AJEHE
Avicenna Journal of Environmental Health Engineering
Avicenna J Environ Health Eng, 2023; 10(2):x-x. doi:10.34172/ajehe.5310
http://ajehe.umsha.ac.ir
UMSHA Press

Original Article

Human Health Risk Assessment of Organophosphorus Pesticides in Tea Leaves Harvested from Farm Regions of Gilan Province, Iran

Alireza Rahmani[1], Fateme Armak[1*], Mohammad Khazaei[1], Ghorban Asgari[1], Mostafa Leili[1], Marjaneh Einipour Rashti[2]

[1]Department of Environmental Health Engineering, School of Public Health and Research Center for Health Sciences, Hamadan University of Medical Science, Hamadan, Iran
[2]Department of Environmental Health Engineering, Giulan University of Medical Sciences, Rasht, Iran

Article history:
Received: September 16, 2022
Accepted: October 31, 2023
ePublished: December 29, 2023

***Corresponding author:**
Fateme Armak,
Email: fateme.armak@gmail.com

Abstract
Tea holds a special place in Iranian culture, ranking as the most widely consumed beverage after water. A significant portion of Iran's domestic tea production (approximately 70%) originates from the lush landscapes of Gilan province. The Iranian Tea Research Center (ITRC) claims that Iranian tea is pesticide-free. However, there is a concern that tea farms in the foothills may be exposed to pesticide spraying from nearby citrus gardens. This study aimed to investigate the presence of the 17 organophosphorus pesticides in tea leaf samples collected from tea plantation areas in Gilan province. Sample preparation was carried out using the quick, easy, cheap, effective, rugged, and safe (QuEChERS) extraction method, followed by the determination of pesticide concentrations using the gas chromatography (GC) method. To assess the non-carcinogenic risk associated with the consumption of black tea due to pesticide residues, we followed the guidelines provided by the United States Environmental Protection Agency (USEPA). The results were striking, revealing the presence of pesticide residues in all tested samples, raising concerns about the purity of Iranian tea. Notably, the hazard quotient (HQ) for children (<15 years old) exceeded that for adults (>15 years old). Furthermore, spring-harvested tea samples demonstrated higher HQs compared to their autumn counterparts.
Keywords: Black tea, Health risk assessment, Pesticide residues, Organophosphorus

Please cite this article as follows: Rahmani A, Armak F, Khazaei M, Asgari G, Leili M, Einipour Rashti M. Human health risk assessment of organophosphorus pesticides in tea leaves harvested from farm regions of Gilan province, Iran. Avicenna J Environ Health Eng. 2023; 10(2):x-x. doi:10.34172/ajehe.5310

1. Introduction

The evergreen tea plant is endemic to tropical and humid regions of China and India. Globally, tea is regarded as the second leading beverage after water, playing a significant role in intestinal microflora, immunity against intestinal disorders, and protection against oxidative damage to cell membranes. In the northern regions of Iran (Gilan and Mazandaran), tea has been cultivated for approximately 100 years, evolving into a strategic commodity due to its economic importance (1). According to reports from the Iranian Tea Research Center (ITRC), tea is the most widely consumed beverage, followed by water. It should be noted that 70% of Iran's domestic tea is grown in the fertile lands of Gilan province. In 2019, per capita consumption of tea was estimated to be 1.04 kg/y in Iran, resulting in an estimated national consumption of around 110000 t/y, 27% of which originating from domestic productions (2). The conditions in which tea is grown contribute to the spread of diseases, but pesticides significantly impact the quality of tea plants (3). Studies have shown that tea leaves are collected and stored in bulk to prepare tea, leading to the accumulation of heavy metals, especially in black tea (4). The widespread reliance on pesticides globally poses a potential health hazard, particularly when they are used excessively. Tea leaves have a greater surface area per unit weight than other agricultural plants. Additionally, the short time gap between pesticide application and harvest heightens human susceptibility to these chemicals (4). There are few registered pesticides used in the cultivation of plants, whose prolonged and repetitive use can cause pest resistance and reduce their effectiveness (5). Numerous countries have enacted strict regulations to curb the use of pesticides. In Japan, for instance, the number of restricted pesticides increased from 34 in 1986 to 276 in 2006. Similarly, the European Union (EU) expanded its list of restricted pesticides from 6 in 1988 to 210 in 2006 (6). Over the past half-century, the use of agrochemicals has increased, leading to heightened human exposure to heavy metals, pesticides, and insecticides, as reported by the National Pesticide Information Center in 2011 (7). Despite the critical importance of monitoring pesticide residues in tea leaves, few studies have been conducted to identify the types of pesticides (e.g., organochlorine or organophosphorus) and the pesticide sources (e.g., direct pesticide application in tea farms, neighboring citrus gardens, adjacent rice farms, etc.) (8). This study aimed to address this gap by investigating the presence of organophosphorus pesticide residues in tea leaves harvested from tea-growing regions of Gilan province. We assessed the potential non-carcinogenic health risks using guidelines provided by both the World Health Organization (WHO) and the United States Environmental Protection Agency (USEPA) for evaluating pollution-related health hazards.

