International Journal of GEOMATE, April, 2020, Vol.18, Issue 68, pp. 54 - 59
ISSN: 2186-2982 (P), 2186-2990 (O), Japan, DOI: https://doi.org/10.21660/2020.68.5618
Special Issue on Science, Engineering and Environment

EFFECT OF ELECTROLYZED REDUCING WATER WITH VARIOUS CONCENTRATION ON CARBENDAZIM FUNGICIDE REMOVAL IN CHERRY TOMATO

*Wanwarang Pattanapo[1,2] Kanda Whangchai[1,2,3] Jamnong Uthaibutra[1,2,3] Tanachai Pankasemsuk [1,2,4,5] and Usawdee Chanasut[1,2,3]

[1]Postharvest Technology Research Center, Faculty of Agriculture, Chiang Mai University, Thailand; [2]Postharvest Technology Innovation Center, Office of the Higher Education Science, Research and Innovation, Thailand; [3]Department of Biology, Faculty of Science, Chiang Mai University, Thailand; [4]Department of Plant and Soil Sciences, Faculty of Agriculture, Chiang Mai University, Thailand; [5]International College of Digital Innovation, Chiang Mai University, Thailand.

***Corresponding Author, Received: 30 June 2019, Revised: 18 Dec. 2019, Accepted: 09 Feb. 2020

ABSTRACT: The effect of electrolyzed reducing water (ER) on a degradation of carbendazim residues degradation of cherry tomato (*Solanum lycopersicum* L. var. *cerasiforme*) was investigated. Electrolyzed water was generated by electrolysis from 5% NaCl solution. The cherry tomato samples were washed in ER water at different concentrations (100, 250, 500 and 1000 mg/L) and in distilled water (control). The standard carbendazim solution (5 mg/L) was treated with the previous concentrations of ER. Reduction of residual carbendazim was determined by high performance liquid chromatography (HPLC) while liquid chromatography-quadrupole-time-of-flight mass spectrometer (Agilent, LC-6545 Q-TOF/MS) was used to determine the concentration of anions as major degradation products. It was found that all concentration of ER water significantly reduced carbendazim solution, compared to control. The ER water at 250 mg/L could effectively remove carbendazim residues from cherry tomato, most of the degradation occurred within 30 min. Residue of carbendazim on cherry tomato were reduced by 60.36%, 80.49%, 67.32%, and 64.68% after 30 min washing in ER 100 mg/L, 250 mg/L, 500 mg/L, and 1000 mg/L, respectively.

Keywords: Electrolyzed reducing water, Carbendazim degradation and *Cherry tomato*

1. INTRODUCTION

Cherry tomato (*Solanum lycopersicum* L. var. *cerasiforme*) is one of the popular tomato varieties in Thailand which is locally consumed and exported. It is a small tomato with high nutritional value and can be consumed as fresh or processed tomato. The cherry tomato growers usually apply fungicides and insecticides many times until the fruit were harvested. As a result, there are excessive amounts of various of pesticides left on the fruits. The pesticide residue is an important problem and becomes seriously related to human health problems and hazardous to the environment. One of the serious problems of harvested tomato is pesticide residue over the Maximum Residues Limits (MRLs). Thai Pesticide Alert Network [1] also reported that 11.11% of tomato samples in Bangkok, Chiang Mai and Ubon Ratchathani province were contaminated with the pesticide residues over the MRLs. It was shown that pesticide residue mostly found in tomato was carbendazim. Carbendazim is a very active systemic broad spectrum fungicide. It is very cheap and effective, so it is used extensively in each harvesting season to control fungal diseases. For example, it is used to prevent Sclerotinia stem rot (caused by *Sclerotinia sclerotiorum*) in various fruits, vegetables, and other plants [2-4]. Carbendazim is classed as a possible human carcinogen [5,6]. It is persistent and very toxic and has been banned in Australia, USA and most European Union countries [7,8]. The MRLs for carbendazim in tomato is 0.5 mg/kg. The carbendazim residues in vegetables affects on the health of customers and causes huge downside for Thai clients and exporters. The residue problem comes from the inefficient processes of planting and cleaning raw materials. The most common practice for reducing contamination is using a sanitizer. Finding the appropriate process to eliminate chemical contaminant which has the least impact on human health and environment would be an ideal requirement. In the past, the researchers applied different techniques to reduce the pesticide residues such as washing with tap water or strong oxidizing agent e.g. ozone and chlorine dioxide.

