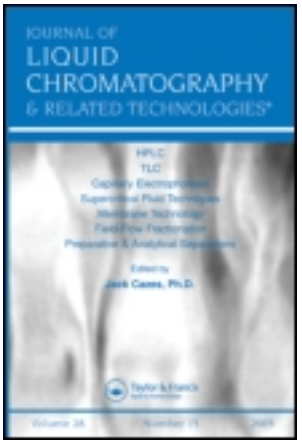

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DETERMINATION OF IMIDACLOPRID RESIDUES IN MULBERRY LEAVES BY QuEChERS AND LIQUID CHROMATOGRAPHY WITH DIODE ARRAY DETECTION

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□ *A simple and sensitive QuEChERS method for the determination of imidacloprid residues in mulberry leaf was developed using high performance liquid chromatography (HPLC). This method was then evaluated for the residual level and dissipation rate of imidacloprid in mulberry leaves. A maximum initial deposits of imidacloprid on mulberry leaves was found to be 3.047 $\mu g\,g^{-1}$ and 6.490 $\mu g\,g^{-1}$ following two application rates of imidacloprid 17.8 SL at 0.6 and 1.2 mL L^{-1}. The calculated half-life of imidacloprid in mulberry leaves was at recommended dose 3.81 d with a dissipation rate of 76% over 7 d; whereas, in double, the dose was 4.93 days with a dissipation rate of 84% over 10 d.*

Keywords dissipation, HPLC, imidacloprid, mulberry leaves, QuEChERS, residue

INTRODUCTION

India is the second largest producer of silk in the world next to China. Mulberry (*Morus* sps) is an important plant in the economy because silk production depends on the quality of the leaves. It is a perennial, evergreen, luxuriant crop cultivated in all types of soils, both under rain-fed and irrigated conditions.[1] The production of mulberry plants is hampered by pests and disease attacks. Recently, the papaya mealy bug (*Paracoccus marginatus*) has been found to attack the mulberry (*Morus* sps) crop,[2] the sole food plant for mulberry silkworm, *Bombyx mori* L. The use of chemical pesticides such as profenofos, imidacloprid, acephate, dimethoate, and dichlorovos have been found to provide effective control of mealy bugs

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on mulberry plants.[2] However, the remaining residue creates a problem of residual toxicity in the treated plants which reduced the palatability of the leaves by silkworm larvae, thereby reducing the growth of larvae and in turn silk production.

Imidacloprid, N-[1-[(6-chloro-3-pyridyl) methyl]-4,5-dihydroimidazol-2-yl] nitramide is a broad spectrum systemic chloronicotinyl insecticide effective against a wide range of pests.[3] It acts by disrupting nicotinic acetylcholine receptors in the insect central nervous system. It is used for controlling sucking insects, soil insects, and some chewing insects.[4] Therefore, it is essential to know the extent to which the pesticide residue remains on mulberry leaves after application. QuEChERS has been applied with success on several food matrices such as fruits and vegetables,[5] and low-fatty (2–20%) food matrices.[6] It has also been used around the world in numerous studies for pesticide residue analysis in different matrix samples.[7–10] On the other hand, studies on the determination of imidacloprid residues in mulberry leaves have not been published.

In this article, a simple analytical method for the determination of imidacloprid residues in mulberry leaves by employing the QuEChERS method and high performance liquid chromatography using diode array detection (HPLC-DAD) is described. In order to provide a scientific basis for setting up a safe harvesting or plucking of mulberry leaves to feed the silkworm larvae, a research program was conducted to study the residues and degradation dynamics of imidacloprid in mulberry leaves.

EXPERIMENTAL

Analytical reference standard of imidacloprid (99.9% purity) were purchased from Sigma-Aldrich, and Imidaclopird (17.8 SL) formulation was purchased from the local market, Coimbatore, (Tamil Nadu). All the other chemicals and solvents were from E. Merck, Mumbai, (India) and were of analytical grade and the Primary secondary amine (PSA, 40 μm, Bondesil) was purchased from Varian (India).

