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Research Article

Dissipation kinetics, decontamination and dietary risk assessment of imidacloprid residue in bitter gourd and soil

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Abstract
Imidacloprid is a broad-spectrum neonicotinoid class insecticide with systemic action, widely used on vegetables in India for the management of sucking insect pests. The overall pesticide usage profile in gourds growing districts of Tamil Nadu showed that imidacloprid as the most commonly used insecticide. The present study aimed to develop and validate an analytical approach for detecting imidacloprid and 6-chloronicotinic acid residues in bitter gourd fruit, juice and soil using LC-EI-MS (liquid chromatography coupled with electron ionization mass spectrometry) was undertaken. The persistence pattern, effect of household processing and risk assessment of imidacloprid on bitter gourd was studied by conducting field trials at single and double doses of 20 and 40 g a.i ha^{-1}. Calibration curves showed a good linear relationship (r^2>0.99) with the concentrations (0.0025–0.5 µg mL^{-1}) of imidacloprid and 6-chloronicotinic acid. The limit of detection and quantification of the method were 0.008 and 0.025 mg kg^{-1}, respectively. Accuracy of imidacloprid and 6-chloronicotinic acid residue recovery was in the range of 88–101 per cent with RSD of less than six per cent in all the matrices of bitter gourd. Initial deposits of imidacloprid at 20 and 40 g a.i ha^{-1} were 0.68 and 1.25 mg kg^{-1} and the residues persisted up to 10 and 15 days with their respective half-lives of 2.51 and 3.13 days. Simple decontamination techniques showed 33 to 80 per cent reduction of residues in samples collected up to 10 days after treatment. The estimated RQ was less than one indicating the level of risk to the consumer is negligible.

Keywords: Bitter gourd, Decontamination, Dissipation, Imidacloprid, LC-MS

INTRODUCTION

Bitter gourd (*Momordica charantia* L., 2n=22) is a very popular Cucurbitaceae vegetable grown worldwide, particularly in India, China and Pakistan (Li *et al*., 2020). It is more rich in glucose, proteins, iron, phosphorus and ascorbic acid than other vegetables (Samadov, 2022). Additionally, the leaves and fruits have been used by Indians for centuries to treat diabetes, skin sores and wounds (Zhuo *et al*., 2021). The area and production of bitter gourd during the year 2020-21 were 1.07 lakh hectares and 12.96 lakh metric tonnes in India, while in Tamil Nadu 0.24 lakh hectares and 0.44 lakh MT were produced (National horticulture board, 2020). Insect pests are major menace starting from vegetative to reproductive stages, which includes fruit fly, leafhoppers, aphids, whiteflies, mites, red pumpkin beetles and thrips (Mawtham *et al*., 2020). Imidacloprid is a chloronicotinyl insecticide of M/S Bayer crop science. It has a high insecticidal potency with low persistence in soil and relatively low toxicity to mammals (Han *et al*., 2018). It is effective against a wide range of sucking pests infesting okra, chilli, citrus, grapes, and tomato (Central Insecticides Board and

Registration Committee, 2021). In the neurological system, imidacloprid works on various types of post-synaptic nicotinic acetylcholine receptors. Nerve impulses are discharged spontaneously after binding to the nicotinic receptor, followed by the neuron's failure to transmit the signal (Casida, 2018). The phytochemical properties of imidacloprid viz., water solubility (0.61 g/L at 20 °C), vapor pressure (3×10^{-12} mmHg at 20 °C), octanol/water partition coefficient (Kow) (pH 7 – 0.57 at 21 °C) and Henry's constant (1.7×10^{-10} Pa·m^3/mol) (Pesticide Properties DataBase, 2022).

Imidacloprid residues in crops like mango, grapes, okra, potato, cucumber, strawberry, apple and soil have been quantified using Liquid chromatography with tandem mass spectrometry (LC-MS/MS), High-performance liquid chromatography (HPLC), Gas chromatography mass spectrometry (GC-MS) and Liquid chromatography with mass spectrometry (LC-MS) (Malhat *et al*., 2021; Hendawi *et al*, 2018; Majed *et al*., 2021; Mohapatra *et al*., 2012; Sahoo *et al*., 2012). While previous research estimated imidacloprid residues on different crops, no systematic study has been carried out on the dissipation kinetics, decontamination and risk assessment of imidacloprid in bitter gourd. The purpose of this study was (i) to develop and validate a method for quantifying imidacloprid residues in bitter gourd fruit, juice and soil, (ii) to evaluate a common household technique such as washing, cooking alone or in combination for the reduction of imidacloprid residue and (iii) to estimate the dietary risk of imidacloprid residue for human consumption.

MATERIALS AND METHODS

Chemicals and reagents

Certified Reference Materials (CRM) of imidacloprid (98.3%) and 6-chloronicotinic acid (6-CNA) (98.9%) were procured from Sigma-Aldrich Pvt. Ltd., Bangalore, India. The commercial formulation, imidacloprid 17.8% SL (local pesticide shop, Coimbatore, Tamil Nadu, India), LC-MS grade acetonitrile, formic acid and ammonium formate (Sigma Aldrich), methanol (MeOH) (Fisher chemical, USA), Sodium chloride (NaCl) (> 99% purity), anhydrous magnesium sulphate ($MgSO_4$) (>99.5% purity) and sodium sulphate (Na_2SO_4) (>99%) (Merck, Mumbai, India) were purchased. Sorbents like graphitized carbon black (GCB) and primary, and secondary amine (PSA, 40 μm) (Agilent Technologies, USA) were procured. Before usage, the $MgSO_4$ was baked in a muffle furnace at 400°C for 4 hours and maintained in an airtight desiccator. Throughout the analysis, purified Millipore water (18.2 MΩ) from a lab-scale (Q3 Merck) Millipore unit was used.

