EFFECT OF COOKING TEMPERATURE AND CLEANING METHODS ON LEVELS OF MANCOZEB AND ETHYLENETHIOUREA IN TOMATO

Karanja E. K., Murungi J. I. & Nyambaka H.

Egoji T.T.C, Private Bag Igoji

Department of Chemistry, Kenyatta University, P.O BOX 43844, 00200, Nairobi, Kenya

elkarunyaki@yahoo.com

Citation:

Karanja E. K., Murungi J. I. & Nyambaka H. (2023). ***Effect of Cooking Temperature and Cleaning Methods on Levels of Mancozeb and Ethylenethiourea in Tomato.*** **In: Isutsa, D. K. (Ed.).** ***Proceedings of the Chuka University 9th Annual International Research Conference held in Chuka University, Chuka, Kenya from 24th to 25th November*, 2022. 460-459 pp.**

ABSTRACT

Pests and diseases in vegetables are controlled using pesticides which include fungicides in the class of dithiocarbamates (DTCs) such as mancozeb which are injurious to the environment, animals and human at high concentrations. Cooking degrades DTCs to metabolites such as ethylenthiourea (ETU) known to be carcinogenic, while proper cleaning removes the residue considerably. This study determined the effect of temperatures achieved by cooking and cleaning methods on the residue levels of mancozeb and the metabolite ETU on tomato (*Lycopersicon esculentum*) sprayed with mancozeb. Samples were subjected to different temperature and cleaning treatments before extraction and method validation together with analysis by HPLC. The method of analysis had The R^2 values (0.948- 0.999) and recoveries ranging from 91.26-95.89 %.The ETU residues rose from a mean of 9.43±0.03 mg/kg at 25℃ to a mean of 12.43±0.38mg/kg at 90℃, while mancozeb levels decreased from 5.23±0.02 mg/kg at 25℃ to non detectable levels at 60℃. The levels of ETU reduced from 46±0.71m/kg to 0.05± 0.00 mg/kg (99.9±0.00 %) when cleaned with sodium hypochlorite while, water alone reduced ETU to 0.27±0.08 mg/kg (99.4±0. 00%).Mancozeb levels in tomato cleaned with sodium hypochlorite, reduced by 95.2±0.00% while cleaning with water alone removed 16.3±0.64%. Cleaning with sodium hypochlorite showed a highly significant removal of fungicides compared to cleaning with water only. The results from this study indicates exposure of mancozeb and ETU in fresh and cooked tomato, hence a need for cleaning with chlorine and rinsing with water before consumption and a need for more alternatives of chemical cleaning.

Key words: Mancozeb, Ethylenethiourea, Temperature, Residue, Tomato, Cleaning.

INTRODUCTION

Vegetable production is one of the major branches of horticulture, they are considered as an asset providing income to the growers and providing a vital part of human diet. Tomato is vegetables which are eaten raw or cooked as the fruit part. Tomato *(L. esculentum*) originated from South America. The tomato crop is affected by various fungal diseases such as leaf spot *(Septoria lycopersici),* powdery mildew *(Leveillula taurica*), late bright *(Phytopthora infestans)*, early bright (*Alternaria Solani*), *Anthracnose* or ripe fruit rot caused by *(Colletotrichum capsici*) to a lesser degree and mainly (*Colletotrichum leosporioides)* among others and hence are usually sprayed for protection or for curative purpose [7]**.** Dithiocarbamates which are commonly used fungicides to control such diseases, are organosulphur compounds that exist as strong complexes with various metal ions in a polymeric form and include ethylenebis-dithiocarbamates (EBDCs) such as mancozeb, In contemporary agriculture over 30% of the produce does not reach the consumer market, and without use of chemical agents the loss would be doubled [7] and yield could drop by as much as a third while food prices could increase by as much as75% [18].World health organization (WHO) and other bodies have set maximum residue levels on vegetables when consumed, however farmers may apply more than the recommended pesticide quantities especially during wet seasons while some may not even wait for Post Harvest Interval (PHI) based on demand. Studies have shown that ethylenebisdithiocarbamates (EBDCs) are in the category of pesticides whose residues are more frequently found in raw agricultural products [12].