The stock solution of the analyte was prepared by diluting 25 mg of the standard in 25 mL of acetonitrile to obtain a concentration of 1000 μg/mL. The working standard solutions were prepared by diluting the stock solutions as required. Fortification levels and calibration solutions for HPLC analysis were prepared by diluting the stock solution using the mobile phase (acetonitrile:water, 20:80, *v*/*v*), and the concentrations ranged from 0.01 to 1 $\mu g\,mL^{-1}$. Standard solutions used for fortification and calibration were stored at −4°C.

Analysis of imidacloprid residues in mulberry leaves was carried out using a High Performance Liquid Chromatography (HPLC) with the following operating parameters: Shimadzu-LC 20AT with PDA detector fitted with

reversed-phase Chromolith Performance column, 100 × 4.6 mm (RP-18c) at ambient temperature; Labsolution software system was used throughout the experiment to acquire and process chromatographic data. The mobile phase was acetonitrile: water (20:80, *v*/*v*), with a flow rate of 1 mL min^{-1}, and a wavelength of 272 nm. When the aforementioned parameters were followed, the imidacloprid was eluted at 9.75 min (Figure 1).

The field trial was conducted in a randomized block design (RBD) at the experimental farm of Tamil Nadu Agricultural University, Coimbatore. Two treatment and one untreated plots in triplicate were laid out. The commercial formulation of imidacloprid 17.8 SL at the recommended dose (0.6 mL L^{-1}) and double the dose (1.2 mL L^{-1}) was manually sprayed onto mulberry plants with a knapsack sprayer. Random samples of approximately 500 g leaves were plugged, leaving the border plant at each sampling date, at namely, 0 (2 hrs), 1, 3, 5, 7, 10, 15, 22, and 30 d after application of the insecticide. Samples were immediately chopped, homogenized, and stored at −4°C until analysis. Imidacloprid residues were determined by the analytical protocol described in the following section, and their log values over times (days after application) were subjected to weighted linear regression. The half-life (time in days required to reduce the residue to half of initial deposits) was calculated from the regression equation.

The homogenized mulberry leaf sample (5 g) was placed in a 50-mL screw capped polypropylene centrifuge tube and 20 mL of acetonitrile was

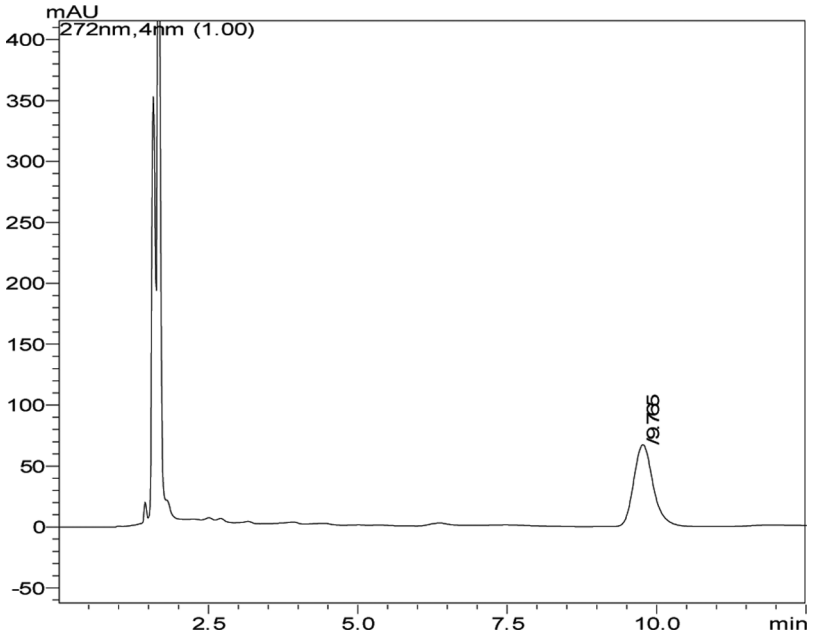

FIGURE 1 HPLC chromatogram of imidacloprid standard 0.1 μg g^{-1}.

