Degradation behaviour, impact of post-harvest processing and dietary risk assessment of frequently detected pesticides in curry leaves

K. Bhuvaneswari*, J. Kousika, P. Anuradha, V. Muralitharan and P. Karthik

Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore 641 003, India

Supervised field experiments were conducted to study the dissipation of ethion, chlorpyriphos, profenophos, carbendazim and cypermethrin in curry leaves. An analytical method was developed in line with the SANTE guideline on the method validation procedure for pesticide residue analysis. The validated method showed good recovery (70–120%) and repeatability (<20%). The limit of detection and limit of quantification were determined as 0.01 and 0.05 mg/kg respectively, for all analytes, except cypermethrin (0.05 and 0.1 mg/kg respectively). The mean initial deposit of pesticides analysed ranged from 9.53 to 93.92 mg/kg with a half-life of <8 days. Except for ethion, the dietary risk assessment for women, men and children was at an acceptable level.

Keywords: Curry leaves, dietary risk, dissipation, method validation, processing factor, residue.

THE curry tree (*Murraya koenigii* L.), a member of the citrus family Rutaceae, is indigenous to Asia. In India, it is widely cultivated in Tamil Nadu (TN), Karnataka, Andhra Pradesh and Telangana¹. Coimbatore, Salem and Tiruchirapalli are the major districts of TN, having 2524 ha under curry leaves cultivation with a production of 35,268.05 MT. Coimbatore alone shares 49% (1240 ha) of area and production². Curry leaf is an essential ingredient used in Indian cuisine for flavouring and seasoning due to its pleasant aroma and flavour. It was valued as a major exported commodity from India during 2016–17. The country has exported 600 tonnes of curry leaves worth Rs 371 lakh, particularly to the European Union (EU)³.

Curry leaves are infested by 12 insect pests belonging to 10 families and five insect orders. Pest management is solely dependent on the application of pesticides. However, no pesticides are registered with the Central Insecticide Board and Registration Committee, Faridabad, Haryana. In order to ensure higher returns, farmers are indiscriminately applying pesticides. Ramakrishnan *et al.*⁴ surveyed pesticides used in curry leaves by farmers in the Coimbatore. According to them, 32% of farmers used profenophos 40% + cypermethrin 4%, followed by dimethoate (12%), profenophos (11%), triazophos (7%), cypermethrin (6%),

malathion (2%) and fenazaquin (1%). The other pesticides were quinalphos, hexythiazox, difenoconazole and Exodus, which is marketed as a plant-based product. The majority of the farmers applied pesticides regularly at a 7–15-day interval.

Consequently, detecting pesticide residues in curry leaves is a major hurdle in trade. The rapid alert system for food and feed of Europe has issued a red alert notice for the detection of pesticides above the legal limit in a shipment of curry leaves and has found high levels of non-compliance for curry leaves exported from India⁵. In 2017–18, the monitoring of pesticide residues at the national level (MPRNL) scheme tested 616 curry leaf samples, of which 438 contained pesticide residues⁶. Curry leaf/powder exported from India is also contaminated with insecticide residues like chlorpyriphos, profenophos, triazophos, cypermethrin, fenvalerate and ethion, which has resulted in the rejection of consignments⁷.

Thus, the present study was conducted to: (i) evaluate the dissipation of five pesticides in curry leaves, (ii) assess the dietary risk to consumer health, and (iii) determine the impact of curry leaf powder processing on pesticide residues.

Materials and methods

Chemicals used in the study

Certified reference materials of ethion (97.60%), chlorpyriphos (99.30%), profenophos (97.60%), carbendazim (97.00%), cypermethrin (99.70%) (Sigma Aldrich, Bengaluru); solvents, viz. acetonitrile, hexane; salts, viz. anhydrous sodium chloride and sodium sulfate (Merck, Bengaluru, India), anhydrous magnesium sulphate (MgSO₄) (Himedia Laboratory, Mumbai), primary, secondary amine (PSA, 40 μm ; Agilent, USA) and graphitized carbon black (GCB) (Agilent, USA) were used in the study. The membrane filter paper (0.45 and 0.20 μm , Ultipor) was procured from Pall Life Science, Mumbai.