Levels ranging from 17.5mg/kg to 10.6 mg/kg have been found in tomatofrom Kenya [10] and varying concentrations in different parts of the world; Congo, 4.65mg/kg of mancozeb in tomato [16], Turkey 0.11mg/kg ETU [6] and many others including Spain [14]. Metabolites of dithiocarbamates have been shown to cause cancer while high toxic residues of the fungicides are injurious to the environment, birds and fish. ETU which is a metabolite of EBDCs has a half-life of 1-7 days in the environment, a long half life in the soil of between 5-10 weeks [14]. Globally there are over 18.1 million new cases of cancer with over 9.6 million deaths translating to over 13% of total deaths [4]. Some of the cancer cases may arise from diets containing carcinogenic compounds like ethylenethiourea. The residue of these fungicides is converted to ethylenethiourea (ETU) during storage. Foods cooked containing their residues decompose increasing levels of ethylenethiourea. In a study done, Thermal treatment of the dithiocarbamate maneb at 100 ºc showed conversion to Ethylenethiourea by 28 % while treatment at 121ºc resulted to a change of

32% [12]. Cleaning vegetables with water and other processing methods is known to affect the residue levels of pesticides. Studies of washing with chemicals like basic sodium hypochlorite (chlorine) have shown a reduction of the residues of EBDCs and ETU considerably [8]. EBDCs such as mancozeb and propylenebis-dithiocarbamates (PBDCs) like propineb. Dithiocarbamates and the metabolites especially ethylenethiourea (ETU), have thyroid effect, are neurotoxic, mutagenic and teratogenic hence suspected to have carcinogenic effects on test animals and can enter through placental barrier hence affecting the unborn by increasing tumor development [17]. Dithiocarbamates in combination with nitrite arising from foliar application of nitrogenous fertilizers with nitrates is of health concern in that the nitrites formed by reduction in vegetables after cooking, in human saliva or in meats especially when cooked, can be converted to N-nitroso which may be a carcinogenic derivative. Such derivatives include N- nitrosodimethylamine (NDMA) and nitrosoethylenethiourea [3]. Mancozeb is also an example of cholinesterase inhibitors which affects the nervous system and also causes skin irritation) [14]. Mancozeb also causes spontaneous abortions in Rabbits at a dose of 80 mg/kg/day [13]. The main aim of the study was to investigate the effect of temperature and cleaning methods on the residue levels of mancozeb and the harmful metabolite ethylenethiourea in tomato.

MATERIALS AND METHODS

Sampling and Sample pre-treatment

Samples for temperature treatment and washing treatment were collected from the

experimental gardens (Figure 1) sprayed with mancozeb by purposive sampling from all the plants one day after spraying in separate one kilogram samples. The samples were packed in a cold box then transported same day and stored in a deep freezer
.

Figure.1: Tomato at the experimental gardens

Cleaning of Glass apparatus

Glass ware was soaked for 10 hours using fresh hydrochloric acid, soaked in distilled and de-ionized water for 8 hours, rinsed with fresh distilled and de-ionized water then dried on a rack in an oven at temperatures of 100℃.

Chemicals and Reagents

All chemicals and reagents used in the study were analytical grade and above, they included,

Analytical standard ethylenethiourea and mancozeb (purities; 98%,96.8%) respectively from Pestanal
Acetonitrile, chloroform, dichloromethane and methanol HPLC grade ,from Chemoquip limited (Nairobi Kenya)
sodium dodecyl sulphate, HPLC grade, Kobian Kenya limited (Nairobi Kenya)
Water (distilled and deionized), HPLC grade.

