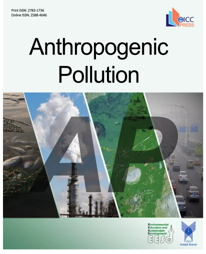

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Determining Chlorpyrifos residue in apples case study: Damavand orchards

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Abstract:
This study was conducted with the purpose of measuring the remaining of Chlorpyrifos insecticide in apples produced in Ahmadabad region of Damavand at the beginning of crop spraying and after its currency period over two periods of the second and third crop spraying in September and October, 2016 and also June, 2017. Samples were collected from three selected stations during September and October, 2016 and also June, 2017. Afterwards, there were some laboratory stages to measure the concentration chlorpyrifos in each sample by means of GC-MASS. According to the calculations made by SPSS software, chlorpyrifos with an average of 1.6917 has a higher level than EPA, ISIRI, CODEX and EFSA standards. Also, in the tests of September and October, chlorpyrifos in all stations has values lower than the EFSA CODEX standard, but in station 1 it is higher than the EPA and ISIRI standards; other stations have values lower than all standards. During the calculations made by SPSS software, it shows that chlorpyrifos with an average of 0.123 has a lower level than CODEX and EFSA standards, but it has a higher level than EPA and ISIRI standards.

Keywords: Phosphorus pesticides; Chlorpyrifos; Currency period; GC-MASS

1. Introduction

The entrance toxic compounds into the environment has unpleasant effects (makvand et al. 2021; Khani et al. 2017). The result of the presence of toxic substances in the environment, is their entry into the food chain. Pests are one of the main issues that endanger the health of human food sources. Therefore, people are always looking for quick and effective ways to deal with them. Producing pesticides was one popular way due to its quick effectiveness and high efficiency. To comprehend the notion of pesticide better, it is suggested that they are found to be natural or artificial combinations. They also have high impacts on controlling and destroying each type of pest, which threatens products during production, storing, and distribution (Sheikhi Gurjan et al., 2013). Pesticides have different classifications, that is to say, depending on the target for which they are produced, pesticides include a wide range, such as herbicides, raticides, and nematicides. Pesticides are also classified based on how they cause poisoning, such as systemic, non-systemic, etc., and how they cause poisoning, such as skin poisoning, inhalation, etc., and their various chemicals (Nasehi and Fataei 2012; Zaefizadeh et al. 2011). Systemic toxins are among those that specifically affect certain parts of the body of the target organism to destroy them (Fataei et al. 2010). These toxins are highly stable and do not disappear by washing the surface of the product (Ajami and Fataei 2015). Absorption and semi-systemic pesticides are less irritating than systemic pesticides. Chemically, pesticides are divided into different groups, such as organic compounds, which include organic compounds of chlorine, phosphorus, carbamate, etc. (Dehghani 2010). There are various elements in a pesticide attracting users when selecting one (Hajjabbari and Fataei 2016). Its amount and speed of impact in the shortest time are the most important factors for attracting pesticide users. Nevertheless, there are also effective factors, such as the percentage of active substance, the easy combinability of Pesticide with water, the extent and severity of its impact on humans' health and the environment, the lack of pests resisting against the pesticide, the maintenance of the plant health, and low shelf life (Sekachaee et al. 2010).

1.1. How pesticides function:

Pesticides have different functions to each other. The first group contains pesticides called digestive pesticides. They are used as food or bait to attract the target pest and they lead to its destruction. The second group is related to those contact pesticides which destroy insects when they contact them by causing poisoning; chlorpyrifos is one of the most important pesticides in this group, chlorpyrifos has the same action as diazinon. Concerning herbal tissues functions, pesticides are subcategorized as systemic and semi-systemic.

After spraying, systemic pesticides enter all parts of the plant through penetrating into plant sap. They are also absorbed quickly, do not vanish by washing, and have fewer hazards for insects. Furthermore, as semi-systematic pesticides have less mobility compared to systematic ones, it is more probable for them to kill useful creatures.