added. The samples were shaken for 2 min. The samples were homogenized for 2 min (2000 rpm) using a silent crusher. Afterwards, 2 g of anhydrous magnesium sulfate and sodium chloride (0.5 g) were added to the tube and contents were shaken vigorously to prevent the agglomeration of anhydrous magnesium sulfate. The samples were vortexed for 2 min and centrifuged at 10,000 rpm for 10 min. The supernatant (6 mL) aliquot was transferred into a small centrifuge tube containing prefilled 25-mg PSA sorbent and 150-mg anhydrous magnesium sulfate and shaken for 30 s. After that, the sample was centrifuged at 5000 rpm for 5 min to separate solids from solution. The supernatant extract (4 mL) was concentrated by purging with a gentle stream of nitrogen (Caliper Life Sciences, Russelsheim, Germany) set at 40°C and 15 psi. The final volume was made up with acetonitrile:water (1:1, *v*/*v*) for HPLC analysis.

Calibration data, precision, accuracy, and the limits of detection and quantification were calculated for this study to validate the methodology. The recovery rate was determined by spiking appropriate standard solutions into untreated mulberry leaf samples (5 g homogenized sample) at three different concentrations (0.01, 0.05, and 0.1 $\mu g\,g^{-1}$). The spiked samples were then equilibrated for 30 min and the residue was determined by the aforementioned method. After determination, the recovery of imidacloprid from mulberry leaves was calculated for each concentration and replication.

RESULTS AND DISCUSSION

A method was optimized for the determination of imidacloprid in mulberry leaves by HPLC-PDA. Liquid chromatographic analysis of imidacloprid was performed on octadecyl reversed-phase. It was found that acetonitrile–water isocratic elution as a mobile phase; a Chromolith Performance column, 100 × 4.6 mm (RP-18c), at 1.0 mL min^{-1}; and the detection at 272 nm was shown to be the best condition with respect to the analysis of imidacloprid. Figure 2 shows chromatograms of the control sample of mulberry leaves and the sample fortified with standard solutions of imidacloprid at 0.1 $mg\,mL^{-1}$, respectively. There are few peaks in the chromatogram of the control sample, but retention times of these endogenous compounds were not matched their with imidacloprid peaks; as a result, they did not interfere with the determination of the imidacloprid. Their identification was realized by the retention time obtained when the standard solution of this pesticide was injected into the liquid chromatograph (Figure 1). The acetonitrile was selected for the experiments because it provided higher or acceptable recoveries for imidacloprid.[3,10]

To determine extraction efficiency, untreated mulberry leaf samples were fortified at three levels, 0.01, 0.05, and 0.1 $mg\,mL^{-1}$; the mean recoveries of fortified mulberry leaf samples ranged from 90% to 101%, which