Preparations of Standards and Mobile Phases

The stock solution of mancozeb standard was prepared by dissolving 10 mg in10 ml acetonitrile while ETU was made by dissolving 12.5 mg in 50 ml of acetonitrile and stored in a freezer. The mobile phases were,95% sodium dodecyl sulphate (SDS)+ 5% Acetonitrile made by adding 50 cm^3 of Acetonitrile to 950cm^3 of 0.1 molar sodium dodecyl sulphate, O.1 Molar SDS made by dissolving 57.676 Gram of sodium dodecyl sulfate per 2 liters of distilled water. Methanol and acetonitrile required no preparation.

Simulated cooking

A one-kilogram sample of tomato obtained from the plot sprayed with mancozeb was cut into small pieces with a knife and homogenized then divided into five equal portions of 200 grams. Each of the 200 gram portions was divided into two, 100 gram portions. From the first portion 3 small portions weighing 1.0 grams of the uncooked tomato were extracted and analyzed while the second portion of 100 grams were placed in a 250 ml round bottomed flask and connected to a vertical condenser. Heating using a mantle was done at 60 ℃ for 10 minutes followed by cooling. Extraction was done on one-gram sub samples in triplicate as shown in the extraction procedure. The procedure was repeated at different temperatures with intervals of 10 ℃ up to 100 ℃ . Solutions obtained were stored in amber colored vials in a deep freezer awaiting analysis. The study was done using three different samples.

Cleaning procedure

The study on the effect of different types of cleaning methods on residue levels involved use of three-kilogram tomato samples collected from the study garden sprayed with mancozeb. The sample was divided into three equal portions (1 kg each) for cleaning with distilled water, sodium hypochlorite solution and one to remain unclean. The first portion of tomato sprayed with mancozeb was cleaned by placing sample in a bowl containing five liters of distilled water for four minutes and rinsed twice. The sample was allowed to dry for one hour then cut into small pieces and homogenized. From the homogenized tomato six different one-gram sub samples were obtained and extracted according to the extraction procedure. The procedure was repeated with the second portion of tomato which was cleaned with five liters of a 1% sodium hypochlorite solution for 4 minutes and rinsed twice with two liters of distilled water. A third portion of tomato which was unclean was processed according to the extraction procedure. The procedures were repeated with six different samples.

Extraction procedure

The tomatoes were finely chopped using a knife. A 1.0-gram sub-sample was placed in a 50 milliliters beaker containing a volume of 3 milliliters of a mixture of acetonitrile, dichloromethane and chloroform in the ratio of 1:1:1. Extraction was achieved by 2 minutes of mechanical shaking. The suspension obtained was filtered through a filter paper in a Buchner funnel and rinsed with 2 ml of extractant followed by addition of 2 milliliters of methanol to filtrate. The mixture was then evaporated to dryness under a gentle stream of nitrogen gas at room temperature. Residues obtained were dissolved in 500 µl of a solution of acetonitrile and water in the ratio of 1:1. The solution was filtered through a Millipore 0.45 µm nylon syringe into sample vials then stored in a freezer before HPLC analysis [5].

Sample analysis using HPLC-DAD

The HPLC analysis was carried out at Jomo Kenyatta University of Agriculture and Technology, Department of Food Science and Technology using the instrument Shimadzu model LC-20A which had a Quaternary pump. The machine was connected to a computer with a software Shimadzu LC solution. The HPLC column used was NUCLEODUR Batch 34500033, which is a stainless steel analytical column packed with C-18 5µm particles and length 250 mm×4.6 mm internal diameter, obtained from Macherey Nagel Germany and supplied by Chemo quip Kenya limited. The mobile phases used were 95% sodium dodecyl sulphate (SDS) + 5% acetonitrile (pump A), 0.1 Molar SDS (Pump B), methanol (Pump C) and acetonitrile (Pump D).Other conditions include a flow rate of 0.9 ml/min and oven temperature of 40 ℃. Quantification was by multiwavelength monitoring done with a band width of 4nm and absorbance spectra recorded from 200-400 nm. The elution program is shown on Table 1