2. Materials and Methods

2.1. Study Area

In 2021, 8 random samples of dry tea were selected from the spring harvest in 8 tea-growing cities in Gilan. Sampling was repeated in the autumn season; in other words, the samples were collected from the same spots as they were collected in spring. Fig. 1 shows the eight counties in Gilan province where tea farms are located. In this study, we collected a dry tea sample from each of these tea-growing counties. Depending on each factory's acceptance based solely on the green tea samples, a dry tea sample can be regarded as representative of the quality of tea in that specific region. It is important to note that the samples we collected were not in the form of packaged tea. Instead, they consisted of freshly harvested green tea leaves collected from the mentioned counties, which were mixed after being obtained from the farmers and used in the dry tea production process.

Fig. 1. Green-Highlighted Counties in Gilan Province for Sample Collection

Following the guidelines of the Iranian Standard Organization, the samples were sent to the laboratory in plastic containers within 48 hours of collection, away from direct sunlight, to determine the 17 organophosphorus pesticides: Beta-mevinphos, trifluralin, diazinon, disulfoton, methyl parathion, ethyl paraoxon, fenitrothion, malathion, fenthion, chlorpyrifos, parathion, bromofos, trans-chlorfenvinphos, butachlor, profenophos, ethion, and Azinphos methyl. Finally, by analyzing the levels of pesticide residues in the samples and concentrations of pesticides transferred to the brewed tea, an assessment of the non-carcinogenic risk of exposure to these pesticides was conducted according to the EPA guidelines.

2.2. Sample Preparation

First, 5 g of each homogenized tea sample was weighed in a 50 mL Falcon tube to extract pesticides. Second, we added 5 µL of TPM (internal standard) to the sample - to reduce work errors and determine the value (quantification) - with a concentration of 1 mg/mL at ambient temperature. The Falcon tube was placed in darkness for 30 minutes so that the pesticide could be thoroughly mixed with the sample and establish a bond (9).

Afterward, 10 mL of 50/50 ethanol/toluene mixture was added and vortexed for 1 minute. Then, 0.5 mg of sodium chloride (NaCl) was added to facilitate the transfer of the pesticide from the aqueous phase into the organic phase and vortexed again for 1 minute. It was then placed in a centrifuge at 4500 rpm to separate the layers for 5 minutes at -5 °C (9).

Purification was performed by transferring the organic phase from the 50 mL Falcon tube to a 15 mL Falcon tube, adding 750 mg of magnesium sulfate, 100 mg of primary-secondary amine (PSA), and 50 mg of graphite carbon black (GCB), during clean-up phase, PSA was used for absorbing proteins and potential sugar and GCB was added to clarify colored samples and vortexing for 1 minute. Then, it was placed in a centrifuge, and after 10 minutes, it was removed from there. Afterwards, 2 mL of the supernatant was transferred into a 4 mL vial with a sampler. Then, 500 µL of toluene solvent was added to the vial. Finally, after 3 minutes of vortexing, 2 µL of the final solution was injected into the GC/MS device (9-12).

2.3. GC/Ms Condition

An Agilent gas chromatography system (GC) with an EI-triple quadrupole detector and a capillary HP-5 ms column (30 m × 0.25 mm × 0.25 um) was used in the study. Helium (He) was used as a carrier gas in this system (Danesh Pazhoohan Amin, Tehran, Iran).

2.4. Non-carcinogenic Risk Assessment

Pesticides provide benefits for increasing agricultural production, but their bioaccumulation through the food chain can ultimately become a threat to animals and humans (13). To determine the health risks of the samples to consumers, it was necessary to calculate the estimated daily intake (EDI) by dividing the average amounts of each pesticide first. The risk assessment was performed based on the guidelines of the EPA. This index was calculated for the age group of adults with an average weight of 70 kg and the age group of children with an average weight of 15 kg according to equation (1) as follows (14-17):

