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Dissipation of Imidacloprid in Greenhouse Cucumbers at Single and Double Dosages Spraying

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ABSTRACT

In this study, residues of imidacloprid (Confidor) were measured in greenhouse cucumbers in Mazandaran Province, Iran. Confidor 200 SL was applied at the recommended rate (30.0 g ai ha^{-1}) and its double (60.0 g ai ha^{-1}). Samples were collected at 1 h to 21 days after application and analyzed to determine the content and dissipation rate of Imidacloprid. . Analysis was carried out by the QuEChERS method using HPLC-UV. The average initial deposits of imidacloprid on the cucumber fruits were found to be 1.93 and 3.65 mg kg^{-1} at the single and double dosages, respectively. Results showed that Imidacloprid was rapidly dissipated in cucumbers following a first order reaction kinetics at both application rates. The amount of dissipation in 21 days was 94.48% and 99.18% for, respectively, the single and double dosages. Residues of imidacloprid dissipated below the maximum residue limit (MRL) of 1 mg kg^{-1} in 3 days. Half-life ($T_{1/2}$) for degradation of imidacloprid in cucumber was observed to be 3.40 and 2.70 days at the single and double dosages, respectively. A waiting period of 3 days is suggested for safe consumption of cucumber. Also, results showed that the dissipation was dependent on the initial application dose and followed a first order rate kinetics.

Keywords: Cucumber, Imidacloprid residue, Pre-harvest interval (PHI), QuECHERS method.

INTRODUCTION

Greenhouse cucumber is considered as a major vegetable crop grown on a large scale in Iran, but, the plant is attacked by many insects, making frequent use of insecticides necessary. To control the various insect pests of greenhouse cucumbers, Imidacloprid is widely used in Iran. Imidacloprid, 1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine (Figure 1), is a systemic chloronicotinyl insecticide with soil, seed, and foliar uses for the control of sucking pests (Elbert *et al*., 1998; Sanyal *et al.*, 2006). The chemical has colourless crystals with weak characteristic odor, melting point of 144˚C, vapor pressure of 4 9 10^{-7} mPa (20˚C), and KOW log *P*= 0.57 (21˚C), and stability to hydrolysis at pH 5–11. It is commonly used on rice, cereals, maize, potatoes, vegetable, sugar beat, fruits, cotton, hops and turfs and is systemic when used as seed or soil treatment. The chemical works by interfering with the transmission of stimuli in the insect nervous system. It causes a blockage in a type of neuronal pathways (nicotinergic) that is more abundant in insects than in warm blooded animals. This blockage leads to accumulation of acetylcholine, an important neurotransmitter, resulting in the insect's paralysis and eventually death (Laurent and Rathahao, 2003; Arora *et al*., 2009). A number of researchers have reported residues of imidacloprid in different agriculture crops substrate (Ishii *et al*., 1994; Anonymous, 2005; Alsayeda *et al*., 2008; Gupta *et al*., 2008). As considerable concern is being expressed by various agencies over the magnitude of pesticides left in foodstuffs following their use

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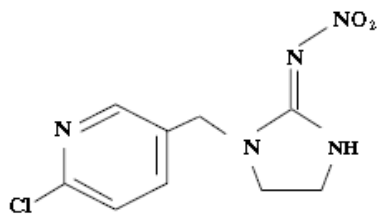

Figure 1. Structure of imidacloprid.

while raising the crops, it is important to ensure that the levels of residues of imidacloprid in cucumbers at harvest time do not pose any hazard to consumers and are admissible in domestic as well as international trade.

Therefore, the present study was undertaken to evaluate the residues of imidacloprid in cucumber following its applications at the single- and double-doses of the recommended rates. In this study, the QuEChERS method was applied using a high performance liquid chromatography-UV detection system for the determination of the residue levels of imidacloprid in cucumbers., Various analytical methods used in neonicotinoid pesticides residues analysis have been reported by different researchers (Ferrer *et al*., 2005; Omirou *et al*., 2009).

Data were obtained to cover a range of pre-harvest intervals (PHIs) 21 days and results were compared with the Codex Maximum Residues Limits (MRLs) established for this insecticide in cucumbers. The purpose of this study was to investigate the dissipation and determination of half-life of imidacloprid residues at different time intervals in cucumbers.

MATERIAL AND METHODS

Preparation of Commercial Pesticide and Application in Greenhouse

Imidacloprid (Confidor 200 SL) was applied at the single- and double-dose of the recommended rate i.e. 30 and 60 g ai ha^{-1}, respectively. The pesticide was applied as high volume spray using Aspee Hand Knapsack Sprayer (capacity 15 l). The first application was made at 50% flowering followed by the second and third applications at 10 days interval. Spray volume used ranged from 75 to 150 l ha^{-1}, depending upon the growth stage of the crop.