Nowadays, the advanced oxidation processes (AOPs) have been proposed for removing pesticide residues from fruits and vegetables such as electrolyzed water. Various studies on the application of AOPs for the pesticide residue reduction have been reported electrolyzed water is a chlorine based solution produced by electrolyzing a diluted salt (usually NaCl) solution in an electrolytic chamber with anode and cathode. ER water is generated from the cathode side. It may also be able to reduce the residues through alkaline hydrolysis. Moreover, it was found that electrolyzed water is an effective treatment to degrade the insecticide residues on agricultural products. For example, Qi [9] suggested that ER water was effective in degrading phosmet and the longer treatment time resulted in the higher reductions of diazinon, cyprodinil, and phosmet. Jianxiong Wuyundalai Liu Chen Zhou Su and Lite [10] also found that using ER water in fresh spinach for 30 mins reduced acephate by 86%, omethoate by 75% and DDVP by 46%. In addition, Tamjapo Uthaibutra and Whangchai [11] indicated that chlopyrifos residues in yard long bean could be degraded using ER water for 30 min. Furthermore, Al-taher Yang Philip and Jack [12] reported the effect of various washing treatments with a combination of the ultrasonication on acephate, malathion, carbaryl, bifenthrin, cypermethrin, permethrin, cyhalothrin, chlorothalonil, and imidacloprid removal from tomatoes. They found that removing of pesticides from tomato surface depended on the washing treatments e.g. washed with water, sodium hypochlorite, peroxyacetic acid, or Tween 20 with and without sonication.

Therefore, this study focused on the effect of ER water on the carbendazim fungicide removal from cherry tomato fruit.

2. MATERIALS AND METHODS

2.1 Plant Material

Organic cherry tomato fruit at a commercial harvesting stage was obtained from a local market, Chiang Mai Province, Thailand and was transported to the Postharvest Technology Research Center, Faculty of Agriculture, Chiang Mai University. It was carefully selected for uniformity in shape, color and size, and insect bites, blemished or diseased fruit was discarded.

2.2 Chemicals Preparation

Standard carbendazim fungicide was purchased from Ehrenstorfer™ with 99.0% purity. Stock solution (1000 mg/L) for the residue analysis was prepared in acetone. The solution was diluted with distilled water to appropriate working concentration (5 mg/L).

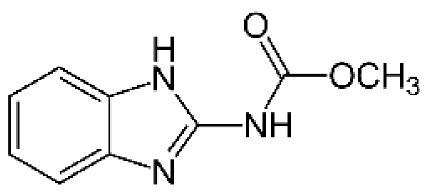


Fig.1 Structure of carbendazim

2.3 Electrolyzed Water Preparation

The ER water was generated by electrolyzing 5% NaCl solution by mixing 500 g NaCl in10 liters of distilled water in an electrolytic chamber with anode and catthode. The electrodes were then subjected to a direct current of 8 A and 8 V using DC power source for 1 hour. The ER water was in the cathode side. The pH and Oxidation Reduction Potential (ORP) were measured using pH/ion meter. The properties of ER water were determined immediately after preparation. The physical and chemical parameters of ER water were pH 11.5, ORP 260 mV. The concentrations of ER water used in this study, were 100, 250, 500 and 1000 mg/L. Ten milliliters of standard carbendazim solution (5 mg/L) was added to 20 mL of ER water at the concentrations previously mentioned and distilled water (control). The removal percentages of each standard carbendazim fungicide sample were determined by high performance liquid chromatography (HPLC).

2.4 Washing Treatments

One kilogram of cherry tomato was divided into 5 groups of 200 g each group were spiked with 5 mg/L carbendazim and 1% tween20 then air dried at room temperature. After that, the fruits were left to soak in different concentrations of ER water (100, 250, 500 and 1000 mg/L) and distilled water (DW) that control for 30 min (without shaking). After treatment, all samples were air dried at room temperature for 1 hour before fungicide extraction.

