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(Original Article)

Dissipation Rates of Certain Insecticides Residues in Eggplant Fruits Grown in Open Field Using QuEChERS Method and HPLC

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Abstract

Pesticides may remain on or in vegetable and fruit crops after application. Due to regular consumption of vegetables and fruits with pesticide residues can pose direct and indirect health concerns, making pesticide residues in vegetable and fruit crops a major global issue. This study investigates the persistence behavior and dissipation rate of chlorpyrifos, emamectin benzoate and imidacloprid in eggplant fruits following application with the label manufactured recommended rate for each pesticide. Samples of eggplant fruits were collected at 0 (1 hr. after spray), 1, 3, 5, 7, 10, and 15 days after treatment. Residues were analyzed using high performance liquid chromatography (HPLC) equipment. Residues of the tested insecticides chlorpyrifos, emamectin benzoate and imidacloprid residues persisted up to the 5th day in eggplant fruits and disappeared by the 7th day after application. No residues were detected in the untreated control fruits. On the first of application, emamectin benzoate and imidacloprid showed the highest rate of dissipation. Then residues of these insecticides declined rapidly in the first three days and then gradually until the fifth day. In contrast, the loss of chlorpyrifos showed only 12.90% dissipation rate on the first day and progressively decreased to 83.87% after five days of treatment.

Keywords: *Chlorpyrifos, Emamectin benzoate, Eggplant, HPLC, Imidacloprid.*

Introduction

Eggplant *(Solanum melongena L.)* is a delicate, tropical perennial plant often cultivated as a tender or half-hardy annual in temperate climates. Eggplant crop is widely cultivated throughout Egypt. An estimated 59,312,600 metric tons of eggplants fruits were produced worldwide in 2022, a 1.0% increase from 58,705,398 tons in 2021, 1.4 million tons of eggplants were produced in Egypt (Anonymous, 2022). Insecticides are widely used in eggplant fruits protection programs. The crop is devastated by weeds, diseases, and insect pest infestations. Whiteflies, aphids, flea beetles and colorado potato beetles are the most traditional insect pests, moreover, spider mites that infest this crop. These pests are serious and cause financial harm; therefore, they need to be managed effectively depending on insecticides.

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More than 1000 pesticides are employed globally to defend crops against various pests. These pesticides are used both before and after harvest to reduce crop loss. Approximately one-third of agricultural products are produced using pesticides (Tudi *et al.,* 2021).

Pesticide residues will be higher in fruits and vegetables that are mostly eaten raw or semi-processed than in food groups derived from plants, such as bread and other foods, according to the World Health Organization (2003) (Claeys *et al*., 2011). Unfortunately, the environment and human health are seriously threatened by the careless use of potentially hazardous pesticides. In order to identify problems associated with chemical control methods, it is advised to monitor insecticide residues on vegetable crops following application and to wait until harvest to ensure that residues are below tolerance levels prior to marketing.

Several insecticides are used to control insect infestation in eggplant fruit crops. Chloropyrifos, emamectin benzoate and imidacloprid are insecticides with a unique chemical configuration. From several years until now, many pests have been controlled with most of them. The primary bottleneck in any analytical process for identifying chemical residues in food products is always sample preparation. The extraction and cleanup processes are made simpler and take less time using the quick, easy, cheap, effective, rugged, and safe (QuEChERS) multi-residue technique. QuEChERS analyses of several kinds of pesticide residues that are frequently used to control eggplant pests based on acetonitrile extraction are described by Angioni *et al.,* (2012); Lehotay *et al.,* (2010) and Allam and Singh, (2016). The methodology's flexibility, high level of selectivity, and sensitivity are its advantages (Anastassiades *et al*., 2003; Lehotay *et al.,* 2005). Since sample throughput is a crucial factor to consider when selecting an analytical method for regular analytical applications, there is currently a growing demand for quick, simple, inexpensive labor, and dependable pesticide analytical methodologies. This challenge can be met and used in agricultural and food production monitoring programs with the QuEChERS sample preparation followed by GC-MS and HPLC chromatography. The aim of this study was conducted to determine the chlorpyrifos, emamectin benzoate and imidacloprid residues as well as the rate of dissipation in eggplant fruits at different days of pre-harvest intervals for safe consumption.

