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Dissipation kinetics, effect of household processing, and dietary risk assessment of the insecticide chlorantraniliprole residue in bitter gourd and soil

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ABSTRACT: A single quadrupole liquid chromatography-mass spectrometry (LC-MS) method was validated to determine the insecticide chlorantraniliprole residues in bitter gourd fruit, juice, and soil, according to the SANTE guidelines. A linear curve was obtained ($R^2 > 0.99$) with LOD and LOQ at 0.003 mg kg^{-1} and 0.01 mg kg^{-1}. The accuracy (87–102%) and precision (RSD <5%) of the method was found to be satisfactory. The dissipation pattern of chlorantraniliprole 18.5% SC was studied by spraying twice at ten days intervals, at the recommended dose (X) (25 g a.i. ha^{-1}), and double the recommended dose (2X) (50 g a.i. ha^{-1}). The initial deposit on bitter gourd was 0.72 and 1.41 mg kg^{-1} and residues persisted up to 15 and 20 days with a half-life of 2.44 and 2.79 days at X and 2X doses, respectively. Simple decontamination techniques were found to reduce residues to the extent of 30 to 80 per cent. The reduction of chlorantraniliprole residues in bitter gourd juice was 40-50 per cent by different washing techniques. The estimated level of Risk quotient indicated (<1) chlorantraniliprole residues pose no dietary risk to consumers at the level detected.

KEY WORDS: LC-MS, validation, residue, persistence, dissipation pattern, decontamination, fruit, juice

INTRODUCTION

Bitter gourd (*Momordica charantia* L.) is a most common and preferred vegetable among the Cucurbitaceae family, grown in India (Singh and Sagar, 2013). The fruit is a rich source of vitamins (B1, B2, B3, B9 and C (88 mg100 g^{-1})), minerals (magnesium, zinc, manganese and phosphorus) and dietary fibre (Krishnendu and Nandini, 2016). The immature fruit contain anticancer and antiviral characteristics, and is useful in treating diabetic diseases (Tan *et al*., 2016). During the year 2020-21 the area and production of bitter gourd were 1.07 lakh hectares and 12.96 lakh metric tonnes (MT) in India respectively, whereas it was 0.24 lakh hectares and 0.44 lakh MT in Tamil Nadu respectively (NHB, 2021). As that of any cultivated vegetables, in bitter gourd also yield is threatened by different insect pests *viz.* aphids, melon fruit fly, hadda beetle, pumpkin caterpillar, and leaf miner. Fruit flies are the major pest of cucurbitaceous

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crops, causing output loss of 30 to 100 per cent depending on crop growth stages and seasons (Dhillon *et al*., 2005), thereby, warranting the application of insecticides for crop protection.

Approximately 13-14 per cent of total pesticide usage (0.678 a.i. kg/ha), including 4 per cent of insecticides is sprayed on vegetables and yields of most vegetables would fall by 50-90 per cent without insecticide (Subash and Kulvir, 2018). Notably, every dollar ($1) spent on pesticide for crops might save up to four dollars ($4) (Zhang, 2018). Inspite of economic protection, frequent application and indiscriminate pesticide usage particularly during the fruiting stage and unsafe waiting periods, may result in an accumulation of pesticide residues in vegetables (Lozowicka *et al*., 2014). The pesticide residues, left in the harvested vegetables may be hazardous to human health and affect trade due to pesticide residues exceeding Maximum Residue Limits (MRL) (Pimentel and Burgess, 2014). The investigation on overall pesticide usage profile in gourds ecosystem showed Chlorantraniliprole 18.50 per cent SC (76.67%) was commonly used insecticide for the management of insect pests (Mawtham *et al*., 2022). Keeping this background, a study was carried out to determine the dissipation and decontamination of chlorantraniliprole in bitter gourd.

Chlorantraniliprole, anthranilic diamide systemic insecticide is effective against Coleopteran, Lepidopteran and few Dipteran pests infesting crops such as bitter gourd, okra, chilli, brinjal and tomato (fruit borers), cabbage (diamondback moth), legumes (pod borers) (CIBRC, 2022). This compound has a unique mode of action and act on ryanodine receptor channels leading to inhibited regulation of muscle contraction due to internal Ca^{2+} store exhaustion (Bentley *et al*., 2010). The greater structural differences at ryanodine receptors between insects and mammals makes the insecticide highly selective and safe (Lahm *et al*., 2007). The physiochemical properties of chlorantraniliprole are water solubility (0.880 mg L^{-1} at 20^0C), vapor pressure (6.3 x 10^{-12} Pa at 20°C, 2.1 x 10^{-11} Pa @ 25°C), octanol/water partition coefficient (P) (pH 7 – 7.24 X 10^2 K_{OW} @ 20^0C) and dissociation constant (10.88 ± 0.71 pKa) (PPDB, 2022). It is an alternative for synthetic pyrethroids to vegetables because it is mentioned as a "low risk" insecticide (USEPA, 2008).

Instrumental analytical procedures for the quantitative analysis of chlorantraniliprole residues in crops like, capsicum, cauliflower, berseem, tomatoes, corn and soil using gas chromatography mass spectrometry, liquid chromatography mass spectrometry, high performance liquid chromatography and liquid chromatography with Orbitrap Mass spectrometry are available (Pathipati *et al*., 2017; Ahlawat *et al*., 2019; Malhat *et al*., 2012; Kar *et al*., 2013; Mandal *et al*., 2014; Dong *et al*., 2011). As there are no available published literature on chlorantraniliprole in bitter gourd, this research on developing and validating an analytical approach for detection and confirmation of chlorantraniliprole residues in bitter gourd fruit, juice and soil using LC-MS (liquid chromatography coupled with mass spectrometry) was undertaken. Furthermore, washing, peeling, cooking, blanching and other household techniques were reported to reduce the residual levels in food (Byrne and Pinkerton, 2004). Therefore, a study was also undertaken to evaluate whether or not simple culinary processes such as washing and cooking can minimise the pesticide residues in bitter gourd fruit and juice.