Preparation of standard solutions

Stock solutions of imidacloprid and 6-CNA (400 mg/L) were prepared in methanol (LC-MS grade) by accurately weighing 10.11 and 10.20 mg of analytical standards into a calibrated class A volumetric flask (25 mL). The intermediate stock solution (40 mg/L) was prepared by transferring 2.5 mL of the stock solution (400 mg/L) to 25 mL volumetric flask and finally, the volume was made up with MeOH. Working standards of 0.0025 – 0.5 mg/L were prepared by serial dilution from the intermediate stock solution. All standard solutions were kept in a deep freezer at -20 ^{0}C for further use.

Method validation

A method was developed and validated for the quantification of imidacloprid and 6-CNA in bitter gourd fruit, juice and soil. The parameters such as linearity, sensitivity, accuracy, precision, uncertainty and matrix effect were estimated as per the guidelines of SANTE (2021). The linearity of the method was measured by evaluating insecticide at seven concentrations ranging from 0.0025 to 0.5 μg/mL with six replications. The linearity of imidacloprid and 6-CNA was measured in both solvent and matrix match standards. Limit of detection (LOD) and limit of quantification (LOQ) were calculated by injecting the matrix match standards of imidacloprid and 6-CNA starting from lower concentration level (0.025 mg kg^{-1}). LOD and LOQ were computed based on the calibration curve using the following formulae as follows:

$$LOD = 3 \times (\text{Standard Deviation/Slope}) \quad \text{Eq. 1}$$

$$LOQ = 10 \times (\text{Standard Deviation/Slope}) \quad \text{Eq. 2}$$

The recovery studies were achieved by spiking three replications of bitter gourd samples like fruit, juice and soil with five varied concentrations of imidacloprid and 6-CNA (0.025, 0.05, 0.075, 0.1, and 0.25 mg kg^{-1}). In order to calculate the per cent recovery, a comparison was made between the peak area of the known quantity of analytes in the spiked sample and the matrix match standard. The relative standard deviation (RSD) for each level of spiking (0.025 to 0.25 mg kg^{-1}) of bitter gourd samples was used to determine the precision of the method. Uncertainty of the method was evaluated using data on repeatability, expected uncertainty of equipment and glassware, LOQ, CRM purity (%) and linearity. The matrix effect (ME) was evaluated using the following equation:

$$ME (\%) = (\text{Peak area of matrix match standard - Peak area of solvent standard})/ \text{Peak area of matrix match standard} \times 100 \quad \text{Eq. 3}$$

Field experiment

A supervised field trial was conducted in Coimbatore, Tamil Nadu, India (11.22^0 N latitude, 77.10^0 E longitude) to investigate the pattern of imidacloprid dissipation and dietary risk in bitter gourd fruit, juice and soil. Bitter gourd (Eastwest F1 hybrid) was raised in 250 m^2 plot/treatment following good agronomic practices. The commercial formulation of imidacloprid 17.8% SL was applied as a foliar spray @ 20 (X) and 40 (2X) g a.i ha^{-1} on bitter gourd along with untreated control (water spray). Two consecutive sprays were given at ten days intervals using 500 L/ha spray fluid and high-volume knapsack compression sprayer. The average maximum (29.0 ^{0}C) and minimum (18.2 ^{0}C) temperatures, as well as the relative humidity (77.2%), were recorded during the field experiment and there was no rainfall.

Sample collection and preparation

Ten kilograms of bitter gourd samples were randomly collected at each sampling interval (0 (2 hours after treatment), 1, 3, 5, 7, 10, 15, 20, 25 and 30 days) from the treated and untreated plot (using water spray only) after the last application of the insecticide. Soil samples collected from each plot were mixed, air-dried, homogenised, crushed and sieved (2 mm pore size). The collected samples were labelled separately and transported to the laboratory for residue analysis. The fruit samples were homogenised using a high-volume blade homogeniser (Robot Coupe, Blixer 6 VVA, France) and a sub sample of 250 g was transferred to a glass storage bottle. Bitter gourd juice was extracted from treated and untreated samples. The 200 mL of homogenised extract was further diluted with water (100 mL) and filtered through a strainer. All the samples were stored at -20 °C until residue analysis.

Extraction and clean up

The imidacloprid and 6-CNA residues were extracted and cleaned up from bitter gourd fruit, juice and soil by modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method (Anastassiades *et al*., 2003). Ten grams sample was taken in 50 mL polypropylene centrifuge tube and along with 20 mL acetonitrile and vortexed for one min. Then four grams of anhydrous magnesium sulphate ($MgSO_4$) and one gram of sodium chloride were added, vortexed and centrifuged for 10 min. at 6000 rpm. After centrifugation, any remaining moisture was removed by passing the top acetonitrile layer (10 mL) through anhydrous sodium sulphate (4 g). A 6 mL of supernatant was transferred into a 15 mL centrifuge tube containing primary and secondary amine (150 mg), graphitized carbon black (25 mg) and $MgSO_4$ (900 mg), vortexed for 1 min. and centrifuged at 3000 rpm for 10 min. A 4 mL aliquot of acetonitrile phase was carefully pipetted into clean glass tube, and was gently evaporated using a turbovap LV (35 ^{0}C) with the stream of nitrogen until nearly dryness. The residues were then redissolved in 1 mL MeOH, filtered through 0.2-micron PTFE syringe filter and transferred into 1.5 mL autosampler glass vials for LC-MS analysis.