Preparation of standard solution

The stock solutions (400 mg/kg) of carbendazim were prepared in acetonitrile for liquid chromatograph-mass

 $[*]For\ correspondence.\ (e-mail:\ bhuvaneswari.k@tnau.ac.in)$

spectrometer (LCMS) analysis, whereas other pesticides were prepared in n-hexane for gas chromatograph (GC) analysis. Intermediate standard (40 mg/kg) and working standards were prepared with appropriate dilution from the stock solution and stored at -20° C.

Method validation

The method validation parameters, viz. linearity, limit of detection (LOD), limit of quantification (LOO), accuracy, and precision, were established in accordance with the SANTE guidelines⁸. A linear relationship was determined by plotting the calibration curve against the response (y) and analyte concentration (x). The lowest concentration of the analyte, which shows three times the response of the blank sample noise, was set as LOD, while the concentration showing signal to noise ratio of 10 was fixed as LOQ. To determine the efficacy of the extraction method, untreated curry leaf samples were fortified at 0.05, 0.25 and 0.5 mg/kg (other analytes), and cypermethrin at 0.10, 0.50 and 1.00 mg/ kg. An untreated control was maintained, and all the treatments were replicated thrice. The samples were processed using QuEChERS method9 with slight modification (addition of GCB) and analysed in gas chromatograph-electron capture detector/flame photometric detector and LCMS. The precision of the method was expressed as a relative standard deviation. Matrix match standard was used to quantify the analytes and to identify interferences, if any, during quantification. Table 1 shows the instrument parameters.

Experimental details

Field experiments were conducted during two seasons, viz. February to March 2017, in Pungampalayam (11.2412°N, 76.9225°E) and October to December 2017, in Velliangadu (11.2144°N, 76.8257°E), Coimbatore district, TN, to study the dissipation pattern of ethion 50 EC at 500 g.a.i. ha⁻¹, chlorpyriphos 20 EC at 300 g.a.i. ha⁻¹, profenophos 50 EC at 500 g.a.i. ha⁻¹, carbendazim 50 WP at 250 g.a.i. ha⁻¹ and cypermethrin 10 EC at 50 g.a.i. ha⁻¹ in the Sengkambu variety. One month after pruning, two sprayings were given at 15 days intervals using knapsack sprayer equipped with a hollow cone nozzle, and the treatments were replicated thrice. After two rounds of spraying, curry leaf samples were collected at 0 (within 2 h) to 40 days from each plot.

Sampling, extraction and clean-up

From each treatment, 1 kg of curry leaves was collected randomly in labelled polythene bags and brought to the laboratory for further analysis. The leaves were removed with minimal handling, homogenized in a high-volume blender (Robot Coupe[®], USA), and a subsample of 250 g was taken for analysis. From each treatment, 5 g of sample

was weighed in a 50 ml centrifuge tube, and 20 ml of acetonitrile was added. After vortexing for 1 min, 1 g of anhydrous NaCl and 4 g of MgSO₄ were added, vortexed and centrifuged at 6000 rpm for 10 min. Then, 9 ml of the supernatant was transferred to the tube containing 4 g of anhydrous Na₂SO₄. The tubes were shaken well and 6 ml of the organic layer was transferred to a 15 ml centrifuge tube containing 100 mg PSA, 600 mg MgSO₄ and 100 mg of GCB. The sample tube was shaken well for 1 min and centrifuged at 3000 rpm for 10 min. The supernatant (4 ml) was transferred to a turbovap tube and kept at 40°C for 20 min. The final residues were redissolved in acetonitrile/*n*-hexane and passed through a nylon filter for analysis in LCMS/GC. The residue data were subjected to statistical analysis to calculate the half-life and safe waiting period ^{10,11}.

Dietary risk assessment

The health risk of pesticide residues in curry leaves was determined by comparing the theoretical maximum residue contribution (TMRC) of the pesticide and the maximum permissible intake (MPI). According to the National Institute of Nutrition, Hyderabad, Telangana the average body weight of men, women and children (7–9 years) is 65, 55 and 25.30 kg respectively, and the average per capita daily consumption of curry leaves is 2 g (ref. 12). The acceptable daily intake (ADI) value of ethion, chlorpyriphos, profenophos, carbendazim and cypermethrin was 0.002 (ref. 13), 0.01, 0.01, 0.03 and 0.05 mg/kg (ref. 14) respectively. To determine the non-carcinogenic effect of the pesticides, the hazard index (HI) was estimated 15.