$$EDI = \frac{C \times IR \times EF \times ED}{BW \times AT} \quad (1)$$

Where EF (exposure frequency) is the frequency of exposure (365 days per year), ED (exposure duration) is the duration of exposure (70 years for adults and 15 years for children), BW (body weight) is the average body weight (70 kg for adults and 15 kg for children) (15,16,18), AT (average time) is the average exposure time to non-carcinogenic effects (365 days in 70 years for adults and 365 days in 15 years for children), IR (intake rate g/d) is the average intake rate (10 g of black tea per day for adults and 5 g/d for children), and C is the concentration of the studied pesticide in tea beverage in mg/g, which is calculated according to equation (2) as follows (19):

$$C = C_t \times Tr \times Inf \quad (2)$$

Where Inf (Infusion Factor, defined as the leaching rate of the pesticides in tea and is the ratio of the transfer of the pesticide from dry tea to the tea infusion), C_t is the concentration of pesticide in black tea, and Tr (transfer rate) is the percentage of pesticide transferred to the tea beverage (an average of 70% was considered in this article).

In this study, to conduct a non-carcinogenic risk assessment, the first step was to determine the EDI based on the type of pesticide.

Based on our analysis of the contexts and interviews with tea planters, experts from Iran's Tea Research Center (ITRC), and consumers in Gilan, 10 g of black tea with a medium particle size (referred to as privileged tea) in 200 mL of boiling water was considered as the consumption unit for adults. Furthermore, the percentage of pesticides transferred to tea beverages is influenced by several variables, including the physicochemical properties of tea, the water quantity, boiling temperature, brewing duration, and water solubility (20). The transfer percentage varies for each type of pesticide, and upon reviewing contexts, 70% was deemed to be the average percentage for all organophosphorus pesticides studied in this research.

The non-carcinogenic risk was assessed using the hazard quotient (HQ) after calculating the EDI value, according to equation (3) as follows:

$$HQ = \frac{EDI}{RfD} \quad (3)$$

Where RfD (Reference dose mg/kg/d) is the reference dose for human consumption of a pesticide in mg/kg/day, obtained from the EPA database. For some pesticides, no information about the RfD value was available in the sources studied, and the EDI calculation was not conducted.

Based on the obtained results and the reviews, the analysis of HQ is as follows:

HQ>1 indicates an unacceptable risk, while HQ<1 is considered an acceptable risk under certain conditions (if the consumption does not exceed the daily dose) (19).

3. Results and Discussion

According to the results, the averages, standard deviations, maximum and minimum amounts of each pesticide in 16 samples are indicated in Table 1. First, the averages were checked in comparison to a normal basis. The normality value obtained from the Shapiro-Wilk test was 0.000 for organophosphorus pesticides in the black tea of the autumn season and 0.237 for organophosphorus pesticides in the black tea of the spring season, respectively. The Nonparametric Mann-Whitney test, along with its corresponding values, was conducted when normality could not be established among the compared groups. This analysis yielded a value of 0.011 for organophosphorus pesticides in black tea collected during autumn and spring.

Tables 2 and 3 present the results of measuring the concentration of 17 organophosphorus pesticides and evaluating the non-carcinogenic risk of consuming black tea harvested in the spring and autumn seasons from the

Table 1. Average, Standard Deviation, Maximum and Minimum Amounts of Pesticides Identified in 16 Black Tea Samples (mg/g)

Pesticides	Mean	SD	Max.	Min.
Beta-Mevinphos	2.86E-05	1.81E-06	4.73E-06	6.06E-08
Trifluralin	3.11E-05	1.86E-06	5.04E-06	1.29E-07
Diazinon	1.47E-05	1.25E-06	2.89E-06	0
Disulfoton	2.04E-05	4.01E-06	1.59E-05	0
Methyl Parathion	1.46E-06	1.18E-07	3.84E-07	0
Ethyl Paraoxon	4.24E-05	2.33E-06	6.33E-06	1.3E-07
Fenitrothion	1.02E-05	6.91E-07	2.45E-06	0
Malathion	2.01E-05	1.85E-06	5.82E-06	0
Fenthion	3.77E-06	4.03E-07	1.27E-06	0
Chlorpyrifos	1.52E-05	1.23E-06	4.18E-06	0
Parathion	1.53E-05	8.01E-07	2.53E-06	0
Bromofos	5.5E-06	1.57E-07	5.51E-07	0
Trans-chlorfenvinphos	2.68E-06	1.43E-07	3.91E-07	0
Butachlor	4.75E-06	3.18E-07	1.22E-06	0
Profenophos	4.02E-05	1.97E-06	6.53E-06	7.74E-08
Ethion	1.58E-05	2.45E-06	1.01E-05	5.17E-08
Azinphos methyl	2.22E-05	1.21E-06	4.02E-06	5.63E-08

