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The Impact of Washing and Peeling of Cucumbers and Tomatoes in Reduction of Daily Intake of Dithiocarbamates

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Abstract: Background: Pesticides residues on foods are potentially toxic to humans. They may induce adverse health effects such as cancer, effects on reproduction, immune and nervous system complications. One of the important goals of public and environmental health officials is reducing exposure to pesticide residues on foods. Materials and methods: In the present study, 80 samples of cucumbers and 60 samples of tomatoes were collected from greenhouses and after preparation, dithiocarbamates (DTCs) residues were analyzed. Dithiocarbamates were determined using spectrophotometric method. During this procedure a mixture of cupper was shaped from the reaction between CS_2 and the cupper after acid decomposition of the DTCs in a 3 trap responsive order. The concentrations of DTCs remnant were compared with MRL_s and their daily intake were calculated and compared with ADI. Results: The outcome depicted that washing with water and peeling may able the reduction of absorption of daily intake of the rest of DTCs from 1.8% (as percent of ADI) in unwashed cucumbers to 0.6% ADI and 0.13% ADI respectively. The DTCs residues daily intake in unwashed tomatoes samples were 0.66% ADI and also in water-washed and peeled tomatoes were 0.43% ADI and 0.23% ADI respectively. Conclusion: The daily intake of DTCs was reduced by washing with water and peeling significantly. Although the estimated daily intakes of DTCs in the studied vegetables do not pose a health risk in consumers, but, because the entrance of fungicides into the human body by other commodities, these daily intakes are considerable.

Keywords: Daily Intake, Dithiocarbamates, Peeling, Washing

1. Introduction

Pesticides are the most widely used chemicals for the pest control in agriculture, gardening, homes and soil treatment. Carbamates, a class of pesticides, were introduced to agriculture market in the 1950 s [1]. Dithiocarbamates (DTC_s) are organosulfur compounds and depending upon their structure can be classified into three subclasses including dimethyl Dithiocarbamates, ethyleneBis Dithiocarbamates ($EBDC_S$) and PropyleneBis-Dithiocarbamates ($PBDC_S$) [2]. Maneb and Mancozeb are two fungicides in the class of $EBDC_S$ which are widely used to control fungal disease of various crops such as cucumber and tomato in Iran. Carbamates are included in the list of endocrine distributor compounds [3]. Although bioaccumulation potential and short-term toxicity of Carbamates are low, according to the priority list of United State Environment Protection Agency (USEPA), they are harmful and hazardous to the Environment and human health. The main portion of all applied pesticides remains as residue in the crops and can find their way into the food chain.

There are many studies about the fungicides residues in fruits and vegetables. Lozowicka et al. (2015) analyzed 974 samples for the presence of over 70 fungicides [4]. They reported that in Poland, boscalid and captan, with the

concentrations of 2.83 mg/kg to 3.31 mg/kg had the highest concentrations.

Jardimet all. (2014) analyzed 238 kaki, cashew apple, guava, and peach fruit and pulp samples, and their results showed that Over 70% of the samples were positive, with DTC present in 46.5% [5].

Due to the perils of DTCs, the exposure studies are needed for safekeeping and advancement of communal sanitation. Many people are mostly faced with pesticides through diet. Diet is the most significant procedure of people's disposal to DTCs by consuming treated fruits, vegetables and polluted water [6].

Due to determine the disposal of people's pesticide, the pesticide remnant in food should be compared with ADI (Acceptable daily intake) or ARFD (Acceptable reference dose) [7].

In different societies different procedures of cleaning on fruits and vegetables are being done. Washing with some chemical materials in order to remove pesticides from them are usually being performed. Chemical materials such as hydroxy peracetic acid, chlorine dioxide, iprodione and some other detergents are completely efficient for that purpose [8],

Washing with water is the most popular cleaning operation which is recommended by health guidelines. The main aim of water washing is prevention of infectious diseases, but these operations on food commodities can reduce the risk of pesticide exposure via reduction of residue concentration of pesticides. FDA recommends washing fruits and vegetables under running water just before eating, cutting, or cooking. Major portions of polar compounds are removed by washing with water in both household and commercial preparation. There are a lot of reports about reduction of pesticide residues by washing. For instance, 50% reduction of enitrothion residues in apples [9] 53% of Azinphos in apples [10] are some reports of water washing of crops.