2.5 Analytical Method

The carbendazim residue in cherry tomato was extracted with the QuEChERS extraction kit (Agilent Quick, USA.) and homogenized according to Anastassiades Lehotay Stajnbaher and Schenck [13]. Tomato sample (15 g) was cut into small pieces and extracted with 15 mL acetronitrile in a 50 mL Teflon centrifuge tube. The Q-sep QuEChERS Extraction Salt was added, immediately shaken on a vortex mixer for 2 min. Then centrifuged at 4,000 rpm for 5 min.

Additionally, a clean-up step was conducted in the optimization procedure. After centrifugation step, the supernatant (about 5 ml) was transferred into a QuEChERS Dispersive SPE 15 mL. After shaking for 2 min and centrifugation at 8,000 rpm for 12 min, 1 mL of the supernatant was taken, filtered through a 0.45 μm nylon filter and injected into the LC–MS/MS system. The samples were analyzed with LC-6545 Q-TOF/MS, each analysis was done with 5 replicates.

For the LC analysis, an Agilent 1260 infinity II with a Poroshell 120 EC-C18 analytical column of 2.1 mm x 100 mm and 2.7 μm particle size was used. The mobile phases, A and B, were H_2O + 5 mM NH_4

formate + 0.1% formic acid and MeOH + 5 mM NH_4 formate + 0.1% formic acid, respectively. The gradient program started with 95% of A for 1.5 min, changed to 5% (10 min) then followed by a return to the initial conditions within 5 min.

A liquid chromatography-quadrupole-time-of-flight mass spectrometer (Agilent, LC-6545 Q-TOF/MS) was used to analyze the mass spectrometry. The electrospray ionization (ESI) source was operated in positive ionization mode and its parameters were as follows: temperature: 150ºC, gas flow of 10 L/min, nebulizer gas: 45 psig, capillary voltage: 3500 V. Nitrogen was served as the nebulizer and collision gas. The multiple reaction monitoring (MRM) was used, with a delta time of 1.0 min for each analyte.

2.6 Statistical Analysis

The data were analyzed by SPSS 16.0, Significant difference was considered at $p<0.05$.

3. RESULTS AND DISCUSSION

It was found that the removal percentage of standard carbendazim was significantly increased at all concentration of ER applied, compared to control. ER water at 250 mg/L was the most effective concentration for removing carbendazim residues, the removal was 52.80%. Washing cherry tomato with ER water was more effective than washing with distilled water. Residue of carbendazim on cherry tomato were reduced by 60.36%, 80.49%, 67.32%, and 64.68% after 30 min washing in ER 100 mg/L, 250 mg/L, 500 mg/L, and 1000 mg/L, respectively (Fig. 2). Similary, Tamjapo Uthaibutra and Whangchai [11] reported that treatment of yard long bean with ER water 250 mg/L for 30 min could degrade chlopyrifos residues. It was also found that the ER water could be used as a cleaning solution to reduce pesticide residues in fruits and vegetables; for example, cabbage, leek [10], beans, grapes [14], and cowpea [15].

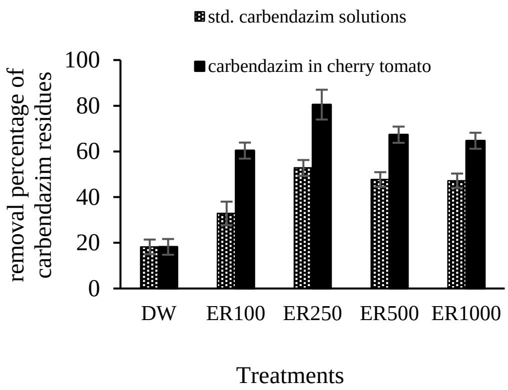
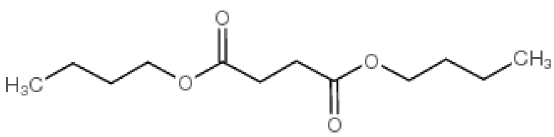


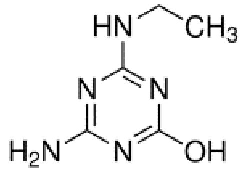
Fig.2 Removal percentage of standard carbendazim solution and carbendazim in cherry tomato after ER water treatment at different concentrations

