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RESIDUE ANALYSIS OF IMIDACLOPRID IN FRUITS OF SWEET PEPPER CARISMA(*CAPSICUM ANNUM* L) USING QUECHERS HPLC METHOD IN GREENHOUSE

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Abstract

The field experiment was carried out at the greenhouse of the Department of Plant Protection College of Agricultural Engineering Science University of Baghdad in Jadriya. It was conducted on the fruits of pepper plant of Carisma in HPLC liquid chromatography method (QuEchERS) and all the Laboratory experiments were conducted to analyze residues of Imidacloprid in the Laboratories of the National Center for Pesticides Control The Ministry Agriculture / Baghdad –Abu Ghreb. The results showed that the highest rate of insecticide recovery was 95%, and the highest level of dissipation curve after 4 days of spraying reached (137 mg/kg) and then began to decline to (0.7 mg/kg) after 8 days below the MRLs limits for insecticide residues, and then decreased in the samples taken on the ninth, tenth, eleventh, twelfth, thirteenth, and fourteenth day as it reached (0.5, 0.2, 0.08, 0.03, 0.009, 0.007 mg/kg) respectively, and reached less than MRLs on pepper plant Carisma. This study showed that the Imidacloprid applicate is safe for the human consumes after 8 days and completely in the twenty-first and twenty-eighth days concentrations respectively they were 0.00 mg/kg and 0.00 mg/kg of spraying.

Key words: QuEchERS, MRLs, residues, Imidacloprid, HPLC, sweet pepper

Introduction

Pepper is one of the most popular spices in the world, and it is a highly antioxidant product containing vitamin C, carotenoids and other essential or beneficial nutrients (Khoshgoftarmanesh *et al.,* 2009). Due to its multiple uses and unique colors, fresh pepper is consumed worldwide (Ozkan, Bindak, Erkmen, 2015). Peppers are plants grown in Iraq in an agricultural way protected in greenhouse at the beginning of autumn (Jafar, 2017). The conditions in the greenhouses causes significant highly increase in temperature and high humidity that help to cause diseases plants, and crop plants grown by insect and nematode injuries. Pepper is infected with many insect pests such as aphids, thrips, and other insect pests, 35 pests are recorded on pepper plants such as Aphid Cotton (*Aphis gossypii* Glover) which is one of the most important pests of pepper plants (Sorensen, 2005). It requires the use of insecticides to high concentrations and lack of commitment by farmers to the pre- harvest interval leading to the accumulation of insecticides residues in crops, Fruits are most suitable to insecticides residues because they are eaten fresh, followed by fresh vegetables, followed by cooked vegetables and cooked grains. Monitoring the chemical insecticides in field crops in greenhouses is useful to identify the dangers of intensive insecticides use and to raise awareness of the need to adhere about the dangers of insecticides residues in peppers. Many chemical insecticides have been used, and the most important insecticides used are systemic insecticides that belong to the group of neonicotinoid insecticide such as Imidacloprid after the emergence of the problem of resistance to insects absorbing insects pests such as aphids of all kinds, especially (*Aphis gossypii*) aphid cotton for many insecticides such as pyrethroids and the organic phosphorus field crops infected by aphid cotton insects in Texas and Alabama farms in United States and Australia (Herron *et al.,* 2000). Imidacloprid is a wide-spread systemic insecticide used against many agricultural insects pests, including aphids and gloves (Sangamithra *et al.,* 2018; Huang *et al.,* 2019). It was discovered that the excessive use of chemical insecticides in the control of insect pests led to the emergence of many environmental and health problems as the case with the studies on most vegetable crops that were controlled by various insecticides to the presence

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of quantities and concentrations of insecticides higher than the limits allowed globally (MRLs) and sometimes within the limits allowed but most times it is higher than the permissible limits and these ranges change from country to another according to the rates of daily food consumption of peoples which is called residues of insecticides and found residues of many insecticides Imidacloprid on agricultural crops, including pepper plant (Jodeh *et al.,* 2016). These chemicals are toxic and harmful and affect the metabolic activities of humans negatively, which reflect the health of humans who eat vegetables containing residues of agricultural insecticides, especially when consumed fresh, as they are estimated by many methods, the most important method is QuEChERS, and it is the best way to detect systemic insecticide such as Imidacloprid in vegetables on pepper plant (Eun - Kyung *et al.,* 2013). Therefore, this study aimed to estimate the residue of Imidacloprid on the fruits of pepper plant in the greenhouse.

