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Observing of the pesticide residues presence and assessment health risk in locally consumed vegetables in Benghazi city

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ABSTRACT:

Background: Although pesticides were used to protect agricultural products and are safe when used appropriately, however, they can become serious health problem when misused. This study aimed at detecting the presence of pesticide residues in some agricultural crops which are widely consumed in Benghazi city, and assessing the health risks of these residues. Methods: One kilogram of five samples of vegetables (cucumbers, Jalu tomatoes, Benghazi tomatoes, squash, eggplant) were randomly collected from the Benghazi local market in three different time periods. They were then transported and analyzed to the lab on the same day to detect the presence of pesticide residues using the High-Performance Liquid Chromatography (HPLC) technique. Results: The result showed that one pesticide residue (2,4 dichlorophenoxyacetic acid) was observed in tomatoes and eggplants in two of the three periods. The highest value was found in eggplant (1.28 and 0.7 ppm) and the lowest in tomatoes (0.525, and 0.5 ppm), in the first and second periods, respectively. Conversely, all samples in third period were free from the studied pesticides, or that the presence of these pesticides was less than the detection limit of the device used. Additionally, the results showed that the value of the long-term hazard quotient (HQ) for residue consumption of the pesticide (2,4 dichlorophenoxyacetic acid) was higher in the case of 39 eggplant consumption in both males (39.7%) and females (47.0%). While the values of chronic risk index (cHI) were 102.5% for males and 121.45% for females, the values of acute risk index (aHI) were below the marginal value in both genders. Therefore, there were no acute health problems that may result from consumption of the highest dose of this pesticide in only one time. Conclusion: This study suggested that there may be long-term (chronic) health problems from consumed vegetables marketing a locally in Benghazi city and contaminated with agricultural pesticides. This requires immediate and strict measures during the use and application of these pesticides in agriculture

Key words: pesticide residues; vegetable contamination; Risk assessment; agricultural products; hazard index; Benghazi city.

1. INTRODUCTION:

Food pollution can be caused by several environment contaminations such as pathogenic germs, radioactive materials or agricultural chemicals1. The main agricultural chemicals using are pesticides. They are commonly adding for protection of agricultural yields such as fruits and vegetables2. It is well-known that there are more than 1,000 types of pesticides that are used all over the world to ensure that no food damage or spoilage due to pests. Furthermore, each of these pesticides has different characteristics. According to the FAO, pesticides are classified into five types depending on the type of pest to be controlled including: insecticides, acaricides, rodenticides, molluscicides, nematicides, fungicides, herbicides. Thus, they are utilized for protective of agricultural products from unwanted grasses, rodents and some insects. These pesticides can be used by several forms; for example,