Table 1 Degradation product of Standard carbendazim after treatment of ER water

Treatments	Compound name	measured mass (m/z)
DW (control)	dibutyl succinate	253
ER100	dibutyl succinate	253
ER250	-	-
ER500	atrazine-desisopropyl-2-OH	173
	dibutyl succinate	253
ER1000	atrazine-desisopropyl-2-OH	173



(a)



(b)

Fig.3 Structure of dibutyl succinate (a) and atrazine-desisopropyl-2-OH (b)

The degradation products that found in each treatment of standard carbendazim were dibutyl succinate and atrazine-desisopropyl-2-OH (Table 1 and Fig.3). The fragment ion at m/z 192 was from molecular ions of carbendazim (Fig.4). After treatment with the various concentrations of ER water, the fragment ion at m/z 192 was loss. It found dibutyl succinate after washing in distilled water, ER 100 mg/L and ER 500 mg/L, but it could not found in ER 1000 mg/L. However, after washing in ER 1000 mg/L and ER 500 mg/L found atrazine-desisopropyl-2-OH. Anywise, in ER 250 mg/L could not found the degradation product. The fragment ion from the 2 degradation product are shown in Table 1 and Figs.5-8. In Michale Ferrer and Amadeo [16] studied in skin of tomato that wash with methanol for remove the white powder, found the ion trap MS/MS analysis in carbendazim was m/z 192 as in these studied. They mentioned that the ion trap MS/MS analysis of the m/z 192 resulted in a m/z 160 ion with a loss of methanol. This loss is consistent with the structure of carbendazim and which the accurate mass neutral loss from m/z 192 to 160. There are carbendazim-methanol (160 m/z), buprofezin (306 m/z), buprofezin fracgment ion (201m/z), thiophanate methyl (343 m/z), thiophanate methyl minus methanol (311 m/z) and basic thiophanate fragmention (151 m/z). But in this study, there found dibutyl succinate (253 m/z), atrazine-desisopropyl-2-OH (173 m/z) and butopyronoxyl (244 m/z) were detected.

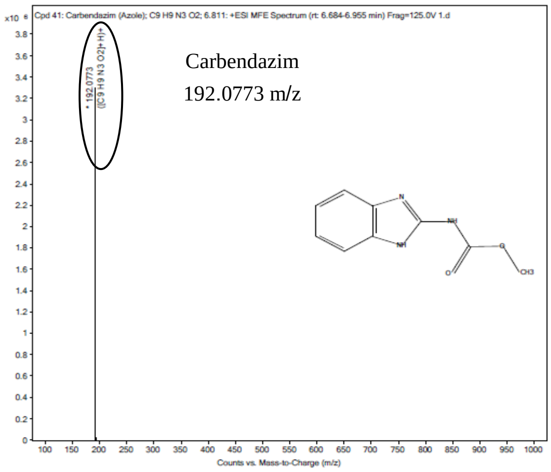


Fig.4 Mass spectrum of carbendazim

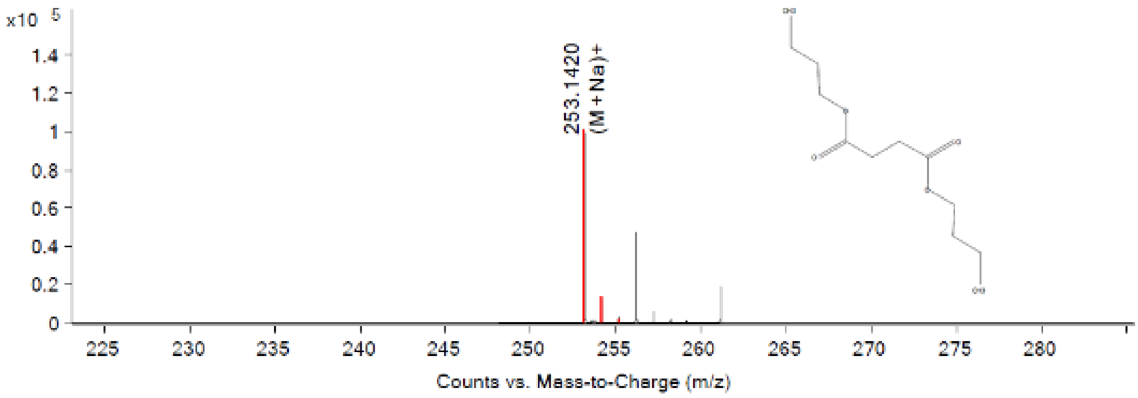


Fig.5 Mass spectrum of dibutyl succinate after treatment of carbendazim with distilled water (DW)

