Journal of Sohag Agriscience (JSAS) 2024, 9(1): 146-152

ISSN 2305-1088
https://jsasj.journals.ekb.eg

JSAS 2024; 9(1): 146-152
Received: 29-06-2024
Accepted: 19-07-2024

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Multi-pesticides Residue Analysis in Cucumber under Greenhouse Conditions using QuEChERS methodology Coupled to GC-MS and HPLC

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Abstract

Pesticides are risk factors that have long-term negative impacts on human health. They are widely employed to advance human pursuits worldwide. In Egypt, government agencies and local farmers frequently utilize insecticides to control pests. Fresh vegetable consumers and their surroundings have been concerned about pesticide residues. Thus, the purpose of this study was determining the residue levels of the pesticides imidacloprid, acetamiprid, malathion, and abamectin in cucumber fruits under greenhouse conditions in Sohag university farm, Egypt. Persistence behavior of imidacloprid, acetamiprid, malathion and abamectin in cucumber fruits was examined following application with the recommended dose for each pesticide. Cucumber fruit samples were collected on 0 (1 hr. after spray), 1, 3, 5, 9, 12 and 15 days after application. Residues were estimated by HPLC and GC-MS. Residues of imidacloprid, acetamiprid and malathion persisted up to the 5^{th} day, while abamectin persisted up to the 1^{st} day in cucumber fruits at recommended dose. There were no residues found in the untreated control. The residues of imidacloprid, acetamiprid, malathion and abamectin in cucumber fruits samples declined progressively with time.

Keywords: QuEChERS, HPLC, GC-MS, residues, imidacloprid, acetamiprid, malathion, abamectin, cucumber.

INTRODUCTION

Cucumber (*Cucumis sativus L.*) is a common creeping vine plant in the Cucurbitaceae family that produces fruits that are typically cylindrical and used as culinary vegetables. Cucumber crops are widely cultivated throughout Egypt. The estimated total world production for cucumbers in 2020 was 91,258,272 metric tons, while Egypt's production was 613,031 metric tons (Anonymous, 2022). Insecticides are widely used in cucumber protection programs. Due to weeds, diseases, and insect pest infestation, the crop can be destroyed. Whiteflies (*Bemisia tabaci* Gennadius) and aphids (*Myzus persicae*) are two insect pests that seriously damage this vegetable and need to be managed effectively. According to the World Health Organization (2003), compared to food groups of plant origin like bread and others, fruits and vegetables that are mostly consumed raw or semi-processed will have higher levels of pesticide residue (Claeys *et al*., 2011). Pesticides are used on cucumber crops to control pest infestation. 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).

Unfortunately, the environment and human health are seriously threatened by the careless use of potentially hazardous pesticides. Insecticide residues on vegetable crops after application should be monitored, and waiting periods between application and harvesting should also be advised to ensure that residues are below tolerance levels before marketing, address these observable issues related to chemical control strategies (Shams EL Din, A. M. *et al*., 2015).

Imidacloprid, acetamiprid, malathion and abamectin are insecticides with a unique chemical configuration and have found a place in the list of chemicals recommended for controlling vegetables insect pests. They exhibit very high activity against a wide range of chewing and sucking pests, particularly, Lepidoptera and coleoptera in public health as a vector control agent. These insecticides have been used for many years to control a variety of arthropod pests on plantation and field crops. The primary obstacle 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 with the QuEChERS multi-residue technique. The results of QuEChERS studies utilizing acetonitrile extraction of various pesticide residue classes that are frequently used to control cucumber pests are presented (Angioni *et al.*, 2012; Lehotay *et al.*, 2010).

This methodology's versatility, 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 take into account when selecting an analytical method for regular analytical applications, there is currently a growing demand for quick, simple, labor-efficient, and reliable pesticide analytical methodologies. The QuEChERS sample preparation followed by GC-MS and HPLC chromatography can meet this challenge and be applied in monitoring programs of agriculture and food production. The aim of this research was to determine the residues of imidacloprid, acetamiprid, malathion and abamectin in cucumber fruits at different days of pre-harvest intervals for safe consumption.

