Determination of Imidacloprid Residue in Okra using Ultra High-Performance Liquid Chromatography Coupled with Photodiode Array Detector

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Abstract
A rapid, sensitive and selective analytical method was validated to determine imidacloprid residue in okra using ultra-high-performance liquid chromatography coupled with a photodiode array detector (UHPLC-PDA). The analytical method showed good linearity, and the limit of quantification was 0.025 mg/kg. Recoveries were ranged from 92.40 to 109.68%, with the relative standard deviation (RSD) of 1.87 to 4.84%. Field results showed that the mean initial deposit of imidacloprid on okra fruits were found to be 0.29 and 0.43 µg.g⁻¹, recommended and doubled the dose, respectively. The residues reached below the detection limit (BDL) of less than 0.025 µg.g⁻¹ on 7 and 10 days after treatment with the recommended and doubled the recommended dose, respectively. The half-life of imidacloprid in okra was 1.58 and 2.02 days, at recommended and double the recommended dose, respectively and a harvest withholding period of one day has been suggested for safe consumption.

Keywords: Dissipation, Imidacloprid, Okra, Residue, UHPLC-PDA.

Introduction
Okra (Abelmoschus esculentus L. Moench), is an important vegetable crop cultivated in tropical countries and India throughout the year. The estimated area and production of okra in India are about 511000 ha and 6219000 MT, respectively, in 2018-19. India is the top producer of okra, contributing 73.25% of the worlds' production and contributing to the country's foreign exchange and accounts for 60% of the export of fresh vegetables (Paramasivam and Bhuvaneswari, 2020). One of the major setbacks identified in the production is the increasing incidence of insect pests and at a conservative estimate cause about 35–40% losses. Several insect pests infest the okra vegetable crop. Among which shoot and fruit borer, leafhopper, whitefly and red spider mite are the most common pests causing substantial crop growth and yield reduction. Synthetic insecticides are the first line of defense in the management of these pests. To overcome these losses, Indian farmer’s excessively rely on new generation chemicals like neonicotinoid insecticide (Imidacloprid).

Imidacloprid is a broad-spectrum systemic chloronicotinyl insecticide effective against a wide range of sucking pests such as leafhoppers, planthoppers, white-flies, aphids, certain coleopterans, and micro lepidopterans at very low dosage having low mammalian toxicity (Elbert et al., 1991). Similar to the naturally occurring nicotinoids, imidacloprid interferes with the transmission of impulses in the nervous system of insects by acting as an antagonist to the nicotinic acetylcholine receptor, thereby resulting in continuous excitation of nerve cells leading finally to the death of the treated insect (Yamamoto, 1999). The imidacloprid residue may present in the food commodities, which may be toxic to humans through dietary intake. The okra grows very fast and is harvested in a tender stage at an interval of 2–3 days; due to its short interval, plucking fruits are likely to present a high level of residues that can pose health hazards to the consumers. In this context, the present work was conducted to evaluate the persistence and dissipation of imidacloprid 17.8 SL in okra for safe consumption.
Materials and Methods

Chemicals and Reagents
The reference standard of imidacloprid (99.5% purity) was obtained from Sigma Aldrich, Bangalore, India. Acetonitrile, methanol, and water for chromatography of HPLC grade, sodium chloride and anhydrous magnesium sulfate of analytical grade were purchased from Merck Life Science, Mumbai, India. Primary Secondary Amine (PSA) (Bondesil 40 µm) and Graphitized Carbon Black (GCB) were purchased from Agilent Technologies, USA.

Preparation of Standard Solutions
The stock solution, 1000 µg mL\(^{-1}\) was prepared by dissolving 24.1 mg imidacloprid standard in 25 mL acetonitrile. An intermediate standard solution of 100 µg mL\(^{-1}\) was prepared by diluting 2.5 mL of the stock solution in 22.5 mL of acetonitrile in a 25 mL volumetric flask. Working standard solutions of imidacloprid (0.025, 0.125, 0.250, 0.5 and 1.0 µg mL\(^{-1}\)) were prepared by diluting a suitable quantity of intermediate stock solution with HPLC grade acetonitrile. These working standards were used to find out the retention time of the respective concentrations and for the quantitative determination of residues in okra fruit samples. All the stock and working standard solutions were stored in the refrigerator at -20°C for further use.