Table 1. The HPLC elution program

Time (min)	A %	B %	C %	D %
0	100	0	0	0
5	0	30	33	37
6	0	30	33	37
10	100	0	0	0

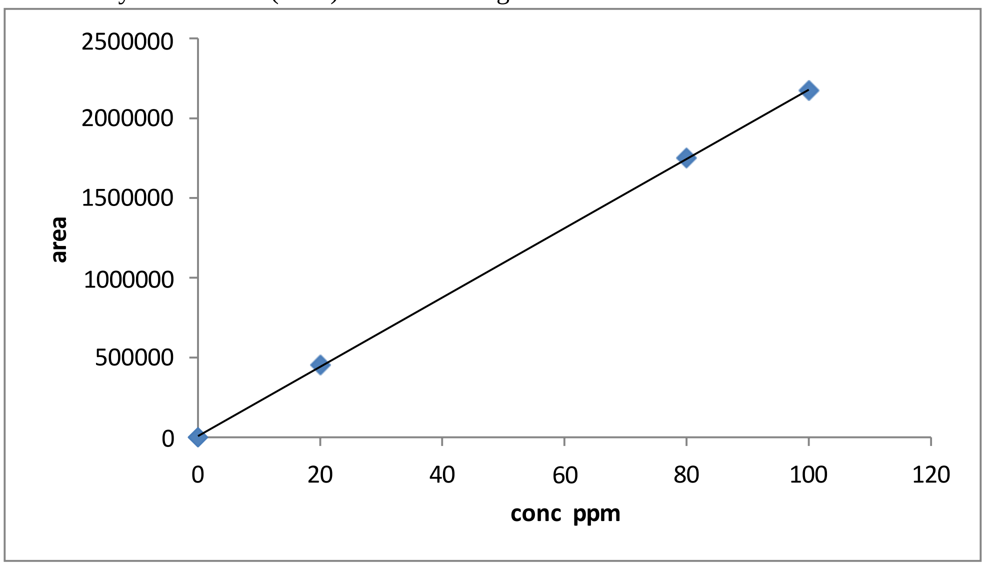
From Table 1, the 95% sodium dodecyl sulphate (SDS) + 5% acetonitrile started at 100 % decreasing steadily to 0 % and isocratic for a minute then increasing to a 100 % steadily, while the pumps B, C and D had followed a linear gradient to 30 % 0.1M SDS, 33 % methanol and 37 % ACN isocratic for a minute before decreasing steadily to 0%. The detector end time was 10 minutes while the LC stop time was 15 minutes**.** The elution program has similarities with another study done on tomato for Maneb and ETU [5].

RESULTS AND DISCUSSIONS

The methods of analysis used were validated and discussed in this section together with chromatographic methods used and the results on the effect of temperature and cleaning on levels of mancozeb and ethylenethiourea in tomato are discussed in the following sections.

Method validation and recovery studies

The methods used in this study were validated by use of calibration curves for the different standards where regression equations, correlation coefficients and limits of detection were obtained. Recovery data, limit of blank and chromatogram separation data are also provided. The calibration curves for mancozeb and ethylenethiourea were obtained. The curve for ethylenethiourea (ETU) is shown in Figure 2.



The curve in Figure 2 shows a linear relationship within a range of different concentrations. The regression equations and correlation coefficients for mancozeb and

ETU are shown in Table 2. Table 2: Calibration data and percentage recovery

Analyte	Reg.equation	R^2	LOD	LOB	% Recovery
Ethylenethiourea	Y=21706X+9159	0.999	0.0100	0.0085	95.89
Mancozeb	Y=2971X+5187	0.948	0.0990	0.0080	91.26