After recognizing the carcinogenic features of organochlorine pesticides and also their role in the creation of resistance in target organisms, some toxins were substituted which were thousands of times stronger than organochlorine pesticides. This group of toxins are called organophosphorus pesticides as phosphorous is a major component in their creation. Even though these pesticides are more toxic than organochlorine pesticides, they are more popular among users as they are not stable in the environment. Depending on the duration of use, the type of material, and the amount of material applied, phosphorous pesticides have various impacts on the environment and people in contact with them. Not only through eating, but also through dermal and inhalant ways phosphorous pesticides cause poisoning. Gene mutations, seizures, tumors, and mortality are among the most common symptoms of poisoning caused by organophosphorus pesticides (Dehghani 2010). Chlorpyrifos, with the generic name of chlorpyrifos ethyl, can cause serious damage to the body by passing through the skin and entering the blood, depending on the amount or dose (Shiran et al. 2017). It is also regarded as a member of pyrimidine organophosphates toxins (Dehghani 2010). Apples are one of the products that are popular in Iran, as well as in the whole world, due to their many uses, pleasant taste and their role in humans' health. Inasmuch as a large number of pests, whether prior or subsequent to flowering

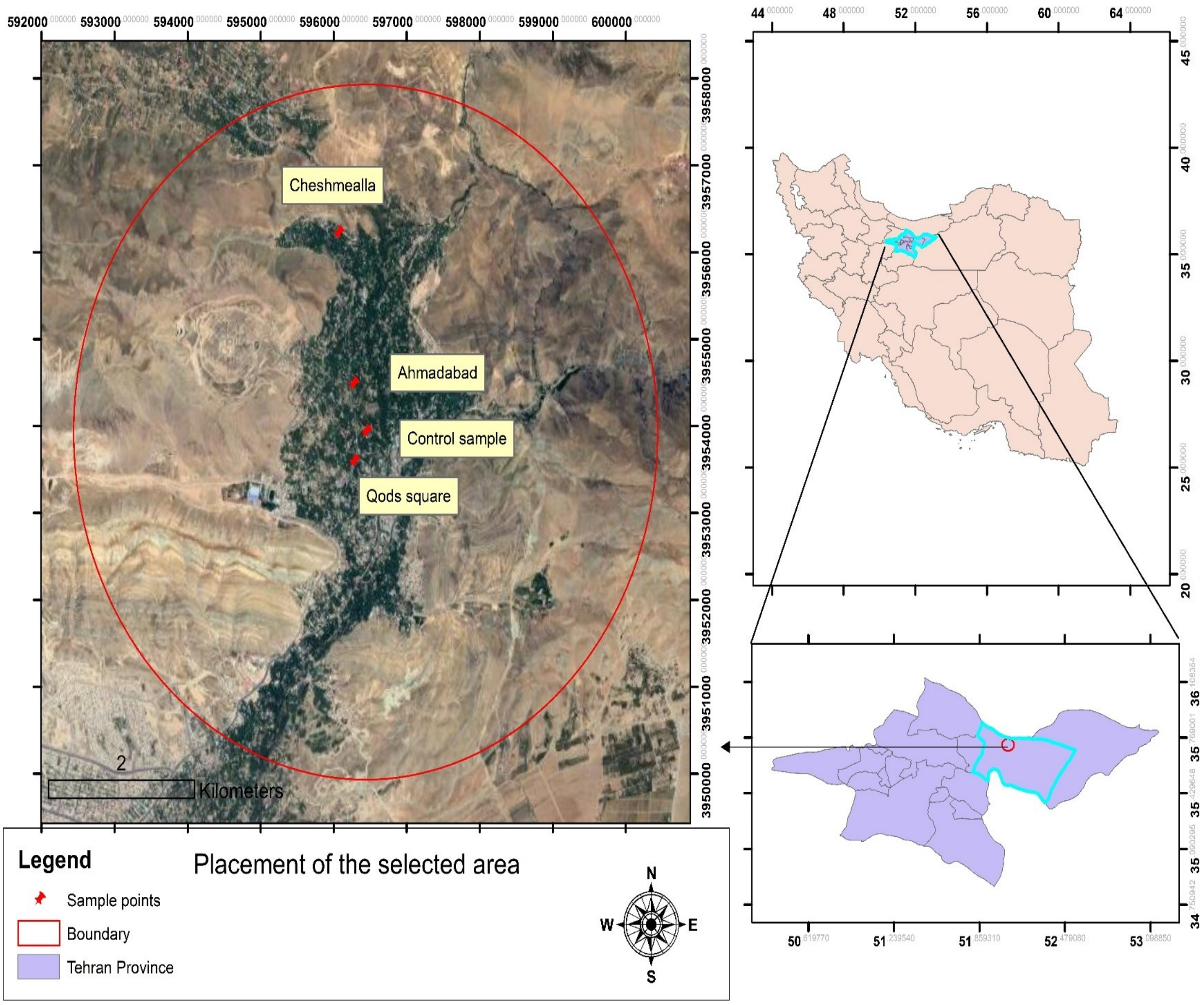

Figure 1. Location of sampling stations on the map.

Table 1. The significance of the difference of the amount of chlorpyrifos concentration.

Sampling time	Station	Chlorpyrifos concentration (ppm)	
		Sample 1	Sample 2
June (first level)	1	4.53	3.19
	2	0.98	1.01
	3	2.49	1.77
June (Second level)	1	2.22	1.56
	2	0.24	0.16
	3	0.85	1.3
September (first level)	1	0.294	
	2	lower than LOQ	
	3	lower than LOQ	
October (Second level)	1	0.285	
	2	lower than LOQ	
	3	lower than LOQ	

