Quality Assurance and Safety of Crops & Foods, 2015; 7 (3): 271-275

Wageningen Academic Publishers

Determination of flubendiamide residues and its safety evaluation for usage in cardamom by liquid chromatography

M. Deepa*, S.A. Jayaprakash, M. Paramasivam, D. Eswar, C. Selvi and S. Chandrasekaran

Tamil Nadu Agricultural University, Department of Agricultural Entomology, Pesticide Toxicology Laboratory, Coimbatore 641003, Tamil Nadu, India; deepabiochem@gmail.com

Received: 26 July 2013 / Accepted: 16 December 2013

RESEARCH ARTICLE

Abstract

Cardamom is a major export oriented commodity in the world and it is susceptible to infestation by various pests which cause considerable economic loss. It is necessary to adopt new chemical insecticides with novel modes of action which control pest infestations. Flubendiamide fits into integrated pest management programmes in a variety of crops and is widely used in crops like tomato, cabbage and brinjal for controlling *Lepidoptera* pests and it is effective when used for cardamom. The major concern is ensuring residue free pods and safe harvest of cardamom for human consumption to prevent any health hazard. The present study investigates the behaviour of flubendiamide in cardamom in the humid subtropical conditions of India. A rapid, simple and selective method has been developed to determine residues of flubendiamide and desiodo flubendiamide in green cardamom, cured cardamom capsule, cured cardamom black seed and soil. The flubendiamide residues were extracted using the QuEChERS principle with acetonitrile and then cleaned up by dispersive solid phase extraction with primary secondary amine sorbent to remove co-extractives, prior to high performance liquid chromatograph-diode array detector analysis. The average recoveries of flubendiamide and desiodo flubendiamide from all matrices were more than 83% and the limit of quantification was 0.05 µg/g. The results of flubendiamide dissipation pattern followed first order kinetics with a half-life of 2.95 to 3.27 days in green cardamom pods. The present study suggests that residues of flubendiamide when applied at the recommended dose does not pose any health risk to consumers if a withdrawal period of 20 days is allowed.

Keywords: flubendiamide, desiodo flubendiamide, cardamom, QuEChERS, withdrawal period

1. Introduction

Cardamom (*Elettaria cardamom* Maton), a perennial, herbaceous and rhizomatous plant, is popularly known as the queen of spices. India is the second largest producer of cardamom after Guatemala. Cardamom, a major export commodity, is widely cultivated in India, especially in southern states viz., Kerala (60%), Karnataka (30%) and Tamil Nadu (10%). There is a worldwide demand for dried cardamom, which is used in food preparation as a flavouring agent in a varity of foods including confectionary, beverages and liquors. The major pests of cardamom are the shoot and capsule borer (*Conogethes punctiferalis* Guenee), thrips (*Sciothrips cardamomi* Ramakrishna), root grubs and hairy caterpillars (Gahukar, 2011). The shoot and capsule borer feeds on the pods and panicles and causes considerable economic loss making it a major pest. To combat the economic losses caused by this major pest, it is necessary to adopt synthetic chemical control measures.

Flubendiamide, N^2-[1,1-dimethyl-2-(methylsulfonyl) ethyl]-3-iodo-N^1-[2-methyl-4-{1,2,2,2-tetrafluoro-1-(trifluoro methyl)ethyl}phenyl]1,2-benzene dicarboxamide), belongs to a new chemical class of phthalic acid diamide compounds (Tohnishi *et al.*, 2005). Flubendiamide activates ryanodine sensitive intracellular calcium release channels in insects by disrupting cellular calcium balance (Masaki *et al.*, 2006) causing contraction of insect skeletal muscle. It has been widely used against a broad spectrum of lepidopterans on a variety of crops as it has a lower application rate and

therefore smaller environmental load. It has low mammalian toxicity, and no reported mutagenic or oncogenic properties. It also fits into integrated pest management programme since it is safe for non-target organisms.

Flubendiamide has been recently introduced in India and residue studies have been conducted in various crops like tomato (Paramasivam and Banerjee, 2012), brinjal (Takkar *et al.*, 2012), cabbage (Mohapatra *et al.*, 2010) and chilli (Sahoo *et al.*, 2009). Recently, flubendiamide has been found to be very effective for the control of lepidopterans attacking cardamom. Cardamom is now considered as the highest pesticide consuming rainfed crop in the world, requiring 15-18 rounds of pesticide sprays per year (Murugan *et al.*, 2011). So the major concern raised about ensuring residue free pods becomes a necessity. There is little information available to our knowledge for the assessment of flubendiamide and its metabolite desiodo flubendiamide residue dissipation in cardamom. Therefore, the present study reports a rapid, simple and sensitive method based on the quick, easy, cheap, effective, rugged and safe (QuEChERS) method (Lehotay, 2004), principle of liquid chromatography for simultaneous determination of flubendiamide and desiodo flubendiamide residues in cardamom pod and soil.

