Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 13, No. 4, p. 38-44, 2018
http://www.innspub.net

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RESEARCH PAPER
OPEN ACCESS

Oxymatrine degradation in cucumber fruit, leaves, and soil in Iraq

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Article published October 06, 2018

Key words: Oxymatrin, Degradation, Cucumber, QuEChERS method.

Abstract

This experiment was conducted in the National Centre for Pesticides Control (NCPC)/Ministry of Agriculture during winter season 2017-2018 Toknow the degredation of Oxymatrine in cucumber fruit ,leaves and soil in greenhouse and the period of this degradation and pre harvest interval for consumers and to know the range of appropriateness the new method of extraction with cucumber, the High performances Liquid Chromatography (HPLC) was employed as analyses equipment and QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) approaches as extraction method. The obtained results were showed that Oxymatrine in cucumber fruit, leaves, and green house soil degradation was acquired in 5^{th}, 7^{th}, and 2^{th} day respectively after the treatment ,then the hplc system did not detected any quantity from the insecticide oxymatrine, 7 day is the pre harvest interval(PHI),The average recoveries of Oxymatrine in cucumber fruit and leaves was 84-86.2%, in soil was 102.3-102.9%, with the relative standard deviation (RSD) 1.1% and 0.9% respectively. Through the results of this achievement, we determing the safety holding periods before the process of fruits reaping which was impossible to predict because the Oxymatrine does not have MRL in the Codex Alimentarius and European Union guideline and United States Environmental Protection Agency (USEPA), and the range of appropriateness the new method of extraction with cucumber as a future study we will expand the study of the degradation of Oxymatrine on other agricultural crops in the food basket for the Iraqi consumer.

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Introduction

Cucumber (*Cucumis sativus L.*) is one member of the family Cucurbitace(Huang *et al*,2009). cucumber consist of vitamins, minerals, amino acids, phytosterols, phenolic acids, fatty acids, and cucurbitacins(D'Amelio,1999).Another source marked the subsistence of essential oil, amino acids, pectins, starch, sugars, vitamin C, and curcurbitacin are found in cucumbers(Anton and Silano.2001). cucumber crop utilize in Popular medicine includes treatment of diarrhea Gonorrhea , diabetes and hypertension have been used to Detoxification, as a fat regulator, anti- inflammatory, serum and antioxidants(Abu-Reidah *et al*, 2012).for these unique benefits ,the cucumber crop is consumed throughout the year, and the Iraqi food basket is not devoid from it ,so farmers cultivate it throughout the agricultural season and in both open and covered crops, , Cucumber crop is mostly exposed to many different pests such as insects and fungi .these pest's can cause several injury to cucumber as a result reduce the quality of the huvest.in order to control pest at cucumber field ,several chemical approaches were used .one of the most environmental friendly insecticide that used in cucumber field Oxymatrine2.4S.L. Oxymatrine (7aS,13aR,13bR,13cS)dodecahydro-1H,5H,10H dipyrido [2,1-f:3′,2′,1′-ij][1,6]naphthyridin-10-one 4-oxide) is a new botanical insecticide with broad spectrum for use on awide range of crops aginst a wide spectrum of pestes and mode of action contact and stomach. It is a tetracycloquinolizindine alkaloid derived from plants roots grows in china called *Sophora flavescens* (Table 1) (Gholami and Sadeghi,2016).*Sophora flavescens*is generally composed of vehicles alkaloidas, lupine alkaloids, quinolizidine and particularly from vehicles Matrine, Oxymatrine, Sophcarpine, Sophramine and Sophoridine (Liu *et al*,2011).

