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The Effect of Washing and Peeling on Reduction of Dithiocarbamates Residues in Cucumber and Tomato

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ABSTRACT

Background: Dithiocarbamates, the main group of fungicides, are used to control about 400 pathogens in more than 70 crops. These pesticides are widely applied to crops including potato, cereal, apple, pear and leafy vegetables throughout the world since 1960. From the late 1980s, using these fungicides has caused much debate among regulators about their long-term effects on consumers and occupational users.

Method: In this study the residues of Dithiocarbamates in cucumber and tomato using the colorimetric method (Keppel method) was measured. Respectively 80 and 45 samples of greenhouse cucumber and tomato were collected from Zanjan vegetables center in autumns and winter 2013. The samples were analyzed in 4 treatments of: unwashed, washing with water, washing whit detergent and peeling.

Result: The results showed that the average concentration of Dithiocarbamates residues in unwashed greenhouse cucumber and tomatoes were 384.5 µg/kg and 65 µg/kg respectively. 35% and 5% of unwashed and water washed cucumber and tomato samples (respectively) had higher Dithiocarbamates residue than MRL recommended by Institute of Standards and Industrial Research of Iran (0.5mg/kg).

Conclusion: The treatments of washing and peeling had significant effect on the reduction of Dithiocarbamates residues in the all samples.

1. Introduction

Dithiocarbamates (DTCs) belong to the family of Carbamate and organophosphate pesticides, which have been used in

agriculture as fungicide for more than 80 years and they widely used as vulcanization accelerators in the

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rubber industry [1-3]. They can control approximately 400 pathogens of more than 70 crops [4].

DTCs are categorized into three groups of Dimethyldithiocarbamate (DMDS), ethylenbis (Dithiocarbamates) (EBDS) and propylenbis (Dithiocarbamates) (PBDS) [5]. EBDS and PBDS are almost insoluble in water and organic solvent. DMDS (e.g. Thiram, Ziram and ferbam) are slightly soluble in water and some polar organic solvents. It is well known that Dithiocarbamates residues in food system are carcinogenic in laboratory animals [6].

Extreme activity of Dithiocarbamates mostly related to their metal-chelating ability and high affinity for proteins containing the SH-groups [1, 7]. The most important transformation products of DTCs are Ethylenthiourea (ETU) and propylene thiourea (PTU) which cause various pathogenic, mutagenic and teratogenic [8, 9]. DTCs are non-systemic fungicides and in use they are sprayed on the plant, so the residue of which mostly remain on the surface of the crop [10]. In recent years the scientific reports show their presence in foods, drinks and environment [11, 12]. DTCs are not stable and cannot be extracted and analyzed directly.

Because of harmful effects of DTCs, national and international health-related organizations are established defined limitations for DTCs. The main limitation is maximum residue levels (MRLs) [13].

The MRLs refer to total DTCs determined as CS₂ evolved during acid digestion and expressed as mgCS₂/kg. According to FAO/WHO standards the MRLs of DTCs in tomato and cucumber are 2 mg/kg [14]. Institute of Standards and Industrial Research of Iran has established the MRLs of DTCs as 0.5 mg/kg in tomato and cucumber [13]. Tomato and cucumber is two main vegetables in Iranian diet. The per capita consumption of cucumber and tomato are 23.078 kg/year and 62.6 kg/year respectively [15]. Insolubility of DTCs in water and other solvents cause some difficulties for extraction and measurement

of their residue on the crops [16]. On the other hand, if the concentration of DTCs residues on the crops be high, there is a doubt about removing of the DTCs residue from the surface of the crop by washing the crops with water and there is no any documented study about it.

In the present experimental study report the effects of washing and peeling on reduction of DTCs residues on the cucumber and tomato is evaluated.

2. Material and Methods

2.1. Chemicals and solutions

All chemicals were analytical grade obtained from Merck. The color reagent (solution of copper Acetate monohydrate) were prepared by 0.024 g copper acetate monohydrate in 55.6 ml of diethanolamine in 500 ml ethanol and stored at 5 °C 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 of 30 g of 30% Pb (C₂H₃O₂)₂ · 3H₂O in 100 ml reagent water.