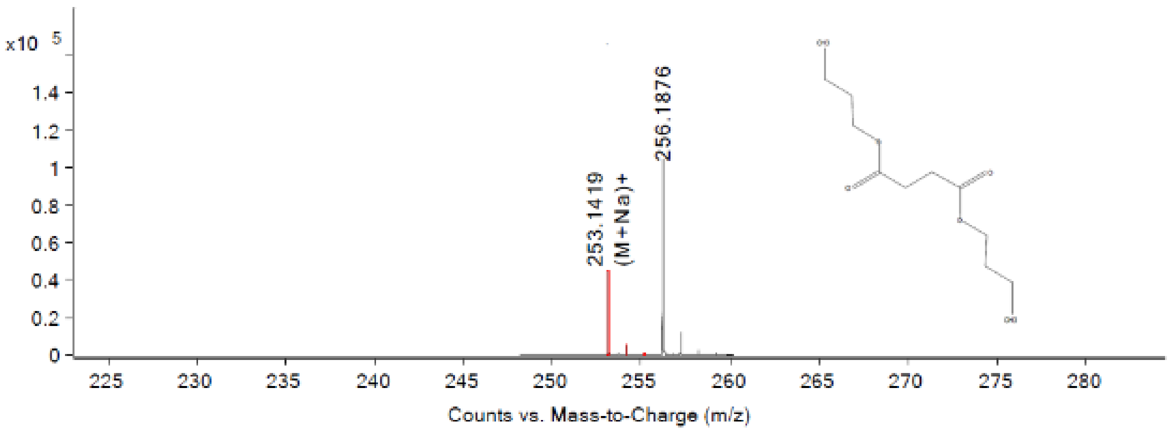


Fig.6 Mass spectrum of dibutyl succinate after treatment of carbendazim with ER water 100 mg/L (ER100)