LC-MS Instrument

Detection of imidacloprid residues was made using a single Quadrupole from Shimadzu 2020 series LC-MS containing reverse phase C18 (Eclipse plus- Agilent) column (250 mm length x 4.6 mm id, 5 µm particle size) with the following instrumental parameters:

LC-MS specifications	
Detector	MS detector
Software	Shimadzu lab solutions software version 5.6
Mobile phase	A (methanol): B (water) at 70:30 with 2 mM ammonium formate with 0.05% formic acid
Flow rate	0.5 mL min^{-1}
Mass ratio (m/z)	256 and 156 for imidacloprid and 6-CNA
Ionizing mode	Positive selected ion monitoring (+SIM) and negative ion monitoring (-SIM) mode for imidacloprid and 6-CNA, respectively
Injection volume	10 µl
Drying gas flow rate	15 L min^{-1}
Column oven temperature	40 °C
Nebulizer gas flow rate	1.5 L min^{-1}
LC-MS pump pressure	48 kg/ cm^2
Heat block temperature	200 ° C
Desolvation line (DL) temperature	250 °C
Capillary voltage	3.5 kV
Run time	15 min
Retention time of analytes	5.87±0.02 and 6.76±0.02 min for imidacloprid and 6-CNA

Data analysis

The concentration of imidacloprid residue was calculated using

$$\text{Residue (mg kg}^{-1}) = A_1 \times C \times I_1 \times F / A_2 \times W \times I_2 \qquad \text{Eq. 4}$$

Where

A_1= Peak area of imidacloprid in the sample solution, A_2 = Peak area of imidacloprid in the standard solution, C = Concentration of standard solution (mg kg^{-1}), I_1 = Injected volume of standard (µL), I_2 = Injected volume of sample (µL), W = weight of the sample (g) and F = Final volume of the sample (mL).

The dissipation of imidacloprid residue was subjected to using equation $Ct = Coe^{-kt}$, where, Ct is the insecticide concentration (mg kg^{-1}) at time t, k is the dissipation rate constant and Co is the apparent initial concentration (mg kg^{-1}) (Mariappan and Kaithamalai, 2020). Half-life of imidacloprid was computed by $T_{1/2}$ = ln (2)/k and pre-harvest interval (PHI) was calculated using the formula PHI = [In Co - In MRL]/k (Hoskins, 1996; Handa *et al*., 1999). The maximum residue limit (MRL) for imidacloprid in bitter gourd fruit is 0.2 mg/kg (Codex Alimentarius Commission, 2016). Under the Food Safety and Standards Authority of India, there is no MRL for imidacloprid in bitter gourd.

Decontamination studies

The effect of simple culinary practices were investigated in order to determine the possibility of removing imidacloprid residues from treated bitter gourd fruit. After the second spraying, samples were collected on 0, 1, 2, 3, 5, 7 and 10 days and subjected to decontamination techniques *viz.,* washing with tap water (pH 7.0), 2% salt solution, lukewarm water (40 ^{0}C), 2% tamarind solution, 2% lemon solution and cooking (10 min.). The combination treatments were evaluated like, tap water washing+ 2% salt/tamarind/lemon solution (30 sec.) + 10 min. cooking.

Washing treatments

Solutions of common salt, tamarind and lemon juice (2%) were prepared in one liter containers separately. The samples were immersed and gently rubbed for a min. using hands. The fruits were spread on blotting paper, air-dried, homogenised and then processed for residue analysis.

Cooking treatment

In the cooking treatment, fruit samples were cut into small pieces and cooked in boiling water (1 L for each 500 g sample) for 10 min. Washed and cooked samples were dried using blotting paper, homogenised and processed for residue estimation.

Combination treatments

Bitter gourd samples (500g) were washed with tap water, dipped in 2 per cent NaCl/tamarind/lemon solution, followed by gentle rubbing for 30 sec. and dried using blotting paper. Washed fruit samples were chopped into small pieces and cooked for 10 min. The samples were then homogenised and processed in a similar manner as explained above.

Processing factor

The Processing factor (PF) is a method of calculating the risk of insecticide residue intake in processed foods. The PF of less than one suggests a decrease in residue in processed food, while a PF of more than one indicates a concentration of residue (Scholz *et al*., 2017).

Dietary risk assessment

To calculate Estimated Daily Intake (EDI) of imidacloprid residue, the maximum residue (mg kg^{-1}) from field experiment was multiplied by the average consumption rate of bitter gourd (60 g/day) (National Institute of Nutrition, 2020) and divided by the average adult male (65 kg) and female body weight (55 kg) (Dong *et al*., 2018). The risk quotient (RQ) was derived by dividing the EDI by the acceptable daily intake (ADI) of the insecticides and expressed in mg kg^{-1} body weight (BW)/day. The ADI for imidacloprid is 0.06 mg kg^{-1} bw/day (Cabrera and Pastor, 2021). The risk of long-term human dietary consumption of imidacloprid was acceptable when RQ was less than one and unacceptable if RQ was more than one.

RESULTS AND DISCUSSION

Method validation

The method optimization results were satisfactory for all validation parameters examined in accordance with the SANTE guidelines (SANTE, 2021). The retention time of imidacloprid and its metabolite were 5.87±0.02 and 6.76±0.02 min, respectively. Linear response was assessed for different standards (0.0025-0.5 mg kg^{-1}) and matrix match standards (0.025-0.25 mg kg^{-1}). Excellent linear relationship and correlation coefficients (r^2) for imidacloprid and 6-CNA in solvent and matrices (fruit, juice and soil) were observed (r^2 >0.99) (Table 1 and Fig. 1). The LOD and LOQ of the method for imidacloprid and in metabolite were determined as 0.008 mg kg^{-1} and 0.025 mg kg^{-1}, respectively, in the five matrix standard solutions. The LOQ (0.025 mg kg^{-1}) estimated using the above method was less than the MRL (0.2 mg kg^{-1}). The overall recovery ranged between 88.20 and 100.71 per cent in bitter gourd fruit, juice and soil, with the RSD of less than five per cent (Table 2). The matrix effect in bitter gourd and soil ranged from -3.49 to 9.23 per cent for all spiked standards and was found to be within the acceptable level of 20 per cent (SANTE, 2021) (Table 1). The possible uncertainty throughout the experiment were included to estimate the uncertainty measurement at LOQ level (0.025 mg kg^{-1}). The uncertainty measurement varied up to 4.4-5 per cent, which was well below the internationally acceptable level of 20%. From this, it is clear that the method has a lower level of uncertainty associated with repeatability and accuracy, which could be due to good recoveries and low RSD (Table 2). These results suggested that the method provided repeatability and reliability with acceptable recovery.