Table 2. Characteristics of Organophosphorus Pesticide Residues in Spring-Harvested Dry Tea Samples (mg/g) and Their Potential Risk Assessment

No.	Pesticides	1	2	3	4	5	6	7	8	Average	*RfD*	HQ (Adult)	HQ (Child)
1	Beta-Mevinphos	2.57E-06	3.41E-06	2.54E-06	3.39E-06	2.15E-06	4.73E-06	4.64E-06	3.88E-06	3.41E-06	0.003	1.14E-04	2.66E-04
2	Trifluralin	2.74E-06	3.88E-06	3.1E-06	3.18E-06	2.54E-06	5.04E-06	4.02E-06	4.64E-06	3.64E-06	0.0075	4.86E-05	1.13E-04
3	Diazinon	1.9E-06	2.87E-06	2.63E-06	2.89E-06	1.59E-06	0	2.71E-06	0	1.82E-06	0.002	9.12E-04	2.13E-03
4	Disulfoton	0	0	0	3.75E-06	0	1.59E-05	0	0	2.45E-06	0.00004	6.13E-03	1.43E-02
5	Methyl parathion	0	0	0	0	0	0	0	0	0	0.00025	0.00E+00	0.00E+00
6	Ethyl paraoxon	3.19E-06	4.72E-06	4.18E-06	5.85E-06	3E-06	5.36E-06	5.23E-06	6.33E-06	4.73E-06			
7	Fenitrothion	5.62E-07	1.37E-06	7.21E-07	1.19E-06	9.19E-07	1.28E-06	6.74E-07	6.91E-07	9.26E-07	0.005	1.85E-05	4.32E-05
8	Malathion	2.6E-06	1.98E-06	2.88E-06	5.82E-06	3.65E-06	3.2E-06	0	0	2.51E-06	0.02	1.26E-05	2.93E-05
9	Fenthion	0	0	0	0	0	0	0	0	0	0.01	0.00E+00	0.00E+00
10	Chlorpyrifos	9.4E-07	1.74E-06	1.24E-06	2.07E-06	6.48E-07	2.2E-06	2.18E-06	4.18E-06	1.89E-06	0.1	1.90E-04	4.43E-04
11	Parathion	6.48E-07	2.48E-06	7.75E-07	1.32E-06	4.38E-07	6.79E-07	1.11E-06	1.21E-06	1.08E-06	0.005	2.17E-05	5.05E-05
12	Bromofos	3.73E-07	2.15E-07	9.33E-08	4.39E-07	4.85E-07	0	4.85E-07	2.99E-07	2.98E-07	0.02	1.49E-06	3.48E-06
13	Trans-chlorfenvinphos	2.89E-07	0	0	2.89E-07	2.61E-07	0	0	0	1.05E-07			
14	Butachlor	6.53E-07	3.39E-07	5.88E-07	4.6E-07	3.52E-07	1.68E-07	3.44E-07	1.22E-06	5.15E-07	0.15	3.44E-07	8.02E-07
15	Profenophos	1.19E-07	1.48E-07	1.61E-07	1.81E-07	7.74E-08	4.94E-06	6.53E-06	3.69E-06	1.98E-06	0.03	6.60E-06	1.54E-05
16	Ethion	7.62E-07	1.04E-06	7.12E-08	1.16E-06	1.78E-07	1.01E-05	3.64E-07	5.17E-08	1.71E-06	0.0005	3.44E-04	8.02E-04
17	Azinphos methyl	2.69E-06	3.45E-07	2.61E-07	7.26E-07	1.18E-07	9.5E-07	1.05E-06	7.39E-07	8.60E-07	0.03	2.87E-06	6.69E-06
HI												7.80E-03	1.82E-02

Table 3. Characteristics of Organophosphorus Pesticide Residues in Autumn-Harvested Dry Tea samples (mg/g) and Their Potential Risk Assessment