Materials and Methods

1. Chemicals and Reagents

Acetone, hexane, and acetonitrile were among the solvents, and sodium chloride (grade C 99.9% ASC reagent) were purchased from Merck in Darmstadt, Germany. Reagent blanks were performed prior to the actual analysis to guarantee the compatibility of the solvents and other compounds. The certified reference standard of chlorpyrifos, emamectin benzoate and imidacloprid (purity 97.4 %) was supplied by Dr. Ehrenstorfer GmbH, Augsburg, Germany. Only chlorpyrifos, emamectin benzoate, and imidacloprid were detected in the formulation's acetonitrile extract, and neither of their metabolic products nor any interfering peaks were seen during the compound's estimated retention time. Furthermore, it was discovered that the amounts of imidacloprid, emamectin benzoate, and chlorpyrifos were accurate in regard to their purity as reported by the manufacturers.

2. Preparation of standard solution

Standard stock solutions (1mg/ml) each of chlorpyrifos, emamectin benzoate and imidacloprid were prepared in acetonitrile. The range of standard solutions used to plot the calibration curve was (0.10 to 5.00 µg ml^{-1}) for chlorpyrifos and emamectin benzoate (0.01, 1.00, 2.5, 5.00, µg ml^{-1}) for imidacloprid were prepared from stock solutions by serial dilutions with acetonitrile. Prior to use, all standard solutions were kept at -4°C.

3. Instrumentation

Analysis of the chlorpyrifos, emamectin benzoate and imidacloprid were carried out on high performance liquid chromatography (HPLC) 1260 in the lab of plant protection department, Faculty of Agriculture, Al-Azhar University, column Eclipse plus C18 , 4.5 * 250 nm , 5 µm .chlorpyrifos HPLC method was water: acetonitrile (65:35) as mobile phase with flow rate 1ml / min and wavelength 270 nm. Emamectin benzoate method was acetonitrile: water (75:25) as mobiles phase with flow rate 0.6 ml /min and wavelength 254nm. imidacloprid method was acetonitrile: methanol: water (45:40:15) mobile phase with flow rate 1ml /min and 245nm.

4. Method Validation

Three spiked levels for chlorpyrifos, emamectin benzoate and imidacloprid (0. 1, 1 and 10 mg. kg^{-1}) to assess the method's recovery. For quantitative analysis calibration curve concentrations ranged from 5 to 0.1 mg kg^{-1} for each tested pesticide (Fig. 1).