and fruit growth, threaten the health of the tree and its apples seriously, 2 or even sometimes 3 times of crop spraying are essential for maintaining the quality of the product and even at times the tree itself. Two major and essential crop spraying periods, which play an important role in the health of the product, happen during early March and June. During the first period of spraying (March), a combination of emulsifiable (pesticide) chlorpyrifos, Penconazole (fungicide) and Nissorun (miticide), and paraffin vegetable oil, in order to increase the shelf life of Pesticides, are mixed and crop spraying begins. In addition to increasing the shelf life of Pesticides, paraffin vegetable oils prevent cold weather from damaging the tree; consequently, they are used in the winter. After the blossoms fall, miticides and fungicides are applied against fruit pests before fruit growth.

In the second period of spraying (June), the major pesticide being practical is chlorpyrifos; nonetheless, depending on the decision made by the veteran in charge of spraying, other toxins, such as chlorpyrifos, phosalone, and ethion might be applied. The important issue in this period is that before harvesting, depending on the existing pests, the pesticides used for them are different. Among pests threatening the crop during this period are myzus persicae and aphid pests against which decis and imidacloprid are used, respectively. Furthermore, zeuzeran pesticide is used against zeuzera pyrina in all seasons. Pesticide residues in food are harmful to human health, Therefore, it is necessary to measure the amount of these substances. Considering that apples produced in Damavand orchards are consumed a lot, it needs to be checked for pesticide residues.

2. Materials and methods

In order to conduct this research, various visitations and analyses were carried out in Damavand region to specify major apple orchards and the sort of pesticides used there. According to the conditions of the region, as well as the abundance and concentration of apple orchards from Ghods Square to Cheshmeh Aala, 3 stations of Ghods, Ahmadabad, and Cheshmeh Aala were selected. Next, a garden was selected randomly from each station and their geographical location was recorded by a GPS device.

