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Estimating the Degradation of the Pesticides Acetamiprid and Thiamethoxam in the Agricultural Soil of the Cabbage Crop using HPLC Technique

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ABSTRACT

Background: The study focuses on the degradation of two pesticides, Thiamethoxam and Acetamiprid, that belong to the Neonicotinoid chemical group. This group is known for its water solubility, making these pesticides possible pollutants in agricultural settings. A research study aimed to estimate the residues of the pesticides Acetamiprid and Thiamethoxam in the soil of the Cabbage crop.

Methods: Fields on the cabbage crop, Globe master variety, with an area of 30×5 m². The soil was preparedand the seedlings were moved from the appellant to the experimental field in three rows, during the autumn season for year 2023, Were used in the experiment Two concentrations of the pesticide acetamiprid, the recommended concentration 0.5 g/L and double the concentration 1 g/L, For Thiamethoxam, used at concentrations of 0.3 g/L and 0.6 g/L.

Result: The findings revealed that Acetamiprid, at concentrations of 0.5 g/L and 1 g/L, decreased significantly over time. One-hour post-application, the residues measured 21.46 mg/kg for the lower concentration and 38.10 mg/kg for the higher concentration. These amounts further declined to 5.83 mg/kg on the ideal full degradation dayand the device could no longer detect the pesticide in subsequent days. After ten days, the highest detectable residue was 3.76 mg/kg. While Thiamethoxam, the residues one hour after application were 1.41 mg/kg and 8.87 mg/kg, respectively. These levels continued to decrease, reaching 5.2 mg/kg by the fifth day. After seven days, only a double concentration of 9.9 mg/kg remained detectable. The recovery rates for Acetamiprid and Thiamethoxam were 90% and 86%, respectively, indicating the efficiency of the methods used to analyze the pesticide residues in the soil samples.

Key words: Acetamiprid, Degradation, HPLC, Thiamethoxam.

INTRODUCTION

One of the major crops in the Cruciferae family is *Brassica oleracea* var.cabatata (Murrison and Napier, 2006). As one of the twenty vegetable crops, it is very significant as a global food source because it contains nutrients like phosphorus, protein calcium and carbohydrate, in addition, it is considered a good source of vitamins such as vitamin C and K (FAO, 1988; Nishant and Upadhyay, 2016). Despite its great nutritional and medicinal value, *B. oleracea* form alba, the white cabbage, is the most common species of the red cabbage, *B. oleracea* form rubra, according to Balkaya *et al*. (2005). According to the Iraqi Ministry of Planning (2020), the country's cultivated area was estimated to be around 3,690 dunumsand its average productivity was 2,381.8 kg/dunum, or an estimated 8,789 tons. The chemical group known as Neonicotinoid, which includes the pesticides Acetamiprid and Thiamethoxam, is characterized by its solubility in water, making it a pollutant. when specific environmental circumstances are met, to soil and surface water (Van Dijk, 2013; Bonmatin *et al*., 2015; Mohammadali *et al*., 2019).

Although these pesticides are very effective at getting rid of pests, one drawback of added pesticides is that their duration of effectiveness in crops and concentrations exceeds what is allowed. This means that using these pesticides against insect pests on agricultural crops can result in some of the pests remaining in the soil, (Abu-Duka and Mohammadali, 2021; Salam and Mohammadali, 2021; Sahoo *et al*., 2024) refer to the plant or soil. Because of its accuracy, the QuEchERS method is a sample preparation technique that vastly simplifies the analysis of pesticide residues. It is a quick, easy, cheap, effective, rugged and safe methods and is one of the techniques used to estimate pesticide residues using an HPLC device that its motion phase passed quickly (Anastassiades *et al*., 2003; Apriyanto and Harmayani, 2017). The aim of this study was to determine the residues of the pesticides Acetamiprid and Thiamethoxam in the soil using High Performance Liquid Chromatography (HPLC) technique.

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MATERIALS AND METHODS

The experiment was conducted at the College of Agriculture /University of Karbala's fields on the cabbage crop, *B. oleraceae*, Globe master variety, with an area of 30×5 m^2. The soil was preparedand the seedlings were moved from the appellant to the experimental field in three rows during the autumn season, 2023. The cabbage crop was treated by spraying pesticides on the shoots including Acetamipride and Thiamethoxam, as shown in Table 1. Three replicates were employed, with each one being divided into five treatments. Four of these treatments involved two pesticides, along with a control treatment that used only distilled water. The pesticide treatments included both the recommended concentration and double the concentration used in the control process, applied with a small 2-liter capacity sprayer. The soil used in agriculture was analyzed in the Field Crops Laboratory of the College of Agriculture - University of Karbala. The results of the analysis were as shown (soil type: sandy mixture, soil pH 7.2, salinity 4.2, organic matter 0.54%, sand 68.2%, silt 17.8%, clay 14%).