Peeling and juicing operations are the main routes which can remove non-polar pesticides [8]. Reduction of different pesticides by peeling was reported by many researchers. Fahey et al. (1970) have reported that a lye peeling process removed more than 99% of tetrachlorvinphos in peaches [11]. Reduction of 99% of fenitrothion residue in potato [12] 80.6% to 89.2% for different pesticides residues such as DDT in tomatoes [13] 91% - 98% reduction of chloropropham residues in potatoes [14], substantial decrease in chlorpropham residues [15] are some of the results of peeling in pesticides residues reduction.

Lee and Lee (1997) could eliminate 45% of rganophosphorous pesticide (OP) residues of foods by washing in water, 56 %with detergent washing, 91% with peeling, 51 %with blanching-boiling and 90% in milling and processing [16].

The removal rates of 8–52% and 19–67%, for pesticide residues of dieldrin and heptachlor epoxide in pumpkins and cucumbers, by washing with water or 0.1% liquid detergent (respectively) have been reported [17].

Non- polar pesticides can dissolve in detergents or soap water but washing with soap and detergents is not recommended for washing and cleaning of fruits and vegetables. The main reason is remaining of detergents on the outer surface of crops. However, there is no documented reports about the concentration of detergent residues in crops. If the reduction of pesticide residues by detergents is significant, more studies about concentration of detergent residues will be needed.

In the recent years, the cultivation of vegetables under greenhouse has been developing in Iran due to water crisis. The cultivation in greenhouse requires less water than open fields.

There are some scientific reports on the influence of purifying vegetables and fruits with detergents to reduce the pesticide of crops planted in greenhouses. The supplied study targeted the assessment of the beneficence of purifying with water and detergent to reduce DTCs health hazard in greenhouse tomatoes and cucumbers.

2. Material and Methods

2.1. Chemicals and Solutions

The entire number of chemicals were analytic levels which had been gotten from Merck. The color reagent (solution of copper Acetate monohydrate) had been gotten ready by 0.024 gcupper ǁ acetate monohydrate in 55.6 ml of diethanolamine in 500 ml ethanol and stored at 5 ċ protected from light. Hydrolysis reagent was prepared with 100 ml hydrochloric acid (37%) and 50 ml reagent water. Lead acetate solution was prepared by dissolving 30 g of 30% Pb $(C_2H_3O_2)_2.3H_2O$ in 100 ml reagent water.

2.2. Sample Preparation

The paradigms of tomatoes and cucumbers planted in greenhouses were gathered from the centers of distribution of fruits and vegetables in Zanjan, Iran. The paradigms were sorted into the categories (treatments) of 4, namely unwashed, washed with water, washed with detergent and peeled categories. An anionic detergent in the name of Jam was used to wash the fruits and vegetables with detergent.

2.3. Analysis

The analytic procedure for evaluation of DTCs in this study was spectrophotometry method. It was according to the alternation of the remnant of DTCs to CS_2 and an adjustment of this design that was recommended by other researchers [18] [19] [20] [21]. This method is recommended for analysis of DTC_s in wastewater by USEPA [22]. Although the analytical methods have been developed in recent years, spectrophotometry method for analysis of DTCs is the most valid method. One of the best evidence is the research of Jardim et al. (2014) in which they analyzed DTC_s in cashew apple, guava, kaki and peach using spectrophotometry method and for analysis of other pesticides, they used GC–lECD, GC–FPD and LC–MS/MS multiresidue methods [5].