Impact of processing steps on insecticide residues in curry leaves

The standardized protocol for the preparation of curry leaf powder was obtained from Post Harvest Technology Centre, Agricultural Engineering College and Research Institute, Tamil Nadu Agricultural University, Coimbatore. Samples were collected, and the impact of each processing step on the level of insecticide residues was evaluated by drawing and analysing samples from each step.

Three kilograms of curry leaves were collected randomly from treated plots @ 1 kg per replication after 1 h of spraying and subjected to the following processing steps. Each treatment was replicated thrice.

A portion of 500 g fresh curry leaves was homogenized (T_1) , and the remaining 2.5 kg was reserved for further treatment during processing according to the protocol. The 2.5 kg curry leaf samples were washed with tap water by making gentle swirls for 2 min, blot-dried on tissue paper and a 500 g subsample was homogenized (T_2) . The remaining 2 kg was then soaked in a solution containing potassium metabisulphate (KMS, 0.5%), NaHCO₃ (0.1%) and MgO (0.1%) in the ratio 1: 8. After 15 min, a 500 g subsample

Table 1. Instrument parameters

Particulars	Carbendazim
Liquid chromatograph mass spectrometer	
Model	LCMS (Shimadzu, series 2020)
Column	Shimadzu shim-pack GIST-HP C18, 100 × 3.0 mm, 3 μm particle size
Detector	Diode array detector (SPD-M20A)
Mobile phase	Acetonitrile: water with ammonium acetate (50:50, v/v)
Column oven temperature	40°C
Flow rate	0.3 ml min^{-1}
Mass ratio (m/z)	192, positive ionization
Injection volume	5 μl
Drying gas flow rate	15 1/min
Nebulizer gas flow rate	1.5
Desolvation line temperature	250°C
Heat block temperature	300°C
Retention time of analytes	5 min
Gas chromatograph	
Model	GC-2010 (Shimadzu)
Column	DB-1, 30 m \times 0.25 mm i.d. \times 0.25 μ m for ethion, chlorpyriphos and profenopho
	DB-5, 30 mm \times 0.25 mm i.d. \times 0.25 μ m for cypermethrin.
Detector	Flame photometric detector (ethion, chlorpyriphos and profenophos)
	Electron capture detector (cypermethrin)
Carrier gas	Nitrogen
Injection port temperature	280°C
Detector temperature	300°C
Injection volume	1 μl
Ethion and profenophos	150°C, held for 0 min; increased @ 10°C/min to 250°C, held for 1 min; increased @ 20°C/min to 280°C, held for 8 min. Run time 20.50 min.
Chlorpyriphos	130°C, held for 0 min; increased @ 10°C/min to 250°C, held for 0 min; increased @ 15°C/min to 280°C, hold for 1 min. Run time 15.00 min
Cypermethrin	150°C, held for 0 min; increased @ 10°C/min to 250°C, held for 1 min; increased @ 20°C/min to 280°C, held for 8 min. Run time 20.50 min.
Column flow	Ethion and profenophos, 0.93 ml/min; chlorpyriphos, 0.85 ml/min; cypermethrin,1.03 ml/min
Retention time	Ethion, 12.05 min; chlorpyriphos, 11.30 min; profenophos, 11.3 min; cypermethrin, 18.08 and 18.47 min
Injection mode	Splitless (ethion, chlorpyriphos and profenophos) Split (cypermethrin)

was drawn and homogenized for analysis (T_3). From the remaining 1.5 kg of curry leaves, 500 g was dried under shade. Once the leaves turned brittle, the sample was powdered and maintained for residue analysis (T_4). The last part of 1 kg of the sample was subjected to cabinet drying at 50°C for 3.30 h, powdered and a subsample of 500 g was drawn for residue analysis (T_5).

Ten grams of the representative sample drawn from each processing step was analysed using the validated QuEChERS method as described earlier.

Results and discussion

Method validation

The analytical method developed for pesticide residue analysis showed good linearity for all the pesticides analysed in GC and LCMS with a satisfactory coefficient of determination (R^2). LOD and LOQ were determined as 0.01 and 0.05 mg/kg respectively, for ethion, chlorpyriphos, profenophos and carbendazim, while for cypermethrin, it was

0.05 and 0.1 mg/kg respectively (Figure 1). Curry leaves have higher chlorophyll content of 9.11 mg/g (ref. 16). Hence, PSA and GCB were added at the rate of 600 and 100 mg during the clean-up process. Per cent recovery achieved was in the range 99.09–119.48 with acceptable RSD <20%. The recovery table and chromatograms are given in Supplementary Table 1. Recoveries and RSD in the acceptable range according to SANTE⁸ confirmed that the validated method is reliable and suitable for analysing residues in curry leaves for five pesticides mentioned in this study.