formulations may be liquid or solid, powders or granules, water-based or oil-based, 3. However, the extensive applying pesticides, misuse, handing lead to health problems. For instance, using extremely dangerous, either internationally prohibited pesticides or using permitted pesticides, but not adhering to the instructions for use. In Addition, utilizing quantities higher than the required limit and repeat spraying in short periods are together caused different diseases. Furthermore, using of inappropriate pesticide and non-compliance with the period of safety or prohibition mixing more than one pesticide in random ways is associated with health concern4, 5. Instance, these chemicals lead to two toxic effects, chronic toxic causing (rashes, nausea diarrhea, dizziness, and headaches) and high acute effects resulting in (cancerous, reproductive problems, kidney dysfunction and liver diseases) even at very low levels of exposure. Consequently, Pesticides are globally considered among the main death causes, 3,6. The dangerous of these pesticides come from their ability in iterance and accumulation inside plant tissues. Especially, there is difficulty in removing them by washing because they need a sufficient period of time for disposal calling safety period or prohibition period4, 7. The processes of Pesticide contamination usually occur by eating polluted agriculture crops or by direct exposure to pesticides, either by inhalation through the respiratory system, or by exposure through the skin and eyes8-14. Accordingly, several studies were carried out for detection of pesticide residues in different types of crops. For example, analyzed of 206 samples of 27 types of vegetables taken from a local market of Karachi city of Pakistan for residues of 24 different types of pesticides, found that a dangerous level of pesticide residues on vegetables where (36%) of these samples had pesticide residues and (46%) of them had pesticide residues level above the maximum residue limits (MRL)15. Similarly, in Polish, the results of study 1026 samples of fruits and vegetables were selected for detecting the level of pesticide residues showed the presence of pesticide residues in 376 samples, i.e. (36.6%) of the total samples analyzed, and in 18 samples, i.e. (1.8%) pesticide residues were in excess of the permissible limit High values were found for dimethoate insecticide residues in apples at a rate of (1.7%) for adults and (6.8%) in children, meaning that long-term exposure constitutes (1%) for adults and (3%) for children, while exposure for short periods was found in high values in apple consumption by (4.5%) for adults and (13.3%) for children16. The result of another study was conducted in Poland to detect 130 types of chemical pesticides belonging to different groups in 365 samples of cabbage plant (chloroorganic, phosphoroorganic, carbamates, strobilurines, neonicotinoids, amides, pyrimidines, benzimidazoles, imidazoles and triazoles), found 15 types of chemical pesticides were discovered in 118 samples, with a percentage of 32%). Other pesticides were detected in about (4%) of the samples. Where, Chlorpyrifos and cypermethrin were the most common, as Chlorpyrifos was found in (27.4%) of the samples, with a residual rate ranging between 0.005 - 1.51 mmol/kg, while cypermethrin was detected in 3.3% of the samples. With a residual rate ranging between 0.02 - 0.19 mg / kg and 33 samples (9%) that exceeded the permissible limit. 17.

The study carried out by Jallow *et al*, In Kuwait, observed of 34 pesticide residues in 150 samples of fruits and vegetables. No pesticide residues were detected in 62 samples (42%), while 88 samples (58%) contained amount of pesticide residues, a total of 32 samples (21%) contained pesticide residues above MRLs While 56 samples (37%) contained pesticide residues at or below the MRLs 18. Alternative study by Ayas *et al*. (2017) also detected pesticide residues in 160 samples of fruits and vegetables in Algeria19, In their study, 42.5% of the samples of did not contain pesticide residues whereas (12.5%) of the samples contained pesticide residues that exceeded the limit. the results also showed that the long-term exposure was less than (1%) of the estimated daily intake. The short-term exposure was evaluated, where the results showed an increase in the acute risk index. In Saudi Arabia a total of 200 samples of dates (1-2 kg) were selected for investigation of the presence of more than 42 types of different pesticides. That study indicated that 36 samples (18%) contained pesticide residues and 15 (7.5%) of the samples contained concentrations greater than the permissible limit according to the EU classification 20. However, there are limited studies in Libya and particularly in Benghazi city regarding to pesticide residues contamination and their health risk assessment. Thus, this study is considered one of the first studies that deal with these issues. There are a wide range of pesticides are used via different forms in various field of use in Benghazi city (21). This may explain high rate of several diseases and health problems that have been recently observed in this area. Thus, the objective of this study is to detect the presence of pesticide residues in some vegetables from a local market of Benghazi. Also, to estimate the health risk assessment linked with the presence of pesticides in local consumed vegetables.

2. MATERIALS AND METHODS:

This study divided into two parts. The first part is a practical part that depend on the use of multi-separation technology by using HPLC device to estimate the percentage of residues in vegetables. The second parts are a theoretical part based on set of equations for the health assessment of pesticide residues in vegetables.

2.1 **Sample collection**

Five samples of vegetables (cucumbers, Jalu tomatoes, Benghazi tomatoes, squash, eggplant) were randomly collected form the local market of Benghazi, at the amount of one kilogram for each type, over three-time period. The first analyzed on12/18/2017, the second on15/2/2018 and the third on 14/5/2018. On the same day, all samples were transported and analyzed in Al-Mukhtar Center for Research as well as Consultations at Omar Al- Mukhtar University in the city of Al-Beyda.

