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Optimization of extraction conditions and determination of the Chlorpyrifos, Diazinon, and malathion residues in environment samples: Fruit (Apple, Orange, and Tomato)

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ABSTRACT

Organophosphate (OP) insecticide, such as diazinon is found in the environments like water which is still approved for agricultural uses. When diazinon residues enter the human body, it functions as an acetylcholinesterase (AChE) inhibitor. This research aims to measure of chlorpyrifos (CPF), diazinon, and malathion residues in fruit such as apple, orange, and tomato after optimizing extraction conditions. Pesticide residues are measured by gas chromatography (GC) technique. Effective variables on pesticide residues are studied including pesticides kind, sampling station, and fruit kind. Results show that average concentration CPF, diazinon, and malathion residues in orange water are 7.05 ± 0.01 mg L^{-1}, 6.66 ± 0.03 mg L^{-1}, and 12.38 ± 0.02 μgL^{-1}, respectively. The average concentration CPF, diazinon, and malathion residues in apple water are 0.74 ± 0.02 mgL^{-1}, 0.70 ± 0.01 mgL^{-1}, and 1.10 ± 0.01 μgL^{-1}, respectively. The average concentration CPF, diazinon, and malathion residues in tomato water are 0.60 ± 0.02 mgL^{-1}, 0.57 ± 0.02 mgL^{-1}, and 0.89 ± 0.01 μgL^{-1}, respectively. The highest CPF concentration is observed in the orange fruit from station 20. Due to an appropriate storage condition and presenting organic fruits in the supermarket, the least mean concentration of pesticides is obtained in studied fruits from station 2. The manner of washing, peeling, and storage period before consuming fruits lead to decreasing studied pesticides concentration about 15–35, 40–50, and 50–60%, respectively. Increasing the fruit shelf-life led to decreasing studied pesticides concentration. Between pesticide concentration and variables: pesticides kind, sampling station, fruit kind, are not seen meaningful statistic relationship ($P > 0.05$). This study showed that pesticide residues in fruits can be decreased by washing, refrigerating, peeling procedures and increase in public surveillance.

Introduction

Food security is an important and global issue. Total government policies programmes for food and nutrition security schedules involve supplying healthy food, economic and social availability for all people (Damari et al., 2018). *Cydia pomonell* L is considered an important pest in terms of producing economic losses in fruit production (Kadoić Balaško et al., 2020). Iran is the fifth-largest producer of citrus in the world. The annual production rate of orange, apple, and tomato in 2016 is about 2,000,000 tons, 3,400,000 tons, and 6,400,000 tons, respectively, in Iran country. (Cilas and Bastide, 2020). The organophosphate (OP) insecticides are organic ester products of phosphorous and side chains of phenoxy, cyanide, and thiocyanate group (Kumar et al., 2016). Applying OP insecticides, as acetylcholinesterase (AChE) inhibitor and fast-acting agent, such as chlorpyrifos (CPF), diazinon, and malathion results to improve the yield of fruits and vegetables (Muhammad et al., 2017). These OP insecticides affect pest's nervous system (King and Aaron, 2015). Annual poison consumption in 2020 is estimated to be about 30000–35000 tons in Iran country. International Agency for Research on Cancer (IARC) has defined that annual mortality rate due to various

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cancers in 2016 is 54,000 people in Iran country (Farhood et al., 2018). Its unreasonable application led to deteriorating environmental quality such as soil and water which is considered an important concern at local, regional, national, and global levels due to biodiversity losses (Alfonso et al., 2017). These pesticides can also be absorbed via human respiratory system. United States Environmental Protection Agency (USEPA) groups most of OP pesticides in toxicity class from I to IV for inhalation and oral exposure (Sidhu et al., 2019). OP pesticides result in more acute toxicity and a more quick decaying than organochlorines (OC) pesticides. Besides, OC pesticides are very persistent in enviroment (Ragab et al., 2016). Diazinon is an OP insecticide which was broadly applied to manage rice striped stem borer in all rice fields. World Health Organization (WHO) groups diazinon in moderately hazardous Class II (Baharum et al., 2020). Diazinon residues enter human body, these function as AChE inhibitors which can lead to various neurological side effects (Esfandian et al., 2016). The increase in OP levels in the environment was mainly attributed to manufacturing, transportation, agriculture utilizations, and wastewater treatment plants (Moussavi et al., 2013). OP insecticides are grouped as moderately hazardous, class II by WHO. The toxicity of CPF was related to neurological dysfunctions, endocrine disruption, and cardiovascular diseases (CVDs) (ur Rahman et al., 2021). The lethal human doses limit is 90–444 $mgkg^{-1}$. It is reported that there are OP pesticides residues in water, soil, sediment, biota, and food chain global (Gobas et al., 2018). OP pesticides fate relates to biodegradation, chemical hydrolyses, photolysis, and volatilization process (Akbar & Sultan, 2016). The person is exposed to pesticides via the consumption of foodstuff. Pesticide residues are measured in foodstuffs, including strawberry (Ghabbour et al., 2012), apple (Mackialeagha and Farahani, 2012), and children herbal medicine (Mosaddegh et al., 2014). This research aims to measure CPF, diazinon, and malathion residues in fruit such as apple, orange, and tomato after optimizing extration conditions.