Dissipation

The mean initial pesticide residues ranged from 13.75-58.55 mg/kg in the first season and 9.53-93.92 mg/kg in the second season (Figure 2a and b). In most of the treatments, >50% of the initial residues were found to dissipate within 7 day after treatment (DAT) during both seasons. When compared to other pesticides analysed, the persistence of carbendazim (40 DAT) on curry leaf samples was high,

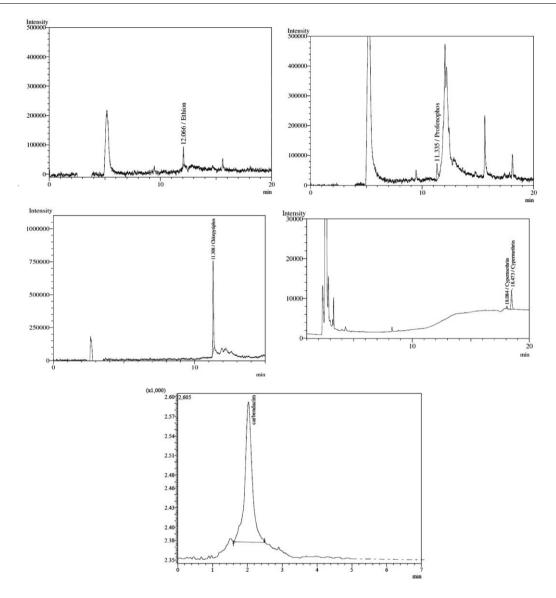


Figure 1. Standard chromatograms at limit of quantification level.

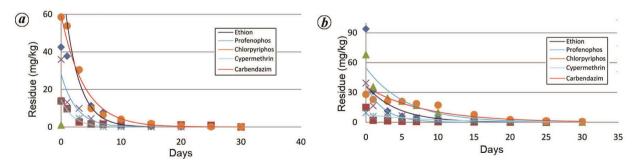


Figure 2. Dissipation curve of selected pesticides in curry leaves. a, season I; b, season II.

while cypermethrin showed quick degradation during the first season (15 DAT), followed by profenophos (20 DAT). In Telangana, the persistence of ethion, chlorpyrifos and profenophos was found up to 25 DAT, while cypermethrin and carbendazim dissipated on 10 and 20 DAT respecti-

vely^{17,18}. The dissipation table and chromatograms are given in <u>Supplementary Table 2 and Figures 1–3</u>.

The half-life period ($t_{1/2}$) was in the range of 1.37–7.92 days. The safe waiting period was worked out based on MRL published by the EU, as no MRL values are available

according to FSSAI and Codex (<u>Supplementary Table 2</u>). The European Food Safety Authority has grouped curry leaves under the category of laurel/bay leaves in the food classification under regulation (EC) No. 396/2005 (ref. 19). The estimated safe waiting period is between 3.81 and 78.54 days and 16.10 and 49.66 days during first and second season respectively.

Among the pesticides analysed, cypermethrin showed fewer initial deposits and rapid degradation. Cypermethrin, a contact insecticide with restricted translocation into the plant system, might have resulted in quick degradation compared to other pesticides. In the present study, during the first season, the dissipation pattern of all the analytes was rapid when compared with the second season. The maximum temperature (33.3°C) that prevailed in the first season is likely to have contributed to the rapid reduction of residues than in the second season (30.42°C; Supplementary Table 3).

The high initial pesticide residues in leaf samples can be explained based on the correlation between surface area and initial deposits, which ultimately favours more droplet collection by curry leaves²⁰. In addition, the essential oils (2.6%) present in curry leaves might have favoured higher retention of pesticides²⁰. Higher wax and oil in citrus fruits resulted in a higher deposition of fungicide pyraclostrobin²¹. Its dissolution into the oil sacs of the fruit resulted in a longer half-life period by hampering direct exposure of the fungicide to the environment²¹. Similarly, the essential oil of green peel-orange combined with prochloraz sprayed on cucumber leaves resulted in greater deposition, spreading and penetration²².

Effect of processing methods on the removal of pesticides

Food crops treated with pesticides may leave some residues, so it becomes imperative to find a suitable processing method. Pesticide residues in food are largely influenced by various factors involved during storage, handling and processing from harvest to consumption, and these steps may significantly impact the removal or reduction of residues²³.