Field Experiment
A separate supervised field trial of 60 square meters was maintained at a farmer’s field at Kaliyavoor in Srivaikundam block of Thoothukudi district, Tamil Nadu, India. Okra (Samrat hybrid) was raised by following recommended agronomic practices. Spray of Emamectin benzoate 5% SG (3.0 g/10lit) was given based on the presence of Fruit and shoot borer infestation. The treatments for the management of sucking pests comprised of imidacloprid 17.8 SL was given at 20 g a.i. ha\(^{-1}\) (recommended dose) and 40 g a.i. ha\(^{-1}\) (double the recommended dose). An untreated control was simultaneously maintained during the study. Treatments were applied using a pneumatic knapsack sprayer equipped with a hollow cone nozzle. The spray volume used was 500 L ha\(^{-1}\) and foliar sprays were made at 50% flowering stage using a hand-operated knapsack sprayer during morning hours, and the remaining sprayings were given at 10 days interval. About 1.0 kg of marketable okra fruits samples were collected randomly from each replicate of the treated and control plots at regular intervals viz., 0 (1 hour after spray), 1, 3, 5, 7, and 10 days after the insecticide application. All the samples were collected in polythene bags and transported to the laboratory for analysis.

Sample Preparation
Okra fruits (1 kg) were taken, and the tip and stalk portions were removed. The fruit portion was chopped into small pieces, homogenized with a mixer grinder, and used for residue analysis.

Extraction and Clean-up
The samples were processed by adopting QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method (Anastassiades et al., 2003). A representative homogenized sample of 10 g from each treatment was taken in a 50 mL poly-propylene centrifuge tube, and 20 mL of acetonitrile was added to it. The mixture was hand-shaken vigorously, followed by vortexing for 2 minutes. Subsequently, 1 g of Sodium Chloride (NaCl) and 4 g of anhydrous Magnesium Sulphate (MgSO\(_4\)) were added to the sample mixture, vortexed for 2 minutes, followed by centrifugation at 6000 rpm for 10 min. The supernatant (9 mL) aliquot was transferred into a test tube containing 4 g of MgSO\(_4\). From this 6 mL of aliquot was transferred to a 15 mL prefixed centrifuge tube with 10 mg GCB, 100 mg PSA sorbent, and 600 µg anhydrous MgSO\(_4\). The mixture was vortexed for one minute and then centrifuged at 3000 rpm for 10 minutes. And 4 mL of supernatant aliquot was transferred into a turbovap tube concentrated to dryness under a gentle stream of nitrogen by using the Turbopav LV set at 40°C. The residue was redissolved using acetonitrile (1-mL) and was filtered by 0.2 µm membrane syringe filter and transferred into a 1.5 mL UHPLC autosampler glass vials for analysis.

UHPLC Instrument Parameters
UHPLC (Shimadzu, I series 2020) is equipped with a diode array detector (SPD-M30A) and auto-sampler to estimate imidacloprid residue. Chromatographic separation was achieved with reverse phase C18 (Shimadzu) column, 250 mm length x 4.6 mm id x 4 µ particle size in a column oven, at 40°C. The low-pressure gradient condition employed with a mobile phase of acetonitrile and water (70:30, v/v) with a flow rate of 1.0 ml min\(^{-1}\) and the injection volume of 10 µL. The wavelength of maximum absorbance (\(\lambda_{\text{max}}\)) was 272 nm. The imidacloprid was eluted at the retention of 5.45 min (Fig. 1). Residues of insecticide were quantified by the comparison of peak area of standards with that of unknown or spiked samples run under identical conditions of operation.

Efficacy of Analytical Method

Linearity
The linearity study was conducted by injecting five different concentrations of working standard solutions following three replications for imidacloprid. The linearity of the calibration curves for imidacloprid was established in the range of 0.025, 0.125, 0.250, 0.5, and 1 µg mL\(^{-1}\) (Fig. 2).

Determination of LOD and LOQ
The limit of detection (LOD) was calculated by considering a signal-to-noise ratio of three with reference to the background.
noise obtained from the blank sample and the limit of quantification (LOQ) by considering a signal-to-noise ratio to ten.

**Recovery Experiment**

Recovery studies were carried out to establish the reliability of the analytical methods and to know the efficiency of extraction and clean-up steps employed for the present study. The homogenized untreated okra fruit samples (10 g) was spiked at three different concentrations level (0.025, 0.125, and 0.250 µg g⁻¹) separately using the standard analytical solution of imidacloprid. The spiked samples were equilibrated for one hour. The amount of residue was determined by comparing the sample response with the standard response under identical operating conditions. Each treatment was replicated three times with untreated control. The percent recovery was calculated using the formula.

\[
\text{Recovery ( % )} = \frac{\text{Residue obtained (µg/g)}}{\text{Spiked concentration (µg/g)}} \times 100
\]

**Statistical Analysis**

Imidacloprid residue was quantified by comparing the sample peaks with that of the standard peaks.

\[
\text{The residue (µg/g)} = \frac{A_1 \times C \times I_1 \times F}{A_2 \times W \times I_2}
\]

Where \(A_1\) = Peak area of the sample, \(A_2\) = Peak area of the standard, \(I_1\) = Injected volume of standard (µL), \(I_2\) = Injected volume of sample (µL), \(C\) = concentration of standard solution (µg/mL), \(F\) = Final volume of the sample (mL) and \(W\) = weight of the sample (g).