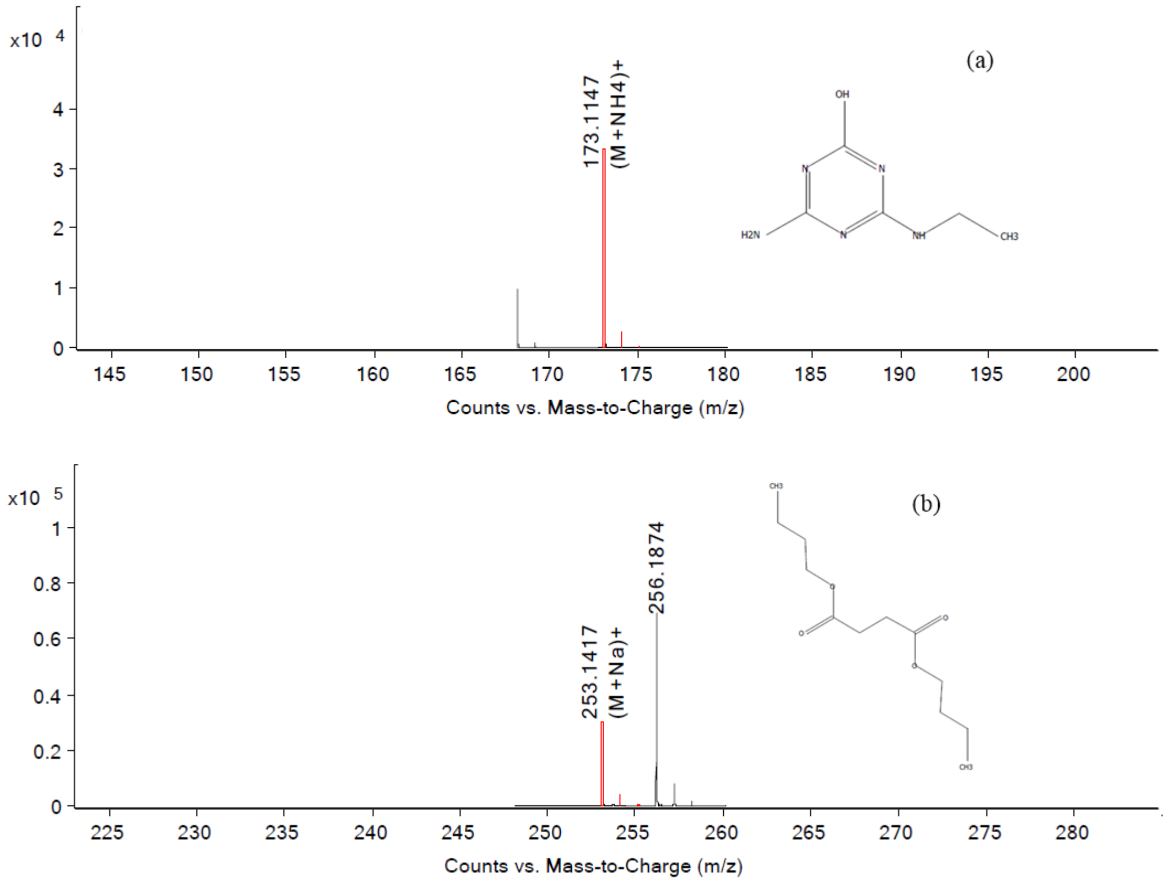


Fig.7 Mass spectrum of atrazine-desisopropyl-2-OH (a) and dibutyl succinate (b) after treatment of carbendazim with ER water 500 mg/L (ER500)

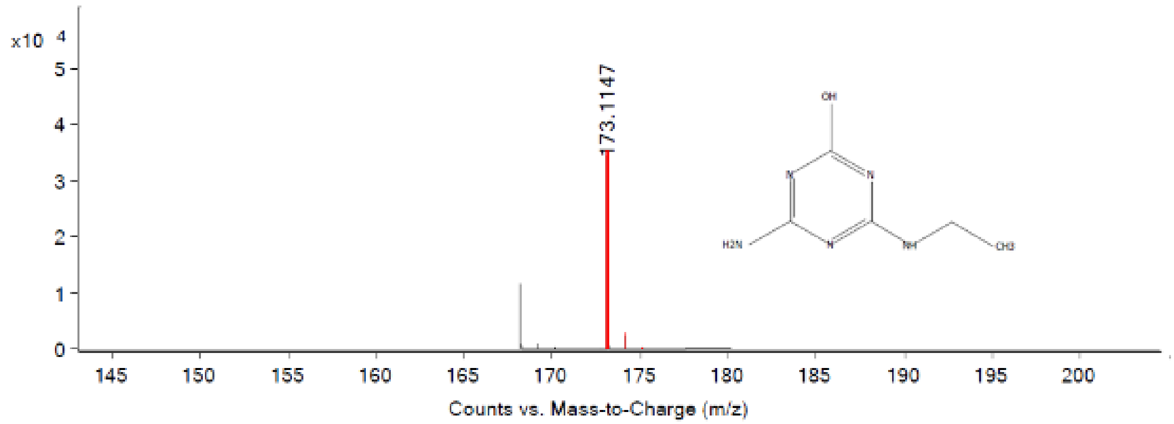


Fig.8 Mass spectrum of atrazine-desisopropyl-2-OH after treatment of carbendazim with ER water 1000 mg/L (ER1000)

4. CONCLUSIONS

ER water at 250 mg/L could effectively remove carbendazim residues from cherry tomato, most of the degradation occurred within 30 min**.** This study demonstrated the ER water could be used to reduced cabendazim residues on tomato and have a positive impact on ensuring safety of tomatoes for consumption**.** Further studies are also needed to investigate the degradation mechanism and possible disinfection of by-products after ER washing**.**

5. ACKNOWLEDGMENTS

We would like to thank the Postharvest Technology Research Center and Department of Plant and Soil Sciences, Faculty of Agriculture, Chiang Mai University; Department of Biology, Faculty of Science, Chiang Mai University and The Graduate School Chiang Mai University, Chiang Mai, Thailand for providing research facilities; Postharvest Technology Innovation Center, Ministry of Higher Education, Science, Research and Innovation, Bangkok, Thailand for a research fund**.**

6. REFERENCES

[1] Thai Pesticide Alert Network. 2016. Surveillance of pesticide residues in fruits and vegetables on 2016. [Online]. Avaliable: http://www.thaipan.org/sites/default/files/file/pesticide_doc24_press_4_5_2559.pdf (15 May 2018).