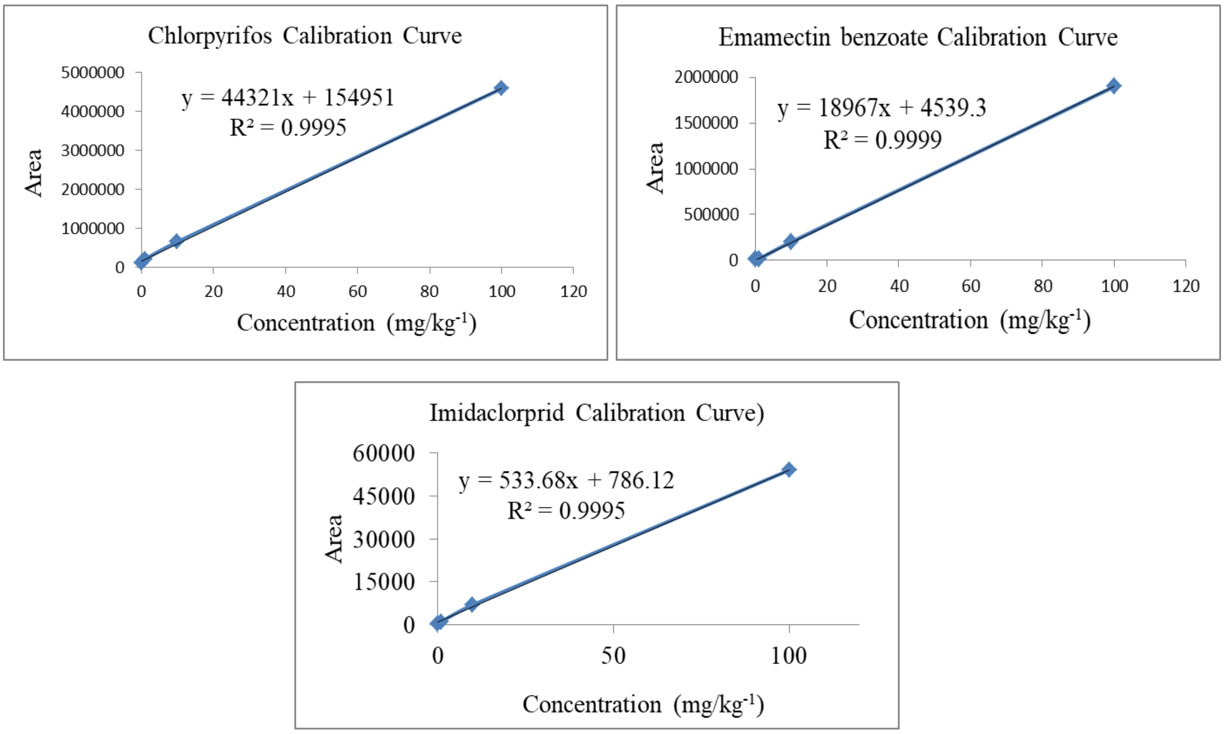

Fig 1. Calibration curves of chlorpyrifos, emamectin benzoate and imidacloprid

5. Field trial

- Crop planting

Eggplant fruits were planted at experimental farm at department of Plant Protection, Faculty of Agricultural, South Valley University, Egypt, according to the recommended agronomic practices and the experiment design was Randomized Complete Block Design (RCBD). Each treatment, including the control, was replicated thrice in separate plots. A buffer area was used to separate different treated plots. The control plots had no history of chlorpyrifos, emamectin benzoate and imidacloprid use. Plots that had not been treated served as a control. A 20-liter backpack sprayer fitted with one nozzle boom was used.

- Application of the insecticide

Chlorpyrifos (Dofos 48% EC) at 50 ml / 100 L water, emamectin benzoate (Robust 50% EC) at 25 ml/ 100 L water and imidacloprid (Avenue 70% WP) at 30g/ 100 L water were applied in the experimental plots. Each insecticide was applied twice: first at the 50% fruit initiation stage and again ten days following the first treatment.

- Sampling

Three replicates' samples (1 kg of the total sample) of treated and untreated samples of eggplant fruits crop were randomly packed up one hour after treatments and then 1, 3, 5, 7, 10 and 15 days after insecticides spraying for residue analysis. After gathering, the marketable fruit samples were promptly taken to the lab in polyethylene pages (kept in a deep freezer at -20 °C for 24 hrs till residue analysis).

- Extraction and cleanup

The QuEChERS method was used to prepare the samples in accordance with (Anastassiades *et al.* 2003). Ten grams of homogenized eggplant fruits sample were weighed into a 50 ml PTFE centrifuge tube, 10 ml of acetonitrile were added, the tube was vigorously hand shaken for 1 minute. Four grams of anhydrous $MgSO_4$ and 1 grams of sodium chloride were added, the tube was hand shaken for 30 seconds. The mixture was then centrifuged at = 5000 rpm for 5 minute. An aliquot of 1.0 ml was transferred into dSPE tubes containing 25 mg PSA and 150 mg $MgSO_4$) for clean-up. After properly capping and vortexing the tubes for 30 seconds, they were centrifuged for 5 minutes at 5000 rpm. The combined eluate was filtered using a 0.22 µm nylon syringe filter the combined elute before being injected into an auto sampler vial.

Results

1. Efficiency of the method

Recovery tests were conducted at various levels in the current studies to determine the validity and dependability of the analytical method as well as the effectiveness of the extraction and cleanup processes. Samples of eggplant fruits from control plots were spiked at levels of 0.1, 1, and 10 mg kg^{-1} for all tested insecticides. These samples were extracted, cleaned, and examined using the previously mentioned methodology. To determine if there was any interference caused by the substrate or reagents, the same procedure was used to process the control samples from untreated plots and reagent blanks.

Table 1. Recovery percentages of cholorpyrifos, emamectin benzoate and imidacloprid in eggplant fruits

Spiking level (mg/kg)	Insecticides					
	Chlorpyrifos		Emamectin benzoate		Imidacloprid	
	Mean recovery ±SD	% RSD	Mean recovery ±SD	RSD%	Mean recovery ±SD	RSD%
0.1	99.00±1.32	1.33	91.46±1.90	2.08	86.10 ±0.92	1.07
1	83.02±2.69	3.24	82.89±7.32	8.83	92.66 ±7.07	7.63
10	93.66±3.64	3.89	89.98±4.46	4.96	84.56 ±7.12	8.42
Average	91.89± 2.55	2.82	88.11 ±4.56	5.29	87.77 ±5.03	5.7

ND: Not detectable; MRL: Maximum Residue Limits; RSD: Relative Standard Deviations; SD: Standard Deviation.

