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Degradation study of thiophanate-methyl residues in cucumber (*Cucumis sativus*)

Ali Bassam Al-Obaidie*[1], Abdul kareem Jawad Ali[2], Saleh Hassan Sumir[1]

[1]*Ministry of Higher Education and Scientific Research, Baghdad, Iraq*

[2]*National Center for Pesticides Control, Ministry of Agriculture, Baghdad, Iraq*

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Abstract

The purpose of the review is to assess the degradation sample of Thiophanate-methyl (70% WP) fungicide, three treatments (1.5gm/L. 3gm/L. and double spray 1.5gm/L) were used on cucumber yield. We carried out the experiment under greenhouse conditions in plant protection department/Ministry of Agriculture during 2017–2018 season. The researchers carried out the quantitative analysis of the fungicide residues as carbendazim was using High-performance liquid chromatography (HPLC) using QuEChERS extraction method. I found the rate of recovery was 92-106% and the comparative standard variation (RSD) were below 3.8%. Next the first order kinetics the fungicide degrades in cucumber found the half life value in cucumber between 12.2–13.4 days unheeding of position and dose.

***Corresponding Author:** Ali Bassam Al-Obaidie ✉ omertofe@yahoo.com

Introduction

Toxic substances released from fungicides industries are One source of environmental pollution (Naqvi *et al.*, 2006) Thiophanate methyl [1, 2-bis-(3-methoxy carboxy-2-thioureidobenzene)] is a large spectrum systemic fungicide which is extreme applied to control the major fungal diseases of different harvest (Maranghi *et al.,* 2003).

This fungicides has ability to absorbed quickly by the plant during spraying it upon them. The compound can easily transported through parts of the plant as methyl benzimidazole carbamates (MBC) which is responsible for the fungicidal activity inside the internal tissue of the plant (Buchenauer *et al.,* 1973). The main key factors for conversing of Thiophanate methyl to biochemical compound inside the plant are the presence of plant enzyme and sunlight (Buchenauer *et al.,* 1973 and Soeda; Kosaka, *et al.,* 1972) (Fig. 1).

Thiophanate methyl transposition to MBC which is considered a chemically stable metabolite and relatively persistent fungicide, which only metabolized to a limited extent in plants and soil. Thiophanate methyl can be determined in the form of MBC after transformation (Muccio *et al.,* 1995). Under acidic status the most cost efficient analytical method that use for control residues of thiophanate-methyl and carbendazim can be shown as the thiophanate-methyl converted to carbendazim by refluxing. UV/VIS spectrophotometer can specify carbendazim (Ono, 1973). This fungicide is applied for protection of several agricultural crops such as grapes, lettuce, citrus fruits, potatoes, cucumber and cereals. Cucumber (*Cucumis sativus*) is the most important cultivated crop belongs to the family of Cucurbitaceae (Heywood *et al.,* 2007). It's a creeping vine vegetables crop. Within cucumber varieties, several cultivars have been created. Cucumber is originally from South Asia, but now it grows in most countries. Many different types of cucumber are traded on the global market. Cucumber is produced in the tropical, subtropical and temperate regions (Heywood *et al.,* 2007; Judd *et al.*, 2008). Each 100g of fresh cucumber contains 96g water, 0.1g fat, 2.6-2.4g sugars and minerals. Fungal diseases and insects infections are the main pests that infect cucumber crop which are responsible for yield losses. Utilizing of different pesticides during pest control gives such wonderful results of the sometime, however the danger of these chemical cannot be ignored due to their residues. (Banks and Soliman, 1997; Urani *et al.,* 1995). The degradation of any complex depends on different factors, including plant species, chemical formulation, climatic conditions, physical phenomenon fundamentally volatilization, application method and chemical degradation, in which sunlight plays a prominent role. Thus, Its important to study the degradation for each crop and fungicides residues under field and protected cultivations appeared that the half-life was 7.2 to 8.3 days in field farming (Abd El-Megeed *et al.,* 2000).

Materials and methods

Chemicals

HPLC grade is provide from National Center for Pesticide Control (NCPC) /Ministry of Agriculture (Baghdad). Acetonitrile purity (99.9%) from Labscan Ltd. (Ireland); Anhydrous magnesium sulfate $MgSO_4$ from Sigma Chemical (U.S.A). Sodium chloride NaCl from Avantor (U.S.A). Primary secondary amine (PSA) (40-60 µm), Graphitized Carbon Black (GCB) (200-400 mesh) were from Restek Qsep. (U.S.A) Thiophanate-methyl reference standard was of 99% purity and obtained from Dr. Ehrenstorfer GmbH Germany. Stock solution was prepared in acetonitrile and stored at –18° C. The standard solutions required for constructing a calibration graph (0.01, 0.25, 0.5, 1, 5, 10 mg/kg) were prepared from stock solution by serial dilution with acetonitrile and were stored at 4° C before use.