In acid decomposition method of DTCs, in stannous chloride presence as a diminishing factor, the cupric complex

is framed from the reaction between CS_2 and the copper (II) acetate monohydrate which is measured at 435 nm by spectrophotometry method. Presented in this study, two boiling flasks in a round-bottom shape were placed in a heating mantel linked to a common system of distillation which was used for acid decomposition of DTCS and a set of CS_2. This system comprised 3 traps which are linked to the condenser.

The starting trap comprised 20 mL of NaOH (4N) solution, the next trap had 10 mL of Lead acetate solution, and the last one also comprised 15 mL of color reagent. Not only NaOH but also Lead acetate solution was used to refine the CS_2 form, omitting interferences, most importantly H_2S and CS_2.

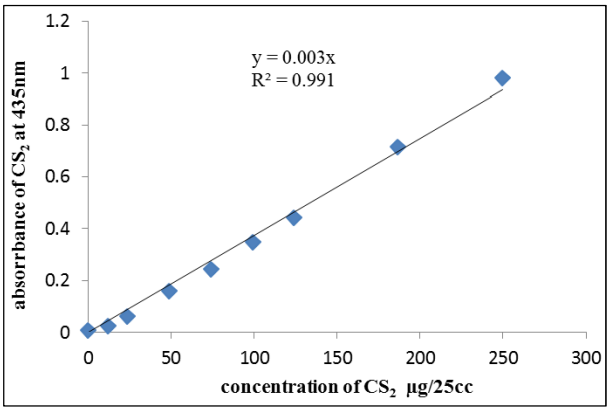

Figure 1. *Standard curve of absorbance against µg CS_2 for determination of DTC_S residues.*

Ground paradigm with the weight of 100 g, 2g of $SnCl_2$, and 100ml of hydrolysis reagent were set in the boiling flasks with a neck round-bottom shape which had direct heat for 45 minutes, and the acid decomposition of DTCs happened in HCI presence. Since the product of distillation had been cooled, the last trap's solution was diluted to 25ml by ethanol. And by passing 15 minutes the sorption of the solution was scaled at 435 nm against the reagent blanks which were prepared in the same manner.

The standard curve of the CS_2 had been gotten ready by CS_2 calibration solution (Figure 1). The famous content of the CS_2 stock solution were altered to 25 mL volumetric flasks, color reagent was increased by 15 ml, the content was completed by ethanol, and the solution was permitted to stand for 15 minutes. The sorption was scaled by spectrophotometer (Hach, DR- 5000).

2.4. Quality Control of the Method

The LOD (limit of detection), LOQ (limit of quantification) and the percentage of recovery were assessed for quality control of the analysis procedure [23].

Paradigms of tomatoes and cucumbers were spiked with a fixed scaled solution of Maneb and Mancozeb and after analysis of the samples the amounts of LOD, LOQ and recovery were specified. The LOD and LOQ of the method in this mentioned order were 18 µg/kg and 60 µg/kg. due to direct statistical analysis of the DTCS, the remnant concentration with values less that LOD, moiety of the assessed LOD are considered in calculations [24].

The outcomes of the test's recovery are illustrated in table 1. The entire number of received recoveries was in the range of 88% and 115%.

Table 1. *The results of the recovery experiments.*

Sample type	Spiked solution	Concentration of standard solution (µg/Kg)	Number Of replicates	Mean recovery (%)
		8.5	3	97
	Commercial Mancozeb	14	3	95
		17	3	96
		14	3	115
Tomato	Standard Mancozeb	17	3	103
		34	3	92
		14	3	110
	Standard Maneb	17	3	110
		34	3	88

3. Estimated Daily Intake

The portion of cucumbers and tomatoes to ADI was defined using the method suggested by by the Pesticides Safety Directorate (PSD) of the Department for Environment, British Food and Rural Affairs [25]. The estimated daily intake (EDI) is calculated using the following formula:

$$EDI = \frac{Fi \times RLi}{MBW}$$

Where: Fi - food consumption data, RLi - residue level to the commodity. MBW- Mean body weight.