Sample Collection

Cucumber crops were raised in a commercial greenhouse located in Mazandaran, Iran. Cucumber samples were collected after a 1.5 month growth period. The row spacing was 40 cm and the plant spacing was 20 cm. The absence of residual pesticides on the samples was confirmed by residue analysis prior to the application of commercial pesticides.

During the experiment, a control sample was taken in each sampling time. The greenhouse relative humidity was 28% with a temperature of 23°C. The maximum, minimum, and average humidity during the experiment were 38%, 21% and 28%, respectively, while the maximum, minimum, and average temperature were 32°C, 14°C and 22°C, respectively. There were three replications for each treatment, including the control, arranged randomly. Control plots were sprayed with water only. Mature samples of cucumber were randomly taken from each treatment plot before and 0 (1 hour after spraying), 1, 3, 5, 7, 10, 13, 17 and 21 days after the third application of the insecticide. About five to six marketable size fruits of cucumber were collected at random from each plot and were wrapped in aluminum foil and placed in polyethylene bags, then, they were transferred to the laboratory and analyzed immediately.

Chemicals and Reagents

Pesticide analytical standard of imidacloprid was purchased from Dr. Ehrenstorfer Inc. with the purity certified by Augsburg, Germany. Individual pesticide stock solutions (1000 µg ml^{-1}) were prepared in pure acetonitrile (MeCN) and stored at

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4°C. Intermediate and working standard solutions of imidacloprid was prepared in acetonitrile. Calibration solutions were prepared with different concentrations just before use. HPLC grade acetonitrile and analytical grade anhydrous magnesium sulfate ($MgSO_4$) and sodium chloride (NaCl) were purchased from Merck (Darmstadt, Germany). Graphitized carbon black (GCB, 400 meshes) was obtained from Supelco. All glassware was rinsed with high purity acetone before use.

Extraction Procedure

The QuEchERS method (acronym for "quick, easy, cheap, effective, rugged, and safe) was used for the extraction of imidacloprid from the cucumber samples. This method was carried out as described by other worker (Dong Nguyen *et al*., 2008). First, the sample (1.0 kg of cucumber) was chopped and homogenized for 5 minutes at high speed in a laboratory homogenizer. Later, 10.0 g of homogenized sample was placed into a 50 ml centrifuge tube with 10 ml of acetonitrile. The screw cap was closed and the tube was shaken vigorously for 1 minute by hand, ensuring that the solvent interacted well with the entire sample. Then, 4.0 g of anhydrous $MgSO_4$ and 1.0 g of NaCl was added repeating the shaking process for 1 minute to prevent coagulation of $MgSO_4$. After centrifuging, the upper layer was cleaned by dispersive solid-phase extraction with 0.5 g of Graphite Carbon Black (GCB) and 1.5 g of anhydrous $MgSO_4$. The mixture was then shaken for 1 minute and centrifuged for 5 minutes at 4,000 rpm. The cleaned extract sample was concentrated to 1.0 ml with a gentle stream of nitrogen and, then, 20 µl of this solution were injected into HPLC.

HPLC Instrumentation

The residues of imidacloprid were determined using Shimadzu HPLC (Shimadzu 10 Ac VP, Kyoto, Japan) equipped with UV detector, dual pump, degasser system, fraction collector FRC-10A and C18 column (150 mm length, 3.9 mm i.d., 5µm particle size). Acetonitrile–water (60:40, v/v) was selected as mobile phase at a flow rate of 1.0 ml min^{-1} using a UV wavelength of 280 nm. Retention time of imidacloprid under these conditions was observed to be 5.01 minutes. Identification of imidacloprid residues were accomplished by retention time (t_R) and compared with a known standard at the same conditions. The quantities were calculated on peak area basis. The injection volume of 20 µl was used in all experiments. The chromatographic apparatus was controlled by LC solution software.

Method Validation

Recovery experiments were carried out by spiking cucumber at different levels to establish the reliability and validity of analytical method adopted. Calibration curves and cucumber fortified samples were prepared by using working standard solutions. Cucumber samples fortified at 0.25, 0.50 and 1.0 mg kg^{-1} were processed as previously described and analyzed by HPLC to evaluate the accuracy and the precision of the analytical procedure. Recovery tests were replicated three times for each fortification level. The limit of detection (LOD mg kg^{-1}) of each analyte was determined as the lowest concentration giving a response three times the standard deviation of the baseline noise defined based on the analysis of three control samples. The limit of quantification (LOQ mg kg^{-1}) was determined as the lowest concentration of a given compound giving a response that could be quantified with relative standard deviation lower than 20% (Vryzas and Papadopoulou-Mourkidou, 2002).