2. 3 **Detection of the pesticide residues presence**

Pesticide residues (2,4 dichlorophenoxyacetic acid, Dicofol and Heptachlor) presence was evaluated using the approach of QuEChERS which assisted in the multiple determinations of pesticides in vegetables. The extraction and clean-up were done based on the QuEChERS method for pesticides. This method was summarized in the following: Samples were cut, then mixed in the blender, 15 grams were taken from the mixture and extracted using 15 milliliters of 5.1+ acetonitrile acetic acid and then centrifuged at 3,700 laps for 5 minutes take 5 ml of sediment + 750 mg anhydrous magnesium sulfate (250) + MgSO4 mg PSA (amine secondary primary), then shake for 20 seconds and centrifuge for 5 minutes. The stencil was taken, and then dried with a light stream of nitrogen, as well as the product was dissolved in 1 ml of acetonitrile, and 1 ul was taken from it for injection into a high-efficiency chromatograph,22 .

2.4 **The risk assessment of pesticide residues on human health**

To assess the risk of pesticide residues on human health, it is necessary to know the daily intake of this person the pesticide, as it is taken on the basis of body weight and nutritional status of the person. Evaluation of the consumption rate plays a major role in assessing the risk of pesticide residues in foods. The risk is calculated by the arithmetic mean of all results obtained by comparing the residues in the food with what is known as the estimated daily intake rate (EDI) with intake daily estimated (ADI) and the acute reference dose in ARFD (acute reference dose). The product ADL and ARFD value is calculated from what was agreed upon at the Joint Meeting of the World Health Organization (WHO) and FAO (Agri- Food Organization) in 2002 in Roma at the Meeting on Pesticide Residues and revised in 2006,23. The estimated daily intake (EDI) of pesticide residues was calculated according to the following equation24: EDI= Fi × RLI /mean bw (1) Where, Fi: is meaning concentration of pesticide residues ($mg{\cdot}kg^{-1}$) in the vegetable samples Mean bw: is Mean body weight which was obtained by average means of a questionnaire for 250 individual in the Benghazi city including 137 females and 113 males. RLI: is Food consumption data that was determined based on the data from survey involving questionnaire for (200) a family in the city of Benghazi. This survey included some question for a head of family related to estimation of the amount of weekly consumption of each of the studies crops; the number of his family members, and the average daily consumption per capita of each crop was extracted. The long-term risk assessment of the intake compared to the data collected from the toxicity of pesticides was done by calculating the hazard quotient (HQ), by dividing the international estimated daily intake with the relevant acceptable daily intake: HQ = IEDI/ADI × 100% (2) where: ADI is the acceptable daily intake. The calculated of the short-run take-in index term estimated was done using the following equation: ESTI= {F × HR.P/ mean body weight where: F: data for fully consumed product, P.HR: the highest level of the residue comparing the calculated intake of pesticides in Food with ARFT to calculate acute health index (AHI) as follows: AHI= ESTI /ARF, where ARFD means acute reference dose, which is an estimate of the amount of chemical substance in food or water which is calculated on the basis of body weight and it is taken in a period of 24 hours or less without any noticeable health symptoms appearing. It is considered that there is an unacceptable health problem if the value of the chronic (CHI) as well as acute risk AHI indicators is greater than 1.

4. RESULT:

The results of the presence of pesticide residues in the studied samples:

The samples collecting on 18/12/2017 revealed presence of residues of 2,4 dichlorophenoxyacetic acid in each of Jalu tomatoes, Benghazi tomato and eggplant as shown in table 1. The highest residual values were detected in Eggplant, 280.1 ppm, followed by Benghazi tomato and Jalu tomato, 735.0, 525.0 ppm respectively. However, all samples were free of the residues of Dicofol and Heptachlor, or may be less than the detection limit of the device used.