Dissipation kinetics of imidacloprid

Bitter gourd and soil samples were collected for residue analysis from 0 to 30 days after the second application. The initial imidacloprid residues in bitter gourd fruit were 0.68 and 1.25 mg kg^{-1} @ 20 and 40 g a.i ha^{-1}, respectively (Table 3). After 3rd day, more than 50 per cent of the initial residue dissipated and reached below the limit of quantification (BLQ) (0.025 mg kg^{-1}) 15 and 20 days after application in X and 2X doses, respectively (Fig. 2). The 6-CNA residue was not detected in any of the samples analysed. The soil sample collected at harvest time showed no detectable residues of imidacloprid and 6-CNA. Initial imidacloprid residues in okra were 0.15 and 0.26 mg kg^{-1} at 24.5 and 49 g a.i ha^{-1}, respectively (Karthik *et al*., 2015). In cucumber, the initial residue of imidacloprid was 0.94 mg kg^{-1} and reached BLQ after 21 days of treatment at 125 g a.i ha^{-1} (Nasr *et al*., 2014). The initial deposit of 1.53 mg kg^{-1} imidacloprid residue in tomato at 150 g a.i ha^{-1} and reached BLQ after 21 days (Badawy *et al*., 2019).

Half-life and pre-harvest interval (PHI)

The findings of determining the imidacloprid dissipation kinetics in bitter gourd by comparing residue level against time are discussed below. Imidacloprid persistence was determined using its half-life ($T_{1/2}$) or DT_{50}. Imidacloprid dissipation on bitter gourd fruit followed first-order kinetics. The half-life (DT_{50}) values of imidacloprid were 2.51 and 3.13 days at 20 and 40 g a.i ha^{-1}, respectively (Table 3). The PHI was found to be 4.45 and 8.30 days for 20 and 40 g a.i ha^{-1} of imidacloprid, respectively. The imidacloprid residues dissipated with a half-life of 3.2 and 3.9 days on *Zizania latifolia* and 5.5 and 3.8 days on sweet potato treated at 31.5 and 47.3 g a.i ha^{-1} (Yu *et al*., 2019). Application of imidacloprid at 150 g a.i ha^{-1} in tomato required a PHI of 6.18 days (Badawy *et al*., 2019). The residual deposition and persistence of insecticides are determined by different factors, *viz.,* type and formulation of insecticide, carrier material, the active ingredient, meteorological parameters, plant growth and plant type (Lavtizar *et al*., 2014).

Decontamination of imidacloprid residues in bitter gourd fruits

The results in Tables 4 and 5 showed that all the decontamination procedures effectively reduced imidaclo-

Table 1. Linearity parameters and matrix effect for imidacloprid and 6-CNA residues in different matrices of bitter gourd

Pesticides	Calibration (matrix)	Calibration range (mg L^{-1})	Regression equation	Correlation coefficient (R^2)	Matrix effect (%)
Imidacloprid	Solvent	0.0025-0.5	y = 2E+06x + 2876.8	0.9975	-
	Fruit	0.025-0.25	y = 2E+06x - 26482	0.9986	2.11-9.23
	Juice	0.025-0.25	y = 2E+06x - 28773	0.9967	-
	Soil	0.025-0.25	y = 2E+06x - 22382	0.9956	0.12-5.74
6-CNA	Solvent	0.0025-0.5	y = 103912x + 624.92	0.9979	-
	Fruit	0.025-0.25	y = 129436x - 952.87	0.9945	-3.49-7.44
	Juice	0.025-0.25	y = 301387x + 1532.3	0.9998	-
	Soil	0.025-0.25	y = 140175x - 1088.2	0.9917	3.73-8.38

Table 2. Recovery percentage of imidacloprid in bitter gourd fruit, juice and soil

Sub-strates	Spiked concentration (mg kg^{-1})	Imidacloprid			6-Chloroniconitic acid		
		Recovered concentration (mg kg^{-1})*	Mean recovery (%) ± SD*	Repeatability (RSD %)	Recovered concentration (mg kg^{-1})*	Mean recovery (%) ± SD*	Repeatability (RSD %)
Fruit							
	0.025	0.02	89.84 ±1.42	1.58	0.02	89.28 ± 0.74	0.83
	0.05	0.05	91.85 ±3.42	3.72	0.05	98.09 ± 2.59	2.64
	0.075	0.07	88.20 ±1.90	2.16	0.07	94.24 ± 1.82	1.93
	0.1	0.1	95.35 ±1.97	2.07	0.1	100.71±1.04	1.03
	0.25	0.23	93.99 ±1.25	1.33	0.25	98.90 ±0.69	0.70
Juice							
	0.025	0.02	90.83 ±3.68	4.05	0.02	92.25 ±5.06	5.06
	0.05	0.05	91.18 ±5.33	5.85	0.05	91.85 ±3.23	3.23
	0.075	0.07	93.17 ±4.76	5.11	0.07	92.63 ±2.04	2.04
	0.1	0.1	96.52 ±2.01	2.09	0.10	97.39 ±3.61	3.61
	0.25	0.23	91.08 ±3.34	0.67	0.23	90.78 ±1.30	1.30
Soil							
	0.025	0.02	90.95 ±1.73	1.91	0.02	94.96 ± 3.31	3.49
	0.05	0.05	90.21 ±0.86	0.95	0.05	90.75 ± 3.04	3.35
	0.075	0.07	94.58 ±1.37	1.44	0.07	97.62 ± 3.07	3.14
	0.1	0.1	99.04 ±2.58	2.60	0.1	98.70 ± 2.98	3.02
	0.25	0.25	98.52 ±1.23	1.25	0.23	92.50 ± 2.77	2.99