RESULTS AND DISCUSSION

Method Performance

The analytical method was developed so as to provide a rapid, accurate, and efficient

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means of determining imidacloprid residues in cucumbers. Mean recovery value obtained for imidacloprid was 104% with relative standard deviation (RSD) values below 15% in the fortification range from 0.25 to 1 mg kg^{-1}. Calibration for quantification was carried out by use of external standard calibration curves; calibration curves were linear with correlation coefficients being better than 0.999 for both analytes. Retention time of imidacloprid under these conditions was observed to be 5.01 minutes. The LOD and LOQ for both analytes in the cucumbers fruits were 0.01 and 0.001 mg kg^{-1}, respectively, ensuring LOQ values significantly lower than the MRLs established by the Codex. Sample chromatogram of imidacloprid from the analysis of cucumber samples is shown in Figure 2.

Imidacloprid Residue Levels in Fresh Harvested Cucumbers

The results of imidacloprid residue analyses and the percent dissipation at different intervals at single and double dosages are presented in Tables 1 and 2. Following three applications at 1 hour intervals, the mean initial amounts of imidacloprid in cucumber fruits were found to be 1.93 and 3.65 mg kg^{-1} for 30 and 60 g ai ha^{-1}, respectively. The imidacloprid residues were dissipated by more than 70% and 90% in, respectively, 7 and 13 days at the recommended dosage. Residue levels of imidacloprid in samples that were collected after the application of the pesticides

Table 1. Residues of imidacloprid in cucumber following its application at 30 g ai ha^{-1}.

Days after treatment	Residue level (mg kg^{-1})		Dissipation (%)
	Replication	Mean±SD	
Before application	BDL[a]		
	BDL	BDL	-
	BDL		
0 (1 hour after spray)	1.90	1.93±0.21	-
	2.10		
	1.80		
1	1.35	1.31±0.15	32.24
	1.21		
	1.36		
3	0.90	1.00±0.15	48.28
	1.00		
	1.10		
5	0.70	0.77±0.12	60.34
	1.00		
	0.60		
7	0.62	0.51±0.08	72.07
	0.41		
	0.59		
10	0.32	0.27±0.01	86.03
	0.28		
	0.21		
13	0.09	0.11±0.01	94.48
	0.14		
	0.11		
17	BDL	BDL	-
	BDL		
	BDL		
21	BDL	BDL	-
	BDL		
	BDL		

[a] Below Detection Limit (< 0.01 mg kg^{-1}).

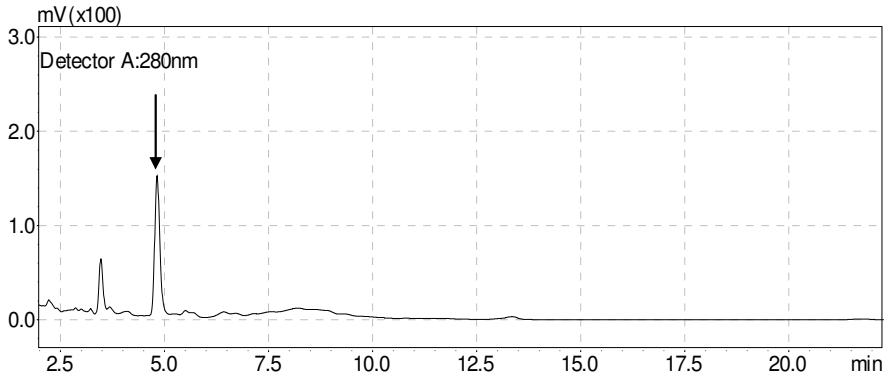

Figure 2. Sample chromatogram of imidacloprid in cucumber samples.

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Table 2. Residues of imidacloprid in cucumber following its application at 60 g ai ha^{-1}.