Materials and Methods

Greenhouse Experiment

A field trail was conducted during (December 2018) at the greenhouses of the Plant Protection Department / College of Agricultural Engineering Science / University of Baghdad, and it is was carried out on pepper plant of the charisma / an area of greenhouse was (125) m². All the experiment was laid out in Randomized Complete Block Design (RCBD) with 3 replicates each experimental plot was 3m×4m and was separated by drip irrigation system by three replications per treatment, the length of the center is 3m and the distance between one center and another is 75cm and between a plant and another 40cm. The species Carisma was distributed randomly within each sector and placed in each bis 30 seedlings and each flag with a specific symbol of the function on the species taking into account all the recommended agricultural operations.

Insecticide Spraying and Sampling

The field trial was performed in a greenhouse at a research station affiliated to the Department of Plant Protection, College of Agriculture Engineering Sciences / University of Baghdad, Iraq in (April - 2019). The plants from which the samples will be collected were marked by marking and a sample was collected for comparison before spraying. The spraying was carried out using the concentration recommended by the producing company at 5ml /10 liters of water. Dated 3/4/2019. Samples were collected according to the schedule in table 1 and time of collected fruits sweet pepper were at 9am fruits were taken 3kg in each samples in the Sweet Pepper cultivar used was Carisma (the seeds from fito seed company, Spain). The fruits was collected from plants before spraying the Insecticide and was sprayed once using (imidacloprid 200SL). It was applied at the dosage recommended by regulation guidelines (5ml / 10L). The samples were collected randomly from the lower, middle, and upper rows of plant and transported to the laboratory by polyethylene plastic bag (Chavarri *et al.,* 2004). Moreover, control samples (without any processing) were taken in each step to compare the results (Table 1).

Table 1: Collecting pepper fruits before and after Imidacloprid spraying.

Date	Period of the sample taken/ hours and days
3/4/2019	Before spraying the insecticide one hour /the control sweet peppers 2.5-3 kg
3/4/2019	After spray 2 hours
4/4/2019	After spray 1 day
5/4/2019	After spray 2 day
6/4/2019	After spray 3 day
7/4/2019	After spray 4 day
8/4/2019	After spray 5 day
9/4/ 2019	After spray 6 day
10/4/2019	After spray 7 day
11/4/2019	After spray 8 day
12/4/2019	After spray 9 day
13/4/2019	After spray 10 day
17/4/2019	After spray 14 day
24/4/2019	After spray 21 day
1/5/2019	After spray 28 day

Extraction

The QuEChERS method (quick, easy, cheap effective, rugged, and safe) was used for the extraction of imidacloprid from the cucumber samples (AOAC 2007; Anastassisdes, *et al.,* 2003). 10gm of homogenized sample of the fruits pepper was placed into a 50ml centrifuge tube with 10 ml of ACN, then at vortex for 5 minutes to ensure that the solvent is interacted well with the entire sample. Then, 4. 0 gm of anhydrous $MgSO_4$, and1 gm ($C_6H5Na_3O_7$. $2H_2O$) and 0.5 gm ($C_6H_6Na_2O_7$.1. $5H_2O$) and 1 gm of NaCl was added repeating the shaking process for 1 minute to prevent coagulation of $MgSO_4$, and put in centrifuge 3500 rpm / 5 min, the upper layer was cleaned by dispersive solid - phase extraction with 0. 5 g of primary secondary amine and 150 mg of anhydrous $MgSO_4$. The mixture was then shaken for 1 minute and centrifuged for 5 minutes at 3500 rpm. Then, the extract was cleaned with a 0.45um filter and, then this solution were injected into HPLC.

