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Residue estimation of chlorpyriphos and lambda cyhalothrin in cardamom [*Elettaria cardamomum* (L.) Maton]

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Abstract

The aim of the study was to validate the method for estimation of residues of chlorpyrifos and lambda cyhalothrin in cardamom. Accordingly, the limit of detection (LOD) arrived at was 0.005 µg kg^{-1} and the limit of quantitation (LOQ) arrived at was 0.01 mg kg^{-1}. Chlorpyriphos and lambda cyhalothrin residues showed a linear response in the range of 0.005-5.0 µg kg^{-1} and the mean recovery obtained was 97.3% for chlorpyriphos and 98.9% for lambda cyhalothrin with satisfactory RSD values. The initial residues of chlorpyriphos applied at a concentration of 0.05% in cardamom was 1.6 µg kg^{-1} and the residue was 5.64 µg kg^{-1} after processing, with a processing factor of 3.53, while the lambda cyhalothrin when applied at 0.0025% resulted in initial residues of 0.26 µg kg^{-1} which got magnified to 1.19 µg kg^{-1}, with a processing factor of 4.58. The processing factor obtained in this study led to the conclusion that the residues of chlorpyriphos were magnified 3.53 times and that of lambda cyhalothrin were magnified 4.58 times of initial residues, mainly due to loss of weight of cardamom due to dehydration during curing.

Keywords: cardamom, chlorpyriphos, *Elettaria cardamomum*, lambda-cyhalothrin, pesticide residue

Introduction

Cardamom [*Elettaria cardamomum* (L.) Maton], the queen of spices is one of the most important commercial spice crops of Kerala. Even though India accounts for largest area under cardamom cultivation, the productivity is low mainly due to the attack of diverse pests at all stages of the crop, necessitating frequent application of pesticides (Kumaresan 2008). The residues of pesticides deposited during plant protection operations are a major concern and pesticide residue in spices have affected our exports (Bhardwaj *et al*. 2011). Chlorpyriphos (Varadarasan *et al*. 1990 & 1991) and lambda cyhalothrin are the most widely used insecticides (Sureshkumar *et al*. 2002; Rajabaskar 2003) in cardamom and residues are frequently encountered in samples (AINP (PR) 2010). On an average, farmers apply 15-20 sprays for pest control in cardamom using any of the pesticides like quinalphos, chlorpyriphos, monocrotophos, fenthion, methyl parathion, endosulfan, cypermethrin, lambda cyhalothrin and emisan, either alone or in combination resulting in a heavy load of the produce with residue of these pesticides (Shetty *et al*. 2008). In addition, there is a likely possibility for contamination of surface and ground water with residues due to leaching (Colborn 2006). Therefore, a study was undertaken to quantify the initial residues and the effect of curing processes on removal of insecticides (chlorpyriphos and lambda cyhalothrin) and to arrive at a suitable processing factor for cardamom.

The analytical grade (>99% pure) standards of chlorpyriphos and lambda cyhalothrin were procured from M/s Sigma-Aldrich. All solvents were purified by glass distillation. All the glass wares were pre washed with chromic acid solution and rinsed with acetone in order to avoid contamination. Other reagents/ adsorbents were activated before use. Stock solution of 1000 µg mL^{-1} for each pesticides was prepared by dissolving the required amount of Certified Reference Material (CRM) in acetone and then made up using Hexane : Toluene (1:1) and 5 mL of the individual stock solution was diluted to 100 mL to get an intermediate stock solution of 50 µg mL^{-1}. Working standard solutions of 10.0, 5.0, 1.0, 0.5, 0.1, 0.05, 0.01, 0.005 and 0.001 µg mL^{-1} were prepared by serial dilution. Standard solutions containing 0.001, 0.005, 0.01, 0.05, 0.1, 0.5, 1.0, 5.0 and 10 µg mL^{-1} of the individual pesticides were injected in to GLC (Shimadzu 2010 A) equipped with ECD. The minimum concentration of pesticide giving response three times that of noise level was considered as limit of detection (LOD). The peak area of pesticides was plotted against concentration and linearity range of each pesticide was determined.