Figure 1 shows the location of sampling stations on the map. After that, the locations of the stations were marked on the map of the region. To determine the amount of pesticide residues, sampling was randomly selected from gardens which were sprayed in the second period in June and the third one in September. In this process, on the first day, 5 cc of chlorpyrifos was mixed with 5 liters of water, and to improve the process of absorption in the samples and maximizing the precision of the test, all the steps of spraying, based on a spraying expert's suggestion, were taken in the evening. The third spraying happened in September with 60% emulsifiable chlorpyrifos toxin. The amount of chlorpyrifos to water ratio, for combining and spraying them according to the spraying expert's instructions, as well as the instructions on the Pesticide, was 1 cc chlorpyrifos for each liter of water (according to the specialist's order, 9 liters of water were needed for 9 cc of chlorpyrifos used for each orchard; moreover, sprayings happened in the evening. Samples were collected during two periods: prior to currency period (72 hours after spraying) on September 16th and also subsequent to

Table 2. The summary of the amount of chlorpyrifos in the samples.

The amount detected
1.6917 ± 0.36

Table 3. The significance of the difference of pesticide in samples.

significant	F	average of squares	Degrees of freedom	The sum of the powers of the squares	Error source
.721	.572	1.982	5	9.908	between groups
		3.466	42	145.566	within groups
			47	155.474	Total

the 21-day currency period on October 8th. During each period, 4 apple samples were randomly selected from the sprayed apple trees in the orchards. The total number of specimens in each period was 12, and in total, 24 samples were taken (for each period 12 samples and for each station 4 samples were collected). Samples were delivered to the laboratory for testing by the GC-MASS within 24 hours after sampling. 90 milligrams of apple skin samples were cut and put in a cylindrical container with a lid. 10 micro liter of methanol solvent with the concentration of 58 ppm was added to the samples. After 10 minutes, 1 ml of hexane solvent was added to the specimens, then it was placed on a magnetic stirrer for 20 minutes and next, as it was in rotation, the drop formed at the end of the cone, resulting from the rotation period, was extracted with a syringe or sampler. The extracted organic phase was poured into a vial. 100, 50, 20 and 10 ppb standards, which include the internal standards and standards for the analyzed compounds required in the concentration range of samples, were equal to the sample size and injected into the machine. The analysis of the samples was done by injecting 1 microgram of the extracted solution into GC/MASS/Agilent 5973 apparatus, which is made in the USA.

The following temperature plan was used for the analysis:
Carrier gas: He (99.999%)
Constant flow: 1 mL/min
Auxiliary: 290°C
Injector (Splitless): 250°C
Initial temperature: 50°C hold 2 min
Temperature program: 50°C to 100 °C at 25 °C /min hold 2 min
Temperature program: 100°C to 290 °C at 25 °C /min
Final temperature: 290°C hold 5 min

The method for calculating the concentration of Chlorpyrifos in GC / MASS apparatus:

$$C_y = \frac{C \times \text{FVol}}{\text{Volume of sample Extracted}} \times \frac{100}{\%\text{Recovery}}$$

In this formula, C = Concentration of compound acquired from cal curve, FVol = Final Volume of the Extract in m, Volume of Sample Extracted in mL l, and C_y = Concentration of Compound Y in the Sample.

3. Results

3.1 The difference of the toxin in various times

Table 1. shows the concentration of chlorpyrifos in sprayed samples at the beginning of spraying and after the carnes period.

3.2 The difference of the amount of the toxin in the samples

Table 2. The summary shows the amount of chlorpyrifos in the samples.

4. Discussion

After specifying the concentration of chlorpyrifos toxin in the sample apples of the 3 stations using GC/MASS, the results of this study was analyzed via Excel 2013 and SPSS applications. For the purpose of examining the possibility of differences in the amount of toxins in samples, tests based on time separation were used through common statistical methods. In the analysis of the difference of this amount, depending on the test and samples (in which there are two levels), one-way ANOVA was used.

4.1 Analyzing the amount of toxin

In samples collected in June, on average, 2.4 grams of toxin was found for each kilogram of apples. The 95% confidence interval for this amount of pesticide is achieved

Table 4. Comparison of chlorpyrifos concentration (first test in June) with domestic and universal standards.