Field Experiment and Sampling

A field study was conducted during winter seasons 2018 at Organic Agriculture Research Centre, Plant Protection department, Baghdad: Abu-Ghreb. The cucumber cultivated seeds were obtained by Dr. Enad Daher University of Baghdad College of Agriculture. I tested Soil before the seedling process in the

laboratories of the soil department of Agricultural Research as the results were as follows: organic matter; 0.86%, pH 7.6, EC; 6.0ms/cm, texture,; sandy Soil, Nitrogen70.0 ppm, Potassium 592ppm, Phosphorus 24.16ppm. I conducted the experiment in randomized block design in three replicates (each replicate considered as 25 plant) during 2017-2018. Treatment was carried out by Assltawakul sprayer (Turkey made 5 Lt.) equipped with one nozzle. I applied the formulation of thiophanate methyl 70% WP in the recommended dose i.e., 1.5 g L-1 (T2) then the recommended dose was doubled to 3 g L-1 (T3) respectively. And double spray in the recommended dose i.e.,1.5 g L-1 (T4). For the degradation study of thiophanate methyl in cucumber, samples were collected periodically by randomly at different time interval zero time, (1 h after the application). 1, 3, 5, 7 and 15, 21, 28 days after the application. A 500 mg of cultivated cucumber was collected as sample from each replicate including control line. These samples were transferred by cooler box to the laboratory for storing at -20° C in frezer.

Sampling preparation

Samples were performed by randomly collecting from different places of the experimental plots according to the FAO/WHO references (Soeda *et al.,* 1972). The samples were extracted according to the QuEChERS method described by Anastassiades (Anastassiades *et al.,* 2003). A 10 g of the homogenized samples from weighted in a 50 ml centrifuge tube and then 10ml of acetonitrile added to the mixture.

The mixture was shacked for 1min using vortex at maximum, Then a 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 at 3500 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 for 10min. at 3500 rpm. The final volume of the matrix was analyzed by HPLC.

HPLC Instrument

A Shimadzu LC-20AD (UFLC) Ultra-Fast Liquid Chromatography, High performance liquid chromatography with UV/visible detector was made in Japan for detecting the fungicide residuesd. The HPLC used reversed phase Kromasil C18 column for Separation the resedues from crops. The quality of coloum was (4.0mm × 250 mm) with a 5µm particle size (France made, Touzart & Matignon,). In order to work, the coloum needs to be thermo stated at 40° C. Acetonitrile is Mobile phase, it consist of: water (95:5 v/v) consisted of: water (95:5 v/v). Anew pump was used at flow rate of 0.5 mL min-1 injection volume was 5µL. The wave length was fixed at 230 nm and the run time was 10 min per sample.

Standard preparation

A 0.02 gm of references standard Thiophanate-methyl 99.96% from dr. ehrenstorfer gmbh company was weighted and dissolved of acetonitrile. The mixture was then transferred into a 100 ml volumetric flask and diluted with acetonitrile to the mark in order to prepare 200 µg.ml1- stock solutions. The stock solution was used then for preparing of calibration curve and recovery test. Acetonitrile in order to obtained the standard solutions for spiking and calibration curve. The standard solutions were freshly prepared, filtered through 0.45 µm membrane filters, and kept at 4° C in the dark.

Recovery assays

Un-treated cucumber plant samples were used. These untreated samples were fortified with 0.01, 0.1mg/ml of standard solution. These fortified samples were extracted and clean up and then analyses by HPLC.

Results and discussion

Linearity, the QuechERS method was used to determine the residue of the Thiophanate - methyl fungicide. It was found that the average recovery rate of all analyzes and external parameters was constant from 92-102% (Fig. 3) , which indicates the efficiency of this method and allowed identification and comparison of the fungicide in the samples.

Table 1. Recovery assays.

Fortified level (mg/kg)	Recovery	RSD
0.01	106	2.3
0.05	92	3.2

The values of the relative standard deviation (RSD) were less than 5% (Table 1). In all cases, and generally acceptable for further analysis, the calibration of the quantitative (LOQ) scale was performed by using the external standard calibration curves, which were linear with a correlation coefficient R2=0.998 for each analysis.