A recovery experiment was planned and conducted to assess the extraction efficiency of different pesticides spiked in cardamom. As such no validated protocol is readily available for the estimation of pesticide residues in cardamom. Hence, the multiresidue estimation procedure recommended for fruits and vegetables as per AOAC (2007) was adopted with slight modifications so as to suit the changed substrate. Control samples of cardamom with no history of pesticide application were used for spiking chlorpyriphos and lambda cyhalothrin each at five different levels. A 10 g coarsely ground sample was spiked separately with chlorpyrifos and lamda cyhalothrin at 0.01, 0.05, 0.1, 0.5 and 1 ppm levels in a 50 mL centrifuge tube to which 4 g of sodium chloride was added. The samples were mixed thoroughly by shaking in a vortex shaker for 5 minutes and the solvent was evaporated. After the addition of 5 mL distilled water, the samples were extracted using 25 mL acetonitrile by shaking for 3 minutes in a vortex shaker followed by centrifugation at 3600 rpm for five minutes. A 10 mL of the supernatant was collected and 1g each of anhydrous sodium sulphate and magnesium sulphate were added, stirred well and kept for 5 minutes. The extract was then subjected to clean up by adding 0.125 g PSA (Primary Secondary Amine) and centrifuged at 2500 rpm for three minutes from which 5 mL supernatant was collected. The extract was then concentrated to 2 mL in a turbovap by repeated addition of n- hexane. The n- hexane extract was used for estimation of residues using GLC (Shimadzu 2010 A) equipped with ECD. Accordingly, the minimum amount of the pesticide that can be quantified by the above procedure was calculated as per the standard procedure.

The reagents used were Acetonitrile (HPLC grade), n- Hexane (HPLC), $MgSO_4$ (AR), Anhydrous Na_2SO_4 (AR), Primary Secondary Amine (PSA Bondesil- Agilent, USA). The operating conditions were Temperature: Injection port (°C) - 250, oven- 160-270 (@ 3 °C min^{-1}), Detector ECD-300°C. The column consisted of fused silica capillary column BPX 5, 30 m × 0.25 mm ID × 0.25 µm film thickness and column flow 1.87 mL min^{-1}. The retention time (min) of chlorpyriphos was 11.71 and that of lambda cyhalothrin was 27.78. The recoveries of each of the spiked insecticides were assessed by comparing the peak area with standards of known concentration (Sharma 2007).

The experiment was conducted at Cardamom Research Station, Pampadumpara, Idukki District of Kerala Agricultural University during August 2009. The site is located at an altitude of 1,068 m above MSL at 9°47' 27" N latitude, and 77° 09' 28" E longitude and enjoys humid tropical monsoon climate. The soil was forest loamy with pH of 5-6. Undamaged cardamom pods collected from control plots with no recent history of insecticide application were used for the study. Three replicate samples (25 g each) of cardamom pods were drawn as control sample from the lot for comparison and to estimate the back ground residue level, if any. 2 kg each of freshly harvested capsules were immersed separately in insecticide solution prepared by dissolving 2.5 mL of chlorpyriphos (Roban 20EC) per litre of water corresponding to a 0.05% concentration and 0.5 mL of lambda cyhalothrin (Karate 5 EC) per litre of water, corresponding to 0.0025% concentration. A total of 4 L of each of these solutions was prepared. The capsules were taken out after 30 seconds and the excess solution was drained completely. The samples were spread and dried in shade for 1 hour. Three replicate samples (25 g each) from each of the treated cardamom capsules were drawn and extracted to assess the initial deposit. The rest of the samples were cured at 50°C for 36 hours and then kept at room temperature for 6 hours. The samples were again cured at 70°C for three hours and polished, when still hot and crispy. The samples were kept for 24 hours at room temperature and three replicates of the cured samples were extracted to determine the residue levels after processing. The freshly treated cardamom capsules were crushed in a pestle using a mortar and a 25 g of the sample (10 g cured sample) was weighed into a 50 mL centrifuge tube to which 4 g of sodium chloride was added. In the case of cured sample a 5 mL distilled water was also added prior to extraction. The extraction, clean up and estimation of residues were performed as per the method described in the recovery experiment. The processing factor (PF) was calculated as, P.F = Residues in dry cardamom (µg g^{-1})/ Residues in fresh cardamom (µg g^{-1}).