From Table 2, the R^2 values (0.948-0.999) correlates with other studies on same compounds [5, 6, and 9]. The correlation coefficients make the study appropriate for use in analysis of residue levels of dithiocarbamate fungicides and the metabolite The R^2 values indicate that the established calibration curves are linear over the respective range of concentrations, explaining that more than 94.8 % correlation between concentration and responses. Recovery studies were evaluated by recovery test, according to Equation 1 [2]

$$\% \textbf{Recovery} =$$

$$C_F - C_U$$

$$\mathbf{C_A \times 100}\ \mathbf{1}$$

Where C_U represents the concentration in unfortified sample; $\mathbf{C_A}$ is the concentration of fortification (spiking solution); C_F is the concentration determined in fortified sample after extraction. The % recoveries together with limits of detection (LOD) and limits of blank (LOB) are shown in Table 2. The percentage recovery lies within the range
91.26 % to 95.26 %. The recovery studies compare favourably with other studies where 94.15 % recovery for Maneb and 96.46 % for ETU was achieved [6] which confirms the method fit for analysis of the above parameters. The limits of blank (LOB) and limits of detection (LOD) were calculated according to Equation 2 and 3[2].

$$\mathbf{LOB = mean}\ \text{Blank}+1.645\ (\text{SD blank})$$

$$\mathbf{LOD = LOB} + 1.645\ (\text{SD low concentration sample})3$$

The limits of blank ranged from 0.0085 mg/kg for ethylenethiourea (ETU) and 0.008 mg/kg for mancozeb while the limits of detection ranged from 0.01 mg/kg for ETU to 0.099 mg/kg for mancozeb. A study done on dithiocarbamates and the metabolites obtained the limit of detection (LOD) of 0.01 mg/kg for ETU and 0.1 mg/kg for maneb [1]. In another study done in Iran, the LOD of 0.1 mg/kg for mancozeb and propineb was attained, [9]. The values show that the method is robust for the analysis of the fungicides and the metabolite in vegetables studied, since results are consistent with other studies. The high pressure liquid chromatographic (HPLC) analysis of the two analyte of interest was done using a simultaneous analysis and peak identification. The retention time for ethylenethiourea (ETU) was
3.2±0.2 minutes while that of mancozeb was 8.7±0.2. The retention times achieved in this study are similar to other studies where; using a C18 column the retention time of ETU was 3.3 minutes, maneb was 4.1 minutes while that of mancozeb was 9.1 minutes [6]. Quantification was done by multiplying the amount obtained from the graph (using the regression equations for standard) with a dilution factor of 0.5 as shown in Equation 4.

$$\mathbf{Q}\left(\frac{\mathbf{mg}}{\mathbf{kg}}\right) \quad \mathbf{P-C}) = (\mathbf{M}) \times \mathbf{0.54}$$

Where Q (X) is the levels in mg/kg, P is the peak area (Y) from the table; C is the Y intercept while M is the gradient of the regression equation

Effect of temperature on ethylenethioure (ETU) and mancozeb residue in tomato

The results showing the effect of temperature on ETU and mancozeb residue in tomato are shown on Table 3 and discussed in this section.

Table 3 Effect of temperature on ETU and mancozeb residues in tomato

TEMPERATURE(℃)	**ETU** mg/kg ± SD (n=3)	**MANCOZEB** mg/kg ± SD (n=3)
25	9.43±0.03^a	5.23±0.03
60	10.48±0.15^b	BDL
70	11.57±0.09^c	BDL
80	11.91±0.01^d	BDL
90	12.43±0.38^e	BDL
100	10.60±0.03^b	BDL
P- value	0.003	

Mean values followed by the same small letter within the same column do not differ significantly from one another

From Table 3, on heating from 25℃ the residue levels of ETU in tomato increased from