MATERIALS AND METHODS

Chemical and reagents: Certified reference material (CRM) of chlorantraniliprole (purity, 98.3%) was procured from Sigma-Aldrich Pvt. Ltd. (Bangalore, India). The commercial formulation (chlorantraniliprole 18.5% SC) was obtained from a local pesticide shop in Coimbatore, Tamil Nadu, India. Acetonitrile, formic acid and ammonium formate of LC-MS grade (Sigma Aldrich), LC-MS grade 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), Sorbents like graphitized carbon black (GCB) and primary secondary amine (PSA, 40 ìm) (Agilent Technologies, USA) were purchased from commercial suppliers as indicated.

Before usage, the magnesium sulphate was baked in a muffle furnace at 400 °C for 4 hours and maintained in an airtight desiccator in order to prevent the moisture absorption. Throughout the analysis, purified Millipore water (18.2 MÙ) from a lab-scale (Q3 Merck) Millipore unit was used.

Preparation of standard solutions: The chlorantraniliprole stock solution (400 mg L^{-1}) was prepared in methanol (LC-MS grade) by accurately weighing 10.17 mg of analytical standard into a calibrated (Class A) 25 ml volumetric flask. The intermediate standard (40 mg L^{-1}) was prepared by transferring 2.5 ml from the stock solution (400 mg L^{-1}) into a 25 ml volumetric flask and the volume was made with methanol. Serial dilution from the intermediate standard solution was made in the range of 0.0025 – 0.5 mg L^{-1} and matrix match standard solutions were prepared at 0.01, 0.025, 0.05, 0.075 and 0.1 mg L^{-1}. All standard solutions were kept in a -20 ^{0}C freezer until further use.

Field experiment: A supervised field trial was carried out in farmer's field at Annur block, Coimbatore district, Tamil Nadu, India (11.22^0 N latitude and 77.10^0 E longitude) from November 2021-March 2022, to study the dissipation pattern and decontamination of chlorantraniliprole in bitter gourd fruit. Bitter gourd (Eastwest F1 hybrid) was raised in 250 m^2 plot/treatment with three treatments following good agronomic practices. Chlorantraniliprole was applied at recommended dose @25 g a.i ha^{-1}(X) and double the recommended dose @50 g a.i ha^{-1} (2X) as per CIB&RC (2022) recommendations. The first spraying was done after 45 days of sowing followed by second spray at 10 days interval. An untreated plot (water spray) was maintained throughout the study period. Two consecutive sprays were done using a 500 L ha^{-1} spray fluid, high-volume knapsack compression sprayer during morning hours. During the field experiment, average maximum (29.0 ^{0}C) and minimum temperature (18.2 ^{0}C) and relative humidity (77.2 %) were recorded and there was no rainfall.

Sample collection and preparation: Two kilograms of bitter gourd fruit samples were randomly collected at each sampling intervals 0 (within 2 hr), 1, 3, 5, 7, 10, 15, 20, 25 and 30 days from treated and control plot after last insecticide application for dissipation study. The soil samples (15 days after spraying) collected at 0-15 cm depth 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 to carry out residue analysis. A high-volume blade homogeniser (Robot Coupe, Blixer 6 VVA, France) was used to homogenise the samples. Bitter gourd juice was extracted from homogenised extract of treated and untreated samples diluted 200 ml with water (100 ml) and filtered through a strainer. All the samples were stored at -20 ^{0}C until residue analysis.

Extraction and clean-up: The chlorantraniliprole residues were extracted and cleaned up from bitter gourd fruit, juice, and soil matrices by modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method (Anastassiades *et al*., 2003). Representative samples (3 replicates each) of 10 g were weighed in a 50 ml polypropylene centrifuge tube, 20 ml of acetonitrile was added and vortexed for one minute. Then four gram of anhydrous magnesium sulphate and one gram of sodium chloride were added, mixed thoroughly using vortex and centrifuged for 10 minutes at 6000 rpm. After centrifugation, the upper acetonitrile layer (10 ml) was passed through anhydrous sodium sulphate (4 g) to remove moisture traces. A six-millilitre supernatant was transferred into a 15 ml centrifuge tube containing PSA (150 mg), GCB (25 mg) and $MgSO_4$ (900 mg), vortexed for one minute and centrifuged at 3000 rpm for 10 min. A four-millilitre supernatant acetonitrile phase was carefully pipetted out to a clean glass tube and evaporated to near dryness at 35°C using turbovap LV with a gentle stream of nitrogen. The residues were then redissolved in one millilitre of methanol, filtered through a 0.2-micron PTFE syringe filter (Millipore, USA) and transferred to 1.5 ml autosampler glass vials for LC-MS analysis.