An analysis of the data on the recovery of chlorpyriphos fortified at 5 different levels indicated a mean recovery of 97.3% (Table 1). The recovery percentage ranged from 84.4 to 104.2. The standard deviation of recoveries ranged from 1.7 to 8.6 and all the RSD values were lower than 9.56. Considering a satisfactory recovery percentage (70-110) and RSD values (<20), the method can be considered appropriate for the estimation of chlorpyriphos residues in cardamom. The data on the recovery of lambda cyhalothrin fortified at five different levels indicated a mean recovery of 98.9% (Table 2).

Table 1. Recovery of chlorpyriphos from spiked samples of cardamom

Level of spiking(µg g^{-1})	Recovery(%)			Mean Recovery(%)	RSD(%)
	Rep I	Rep II	Rep III		
0.01	85.8	82.6	84.9	84.4 ± 1.65	1.95
0.05	80.1	95.7	94.2	90 .0 ± 8.61	9.56
0.1	101.6	105.8	104.2	103.9 ± 2.12	2.04
0.5	104.9	106.2	101.1	104.1 ± 2.65	2.55
1.0	98.2	104.2	110.2	104.2 ± 6.00	5.76

Table 2. Recovery of lambda cyhalothrin from spiked samples of cardamom

Level of spiking(µg g^{-1})	Recovery(%)			Mean Recovery(%)	RSD(%)
	Rep I	Rep II	Rep III		
0.01	87.6	95.8	120	101.1 ± 16.85	16.7
0.05	89.1	98.5	97.3	95.0 ± 5.12	5.4
0.1	96.5	108.7	99.4	101.5 ± 6.37	6.3
0.5	93.5	95.7	89.5	92.9 ± 3.14	3.4
1.0	105.9	95.7	109.8	103.8 ± 7.28	7.0

The recovery percentage ranged from 92.9 to 103.8. The standard deviation of recoveries ranged from 3.1 to 16.8 and all the RSD values were lower than 16.7. Considering a satisfactory recovery percentage (70-110) and RSD values (< 20), the method can be considered appropriate for the estimation of lambda cyhalothrin residues in cardamom. The lowest level of the pesticides which can be detected by the instrument obtaining a response equivalent to three times the area of noise peak has been calculated as 0.005 mg kg^{-1}. Based on the instrument detection limits, the LOD value for chlorpyrifos and lambda cyhalothrin were fixed as 0.005 µg g^{-1}. The lowest level of the pesticides which can be satisfactorily recovered following the adopted procedure is fixed as 0.01 µg g^{-1} and the LOQ value arrived from the recovery experiment for chlorpyriphos and lambda cyhalothrin was 0.01µg g^{-1}. The response of the instrument was linear over 0.01-5.0 µg g^{-1} range, wherein the response was in proportion to the concentration.

The results of the analysis of fresh cardamom samples after treatment revealed that the initial deposits of chlorpyriphos (20 EC) and lambda cyhalothrin (5 EC) in fresh cardamom were 1.6 and 0.26 µg kg^{-1} respectively (Table 3). A higher residue of chlorpyriphos was observed presumably due to higher concentration of the insecticide solution (0.05%) compared to that of lambda cyhalothrin (0.0025%). In the case of dry cured cardamom, the residues present were 5.64 and 1.19 µg g^{-1} respectively, for chlorpyriphos and lambda cyhalothrin (Table 3). During the curing process, 75.0%–78.0% of moisture was depleted which would have resulted in the accumulation of residues and a portion of residues also would have dissipated due to the effect of heat and rubbing. Hence, the level of residues in cured sample was magnified a 3.53–4.58 times the original residue. The processing factor arrived at from the above experiment were 3.53 and 4.58 for chlorpyriphos and lambda cyhalothrin, respectively. A lower processing factor observed for chlorpyriphos is indicative of its higher proneness to forces of degradation while a higher value for lamda cyhalothrin could be due to its higher stability (Shah *et al*. 2009). The result obtained in this study corroborated the findings of Pathan *et al*. (2009) wherein the effect of processing on the dissipation of residues of dicofol, ethion and cypermethrin on chilli was reported with processing factor of 5.59, 3.52 and 7.50 respectively.