Days after treatment	Residue level (mg kg^{-1})		Dissipation (%)
	Replication	Mean±SD	
Before application	BDL [a]		
	BDL	BDL	-
	BDL		
0 (1 h after spray)	3.59	3.65±0.22	-
	3.79		
	3.59		
1	2.41	2.54±0.18	30.41
	2.57		
	2.69		
3	1.81	1.89±0.11	48.22
	1.95		
	1.91		
5	1.43	1.41±0.08	61.37
	1.39		
	1.42		
7	0.97	1.01±0.08	72.33
	0.98		
	1.10		
10	0.46	0.49±0.05	86.58
	0.51		
	0.50		
13	0.21	0.21±0.02	94.25
	0.23		
	0.21		
17	0.03	0.03±0.01	99.18
	0.03		
	0.02		
21	BDL	BDL	-
	BDL		
	BDL		

[a] Below Detection Limit (< 0.01 mg kg^{-1}).

throughout a period of 21 days showed a gradual and significant ($P< 0.05$) decrease in content. These results agree with the findings of other researchers regarding dissipation rate of imidacloprid in different agriculture crops substrate (Shim *et al.*, 2009; Arora *et al.*, 2009; Omirou *et al.*, 2009).

The data revealed that there is a rapid loss of this pesticide from the first few hours/days after application to the end of the periodic interval, because the pesticide residues are rapidly lost from plant surfaces by volatilization or some other process (Elkines, 1989). Similar initial rapid losses have been reported for many insecticides (Kumar *et al.*, 2000; Hassanzadeh *et al.*, 2010). Imidacloprid residue concentrations in cucumber fruits obtained in the dissipation study with the corresponding first-order decay fits are presented in Figure 3. Half-life of imidacloprid in different matrices was calculated using the first order rate equation:

$C_t = C_0 e^{-kt}$

Where, C_t represents the concentration of the pesticide residue at time t, C_0 represents the initial concentration, and k is the rate constant per day. The half life ($t_{1/2}$) was determined from the k value for each experiment, where $t_{1/2}= ln2/k$. The fitness of the data to first order kinetics was confirmed by testing the statistical significance of correlation coefficient. The degradation kinetics of this insecticide deposit was well described by first-order decay equation, ($C(t)= 1.9192\times e^{-0.2068\times t}$, $r^2= 0.978$) and ($C(t)= 4.4124\times e^{-0.2578\times t}$, $r^2= 0.950$) for Imidacloprid at single and double dosages, respectively. According to our experimental results, the half-lives ($T_{1/2}$) of imidacloprid are 3.4 and 2.7 days if applied on cucumber fruits. The half-life of imidacloprid was calculated according to Juraske *et al*. (2007) method.

Many researchers have calculated the half-lives of imidacloprid in different fruits (Gupta *et al*., 2008; Arora *et al*., 2009). These studies indicated that residues of imidacloprid on different fruits were rapidly lost in 2 and 4.3 days after application at the recommended and used dosage. Also, Sanyal *et al.* (2006) reported the half lives of imidacloprid in CTC tea in the range of 0.91–1.16 days. The study revealed that the dissipation rate was dependent on the initial application dose and the half-life ($t_{½}$) values of imidacloprid in cucumbers.

The theoretical dissipation models established through regression between time after spray application and the corresponding residues in cucumber, correlation coefficient, and half-lives are presented in Table 3. The dissipation rate constants of 0.2068 and 0.2578 day^{-1},

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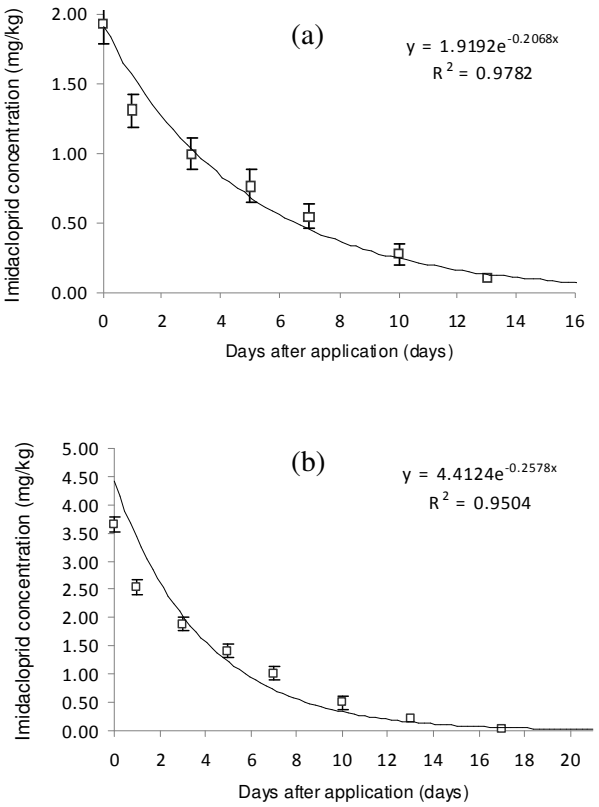

Figure 3. Dissipation of imidacloprid from cucumber following its application at 30 g ai ha^{-1} (a) and 60g ai ha^{-1}(b).