Weights of the following buffer salts in this experiment extraction by using QuEChERS method

(4) gm Magnesium Sulfate Anhydrous ($MgSO_4$)

(1) gm Sodium Citrate Dihydrate ($C_6H5Na_3O_7$. $2H_2O$) (0.5) gm Di Sodium Hydrogen Citrat Sesquihydrate ($C_6H_6Na_2O_7$.1. $5H_2O$)

(1) gm Sodium Clorid (NaCl)

Chemicals, Reagents and Apparatus

Analytical standard imidacloprid (99. 5% purity) Dr. Ehrenstrofer / Germany and Imidacloprid (200 SL) systemic insecticide from local market, rate of application: 5ml/10L water from Bayer company (Germany), Acetonitrile (ACN) (liquid chromatography grade) Anhydrous magnesium sulphate ($MgSO_4$), and water ionized (HPLC grade) from ROMIL company (England), Sodium chloride (NaCl) (99. 5% purty) were purchased from Sigma - Aldrich (Germany) and primary secondary amine (PSA) was provided by Agilent Technology. HPLC (CTO - 20AC) Shimadzu Corporation Kyoto, Japan.

HPLC Conditions

The HPLC (model Shimadzu LC - 20AD Koyto, Japan) equipped with an UV detector at a wavelength of 230 nm and Waters column C18 25 mm x 4. 6 mm was used to measure imidacloprid residue with the mobile phase of acetonitrile / water (10/90v/v) with flow rate was 0.55 ml / min, and total flow= 1 ml/min, end time = 10 min Oven temperature was 40 C and last injection volume was, L 5 micro letters.

Recovery Efficiency

The purpose of recovery efficiency is to verify that the extraction method is suitable for the determination of the insecticide Imidadacloprid and to determine the efficacy of the extraction method used in the experiment by taking three different concentrations of the standard active ingredient Standard Imidadacloprid (200, 12.5, 1.56 mg/kg). Active substance is produced by laboratories Dr. Ehrenstorfer GmbH / Germany, placed in a 10 ml Volumetric Flask glass tube added to the models of unsprinkled pepper fruits to control the weight of pepper 10g, and then left for two hours after the extraction and purification process was carried out in all its steps as in the method used to extract the insecticide Pepper using QuChERs method and making five replicates for each sample and the replicates of each model were injected into the HPLC and the area extraction for each model (5 replicates) was then taken and the rate was placed on a calibration curve to calculate the concentrations and use the Excel program programmed with the HPLC device to calculate the recovery efficiency. The concentrations were calculated from the program and were respectively (190, 11, 1.3mg/kg) using recovery efficiency equations equal to the extracted concentration of the insecticide divided by the concentration Standard information 100 times. (Lesueuretal *et al.,* 2008).

Recovery efficiency =

$$\frac{extracted\,concentration}{concentration\,S\tan dard\inf ormation}\times 100$$

Study of the Analysis of Residues of Imidadacloprid on the Fruits of Sweet Pepper (Carisma) *Capsicum annum*

Preparation of the standard solution of the insecticide Imidadacloprid Vegetable standard solution volume of 50 ml at a concentration of 400 mg/kg of the standard substance of the insecticide Imidadacloprid and stored in the refrigerator at a temperature of 4 m away from light. All laboratory experiments were carried out in the laboratories of the National Center for Residues Pesticides Control / The Ministry Agriculture of Iraq/Baghdad –Abu Gareeb.

Preparation of Standard Curve Solutions.

Preparing a standard solution is to make the standard curve in the volume of 50 ml at a concentration of 400 mg/kg of the standard substance of the insecticide Imidacloprid and kept in the refrigerator at a temperature of 4 m away from light. The concentrations of solutions prepared from the standard solution were (100, 50, 0, 78, 1, 56, 6, 25, 12, 5, 25, 0, 19, 0). The purpose of the preparation of these solutions was to find out the coefficient of correlation between the calibration curve points and the retention time (the time when the representative top of the insecticide appears on the datasheet and the LOD limit), which is the minimum amount of Standard solutions that can be detected in an HPLC (LOQ) are the minimum amount of material required in the sample that can be quantified The injection process was repeated twice for each concentration and LOD, LOQ and half-life were calculated according to Al Kraawi (2018) from the following equations: LOD = 3 × (sd/slope) LOQ = 10 × (sd/ slope) Sd: Standard error, slope: represents the regression coefficient.