Table 3. Processing factor of chlorpyriphos and lambda cyhalothrin in cardamom

	Chlorpyrifos	Lambda cyhalothrin
Residues µg g^{-1} in fresh sample	1.6±0.025	0.26±0.004
Residues µg g^{-1} in cured sample	5.64±0.043	1.19±0.017
Processing factor	3.53	4.58

The processing factor calculated in this study led to the conclusion that application of chlorpyriphos at a concentration of 0.05% on cardamom followed by curing as per the farmers adopted practice followed by polishing resulted in 3.53 times more residues in the cured product. Similarly, lambda cyhalothrin when applied at 0.0025% in fresh cardamom followed by curing and polishing resulted in a 4.6 times more residues in the cured product. This could be due to a relatively high resistance of the compounds to degradation forces like high temperature as well as loss of moisture leading to decrease in the weight of the fresh product by 75%–78%. The residues in fresh product were estimated on fresh weight basis while that on cured product was estimated and expressed on dry weight basis. Fresh cardamom contains 75%–80% moisture and the dry yield of cardamom is generally in the range of 20%–25%. Assuming zero dissipation of the insecticide during curing the residues after curing would have been 6.4–8 ppm for chlorpyriphos and 1.04–1.30 ppm for lambda cyhalothrin. In the present study, residue obtained in the cured produce was 5.64 and 1.19 respectively for chlorpyriphos and lambda cyhalothrin indicating lower residue than the maximum possible. The difference of the two corresponds to the residue lost during curing. The curing processes in cardamom ultimately resulted in a magnification of residues in the final product, which could be due to the moisture loss. The processing factor can be utilised to establish MRL's for processed commodities and or to conduct dietary intake assessments. Cardamom being the most important and exported spices from India, more location specific studies are required to derive authentic processing factors for different pesticides used.

References

AINP (PR) 2010 Annual report of All India Network Project on Pesticide Residues, College of Agriculture, Vellayani.

AOAC (18th Edition) Official Methods of Analysis 2007 AOAC Pesticides and Industrial Chemical Residues- Fruits and vegetables, pp.17–26.

Bhardwaj R K, Sikka B K, Ashutosh Singh, Sharma M L, Singh N K & Rahul Arya 2011 Challenges and constraints of marketing and export of Indian spices in India. In: Proc. International conference on technology and business management, 28–30 March 2011, Dubai.

Colborn T 2006 A case for revisiting the safety of pesticides: A closer look at neurodevelopment. J. Environ. Health Perspect. 114: 10–17.

Kumaresan D 2008 Pest problem and their ecofriendly management techniques in cardamom (*Eletteria cardamomum* (L) Maton). Spice India 21: 28–34.

Pathan A R K, Parihar N S & Sharma B N 2009 Effect of drying on the residues of dicofol, ethion and cypermethrin in chilli (*Capsicum annum* L.) Pest Manage. Hort. Ecosystem. 15: 167–169.

Rajabaskar D 2003 Studies on the evaluation of IPM modules against *Conogethes punctiferalis* Guenee and *Sciothrips cardamom* Ramk. on cardamom. Ph.D Thesis, Tamil Nadu Agricultural University, Coimbatore, India, p.198.

Shah P G, Diwan K, Raj M F & Patel A R 2009 Effect of processing factor of turmeric on chlorthalonil, chlorpyriphos and endosulfan. Pestic. Res. J. 21: 86–88.

Sharma K K 2007 Pesticide residue analysis manual. Directorate of information and publication of Agriculture, New Delhi, pp.294.

Shetty P K, Murugan M & Sreeja K G 2008 Crop protection stewardship in India: Wanted or unwanted. Current Sci. 95: 457–464.

Sureshkumar R S D, Rajabaskar K, Chozhan & Regupatham A 2002 Efficacy of lambda cyhalothrin (Karate® 5EC) against cardamom thrips, *Sciothrips cardamomi* (Ramk.). In: National Seminar on Changing Scenario in the Production Systems of Hill Horticultural Crops, Ooty, 20–21 February 2002.

Varadarasan S, Kuamresan D & Gopakumar B 1990 Bi- annual report 1987–1988 and 1988–1989. Indian Cardamom Research Institute, Spices Board, Myladumpara, India, 65–66.

Varadarasan S, Kuamresan D & Gopakumar B 1991 Cardamom root grub. Spice India 4: 6–10.