Preparing the calibration curve for the standard substance for the pesticides Acetamipride and Thiamethoxam

Ten grams of the standard substance for the two pesticides were weighed and placed in a 25 ml plastic container. High-purity methanol was then added until the initial concentration reached 400 ppm. Using the dilution equation $C_1V_1 = C_2 V_2$, four concentrations were prepared for each pesticide. The prepared concentrations for Acetamipride were 50, 75, 100 and 125 ppm, while the concentrations for Thiamethoxam were 20, 40, 60 and 80 ppm. These concentrations were then placed in the HPLC device.

Three replicates were used to calculate the average and plot the graph of the standard solution, showing the relationship between pesticide concentration and the area of the triangle to assess the device's accuracy. The model was then injected to diagnose pesticide residue based on the retention time of the standard substance, compared with the retention time observed in the model (FDA, 2018).

Determining the residues of the pesticides Acetamipride and Thiamethoxam in the agricultural soil of the cabbage crop

To estimate pesticide residues in the soil, samples were collected from agricultural soil, as detailed in Table 2. The model used represents the same time and one pesticide from each treatment, sourced from various locations (EPA, 1998). Equal-sized portions of the collected samples were placed in black polyethylene plastic bags, tightly sealed, labeledand stored in a freezer at -4°C. This method ensured the samples remained uncontaminated until they were analyzed for pesticide residues using an HPLC device (Sivaperumal *et al*., 2015).

Qualitative and quantitative evaluation of the pesticide residues acetamipride

Sample preparation

The plastic bags containing the frozen samples were extracted. then, weighing 10 grams from each sampleand mixed well. The QUECHERS method was used for extraction, purification and detection (Nwasa *et al*., 2015).

The extraction

The sample was extracted using a solvent (Ethyl acetate+ Dichloromethane+Acetone) in proportions (2: 8: 10) ml respectively. Activated carbon and (florisil) were added and

Table 1: Shows the chemical pesticides and concentrations that used in the field experiment.

Common name	Dosage Rate/ L.	Company	Trade name
Acetamipride	0.5 g.	Nipon soda	Mospilan 20% SP
	1.0 g.	Japan	
Thiamethoxam	0.3 g.	Agrochem	Tiam 25% WG
	0.6 g	Australia	

Table 2: Indicated the periods for collecting samples of the cabbage crop after treating with the pesticides acetamiprid at the recommended concentration (0.5 g/L of water) and double the concentration (1 g/L of water) and for the pesticide Thiamethoxam at the recommended concentration (0.3 g/L of water) and double the concentration (0.6 g/L of water).

No. of sample	Date of collected	Time
1	1/11/2023	One hour after treatment
2	2/11/2023	One day after treatment
3	3/11/2023	Tow day after treatment
4	4/11/2023	Three day after treatment
5	6/11/2023	Five day after treatment
6	8/11/2023	One week after treatment
7	11/11/2023	Ten day after treatment
8	15/11/2023	Tow week after treatment

placed in the ultrasound machine for 5 minutes. After that it has placed in the centrifuge at 4000 rpm for 10 minutes.

Purification

The upper layer, or clear solution, was filtered through a 0.45 µm fine filter. The sample was then dried with a rotary evaporatorand 20 mL of methanol was added and being refrigerated.

Detection and analysis

The high-performance liquid chromatography (HPLC SYKAMN) in the laboratories of the Ministry of Science and Technology / Department of Environment and Water was used. moreover methanol: distilled water as a carrier phase with a ratio of 90:10 ml, a flow rate of 1.2 ml/min, a separation column C_{18} - ODS 25 cm * 4.6 mm and a detector. Ultraviolet radiation with a length of 254 nanometers. The oven temperature was 37°C and the pH was 7.0.

Qualitative and quantitative estimation of the persistence of the pesticide Thiamethoxam

Sample preparation

The plastic bags containing the frozen samples were extracted. then, weighing 10 grams from each sample and mixed well. The QUECHERS method was used for extraction, purification and detection (Kumar *et al*., 2014).