Results and Discussion

The Calibration Curve of Imidacloprid

The results showed that the Imidacloprid standard calibration curve of the first degree straight line formula which reflects the relationship between the insecticide concentration (mg / kg) and the peak area shown in the datasheet was (= 0.9949). (The calibration curve was used to determine the detection limit (LOD) and had a value of 0.003 mg/kg). The quantitative estimate limit (LOQ) was calculated and its value (0.1 mg/kg). The ability of HPLC to detect low insecticide concentrations, and LOD and LOQ are essential elements for verifying the efficiency of the method. It has been shown from the above values that the device is able to estimate the insecticide residues to a high degree (Yadav and Bharkatiya, 2017).

Recovery Efficiency

The recovery rate of Imidacloprid ranged from pepper fruits respectively was (83, 95, 88) % after taking the rate for five replicates of the three concentrations (200, 12.5, 1.56) mg/kg and this falls within the acceptable range in the analytical method that is (70 - 120)% (Zhao *et al.,* 2014) which confirms that the extraction method used in this study is highly efficient and acceptable in the recovery of the insecticide from the fruits of pepper.

Imidacloprid Residues in Pepper Fruits through Retention Time.

The sample of commercial model of Imidacloprid was analyzed in HPLC analysis and separation device which was analyzed to retention time; Retention Time to find