2. Materials and methods

Reference standard

The reference standard of flubendiamide (99.5%) and desiodo flubendiamide (99.2%) and the formulation (Fame 480 SC) was supplied by Bayer CropScience Ltd. (Mumbai, India). A stock standard solution (1000 µg/g) was prepared in acetonitrile separately for high performance liquid chromatography (HPLC) analysis. Mixture standard solutions were prepared by mixing and diluting the individual standard stock solutions. Spiking and calibration standard solutions for HPLC analysis were prepared by diluting the stock solution. The standard solutions were kept under refrigerated condition (-4 °C) and protected from light. All the other organic solvents and reagents were of HPLC grade, and anhydrous magnesium sulphate ($MgSO_4$) and sodium chloride (NaCl) were purchased from Merck (Mumbai, India). Primary secondary amine (PSA) (40 µm Bondesil) was purchased from Agilent Technologies India Pvt. Ltd. (New Delhi, India).

Field experiment

The field experiments were carried out in two different locations: (1) Kumuli during August-October, 2010; and (2) Nedunkandam during August-October, 2011 with Vazhukka and Green gold variety, respectively. The experiments were laid out in randomised block design with 90 m^2 plots for each treatment containing approximately 30 plants per plot following good agricultural practices, with three treatments replicated in triplicate. The treatments details are as follows: (1) flubendiamide 480 SC, 0.72 gram active ingredient (g.a.i.)/10 l water (recommended dose); (2) flubendiamide 480 SC, 1.44 g.a.i./10 l water (double the recommended dose); and (3) untreated control (water spray). The first spray application was given at pod formation stage further 2 sprays were given at 21 days intervals with a rocker sprayer. It was ensured that flubendiamide was not used earlier in the experimental plots. For every treatment 5 plots were taken. On every sampling day approximately 250 g of pods were collected from each plot. Samples from all 5 plots were pooled together. The fresh green cardamom pods (a total of 1.25 kg) were collected randomly from each treatment after the last application at 0 (2 h after spraying), 3, 5, 7, 10, 15 and 20 days after flubendiamide 480 SC application. Immediately after collecting the samples, they were brought to the laboratory for analysis in ice boxes to keep the temperature at 4 °C. The green cardamom pod samples collected on the 20^{th} day were dried at 100 °C for 10 h in a hot air oven. The dried black seeds were separated from dried cardamom pods. Soil samples (1 kg) for residue analysis were collected from all replicated plots by using a hand held auger driven to a plough depth of 15 cm. Soil was collected from a minimum of 10 cores across the treatment plot and bulked together from which a representative sample of 500 g was taken by quartering technique and screened through a 2 mm sieve.

Sample extraction

Flubendiamide and desiodo flubendiamide residue extraction was carried out by QuEChERS method as described by Paramasivam and Banerjee (2011). Control samples were analysed before the analysis of fortified samples or samples from treated plots in the order of lower to higher concentration. For the extraction of flubendiamide and its metabolite residue from green cardamom capsule, cured pod and black seed is as follows. A 10 g of sample was weighed into a 100 ml conical flask, 20 ml of acetonitrile was added and the mixture was allowed to stand for 20 min. The solvent mixture was shaken for 30 min at 250 rpm on a mechanical shaker. The acetonitrile solvent was decanted into a 50 ml screw-capped polypropylene centrifuge tube and 1 g of sodium chloride and 4 g of anhydrous magnesium sulphate were added. The mixture was shaken vigorously by hand for 1 min to prevent coagulation of magnesium sulphate. The content of the centrifuge tube was again vortexed for another 1 min and the extract was centrifuged (6,000 rpm) for 10 min at room temperature.

Dispersive solid phase extraction clean-up

The dispersive solid phase extraction (d-SPE) cleanup was performed with Bondesil PSA. An aliquot (6 ml) was transferred from the supernatant to new clean centrifuge tube containing 100 mg of PSA and 600 mg magnesium

sulphate. The tube was shaken well and then centrifuged at 3,000 rpm for 10 min. An aliquot of 4 ml of the supernatant was taken and concentrated to dryness using a Turbovap® LV (Caliper Life Sciences, Russelsheim, Germany) set at 40 °C, under a gentle stream of nitrogen (15 psi) and the residues were reconstituted in 1 ml of mobile phase acetonitrile : water (65:35, v/v). Representative 10 g soil samples (3 replicates) were taken in centrifuge tubes, 20 ml of acetonitrile was added and the tubes were vortex mixed. Then, 1 g of sodium chloride and 4 g of anhydrous magnesium sulphate was added, the tubes were vortexed again and centrifuged for 10 min at 6,000 rpm. The other steps were same as those of cardamom pod samples with d-SPE clean-up.

High performance liquid chromatography analysis

Flubendiamide and desiodo flubendiamide residues were determined by HPLC (Shimadzu LC 20 AT; Shimadzu, Kyoto, Japan) equipped with photo diode array detector (PDA-SPD M20 A; Shimadzu) and Chromolith® Performance, RP-18, 250×4.6 mm i.d., 5 µm particle size. The mobile phase was acetonitrile: water (65:35, v/v), with a flow rate of 1 ml/min and the injection volume was 20 µl. The detector wavelength was set at 230 nm. The retention time of flubendiamide and desiodo flubendiamide was 8.5 and 6.5 min, respectively. The concentration in the samples was identified by comparing the retention time of the sample peak with the retention time of the injected standards.