2.2. Sample preparation

The greenhouse tomato and cucumber samples were collected from the distribution center of fruit and vegetables of Zanjan in December, January and February of 2012. Samples were divided into 4 groups (treatments) including unwashed, washed with water, washed with detergent and peeled. For washing with detergent an anionic detergent which is used for dish washing and in Iran named Jaam was used.

2.3. Analysis

The analytical method for measurement of DTCs in this research was based on the conversion of the residue of the DTCs to CS₂ (Cullen method) [17]. A modification of this design was proposed by Thier and Zeumer [18-20].

In the process of acid decomposition of DTCs, in the presence of stannous chloride as a reducing agent, the cupric complex is formed from the reaction between CS₂ and the copper (II) acetate monohydrate which is measured at 435 nm by spectrophotometry method. In this

research, a two neck round- bottom boiling flask placed in a heating mantel connected to a traditional distillation system was used for acid decomposition of DTCS and CS₂ collection. The distillation system consisted of 3 traps which are connected to the condenser.

The first trap contains 20 mL of NaOH (4 N) solution, the second trap contains 10 mL of Lead acetate solution, and the third trap contains 15 mL of color reagent. Both NaOH and lead acetate solutions were used to purify the CS₂ formed, removing volatile interferences, mainly H₂S and CS₂.

100 gr of grinded sample, 2g of SnCl₂, and 100ml of hydrolysis reagent were put in the tow- neck round bottom boiling flask which was heated for 45 min. and the

acid decomposition of DTC_s took place in the presence of HCl.

After cooling of product of distillation, the solution of trap3 was diluted to 25ml by ethanol. After 15 minute, the absorbance of the solution was measured at 435 nm against the reagent blanks prepared in the same way.

The standard curve of the CS₂ was prepared by CS₂ calibration solution (Fig. 1). Known volumes of the CS₂ stock solution were transferred to 25 mL volumetric flasks, 15 mL of the color reagent was added, the volume was completed with ethanol, and the solution was allowed to stand for 15 min. The absorbance was measured by spectrophotometer (DR- 5000).

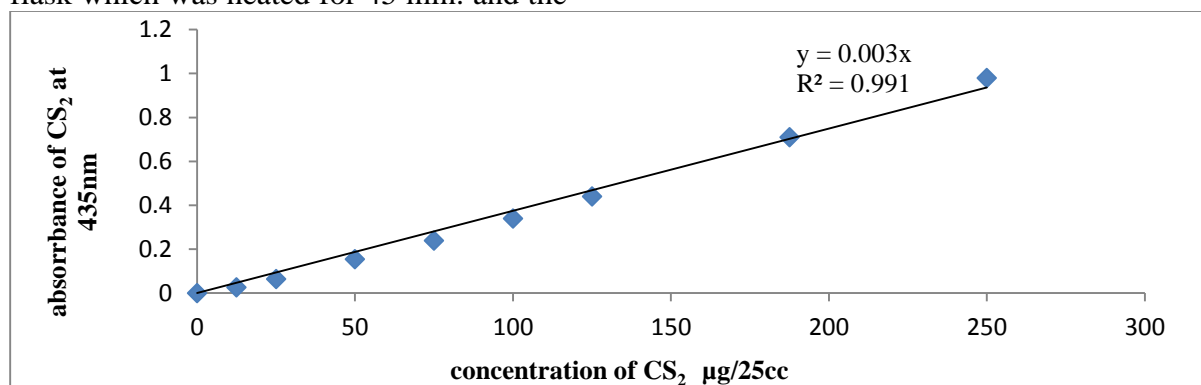


Fig.1: Standard curve of absorbance against µg CS₂ for determination of DTC_s residues.

2.4. Quality control of the method

The LOD (limit of detection), LOQ (limit of quantification) and the percent of recovery were determined for quality control of the analysis method [21].

The tomato and cucumber samples were spiked with standard solution of Maneb and Mancozeb and after analysis of the samples the amounts of LOD, LOQ and recovery were determined. The LOD

and LOQ of the method were 18 µg/kg and 60 µg/kg respectively. In order to statistical analysis of the DTC_s residues concentrations with values less than LOD, half of the determined LOD (1/2 LOD) was considered in calculations [22].