The extraction

Ten gram of the sample was placed in a 50 mL tube with 5 mL of acetonitrile and 15 mL of water. Following that, 8 grams of anhydrous magnesium sulfate and 7 grams of sodium chloride were added. Then, use the Vortex instrument for 5 minutes to combine the sample with the added chemical compounds. The mixture was placed in a centrifuge and spun at 5000 rpm for 5 minutes. The extract is filtered through a column containing anhydrous sodium sulfate to remove moisture from the solution.

Purification

Two milliliters (ml) of the solution were extracted from the preceding filter with 0.45 µm size. The 1.5 ml vials were specifically designed for the HPLC device and stored in the refrigerator.

Detection and analysis

The high-performance liquid chromatography technique (HPLC SYKAMN) at the Ministry of Science and Technology /Department of Environment and Water was used. A separation column C_{18} - ODS 25 cm * 4.6 mm, a detector and distilled water: Aceyonitrile as a carrier phase with a ratio of 30:70 ml were prepared. The flow rate was 0.7 ml/ min. ultraviolet radiation has a wavelength of 290 nm. The oven temperature was 37°C and the pH level was 7.0. The sample injection volume is 100 microliters.

Recovery efficiency

A 10-gram soil sample without pesticide (for comparison) was taken. The pesticide was then applied at a concentration of 50 ppm, followed by extraction, analysisand detection according to the specified conditions. The sample was found to contain 45 ppm of the pesticide Acetamipride and 43 ppm of the pesticide Thiamethoxam (Telo *et al*., 2015).

Statistical analysis

A factorial experiment was carried out using a completely randomized block design (R.C.B.D. Factorial Experiment conducted for the field experiments, while a complete randomized design (CRD) was used in the laboratory experiments. The results were compared using the least significant difference (LSD) at the probability level of 0.05, as the (SAS) Statistical program was used for the purpose of statistical analysis (Arthur, 2013).

RESULTS AND DISCUSSION

Prepare a calibration curve for the standard substance

The prepared concentrations were added to the standard solutions of the pesticides Acetamipride and Thiamethoxam, which are (50, 75, 100, 125) and (20, 40, 60, 80) mg/liter, respectively to prepare the standard curve in the HPLC device. To obtain a linear relationship for the two pesticides, their concentrations are dropped along the axis. The sigmoid is in mg/L and the peak area on the y-axis, as shown in Fig (1A,B), which was linear with a correction factor (R2) of 0.999 for acetamipride and 0.998 for

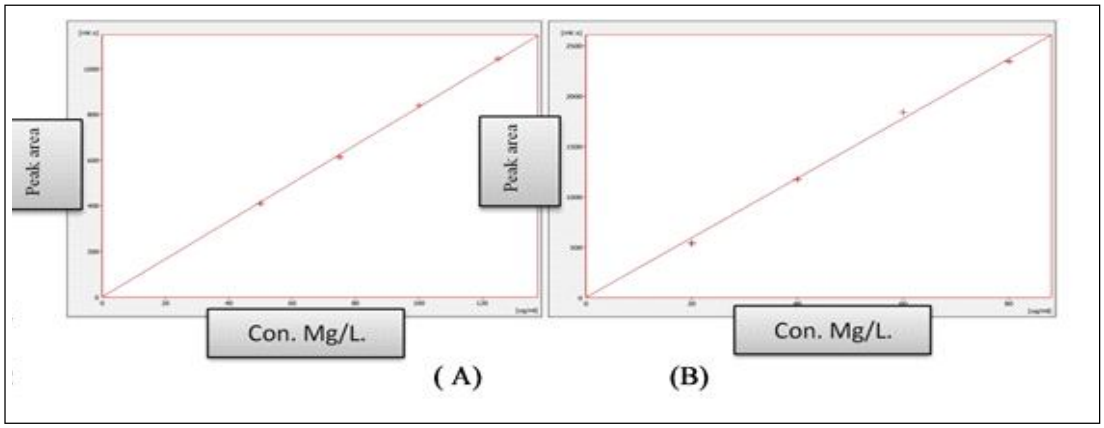


Fig 1: Calibration curve for the two pesticides: A- Acetamipride B- Thiamethoxam.

thiamethoxam, indicating the determination of retention time and accuracy. Preparing the standard materials.