The dissipation of imidacloprid in okra fruit was calculated using the following formulae

\[
\text{Dissipation ( % )} = \frac{\text{Initial residue (µg/g) - Residue at a given time (µg/g)}}{\text{The initial residue (µg/g)}} \times 100
\]

The imidacloprid residue data was fitted into first-order dissipation kinetics \(C= Co e^{-kt}\) and \(t_{1/2}= \ln 2/k\), where, \(C\) is pesticide concentration in mg g⁻¹, at the time \(t\) in days, \(Co\) is apparent initial concentration (mg g⁻¹), \(k\) is rate constant and \(t_{1/2}\) is pesticide half-life in okra (Paramasivam and Bhuvaneswari, 2020 and Sakthiselvi et al., 2020).

**Waiting Period/ Pre-harvest Interval (PHI)**

The maximum residue limit (MRL) has been published by food safety and standards authority of India (FSSAI) for imidacloprid in okra was 2.0 mg/kg (FSSAI, 2021), and pre-harvest interval (PHI) of imidacloprid was calculated using \(\text{PHI}= [\ln C_0 - \ln \text{MRL}] / k\) (Paramasivam and Bhuvaneswari, 2020 and Sakthiselvi et al., 2020).

**RESULTS AND DISCUSSION**

**Efficiency of the Analytical Method**

The method linearity ranged from 0.025 to 1.0 mg/L concentration with a correlation coefficient greater than 0.9999, indicating good linearity (Fig. 2). The LOD and LOQ were 0.007 and 0.025 mg/kg, respectively. The LOQ of the proposed analytical method was below the maximum residue limit (MRL) value fixed by FSSAI (2.0 mg/kg). The mean recovery of imidacloprid was 100.89, 104.33, and 107.42% in okra fruits with the Relative Standard Deviation (RSD) of 4.84, 3.34, and 1.87% at spiking levels of 0.025, 0.125, and 0.25 µg g⁻¹, respectively (Table 1).

**Persistence and Dissipation of Imidacloprid on Okra**

The data on persistence and dissipation of imidacloprid in okra fruits sprayed at 20 g a.i. ha⁻¹ (recommended dose) and 40 g a.i. ha⁻¹ (double the recommended dose) are presented (Table 2). The mean initial deposit (1 hour after spraying) of imidacloprid on okra fruits was found to be 0.29 and 0.43 µg g⁻¹ in the recommended and double the dose, respectively. At the recommended dose, imidacloprid dissipated to 0.16, 0.08, and 0.03 µg g⁻¹ on the first, third, and fifth day after treatment with the dissipation of 44.83, 72.41, and 89.66% respectively (Table 3). The imidacloprid reached BDL 10 days after treatment at double the recommended dose. The rapid dissipation of the insecticide in subsequent sampling after spraying might be due to dilution of the toxicant because of plant growth. Environment factors such as radiation, temperature, and relative humidity also would have played a significant role in the dissipation of imidacloprid residue. The results are in agreement with those of Karthik et al. (2015), who reported an initial deposit of 0.15 µg g⁻¹.

**Table 1:** Recovery of imidacloprid on okra fruit

<table>
<thead>
<tr>
<th>Spiking level (µg g⁻¹)</th>
<th>Recovery (%)</th>
<th>Mean* ± SD</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.025</td>
<td>109.08</td>
<td>101.21</td>
<td>100.89 ± 4.73</td>
</tr>
<tr>
<td>0.125</td>
<td>99.38</td>
<td>108.10</td>
<td>104.33 ± 3.74</td>
</tr>
<tr>
<td>0.250</td>
<td>106.77</td>
<td>109.68</td>
<td>107.42 ± 2.01</td>
</tr>
</tbody>
</table>

*Mean of three replicates, SD – Standard Deviation, RSD – Relative Standard Deviation

**Fig. 2:** Linearity calibration curve of imidacloprid – UHPLC
Determining Imidacloprid Residue in Okra using UHPLC

Table 2: Persistence and dissipation of imidacloprid residue in okra fruits at 20 g a.i./ha