MATERIALS AND METHODS

1. Chemicals and Reagents

Solvents like acetone, hexane and acetonitrile were procured from Merck, Darmstadt, Germany. Sodium chloride (ASC reagent grade C 99.9 %) was also obtained from Merck, Darmstadt, Germany. Before conducting the actual analysis, reagent blanks were performed to verify the compatibility of the solvents and other compounds. The certified reference standard of Imidacloprid, acetamiprid, malathion and abamectin (purity 97.4 %) were supplied by Dr. Ehrenstorfer GmbH, Augsburg, Germany. Only imidacloprid, acetamiprid, malathion, and abamectin were detected in the acetonitrile extract of the formulation, with none of their metabolic products and no interfering peaks under the retention time of the chemical being assessed. Moreover, the concentration of imidacloprid, acetamiprid, malathion and abamectin were found to be accurate with respect to their purity as claimed by the manufacturers.

2. Preparation of standard solution

Standard stock solutions (1mg/ml) of imidacloprid, acetamiprid, malathion and abamectin were prepared in acetonitrile. The stock solutions were serially diluted with acetonitrile to provide the standard solutions used for the calibration curve plotting, which ranged from (5.00 to 0.10 µg ml-1) for imidacloprid, acetamiprid, and malathion, and (5.00, 2.5, 1.00, 0.01 µg ml-1) for abamectin. Prior to use, all standard solutions were kept in storage at -4 °C.

3. Instrumentation

Analysis of imidacloprid, acetamiprid and abamectin were carried out using high performance liquid chromatography (HPLC) 1260, with a column Eclipse plus C18, 4.5 * 250 nm, 5 µm. The HPLC method for imidacloprid used a mobile phase of water: acetonitrile (65:35) with a flow rate of 1ml / min and a wavelength of 270 nm. The acetamiprid method used acetonitrile: water (75:25) as a mobiles phase with flow rate of 0.6 ml /min and a wavelength of 254nm. The abamectin method used acetonitrile: methanol: water (45:40:15) as the mobile phase with a flow rate of 1ml /min and a wavelength of 245nm. Malathion was analyzed by gas chromatography (HP6890 Series GC system) connected to a 5973-mass selective detector (Agilent Technologies, Inc., CA, USA) with the detection system set to the selective ion-monitoring mode (SIM). The ions selected for analysis were 125, 173, and 93 m/z. Sample ionization was achieved by electron impact at 70 k eV. The column used was an HP-5, 5% phenyl methyl siloxane (30 m * 0.25 mm * 0.25 µm). The oven was programmed to start at 120 ºC for 2 min, ramp at 10 ºC/min until 220 ºC for 2 min, ramp at 10 ºC/ min until 260 ºC for 2 min, ramp at 10 ºC/ min until 280 ºC for 2 min. with flow rate of 1.5 µl/min.

Method Validation

Three spiked levels for imidacloprid, acetamiprid and malathion, 1, 0.5 and 0.1 mg kg^{-1} each. abamectin spiked with 4 levels 1, 0.5, 0.1, 0.01 mg kg^{-1}. For quantitative analysis Calibration curve concentrations ranged from 5 to 0.01 mg kg^{-1} for each tested pesticide (Fig. 1).