The effect of processing aspects such as washing, dipping and dehydration on removing insecticide residues from curry leaves was assessed. The residues were estimated, and the per cent reduction of insecticide residues was obtained by comparing the residues in the samples drawn after each processing step with those of the previous step.

In the present study, the initial deposits of pesticides in raw curry leaf samples were in the range of 2.99–99.20 mg/kg (Supplementary Table 4). By washing the samples under running tap water for 2 min, the pesticide residues were removed to the extent 18.06–40.89%. Dipping of curry leaf samples in soak solution (KMS (0.5%), NaHCO₃ (0.1%) and MgO (0.1%)) removed pesticide residues to the extent of 21.28–45.58%. Shade drying resulted in a 32.39–74.46% loss of the initial residues from the curry

leaf samples. The cabinet drying method removed pesticide residues from curry leaf samples in the range of 7.11–79.25%. For all the pesticides analysed, the processing factor was found to be <1 for both shade and cabinet-dried samples, indicating a reduction in pesticide residues due to processing²⁴.

Different types of washing techniques can remove pesticide residues that are loosely bound²⁵. Moreover, the majority of pesticides sprayed on crops are restricted to the outer surface and undergo negligible penetration of the cuticle. Consequently, they are amenable to removal by washing, peeling and cutting. In the present study, the pesticides were removed to the extent of 40% by washing in tap water. The solubility of the pesticides in water (1.4–29 mg/l at 25°C) helps remove them from the curry leaf samples when subjected to washing treatment.

The curry leaf samples treated with washing followed by dipping in soak solution resulted in the reduction of pesticides to the extent of 45.58%. The reduction of pesticides by NaHCO₃ in the soak solution can be compared with the log K_{ow} (n-octanol/water partition coefficient) value of the pesticides (ethion, profenophos, chlorpyriphos and cypermethrin, 5.07, 4.68, 4.66 and 6.94 respectively)²⁶. The results obtained in the present study showed a greater reduction of pesticides with a decrease in log K_{ow} values. In addition, when NaHCO₃ interacts with water, it forms H_2CO_3 owing to oxidation and removal of pesticides²⁷.

During the drying process, reduction in pesticide residues has been attributed to evaporation, degradation and codistillation²⁸. In the present study, shade drying removed a maximum amount of pesticides from curry leaves than cabinet drying; possibly due to the time difference in drying. Maximum removal of pesticides in prune processing was during sun-drying compared to oven²⁹. Loss of residues of selected insecticides in shade drying > cabinet drying > dipping in soak solution > tap water washing was observed to a certain extent in each step. Among the steps, shade drying of curry leaves resulted in the removal of maximum pesticides, which can be identified as the critical control point in curry leaf powder preparation.

Dietary risk assessment

The aim of health risk assessment is to evaluate the safe level of dietary exposure to pesticides in humans. The TMRC values of chlorpyriphos and carbendazim were less than MPI in both seasons. In the case of ethion, the TMRC values were greater than MPI in the second season (<u>Supplementary Table 5</u>). Chlorpyriphos, profenophos, cypermethrin and carbendazim, showed HI values <1 during both seasons, indicating low health risk to the consumers, except ethion.

Conclusion

The analytical method developed for the determination of pesticide residues in curry leaves gave satisfactory results

in the acceptable range of recovery (70-120%) and RSD (>20%). Field experiments to evaluate the dissipation of pesticides in curry leaves showed persistence from 10 to 25 DAT in the first season and 15 to 35 DAT in the second season. The estimated half-life period was 1.37-7.92 and 1.75-6.02 days in the first and second seasons, respectively. The safe waiting period estimated based on EU MRL was 3.81-78.54 days during the first season and 13.56-49.66 days during the second season. The processing factor was <1, indicating a loss of residues during the processing of curry leaf powder. The safety factors, viz. TMRC and HI values of chlorpyriphos, profenophos, carbendazim and cypermethrin were within the acceptable limit, except for ethion. The long persistence of the selected pesticides, particularly organophosphorus compounds in the study location, warrants training on selecting suitable pesticides and their safe use to farmers to manage pesticide residues in curry leaves. Expanding the label claim of already registered pesticides will favour farmers to adopt good agricultural practice and to handle the issues with residues detected in fresh curry leaves and curry leaf powder for the domestic and export market.

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