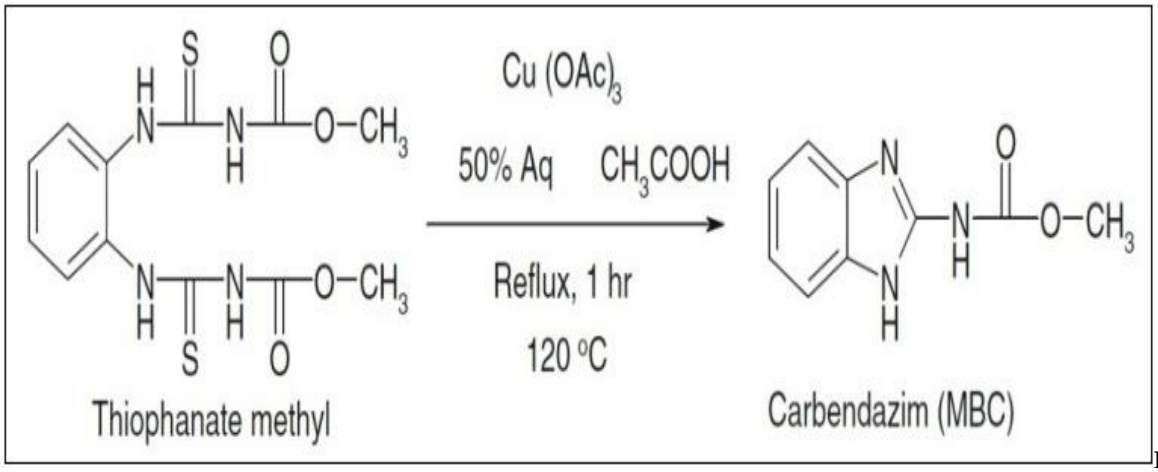

Fig. 1. Transformation of Thiophanate- methyl carbendazim (MBC).

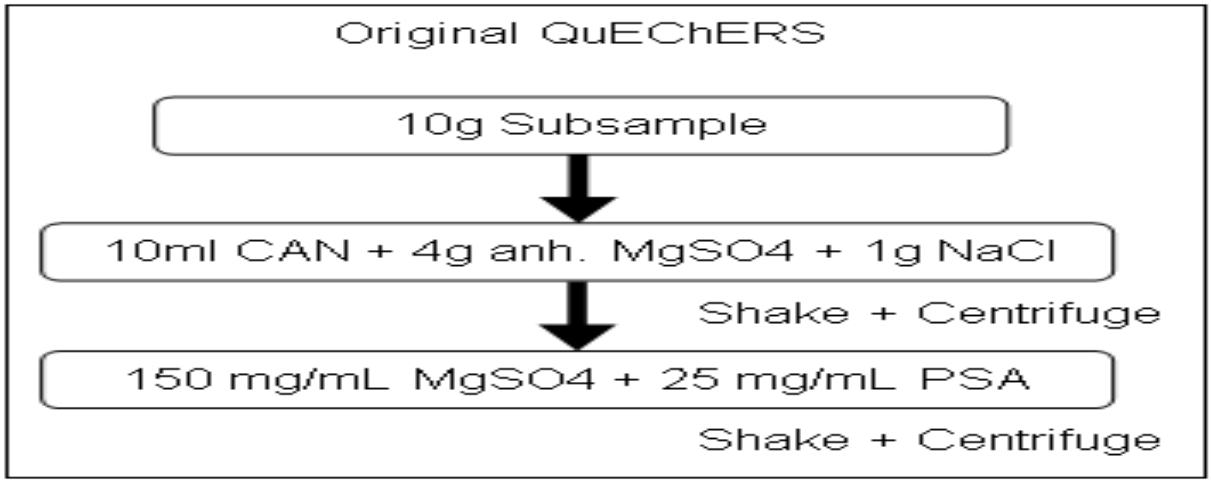

Fig. 2. The QuEChERS method for cucumber plant.

The Limit Of Detection (LOD) level for each analysis in the cucumber samples ranged from 0.001 mg to 1 kg, ensuring that the values were significantly lower than the allowable residue values on the cucumber. Then known concentration of reference slandered was added to each sample (0.05,0.01)ml/L. respectively. For the degradation curve in (Fig. 4). As a result, the Degradation of Thiophanate – methy was recorded at between 25 - 27 days. Based on a study was conducted by Abd El-Megeed *et al.,* 2000 that the Half Time of Thiophanate - methyl was 7.2 to 8.3 days for both cultivated approach greenhouse and open field in addition the average of separation of the fungicide was one application during control pest.