Chlorantraniliprole residue was estimated using 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) at a column oven temperature of 40°C. Solvents A (ultra-pure

water with two mM ammonium formate, 0.05 per cent formic acid) and B (Methanol with two mM ammonium formate, 0.05 per cent formic acid) were used as mobile phase (30:70 V/V). Before use, mobile phases were degassed for 20 min in a sonicator. Then, the mobile phase was discharged at a constant isocratic flow rate of 0.5 ml/min using the LC-MS pump in binary mode at a pressure of 48 kg/cm^2. Shimadzu lab solutions software version 5.6 was used to operate the instruments and analyse the chromatograms. The chromatograms and sample were ionized by positive electrospray ionization (ESI+) in selected ion monitoring (SIM) at m/z 483 and interface mode with 0.1 ìA^o of interface current. The optimised instrument parameters were capillary voltage 3.5 kV; heat block temperature 200 ^{0}C; desolvation line temperature 250 ^{0}C; nebulizer gas (N_2-99.99%) flow (1.5 lmin^{-1}), drying gas (15 lmin^{-1}), injection volume was 10 µl and 15000 sec scan speed. The residue detection method was developed and validated for the parameters such as linearity, sensitivity, accuracy, and precision and matrix effect (SANTE, 2021).

Linearity: Chlorantraniliprole linearity curves were obtained for solvent and matrix match calibration standards by injecting seven concentrations ranging from 0.0025 to 0.5 mg kg^{-1} in LC-ESI-MS with six replications. The linear relationship between the concentration and signal area were calculated.

Sensitivity: Limit of detection (LOD) and limit of quantification (LOQ) were calculated by injecting the matrix match standards of chlorantraniliprole starting from lowest concentration level (0.01 mg kg^{-1}). Based on the calibration curve, LOD and LOQ were calculated.

LOD = 3 x (Standard Deviation/Slope)
LOQ = 10 x (Standard Deviation/Slope)

Accuracy (recovery): Experiments were carried out by spiking five different concentrations of chlorantraniliprole (0.01, 0.025, 0.05, 0.075 and 0.1 mg kg^{-1}) in bitter gourd fruit, juice and soil samples with six replications. Recovery (%) was calculated by comparing the peak area of the known quantity of analytes in the spiked sample (prior extraction) and matrix match standard.

Precision (repeatability): The precision of the method was evaluated through relative standard deviation (RSD) for each spiking level (0.01 to 0.1 mg kg^{-1}) of bitter gourd fruit, juice and soil matrix.

Matrix effect (ME): The peak area of the matrix standard was compared with the peak area of the solvent standard to measure matrix effects (Mariappan and Kaithamalai, 2020). The ME was calculated using the following equation:

$$ME\ (\%) = \frac{\text{(Peak area of matrix standard - Peak area of solvent standard)}}{\text{Peak area of matrix standard}} \times 100$$

Data Analysis: The concentration of chlorantraniliprole residue was calculated using—

$$\text{Residue (mg kg}^{-1}) = \frac{A_1 \times C \times I_1 \times F}{A_2 \times W \times I_2}$$

Where A_1= Peak area of chlorantraniliprole in the sample solution,

A_2 = Peak area of chlorantraniliprole in the standard solution,

C = Concentration of standard solution (mg kg^{-1}),

I_1 = Injected volume of standard (ìl), I_2 = Injected volume of sample (ìl), W = mass of the sample (g) and F = Final volume of the sample (ml).

The dissipation of chlorantraniliprole residue followed first-order rate of kinetics 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). $T_{1/2} = \ln (2)/k$ was used to compute the half-life of chlorantraniliprole and pre-harvest interval (PHI) was calculated using the formula PHI = [ln Co - ln MRL]/k (Hoskins, 1961; Handa *et al*., 1999). According to the Codex Alimentarius and the European pesticide (EU) database, the maximum residue limit (MRL) for chlorantraniliprole in bitter gourd fruit is 0.3 mg kg^{-1}.

Decontamination studies: The effect of simple culinary practices in removing chlorantraniliprole residues from bitter gourd fruit was assessed through laboratory experiment. After second spraying, samples were collected at 0, 1, 2, 3, 5, 7 and 10 days. For each treatment, a sample of one kilogramme of bitter gourd fruit was taken. The fruits were then subjected to decontamination methods *viz*., washing with tap water (pH 7.0), 2% salt solution, lukewarm water (40 ^{0}C), 2% tamarind solution, 2% lemon solution, and also subjected to cooking. Washing was done for one min which cooking was done for 10 min under open cook method. The treatments were also combined and evaluated as methods like, tap water washing + 2% salt solution + 10 min cooking, tap water washing + 2% tamarind solution + 10 min cooking, tap water washing + 2% lemon solution + 10 min cooking. Decontamination solutions (2%) were made by mixing 20 g of each common salt, tamarind and lemon juice in one litre of water and the fruits were gently rubbed in the solution for about 1 min. In lukewarm (40 ^{0}C) and tap water (1L) treatment, fruit were immersed for 1 min and then gently rubbed with hands. In cooking treatment, part of fruit sample was 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 and then homogenised. Chlorantraniliprole residues were estimated by following the above standardized methodology.

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

$$PF = \frac{\text{Residue of processed product (mg kg}^{-1})}{\text{Residue of raw agricultural commodity (mg kg}^{-1})}$$

Dietary risk assessment: The maximum residue obtained from the field trial was multiplied by the average food consumption rate (60 g day^{-1}) (NIN, 2020) divided by the average adult male (65 kg) and female weight (55 kg) to compute the estimated daily intake (EDI) of chlorantraniliprole residue (Dong *et al*., 2018). The risk quotient (RQ) was derived by dividing the EDI by acceptable daily intake (ADI) of the insecticide and expressed in mg kg^{-1} body weight (BW)/day. The ADI for chlorantraniliprole is 1.58 mg kg^{-1} BW day^{-1} (EFSA, 2012). The risk of long-term human dietary consumption of chlorantraniliprole residues in food is acceptable when RQ is less than one and unacceptable if RQ is more than one.