Table (1): the results of Pesticides residues in different type of vegetables

Sample collection date	Common name of vegetables	Types of test and result (Pesticides residues)/ ppm		
		2,4 dichlorophenoxyacetic acid	Dicofol	Heptachlor
First Sample December 18,2017	Jalu tomatoes	0.525 ppm	LOD*	LOD
	Benghazi tomatoes	0.735 ppm	LOD	LOD
	Cucumbers	LOD	LOD	LOD
	Squash	LOD	LOD	LOD
	Eggplant	1.280 ppm	LOD	LOD
Second Sample February 15, 2018	Jalu tomatoes	LOD	LOD	LOD
	Benghazi tomatoes	0.3 ppm	LOD	LOD
	Cucumbers	LOD	LOD	LOD
	Squash	LOD	LOD	LOD
	Eggplant	0.7 ppm	LOD	LOD
Third Sample May 14,2018	Jalu tomatoes	LOD	LOD	LOD
	Benghazi tomatoes	LOD	LOD	LOD
	Cucumbers	LOD	LOD	LOD
	Squash	LOD	LOD	LOD
	Eggplant	LOD	LOD	LOD

***LOD= Limit of Detection**

As for the second sample collected on 15/2/2018, it also showed the presence of remnants of (2,4 dichlorophenoxyacetic acid). The highest percentage of residuals in Eggplant was 7.0 ppm, followed by Residual in Benghazi tomato 3.0ppm as shown in Table 1. Nevertheless, there were no any pesticides residuals in the third sample of vegetable collecting on 14/5/2018, or that the percentage of these pesticides is below the limit of detection (LOD) for HPLC as presented in Table 1.

2.1 Assessing the risk of pesticide residues on human health

2.2.1 Calculate the estimated daily intake (EDI):

Table (2): Estimated daily intake (EDI) of pesticides residues and consumption value of the crop in different vegetables of studied samples

Level of pesticides in crop (mg/ kg/day)	consumption value of the crop	Average weight of male	Average weight of female	Estimated daily intake (EDI)	
				Male (mg/ kg)	Female (mg/ kg)
Benghazi tomatoes 0.5175	0.428	71	60	3.12×10^{-3}	3.7×10^{-3}
Jalu tomatoes 0.525	0.428	71	60	3.16×10^{-3}	3.745×10^{-3}
Eggplant 0.99	0.285	71	60	3.97×10^{-3}	4.7×10^{-3}

The above table shows the estimated daily intake in the studied samples and the consumption value of the crop with an indication Average body weight for males and females, the results showed that the estimated daily intake of (2,4 dichlorophenoxyacetic acid) was higher in the eggplant crop for both males and females, while the lowest value of daily intake of the pesticide was in the tomato crop at Males).

4.2. Estimate the long-term index (Health Quotient (HQ))

The value of the long-term hazard ratio (HQ) for the consumption of pesticide residues (2,4 dichlorophenoxyacetic acid) shoed in table 3. The technician declared the consumption of the eggplant crop, the technician declared that the males were (7.39%) and the females were (0.47%, 0.47%). Females were also more sensitive than males in other crops. By collecting the coefficient of risk coefficient (HQ) values, the chronic risk index (cHI) is shown. Indicator and mean curricular index for males, females, = 5.102 (<1%) and for males, females, = 5.102 121.45 (<1).

Table (3): Estimate the long-term index (Health Quotient (HQ))

Crops	Estimated daily intake (EDI)		Acceptable daily intake of the pesticides (ADI)	Health Quotient (HQ)
Benghazi tomatoes	**Female**	3.7×10^{-3}	0 - 0.01	37.0%
	Male	3.12×10^{-3}	0 - 0.01	31.2%
Jalu tomatoes	**Female**	3.745×10^{-3}	0 - 0.01	37.45%
	Male	3.16×10^{-3}	0 - 0.01	31.6%
Eggplant	**Female**	4.7×10^{-3}	0 - 0.01	47.0%
	Male	3.97×10^{-3}	0 - 0.01	39.7%

4.2.1 Evaluate of short-term intake (ESTA)

Table (4): Evaluation of short-term intake (ESTA)

Consumer details (mg/Kg)		High Level of residuals (mg/Kg)	Average of male body weight (Kg)	Average of female body weight (Kg)	evaluate of Short Time Intake (ESTA)	
Crops	**Consumption**				**Male**	**Female**
Benghazi tomatoes	0.428	0.735	71	60	0.004431	0.005243
Jalu tomatoes	0.428	0.525	71	60	0.003165	0.003745
Eggplant	0.285	1.28	71	60	0.005138	0.00608

Table (4), shows that the amount of intake in the short term (ESTI) is higher in the eggplant crop for females (0.00608) and males (0.005138) compared to the rest of the crops, while the lowest value was in Jalu tomato yield for males (0.003165) and females (0.003745) as well.