9.43±0.03 to 12.43±0.38 at 90℃ and then decreased to 10.60±0.03mg/kg at 100℃ showing a conversion of 57.36 % of the initial mancozeb. On heating tomato from 25℃ to 60℃ levels of ETU rose from 9.43±0.03 mg/kg to 10.48±0.15 mg/ kg showing that some mancozeb was converted to ETU by 20.07% at that temperature.The results indicated a significant difference on comparing different temperature conditions.The ETU residues decrease at 100℃ due to decomposition of ETU at that temperature and there was also a complete decomposition of mancozeb to levels below detection limit from the initial 5.23±0.03 mg/kg at 25℃ when the tomato were heated to 60℃ .The results from this study compares favourably with other studies done such as [12] where it was found that there was no decline in ethylenethiourea recovery even on heating at high temperatures such as 90℃ even for upto 80 minutes however a conversion of dithiocarbamates to

ethylenethiourea was as high as 32±1%. The results also compare with other studies which notes that mancozeb like other ethylenebisdithiocarbamates decomposes at cooking temperatures to very low levels while ETU levels rises[11].

Effect of cleaning on residue levels of mancozeb and ETU in tomato

The results showing cleaning effects are shown in Table 4 and

discussed in this section. Table 4 Effect of cleaning on residue levels of mancozeb and ETU in tomato

CLEANING TREATMENT	MANCOZEB mg/kg±SD (n=6)	% Reduction	ETU mg/kg±SD (n=6)	% Reduction
UNCLEANED	5.23±0.02^{c}	0.0	46.71±0.71^{b}	0.0
WATER ONLY	4.37±0.06^{b}	16.3±0.64	0.27±0.08^{a}	99.4±000
CHLORINE	0.25±0.00^{a}	95.2±0.00	0.05±0.00^{b}	99.9±0.00
P-value	<0.001	-	<0.001	-

Mean values followed by the same small letter within the same column do not differ significantly from one another ($P< 0.001$, $\alpha=0.05$)

From Table 4, the levels of mancozeb residues in tomato decreased from a mean of 5.23±0.02 mg/kg in unclean tomato to 0.25±0.00 mg/kg in that cleaned with chlorine, removing a high amount of mancozeb totaling to 95.2±0.00 % while water cleaning reduced the residues to 4.37± 0.06 mg/kg translating to 16.3±0.64 % reduction. Ethylenethiourea (ETU) residues changed from a mean of 46.71±0.71 mg/kg in unclean tomato to 0.05±0.00 mg/kg in chlorine cleaned tomato which showed a reduction by 99.9±0.00%. Cleaning tomatoes with water reduced the mean residues of ETU to a mean of 0.27±0.08 mg/kg which indicated a reduction in residues by 99.4±0.00%. The results shows that cleaning with water and cleaning with detergent (chlorine) gave results which differed significantly for the two cleaning agents under study though the % removal of ETU was slightly higher than that of mancozeb most likely because ETU is more water soluble. The results indicate that chlorine wash reduced mancozeb and ETU residues on tomato more efficiently than water cleaning alone. The results from this study are further supported by other studies such as one done in Turkey where a reduction of the residues of mancozeb by 65 % on tomato was achieved with chlorine wash [15], while a reduction of 82 % of the residues of mancozeb on apples was achieved with chlorine wash [8]. Others include earlier work where water wash alone led to reduction of ETU residues on tomato by 70 % [11], while in another study washing tomato with 0.1 % sodium hypochlorite reduced residue levels of ETU by almost 100% [12]

CONCLUSIONS

The results of this study indicate that the residue levels of mancozeb and ETU in tomato are affected by cooking temperature with a detrimental consequence to consumers who should not just wash vegetables with water but use chlorinated water then rinse with boiled water to remove chlorine before consumption or processing. The Farmers, vendors and other value addition processors should wash the vegetables similarly with a recommendation for regular surveillance of residue levels at all levels and conditions in the consumer and processing chain

Data availability

The authors declare that the summarized data used to support the findings of this study are included within the article.

Conflict of interest

The authors declare there are no conflicts of interest regarding the publication of this paper.

Funding statement

The project was funded partly by the authors and Kenyatta University

Acknowledgement

We acknowledge the Kenyatta University, Chemistry Department and Jomo Kenyatta University, Department of Food Science and Technology for material support.