<table>
<thead>
<tr>
<th>Days after application</th>
<th>Residues (µg g(^{-1}))</th>
<th>Dissipation (%)* Mean (\pm SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>0 (1 hr)</td>
<td>0.28 ND 0.26 ND 0.32 ND</td>
<td>0.29 ± 0.03 -</td>
</tr>
<tr>
<td>1</td>
<td>0.19 0.13 0.16 0.16</td>
<td>0.16 ± 0.03 44.83</td>
</tr>
<tr>
<td>3</td>
<td>0.07 0.08 0.07 0.08</td>
<td>0.08 ± 0.01 72.41</td>
</tr>
<tr>
<td>5</td>
<td>0.03 0.03 0.03 0.03</td>
<td>0.03 ± 0.00 89.66</td>
</tr>
<tr>
<td>7</td>
<td>BDL BDL BDL BDL</td>
<td>100.00</td>
</tr>
<tr>
<td>10</td>
<td>BDL BDL BDL BDL</td>
<td>-</td>
</tr>
</tbody>
</table>

*Mean of three replicates; ND – Not Detected; BDL – Below Detectable Limit (< 0.025 µg g\(^{-1}\))

Table 3: Persistence and dissipation of imidacloprid residue in okra at 40 g a.i./ha

<table>
<thead>
<tr>
<th>Days after application</th>
<th>Residues (µg g(^{-1}))</th>
<th>Dissipation (%)* Mean (\pm SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>0 (1 hr)</td>
<td>0.42 0.40 0.48 0.43</td>
<td>0.43 ± 0.04 -</td>
</tr>
<tr>
<td>1</td>
<td>0.36 0.25 0.29 0.30</td>
<td>0.30 ± 0.06 30.23</td>
</tr>
<tr>
<td>3</td>
<td>0.12 0.13 0.12 0.12</td>
<td>0.12 ± 0.01 72.09</td>
</tr>
<tr>
<td>5</td>
<td>0.08 0.07 0.07 0.07</td>
<td>0.07 ± 0.00 83.72</td>
</tr>
<tr>
<td>7</td>
<td>0.03 0.04 0.03 0.04</td>
<td>0.04 ± 0.01 90.70</td>
</tr>
<tr>
<td>10</td>
<td>BDL BDL BDL BDL</td>
<td>100.00</td>
</tr>
</tbody>
</table>

*Mean of three replicates; ND – Not Detected; BDL – Below Detectable limit (< 0.025 µg g\(^{-1}\))

Fig. 3 Persistence and dissipation of imidacloprid on okra

and 0.26 mg kg\(^{-1}\), following application of imidacloprid 70 WG at 24.5 and 49 g a.i. ha\(^{-1}\), respectively in okra fruits. Similarly, Ilango and Devaraj, (2003) reported that imidacloprid applied at 20 g a.i. ha\(^{-1}\) on okra dissipated and reached the BDL on 7 days after application. On the contrary, Dikshit et al. (2000) and Sivaveerapandian et al. (2002) reported the residues of applied imidacloprid at 20 g a.i. ha\(^{-1}\) and 40 g a.i. ha\(^{-1}\) on okra dissipated and reached BDL on 3 and 15 days after treatment, respectively. Tirthankar et al. (2012) reported that imidacloprid residues persisted up to 5 and 7 days after treatment with the combination product Solomon ® 300 OD (betacyfluthrin 9pimi-
dacloprid 21) at 60 and 120 g a.i. ha\(^{-1}\), respectively in okra fruits. The persistence of this insecticide was found to be highest in brinjal followed by tomato and least in okra when compared between different vegetables, the probable clue of which was proposed based on the non-enzymatic antioxidant content of the fruits (Tirthankar et al., 2012). Also, Karthik et al. (2015) found no detectable residues of imidacloprid seed treatment formulations in okra fruits harvested at 5 days after application of imidacloprid.

The kinetic equation and correlation coefficient of imidacloprid were calculated from the experimental data presented in Fig. 3.

The dissipation pattern followed first order kinetics with a half-life of 1.58 and 2.02 days for recommended and double the recommended doses, respectively. The half-life value of imidacloprid was almost the same as that observed by Patel et al. (2012), Karthik et al. (2015), and Gautam et al. (2016) who evaluated the residue dynamics of imidacloprid in okra fruit. The Maximum Residue Limit (MRL) for imidacloprid on okra given by Food Safety Standards Authority of India (FSSAI) MRL was 2.0 µg g\(^{-1}\). Both the recommended and double the recommended dose of imidacloprid 17.8 SL was found to be less than the given MRL at initial application. Hence the safe waiting period for okra after application of imidacloprid 17.8 SL was calculated to be one day after application for safer consumption.

CONCLUSION

An efficient alternate, simple UHPLC-PDA method was developed, validated, and successfully evaluated for its efficiency in determining imidacloprid residue in okra. The LOQ of the method was much lower than the MRL value fixed by FSSAI. The half-life of imidacloprid residue in okra fruit was 1.58 and 2.02 days, respectively for 20 and 40 g a.i./ha. The okra fruit can be consumed safely one day after the application of imidacloprid.

REFERENCE