*Mean of six replications, SD- Standard Deviation, RSD- Relative Standard Deviation prid residues from bitter gourd fruits collected from 0 to 10 days after the last application. The average deposit of imidacloprid residues at 20 g a.i ha^{-1} were 0.67, 0.39, 0.25 and 0.09 mg kg^{-1} and at 40 g a.i ha^{-1} were 1.23, 0.80, 0.51 and 0.19 mg kg^{-1} in the bitter gourd treated samples collected on 0, 2, 5 and 7 days, respectively. Among the decontamination methods, tap water washing and cooking combined with salt, lemon, and tamarind solution were very effective in removing imidacloprid residues to the extent of 70.23-87.04 per cent in both doses. On 7^{th} day imidacloprid residue in a single dose reached BLQ (0.025 mg kg^{-1}), whereas in a higher dose, the BLQ was observed at 10 days of the combined treatments. The 6-CNA residue was not found in any of the bitter gourd samples.

Thermal treatment by cooking process showed a high reduction (60.28-75.21%) of imidacloprid residues in both doses. After cooking, imidacloprid levels were reduced by 70.09, 64.58 and 60.28 per cent in the X dose and 75.21, 64.42 and 64.88 % in the 2X dose in 0, 3 and 7 days after last application. At X dose, 2 % of NaCl, lemon and tamarind solution removed 37.56-56.62 per cent of imidacloprid residues, whereas in 2X dose, the %e reduction was 37.56-54.72 days, respectively. In lukewarm water, the % loss of imidacloprid residues at X dose was 53.62-39.64, while at 2X dose, 50.63-38.84% in 0 to 10 days, respectively. Washing with tap water removed 33.0-37.0 % of imidacloprid residues in X and 2X doses.

In fruit juice extracted from untreated samples collected from 0, 1 and 2 days imidacloprid residues quantified were 0.12, 0.09 and 0.04 mg kg^{-1} in treatments at X (20 g a.i ha^{-1}) dose and 0.28, 0.20, 0.10, and 0.04 mg kg^{-1} in 2X (40 g a.i ha^{-1}) dose, respectively (Table 6 & 7). The washing treatments reduced residues to BLQ (0.025 mg kg^{-1}) in single and double doses after 2 and 3 days. At X dose, 60.84-42.89 per cent residues were eliminated by salt, lemon, tamarind, lukewarm and tap water washing on 0 day, whereas in 2X dose 59.01-44.24 per cent reduction was observed. In tomatoes, 38.87 per cent imidacloprid residues were reduced by tap water washing and 65.93 per cent by combination treatment (washing+cooking) (Dharumarajan and Dikshit, 2010). Imidacloprid residues were eliminated up to 61.89 per cent by NaCl (2%) in field beans followed by plain tap water and baking soda (0.1 %) treatment which was in the range of 22.74 and 56.11 per cent (Srinivasa *et al.*, 2018).

Imidacloprid is a systemic insecticide with moderate solubility (0.61 g/L at pH 7) in water. The octanol/water partition coefficient (Kow) ratio is dependent on the solubility of pesticide compounds in polar and non-polar solvent. The higher the Kow value, the greater the absorption and persistence (Finizio *et al.*, 1997). Imidacloprid has a moderate Kow value (0.57 at 21 ^{0}C). Hence

Table 3. Persistence and dissipation of imidacloprid 17.8% SL residues in/on bitter gourd

	Imidacloprid @ 20 g a.i ha^{-1} (X dose)						**Imidacloprid @ 40 g a.i ha^{-1} (2X dose)**					
Residues (mg kg^{-1})							**Residues (mg kg^{-1})**					
Days after treatment	**R1**	**R2**	**R3**	**Mean± SD**	**RSD (%)**	**Dissipation (%)**	**R1**	**R2**	**R3**	**Mean ± SD**	**RSD (%)**	**Dissipation (%)**
0 (2hrs)	0.68	0.66	0.69	0.68 ± 0.02	2.71	-	1.23	1.26	1.27	1.25 ± 0.02	1.79	-
1	0.45	0.47	0.47	0.46 ± 0.01	2.53	31.84	0.87	0.88	0.90	0.88 ± 0.02	1.87	29.39
3	0.33	0.34	0.35	0.34 ± 0.01	4.13	49.44	0.71	0.73	0.76	0.74 ± 0.02	3.10	41.32
5	0.26	0.25	0.23	0.25 ± 0.01	5.89	63.17	0.56	0.57	0.58	0.57 ± 0.01	1.71	54.20
7	0.10	0.10	0.09	0.10 ± 0.01	4.81	85.52	0.30	0.27	0.28	0.29 ± 0.02	5.54	77.10
10	0.04	0.03	0.04	0.04 ± 0.01	4.11	93.94	0.11	0.10	0.12	0.11 ± 0.01	8.58	91.30
15	BLQ	BLQ	BLQ	BLQ	-	-	0.04	0.05	0.05	0.05 ± 0.00	7.08	96.24
20	BLQ	BLQ	BLQ	BLQ	-	-	BLQ	BLQ	BLQ	BLQ	-	-
Harvest	-	-	-	ND	-	-	-	-	-	ND	-	-
Kinetic equation	$Y=0.7162e^{-0.275x}$						$Y=1.3018e^{-0.221x}$					
R^2 value	0.968						0.961					
Half-life	2.51 days						3.13 days					
PHI	4.45 days						8.30 days					