Station	Sample concentration 1 (ppm)	Sample concentration 2 (ppm)	ISIRI (ppm)	EPA (ppm)	EFSA (ppm)	CODEX (ppm)
1	4.53	3.19	0.05	0.01	0.5	1
2	0.98	1.01	0.05	0.01	0.5	1
3	2.49	1.77	0.05	0.01	0.5	1

Table 5. Comparison of chlorpyrifos concentration (second test in June) with domestic and universal standards.

Station	Sample concentration 1 (ppm)	Sample concentration 2 (ppm)	ISIRI (ppm)	EPA (ppm)	EFSA (ppm)	CODEX (ppm)
1	2.22	1.56	0.05	0.01	0.5	1
2	0.24	0.16	0.05	0.01	0.5	1
3	0.85	1.3	0.05	0.01	0.5	1

at ±0.2625. In samples of September and October too, for each kilogram of apples, 131.42 grams of toxin was detected at 40.2 ± units.

4.2 The difference of the toxin in various times

Table 1. shows the concentration of chlorpyrifos in sprayed samples at the beginning of spraying and after the carnes period. In the samples of June month, which Pesticide was done in the initial stage and after the 6-day carnes period, we found that this amount has decreased significantly. Also, the amount of Pesticide has decreased from about 4.53 grams to 2.22 grams. In September and October samplings, the reduction rate was measured on the first day of spraying and after the 21-day carnes period. It can be seen that this amount has decreased.

4.3 The difference of the amount of the toxin in the samples

Table 2. In the analyses of June, 6 sample apples and in those of September and October, 3 different sample apples were used. This difference found in behavior may be due to the difference in the type of apples used in the test. The difference in Pesticides in apples is shown in Table 2, based on their type.
It can be seen that different specimens have different levels of toxicity. However, the important point is that although the amount of Pesticide is greatest in June at Station 1, and in September at Station 1, the differences can be ignored. This is concluded from the analysis of one-way ANOVA revealed in Table 3.

4.4 The result of the comparison

In this analysis, two sets of data were compared with each other: one was data confronted with little Pesticide and the other was data in which at least 40 grams of toxin existed per a kilogram (higher than the LOQ40 detection index). From the comparison of parts and analyses carried out, the results showed that in both data sets the amount of Pesticides was reduced with time. In the first test, time significantly reduced the amount of Pesticide, while in the second one, this decrease was not significant. In the first test, the type of test had a significant effect on the results; nevertheless, in the second one, this difference in function was not significant. The highest concentration of chlorpyrifos during the sampling period of June tests was in the first station and then the third station in both tests (Table 1). Similar results in another study also show that things such as excessive spraying, failure to observe the carnes period during harvest can be among the factors of the high level of residual chlorpyrifos Pesticide from international standards (Mackialeagha and Farahani 2012). According to Table 4, the comparison of the results of this study based on the existing international standards in this field shows that the concentration of chlorpyrifos in all samples of the standards has values higher than the permissible levels of the EPA standards, the Iranian standard (ISIRI) and the standard of the European countries (EFSA), CODEX. Also, in the second test Table 5, which was conducted after the carnes period, the results showed that all the samples from the first station have higher concentrations than EPA, ISIRI, EFSA, CODEX standards. Also, all the samples of the second station have higher concentrations than the standards allowed by EPA, ISIRI, and lower than EFSA and CODEX standards. In the first example, the third station is lower than the CODEX standard, but higher than the EFSA standard. During the calculations made by SPSS software, it shows that chlorpyrifos has a higher level than EPA, ISIRI, CODEX and EFSA standards with an average of 1.6917. The highest amount of chlorpyrifos in the tests of September month and October month is related to Quds Square station Table 6 and Table 7. Also,

Table 6. Comparison of chlorpyrifos concentration (test of September) with domestic and universal standards.