The mean percent recoveries of chlorpyrifos, emamectin benzoate and imidacloprid from eggplant fruits samples at the fortification levels ranged from 83.02 to 99.0%, 82.89 to 91.46 % and 84.56 to 92.66 %, respectively (Table 1). The results have been provided without the use of a correction factor because the average recovery values were determined to be more than 82%.

2. Persistence of chlorpyrifos, emamectin benzoate and imidacloprid in eggplant fruits

The residue data of chlorpyrifos, emamectin benzoate and imidacloprid in eggplant fruits at various intervals of days are shown in (Tables 2, 3, 4). The initial deposits (1 h after spraying) of chlorpyrifos in eggplant fruits samples were found to be 3.1±0.03 mg kg^{-1}. The residues persisted for up to 5 days. The untreated control showed no residue. The residues of emamectin benzoate and imidacloprid in eggplant fruits samples also declined progressively with time. After 5 days, the residues in samples of eggplant fruits fell below the detection level for all pesticides.

Table 2. Dissipation rate of chlorpyrifos on eggplant edible parts

Time after treatment (Days)	Residues ± SD (mg kg^{-1})	RSD%	Dissipation%
0	3.1 ±0.03	0.97	0.00
1	2.7 ±0.04	1.48	12.90
3	1.6 ±0.25	15.62	48.38
5	0.5 ±0.42	84.00	83.87
7	nd	nd	nd
10	nd	nd	nd
15	nd	nd	nd
MRL	0.4 mg kg^{-1} EU (2017)		

ND: Not detectable; MRL: Maximum Residue Limits; RSD: Relative Standard Deviations; SD: Standard Deviation.

Table 3. Dissipation rate of emamectin benzoate on eggplant edible parts

Time after treatment (Days)	Residues ± SD (mg kg^{-1})	RSD%	Dissipation%
0	0.37 ±0.033	8.92	0.00
1	0.21 ±0.026	12.3	43.24
3	0.093 ±0.021	22.6	74.86
5	0.065 ±0.01	15.4	82.43
7	nd	nd	nd
10	nd	nd	nd
15	nd	nd	nd
MRL	0.02 mg kg^{-1} EU (2017)		

ND: Not detectable; MRL: Maximum Residue Limits; RSD: Relative Standard Deviations; SD: Standard Deviation.

Maximum residues of chlorpyrifos, emamectin benzoate and imidacloprid were found to be 3.1 ±0.03, 0.37 ±0.033 and 1.22 ±0.09 mg kg^{-1} in fruits samples collected at 0 day (1 hr after spray). These residues declined to 2.7 ±0.04, 0.21 ±0.026 and 0.41

±0.06 mg kg^{-1} in the samples that were gathered following 1 day of application of chlorpyrifos, emamectin benzoate and imidacloprid, respectively. The residues were further declined to 1.6 ±0.25, 0.093 ±0.021 and 0.26 ±0.11 mg kg^{-1} after 3 days and become 0.5 ±0.42, 0.065 ±0.01 and 0.08 ±0.05 after 5 days of application and below detectable limits mg kg^{-1} after 7 days of application in case of chlorpyrifos, emamectin benzoate and imidacloprid respectively.

Table 4. Dissipation rate of imidacloprid on eggplant edible parts

Time after treatment (Days)	Residues ± SD (mg kg^{-1})	RSD%	Dissipation%
0	1.22 ±0.09	7.3	0.00
1	0.41 ±0.06	14.6	66.39
3	0.26 ±0.11	42.3	78.68
5	0.08 ±0.05	62.5	93.44
7	nd	nd	nd
10	nd	nd	nd
15	nd	nd	nd
MRL	0. 5 mg kg^{-1} EU (2017)		

ND: Not detectable; MRL: Maximum Residue Limits; RSD: Relative Standard Deviations; SD: Standard Deviation.