ND- Not Detected, BLQ-Below the Limit of Quantification (0.025 mg kg^{-1}), PHI- Pre-Harvest Interval

Table 4. Effect of different decontamination techniques on residues of imidacloprid @ 20 g a.i ha^{-1} on bitter gourd

Treatments	Residues in mg kg^{-1} and reduction (%)*							Mean Per cent reduction
	0 day (2 hr)	1 day	2 day	3 day	5 day	7 day	10 day	
T1	0.40 (40.38)	0.29 (37.61)	0.26 (33.01)	0.22 (31.62)	0.18 (30.10)	0.06 (30.66)	BLQ	33.90
T2	0.31 (53.62)	0.25 (45.88)	0.22 (42.65)	0.20 (39.90)	0.16 (37.57)	0.06 (39.64)	BLQ	43.16
T3	0.29 (56.62)	0.22 (52.66)	0.20 (48.61)	0.18 (46.09)	0.14 (44.19)	0.05 (43.93)	BLQ	48.68
T4	0.34 (48.91)	0.25 (45.82)	0.23 (41.33)	0.20 (39.39)	0.16 (38.34)	0.06 (37.83)	BLQ	41.94
T5	0.30 (54.38)	0.23 (50.46)	0.21 (46.48)	0.18 (44.91)	0.14 (43.33)	0.05 (43.31)	BLQ	46.65
T6	0.20 (70.09)	0.16 (66.19)	0.14 (63.56)	0.13 (61.58)	0.10 (60.04)	0.04 (60.28)	BLQ	63.79
T7	0.09 (87.04)	0.09 (81.10)	0.08 (77.26)	0.07 (76.59)	0.05 (75.27)	BLQ	BLQ	78.77
T8	0.14 (79.51)	0.11 (76.58)	0.10 (73.96)	0.09 (71.61)	0.07 (70.23)	BLQ	BLQ	75.10
T9	0.11 (83.75)	0.10 (79.33)	0.09 (76.74)	0.08 (75.38)	0.06 (73.70)	BLQ	BLQ	78.62
T0	0.67	0.47	0.39	0.33	0.25	0.09	0.04	-

* Mean of three replications, BLQ-Below the Limit of Quantification (0.025 mg kg^{-1}), Figures in parentheses reduction percentage

Table 5. Effect of different decontamination techniques on residues of imidacloprid @ 40 g a.i ha^{-1} on bitter gourd

Treatments	Residues in mg kg^{-1} and reduction (%)*							Mean Per cent reduction
	0 day (2 hr)	1 day	2 day	3 day	5 day	7 day	10 day	
T1	0.78 (36.78)	0.59 (36.45)	0.51 (35.58)	0.42 (33.22)	0.35 (32.49)	0.13 (32.96)	0.07 (32.64)	34.30
T2	0.61 (50.63)	0.51 (45.64)	0.45 (43.33)	0.37 (41.64)	0.32 (38.64)	0.11 (40.15)	0.06 (38.84)	42.70
T3	0.56 (54.72)	0.46 (50.61)	0.42 (47.19)	0.34 (46.08)	0.28 (46.22)	0.10 (46.42)	0.04 (45.13)	48.05
T4	0.65 (46.75)	0.55 (41.35)	0.48 (39.75)	0.39 (38.58)	0.34 (34.16)	0.12 (37.53)	0.06 (37.56)	39.38
T5	0.61 (50.60)	0.50 (46.71)	0.45 (43.47)	0.36 (43.32)	0.29 (42.83)	0.11 (43.88)	0.05 (42.19)	44.71
T6	0.30 (75.21)	0.29 (69.17)	0.28 (65.36)	0.23 (64.42)	0.19 (63.90)	0.07 (64.88)	BLQ	67.16
T7	0.16 (86.66)	0.15 (82.18)	0.14 (79.07)	0.12 (77.59)	0.12 (77.32)	0.04 (76.52)	BLQ	79.89
T8	0.23 (81.43)	0.22 (76.28)	0.20 (72.86)	0.19 (70.36)	0.16 (69.81)	0.05 (71.41)	BLQ	73.69
T9	0.19 (84.56)	0.18 (80.37)	0.16 (77.28)	0.15 (75.85)	0.12 (75.87)	0.05 (74.30)	BLQ	78.04
T0	1.23	0.93	0.80	0.63	0.51	0.19	0.10	-

* Mean of three replications, BLQ-Below the Limit of Quantification (0.025 mg kg^{-1}), Figures in parentheses reduction percentage

Table 6. Effect of washing on the reduction of imidacloprid residue in bitter gourd juice at 20 g a.i ha^{-1} (X)

Treatments	Residues in mg kg^{-1} and reduction (%)*						Reduction (%)
	0 day (2 hr)		1 day		2 day	3 day	
	Residues	PF	Residues	PF			
Washing in tap water	0.07 (42.89)	0.58	0.05 (41.86)	0.55	BLQ	BLQ	42.37
Washing in lukewarm water	0.06 (47.93)	0.50	0.05 (45.26)	0.55	BLQ	BLQ	46.59
Washing in salt solution (2%)	0.05 (60.84)	0.42	0.04 (57.42)	0.44	BLQ	BLQ	59.13
Washing in tamarind solution (2%)	0.05 (55.35)	0.42	0.04 (52.42)	0.44	BLQ	BLQ	53.88
Washing in lemon juice (2%)	0.05 (58.10)	0.42	0.04 (54.57)	0.44	BLQ	BLQ	56.34
Untreated (control)	0.12	-	0.09	-	0.04	BLQ	