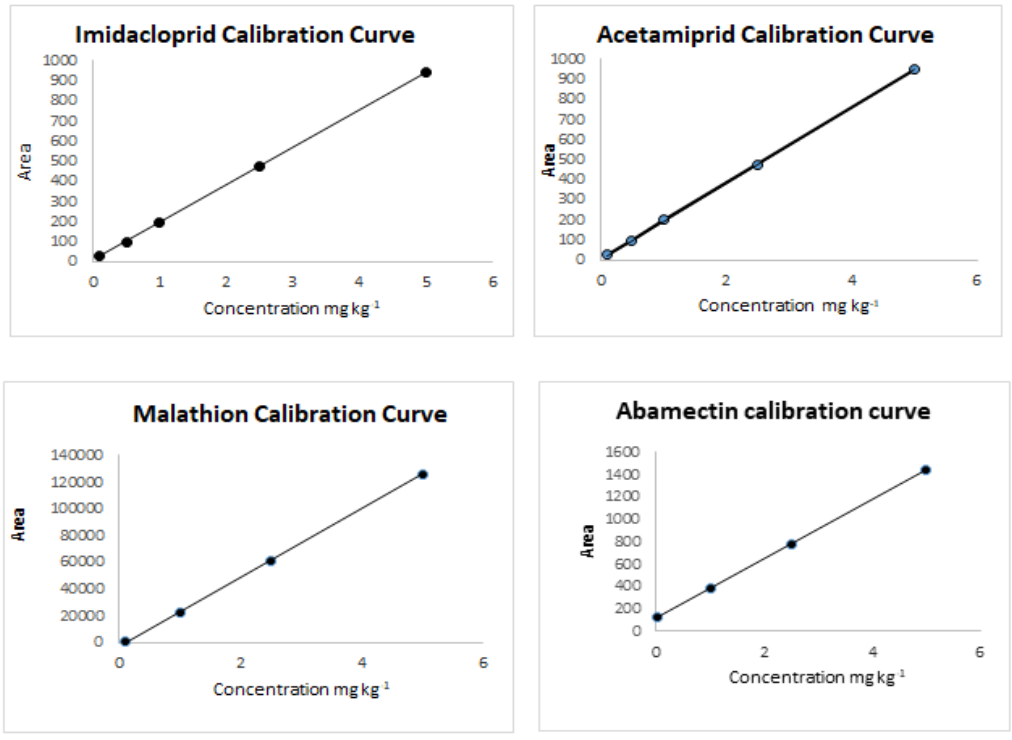

Fig (1) Calibration curves of imidacloprid, acetamiprid, malathion and abamectin

4. Field trials

4.1. Crop planting

In compliance with suggested agronomic techniques, cucumber (variety Sultan) was planted in the experimental farm of the Faculty of Agricultural, Sohag University, Egypt. Three independent plots were used to replicate each treatment, including the control. A buffer area was used to separate the different treated plots. The control plots had no history of imidacloprid, acetamiprid, malathion and abamectin use. Untreated plots were left as control check. A knapsack hand sprayer fitted with a single nozzle boom was used.

4.2. Application of the insecticides

Imidacloprid (Imidachem 35% SC) at 75 ml / 100 L water, acetamiprid (mosiplan 20% SP) at 25 g / 100 L water, malathion (malason/cormandel 57 EC) at 100 ml / 100 L water, and abamectin (vertimec 1.8 EC) at 40 ml / 100 L water, were applied at the recommended dose in the experimental plots. Two applications of each pesticide were sprayed, with the first application at 50 percent fruit initiation stage followed by the second one after 10 days of the first application.

4.3. Sampling

For residue analysis, three replicates of treated and untreated cucumber crop samples were randomly packed up one hour after treatments and then on days 1, 3, 5, 9, 12 and 15 following pesticide spraying. After being collected in polyethylene bages, the marketable size fruit samples were sent directly to the laboratory (kept in a deep freezer at -20 °C for 24 hours until residue analysis).

2.5. Extraction and cleanup

The samples were prepared using the QuEChERS method according to Anastassiades et al. (2003). Ten grams of homogenized cucumber sample were weighed into a 50 mL PTFE centrifuge tube, Ten mL of acetonitrile was added, the tube was vigorously hand shaken for 1 minutes, 4 g of anhydrous MgSO4 and 1 g of sodium chloride were added, and the tube was hand shaken for 30 s. The mixture was then centrifuged at = 5000 rpm for 5 minutes. An aliquot of 1.0 mL was transferred into dSPE tubes containing 25 mg PSA and 150 mg MgSO4) for clean-up. The tubes were capped vortexed for 30 seconds and then centrifuged for 5 minutes at = 5000 rpm. The combined eluate was filtered through a 0.22-µm nylon syringe filter into an auto sampler vial for injection.

RESULTS

1. Efficiency of the method

Recovery tests were conducted at various levels to determine the validity and dependability of the analytical approach as well as the effectiveness of the extraction and cleanup processes. The recovery experiments were carried out in order to evaluate the effectiveness of extraction and cleanup. Cucumber samples and soil samples from control plots were tampered with at a level of 1.00, 0.50 and 0.1 mg kg^{-1} for all tested pesticides except abamectin spiked levels are 1,0.5,0.1,0.01 mg kg^{-1}. These were taken out, cleaned, and examined using the previously mentioned methodology. The same procedure was also applied to the control samples from the untreated plots and the reagent blanks to identify any interferences caused by the substrate or reagents, respectively. The mean percent recoveries of imidacloprid, acetamiprid, malathion and abamectin from cucumber samples at the fortification levels ranged from 91.0 to 100.0%, 85.0 to 100.0%, 100.3 to 102.0% and 89.0 to 101.0%, respectively (Tables 2,3,4 and 5). Since the average recovery values were determined to be greater than 85%, no correction factor was used, and the findings were reported as such.