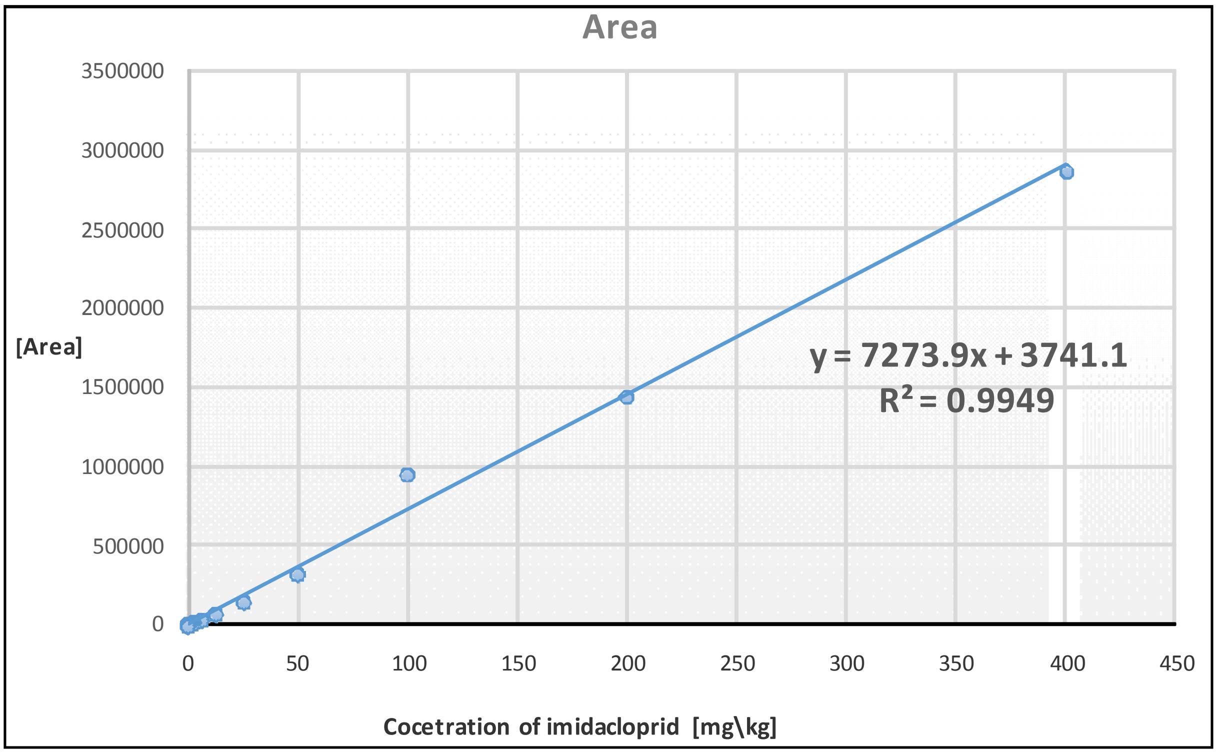

Fig. 1: The Calibration curve of Imidacloprid

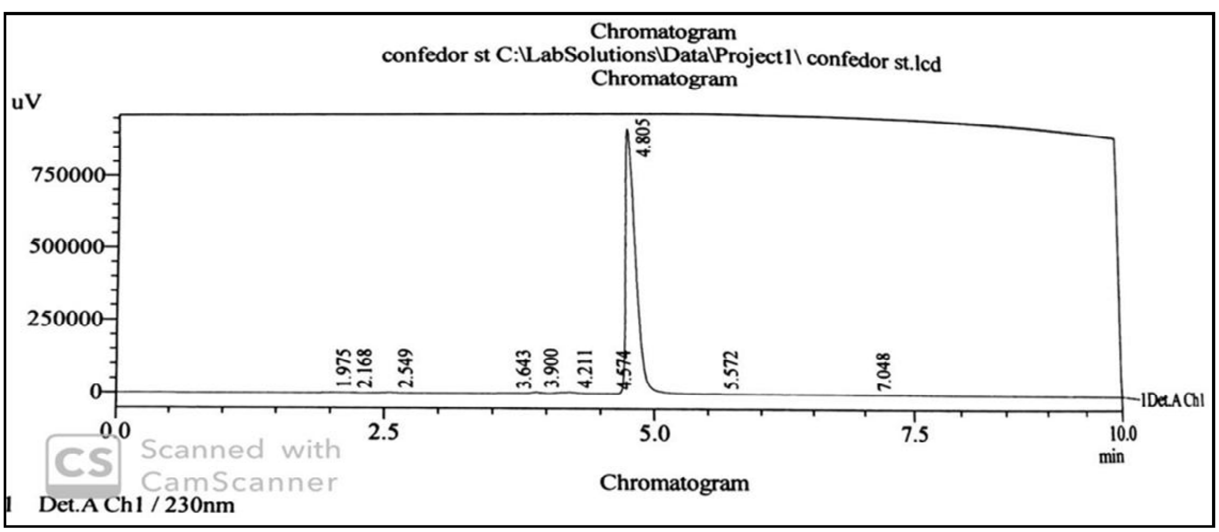

Fig. 2: Imidacloprid / Slandered 4. 805 compared to retention time

out whether the retention time of the standard active ingredient standard is matched with the retention time of the sample of the commercial formulation used in the experiment. The efficacy of the active ingredient in the insecticide sample used in the experiment. The results in Fig. 3 showed the time of retention of the active ingredient for the commercial preparation. The user's hand in the experiment reached 4.779, It was compared to the time of retention of the standard active ingredient Standard as the concentration of 200 mg/kg was taken, and the time of retention of the standard active substance of the insecticide was (2) Imidacloprid/Slandered 4.805 compared to retention time, Imidacloprid Sample (4.778).

The results showed that the active ingredient of the commercial sample was matched with the time of the Imidacloprid retention in pepper fruits. 4.779 and compared with the time of retention of the insecticide in pepper fruit samples, the concentration of 100mg/kg was taken, as the time of retention in pepper fruits four days after spraying 4.778 and the highest peak area of the insecticide, the highest concentrations on the fourth or fifth day after spraying.