Materials and methods

Sampling method

In this descriptive-analytical study, fruits in 5 supermarkets where are situated in north, central, west, east, and south of Tehran in March 2020 are selected. 15 fruits (apple, orange, and tomato) are selected in random sampling. All tests are performed in triplicate, and the mean data values are reported. The net weight of fruits applied for sampling is between 30 and 33 g. After transferring collected samples to the laboratory, measurements are performed, including total weight and texture. The samples are stored in 4 °C.

Mesuerment pesticide residues

The described procedure for sample preparation including separation, extraction, and cleanup is performed based on modified the proposed method associated of official analytical chemistry (AOAC). Before residual pesticides (CPF, diazinon, and malathion) analysis, the fruits are powdered crushed by a high-speed electrical blender (MATER, Italy) for 5 min and 7000 rpm, and their pulps are collected. Then, the solution is filtered which is concentrated using a vacuum evaporation apparatus and milipore filter (Whatman, England) with a pore size of 0.45 μm. 7 ml of sample is poured into laboratory tube. Pesticide residues is extracted by solid phase extraction method. 5 ml of acetone, as the best extracting solvent for pesticide residues in studying fruits (foods have quantities high moisture and low fat), are added and centrifuged for 15 min and 4000 rpm into an electrical centrifuge (MATER, Italy). The organic phase is separated from the aqueous phase by the decanter, poured into round bottom balloon, and transfer to a rotary evaporation apparatus until evaporating solvent and concentrating the samples under vacuum (Rota vapor B480, Buchi, Switzerland) at 40 °C and 100 rpm. The evaporated and concentrated sample volume is 2 ml. Gas chromatography (GC) and liquid chromatography with a mass spectrophotometer (LC/MS) are considered a golden method to assess pesticides residues in fruit juice such as apple, orange, and tomato because they cover a wide range of pesticides (Hakme et al., 2018). The sample is prepared for injecting into GC equipped with capillary column non-polar BP5 (5% phenyl/95% dimethyl polysiloxane, ID: 0.25 mm, film thickness: 0.5 μ, length: 30 m). GC (GC 6890 N, AGILENT), with nitrogen gas as a carrier gas, 50 ml min^{-1} of flow, 30 min of retention time, injection temperature of 250 °C and phosphorous pesticide- thermal specific detector (TSD) at 280 °C, and sensitivity of 4×10^{-10} and the column temperature of 230 °C, is calibrated and prepared to receive the sample. The standards of pesticides with a certain concentration (0.1, 1, 10, 100, and 1000 ppm of the pesticide.) are prepared (Razzaghi et al., 2018). Extraction and purification steps are performed on spiked samples, and the extracted pesticides solution is injected into the GC (Fig. 1) (Amirahmadi et al., 2013; Jahanmard et al., 2016). This method used to detect pesticides has a good recovery of 88–121% and a relative standard deviation (SD) of <18%. The limit of detection (LOD) and limit of quantification (LOQ) is obtained at 3.50–14.90 μgL^{-1} and 11.80–49.90 μgL^{-1}, respectively. LOD and LOQ factors are computed 3 and 10 times the SD on the slope of the calibration curve, respectively.

Statistical analysis

The collected data are analyzed by SPSS 18 software using ANOVA and descriptive statistics. A $p \leq 0.05$ is considered significant. The statistical analysis is done based on quantitative variables after transforming all quantitative and qualitative variables into quantitative.

Results and discussion

Description of the study area (Tehran city)

Fig. 2 shows the location of Tehran city, Tehran province, Iran. The study area is located from 80°14′3″ to 80°18′15′ East longitude and 59°35′41″ and 13°08′36″ North latitude. The area of the city is 730 km^2. The estimated Tehran population in 2016 was 13,260,000 people.