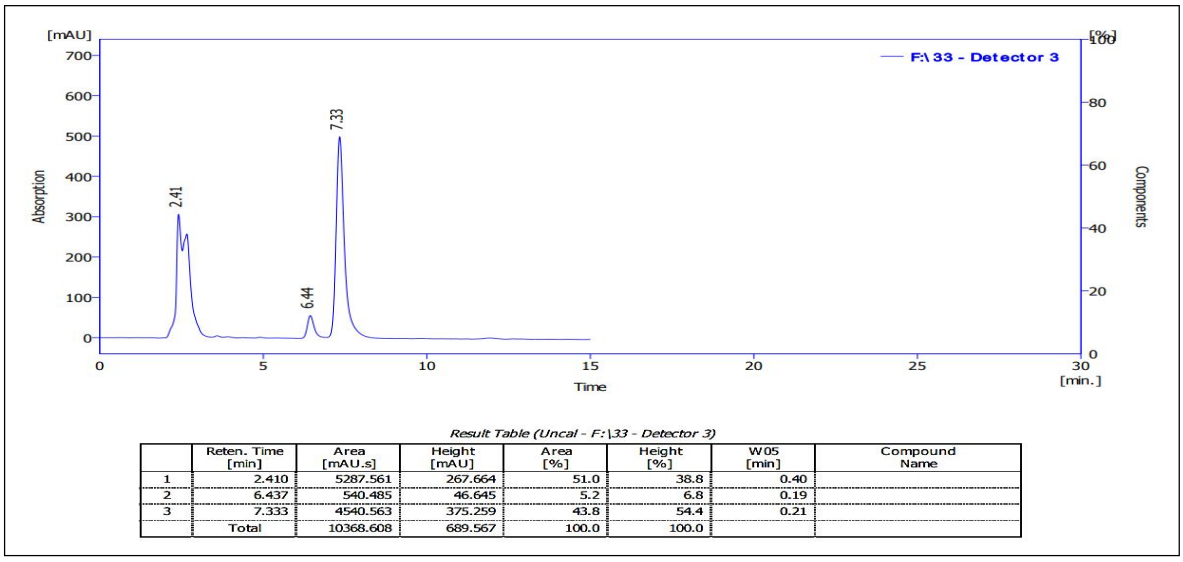
Determination of the remaining elements of Acetamipride and Thiamethoxam in the agricultural soil of the cabbage crop

Disappearance of the pesticide acetamipride from agricultural soil

The standard solution of the pesticide Acetamipride, at a concentration of 50 mg/L, was injected into the HPLC instrument. The resulting curve, observed at 7.33 minutes, served as a reference for detecting the active ingredient in samples collected at various time intervals, as shown in Fig 2.

Fig (3), which shows the pesticide's disappearance curve from agricultural soil, shows that the quantity of pesticide one hour after treatment was 21.46 mg/kg for the authorized dosage and 38.10 mg/kg for double the dose. The pesticide concentration began to decrease and continued until after seven days of treatment, when it reached 5.83 mg/kg, after which the HPLC device detected no amount of pesticide for the remaining days at the recommended concentration, while the last amount sensed by the HPLC device was 3.76 mg/kg after ten days for double concentration.

There are several factors that influence the behavior, degradation and fading of pesticides applied to agricultural crops, including the physical and chemical properties of each pesticide (vapor pressure, solubility in water and



Result Table (Uncal - F:\33 - Detector 3)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	2.410	5287.561	267.664	51.0	38.8	0.40	
2	6.437	540.485	46.645	5.2	6.8	0.19	
3	7.333	4540.563	375.259	43.8	54.4	0.21	
	Total	10368.608	689.567	100.0	100.0		

Fig 2: Standard curve for the pesticide Acetamipride at a concentration of 50 mg/L. (devise data for HPLC).