Station	Sample concentration (ppm)	ISIRI (ppm)	EPA (ppm)	EFSA (ppm)	CODEX (ppm)
1	0.294	0.05	0.01	0.5	1
2	lower than LOQ	0.05	0.01	0.5	1
3	lower than LOQ	0.05	0.01	0.5	1

Table 7. Comparison of chlorpyrifos concentration (test of September) with domestic and universal standards.

Station	Sample concentration (ppm)	ISIRI (ppm)	EPA (ppm)	EFSA (ppm)	CODEX (ppm)
1	0.285	0.05	0.01	0.5	1
2	lower than LOQ	0.05	0.01	0.5	1
3	lower than LOQ	0.05	0.01	0.5	1

in the tests of September and October, chlorpyrifos in all stations has values lower than the EFSA CODEX standard, but in station 1 it is higher than the EPA and ISIRI standards, other stations have values lower than all standards. During the calculations made by SPSS software, it shows that chlorpyrifos with an average of 0.123 has a lower level than CODEX and EFSA standards, but it has a higher level than EPA and ISIRI standards.
In another study that deals with the effect of different insecticide combinations on shielding and parasitoid on kiwifruit trees, the results show that chlorpyrifos insecticide with a rate of 1.5 per thousand, in combination with 1% and 2% oils in winter spraying and spring has the greatest effect in reducing the shield and parasitoid pest, which is considered its enemy (Gholamian et al. 2013). Chlorpyrifos has a great ability to disinfect the soil, its vapors cause the destruction of soil pests. This Pesticide has a shelf life of 8 to 12 weeks in the soil (Arzhengi 2013), the results of another study on the environmental hazards of insecticides registered in Iran show that Pesticides include 10 insecticides, including chlorpyrifos, including They are the Pesticides that have the most potential toxicity (Moinoddini et al. 2014). The results of other studies that examined 747 samples of 39 different types of fresh fruits and vegetables for pesticide residue