* Mean of three replications, PF- Processing Factor, BLQ-Below the Limit of Quantification (0.025 mg kg^{-1}), Figures in parentheses reduction percentage

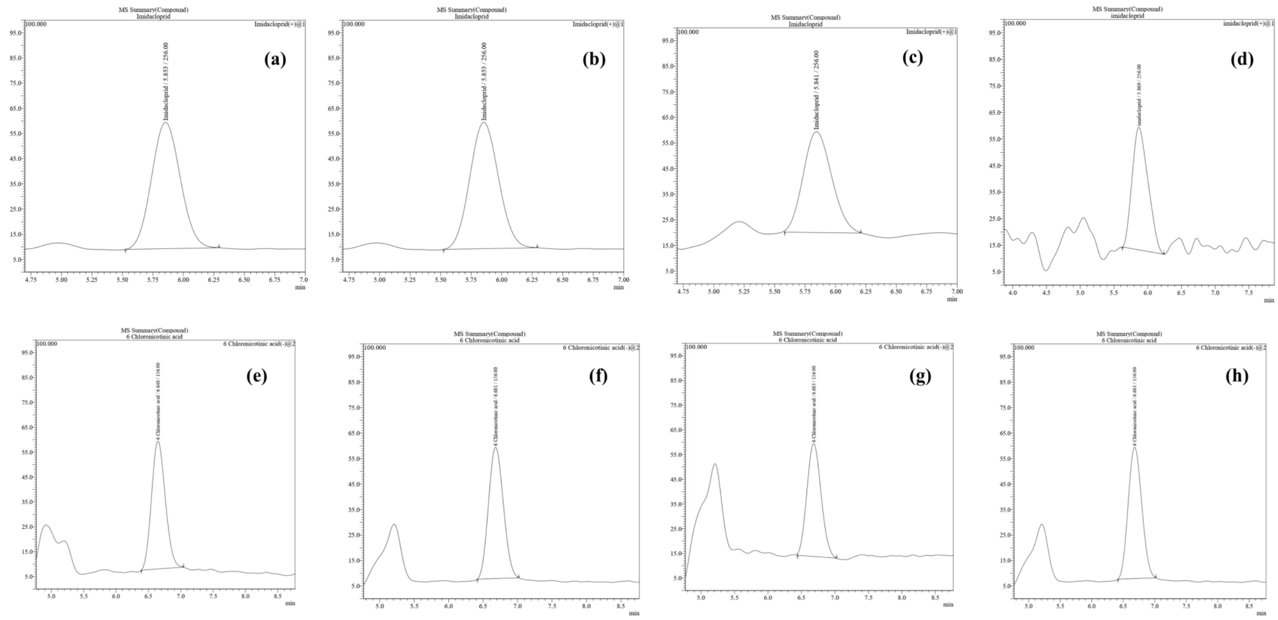

Fig. 1. *LC-MS chromatogram of imidacloprid standard (0.025 mg kg^{-1}) (a), fruit recovery (b), juice recovery (c), soil recovery (d), 6 CNA standard (0.025 mg kg^{-1}) (e), fruit recovery (f), juice recovery (g) and soil recovery (h)*

Table 7. Effect of washing on the reduction of imidacloprid residue in bitter gourd juice at 40 g a.i ha^{-1} (2X)

Treatments	Residues in mg kg^{-1} and reduction (%)*							
	0 day (2 hr)		1 day		2 day		3 day	Reduction (%)
	Residues	PF	Residues	PF	Residues	PF		
Washing in tap water	0.16 (44.24)	0.57	0.11 (43.61)	0.55	0.06 (40.50)	0.60	BLQ	42.37
Washing in luke-warm water	0.14 (48.24)	0.50	0.11 (45.89)	0.55	0.06 (45.50)	0.60	BLQ	46.59
Washing in salt solution (2%)	0.11 (59.01)	0.39	0.09 (55.16)	0.45	0.05 (56.02)	0.50	BLQ	59.13
Washing in tama-rind solution (2%)	0.14 (51.54)	0.50	0.09 (52.97)	0.45	0.05 (53.23)	0.50	BLQ	53.88
Washing in lemon juice (2%)	0.12 (58.06)	0.43	0.09 (53.01)	0.45	0.05 (55.12)	0.50	BLQ	56.34
Untreated (control)	0.28	-	0.20	-	0.10	-	BLQ	

* Mean of three replications, PF- Processing Factor, BLQ-Below the Limit of Quantification (0.025 mg kg^{-1}), Figures in parentheses reduction percentage

washing removed less concentration (48%) of the residue. Bitter gourd treated with chlorpyriphos (4.70 X 10^{2}), phorate, parathion (2.5 x 10^{4}), captafol and permethrin (6.1 X 10^{1}) showed 17.0 to 78.89 per cent removal of residues by washing (Joshi *et al*., 2015). The degradation or breakdown rate of the pesticide residue is highly dependent on the physico-chemical properties. The vapour pressure of imidacloprid is moderate (3 x 10^{-12} mmHg at 20 ^{0}C) and hence, the cooking method has resulted in moderate reduction of pesticide residues in the sample (Kwon *et al*., 2015). In comparison to other salts, sodium chloride has a high pesticide reduction potency and its high solubility in water might have resulted in higher removal of pesticide residues. The active ingredient citric acid in lemon juice is a chelating agent, contribute to loss of residues from treated surface (Mariappan and Kaithamalai, 2020). The tamarind solution has an acidic pH (1.8 to 3.7) with higher rate of furan derivatives and carboxylic acids (44.4 and 38.2 %). These volatile constituents and the acidic nature of tamarind might have contributed to the removal of chlorantraniliprole and imidacloprid residues (Nowowi *et al*., 2016).