Table (5): Acute health index (aHI)

Consumer details (mg/Kg)	evaluate of Short Time Intake (ESTA)		Acute reference Dose (ARfD)	Acute health index (aHI)	
Crops	**Male**	**Female**		**Male**	**Female**
Benghazi tomatoes	0.004431	0.005243	0.8	0.005539	0.006554
Jalu tomatoes	0.003165	0.003745	0.8	0.003956	0.004681
Eggplant	0.005138	0.00608	0.8	0.006423	0.0076

According to above table by comparing short-term intake (ESTI) with acute reference doses (ARfDs) to determine the Acute Hazard (aHI), the results showed that the highest value of the acute hazard index is in the short term in the eggplant crop in females (0076.0), the smallest value of the indicator was in the males in the crop Jalu tomatoes (003956.0). However, all values were less than the limit value, 1.

5. DISCUSSION

5.1. Pesticide residue

Three types of pesticides have been studied: 2,4 dichlorophenoxyacetic acid, Dicofol and Heptachlor.

Although one of the objectives of this study was to reveal the largest number of pesticides using in the Benghazi city, especially the organophosphorous pesticides, which are highly toxic to humans and most frequently used in Libya, the apparatus using in the investigation of the residual effect had no ability to detect the standard values for organophosphorous pesticides. This device can investigate 2,4 dichlorophenoxyacetic acid, Dicofol, along with DDT and DDD but the last two pesticides are not communally used in Libya. The results showed that the collected samples on 12/18/2017, contained pesticide residues (2,4 dichlorophenoxyacetic acid) in each of Jalu tomatoes, Benghazi tomato, and eggplant (525.0, 735.0, 280.1 ppm) respectively. In contrast, the residues of Dicofol and Heptachlor are less than the detection limit of used device (LOD). As for the second sample that was taken on 15/2/2018, it also showed the presence of residues of (2,4 dichlorophenoxyacetic acid) pesticides, and the highest percentage of residues was in (eggplant 7.0 ppm), followed by the percentage of residues in (Tomato Benghazi 3.0 parts per million). These results support the view of (7) where found high residue levels of pesticides detected in tomato samples. In the third sample which was taken on 05/14/2018, no pesticides were observed in it or that the presence of pesticides proportion was below the limit of detection (LOD) for the user's device in that sample. It's likely that there were residues of 2,4 dichlorophenoxyacetic acid in the first and second sample however not in the third one. This may be because of the period of taking the first and second samples which was in the winter and spring seasons. In both seasons, the temperature drops and the most of vegetable were commonly produced in that period by all greenhouses where the heat and humidity rise. Moreover, there is a suitable environment for the growth of pests, weeds, and consequently to the excessive use of pesticides, which increases the possibility of the presence of residuals on these vegetable leading to health effects in the long and short term. As shown in the results, the cucumber and zucchini crops were free of pesticides residues in the studies samples. This may be the result of the fact that the samples collected from two different species, tomatoes and eggplants that are from the Salicaceae family, while squash and cucumbers are form 27 Cucurbitaceous families. The absence of pesticide residues in these species may be due to the ability of the Cucurbitaceous family to destroy or dispose of pesticides more quickly than the nightshade family, or as a result of that the pesticide (2,4 dichlorophenoxyacetic acid) was not used at all. As comparing the results with the permissible limit for this pesticide in different crops showed that residual concentrations of (2,4 dichlorophenoxyacetic acid) are much higher than the maximum permissible limits in these vegetables, as its concentrations in the Jalu tomatoes represent more than 11 times the maximum allowed for its presence in these vegetables, besides its concentrations in Benghazi tomatoes were higher, representing more than 15 times the maximum allowable presence in this crop, while its concentrations in eggplant were higher than other crops, which represent almost more than 26 times the permissible limit in eggplant, according to the World Health Organization and the United Nations Environmental Protection Agency for consumers. This contrasts with many Studies that were carried out in different countries of the world (15-21). Those studies found remnants of pesticides but their concentrations were less than the maximum permissible limit or did not to reach such the large concentrations as in the present study. Consequently, this clearly shows how dangerous the application of this pesticide on the vegetables growing or marketing in the city of Benghazi and the serious health problems resulting from them that often appears after a period time. This is often the result of a lack of awareness among farmers, a lack of adherence to the proper instructions for the use of pesticides and a lack of oversight from various responsible authorities. This ultimately leads to the accumulation of large quantities from these chemicals in the human body, reaching often to the toxic limit effect. This effect, according to previous studies, can be a cause of serious health problems which some of them have been seen in a Libya, especially in Benghazi city, such as liver diseases, kidney failure and cancers. Therefore, the result of this study may help in understanding the reasons behind to such diseases by introducing pesticides as one of the most important causes of such diseases.