RESULTS AND DISCUSSION

Method validation

The results of method optimization were satisfactory for all validation parameters studied following the SANTE guidelines (SANTE, 2021). Linear response was assessed for different solvent concentrations (0.0025-0.5 mg kg^{-1}) and matrix match standard concentration (0.01-0.1 mg kg^{-1}) (Fig. 1). Good linear curve and correlation coefficient (R^2) values of chlorantraniliprole in solvent (0.999), fruit matrix match standards (0.997), fruit juice (0.998) and soil (0.998) (Fig. 2) were obtained. LOD was 0.003 mg kg^{-1} and LOQ was 0.01 mg kg^{-1}. In bitter gourd fruit, juice, and soil, the recovery was within 87.45-101.08 per cent and RSD was 1.02-4.22 per cent (Table 1 and Fig. 3 (a,b,c)). The LOQ estimated using above method was less than the MRL value (0.3 mg kg^{-1}). The matrix effect was within 0.35-9.86 per cent of the spiked chlorantraniliprole standards in the bitter gourd fruit and soil. Matrix effect was not found in fruit juice. Matrix effect values were less than 20 per cent in both matrices, indicating that the samples had no apparent matrix effect and that the purification effect was acceptable.

Dissipation of chlorantraniliprole in bitter gourd and soil

Bitter gourd and soil samples were collected at periodical interval from 0 to 30 days after the last application for residue analysis. The average residues of chlorantraniliprole in bitter gourd fruits were 0.72 and 1.41 mg kg^{-1} as initial deposit at single and double the doses (Table 2 and Fig. 3d). After third day, more than 50 per cent of the residues

Table 1. Recovery percentage of chlorantraniliprole in different matrices of bitter gourd

Spiked (mg kg-1)	Recovered conc	Recovery* (mg kg-1)	Repeatability (RSD %)
		Fruit	
0.01	0.01	101.08 ± 1.03	1.02
0.025	0.03	100.03 ± 1.66	1.66
0.05	0.05	96.16 ± 3.48	3.62
0.075	0.07	99.21 ± 2.56	2.58
0.10	0.10	96.97 ± 2.70	2.78
		Juice	
0.01	0.01	100.86 ± 3.90	3.87
0.025	0.02	96.72 ± 4.08	4.22
0.05	0.04	93.97 ± 3.47	3.69
0.075	0.06	92.57 ± 4.18	4.51
0.10	0.09	90.94 ± 3.04	3.34
		Soil	
0.01	0.01	92.62 ± 1.50	1.62
0.025	0.02	92.82 ± 1.96	2.12
0.05	0.04	87.45 ± 1.26	1.44
0.075	0.07	94.50 ± 2.68	2.83
0.10	0.09	92.49 ± 2.63	2.85

*Mean of six replications, RSD- Relative Standard Deviation

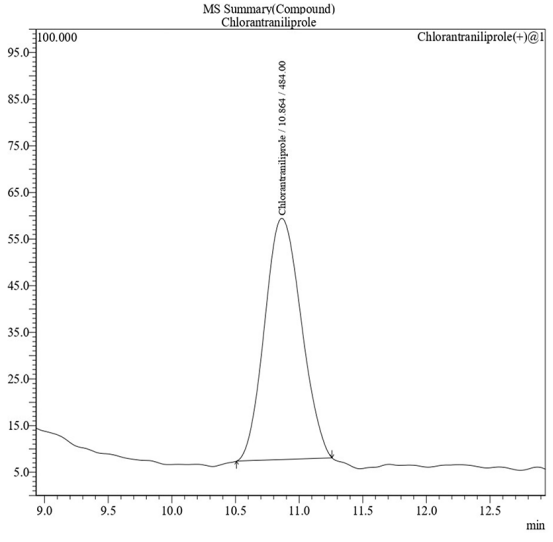

Fig. 1. LC-MS chromatogram of chlorantraniliprole standard (0.01 mg kg^{-1})

dissipated from bitter gourd and reached BLQ (0.01 mg kg^{-1}) on 15 and 20 days after application in single and double the doses, respectively (Fig. 4). The chlorantraniliprole residue was not detected in soil samples collected at harvest (15 days after second spray). The residual deposition and persistence of pesticides are affected by several factors: type of pesticide, its formulation, active ingredient, carrier material, meteorological parameters, plant growth, and type of plant (Lavtizar *et al*., 2014). Initial concentration and persistence of chlorantraniliprole residues varied with crops. In capsicum, chlorantraniliprole residue levels were 3.16 and 4.18 mg kg^{-1} on 0 day after treatment at 30-60 g a.i. ha^{-1} (Ahlawat *et al*., 2019). In tomato fruit the initial residue concentration of chlorantraniliprole was 2.31 mg kg^{-1} and reached BLQ (0.01 mg kg^{-1}) after 21 days of application at 30 g a.i. ha^{-1} (Malhat *et al*., 2012). Chlorantraniliprole initial residues were 0.18 and 0.29 mg kg^{-1} and reached BLQ after 5 and 7 days from last application on cauliflower (Kar *et al*., 2013). In okra, chlorantraniliprole residue in soil was BLQ after 15 days of last application and was attributed to microbial and chemical degradation (Singla *et al*., 2020).