[2] Ma, H. X., Yu, C., Wang, J. X., Yu, W. Y., Tang, Z. H., Chen, C. J. Activity of carbendazim, dimethachlon, iprodione, procymidone and boscalid against sclerotinia stem rot in jiangsu province of China. Phytoparasitica, Vol. 37, No.5, 2009, pp. 421-429.

[3] Tortella, G. R., Mella-Herrera, R. A., Sousa, D. Z., Rubilar, O., Briceno, G., Parra, L. Carbendazim dissipation in the biomixture of on-farm biopurification systems and its effect on microbial communities. Chemosphere, Vol. 93, No.6, 2013, pp. 1084-1093.

[4] Devi, P. A., Paramasivam, M., and Prakasam, V. Degradation pattern and risk assessment of carbendazim and mancozeb in mango fruits. Environmental Monitoring and Assessment, Vol. 187, 2015, pp. 1-6.

[5] Goodson, W. H., Lowe, L., Carpenter, D. O., Gilbertson, M., Ali, A. M., de Cerain Salsamendi, A. L. Assessing the carcinogenic potential of low-dose exposures to chemical mixtures in the environment: The challenge ahead. Carcinogenesis, Vol. 36, No.1, 2015, pp. 254-296.

[6] Selmanoglu, G., Barlas, N., Songür, S., and Koçkaya, E. A. Carbendazim-induced haematological, biochemical and histopathological changes to the liver and kidney of male rats. Human and Experimental Toxicology, Vol. 20, No.12, 2001, pp. 625-630.

[7] Huan, Z., Luo, J., Xu, Z., & Xie, D. Acute toxicity and genotoxicity of carbendazim, main impurities and metabolite to earthworms (*eisenia foetida*). Bulletin of Environmental Contamination and Toxicology, Vol. 96, No.1, 2016, pp. 62-69.

[8] Zhang, X., Huang, Y., Harvey, P. R., Li, H., Ren, Y., Li, J., Wang, J. and Hetong Y. Isolation and characterization of carbendazim-degrading *Rhodococcus erythropolis* djl-11. PLoS One, Vol. 8, Issue 10, 2013, pp. e74810.

[9] Qi, H. Efficacy of electrolyzed water in degrading and removing pesticide residues on fresh produce. M.S. Thesis. Faculty of the University of Georgia, Georgia, 2015, 122 pp.

[10] Jianxiong H., Wuyundalai, H. Liu, T. Chen, Y. Zhou, Y. Su, and Lite L. Reduction of pesticide residues on fresh vegetables with electrolyzed water treatment. Journal of Food Science Vol. 76, Issue 4, 2011, C520- C524.

[11] Tamjapo, A., J. Uthaibutra, and K. Whangchai. Effect of electrolyzed oxidizing and reducing water on the reduction of pesticide residue and microbial contamination in yardlong bean. Agricultural Science Journal, Vol.47, No.3 (Suppl), 2016, pp. 39-42.

[12] Al-taher, F., C. Yang, W. Philip and C. Jack. Reduction of pesticide residues in tomatoes and other produce. Journal of Food Protection, Vol. 76, No.3, 2013, pp. 510-515.

[13] Anastassiades, M., S.J. Lehotay, D. Stajnbaher and F.J Schenck. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction for determination of pesticide residue in produce. Journal of AOAC International, Vol. 86, No.2, 2003, pp. 412-431.

[14] Qi, H., Q. Huang, and Hung Y.C. Effectiveness of electrolyzed oxidizing water treatment in removing pesticide residues and its effect on produce quality, Food Chemistry, Vol. 239, 2018, 561–568.

[15] Han, Y., L. Song, Q. An, and Pan C. Removal of six pesticide residues in cowpea with alkaline electrolyzed water, Journal of the Science of Food and Agriculture, Vol. 97, 2017, pp. 2333–2338.

[16] Michael E. T, I. Ferrer, Amadeo R. F. Matching unknown empirical formulas to chemical structure using LC/MS TOF axxurate mass and database searching: example of unknown pesticides on tomato skins, Journal of Chromatography A, Vol. 1067, 2005, pp.127-134.

Copyright © Int. J. of GEOMATE. All rights reserved, including the making of copies unless permission is obtained from the copyright proprietors.