Fruit samples taken 7 days following the application of imidacloprid, emamectin benzoate and chlorpyrifos, respectively, showed residues below the detection limit (0.01 mg kg-1) (Tables 2, 3, 4). The untreated control fruit samples showed no residue.

3. **Dissipation of pesticides in eggplant fruits.**

The dissipation pattern of chlorpyrifos, emamectin benzoate and imidacloprid in eggplant at various sampling intervals (0, 1, 2, 3, 5, 7, 10, and 15 days) is presented in table (2, 3, and 4). The dissipation rate of imidacloprid and emamectin benzoate per day was highest on the first day, and the concentration of these compounds decreased quickly during the first three days of treatment, then gradually until it was undetectable on the fifth day. On the other hand, the dissipation rate of chlorpyrifos in eggplant fruits was 12.90% on the first day and then gradually decreased until 83.87% loss after five days of application.

Discussion

1. **Persistence of chlorpyrifos, emamectin benzoate and imidacloprid in eggplant fruits**

The aforementioned results showed that higher residues were produced by applying chlorpyrifos, emamectin benzoate, and imidacloprid at higher rate. Similar to the other insecticides, imidacloprid, emamectin benzoate, and chlorpyrifos residues on fruit samples decreased over time, and a relatively high rate of dissipation was noted. The residues of chlorpyrifos, emamectin benzoate and imidacloprid in Eggplant fruits samples declined progressively with time. Beyond 5^{th} day the level of residues were below the determination limit and the residues were no longer detectable (< 0.01 mg kg^{-1}) at the recommended rate.

Nonetheless, the studies agree with that of Momtaz and Khan, (2024) who reported that chlorpyrifos residue was found in 80% of eggplant samples with 65% of samples higher than MRLs. The eggplant samples had the highest residue level, 0.87 mg/kg, which is roughly twice as high as the MRLs. The results are consistent with Mostafa *et* *al.* (2016) determines the residual concentrations of ethion and imidacloprid in Eggplant fruits grown in greenhouse; ethion's and imidacloprid's maximum residue levels (MRLs) exceeded the Codex standard level. The levels of ethion and imidacloprid were reduced by approximately 35 and 31%, respectively; one day after the pesticide was applied. Al-Hawadi *et al*., 2023 examined imidacloprid in 300 vegetable and fruit samples that were acquired from 15 major wholesalers spread over four Amman locations. Notably, the highest average concentrations were found in apples and eggplant (0.40 and 0.25 mg kg^{-1}, respectively).

Vahideh *et. al.,* (2022) examines 56 pesticide residues in 150 cantaloupe and melon samples and 100 greenhouse eggplant fruits that were gathered from Iranian marketplaces using the QuEChERS extraction technology. According to Iranian regulation, at least one pesticide was present in 22% of the cantaloupe and melon samples and 18% of the eggplant fruits. For eggplant fruits, the HQ ranking for pesticides was diazinon > thiacloprid > imidacloprid tebuconazole. Nasr *et. al*., 2009, found that after 1hour, 1,3 and 6 days, the residues and degradation rates of emamectin benzoate in tomato fruit samples were, respectively, 0.22, 0.05, 0.01 and 0.01 ppm. After 10 and 15 days of treatment, no emamectin benzoate was found in the samples. The pre-harvest intervals (PHI) for tomatoes treated with difenoconazole, emamectin benzoate, and fenazaquin were found to be 8, 3, and 1 days, respectively. Shiboob (2001) discovered that the residual half-lives were 1.74, 1.83, and 1.95 days, respectively, and that the acceptable duration for profenofos was 10 days on sweet pepper fruit and 14 days on hot pepper and eggplant fruits. The edible safety period of profenofos was discovered by Mohamed *et. al*., (2004) to be 10 days for sweet pepper and 14 days for hot pepper and eggplant fruits.

