, Iran; [3] Department of Epidemiology and Statistic, School of Health, Shahrekord University of Medical Sciences, Shahrekord, Iran; [4] Organization of Agricultural Jahad, Plant Protection Management, Shahrekord, Iran.

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Corresponding author:
ramezansadeghi@yahoo.com
Department of Environmental Health Engineering, School of Health, Shahrekord University of Medical Sciences, Shahrekord, Iran.

Postal code: 8813733445
Tel: +98 9132829853

ABSTRACT

Background: Nowadays, increasing the use of pesticides to manage the agriculture production resulted in increased pesticide residues in final products and concerns about environmental pollution and prevalence of emerging diseases. This study aims to investigate the residual amount of Phosalone toxin in the early and late varieties of peach orchards in Saman city located in Chaharmahal and Bakhtiari province. **Methods**: In this study, 60 samples of early and late peach varieties of peach orchards were selected randomly. Phosalone residues in early and late peach varieties before and after washing, and after peeling were extracted by QUECHERS method and the amount of extracted toxin were measured by high-performance liquid chromatography (HPLC). The data were analyzed using SPSS v.24 software. **Results**: Phosalone residues in early peach varieties before and after washing were 3.55 ± 0.94 and 0.31 ± 0.29 mg/kg, respectively. The concentration of Phosalone residues in unwashed early and late peach varieties before washing was 3.17 ± 0.97 mg/kg but decreased to 0.64 ±0.74 mg /kg after washing. Peeling reduced 97.79% of the Phosalone residues in late peach. Early and late peaches covered by the plant pathology clinic and unwashed late peaches not covered by plant pathology clinic were significantly ($P < 0.05$) higher than the Iranian national standard (2 mg/kg). **Conclusion:** Washing and peeling the peach significantly decreased the toxin residues. In order to minimize the risk of exposure to Phosalone residues, integrated pest control management programs are essential.

Keywords: *Toxin Residues; Phosalone; Peach*

Introduction

Agricultural development is one of the most important ways of food supply. Today, in order to increase the production of agricultural products, large volumes of pesticides and chemical fertilizers are added to the environment, the negative consequences of which have led to concerns about food safety and environmental pollution in modern agriculture (Chowdhury *et al.*, 2013).

Improper and excessive use of these compounds in the production cycle of agricultural products causes the presence of pesticide residues in the food chain of humans and other organisms (Keikotlhaile and Spanoghe, 2011).

Residues of pesticides accumulate in the human body through direct contact or use of contaminated that resulted in immune system problems,

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reproductive and endocrine disorders, cancer, and chronic kidney disease (Hadian *et al.*, 22020, Mostafalou *et al.*, 2013, Qin *et al.*, 2016).

The Phosalone is an insecticide and belongs to a member of the family of organic phosphorus toxins. Phosalone is used to control the pests on the fruit trees such as apples, pears, grapes, cherries, sour cherries, and peaches (Pirsaheb *et al.*, 2013).

This insecticide does not penetrate into the plant and accumulates in the skin of the fruit and leaf cuticle. The maximum residue of Phosalone according to the Iranian National Standard No. 13117 (Revised 2015), for peach fruit is equal to 2 mg/kg and the allowable daily intake (ADI) is 0.02 mg/kg body weight (Iran National Standards Organization (INSO), 2016).

The oral LD50 of Phosalone is 120 mg/kg. This compound has acute toxicity in oral exposure and is slowly hydrolyzed in the liver. Phosalone is converted to Phosalone exon by environmental factors such as oxygen and light, as well as liver microsomal enzymes, and its toxicity increases (Roberts and Reigart, 2013).

Studies have determined the residual concentration of toxins in peach cultivars. Zioris *et al.* examined the residual toxins in a sample of freshly produced peaches in northern Greece (Zioris *et al.*, 2009). Quinjano in 2016 addressed the issue of cumulative risk assessment of organophosphates in peach samples (Quijano *et al.*, 2016).

According to statistics and information of the Plant Protection Department of Jihad Agricultural Organization of Chaharmahal and Bakhtiari Province, 4500 hectares of land are under fruit trees including peaches, apples, etc. Eighty percent of the peach orchards in the province are located in Saman and Zayandehrud river. Annually, more than 50,000 tons of peach fruits are harvested from all peach orchards in Chaharmahal and Bakhtiari province and exported to neighboring and other provinces.

According to the probability of the presence of Phosalone toxin in late cultivars spring, to control peach branching pest in early cultivars (saffron) and in early summer for late cultivars and considering that the study of pesticide residues in crops is an important priority for consumer food security, the aim of this study was to survey the presence of Phosalone and determine the amount of Phosalone toxin in early and late peach cultivars in certain orchards of Saman city and compare the mean residual concentration of Phosalone in samples with Iranian standard (2 mg/kg).