The *Sophora flavescens*, which is a Chinese traditional herb used to control viral hepatitis and combat arthritis and other diseases, also contains many biologically active compounds, including matrine and Oxymatrine, known as biologically active substances against insect pests. Different, fungi, bacteria, and nematodes, because of this scientific evidence, the Korea Rural Development Administration has approved the manufacture, use and marketing of 21 commercial biocide *Sophora flavescens*(Lim *et al*, 2014). The oxymatrine is an environmentally friendly pesticide since it has low toxicity to humans and livestock as it is classified by the World Health Organization (WHO) under section five U in non-toxic substances, which is non-carcinogenic and has non-teratogenic effect and congenital or genital malformations non-mutagenic or Reproductive(Sineria,2016).Also, the pesticide Oxymatrine has a wide and rapid effect, as well as not harmful to experimental animals and its residue non-toxic(Sineria,2016). Far their more Oxymatrine has weak effects on honey bees in acetylcholinesterase (AChE) and adenosine triphosphatase (ATPase) activities as biochemical indicators (Rabea *et al* 2010). Oxymatrine used for protection of various agricultural crops such as citrus fruits , Ornamentals, Tobacco, cotton, and cucumber ...due to lack of studies are available on this insecticide, this study was applied evaluate the degradation pattern of oxymatrine in cucumber fruit and soil by using high performance liquid chromatography(HPLC)based on QuEChERS(Quick, Easy, Cheap, Effective, Rugged and Safe) method for extraction (Anastassiades *et al*,2003). The pesticide residues caused health and environmental problems in Iraq.

This is due to the lack of knowledge and restriction of the farms in the pre-harvest period of the pesticide. This study was used to determine the time period for the pesticide to be destroyed in fruits, leaves, soil, and the safety period. Fit the new extraction method for the cucumber crop or not.

Materials and method

Chemicals

Chemicals and solvents used in this study are HPLC-grad supplied by :Acetonitrile HPLC grad (99.9%) purchased from Labscan Ltd. (Ireland); Sodium chloride NaCl from Avantor (U.S.A); Anhydrous

magnesium sulfate $MgSO_4$ from Sigma Chemical (U.S.A) ; Primary secondary amine (PSA) (40-60 µm),and C18 (50 µm, 60A) were from Restek Qsep (U.S.A). Sodium chloride were astimulate by heating at 250°C for 4h in the oven before use and maintained in desiccators. Stock solution was drawn upin acetonitrile and stored at –18°C.

HPLC apparatus

The HPLC analysis was implemented with device type a Shimadzu LC-20AD (UFLC) Ultra Fast Liquid Chromatography, High performance liquid chromatography with UV/visible detector were used for determination of Oxymatrine.

The column was C18 (4.0mm ×250 mm) with a 5µm particle size (Touzart & Matignon, France). The column was thermo stated at 40°C. Mobile phase comprised of acetonitrile: water (95:5 v/v) as isocratic elution was infusion at flow rate of 0.5 mL min^{-1}, injection volume was 5 µL, the wavelength of the UV/visible detector was steady at 230 nm and the run time was 10 min per sample.

Field Experiment and Sampling

During winter season 2017-2018afield study was conducted of cucumber growing in Iraq. The experiment was applied at plant protection research station that in west of Baghdad (Abu-Ghraib) Ministry of Agriculture. The soil was inspect before the cultivation process at laboratories of the soil department of Agricultural Research the results were collected as: organic matter 0.86%; pH 7.6;texture, sandy Soil; sand 88.4%;silt 9.6%;clay 2.0%; .the experiment was conducted in randomized Complete block design (RCBD) in three replicates.

The formulation of Levo2.4% S.L (active ingredient :Oxymatrine) was applied in the recommended dose i.e., 0.3 g. $L^{-}1$. For the degredation study of Oxymatrinein cucumber, samples of cucmber fruie, leaf and soil were collected periodically by randomly at different time interval zero time, (1 hour after the application). 1, 2, 3, 4, 5, 6 and 7 days after the application. the samples werestorageat (-20C°) in deep freezertill the analysis procedure.

Extraction and clean up

The extraction of all samples of homogenized was relied on QuEChERS(Quick, Easy, Cheap, Effective, Rugged and Safe)methods. A 10 g of the samples(cucumber fruits ,leaves and soil) was weighted by using sensitive balance (Sartorius, Germany) and add in a50 ml centrifuge tube and then 10ml acetonitrile added to the mixture.