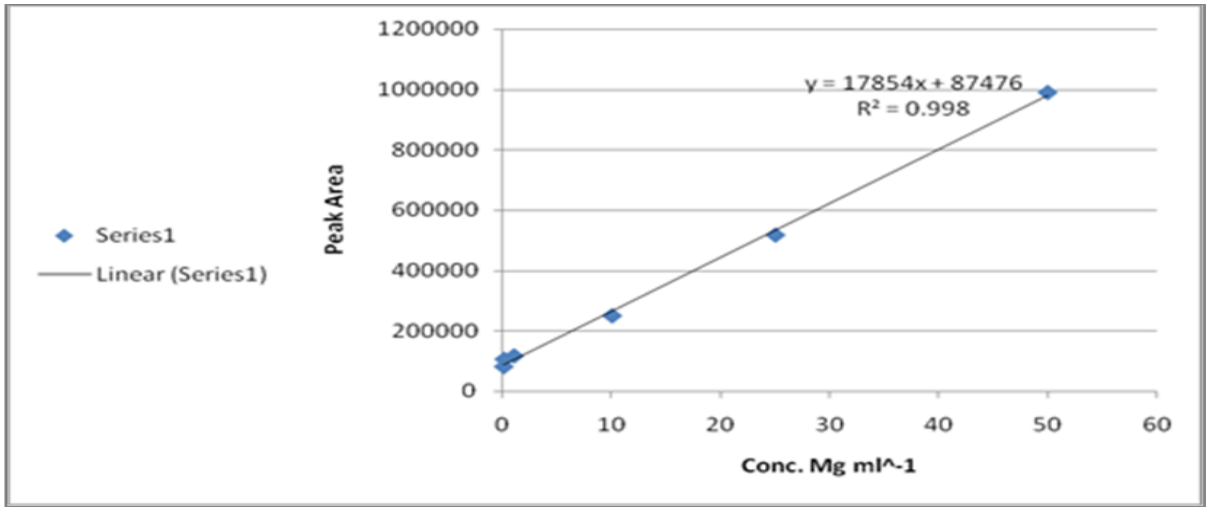

Fig. 3. Calibration curve of Thiophanate-Methyl from 50 to 0.05.

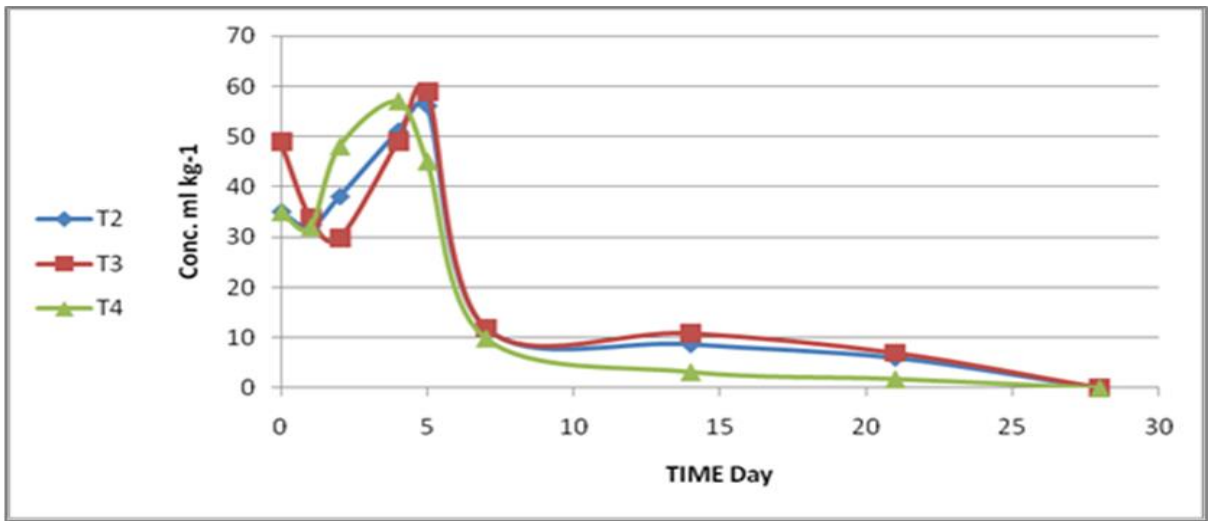

Fig. 4. Degradation curve of Thiophanate-Methyl.

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