These findings clearly depict that, all the decontamination procedures utilized in the study were more efficient in eliminating the pesticide residues. Imidacloprid residues were below MRL level (0.2 mg kg^{-1}) after 3 and 5 days of washing treatments and after 0 and 1 day for combined treatments in single and double doses. Hence, it is concluded that bitter gourd treated with imidacloprid at 20 and 40 g a.i ha^{-1} are safe for consumption when subjected to combined decontamination

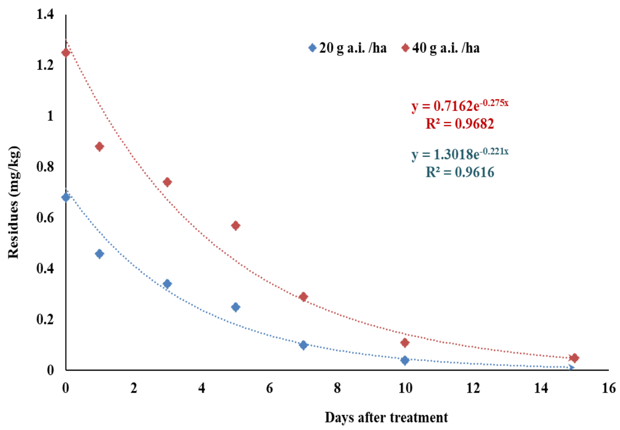

Fig. 2. *Dissipation kinetics of imidacloprid in bitter gourd fruit*

treatments. Bitter gourd juice consumption in 0 days poses no risk to the consumer at imidacloprid X and 2X doses when washing treatments were followed.

Risk assessment

The PHI for imidacloprid applied at 20 and 40 g a.i ha^{-1} was estimated as 4.45 and 8.30 days. The residues exceeded the MRL for up to 7 days in the X dose and up to 10 days in the 2X dose. Consequently, the evaluation of dietary risk to the consumer is crucial. The RQ calculated for the adult male and female with the body wt. of 65 and 55 showed no risk. The RQ value was found to be less than one in both single and double doses, even on 0 days of spraying (2 hours). This indicates that imidacloprid is safe for consumption and the risk is acceptable (Tables 8 and 9). The Processing factor calculated was in the range of 0.39 to 0.60 for bitter gourd juice (Tables 6 and 7) and PF was less than one in juice extracted from all various washing treatments, indicating safety to the consumers.

Conclusion

In the present study, a simple analytical technique was developed with high repeatability for the determination of imidacloprid and 6-CNA residues in bitter gourd fruit and soil using LC-MS to work out the dissipation pattern, half-life, PHI and decontamination of imidacloprid in bitter gourd (Eastwest F1 hybrid). Imidacloprid residues dissipated to below the LOQ level in 15 and 20 days at 20 and 40 g a.i ha^{-1} with half-life of 2.51 and 3.13 days, respectively. The preharvest interval was found to be 4.45 and 8.30 days, respectively. The metabolite 6-CNA was not detected in any of the samples collected at different intervals after spraying. The RQ was less than one and the risk to the consumer was at an acceptable level. Washing and cooking treatments

Table 8. Dietary risk assessment of imidacloprid in bitter gourd at 20 g a.i ha^{-1} (X)

Days after treatment	Imidacloprid residues* (mg/kg)	Dietary risk assessment (Male-65kg) ($\times 10^{-3}$)		Dietary risk assessment (Female-55kg) ($\times 10^{-3}$)	
		EDI (mg/kg/ bw/day)	Risk quotient (RQ)	EDI (mg/kg/ bw/day)	Risk quotient (RQ)
0 (2hrs)	0.68	0.6276	10.465	0.741	12.363
1	0.46	0.4246	7.079	0.508	8.363
3	0.34	0.3138	5.230	0.371	6.181
5	0.25	0.2307	3.846	2.73	4.545
7	0.10	0.0923	1.538	0.109	1.818
10	0.04	0.0369	0.615	0.043	0.727
15	BLQ	-	-	-	-

EDI-Estimated Daily Intake, BLQ-Below the Limit of Quantification (0.025 mg kg^{-1}), * Mean of residues

Table 9. Dietary risk assessment of imidacloprid in bitter gourd at 40 g a.i ha^{-1} (2X)

Days after treatment	Imidacloprid residues* (mg/kg)	Dietary risk assessment (Male-65kg) ($\times 10^{-3}$)		Dietary risk assessment (Female-55kg) ($\times 10^{-3}$)	
		EDI (mg/kg/ bw/day)	Risk quotient (RQ)	EDI (mg/kg/bw/day)	Risk quotient (RQ)
0 (2hrs)	1.25	1.154	19.231	1.364	22.727
1	0.88	0.812	13.528	0.960	16.000
3	0.74	0.683	11.384	0.807	13.455
5	0.57	0.526	8.769	0.621	10.363
7	0.29	0.267	4.461	0.316	5.272
10	0.11	0.101	1.692	0.120	2.000
15	0.05	0.046	0.769	0.054	0.909
20	BLQ	-	-	-	-

EDI-Estimated Daily Intake, BLQ-Below the Limit of Quantification (0.025 mg kg^{-1}), * Mean of residues minimized the imidacloprid residues ranging from 33.90 to 79.89 per cent. The decontamination study revealed that combining tap water and salt solution with cooking treatment was more efficient than the washing technique alone. Bitter gourd juice preparation considerably reduced imidacloprid residues after washing with NaCl solution and minimised the risk to consumers.

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Conflict of interest

The authors declare that they have no conflict of interest.

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