Materials and Methods

Sampling: In order to sample and investigate the residual amount of Phosalone insecticide in early and late cultivars of peach orchards in Saman city, Chaharmahal and Bakhtiari province in 2019, cluster sampling was used. Three designated areas of Bagh Gostaran, Oman Samani and Hooreh, which used Phosalone to control pests by spraying, were considered as the main clusters. In each main cluster, four orchards with early peach cultivars were randomly selected. Each garden was divided into 4 blocks and one of them was randomly selected. In each block, 30 trees were randomly selected and from each tree, 4 fruits with an approximate weight of 100 g per fruit were collected or approximately 12 kg of peach fruit was harvested from each garden, taking into account the Carnes period and completely random. For sampling of late cultivars after two months, sampling was done according to the sampling method of early cultivars. Finally, 60 samples of early and late cultivars were randomly selected from the blocks.

Measurements: Due to the effect of light and temperature on the residual stability of toxins, the samples were transferred to the laboratory in black plastic bags and stored in the refrigerator until the test. After transferring the samples to the laboratory, the early samples collected from each garden were divided into two equal parts (approximately 200 g per part), unwashed and washed by tap water. Then, 3 pieces (around 15 g) of each sample including washed and unwashed samples (3 washed and 3 unwashed samples) were taken. Late cultivars of peach

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samples collected from each garden were also divided into two equal parts (approximately 200 g per part), unwashed, washed and washed and peeled samples. Tap water was used for washing samples. From each section, three samples of 15 g for washed, unwashed and washed and peeled samples were separately taken.

Extraction and purification of Phosalone residues in peach cultivars was performed using the Catchers method (The Association of Official Analytical Chemists (AOAC), 2017) and according to standard 12581 (Fenik *et al.*, 2011). For preparation, peach samples were thoroughly homogenized by a mixer. Then, 15 g of peach sample was poured into a 50 ml centrifuge tube with a volume of 50 ml (pH = 3.95), then 15 ml of acetonitrile (HPLC Grade) and 1% acetic acid were added to the tubes and shaken for one minute, then centrifuged at 3450 rpm for 5 min.

After that 5 ml of the solution was removed from the tubes and 100 mg of PSA adsorbent and 300 mg of magnesium sulfate were added for purification. The tube containing the sample was shaken again for one minute and then centrifuged at 3450 rpm for 5 min. Finally, 1 ml of the surface extract of the tubes was transferred to the capped vials (Fenik *et al.*, 2011, Jardim *et al.*, 2014, Słowik-Borowiec *et al.*, 2012).

After preparation, the samples were passed through a syringe filter and transferred to microtubes to prevent possible suspended solids from entering the device. After preparation and extraction of samples by catheter method, early and late harvested peach samples were injected into Agilent1200 high performance liquid chromatography (HPLC) to identify the Phosalone residues.

Data analysis: The data were analyzed using SPSS v.24 software and t-test to compare the mean values and p-values less than 0.05 were considered as significant levels. For calibration and accuracy of the device, concentrations of 0.01, 0.25, 1, 2.5, and 6.25 mg / l of Phosalone were injected into the device and a standard calibration curve was drawn (**Figure 1**).

Ethical considerations: This manuscript was derived from a Master's thesis of Environmental Health Engineering approved by Ethics Committee, Shahrekord University of Medical Sciences with the ethics code of IR.SKUMS.REC.1397.308.

Results

Based on the results in **Table 1**, the mean residual concentrations of Phosalone in early washed and unwashed peach cultivars were 3.55 ± 0.94 and 0.31 ± 0.29 mg/kg, respectively. In the case of early peach cultivars, the concentration of Phosalone in orchards covered by Plant Pathology Clinics was on average higher than that of orchards not covered by Plant Pathology Clinics.

The results of the study of residual Phosalone concentration in late peach cultivars are shown in **Table 2**. The concentration of Phosalone in late unwashed peaches was 3.17 ± 0.97 mg/kg and this concentration decreased to 0.64 ± 0.74 and 0.07 ± 0.94 mg/kg after washing and peeling, respectively. Peeling reduced 97.79% of the residual concentration in late peaches.