No.	Pesticides	1	2	3	4	5	6	7	8	Average	*RfD*	HQ (Adult)	HQ (Child)
1	Beta-Mevinphos	7.10E-08	1.79E-07	6.06E-08	1.77E-07	6.64E-08	1.01E-07	3.28E-07	2.68E-07	1.56E-07	0.003	5.21E-06	1.22E-05
2	Trifluralin	1.42E-07	1.37E-07	2.75E-07	2.68E-07	3.30E-07	1.29E-07	3.97E-07	2.97E-07	2.47E-07	0.0075	3.29E-06	7.68E-06
3	Diazinon	1.18E-08	3.79E-09	0	2.78E-08	4.21E-08	1.16E-08	2.36E-08	1.24E-08	1.66E-08	0.0002	8.31E-06	1.94E-05
4	Disulfoton	0	0	0	9.75E-08	1.25E-07	3.25E-07	6.18E-08	9.09E-08	8.75E-08	0.00004	2.19E-04	5.11E-04
5	Methyl Parathion	6.64E-08	5.97E-08	1.00E-07	1.25E-07	2.24E-07	3.84E-07	2.11E-07	1.91E-07	1.83E-07	0.000025	7.31E-04	1.71E-03
6	Ethyl paraoxon	1.52E-07	1.30E-07	7.23E-07	1.79E-07	1.22E-06	3.98E-07	1.44E-06	2.60E-07	5.64E-07			
7	Fenitrothion	3.61E-07	0	0	2.45E-06	0	0	0	0	3.52E-07	0.005	7.04E-06	1.64E-05
8	Malathion	0	0	0	0	0	0	0	0	0	0.02	0.00E+00	0.00E+00
9	Fenthion	8.11E-07	7.53E-07	0	6.37E-07	1.27E-06	0	3.02E-07	0	4.71E-07	0.01	4.71E-06	1.10E-05
10	Chlorpyrifos	0	0	0	0	0	0	0	5.62E-09	7.02E-10	0.001	7.03E-08	1.64E-07
11	Parathion	0	3.40E-07	5.97E-07	0	1.79E-06	2.53E-06	0	1.39E-06	8.30E-07	0.005	1.66E-05	3.87E-05
12	Bromofos	4.30E-07	4.95E-07	4.58E-07	0.00	5.51E-07	3.73E-07	2.61E-07	1.78E-07	3.9E-07	0.02	1.94E-06	4.53E-06
13	Trans-chlorfenvinphos	8.67E-08	7.44E-08	2.72E-07	1.38E-07	2.71E-07	3.91E-07	3.20E-07	2.92E-07	2.30E-07			
14	Butachlor	1.40E-07	1.52E-07	3.35E-08	4.99E-08	7.93E-08	0	7.20E-08	9.54E-08	7.78E-08	0.15	5.18E-08	1.21E-07
15	Profenophos	3.70E-06	3.97E-06	4.23E-06	2.80E-06	2.45E-06	2.39E-06	2.73E-06	2.04E-06	3.04E-06	0.03	1.01E-05	2.36E-05
16	Ethion	2.8566E-07	1.42E-07	1.81E-07	1.88E-07	2.50E-07	5.70E-07	1.99E-07	2.38E-07	2.57E-07	0.0005	5.13E-05	1.20E-04
17	Azinphos methyl	5.63E-08	4.02E-06	2.06E-07	1.61E-06	3.17E-06	1.98E-06	2.54E-06	1.70E-06	1.91E-06	0.03	6.36E-06	1.49E-05
HI												1.07E-03	2.49E-03

tea-growing areas of Gilan province in 2021 for children and adults.

The results of the risk assessment indicate that the non-carcinogenic risk of exposure to organophosphorus pesticides was highest when consuming a minimum of 10 g of spring-harvested black tea per day for adults (5 g for children) in conjunction with an average body weight of 70 kg for adults (15 kg for children). Approximately 70% of the transfer rate (TR) is attributed to a pesticide known as Disulfoton, with a value of 6.13E-03 ppb (1.43E-02 ppb in children). The lowest values were observed in Methyl Parathion and Fenthion pesticides, both of which exhibited a concentration of zero.

Among individuals with an average body weight of 70 kg for adults (15 kg for children), the non-carcinogenic risk of exposure to organophosphorus pesticides reached its highest level when consuming at least 10 g of black tea per day for adults (5 g for children) during the autumn season. In this case, Methyl Parathion stood out with a TR of 70% and a value of 7.31E-04 ppb (1.71E -03 ppb in children), while the lowest value (zero) was reported for Malathion.

In Iran, black tea harvested in spring is more popular among consumers. However, there have been no studies conducted to evaluate the quality of tea harvested in spring and autumn concerning residual pesticides, nor have any investigations been carried out to determine the amount of pesticides present in black tea produced in Iran. According to the study done by Heshmati et al in 2020, Chlorpyrifos is transferred to tea during the preparation process, and its concentration increases during brewing (21). Furthermore, research by Amirahmadi et al on pesticide residues in packaged tea in Tehran markets revealed that Endosulfan was uncontrollably present in the samples (10).

In a study, Szternfeld et al analyzed contaminant levels in selected tea samples sold in Belgian retail markets in 2022 and reported that more than 38% of the tested dry leaf samples contained at least one pesticide exceeding the maximum residue level set by the EU. However, subsequent risk assessments have confirmed that brewed tea poses no health threat to consumers (22). Meanwhile, Yao et al conducted investigations involving 42 pesticide residues in 90 tea samples produced in Fujian, China, and reported that Bifenthrin was identified in nearly 9% of the samples. Notably, the presence of this residue varied significantly across different seasons and regions (23).