The mixture wasshocked by vortex (REAX top,Hei Heidolph–Germany) for 1min. Then 4 g of anhydrous magnesium sulfate ($MgSO_4$) and 1 g of sodium chloride (NaCl) were added to the tube. The mixture was extracted and centrifuged (4K 15, Sigma – Germany) at 4000 rpm for (5 min).

The matrix was cleaned up by dispersive solid-phase extraction. A 50 mg/ml Primary Secondary Amine (PSA) and 150mg/ml magnesium sulfate ($MgSO_4$) were added to the tube and then the matrix was centrifuged (Micro ceterifuges, Hettich zentrifugen – Germany) for (3 min) at 6000 rpm. The final volume of the matrix was filtered through a 0.45 µm membrane filter (polymide membrane filter, Cchromafil xtra pa-45/25 -Germany) for injected to analyzed by HPLC(Fig. 1).

Standard curve

To prepare stock solution, 0.0076 gm of repertoire standard Oxymatrine was weighted and melted in acetonitrile.

The blend was transferred into a 100 ml volumetric flask and diluted with acetonitrile to the mark for preparation 200 µg.ml^{-1} stock solutions. then The stock solutions was used for preparing of calibration curve and recovery test.

Then the standard solutions were prepared, and filtered through 0.45 µm membrane filters, then stored in a refrigerator at 4 C° until analysis.

Results and discussion

The Oxymetrin pesticide used in this study was subjected to a basic test to determine what was called the calibration curve, which is to compare the insecticide used in the study with a specific and known concentration of a set of standard samples by High Performance Liquid Chromatography (HPLC) (fig.2).

Table 1. Average recovery and RSD of Oxymatrine in samples(cucumber fruit ,leaves and soil)at various fortification levels.

Sample	Fortified level(mg/kg)	Recovery%	RSD%
Cucumber fruitand leves	0.01	86.2	0.5
	0.05	84.0	0.6
Soil	0.01	102.9	0.3
	0.05	102.3	0.6

Table 2. The values of the pesticide degredation in (fruit, leaves and field soil).

Time(day)	Conc.Mg.ml^{-1}		
	Fruit	Leaves	Soil
1hour	61.3	46.1	27.2
1day	41.8	34.3	11.5
2day	24.5	23.1	4.7
3day	19.1	18.4	0
4day	12.1	14.3	0
5day	3.2	9.2	0
6day	0	7.1	0
7day	0	5.7	0
8day	0	0	0

Determine the calibration curve by the areas revealed at each user concentration corresponding to the area of the standard sample of the oxymetrine pesticide (Fig. 3). The form obtained for the standard calibration is the achievement of the basic determinant of the efficiency of the model used by the pesticide, known as the linear test.in this study better linearity was scope from 0.1 µg.ml^{-1}to 10µg.ml^{-1}with the determination coefficient R2 = 0.9982.detection limits: limit of detection (LOD) and limit of quantification (LOQ) considered when signal to noise ratio of 3:1 and 10:1, respectively.