According to **Figure 2**, the mean concentration of Phosalone in early peach cultivars was higher than late cultivars; however, this difference was not significant (P = 0.33). In early cultivars, there was no significant difference between the concentration of Phosalone in the orchards covered by the Plant Pathology Clinic and the orchards not covered by the Plant Pathology Clinic (P = 0.07). However, in late cultivars, the residual concentration in the orchards covered by the Plant Pathology Clinic was significantly (P = 0.004) lower than the orchards that were not covered by the Plant Pathology Clinic. In both peach cultivars, there was a significant difference between the concentration of Phosalone residues in unwashed and washed peaches (P <0.05). There was also a significant difference (P = 0.01) in the late type between washed peaches and washed and peeled peaches (**Table 3**).

The results of comparing the mean residual concentrations of Phosalone in unwashed early and late peach samples with Iranian standard (2 mg/kg) are shown in **Table 4**. According to **Table**

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4, Phosalone residues in unwashed early and late peaches covered by the Plant Pathology Clinic and unwashed late peaches not-covered by Plant Pathology Clinic were significantly ($P < 0.05$) higher than the Iranian national standard.

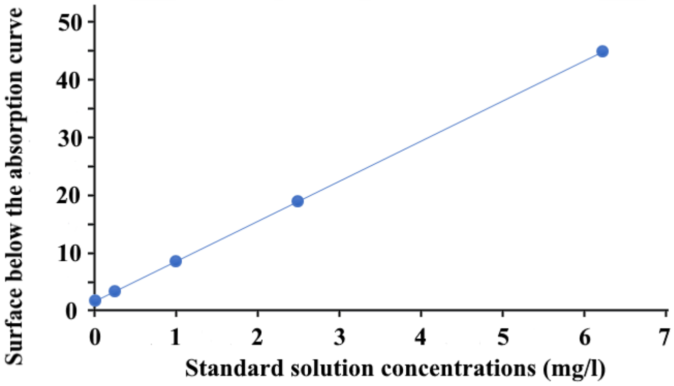

Figure 1. Calibration curve of Phosalone measurement by HPLC method.

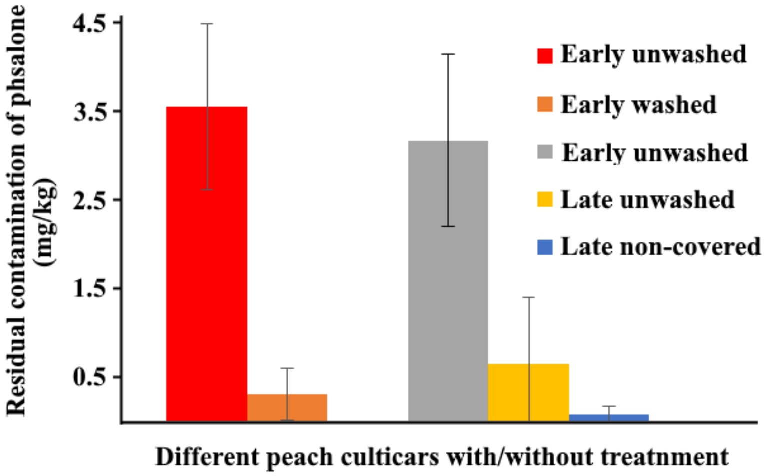

Figure 2. Comparison of mean residual concentrations of Phosalone toxin in late and early peach cultivars.

Table 1. Mean ± SD of Phosalone residual concentration (mg/kg)in early peach cultivars with skin.

Covered by the Plant Pathology Clinic[a]		Not covered by Plant Pathology Clinic[b]		Early peach product		Overall reduction of toxin after washing (%)
Unwashed	Washed	Unwashed	Washed	Unwashed	Washed	
4.02 ± 0.55	0.24 ± 0.02	3.07 ± 1.04	0.36 ± 0.37	3.55 ± 0.94	0.31 ± 0.29	91.27

[a]: Gardens that are under the supervision of experts in plant pathology clinics and all matters related to them such as fertilization, selection of pesticides, spraying, and watering are done under the supervision of experts of these centers; [b]: Gardens that are not under the supervision of experts in Plant Pathology Clinics, so all matters related to them such as fertilization, selection of pesticides, spraying, and watering are done without the supervision of experts in these centers.

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Table 2. Mean ± SD of Phosalone residual concentration (mg / Kg) in late peach cultivars.

Unwashed peaches with skin	Washed Peaches with skin	Peach without skin	Reduction of toxin after washing (%)	Reduction of toxin after peeling (%)
3.17 ± 0.97	0.64 ± 0.74	0.07 ± 0.94	79.81	97.79

Table 3. Comparison of mean residual concentrations of Phosalone in peach samples.