In a study conducted by Lin et al in 2023 on the residue of four common pesticides in tea from a specific region of China, it was observed that chlorpyrifos was detected in 4.35% of the samples, in accordance with China's standard restrictions. Although the risk levels for all four pesticides were below 1 and within a safe range, it is crucial to consider the dietary habits of future generations (24).

Based on the hazard index (HI), calculated as the sum of the HQ in each study group, as shown in Table 4, the highest HI value (1.82E-02) was observed in spring-harvested samples, particularly for organophosphorus pesticides in the children group. Although the HI level was below 1, suggesting a low risk, comparing HI values revealed that it was substantially higher in children than in adults. It should be noted that children's consumption of this beverage is substantial in Iran. Therefore, the results of investigating tea consumption patterns in children and adults, considering factors such as weight and age groups, can provide valuable insights for future research studies.

Table 4. Hazard Index (HI) Values of Organophosphorus Pesticides in Samples Harvested in Spring and Autumn among Two Age Groups of Adults and Children

	Age Groups	Value
Spring	Adult	7.80E-03
	Children	1.82E-02
Autumn	Adult	1.07E-03
	Children	2.49E-03

Based on the research results regarding the average amount of each pesticide in the defined groups, it was observed that the HQ did not fall within the risk range for any of the sample groups. Fig. 2 reveals that the HI for each age group and season under study remained below 1, indicating no hazard. However, the HI for children was higher than that for adults. Additionally, the spring-harvested dry tea demonstrated higher HI compared to autumn-harvested tea.

Since there was a limited number of samples, we were unable to conclusively assert that consumers' health would not be affected. Therefore, it is imperative to evaluate the cumulative risk using appropriate procedures based on a more extensive dataset and investigate consumers' dietary habits (25). Gilan province is home to approximately 70% of the tea plants, according to the Agricultural Jahad Organization statistics, planted under citrus trees due to coexistence. The application of pesticides to citrus trees is inevitable. Personal interviews with farmers have revealed that due to the significant climatic fluctuations in Gilan province, the timing of pesticide application to citrus trees often coincides with the time of tea harvesting in spring and autumn. The possible reasons for the presence of pesticide residues in tea leaves can be the close proximity and overlap of the timing of pesticide use in citrus trees with the time of harvesting green tea leaves. In some cases, this overlap has been observed during sampling.

Meanwhile, due to the increasing number and diversity of pests present in tea plants and the limited knowledge of farmers, citrus and rice pesticides are sometimes used against tea pests. In addition, there is no supervision regarding this issue; therefore, relying on the results and the presence of organophosphorus pesticide residues in all tea-growing areas of Gilan province, the claim that pesticides are not used for tea in Iran can be ruled out. This research can be a prelude to further and deeper investigations, especially by relevant organizations, such as Agricultural Jahad, and the Tea Research Center of Iran. Among the investigated pesticides, Ethion, Diazinon,

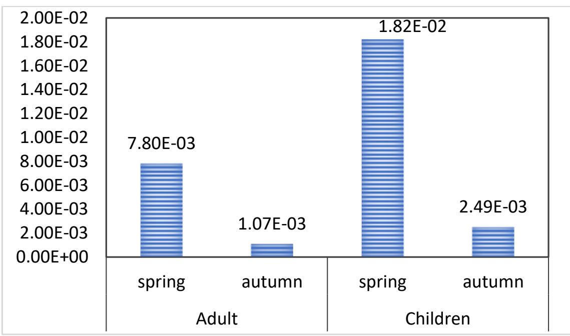

Fig. 2. Hazard Index (HI) Values of Organophosphorus Pesticides in Samples Harvested in Spring and Autumn among Two Age Groups of Adults and Children

Fenthion, Fenitrothion, Chlorpyrifos, and Malathion, along with the herbicide Butachlor, were included in the permitted pesticides category of the Plant Protection Organization of the Jahad Agricultural Ministry of Iran, according to the statistics of 2021, while the remaining 10 examined pesticides were illegal.