Half-life and pre-harvest interval (PHI)

The half-life (DT_{50}) values of chlorantraniliprole were 2.44 and 2.79 days at 25 and 50 g a.i. ha^{-1}, respectively (Table 2). The pre-harvest interval (PHI) was calculated using the kinetic equation obtained from a graph of residues vs time. The PHI was found to be 3.09 and 6.24 days for 25 and 50 g a.i. ha^{-1} of chlorantraniliprole, respectively (Table 2). This shows that consumption of bitter gourd is safe at 3.09 days if recommended dose is followed and at higher doses of chlorantraniliprole 6.24 days is recommended for safe harvest of bitter gourd fruit. The chlorantraniliprole residues dissipated with the half-life of 2.70 days in grapes and 10.0 and 15.2 days in cabbage (Malhat, 2012; Lee *et al*., 2019). Treatment of chlorantraniliprole at 30 and 60 g a.i. $ha^{"1}$ in brinjal required a PHI of 0.69 and 2.38 days (Vijayasree *et al*., 2015).

Decontamination of chlorantraniliprole residue in bitter gourd fruits and juices

The effect of different household techniques on the reduction of chlorantraniliprole residues was studied. All decontamination procedures were effective in terms of reducing chlorantraniliprole residues from the bitter gourd fruits (Table 3,4). Among the various decontamination methods, tap water washing + salt solution+ cooking, tap water washing + lemon solution + cooking and tap water washing + tamarind solution + cooking were all very effective in removing chlorantraniliprole residues to the extent of 75.10 - 80.70 per cent in both doses of treatments. In cabbage and cauliflower (17-40%), reduction of chlorantraniliprole residues was observed by washing with tap water and above 90 per cent by boiling (Kar *et al*., 2012). Chlorantraniliprole is a systemic insecticide with moderate solubility (pH 7- 0.880 mg/L) in water. Solubility of pesticide compound in polar and non-polar solvent determines the octanol/water partition coefficient (Kow) ratio. Higher the Kow value higher will be the absorption and retention (Finizio *et al*., 1997). The Kow value of chlorantraniliprole is moderate (7.24×10^2) and hence less removal by washing was expected (<48%). Bitter gourd treated with, phorate, chlorpyriphos (4.70×10^2), parathion (2.5×10^4), permethrin (6.1×10^1) and captafol showed 17.0 to 78.89 per cent loss of residues by washing (Joshi *et al*., 2015). Among the household techniques, cooking process caused the maximum reduction (62.42-71.21%) of chlorantraniliprole residues. The rate of degradation or break down of the pesticide residue is highly dependent on the physico-chemical properties of chemical. The vapour pressure of chlorantraniliprole is moderate (6.3×10^{-12} Pa at 20°C) and hence, the cooking method has resulted in moderate reduction of pesticide residues in the sample (Kwon *et al*., 2015).

Among the washing treatments, 2 per cent salt solution was the most effective, by eliminating 46.40 to 48.68 per cent of residues in bitter gourd fruit. Washing with 2 per cent lemon solution or 2 per cent tamarind solution reduced chlorantraniliprole residue in bitter gourd fruit by 38.49 to 46.65 per cent, whereas washing with lukewarm water reduced chlorantraniliprole by 43 per cent and washing with tap water reduced 33-37 per cent of chlorantraniliprole residues. In bitter gourd juice, sodium chloride washing effectively influenced the loss of residues to an extent of 54 per cent followed by tamarind solution (51%), lemon solution (50%), lukewarm water (42%) and tap water (40%) (Table 5&6). The Processing Factor (PF) calculated was in the range of 0.42-0.67 for bitter gourd juice (Table 5&6). PF was less than one in juice extracted from all washing treatments indicating insignificant transfer of residues from raw fruit into juice. After three days of insecticide application, the washing treatments decreased the residues below the limit of quantification (0.01 mg kg^{-1}) in both 25 and 50 g a.i. ha^{-1}. In capsicum, chlorantraniliprole residues were eliminated up to 68 per cent by NaCl (5%) followed by hot water and plain tap water treatment which were in the range of 55-58 per cent (Ahlawat *et al*., 2019). In vegetable cowpea, lime and vinegar were more effective in removing chlorantraniliprole residues (87.47-91.70%) (Vijayasree *et al*., 2013). In comparison to other salts, sodium chloride has a high reduction potency and its high solubility in water might have resulted in a higher removal of pesticide residues. Citric acid in lemon is a chelating agent and effectively eliminates pesticide residues from bitter gourd fruit (Chandra *et al*., 2015). 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 along with acidic nature of tamarind, might have contributed for the removal of chlorantraniliprole residues (Nowowi *et al*., 2016).

In bitter gourd, a combined treatment of tap water + salt solution + cooking, one followed by another, eliminated 86 per cent of chlorantraniliprole residues on 0 day, more than 70 per cent on second and seventh days after application. The other treatments, tap water + lemon and tamarind solution + cooking caused maximum reduction on 0 day (79.94 to 84.86%), followed by second (69.83-77.50%) and seven (68.46-77.01%) days after second application. On 10^{th} day in both single and double the doses, chlorantraniliprole residue reached BLQ (0.01 mg kg^{-1}) in the combined

Table 2. Persistence and dissipation of chlorantraniliprole 18.5% SC residues in/on bitter gourd