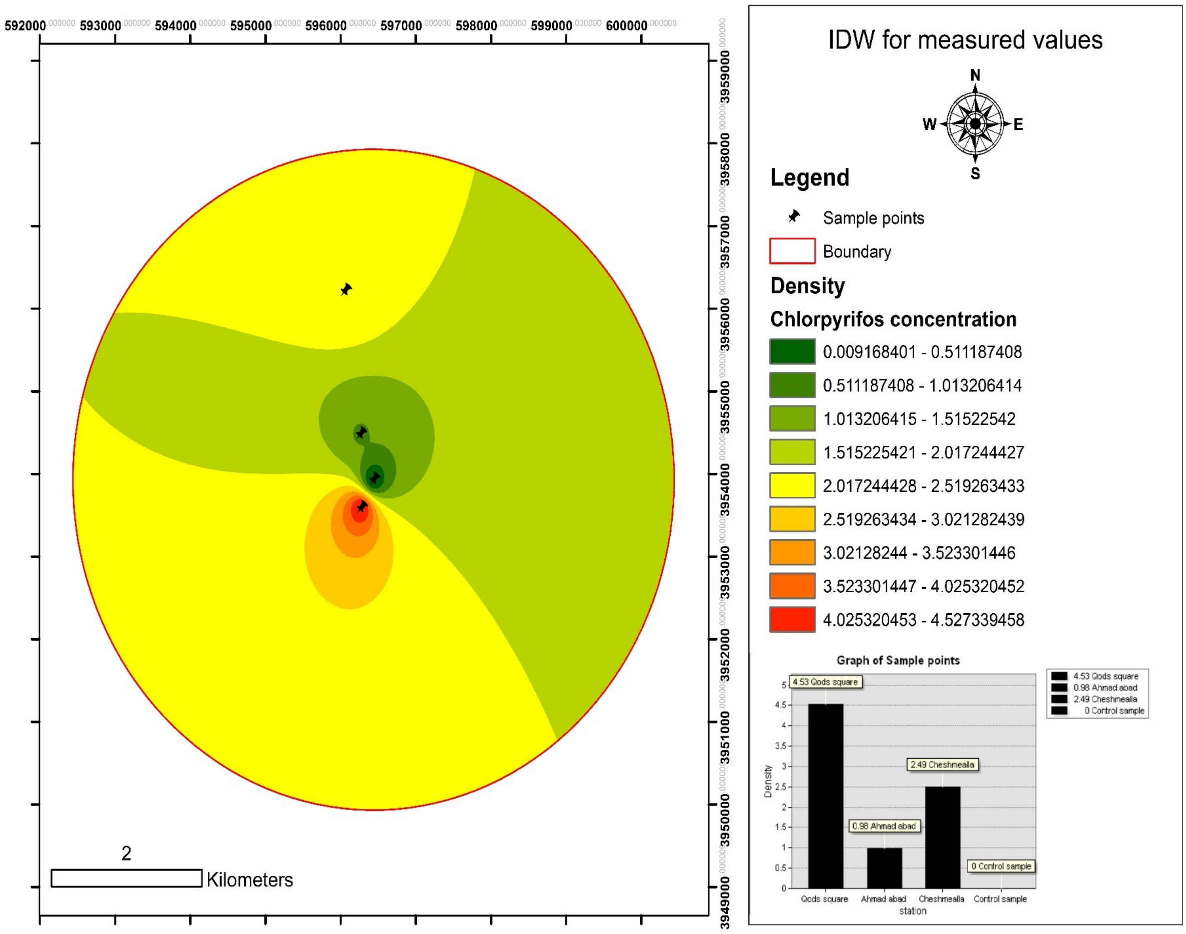

Figure 2. Zoning of pesticide accumulation in the examined orchards.

content. It shows that the highest amount of residues were: bupirimate residues (2.19 mg/kg), captan residues (I, 82 mg/kg), ethylene bis dithiocarbamate residues (1.6 mg/kg), tolyfuanid residues (1.44 mg/kg), procymidone residues (1.19 mg/kg) and chlorpyrifos residues (1.01 mg/kg). In 27 samples (3.6°/4) the remainder was more than the national size (Sadlo et al. 2007).
Figure 2 shown th zoning of pesticide accumulation in the examined orchards. As can be seen in the GIS map, station number 1 (Quds Square station) has the highest amount of chlorpyrifos pesticide, this amount is higher than the standard of ISIRI, EPA, EFSA.
In a similar study conducted by Shahyan and Sheikhloie in Lebanon; the amount of chlorpyrifos in Lebanese red apples was measured in three states: washed with the peel, not washed with the peel, and washed without the peel (Shahyan and Sheikhloie 2017). The results of their investigation showed that the residual amount of chlorpyrifos poison in all three cases is higher than the (EPA) standard (Shahyan and Sheikhloie 2017). Also, in another research conducted by Meki Al-Agha and Farahani, it showed that the residual amount of chlorpyrifos in Golden Word apple varieties of Damavand city is more than the allowed amount (Mackialeagha and Farahani 2012). In another similar study conducted by Mohammadi and Imani on greenhouse tomatoes in Karaj city, it shows that the tested samples have higher amounts of pesticides compared to the Codex standard and the national standard of Iran (Mohammadi and Imani 2012).

5. Conclusion

With respect to the high average of chlorpyrifos over the seven-day currency period of June, 2017 compared with the above-mentioned standards, a seven-day currency period is not recommended for harvesting apples. Moreover, even though the average of the toxin in September and October, 2016 was less than the universal standards, due to the first station being toxic subsequent to a 21-day currency period and also the existence chlorpyrifos in samples from other stations, more care and supervision with more frequency during harvest is essential in order to maintain the health of human societies.

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Author contributions
Zahra haddadnejad, Omid mohammadalikhan, Shahrzad Khoramnejadian: Prposed the plan, conceived the experiments, analyzed the data, authored or revised drafts of the paper, approved the final draft.
Ethics statement
Shahrzad khoramnejadian(department of environment, Islamic azad university, Damavand branch).

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