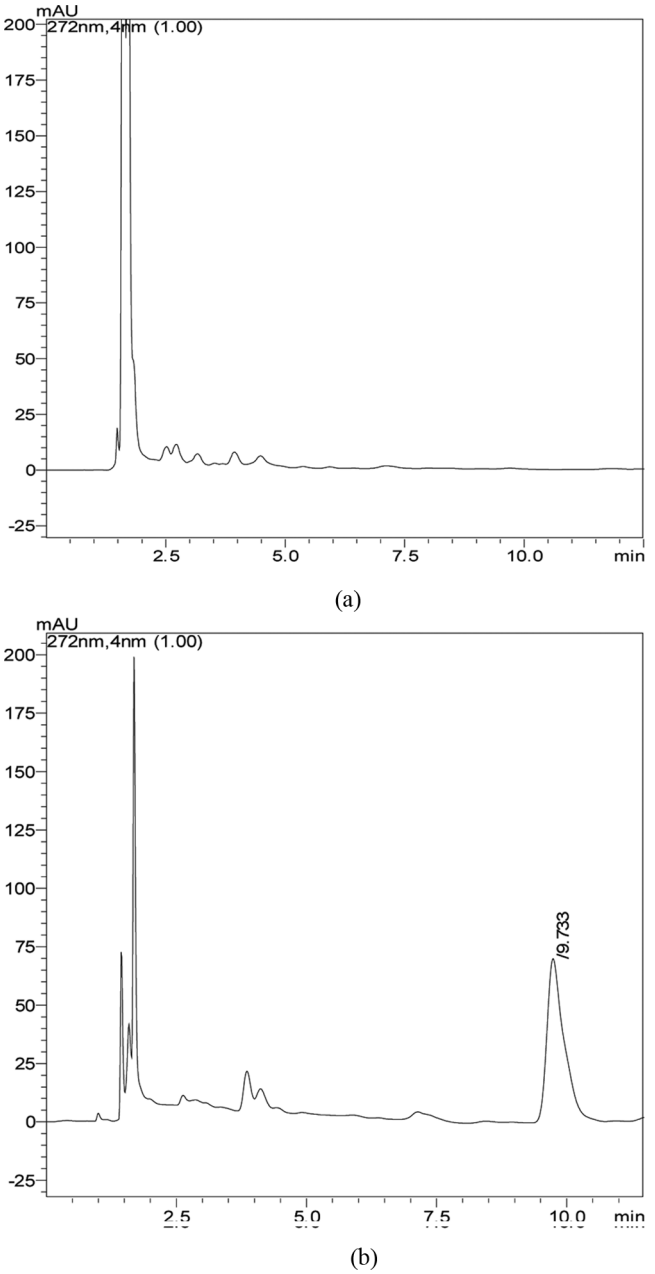

FIGURE 2 HPLC chromatogram of mulberry leaves control (a) and spiked extract of untreated mulberry leaves (b) at 0.1 $\mu g\, g^{-1}$.

is a higher recovery percentage than previously reported,[3,10] as shown in the Table 1. Standard solutions were injected after every ten samples to monitor changes in chromatographic conditions. The precision of the

TABLE 1 Fortified Recoveries of Imidacloprid in Mulberry Leaves

Fortified Concentration ($\mu g\,g^{-1}$)	Recovery (%) ± SD				
	R1	R2	R3	Mean ± SD	RSD (%)
0.01	89.75	91.54	90.25	90.51 ± 0.92	1.02
0.05	94.65	97.84	96.25	96.25 ± 1.60	1.66
0.10	99.74	101.25	102.84	101.28 ± 1.55	1.53

SD, standard deviation; RSD, relative standard deviation.

method was checked by calculating the RSD values and which showed an acceptable range (<2%). As the recovery percentage is more than 85%, this method can be adopted for residue and dissipation study of imidacloprid in mulberry leaf samples.

Quantification of imidacloprid was performed by the external standard. The calibration graphs obtained by plotting concentration versus peak area were linear over the range of 0.01–1 $\mu g\,mL^{-1}$. The equation of calibration curve was obtained by plotting peak areas in "y" axis against concentrations of imidacloprid in "x" axis, which was $y = 15563x + 908.4$, with a correlation coefficient (r^2) of 0.999. The sensitivity of the method was expressed in terms of the attained limit of detection (LOD), which was evaluated as three times the signal-to-noise ratio, 0.003 $\mu g\,g^{-1}$. The limit of quantification (LOQ) of the method as a signal-to-noise ratio from untreated samples equal to ten was calculated as 0.01 $\mu g\,g^{-1}$.