4. Conclusion

The purpose of this study was to identify and evaluate the non-carcinogenic risks associated with 17 organophosphorus pesticide residues in 16 black tea samples collected from gardens in Gilan during the autumn and spring seasons of 2021(from all the tea-growing cities). According to official statistics from the Iranian Ministry of Agriculture, approximately 70% of tea gardens are located in Gilan province, and no similar scientific studies have been conducted in the country thus far. The results obtained indicate that all samples were contaminated with residual organophosphorus pesticides, some of which were not on the list of legal pesticides approved by the Iran Agricultural Ministry. Combining these two facts can highlight the urgent need for serious attention and investigation of this strategic product in Iran. In light of the findings of this study, the HQ was determined to be within the safe range, based on the average concentration of each pesticide in the samples. Taking into account the unique parameters of indirect tea consumption, such as TR, this safe range is only achievable under certain assumptions. Although tea is the second most widely consumed beverage after water, especially in Iran, it is a product that humans consume indirectly through various methods. The advantage of this process (from the time and method of leaf harvesting to drying in a factory, the process of brewing and mixing with water, as well as the effect of temperature on it) is to minimize the remaining pesticides that consumers are exposed to. Ultimately, due to the presence of a wide range of pesticides in the samples and the farmers' lack of adequate awareness, it is recommended that relevant authorities should strengthen their supervision of stores selling pesticides, monitor the use of pesticides by farmers, and assess agricultural pesticide residues in food products more rigorously to ensure food safety.

Acknowledgments

The authors would like to express their sincere gratitude to the people of Gilan, especially the tea farmers working tirelessly in the fields, for their unreserved support and cooperation.

Authors' Contribution

Conceptualization: Fateme Armak.
Data curation: Fateme Armak.
Formal analysis: Alireza Rahmani, Mohammad Khazaei, Ghorban Asgari, Mostafa Leili, Fateme Armak.
Funding acquisition: Alireza Rahmani.
Investigation: Fateme Armak.
Methodology: Alireza Rahmani, Mohammad Khazaei.
Project administration: Alireza Rahmani.
Resources: Fateme Armak.
Software: Fateme Armak.
Supervision: Alireza Rahmani.
Validation: Mohammad Khazaei.
Visualization: Fateme Armak.
Writing–original draft: Fateme Armak, Marjaneh Einipour Rashti.
Writing–review & editing: Alireza Rahmani, Mohammad Khazaei, Ghorban Asgari, Mostafa Leili.

Competing Interests

Not applicable.

Ethical Approval

This study was approved by Iran National Committee for Ethics in Biomedical Research. (Code: IR.UMSHA.REC.1399.722).

Funding

This study was supported by Hamadan University of Medical Science (Grant No. 9909116217).

References

1. Tea Organization of Iran. Available from: https://www.irantea.org/fa/.
2. Iran Agriculture Statistics, 2021. Ministry of Agriculture Jihad. Avaliable at: https://jkgc.ir/fa/Page-346.
3. Zongmao C. Pesticide Residue in Tea And its Risk Assessment. Hangzhou, China: Tea Research Institute, Chinese Academy of Agricultural Sciences; 2013.
4. Kharazi A, Leili M, Khazaei M, Alikhani MY, Shokoohi R. Human health risk assessment of heavy metals in agricultural soil and food crops in Hamadan, Iran. J Food Compost Anal. 2021;100:103890. doi: 10.1016/j.jfca.2021.103890.
5. Li H, Zhong Q, Wang X, Luo F, Zhou L, Chen Z, et al. Simultaneous quantitative determination of residues of pyriproxyfen and its metabolites in tea and tea infusion using ultra-performance liquid chromatography-tandem mass spectrometry. Agronomy. 2022;12(8):1829. doi: 10.3390/agronomy12081829.
6. Pang GF, Fan CL, Zhang F, Li Y, Chang QY, Cao YZ, et al. High-throughput GC/MS and HPLC/MS/MS techniques for the multiclass, multiresidue determination of 653 pesticides and chemical pollutants in tea. J AOAC Int. 2011;94(4):1253-96.
7. Yaqub G, Ilyas F, Idrees M, Mariyam V. Monitoring and risk assessment due to presence of heavy metals and pesticides in tea samples. Food Sci Technol. 2018;38(4):625-8. doi: 10.1590/fst.07417.
8. Hou X, Lei S, Guo L, Qiu S. Optimization of a multi-residue method for 101 pesticides in green tea leaves using gas chromatography-tandem mass spectrometry. Rev Bras Farmacogn. 2016;26(4):401-7. doi: 10.1016/j.bjp.2016.03.007.
9. Iran National Standards Organization. Foods of Plant Origin: Determination of Pesticide Residues Using GC-MS and/or LC-MS/MS Following Acetonitrile Extraction/partitioning and Cleanup by Dispersive SPE-QuEChERS-Method. Iran: INSO; 2013.
10. Amirahmadi M, Shoeibi S, Abdollahi M, Rastegar H, Khosrokhavar R, Pirali Hamedani M. Monitoring of some pesticides residue in consumed tea in Tehran market. Iranian J Environ Health Sci Eng. 2013;10(1):9. doi: 10.1186/1735-2746-10-9.
11. Areo OM, Olowoyo JO, Sethoga LS, Adebo OA, Njobeh PB. Determination of pesticide residues in rooibos (*Aspalathus linearis*) teas in South Africa. Toxicol Rep. 2022;9:852-7. doi: 10.1016/j.toxrep.2022.04.001.
12. Wang W, Cho YJ, Song JW, Kim YJ, Seo JS, Kim JH. Residue behavior of methoxyfenozide and pymetrozine in Chinese cabbage and their health risk assessment. Foods. 2022;11(19):2995. doi: 10.3390/foods11192995.
13. Moinfar S, Milani Hosseini MR. Development of dispersive