Chlorantraniliprole @ 25 g a.i ha^{-1} (X dose)				Chlorantraniliprole @ 50 g a.i ha^{-1} (2X dose)		
Days after treatment	Mean residues (mg kg^{-1}) ± SD*	RSD (%)	Dissipation (%)	Mean residues (mg rkg^{-1}) ± SD*	RSD (%)	Dissipation (%)
0 (2hrs)	0.72 ± 0.02	3.16	-	1.41 ± 0.04	3.13	-
1	0.48 ± 0.01	1.62	33.83	0.96 ± 0.01	0.86	32.09
3	0.30 ± 0.01	2.33	58.03	0.62 ± 0.03	4.37	55.85
5	0.19 ± 0.01	3.37	73.25	0.45 ± 0.01	1.64	68.07
7	0.10 ± 0.01	7.98	86.48	0.27 ± 0.01	2.93	80.75
10	0.04 ± 0.01	13.62	94.71	0.11 ± 0.01	6.03	92.54
15	0.01 ± 0.00	6.83	99.19	0.03 ± 0.00	6.64	98.01
20	BLQ	-	-	0.01 ± 0.00	6.64	99.57
25	BLQ	-	-	BLQ	-	-
Kinetic equation	$y = 0.703e^{-0.283x}$			$y = 1.3723e^{-0.248x}$		
R^2 value	0.992			0.990		
Half-life	2.44 days			2.79 days		
PHI	3.09 days			6.24 days		

*Mean of three replications, SD- Standard Deviation, RSD- Relative Standard Deviation, ND- Not Detected, BLQ-Below the Limit of Quantification (0.01 mg kg^{-1}), PHI- Pre-Harvest Interval

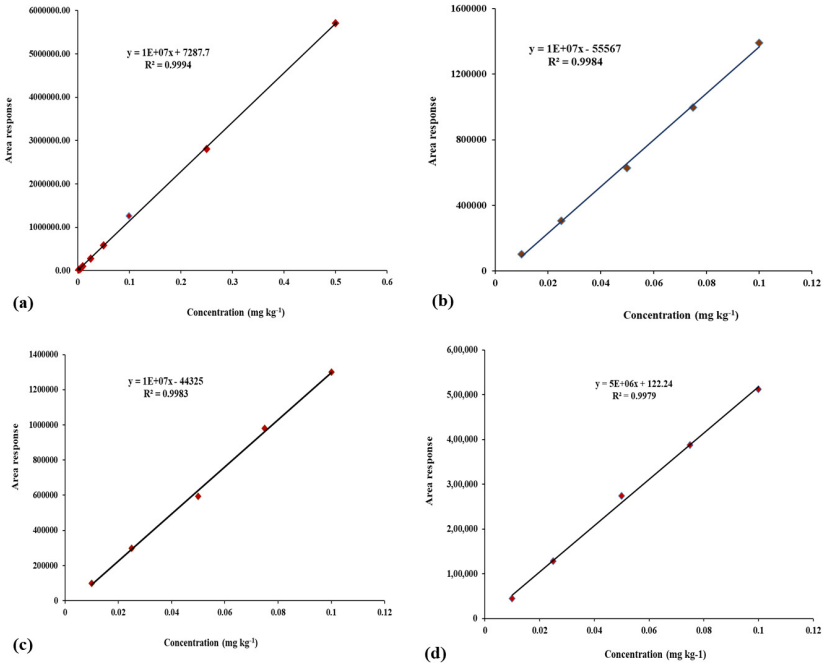

Fig. 2. Calibration curve for the chlorantraniliprole in methanol solvent (a), bitter gourd fruit matrix (b), soil matrix (c) and bitter gourd juice matrix (d)

Table 3. Effect of different decontamination techniques on residues of chlorantraniliprole @ 25 g a.i ha^{-1} on bitter gourd fruit

Treatments	Residues in mg/kg and reduction (%)*							Mean Per cent reduction
	0 day (2 hr)	1 day	2 day	3 day	5 day	7 day	10 day	
Washing in tap water	0.39 (42.83)	0.28 (39.25)	0.24 (37.96)	0.22 (31.62)	0.12 (34.58)	0.07 (33.87)	0.03 (34.82)	36.99
Washing in lukewarm water	0.33 (50.84)	0.24 (46.83)	0.22 (43.44)	0.20 (39.90)	0.11 (40.27)	0.07 (40.46)	0.02 (39.47)	43.23
Washing in (2%) salt solution	0.32 (52.41)	0.23 (49.36)	0.21 (46.41)	0.18 (46.09)	0.11 (43.35)	0.06 (44.19)	0.02 (44.78)	46.40
Washing in tamarind solution (2%)	0.37 (45.36)	0.27 (40.73)	0.24 (37.60)	0.20 (39.39)	0.12 (36.26)	0.07 (36.77)	0.03 (36.06)	38.49
Washing in lemon juice (2%)	0.33 (51.63)	0.25 (46.46)	0.21 (45.45)	0.18 (44.91)	0.11 (42.14)	0.06 (43.72)	0.02 (42.15)	45.02
Cooking	0.19 (71.21)	0.13 (71.23)	0.14 (63.56)	0.11 (65.44)	0.07 (64.32)	0.04 (64.23)	BLQ	67.47
Tap water washing + salt solution (2%) + cooking	0.09 (86.43)	0.08 (82.23)	0.08 (77.26)	0.05 (78.85)	0.04 (78.10)	0.02 (78.33)	BLQ	80.70
Tap water washing + tamarind solution (2%) + cooking	0.12 (82.88)	0.10 (77.19)	0.10 (73.96)	0.07 (72.31)	0.05 (72.40)	0.03 (72.70)	BLQ	75.78
Tap water washing + lemon juice (2%) + cooking	0.10 (84.86)	0.09 (80.23)	0.09 (76.74)	0.06 (77.83)	0.04 (76.39)	0.03 (77.01)	BLQ	79.33
Untreated (control)	0.68	0.46	0.38	0.32	0.19	0.11	0.04	

* Mean of three replications, BLQ-Below the Limit of Quantification (0.01 mg kg^{-1}), Figures in parentheses are reduction percentage

treatments. Chlorantraniliprole residues were found to be less than the MRL (0.3 mg kg^{-1}) after 1 and 3 days of individual washing treatments and after 0 and 1 day when treatments were combined in single and double doses. Hence concluded that safe consumption of raw fruit after 0 and 1 day subjecting to combined treatments at chlorantraniliprole 25 and 50 g a.i. ha^{-1}. Bitter gourd juice consumption after 0 day poses no risk to the consumer at chlorantraniliprole 25 and 50 g a.i. ha^{-1} when washing treatments were followed.