Table 3. Theoretical dissipation models for Imidacloprid in cucumber after foliar treatment.

Treatment (g ai kg^{-1})	Correlation coefficient	Dissipation model	Half-life (Days)
30	-0.9782	*Y=1.9192-0.2068X*	3.40
60	-0.9504	*Y=4.4124-0.2578X*	2.70

corresponding to half-lives of 3.40 and 2.70 days, suggested that the dissipation was dependent on initial dose of imidacloprid and followed a first order rate kinetics. These results are consistent with those of Omirou *et al*. (2009).

Codex maximum residue limit (MRL) of imidacloprid for cucumber has been established as 1 mg kg^{-1} (Anonymous, 2004). Residues of imidacloprid in cucumber fruits were less than the MRL value after 3 days of its application at the recommended dosage. Consequently, a waiting period of 3 days is suggested for safe consumption of cucumber. The use of pesticides on food crops leads to unwanted residues, which may constitute barriers to exporters and domestic consumptions when they exceed MRLs. Gonzalez (2004) studied dissipation of abamectin, spinosad, imidacloprid and thiacloprid on horticultural crops following FAO guidelines and local good agricultural practices and reported negligible residues in all instances. Further, it was also revealed that non-detection residue level was achieved within about 30 days after the last spray. In the cucumber samples collected in the greenhouse, imidacloprid residues higher than their MRL values were observed in initial days. When the pre-harvest intervals between pesticide applications and crop harvest are not

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observed by the farmers, the risk of having higher pesticide levels is likely to increase. In this case, the higher levels of pesticide residues can cause considerable consumer health risks and environmental pollution. Therefore, it is suggested that a waiting period of 3 days should be observed before consumption of cucumber fruits, as it will be safe for the consumers' health. In the light of the above results, it is clear that the advantages of applying pesticides in agriculture for producing better crops must be weighed against the possible health hazard arising from the toxic pesticide residues in food. Pesticides should be applied correctly, according to good agricultural practice, using only the required amounts.

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بررسی نرخ کاهش حشره کش ایمیداکلوپرید در خیار گلخانه ای در شرایط سمپاشی معمول و مضاعف

ن. حسن زاده، ع. اسماعیلی ساری، و ن. بهرامی فر

چکیده

در این مطالعه باقیمانده حشره کش ایمیداکلوپرید (کونفیدور) در خیار گلخانه ای در مازندران بررسی شد. در ایتدا کونفیدور محلول ۲۰۰ با دو غلظت معمول و مضاعف ۳۰ و ۶۰ گرم ماده موثر در هکتار در گلخانه ها سمپاشی شد .نمونه ها از یک ساعت پس از سمپاشی تا ۲۱ روز بعد از سمپاشی به منظور بررسی میزان و مقدار کاهش کونفیدور برداشت شدند و سپس با استفاده از روش QuEChERSو با استفاده از دستگاه کروماتوگرافی مایع با کارآیی بالا آماده سازی شدند. میانگین غلظت باقیمانده کونفیدور در میوه خیار در شرایط سمپاشی معمول و مضاعف به ترتیب ۱/۹۳ و ۳/۶۵ میلی گرم بر کیلو گرم تعیین شد. نتایج نشان داد که کونفیدور در دوره ۲۱ روزه پس از سمپاشی بسیار سریع کاهش می¬یابد. میزان کاهش کونفیدور در نمونه ها در شرایط سمپاشی معمول و مضاعف به ترتیب به میزان ۹۴/۴۸ و ۹۹/۱۸ درصد تعیین شد. همچنین باقیمانده کونفیدور در روز سوم بعد از سمپاشی به میزان کمتر از استاندارد مجاز یعنی ۱ میلی گرم بر کیلوگرم رسید .در این تحقیق نیمه عمر کونفیدور به مقدار ۳/۴۰ و ۲/۷۰ روز در شرایط سمپاشی معمول و مضاعف تعیین شد. با توجه به غلظت مجاز باقیمانده کونفیدور در خیار نتایج این تحقیق نشان داد که رعایت یک دوره زمانی۳ روزه پس از سمپاشی برای برداشت خیار برای ایمنی مصرف کنندگان ضروری است و همچنین نتایج ارائه شده نشان داد که میزان کاهش بقایای کونفیدور از خیار به میزان غلظت سمپاشی بستگی دارد.

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