Groups		Mean ± SE	P-value[a]	95% Confidence interval
Early and late cultivars	Early Late	1.92±0.36 1.30±0.25	0.15	-0.24, 1.48
Early covered and not-covered cultivars of Plant Pathology Clinic	Covered not-covered	2.16±0.45 1.17±0.45	0.249	-0.74, 2.73
Late cultivars covered and not covered by the Plant Pathology Clinic	Covered not-covered	1.01±0.23 2.15±0.68	0.054	-2.28, 0.01
Early cultivars are unwashed and washed	Washed Unwashed	0.28±0.08 3.55±0.27	<0.0001	-2.85, -2.67
Late cultivars are unwashed and washed	Washed Unwashed	0.35±0.12 3.18±0.27	<0.0001	-3.35, -2.29
Late cultivars are washed and peeled	Washed Peeled	1.91±0.31 0.07±0.20	<0.0001	-2.75, -0.92

[a]: test?

Table 4. Comparison of mean residual concentration of Phosalone in unwahsed peach samples with Iranian standard (2 mg/kg)

Groups	Mean ±SE	P-value[a]	%95 Confidence interval
Unwashed early covered cultivars of Plant Pathology Clinic	4.01±0.44	<0.0001	1.67, 2.35
Unwashed late covered cultivars of Plant Pathology Clinic	2.69±0.12	<0.0001	0.41, -0.97
Unwashed early not- covered cultivars of Plant Pathology Clinic	2.15±0.4	0.5	-0.83, 1.15
Unwashed late not-covered cultivars of Plant Pathology Clinic	4.66±0.4	0.007	1.66, 2.66

[a]: test?

Discussion

The use of chemical pesticides is always a priority among various methods of pest control. The residues of chemical pesticides in agricultural products can affect the economic-political conditions and trade relations of countries; moreover, it can also affect food security and health inside the country (Asgari *et al.*, 2013). Considering the fact that a significant percentage of the peaches produced in Saman city are exported to other provinces such as Isfahan, Tehran, and Khuzestan, determining the residues of pesticide used as well as the quality and health of the product is of particular importance.

The results showed that the residual concentration of Phosalone despite the expiration of the the pre-harvest interval (PHI) period in both peach cultivars was higher than the national limit set by the Codex (Food and Agriculture Organization and World Health Organization, 2016). Nowadays, due to the increase in pest resistance to pesticides, gardeners use more concentrations of toxins or more spraying times to control pests and cause the standard concentration residues in the crop to increase too much (Soheil Sobhani Ardakani SDS and Saeed Jame Bozorgi,

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2014). Since late cultivars are sprayed in two stages, the residues seemed to be higher than early cultivars, which were sprayed in one stage; however, the results of this study did not confirm this hypothesis, since the results of statistical tests showed that the average residual concentration of Phosalone in early cultivars was slightly higher than late cultivars. Various factors such as crop type, amount of pesticide used, formulation, number of sprays, climatic conditions, cultivated crop, and planting time can play a role in the amount of pesticide residues in the crop (Rafiei *et al.*, 2016).

There was no significant difference (P = 0.07) between the residual concentration of Phosalone in early peach orchards covered by the Plant Pathology Clinic and not-covered orchards and it seems that the production instructions were not implemented according to the recommendations of the Plant Pathology Clinic supervisors. However, the residual amount of pesticide studied in late peach orchards covered by Plant Pathology Clinic was significantly (P = 0.004) less than orchards not-covered. It has been in the pre-harvest stages.

In early cultivars after fruit washing, 91.27% of Phosalone residue was significantly reduced (P < 0.05) and the amount of Phosalone residues reduced to less than the allowable level. One of the simplest ways to clean fruits and vegetables before consumption is always to wash them for a maximum of 5 min. Higher octanol/water (KOW) will not be completely effective and high polarity systemic toxins cannot be removed by washing process at all (Chung, 2018). Due to the fact that Phosalone is a non-systemic toxin, leaching is effective in reducing the residual toxin (Kazemi *et al.*, 2017).

The results of Nasehi F *et al* study on Phosalone residue in Lebanese red apples showed that the mean concentration of Phosalone in unwashed state was 17.50 and in washed state was 10.73, but in both cases they were higher than the codex standard limit (Nasehi F, 2020). Kong *et al.*, after examining methods for reducing pesticide residues in post-harvest apples, concluded that the residual amount of chloropyrifos in the washed state reduced by 17 to 21% compared to the unwashed state (Kong *et al.*, 2012).

Rasmussen *et al.* studied the pesticide residues after home processes and showed that the mean residual concentrations of chlorpyrifos in unwashed and washed apples in the cultivars of the exploratory cultivars were 0.372 and 0.365, respectively, and in the apple cultivars under the name of Jonagold. Residues in unwashed and washed apples were 0.603 and 0.543, respectively, and washing alone did not have a significant effect on reducing chloropyrifos residues (Rasmusssen *et al.*, 2003).