Risk assessment

The PHI for chlorantraniliprole applied at 25 and 50 g a.i. ha^{-1} was estimated at 3.09 and 6.24 days. In bitter gourd, the MRL for chlorantraniliprole is 0.3 mg kg^{-1} (Codex). Though the residues were exceeding MRL up to 3 days in X dose and up to 5 days in 2X dose, RQ calculated taking into the quantity consumed (60 g/day), showed no risk. The

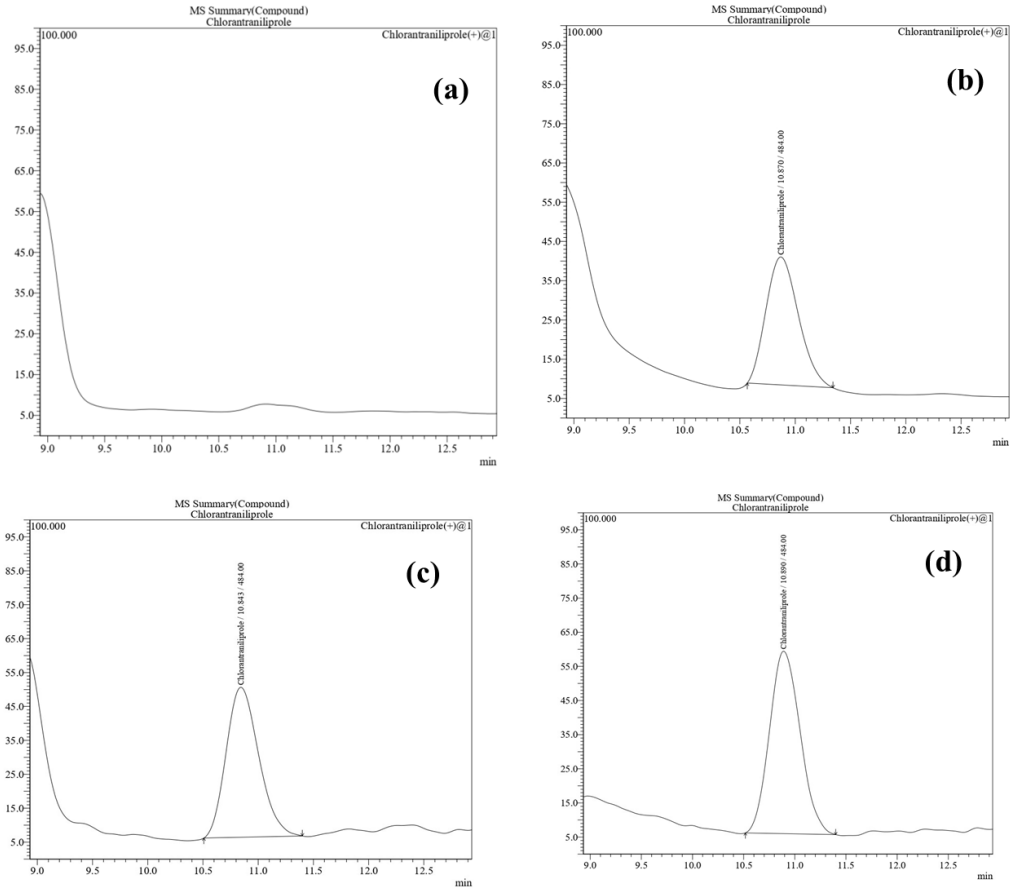

Fig. 3. LC-MS chromatogram of chlorantraniliprole bitter gourd control (a), fruit matrix match (b), fruit recovery (c) and treated field sample (d)

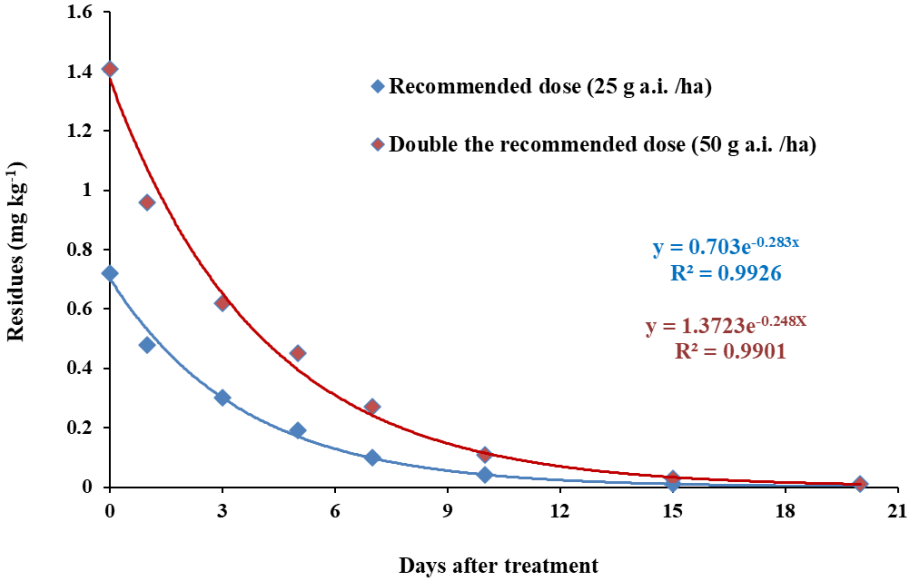

Fig. 4. Dissipation kinetics of chlorantraniliprole in bitter gourd fruit

Table 5. Effect of washing on the reduction of chlorantraniliprole residue in bitter gourd juice at 25 g a.i ha^{-1}