liquid-liquid microextraction method for the analysis of organophosphorus pesticides in tea. J Hazard Mater. 2009;169(1-3):907-11. doi: 10.1016/j.jhazmat.2009.04.030.

14. Chen L, Wang J, Beiyuan J, Guo X, Wu H, Fang L. Environmental and health risk assessment of potentially toxic trace elements in soils near uranium (U) mines: a global meta-analysis. Sci Total Environ. 2022;816:151556. doi: 10.1016/j.scitotenv.2021.151556.
15. Rafiee Jolodar N, Karimi S, Bouteh E, Balist J, Prosser R. Human health and ecological risk assessment of pesticides from rice production in the Babol Roud River in Northern Iran. Sci Total Environ. 2021;772:144729. doi: 10.1016/j.scitotenv.2020.144729.
16. Rakib MR, Jolly YN, Enyoh CE, Khandaker MU, Hossain MB, Akther S, et al. Levels and health risk assessment of heavy metals in dried fish consumed in Bangladesh. Sci Rep. 2021;11(1):14642. doi: 10.1038/s41598-021-93989-w.
17. Wang Y, Gou Y, Zhang L, Li C, Wang Z, Liu Y, et al. Levels and health risk of pesticide residues in Chinese herbal medicines. Front Pharmacol. 2021;12:818268. doi: 10.3389/fphar.2021.818268.
18. Mohammadi AA, Zarei A, Majidi S, Ghaderpoury A, Hashempour Y, Saghi MH, et al. Carcinogenic and non-carcinogenic health risk assessment of heavy metals in drinking water of Khorramabad, Iran. MethodsX. 2019;6:1642-51. doi: 10.1016/j.mex.2019.07.017.
19. Lu EH, Huang SZ, Yu TH, Chiang SY, Wu KY. Systematic probabilistic risk assessment of pesticide residues in tea leaves. Chemosphere. 2020;247:125692. doi: 10.1016/j.chemosphere.2019.125692.
20. Cao P, Yang D, Zhu J, Liu Z, Jiang D, Xu H. Estimated assessment of cumulative dietary exposure to organophosphorus residues from tea infusion in China. Environ Health Prev Med. 2018;23(1):7. doi: 10.1186/s12199-018-0696-1.
21. Heshmati A, Mehri F, Mousavi Khaneghah A. Simultaneous multi-determination of pesticide residues in black tea leaves and infusion: a risk assessment study. Environ Sci Pollut Res Int. 2021;28(11):13725-35. doi: 10.1007/s11356-020-11658-3.
22. Szternfeld P, Montalvo D, Broos J, Cheyns K, Joly L, Vanhee C. Pesticides, trace elements and pharmaceuticals in tea samples available in Belgian retail shops and the risk associated upon acute and chronic exposure. Food Addit Contam Part B Surveill. 2023;16(1):58-68. doi: 10.1080/19393210.2022.2145617.
23. Yao Q, Yan SA, Li J, Huang M, Lin Q. Health risk assessment of 42 pesticide residues in Tieguanyin tea from Fujian, China. Drug Chem Toxicol. 2022;45(2):932-9. doi: 10.1080/01480545.2020.1802476.
24. Lin T, Chen XL, Guo J, Li MX, Tang YF, Li MX, et al. Simultaneous determination and health risk assessment of four high detection rate pesticide residues in Pu'er tea from Yunnan, China. Molecules. 2022;27(3):1053. doi: 10.3390/molecules27031053.
25. Zhang X, Zhang L, Zhou T, Zhou Y. Fungal flora and mycotoxin contamination in tea: current status, detection methods and dietary risk assessment - a comprehensive review. Trends Food Sci Technol. 2022;127:207-20. doi: 10.1016/j.tifs.2022.05.019.