REFERENCES

Blasco, C., Font, G. and Pico, Y. (2004) Determination of dithiocarbamates and metabolites in plants by liquid chromatography-mass spectrometry. *Journal of Chromatography A.* **1028**:267-276

David, A. and Terry, P. (2008). Limit of blank, limit of detection and limit of quantification. *Journal of Clinical Biochemistry* **29(1)**:49-52.

Debbar and Moore.N (2002) A simple method for the determination of ethylenethiourea (ETU) in biological samples. *Journal of Analytical Toxicology* **26(4):**216-221

Freddie, B., Jacques, F., Isabella, S., Rebecca, L and Lindsey, A. (2018). Global Cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *Journal for Clinicians* **68 (6):**394-424

Garcinuno, R. Fernandez, P. and Camara, C. (2004). Simultaneous determination of Maneb and its main metabolites in tomatoes by liquid chromatography using diode array-UV absorbance detection. *Journal of Chromatography A.* **1043**:225-229.

Gul, O. and Buket, A. (2008) Liquid chromatographic analysis of maneb and its main degradation product, ethylenethiourea in fruit juice. *Journal of Food Additives and Contaminants* **25**:961-970.

Gupta, S. and Thind, T. (2006). Disease problems in vegetable production. Jodhpur.pg 10-79.

Hwang, E. Cash,J. and Zabik,M. (2001) Postharvest treatment for reduction of mancozeb in fresh apples. *Journal of Agriculture and Food Chemistry* **49**:3127-3132.

Jafari, A. Shoeibi, S., Amini, M., Amirahmadi, M., Rastega, H., Ghaffrian, A. and Ghazi, M. (2012) Monitoring dithiocarbamate fungicide residues in green house and non-green house tomatoes in Iran by HPLC-UV. *Journal of Food Additives and Contaminants* **B**: 1-6

Kariuki, J. (2008). Investigation of a hazardous metabolite, ethylenethiourea, in ethylenebis-dithiocarbamates, fresh and processed tomatoes: case of Mwea division, Kirinyaga district. Institutional repository, Jomo Kenyatta university of Agriculture and Technology. Google scholar

Knio, K., Dagher, S. and Saad, A. (2000). The fate and persistence of zineb, maneb, and ethylenethiourea on fresh and processed tomatoes. *Journal of Food Additives and Contaminants.***17 (5)**: 393-398

Kontou, S., Tsipi, D. and Tzia, C. (2004). Stability of the dithiocarbamate pesticide maneb in tomato homogenates during cold storage and thermal processing. *Journal of Food Additives and Contaminants.* **21**:1083-1089.

Kroes, R., Renwick, G., Cheeseman, M and Wurtzen, G. (2004). Structure based thresholds of toxicological concern (TTC): guidance for application to substances present

at low levels in diet. *Journal of food and chemical toxicology*.**42**:65-83
Lopez, O., Gonzalez, C., Barreiro, J and Simal, G. (2012). Surveillance of fungicidal dithiocarbamates residue in fruits and vegetables. *Journal of Food Science.* **134:**366-374.
Mehmet, F. and Muharrem, C. (2014). Effect of chlorine, hydrogen peroxide and ozone on the reduction of mancozeb residues on tomatoes. *Turkish Journal of Agriculture and Forestry*. **38**:371-376.
Mpiana, P., Waswa, J., Kiremire, B. and Muyisa, S. (2014) Dithiocarbamate residues in selected vegetables from Bukavu, Democratic Republic of Congo. Journal *of Physical and Chemical Sciences.***1**:327-348
Shukla, Y. and Arora, A. (2001). Transplacental carcinogen potential of the carbamate Fungicide Mancozeb. *Journal of Chromatography B.* **20**: 127-131.
Yadav, S. (2010). Pesticide application-Threat to ecosystems. *Journal of Human Ecology.* **32(1)**: 37-45.