2. Dissipation of pesticides in eggplant fruits.

The experimental results showed that the dissipation of pesticides was observed in the first three days after application, gradually declining until the 5th day. These findings are consistent with those of Vinothkumar *et. al.,* (2018), who reported that at the recommended dosage, the residual of emamectin benzoate decreased to 0.209 and 0.091 µg g-1 on days 1 and 3 of treatment, with dissipation percentages of 41.73 % and 74.68 % respectively. Five days following treatment, the residue reached the Below Detectable Limit (BDL) of less than 0.05 µg g^{-1}. The mean residues of emamectin benzoate at twice the recommended dosage were 0.334, 0.130, and 0.056 µg g-1, with loss percentages of 39.29, 76.29, and 89.79 % after 1, 3, and 5 days after spraying, respectively. Seven days after treatment, the residues were below detectable limits. Minghui *Li et al.,* (2011), found that emamectin benzoate had half-lives of 2.04–8.66 days, 2.89–4.95 days, and 3.65–5.78 days in paddy plants, water, and soil, respectively, with a 90% dissipation rate during the 7 days following treatment.

Pre-harvest interval (PHI), half-life time, residues, and rate of dissipation of acetamiprid in tomato fruits were also evaluated by Abdelfatah *et al*., (2020). The results showed that the rates of dissipation were 54.55, 69.09, 87.00, 99.55, and 99.64 % after 1, 3, 5, 7, and 10 days after treatment, respectively. After 15 days of treatment, acetamiprid was not found in tomato fruits. On the other hand, El-Desouki and El-Hefny (2021) studied the dissipation of cyflumetofen in strawberry fruits for 14 days.

According to the results, there was a significant decrease of 45.16% within the first 24 hours of spraying the strawberries. On the second day following spraying, rapid dissipation was observed, with an 82.66% reduction in cyflumetofen residue, reaching 0.43 mg/kg.

Conclusion

In this study, we determined chlorpyrifos, emamectin benzoate, and imidacloprid residues in eggplant fruits using an HPLC analytical approach based on QuEChERS sample preparation procedures. The mean recoveries ranged from 87.77 %, to 91.89 %. For all pesticides, the residues remained detectable for 3 to 5 days following treatment before dissipating to below the detectable limit 7 days later. It is necessary and must not use eggplant fruits before the PHI days off and apply all pesticides safety recommendations. Also, chlorpyrifos is banned used in vegetables, it may be illegally applied.

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دراسة معدلات اختفاء بقايا بعض المبيدات الحشرية في ثمار الباذنجان المزروعة في الحقول المفتوحة باستخدام طريقة QuECHERS، HPLC

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[3]قسم وقاية النبات، كلية الزراعة، جامعة جنوب الوادي، قنا، مصر.

الملخص

قد تبقى المبيدات على أو في محاصيل الخضروات والفواكه بعد التطبيق. ونظرًا لأن الاستهلاك المنتظم للخضروات والفواكه التي تحمل بقايا المبيدات يشكل مخاوف صحية مباشرة وغير مباشرة، فقد كانت بقايا المبيدات في محاصيل الخضروات والفواكه مصدر قلق عالمي كبير. لذلك، هدفت هذه الدراسة إلى معرفة سلوك الاستمرار ومعدل اختفاء ثلاث مبيدات وهي كلوروبيرفوس وإيمامكتين بنزوات وإيميدا كلوربريد في ثمار الباذنجان بعد التطبيق بالجرعة الموصي بها لكل منهما. تم جمع عينات من ثمار الباذنجان في صفر (1 ساعة بعد الرش)، 1، 3، 5، 7، 10، 15 يومًا بعد تطبيق المبيدات. تم تقدير البقايا بواسطة جهاز الكروماتوجرافى السائل العالي الأداء. اوضحت النتائج استمرار وجود بقايا مبيد كلوروبيرفوس ومبيد إيمامكتين بنزوات ومبيد إيميدا كلوربريد حتى اليوم الخامس في ثمار الباذنجان بالمعدل الموصي به، واختفت في اليوم السابع بعد التطبيق. لم يتم الكشف عن أي بقايا في المجموعة الضابطة غير المعالجة. وقد أظهر اليوم الأول أعلى معدل لاختفاء الإيماميكتين بنزوات والإيميدا كلوربريد في يوم واحد. وانخفضت تركيزات هذه المركبات بسرعة خلال الأيام الثلاثة الأولى ثم تدريجيا حتى اليوم الخامس. ومن ناحية أخرى، بلغ نسبة الفقد من مبيد الكلوروبيروفوس 12.90% في اليوم الأول وانخفض تدريجيا إلى 83.87% بعد خمسة أيام من التطبيق.

الكلمات المفتاحية: *ايميداكلوربريد، امامكتين بنزوات، HPLC، الباذنجان، كلوروبيرفوس.*