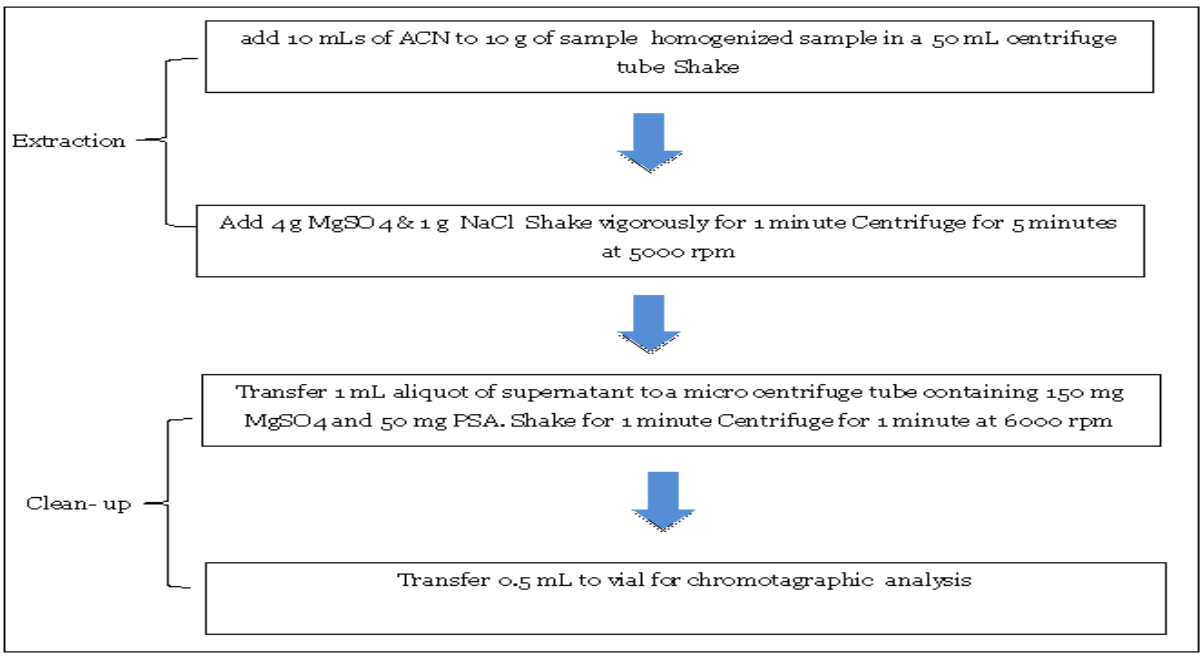

Fig. 1. Flow-chart of QuEChERS method Flow-chart of QuEChERS method for extraction and clenup oxymatrine from cucumber frute, leaves and soil.

LOD and LOQ were determined as 0.006 µg.ml^{-1}and 0.01µg.ml^{-1}for Oxymatrine respectively.

Recovery study: to prove the validity of the analytical method and to know the effectiveness of extraction and clean up steps appoint in this study, recovery studies were carried out by fortifying the cucumber plant and grrnhouse soil samples with different levels of the analytical standard solution. Untreated cucumber samples and soils fortified with 0.01, 0.05mg/ml of standard solution, These fortified samples were Extracted and clean up and then analyses by HPLC. The average recoveries of Oxymatrine in cucumber fruit and leaves was84-86.2%, in soil was 102.3-102.9%, with the relative standard deviation (RSD) 1.1% and 0.9% respectively were listed in (Table 1).