The proposed methodology was applied to a preliminary dissipation study of imidacloprid in mulberry leaves under field condition. Table 2 shows the results obtained from this experiment. A typical HPLC-DAD chromatogram of mulberry leaf samples obtained from field trials collected after

TABLE 2 Residues of Imidacloprid in Mulberry Leaf Sample

Days After Treatment	Residues Recovered ± SD ($\mu g\,g^{-1}$)							
	Standard Dose @ 0.6 $mL\,L^{-1}$				Double Dose @ 1.2 $mL\,L^{-1}$			
	R1	R2	R3	Mean ± SD	R1	R2	R3	Mean ± SD
0	3.02	3.08	3.04	3.05 ± 0.03 (−)	6.61	6.28	6.58	6.49 ± 0.18 (−)
1	1.95	1.89	1.97	1.94 ± 0.04 (36.44)	3.70	3.73	3.79	3.74 ± 0.05 (42.37)
3	1.61	1.48	1.52	1.54 ± 0.07 (49.57)	2.76	2.79	2.71	2.75 ± 0.04 (57.58)
5	1.02	0.98	0.97	0.99 ± 0.03 (67.51)	1.78	1.79	1.81	1.79 ± 0.02 (72.37)
7	0.76	0.68	0.72	0.72 ± 0.04 (76.37)	1.29	1.35	1.34	1.33 ± 0.03 (79.56)
10	0.49	0.44	0.46	0.46 ± 0.03 (84.79)	0.95	0.97	1.09	1.00 ± 0.08 (84.54)
15	0.21	0.17	0.15	0.18 ± 0.03 (94.20)	0.59	0.63	0.67	0.63 ± 0.04 (90.29)
22	0.05	0.05	0.04	0.05 ± .016 (98.46)	0.19	0.21	0.23	0.21 ± 0.02 (96.76)
30	BDL	BDL	BDL	BDL (100)	0.061	0.062	0.064	0.06 ± 0.019 (99.04)

Figures in parenthesis dissipation percentage, BDL, below detection limit; SD, standard deviation.

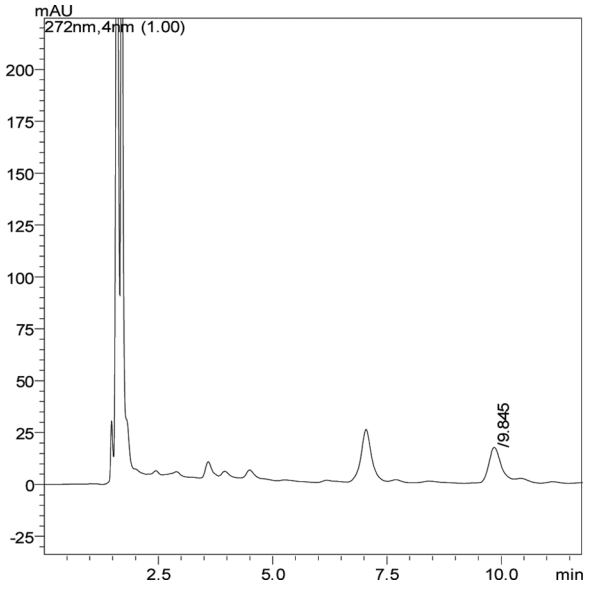

FIGURE 3 HPLC chromatogram of treated mulberry leaf sample.

imidacloprid application (10 day) is shown in Figure 3. The residue of imidacloprid was estimated in mulberry leaves over a period of 30 d. Initial residue deposit of imidacloprid on the mulberry leaf was found to be 3.047 and 6.490 $\mu g\,g^{-1}$ from treatment at recommended and double recommended doses, respectively (Table 2). The residues of imidacloprid in leaf samples declined progressively with time. Approximately 35–70% of the initial residue was dissipated after 5 d of application which further increased to 90–94% after 15 d irrespective of the application doses. The imidacloprid residues remained on leaves for 22 and 30 d, respectively, for both treatments. The residues gradually declined thereafter and reached below determination level (0.01 $\mu g\,g^{-1}$) on 30 d for recommended dose.

Imidacloprid was found to follow first-order dissipation kinetics (Table 3). The calculated half-life was 3.81 and 4.93 d for recommended and double the recommended doses, respectively. These results suggested that imidacloprid can be used on mulberry safely with a preharvest interval of 30.6 d.