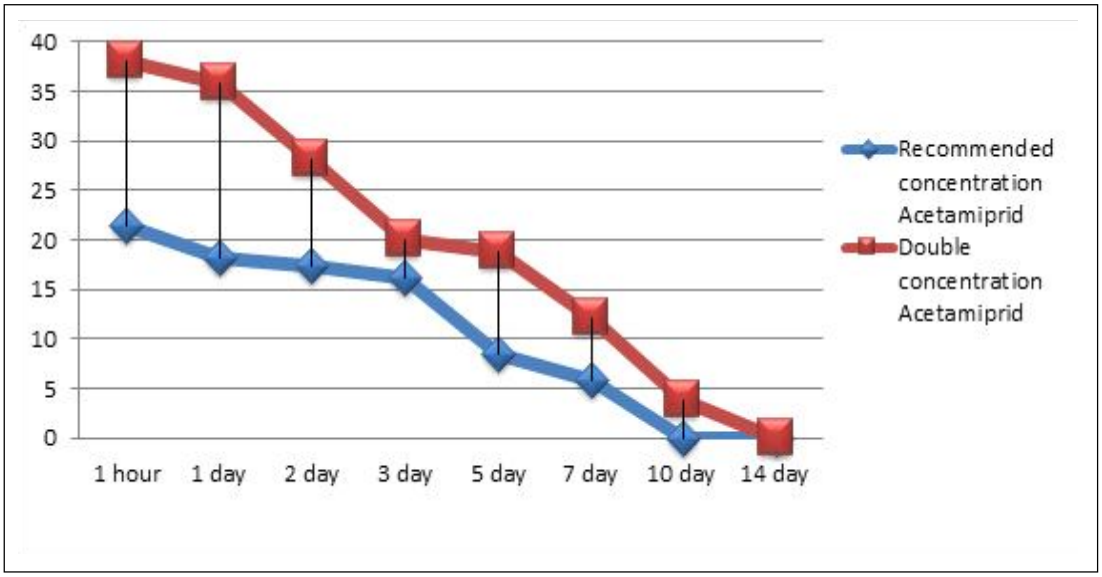


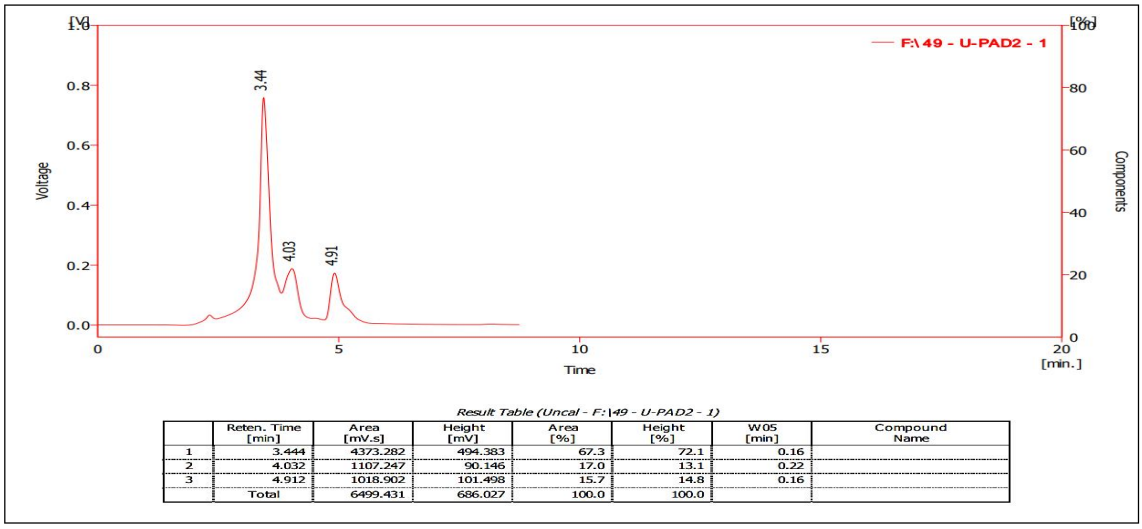
Fig 3: Time period for the disappearance of quantities of the pesticide Acetamipride from soil of the cabbage crop (mg/kg) using the recommended concentration and double the concentration. LSD for pesticide amounts 0.3199.

hydrolysis) as well as crop characteristics (growth rate, ease of penetration, transportand excretion). Microbial activity, environmental conditions (such as rain, temperature, sunshineand humidity), treatment technique, plant species, dose, treatment intervaland harvest time all contribute to pesticide destruction (Fujita *et al*., 2012; Tewary *et al*., 2005; Khay *et al*., 2008).

Disappearance of the pesticide Thiamethoxam from soil

The standard substance for the pesticide, Thiamethoxam, at a concentration of 20 mg/L was injected into the HPLC deviceand its curve showed at the time (3.44 minutes) and was used as an indicator to detect the active substance of the pesticide for samples taken at different time periods, Fig 4.