5.2 Health evaluation of pesticide residues:

The result of the health evaluation of acute health problems (which resulted from consuming a certain of this pesticides once a day without the appearance of noticeable disease symptoms for the residues of this pesticide) showed that the acute toxicity of the pesticide at the concentrations found in the different crops in this study may not constitute a major health problem as the acute hazard index was less than 1 for both males and females 25. However, with regard to the evaluation of the chronic risk of consuming 2,4 dichlorophenoxyacetic acid pesticide, the result of the health evaluation of the residues of this pesticide in this study indicated that this pesticide may be an important cause of long-term (chronic) health problems. This might as a result of the consumption of agricultural crops that contain as high as that found in this study from the residual 2,4 dichlorophenoxyacetic acid, where the chronic risk index

in males was 102.5 (<1%) and in females it was equal to 121.45 (<1%), which indicates the presence of an unacceptable health problem that depends on the type of pesticide used.

Accordingly, toxicological studies on animals and humans (33,32,31,30,29,28,27,26,25,14,8) showed that the (2,4 dichlorophenoxyacetic acid) pesticide causes many chronic diseases such as cancer, liver and kidney diseases, this is also evidence of the possibility that the use of pesticides in the city of Benghazi is a major and important reason for the occurrence of such diseases, especially in the almost complete absence of regulatory bodies and the lack of epidemiological studies linking the use of pesticides with such diseases. Also, the results of the remaining pesticide residues studied in these crops, which appeared as less than the detection limits of the device used (LOD) in this study, does not mean that these crops are free of these pesticides, as they may be present, but in quantities less than the amount that the device can detect. Furthermore, they may pose health damage even at such low levels. Besides, the detection in these crops of the residuals of these studied pesticides doesn't mean that they are free other types of pesticides, which are often more toxic than the studied pesticides such as organophosphorous pesticides in Benghazi and the rest cities of Libya.

6. Conclusion

It's clear from this study that there may be chronic health problems in the city of Benghazi that may be caused by the use of agricultural pesticides, which requires immediate, strict measures to regulate the use in additionally application of pesticides on agricultural crops. Furthermore, the presence of one type of pesticides in the studied samples doesn't means that it is free from the rest of the other pesticides used extensively in the Benghazi. Thus, more researches are needed to determine the size of the problem in the city in particular, and the rest of the Libyan cities in general and the necessity of raising awareness and educational level related to pesticides application among worker who dealing with them and for the general public.

7. Research limitations

There was some difficulty facing this study including: the lack of the ability to detect the remnants of other group of agricultural pesticides such as the organophosphate group. These pesticides were widely used in the city of Benghazi and pose great health risks as a result of their high toxicity to mammals. Also, the high cost of analyzing pesticide residues in crops.

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