Table 1: Fortification levels and recovery for imidacloprid, acetamiprid, malathion and abamectin

Fortified Level	Pesticides							
	Imidacloprid		Acetamiprid		Malathion		Abamectin	
mg kg^{-1}	Rec. % (n=6)	RSD %	Rec. % (n=6)	RSD %	Rec. % (n=6)	RSD %	Rec. % (n=6)	RSD %
1	100.0%	13.0%	100.0%	11.8%	100.3%	5.3%	101.0%	6.8%
0.5	90.0%	11.4%	102.0%	4.7%	108.0%	9.5%	99.0%	6.8%
0.1	91.0%	9.5%	85.0%	11.8%	102.0%	10.8%	96.0%	2.5%

2. Persistence of imidacloprid, acetamiprid, malathion and abamectin in cucumber fruits

The residue data of imidacloprid, acetamiprid, malathion and abamectin in cucumber fruits at different day interval are represented in Tables 2, 3, 4 and 5. After applying imidacloprid, acetamiprid, and malathion at the recommended dosage for 5 days, fruit samples were collected. The residues in these samples were found to be below the detection limit (0.01 mg kg-1). When fruit samples were taken 1 day after applying approved dosages of abamectin, the residues were found to be below the determination limit (0.01 mg kg-1). (Tables 2,3,4,5). No residue was detected in the untreated control samples of fruits. The residues persisted up to 5 days. In cucumber fruit samples, the levels of acetamiprid, malathion, and abamectin residues likewise gradually decreased with time. Almost 47.20 % of the initial malathion residues dissipated during just one day of treatment. After 5 days for imidacloprid, acetamiprid, and abamectin, and one day for malathion spraying, the residues in cucumber fruit samples were no longer detectable. The nature of dissipation of malathion in cucumber fruits samples could be explained in terms of first order reaction.

Table 2: Dissipation rate of imidacloprid on cucumber edible parts

Imidacloprid			
Time after treatment(days)	**Residues ±SD mg kg^{-1}**	**Dissipation (%)**	**RSD (%)**
0	0.33±0.021	0	6.25
1	0.22±0.009	33.10	4.21
3	0.04±0.003	87.57	7.33
5	0.02±0.003	91.84	12.84
9	nd	nd	nd
12	nd	nd	nd
15	nd	nd	nd
MRL	1 mg kg^{-1}		

MRL according to Codex. (2014)

Table 3: Dissipation rate of acetamiprid on cucumber edible parts

Acetamiprid			
Time after treatment (days)	**Residues ±SD mg kg^{-1}**	**Dissipation (%)**	**RSD (%)**
0	1.35±0.04	0	2.9
1	0.18±0.010	86.66	5.5
3	0.036±0.003	97.33	10.5
5	0.022±0.003	98.3	15.4
9	nd	nd	nd
12	nd	nd	nd
15	nd	nd	nd
MRL	0.3 mg kg^{-1}		

MRL according to Codex (2016).

Table 4: Dissipation rate of malathion on cucumber edible parts

Malathion			
Time after treatment (days)	**Residues ±SD mg kg^{-1}**	**Dissipation (%)**	**RSD (%)**
0	1.42±0.020	0	2.8
1	0.35±0.010	75.30	5.4
3	0.32±0.010	77.2	9.4
5	0.27±0.010	80.5	11
9	nd	nd	nd
12	nd	nd	nd
15	nd	nd	nd
MRL	0.2 mg kg^{-1}		

MRL according to Codex (2004).

Table 5: Dissipation rate of abamectin on cucumber edible parts

Abamectin			
Time after treatment (days)	**Residues ±SD mg kg^{-1}**	**Dissipation (%)**	**RSD (%)**
0	0.44±0.04	0	8.0
1	0.23±0.010	47.20	4.4
3	nd	nd	nd
5	nd	nd	nd
9	nd	nd	nd
12	nd	nd	nd
15	nd	nd	nd
MRL	0.03 mg kg^{-1}		

MRL according to Codex (2016).