The pesticide's amount one hour after treatment was 41.1 mg/kg for the recommended concentration and 87.8 mg/kg for the double concentration, as shown in Fig 5, which shows the pesticide's disappearance curve from soil. The pesticide amount decreased until the fifth day, when it reached 5.2 mg/kg. After that, the instruments was not sensitive to any amount of pesticide at the recommended concentration and the lowest amount that the instruments was sensitive to due to double concentration was 9.9 mg/kg after seven days of treatment. This is consistent with the study conducted by Abd-Alrahaman (2014), where found that the concentrations of Thiamethoxam pesticide residues in the soil planted with potato were (7.4, 4.85, 3.15, 1.45, 0.42, 0.03 mg/kg after treatment (2 hours), 1 3, 5, 7 and 14 days, respectively. Hafez and Sing (2016) also indicated, through the study conducted to estimate pesticide residues in soil planted with tomato, that the recommended pesticide concentrations of 50 g active ingredient/ha one hour after spraying



Result Table (Uncal - F:\49 - U-PAD2 - 1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	3.444	4373.282	494.383	67.3	72.1	0.16	
2	4.032	1107.247	90.146	17.0	13.1	0.22	
3	4.912	1018.902	101.498	15.7	14.8	0.16	
	Total	6499.431	686.027	100.0	100.0		

Fig 4: Standard curve for the pesticide Thiamethoxam at a concentration of 20 mg/L. (devise data for HPLC).

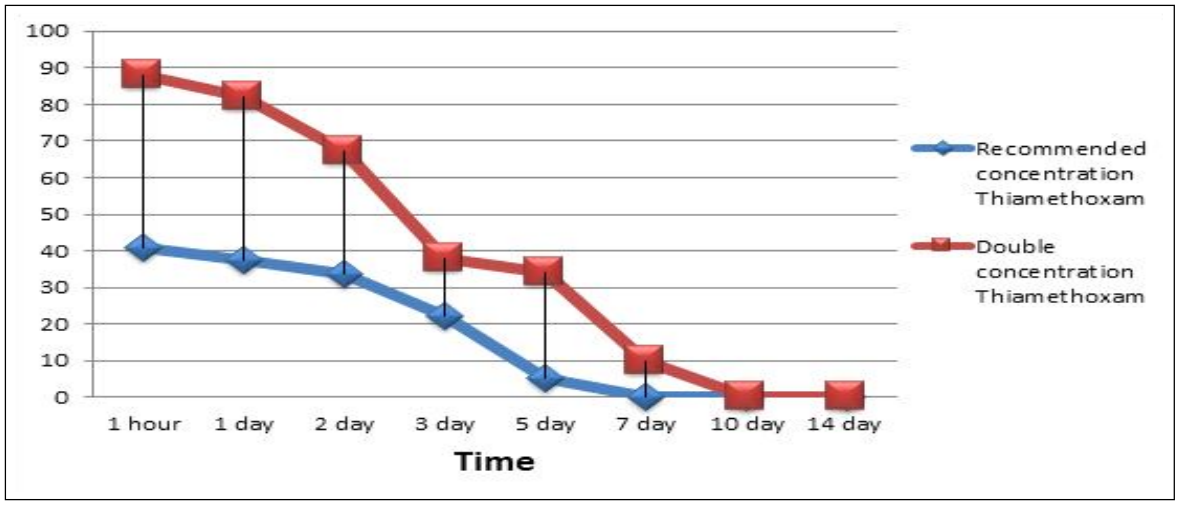


Fig 5: Time period for the disappearance of quantities of Thiamethoxam pesticide from the agricultural soil of the cabbage crop (mg/kg) using the recommended concentration and the double concentration. LSD for pesticide amounts 1.5857.

amounted to 0.06. mg/kgand the dissipation continued until the fifth day, reaching 0.01 mg/kg.

Furthermore, the double concentration of 100 g active ingredient/ha was 0.11 mg/kg an hour after treatment and the pesticide residue continued to deteriorate until the seventh day after treatment, which reached 0.01 mg/kg, after which the device was not sensitive.

Recovery efficiency

To evaluate the extraction efficiency of pesticide residues in agricultural soil, the same two extraction methods were used for Acetamipride and Thiamethoxam, based on the recovery formula:

$$\text{Recovery} = \frac{\text{Residual}}{\text{Total}} * 100$$

The recovery rates for Acetamipride and Thiamethoxam were 90% and 86%, respectively, indicating the effectiveness of the two methods for comparing and identifying the pesticides present in the samples. This study is consistent with Abu-Duka (2021).

CONCLUSION

The results of the study showed that the residues of the insecticides Acetamiprid and Thiamethoxam decompose be faster degraded from the soil due to many environmental factors such as wind, air temperature and sunlight during the day, especially in Iraq, where the intensity and duration of sunlight are a decisive factor. Moreover, the determination of these residues recorded a significant decrease whether in leaves and soil owing to the harsh climate in Iraq, in particularly during the summer.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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