Efect of optimazation

The design of experiments (DOE) approach is used to find the optimum values of the operational variables such as solvent and retention time and to show the relationship between two operational variables and three levels on extraction efficiency. According to the results of pretests, although the system GC reaches the highest efficiency at methanol solvent but the application of methyl alcohol solvent leads to producing neurotoxin and eye blindness syndrome. The effect of retention time and solvent including acetone (propanone as a non-protonated

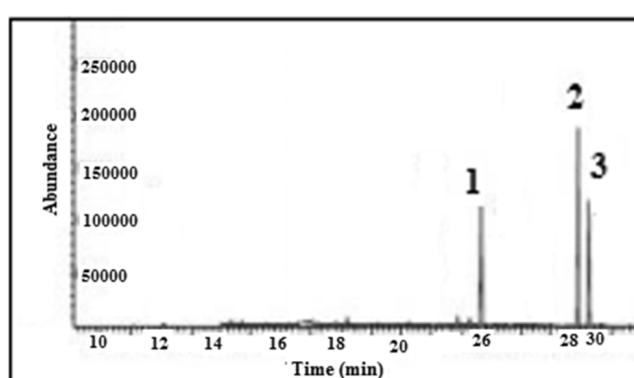

Fig. 1. Chromatogram of standard pesticide solutions with a concentration of 500 μgkg^{-1} using the internal standard (1- Diazinon, 2- Malation, 3- CPF).

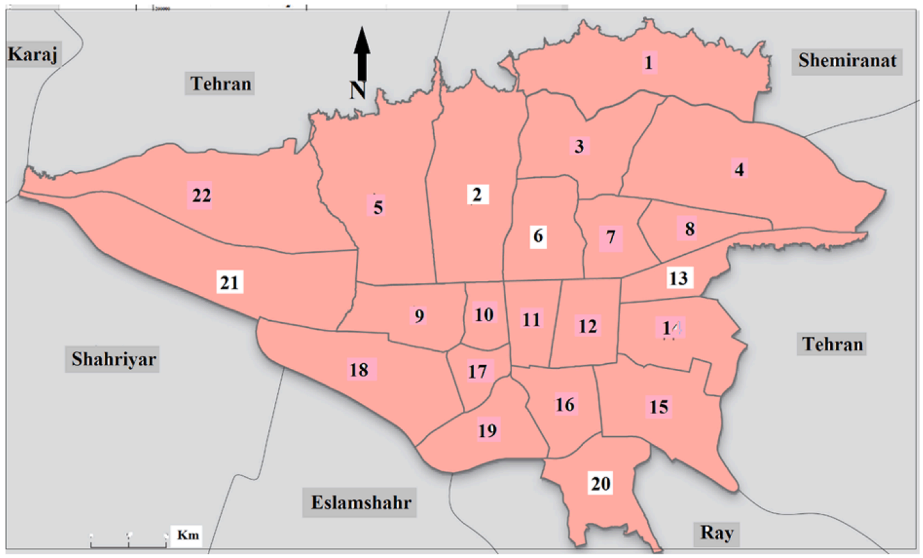

Fig. 2. Location map of Tehran city.

polar ketones) toluene (methylbenzene, as a non-polar solvent), and acetonitryle (methyl cyanide as an aprotic and polar solvent) on the measurement of insecticides (5 mgL^{-1}) is shown in Figs. 3 and 4. The insecticide amount (5 mgL^{-1}) is decreased by increasing the retention time from 30 to 45 min.

Effect of pesticides

According to pretest results, pesticides application plays an important role to increase the productivity of agricultural products (Jallow et al., 2017). In fact, pesticides should be used based on good agriculture practices (GAP) and as needed. According to the pretest, excessive amounts of pesticides residue can be a major health hazard, especially in sensitive groups such as children, the elderly, and infants. According to the national standard of Iran, the maximum residue limit (MRLs) of pesticides-fruit vegetables for CPF, diazinon, and malathion pesticides are 50, 50, and 200 μgkg^{-1}, respectively. The concentration of pesticides (CPF, diazinon, and malathion) in fruit samples (apple, orange, and tomato) from five supermarkets are analogized. The results indicate that the concentration of CPF is more than the concentrations of two other pesticides in the samples from station 20 (Table 1). The concentration of malathion is lower than the concentrations of two other pesticides in the samples from station 2 (Table 1). Results show that average concentration CPF, diazinon, and malathion residues in orange water are 7.05 ± 0.01 (7.03–7.07) mgL^{-1}, 6.66 ± 0.03 (6.64–6.68) mgL^{-1}, and 12.38 ± 0.02 (12.36–12.40) μgL^{-1}, respectively. The average concentration CPF, diazinon, and malathion residues in apple water are 0.74 ± 0.02