The maximum residues of imidacloprid, acetamiprid, malathion and abamectin were found to be 0.33 ±0.021, 1.35 ±0.04, 1.42 ±0.02 and 0.44 ±0.04 mg kg^{-1} in fruits samples collected at 0 day (1 hr after spray). These residues declined to 0.22 ±0.009, 0.18 ±0.01, 0.35 ±0.01 and 0.23 ±0.01 mg kg^{-1} in the samples collected after 1 day of application of imidacloprid, acetamiprid, malathion and abamectin, respectively. The residues further declined to 0.02 ±0.003, 0.022 ±0.003 and 0.27 ±0.01 mg kg^{-1} after 5 days of application in the case of imidacloprid, acetamiprid and malathion respectively.

DISCUSSION

Persistence of imidacloprid, acetamiprid, malathion and abamectin in cucumber fruits

The results above show that residues of imidacloprid, acetamiprid, malathion, and abamectin on fruit samples decreased over time, similar to other insecticides, and a relatively high rate of dissipation was noted. The residues of imidacloprid, acetamiprid, malathion and abamectin in cucumber fruit samples declined progressively with time. Approximately 33.1, 86.66, 75.30 and 47.20% of the initial residue dissipated after the 1st day of application for imidacloprid, acetamiprid, malathion and abamectin, respectively. By the 5th day, the residual level dropped below the determination limit, and at the prescribed dose treatment, the residues were no longer traceable (< 0.01 mg kg^{-1}).

Nonetheless, the research findings align with those of Jayakrishnan et al. (2005) who revealed that there was no residual issue in fruits when lambda cyhalothrin was applied at the dosages (15 and 30 g ai/ha) that were efficient in controlling tomato fruit borer. Most insecticides suffer rapid loss of surface residues from foliage and fruits in initial stages due to the evaporation of the chemical under the influence of strong wind current, sunlight, high humidity and temperature. Dissipation is also affected by the nature of the chemical and substrate (Ebling, 1963). The results are consistent with Mostafa *et. al.,* (2016), who determined the residual concentrations of ethion and imidacloprid in cucumbers grown in greenhouse, Ethion and imidacloprid had maximum residue levels (MRLs) that exceeded the Codex standard limit. The levels of pesticides were reduced by approximately 31% and 35%, respectively, for imidacloprid and ethion one day after their application. Vahideh, *et.al*., (2022) analyzed 56 pesticide residues in 100 green-house cucumber and 150 cantaloupe and melon samples collected from markets in Iran using the QuEChERS extraction method based on analysis with ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS). Furthermore, using the Monte Carlo Simulation (MCS) method, the Hazard Quotient (HQ), Hazard Index (HI), and Carcinogenic Risk (CR) were used to evaluate probabilistic health risk assessments that were both carcinogenic and non-carcinogenic. According to Iranian regulations, at least one pesticide was found to have contaminated 18% of cucumber samples and 22% of cantaloupe and melon samples. The pesticides were ranked in order of HQ: for cucumbers, diazinon > thiacloprid > imidacloprid tebuconazole. Miguel *et. .,al* (2022) stated that because of its inherent benefits, which include quick sample preparation, low cost, simplicity, and minimal use of hazardous reagents and solvents, the QuEChERS method standing for quick, easy, cheap, effective, rugged, and safe has emerged as one of the most environmentally friendly and sustainable alternatives in this field.

Gu *et. al.,* (2008), reported that, the disintegration and persistence of artificial pyrethroids in the Yangtze River Delta are discussed. Additionally, information is available regarding the persistence and breakdown of synthetic pyrethroids in tropical aquatic environments and soil (Awasthi and Prakash, 1997).

CONCLUSION

The results indicate that in cucumber fruits at recommended doses, residues of imidacloprid, acetamiprid, and malathion lasted until the fifth day, but abamectin lasted until the first day. We mentioned MRL as codex with different levels. On one day, at the indicated dosage, the initial deposits of imidacloprid, acetamiprid, malathion, and abamectin in cucumbers were found to be below the MRL. These findings imply that, if a one-day waiting period is followed, using imidacloprid, acetamiprid, malathion, and abamectin at the lowest effective dosages does not appear to provide any risks to consumers.

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