Analysis of the insecticide model in the HPLC device to extract retention time was adopted to determine the extent of the retention time in pepper fruit samples with the time of retention of the sample of the insecticide used in the experiment. The results showed that there is a match with the active substance of the imidacloprid, match the time of retention of the insecticide in the fruits of the pepper and that the retention time of the insecticide. The commercial Sample Imidacloprid was taken from the package of the insecticide used in the experiment reached (4.779). It was compared with the retention time of samples taken from pepper sprayed with insecticide and

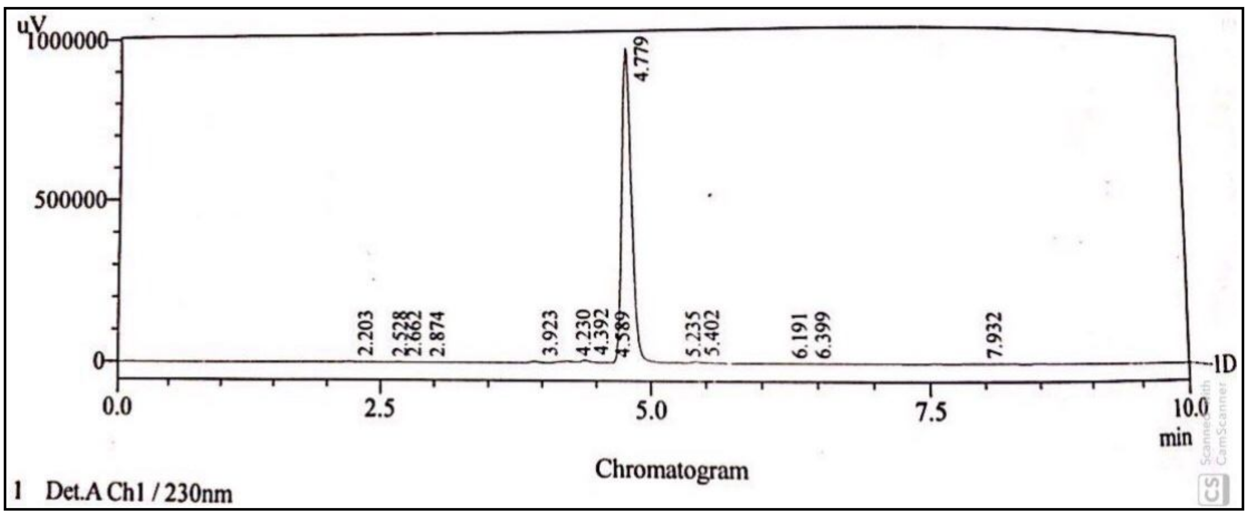

Fig. 3: the Retention time of the Imidacloprid \ sample

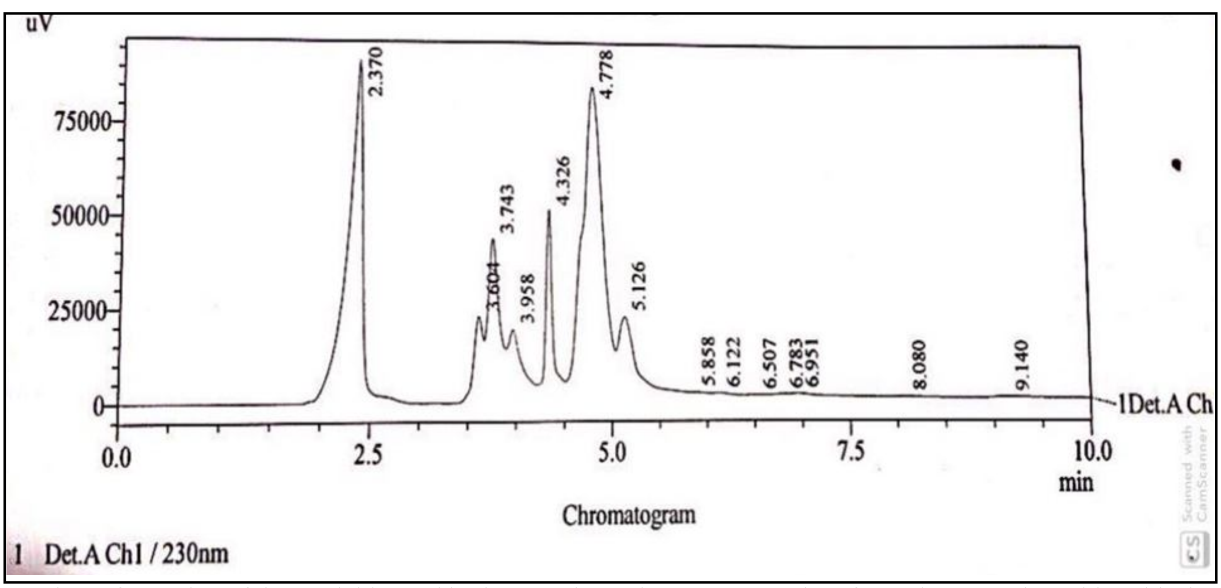

Fig. 4: Time of retention of the pesticide used in the experiment in pepper samples after 4 days spraying with Imidacloprid.

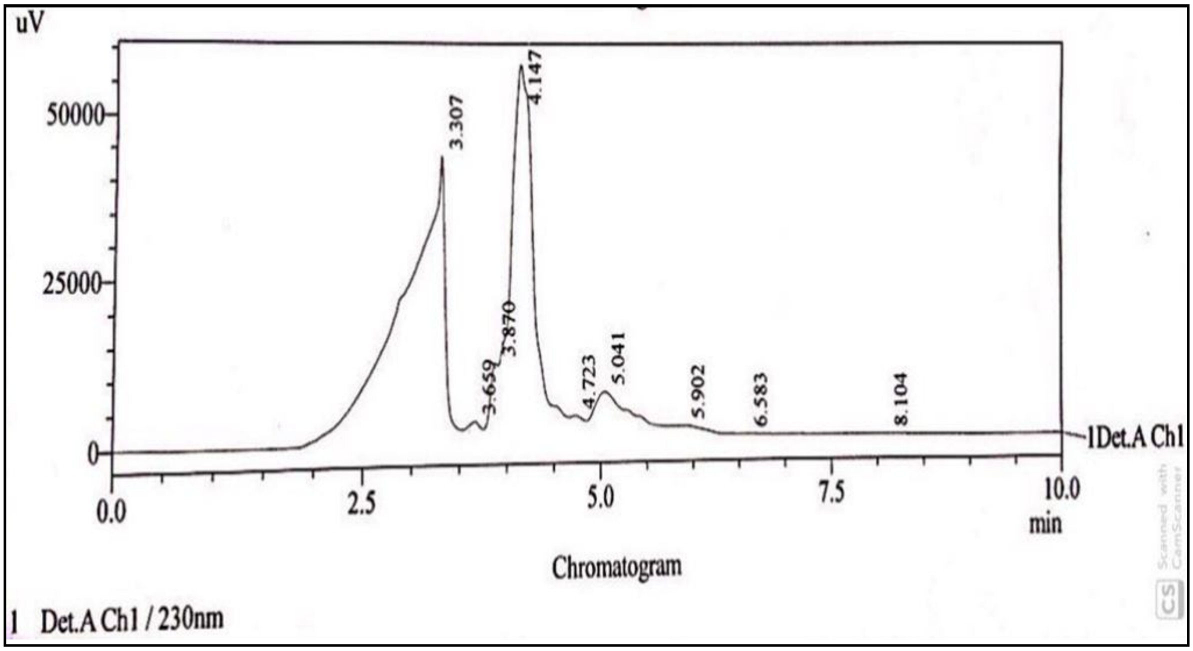

Fig. 5: Time of retention of the pesticide used in the experiment in pepper samples after 8 days spraying with Imidacloprid.

the time of retention in pepper fruits after spraying four days (4.723). A decrease in the height and area of the peak area can be observed, the lowest concentration of the insecticide was on the eighth day after spraying.