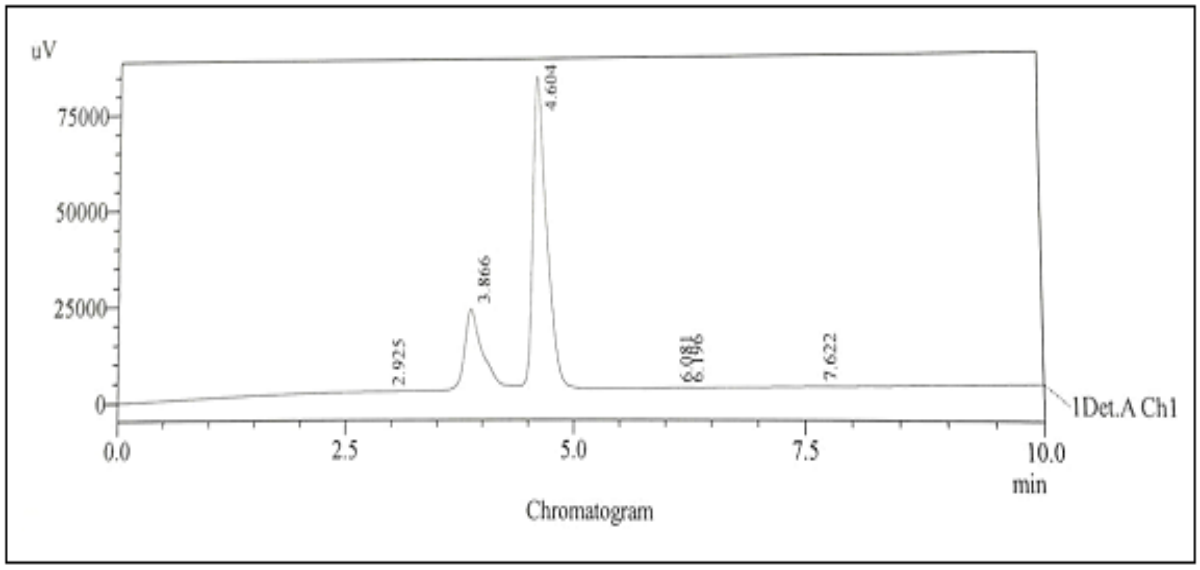

Fig. 2. Standard concentration for Oxymatrine which Analysis by High Performance Liquid Chromotography (HPLC).

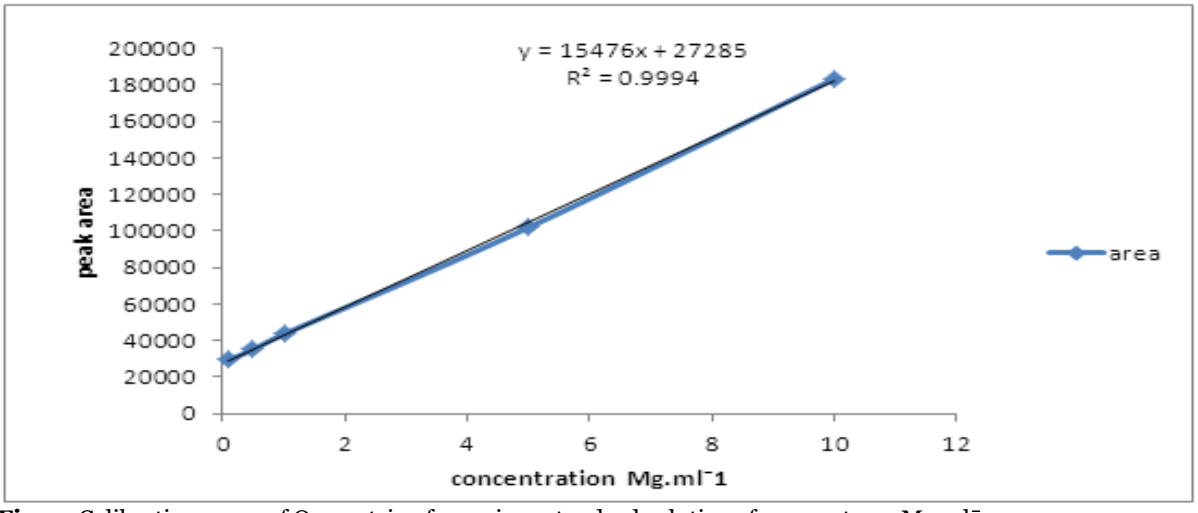

Fig. 3. Calibration curve of Oxymatrine for various standard solutions from 0.1 to 10 Mg.ml¯1.

The degredation trends of Oxymatrine in cucumber (fruit, leaves and greenhouse soil) were shown as in (fig.4). The results showed that Oxymatrine degradation during (5, 7, 4)days respectively in (fruit, leaves and greenhouse soil) after application then the hplc system did not detected any quantity from

the insecticide oxymatrine, and (Table. 2) shows the values of the pesticide degredation in (fruit, leaves and greenhouse soil) .this is the first study on oxymatrine degeadation in cucumer fruit, leaves and soil greenhouse in iraq conditions and the first study in the world.