In late cultivars of peach, washing and peeling of peach led to a decrease of 79.81 and 97.79% of the Phosalone residues, respectively, so that the amount of residue reached below the allowable level. Although the peeling process has little effect on the reduction of nutrients and vitamins in the skin of fruits, it is also an effective way to reduce the residual toxins that accumulate directly in the skin of the fruit. A study found that soaking an apple in water for 10 min reduced the Phosalone residual toxin by 30 to 50%, and washing fresh peaches in water for just 1 min reduced the residual non-systemic fluodioxonyl toxin by 72% (Bhilwadikar *et al.*, 2019).

Sheikh Louie *et al.,* by examining the residual levels of diazinon and chlorpyrifos in Lebanese red apple tree yield, concluded concluded that the residual levels of pesticide residue in unwashed apples with skin, washed with skin, and peeled apples were peeled apples were 0.68, 0.31 and 008 ppm, respectively. 0.98 for diazinon and 0.98, 0.54 ppm and 0.24 for chlorpyrifos. They also showed that the residues of both diazinon and chlorpyrifos were higher in unwashed fruits than in peeled fruits (Shahyan and Sheikhloie, 2017), which is in line with the present study.

Sudesh *et al.* studied the effects of decontamination processes on fruits and vegetables and concluded that the residual chlorpyrifos insecticide in apple fruit was reduced by 52% during the peeling process (Anita and Devi, 2018). In a study by Stapan *et al*. on the amount of residue in the process of preparing apple puree, they concluded that washing only reduced the residue of Phosalone by 3%. Given that the amount of residue detected was much less than the maximum allowed, this small amount of residual reduction will be of no concern (Štěpán *et al.*, 2005).

Mergnat *et al*., after examining the methods of reducing residues in apples, concluded that washing process reduces only 30 to 50% of Phosalone residuals, which can be attributed to the low solubility of Phosalone (Mergnat *et al.*, 1995). Ahmed *et al.* in their study concluded that washing and peeling have little effect on the removal of residues of systemic toxins, since these toxins also penetrate into the fruit tissue (Ahmed *et al.*, 2011).

In addition to washing, water temperature also plays an important role in removing pesticides, so the solubility of toxins in water will increase by increasing water temperature (Bhilwadikar *et al.*, 2019, Chung, 2018). However, soaking fruits in water for 10 to 30 min, or washing and peeling afterwards, has a greater effect on reducing or removing toxin residues than other methods (Saeedi Saravi and Shokrzadeh, 2016).

Dasika *et al.* analyzed residues in fruits and vegetables and reported that the measured concentrations of the insecticide chlorpyrifos in Smith green apple cultivars in the unwashed, lukewarm and lukewarm salts had a decreasing trend equal to 2.8, 1.7 and 1.1 and in Michigan golden yellow apple cultivars, it had a decreasing trend in unwashed, washed with lukewarm and washed with lukewarm salt water equal to 3.4, 1.9 and 0.8, respectively. They stated that in addition to water solubility and temperature, the concentration of salt (sodium chloride) in water can also affect the residual reduction of some pesticides such as chlorpyrifos (Dasika *et al.*, 2012).

Conclusion

The present study investigated the concentration of residual Phosalone in different peach cultivars under any conditions. The overall results showed that the residual concentration in the peach crop was higher than the standard codex; however, washing and peeling significantly reduced the Phosalone residues in early and late cultivars. In order to minimize the risks of exposure to pesticide residues in crops that are consumed raw and immediately after harvest, it is necessary to expand integrated pest management programs and use biological pesticides along with increasing farmers' education. This study unveiled Phosalone residues in early and late peach cultivars, before and after washing. Also, Phosalone residues in the late variety after washing and peeling were investigated. Due to the restrictions on the supply of chemicals such as pesticide standards, the researchers could not study imported products in Shahrekord marketing from other provinces and make a comparison between them. Furthermore, we were unable to determine the other pesticides that could increase the risk of adverse effects in people. Therefore, considering the results and the importance of food health, it is necessary to evaluate other pesticides in fruits.

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Authors' contributions

Faraji R, Sadeghi M, and Sadeghi R participated in analysis, preparing the concept and design. Mardani M and Shabani Borujeni F collected data and wrote the manuscript. Sedehi M assisted in statistical analysis. All authors read, reviewed, and approved the final manuscript.

Conflict of interest statement

The authors declare no conflict of interest.

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