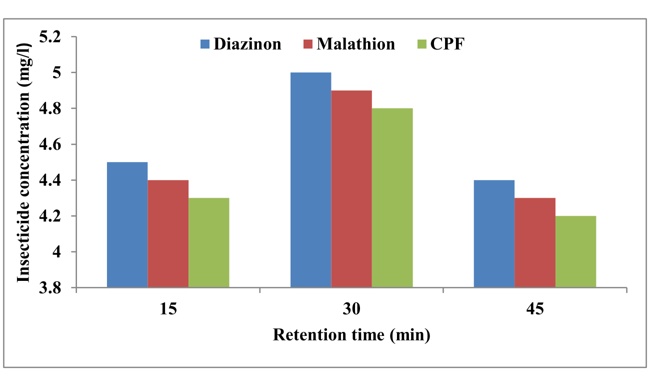

Fig. 3. The effect of retention time on the measurement of the insecticides.

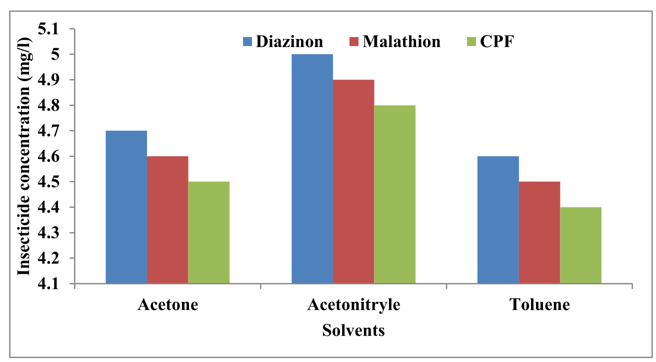

Fig. 4. The effect of solvent on the measurement of the insecticides.

(0.72–0.76) mgL^{-1}, 0.70 ± 0.01 (0.68–0.72) mgL^{-1}, and 1.10 ± 0.01 (1.08–1.12) μgL^{-1}, respectively. The average concentration CPF, diazinon, and malathion residues in tomato water are 0.60 ± 0.02 (0.58–0.62) mgL^{-1}, 0.57 ± 0.02 (0.55–0.59) mgL^{-1}, and 0.89 ± 0.01 (0.87–0.91) μgL^{-1}, respectively. Between pesticide concentration and variables: pesticides kind, sampling station, fruit kind are not seen meaningful statistic relationship ($P > 0.05$) (Table 2). It is concluded that the concentration of pesticide in all samples is higher than permissible limits. The highest CPF concentration is observed in the orange fruit from station 20. According to permissible limit, due to high toxicity for human, CPF is announced as the most dangerous investigated pesticide amongst others. This finding is the same as mullet (Mashian Moradi and Golshani, 2012) and fish (Fianko et al., 2011). Toboconazole and carbofuran pesticide residue in 10 cucumber samples in supermarkets in China is announced more than MRLs (200 μgkg^{-1}) (Wang et al., 2012). Diazinon and oxydemeton-methyl pesticides residue in cucumber samples in Kerman city in Iran country are announced 0.582 and 1.910 μgkg^{-1} and 11.64 and 1.91 times the MRLs, respectively (Rohani et al., 2014). CPF pesticides residue in 28 cucumber samples in Turkish country are announced more than MRLs (Bakırcı et al., 2014). The results indicate a strong positive correlation ($p < 0.081$) between the concentration of studied pesticides and the texture of studied fruits. The highest mean concentration of diazinon is obtained in apple fruit from station 20 with a concentration of 0.72 mgL^{-1}. Due to an appropriate storage condition, sanitation strength, and presenting organic fruits in the supermarket, the least mean concentration of pesticides is obtained in studied fruits from station 2. These concentrations

Table 1
Determination of studied pesticides in samples.

Station	Diazinon concentration mgL^{-1} Fruit			Malathion concentration μgL^{-1} Fruit			CPF concentration mgL^{-1} Fruit		
	Apple (PL = 0.5 mgL^{-1})	Orange (PL = 0.7 mgL^{-1})	Tomato (PL = 0.5 mgL^{-1})	Apple (PL = 0.5 mgL^{-1})	Orange (PL = 0.7 mgL^{-1})	Tomato (PL = 0.5 mgL^{-1})	Apple (PL = 0.01 mgL^{-1})	Orange (PL = 0.02 mgL^{-1})	Tomato (PL = 0.01 mgL^{-1})
2 (north)	0.68	6.64	0.55	1.08	12.36	0.87	0.72	7.03	0.58
6 (central)	0.69	6.65	056	1.09	12.37	0.88	0.73	7.04	0.59
13 (east)	0.71	6.67	058	1.11	12.39	0.90	0.75	7.06	0.61
20 (sought)	0.72	6.68	0.59	1.12	12.40	0.91	0.76	7.07	0.62
21 (west)	0.70	6.66	0.57	1.10	12.38	0.89	0.74	7.05	0.60
Mean ± SD	0.7 ± 0.01	6.66 ± 0.03	0.57 ± 0.02	1.1 ± 0.01	12.38 ± 0.02	0.89 ± 0.01	0.74 ± 0.02	7.05 ± 0.01	0.6 ± 0.02

Table 2
Correlations among study variables.