The results of the recovery experiments are shown in table 1. All of the earned recoveries were between %88 and %115.

Table 1: The results of the recovery experiments.

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

3. Results

After quality control tests, the samples were divided into the 4 treatments (unwashed, water washed, washing with detergent and peeled) and the DTC_s

residues were measured. Tables 2 and 3 shows the mean concentrations of DTC_s residues in the samples briefly. In the table 4 the reduction of DTC_s residues, after washing and peeling of samples are shown.

Table 2: The concentrations of DTC_s residues in the cucumber samples as (µg/kg) CS₂.

Treatment	Sample size	Mean (µg/kg)	Maximum (µg/kg)	Minimum (µg/kg)	Standard error	Mod (µg/kg)
Unwashed	80	384.5	2233	9(1/2 LOD)	119	183
Washed with Water	80	124	663	9(1/2 LOD)	36.4	43
Washed with Detergent	80	52	293	9(1/2 LOD)	15.6	14.5
Peeled	80	17.5	53	9(1/2 LOD)	3.7	9

Table 3: The concentrations of DTC_s residues in the tomato samples as (µg/kg) CS₂.

treatment	Sample size	Mean (µg/kg)	Maximum (µg/kg)	Minimum (µg/kg)	Standard error	Mod (µg/kg)
Unwashed	45	65	190	9(1/2 LOD)	16	33
Washed with Water	45	22	93	9(1/2 LOD)	6.3	9(1/2 LOD)
Washed with Detergent	45	11	40	9(1/2 LOD))	2	9(1/2 LOD)
Peeled	45	10	26	9(1/2 LOD)	1.1	9(1/2 LOD)

Table 4: The reduction of DTC_s residue in different treatments in compare with unwashed treatment.

Sample	Washed with Water	Concentration Washed	Peeled
Cucumber	67.75	86.47	95.4
Tomato	66.3	83	84.6

The mean concentrations of DTC_s residues in unwashed cucumber and tomato samples were 384.5 (µg/kg) and 65(µg/kg) respectively. The minimum DTCS residues concentrations in the all of

the samples were lower than the measured LOD value but in statistical calculations 1/2 LOD was considered.

In 35% and 5% of unwashed and

washed with water greenhouse cucumber samples, DTCS residues were higher than the recommended MRLs of Institute of Standards and Industrial Research of Iran and none of samples were higher than the codex recommended MRLs.

The results showed that, the washing with water, with detergent and peeling can reduce the DTCS concentration in cucumber by 68%, 86% and 95% respectively. These reductions in tomato samples were 66%, 83% and 84% respectively. Washing with detergent reduced the DTCS residues only about 18% more than washing with water, so it can be conclude that water has the main role in cleaning of DTCS residues from the surface of the crops.

In order to determining the differences between the results in different treatments, statistical analysis of post Hoc-Bonferrony was used. This test showed that washing with water and detergent and peeling reduce the DTCS residue significantly. On the other hand in comparison with unwashed samples, the DTCS residue in washed and peeled samples are low ($P < 0.05$).

The results showed that there is no significant decreasing between treatments of washed with detergent and peeled with treatment of water washed samples ($P > 0.05$) as well as there is no significant decrease between treatments of peeled and washed with detergent ($P > 0.05$).

As mentioned before, it means that the most of DTCS residues is washed with water. Peeling cause the most decreasing in DTCS residue in tomato and cucumber and this reduction shows that the most values of DTCS concentrations is accumulated on the surface of the samples. The results showed that washing can reduce the concentration of DTCS by 85 percent but the best way of control is peeling.

4. Discussion and Conclusion

The study showed that the measurement of DTCS residues in cucumber and tomato

using Cullen method is simple and valid with the method detection limit of 18 $\mu\text{g/kg}$. The results showed that 35% of the cucumbers had DTCS residues higher than recommended MRL of Institute of Standards and Industrial Research of Iran. This is an important alarm and establishing a methodological program for sampling and analysis of the crops in Iran.

If the concentration of residue be very high, washing with water or detergent cannot decrease the residue, lowers than recommended MRLs. Meanwhile it is recommended that in any cases, washing with water is necessary and peeling is the most efficient way for reduction of DTCS residues in cucumber and tomato.

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