Treatments	Residues in mg kg^{-1} and reduction (%)*								
	0 day (2 hr)		1 day		2 day		3 day	5 day	Reduction (%)
	Residues	PF	Residues	PF	Residues	PF			
Washing in tap water	0.10 (42.39)	0.59	0.06 (41.58)	0.6	0.04 (36.58)	0.57	BLQ	BLQ	40.19
Washing in lukewarm water	0.09 (44.27)	0.53	0.06 (43.28)	0.6	0.04 (39.99)	0.57	BLQ	BLQ	42.52
Washing in salt solution (2%)	0.08 (54.76)	0.47	0.05 (56.47)	0.5	0.03 (51.49)	0.43	BLQ	BLQ	54.24
Washing in tamarind solution (2%)	0.08 (52.55)	0.46	0.05 (52.85)	0.5	0.03 (48.43)	0.43	BLQ	BLQ	51.28
Washing in lemon juice (2%)	0.08 (50.93)	0.47	0.05 (51.52)	0.5	0.03 (49.31)	0.43	BLQ	BLQ	50.59
Untreated (control)	0.17	-	0.10	-	0.07	-	0.01	BLQ	

* Mean of three replications, PF- Processing Factor, BLQ-Below the Limit of Quantification (0.01 mg kg^{-1}), Figures in parentheses are reduction percentage

Table 6. Effect of washing on the reduction of chlorantraniliprole residue in bitter gourd juice at 50 g a.i ha^{-1}

Treatments	Residues in mg kg^{-1} and reduction (%)*									
	0 day (2 hr)		1 day		2 day		3 day		5 day	Reduction (%)
	Residues	PF	Residues	PF	Residues	PF	Residues	PF		
Washing in tap water	0.22 (41.26)	0.58	0.13 (40.89)	0.56	0.09 (39.22)	0.6	0.04 (36.09)	0.67	BLQ	39.37
Washing in lukewarm water	0.20 (47.13)	0.53	0.13 (44.62)	0.56	0.09 (41.60)	0.6	0.04 (37.57)	0.67	BLQ	42.73
Washing in salt solution (2%)	0.16 (57.90)	0.42	0.10 (56.50)	0.43	0.07 (53.51)	0.47	0.03 (51.88)	0.50	BLQ	54.95
Washing in tamarind solution (2%)	0.18 (53.34)	0.47	0.11 (52.55)	0.47	0.07 (50.33)	0.47	0.03 (46.14)	0.50	BLQ	50.60
Washing in lemon juice (2%)	0.17 (53.71)	0.44	0.11 (51.95)	0.48	0.08 (49.1)	0.53	0.03 (47.72)	0.50	BLQ	50.63
Untreated (control)	0.38	-	0.23	-	0.15	-	0.06	-	BLQ	

* Mean of three replications, PF- Processing Factor, BLQ-Below the Limit of Quantification (0.01 mg kg^{-1}), Figures in parentheses are reduction percentage

Table 7. Dietary risk assessment of chlorantraniliprole in bitter gourd at 25 g a.i ha^{-1} (X) and 50 g a.i ha^{-1} (2X)

Days after treatment	X dose				2X dose			
	Dietary risk assessment (Male-65kg)		Dietary risk assessment (Female-55kg)		Dietary risk assessment (Male-65kg)		Dietary risk assessment (Female-55kg)	
	EDI (mg/kg/ bw/day)	Risk quotient (RQ)	EDI (mg/kg/ bw/day)	Risk quotient (RQ)	EDI (mg/kg/ bw/day)	Risk quotient (RQ)	EDI (mg/kg/ bw/day)	Risk quotient (RQ)
0 (2hrs)	0.000665	0.000421	0.000785	0.000497	0.001301	0.000823	0.001538	0.000974
1	0.000443	0.000280	0.000524	0.000331	0.000886	0.000561	0.001047	0.000663
3	0.000277	0.000175	0.000327	0.000207	0.000572	0.000362	0.000676	0.000428
5	0.000175	0.000111	0.000207	0.000131	0.000415	0.000263	0.000491	0.000311
7	0.000092	0.000058	0.000109	0.000069	0.000249	0.000157	0.000295	0.000186
10	0.000037	0.000023	0.000044	0.000027	0.000102	0.000064	0.000120	0.000076
15	0.000009	0.000006	0.000011	0.000007	0.000027	0.000018	0.000033	0.000021
20	-	-	-	-	0.000009	0.000006	0.000011	0.000007
25	-	-	-	-	-	-	-	-

EDI-Estimated Daily Intake, BLQ-Below the Limit of Quantification (0.01 mg kg^{-1})

risk quotient (RQ) was calculated by dividing the EDI by ADI in mg kg^{-1} body weight (BW)/day. Even on the 0 (within 2 hr) day of spraying, the RQ value was found to be less than one in both single and double the doses, indicates that chlorantraniliprole is safe for consumption and the risk is acceptable (Table 7).

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