A. Imidacloprid Dissipation Curve

The results of the experiment showed Fig. 6 the curve of the fumigation of Imidacloprid in the fruits of pepper after spraying at the recommended concentration during the study period of 28 days. The rise from the first day was 87 mg/kg to reach its highest level after 4 days of spraying and reached (137 mg/kg), then dropped on the fifth day to (102 mg/kg) and reached after 8 days below the permissible limits for the residues of Imidacloprid on the fruits of pepper, as amounted to zero. The pesticide decreased in the samples taken on the ninth, tenth, eleventh, twelfth, thirteenth and fourteenth days as it reached (0.5 , 0.2 , 0.08 , 0.03 , 0.009 , 0.007 mg/kg) respectively, completely finished on the twenty-first and twenty-eighth days and the concentrations were respectively (0.00 and 0.00 mg/kg) of the spray, as no trace of the pesticide was detected in the HPLC. Jodeh *et al.,* (2016) found out that when analyzing pepper fruit samples with the pesticide in HPLC, the Imidacloprid fades really quickly. It reached its highest concentration on the fourth and fifth days and being higher than MRLs, they observed that the limit was deteriorated until it began to decrease significantly on the sixth day until up to the lowest concentration in the eighth or tenth days as it is lower or within the MRLs. But sometimes it may continue to exist at low concentrations in the fruits until the day 14 after spraying and that is attributed to the variation of digestive enzymes and their activity in the analysis and metabolism of the pesticide in the parts of the plant where the presence in fruits less than the rest of its activity and its presence in other parts. The results showed that the amount of the pesticide after an hour of spraying was higher than the permissible limits of the pesticide on pepper and was comparable to the results of other studies where the concentration of the pesticide in option (0.943 mg/kg) (Nasr *et al.,* 2014), The initial concentrations of the pesticide Imidacloprid varied in the option between (1.80-1.586 mg/kg) (Lieli *et al.,* 2016), while the amount of the pesticide Imidacloprid in another experiment was significantly higher than these levels. The remaining

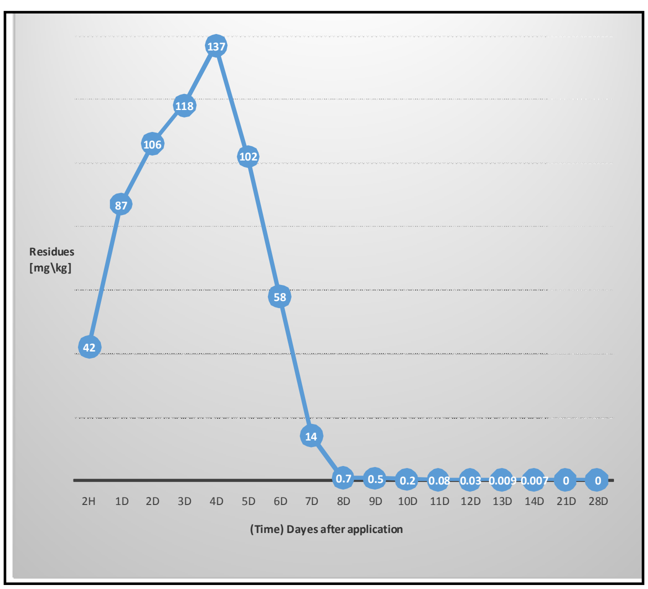

Fig. 6: Imidacloprid dissipation curve

amount was more than (8.4mg/kg) when comparing Imidacloprid with Abamectin in residues and dissipation (Jodeh, 2016). The differences in the effects of Imidacloprid in pepper fruits may be attributed mainly to a number of factors including study conditions, surface-to-mass ratio, the nature of the treated surface, the high wax content of the fruit surface and the difference in balance between lipophilic and hydrophilic substances (Nasr *et al.,* 2014). In a study conducted by Hussein (2019) when studying the residues of the pesticide Imidacloprid on the fruits of cucumbers, it was found that the pesticide may need 10 days to fade to be a safety period of 12 days.

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