The EDI can be calculated in mg/person/day or µg/Kg.bw.d and uses the body weight of an adult as 60kg.

The hazard quotient (HQ) was computed by admeasuring the estimated daily intake with the related admissible daily intake:

$$\text{HQ} = \frac{EDI}{ADI} \times 100\%$$

The HQ for treatments of unwashed, washed with water and detergent and for pilled samples were calculated and the effect of preparation methods on reduction of DTC_s exposure was evaluated.

The MRL of DTCs has been established by many scientific counters and organizations. WHO and FAO have determined the DTC_s MRL as 2000 µg/kg for cucumbers and

tomatoes.

Iranian National Standards Organization (INSO) has established the DTC_s MRL of 500 µg/kg for cucumbers and tomatoes. An ADI of 0.03 µg/Kg.bw.d is determined for DTCs by WHO/FAO and INSO [26]. These values were used for daily intake calculation in this study.

4. Results

The outcome depicts that the concentrations of the rest DTCs in 75% of the cucumber samples were higher than LOQ and 35% of them were higher than INSO's MRL (500 µg/kg). The outcomes were stated in table 2.

Table 2. *The results of analysis of DTCs in the studied vegetables.*

Commodity treatments	No. of samples	Samples with residue>LOQ No. (%)	Samples with residue exceeding MRL (INSO) No. (%)	Samples residues exceeding MRL (WHO/FAO) No. (%)
Cucumber				
Unwashed	20	15 (75)	7 (35)	1 (5)
Washed with water	20	14 (70)	1 (5)	0
Washed with detergent	20	10 (50)	0	0
peeled	20	4 (20)	0	0
Tomatoes				
Unwashed	15	12 (80)	0	0
Washed with water	15	5 (33)	0	0
Washed with detergent	15	1 (6.6)	0	0
peeled	15	1 (6.6)	0	0

In the 80% of the unwashed tomato samples, the concentration of DTCs remnant was higher than LOQ and no paradigms were higher than MRL. By paying attention to all the analyzed outcomes 25% and 20% of the unwashed cucumbers and tomato samples were attached with undetected remnants (< LOQ) respectively. The DTCs remnants were lessened in tomatoes samples which were washed or pilled.

The mean concentration of DTCs remnants were 505 and 72 µg/kg in unwashed cucumbers and tomato paradigms respectively. Washing with water and detergent has positive impact on reduction of DTCs remnants concentration especially in cucumber samples. 66% and 81% of DTCs residues concentration were reduced in cucumber samples which were washed using water and detergent respectively. It is because the DTCs fill on the surface of fruits and vegetables. The mean concentration of DTCs remnants in peeled cucumber samples was 38.2µg/kg with 92% reduction. The residue dissipation by washing and peeling was observed in tomatoes samples too.

As it can be seen in table 3, adult's exposure to DTCs does not exceeded the ADI in the reported cases. The results show that the chronic dietary exposure is pretty low. In unwashed cucumber samples the HQ values was 1.8% and in unwashed tomato samples this value was 0.66%. Peeling of the samples could reduce HQ values to 0.13% and 0.23% in cucumbers and tomatoes samples respectively. In the cucumber samples more than 65% of residues and HQ values are reduced when the samples were washed with water alone whereas in tomatoes samples the residues and HQ values reduction by water were about 34%.

Table 3. *The intake of detected DTCs by adults.*

Treatment	Average concentration of DTCs in samples with a residue>LOQ (µg/kg)	Reduction of concentration compared to unwashed treatments (%)	ADI (µg/Kg.bw.d)	EDI (µg/Kg.bw.d)	HQ (%)
Cucumber					
Unwashed	505.2	-	30	0.53	1.8
Washed with water	173.2	65.75	30	0.18	0.6
Washed with detergent	95.4	81	30	0.10	0.33
peeled	38.2	92.5	30	0.04	0.13
Cucumber					
Unwashed	72.4	-	30	0.2	0.66
Washed with water	47.6	34	30	0.13	0.43
Washed with detergent	40	44.7	30	0.11	0.36
peeled	26	64	30	0.07	0.23