TABLE 3 Results of Statistical Interpretation of Dissipation Data of Imidacloprid in Mulberry Leaves

Treatment	Regression Equation	R^2	$T_{(1/2)}$ Days
0.6 mL L^{-1}	$y = 3.422 - 0.079x$	0.996	3.81
1.2 mL L^{-1}	$y = 3.650 - 0.061x$	0.984	4.93

CONCLUSIONS

The proposed method allows a simple and rapid determination of imidacloprid residues in mulberry leaves. The mean recovery of imidacloprid for the present methodology was in the range of 90–101%. The present studies suggest that the use of imidacloprid at the recommended dose does not seem to pose any hazards to the silkworm if a waiting period of 31 d is observed before harvesting the mulberry leaves for silkworm feeding.

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REFERENCES

1. Subramanian, A. Management of Mulberry Thrips Pseudodendrothrips Mori (Nawa) by Chemical Method. *Madras Agric. J.* **2003**, *90* (10–12), 746–748.
2. Mahalingam, C. A.; Suresh, S.; Subramanian, S.; Murugesh, K. A.; Mohanraj, P.; Shanmugam, R. Papaya Mealybug, Paracoccus Marginatus-A New Pest on Mulberry, Morus spp. *Karnataka J. Agric. Sci.* **2010**, *23* (1), 182–183.
3. Mandal, K.; Chahil, G. S.; Sahoo, S. K.; Battu, R. S.; Singh, B. Dissipation Kinetics of β-Cyfluthrin and Imidacloprid in Brinjal and Soil Under Subtropical Conditions of Punjab, India. *Bull. Environ. Contamin. Toxicol.* **2010**, *84* (2), 225–229.
4. Ishii, Y.; Kobori, I.; Araki, Y.; Kurogochi, S.; Iwaya, K.; Kagabu, S. HPLC Method Determination of the New Insecticide Imidacloprid and Its Behavior in Rice and Cucumber. *J. Agric. Food Chem.* **1994**, *42* (12), 2917–2921.
5. Anastassiades, M.; Lehotay, S. J.; Stajnbaher, D.; Schenck, F. J. Fast and Easy Multiresidue Method Employing Acetonitrile Extraction/Partitioning and "Dispersive Solid-Phase Extraction" for the Determination of Pesticide Residues in Produce. *J. AOAC Int.* **2003**, *86* (2), 412–431.
6. Lehotay, S. J.; Mastovska, K.; Yun, S. J. Evaluation of Two Fast and Easy Methods for Pesticide Residue Analysis in Fatty Food Matrices. *J. AOAC Int.* **2005**, *88* (2), 630–638.
7. Aysal, P.; Ambrus, A.; Lehotay, S. J.; Cannavan, A. Validation of an Efficient Method for the Determination of Pesticides Residues in Fruits and Vegetables Using Ethyl Acetate for Extraction. *J. Environ. Sci. Health B* **2007**, *42* (5), 481–490.
8. Nguyen, T. D.; Yu, J. I.; Lee, D. M.; Lee, G. H. A Multiresidue Method for the Determination of 107 Pesticides in Cabbage and Radish Using QuEChERS Sample Preparation Method and Gas Chromatography Mass Spectrometry. *Food Chem.* **2008**, *110* (1), 207–213.
9. Paramasivam, M.; Banerjee, H. Simultaneous Determination of Flubendiamide its Metabolite Desiodo Flubendiamide Residues in Cabbage, Tomato and Pigeon Pea by HPLC. *Bull. Environ. Contamin. Toxicol.* **2011**, *87*, 452–456.
10. Arora, P. K.; Jyot, G.; Singh, B.; Battu, R. S.; Singh, B.; Aulakh, P. S. Persistence of Imidacloprid on Grape Leaves, Grape Berries and Soil. *Bull. Environ. Contamin. Toxicol.* **2009**, *82*, 239–242.