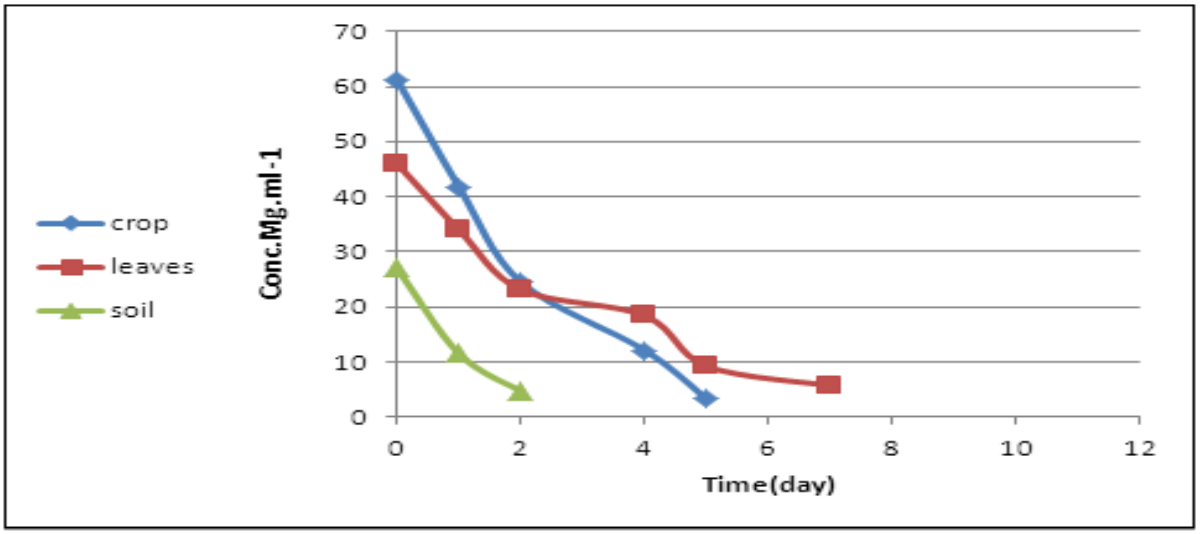

Fig. 4. Dissipation curve of Oxymatrine in cucumber fruit, leaves and soil.

Conclusion and recommendation

In this study, we conclude that botanical pesticide are rapidly dissipation in cucumber, leaves and soil in the greenhouse under these conditions. This requires many studies of different conditions, other countries and many crops.

Acknowledgments

I would like to thank all the employed in the national center for pesticide control for help us to this achievement.

Refrences

Huang S, Li R, Zhang Z, Li L, Gu X, Fan W, Lucas WJ, Wang X, Xie B, Ni P, Ren Y. 2009. The genome of the cucumber, Cucumis sativus L. Nature genetics, **41(12),** p 1275.

Anton R, Patri F, Silano V. 2001. Plants in cosmetics: Plants and plant preparations used as ingredients for cosmetic products. Volume II. Council of Europe Publishing.

Abu-Reidah I, Arráez-Román D, Quirantes-Piné R, Fernández-Arroyo S, Segura-Carretero A, Fernández-Gutiérrez A. 2012. HPLC–ESI-Q-TOF-MS for a comprehensive characterization of bioactive phenolic compounds in cucumber whole fruit extract. Food Research International. **46,** 108-117.

Gholami Z, Sadeghi A. 2016. Management Strategies for Western Flower Thrips in Vegetable Greenhouses in Iran: a Review. Plant Protect. Sci. **52(2),** 87-98.

Liu G, Dong J, Wang H, Hashi Y, Chen S. 2011. Characterization of alkaloids in Sophora flavescens Ait. by high-performance liquid chromatography–electrospray ionization tandem mass spectrometry. Journal of pharmaceutical and biomedical analysis **54(5),** p 1065-1072.

Lim SJ, Jeong DY, Choi GH, Park BJ, Kim JH. 2014. Quantitative analysis of matrine and oxymatrine in Sophora flavescens extract and its biopesticides by UPLC. Journal of Agricultural Chemistry and Environment **3(02),** p.64.

Sineria company Ltd.cyprus. 2016. Levo 2.4 S.L. www.sineria.org .

Rabea EI, Nasr HM, Badawy MEI. 2010. Toxic Effect and Biochemical Study of Chlorfl uazuron, Oxymatrine, and Spinosad on Honey Bees (Apis mellifera). Arch. Environ. Contam. Toxicol, **58,** 722-732.

Anastassiades M, Lehotay SJ, Štajnbaher D. Schenck FJ. 2003. Fast and easy multiresidue method employing acetonitrile extraction/ partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. Journal of AOAC international **86(2),** p 412-431.