Number	Variable	P Value*	R^2
1	Station	0.60	0.006
2	Fruit	0.08	0.070
3	Pesticide	0.09	0.066
4	Vapor pressure	0.07	0.075
5	Temperature	0.07	0.075
6	Texture of fruit	0.08	0.070

*A $p \leq 0.05$ is considered as significant.

are higher than the permissible limit and may be considered an urgent health concern and a threat to human life in Iran due to the highly toxic level. The presence of difenoconazole residue in tomatoes is reported (Kong et al., 2012). The fruit peel thickness and peeling method is a significant variable affecting the adsorption of insecticides. A 28 ± 7% decrease of CPF and malathion is reported 3 days after harvesting (Reiler et al., 2015). According to the results of pretests, the manner of washing, peeling, and storage period before consuming fruits lead to decreasing studied pesticides concentration. According to the results of pretests, increasing the fruit shelf-life led to decreasing studied pesticides concentration. This finding is the same as eggplant, cabbage, and yard-long been (Prodhan et al., 2018). It was concluded that concentration due to loss of active water during fruit storage led to increasing studied pesticide concentration. This finding is the same as tomato paste (Baratian Ghorghi et al., 2014). According to the results of pretests, fruit storage at 4 °C in the refrigerator and peeling before consuming fruits led to decreasing studied pesticides concentration about 50–60, and 40–50%, respectively. Therefore, it is concluded that peeling before consuming fruits is a more effective variable than fruit storage at 4 °C in the refrigerator due to decreasing higher studied insecticides residues. An 85–95% decrease of CPF in apples is reported during juice processing (Li et al., 2015). It is concluded that utilizing appropriate doses of seed, fertilizer, and pesticides leads to decreasing studied pesticides residues in fruits, especially orange. It is concluded that vapor pressure, as physicochemical properties of studied pesticides, relates to pesticides residue of fruits. This finding is reported (Yadav and Devi, 2017). An increase of malathion residue of studied fruits is attributed to lower vapor pressure than diazinon pesticides (Nguyen et al., 2020). It is concluded that studying pesticide residues in studying fruits decreased by an increase in public surveillance. This finding is the same as a cucumber.

Conclusion

Due to an appropriate storage condition and presenting organic fruits in the supermarket, the least mean concentration of pesticides is obtained in studied fruits from station 2. According DOE approach, the system GC reaches the highest efficiency at acetone solvent and the insecticide amount (5 mgL^{-1}) is decreased by increasing the retention time from 30 to 45 min. Application of bio-pesticides for pests control and the training of farmers to use appropriate amounts of pesticides would guaranty the safety and health of consumers of agriculture products. Since the concentration of pesticide in all samples is higher than permissible limits and the OP poisons are often concentrated in the skin and the surface parts are fruits so most of the poison residues are removed by peeling and washing and it is the best way to reduce pollution. This research showed strong positive correlation ($p < 0.081$) between the concentration of studying pesticides and the texture of studied fruits, such as the fruit peel thickness and percentage of active water. This research showed that practices, including the manner of washing, peeling method, an appropriate storage condition, storage period before consuming fruits, the fruit shelf life, the sanitation strength, and presenting organic fruits in the supermarket lead to reducing the amount of studied pesticides residues in the studied fruits. This research showed that physicochemical properties of studied pesticides such as vapor pressure reduce the amount of studying residue residues in the studied fruits. This study showed that pesticide residues in fruits can be decreased by washing, refrigerating, peeling procedures and increase in public surveillance. It is suggested that more detailed studies about residues of high-consumption agricultural pesticides in the other cities of Tehran province and in a variety of products agriculture periodically be done. It is suggested that this method of preparation used as a pre-preparation step for other laboratory apparatuses.

CRediT authorship contribution statement

Giti Kashi: Investigation, Writing – original draft, Writing – review & editing, Supervision. **Nafiseh Nourieh:** Writing – original draft, Writing – review & editing. **Parisa Mostashari:** Writing – original draft, Writing – review & editing. **Fariba Khushab:** Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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