5. Discussion

The individual component of dietary intakes in different age groups is the first step to assess the risk of human health in food toxicology. In the present study, the DTCs residues concentration was used for the assessment of human consumer's risk on the basis of the consumption of 63.2 and 170g/d of cucumbers and tomatoes in Iran, (respectively) and considering the weight of 60 kg for adults. The sole consumption data of vegetables in Iran is reported by INSO (63.2 and 170g/d for cucumbers and tomatoes respectively) and there is no any exact data of individual component of dietary intakes in different age groups. From a risk assessment perspective these data might be insufficient but considering to the goal of the present investigation that was

evaluation of food preparation methods, these data seemed to be adequate. The consumption of tomatoes is higher than cucumbers because different tomatoes by- products such as several kinds of sauces are consumed in Iranian diets.

The results showed that the pesticides residues concentration in fruits and vegetables can influenced by washing and peeling. The placement of the remnant in the plant, age, water solubility, temperature and kind of washing are major factors which determine the effectiveness of washing in removing pesticide residues. Major portions of polar compounds such as carbaryl can remove by washing. Non-polar pesticides tend to accumulate on the waxy layers of the peel of fruits and vegetables [27]. Most of DTCs are soluble in water but insoluble in non- polar solvents therefor, washing can remove them from the surface of fruits and vegetables. Washing with detergent has not significant effect on the residues and HQ reduction, in addition harmful effects of detergent residues should be considered too.

Significant reduction of DTCs by peeling off cucumbers samples skin shows that DTCs are accumulated in fruit pericarp only and no movement to fruit pulp. The skin of tomatoes is very thin, and this is the main reason of lower reduction of residues in tomatoes samples than in cucumber samples. Furthermore, DTCs remnants would be able to be found in the pulp of tomatoes and tomatoes by- products. The outcome of this analysis, accept that washing with water and detergents lessens the health danger of daily intake of DTC_s, but In the case of using detergents, more precautions should be considered about the detergent remnants on the surface of vegetables and fruits.

An important diminution of DTCs by peeling of cucumber samples skin illustrates that most of DTCs are accumulated in fruit skin. The outcomes demonstrated that even in peeled vegetables the DTCs remnant is detected. The dithiocarbamates remnants in Brazilian food have been stated by Caldas et al., their outcomes mentioned that detectable DTCs remnants were found in the pulp of banana, papaya (including the seeds) and orange (50–62% of the analyzed samples) [20]. The outcomes of our study showed that 38.2 and 26 µg/Kg DTCs remnants were caught in the pulp of cucumber and tomatoes respectively. These values are 7.5% and 36% of total residues in unwashed studied vegetables. The skin of tomatoes is very thin, and this is the main reason of higher residues level in the pulp of tomatoes samples. On the other hand, DTCs residues can be found in the pulp of tomatoes and tomatoes by- products.

The calculated EDI_s of DTCs residues in water washed vegetables of the present study are lower than ADI, therefor do not pose a health risk but, because that the DTCs residues can be detected in the other food commodities, these values are high and considerable. For instance, according to the results of this study, the estimated daily intakes of DTCs residues in water washed cucumber and tomatoes were 0.6% ADI and 0.43% ADI. Cucumber and tomatoes are not the main food commodities, and have a small portion in the Iranian's diet, therefore, these values should be controlled and reduced.

6. Conclusion

In conclusion, Washing with water and peeling can lessen the intake of DTCs significantly. The estimated dietary intake of DTCs residues levels detected in the cucumber and tomato samples do not represent a health risk to the consumers but because the existence of pesticide residues in other commodities, every cleaning method before consumption which can reduce the pesticide residues is recommended. Food safety precautions should be more considered in tomatoes and tomatoes by- products consumption because the thin skin of tomatoes and high level of consumption in Iranian dietary, to keep the consumer safe for indiscriminate disposed with pesticides.

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