Recovery studies

Recovery studies were carried out to assess the efficiency and reliability of the method. Fresh untreated green cardamom pods, cured cardamom, black seed and soil samples were fortified in triplicate with analytical standard of flubendiamide and desiodo flubendiamide at the level of 0.05 (limit of quantitation (LOQ) level), 0.25 (5 times LOQ level) and 0.50 µg/g (10 times LOQ level) as described by Doyeli *et al.* (2009). The fortified samples were equilibrated and processed by following the above described analytical method. The residue data was subjected to statistical analysis according to Hoskins (1961) to compute the residual half-life (t½) and pre-harvest interval.

3. Results and discussion

For the preparation of calibration curve, flubendiamide and desiodo flubendiamide standards were diluted with mobile phase in series from 0.01 to 1 µg/ml. Calibration curves for flubendiamide and desiodo flubendiamide were developed by plotting the mean peak area of analyte as recorded in the HPLC chromatogram against concentration. Good linearity was achieved in the range of 0.01 to 1 µg/g. The limit of detection and limit of quantification in this study were determined as 0.01 and 0.05 µg/g, respectively, for all the substrates. The limit of detection and LOQ were established at the signal-to-noise ratio of 3:1 and 10:1, respectively. The recovery results of the analytical method validation are presented in Table 1. The average recoveries of flubendiamide in green cardamom, cured cardamom, black seed and soil samples were in the range of 94.91-97.32, 92.08-99.70, 83.25-97.94 and 92.87-98.37%, respectively, while corresponding values of desiodo flubendiamide were 90.94-98.47, 89.44-97.71, 87.67-96.93 and 90.52-97.33%, respectively. Recovery for the second season was validated and the recovery ranges were similar to those obtained for the first season.

The dissipation pattern of flubendiamide in green cardamom pods during 2010-2011 is shown in Table 2. The average initial deposits (2 h after application) of flubendiamide in green cardamom pod were in the range of 1.354 and 2.309 µg/g in 2010 and 1.439 and 2.768 µg/g in 2011 for recommended and double the recommended dose, respectively. No residues were detected in the control samples. The residue of flubendiamide in green cardamom pod declined steadily with time. The dissipation of flubendiamide was recorded 66-78% after 7 days of last spray application irrespective of experimental locations and doses, which further increased to above 96% after 15 days (Figure 1). The residues of flubendiamide were dissipated below

Table 1. Recovery of flubendiamide and desiodo flubendiamide on green cardamom capsule, cured cardamom capsule, cured black seed and soil at various fortification levels. Data are averages of three replicates.

Fortified concentration (µg/g)	Recovery (%) ± standard deviation							
	Green cardamom capsule		Cured cardamom capsule		Cured black seed		Soil	
	Flubendiamide	Desiodo flubendiamide	Flubendiamide	Desiodo flubendiamide	Flubendiamide	Desiodo flubendiamide	Flubendiamide	Desiodo flubendiamide
0.05	94.91±2.69	98.47±1.55	99.70±0.86	97.71±1.68	97.94±0.81	94.52±3.10	98.37±3.52	97.33±4.72
0.25	95.92±2.75	90.94±3.75	98.79±2.64	97.59±3.05	83.25±2.85	87.67±1.09	97.36 ±1.51	94.99±2.51
0.50	97.32±1.24	97.86±0.85	92.08±2.09	89.44±2.26	95.92±0.77	96.93±3.41	92.87±2.90	90.52±1.15

Table 2. Dissipation of flubendiamide in green cardamom pods for location I and II. Figures in parenthesis show % dissipation.

Days after treatment	Control	Residues recovered (µg/g) ± standard deviation			
		Location I		Location II	
		0.72 g.a.i./10 l water[1]	1.44 g.a.i./10 l water[2]	0.72 g.a.i./10 l water	1.44 g.a.i./10 l water
0	ND	1.354±0.016 (-)	2.309±0.053 (-)	1.439±0.048 (-)	2.768± 0.145 (-)
3	ND	0.983±0.061 (27.40)	1.412±0.110 (38.85)	1.098±0.016 (23.70)	1.736±0.009 (37.28)
5	ND	0.564±0.020 (58.34)	1.242±0.019 (46.21)	0.771±0.029 (46.42)	1.315±0.009 (52.49)
7	ND	0.292±0.037 (78.43)	0.629±0.063 (72.76)	0.461 ±0.022 (67.96)	0.924±0.035 (66.62)
10	ND	0.179±0.019 (86.78)	0.269±0.015 (88.35)	0.181±0.011 (87.42)	0.380±0.038 (86.27)
15	ND	0.057±0.007 (95.71)	0.084±0.012 (96.36)	0.050±0.001 (96.52)	0.123±0.001 (95.56)
20	ND	BDL (100)	BDL (100)	BDL (100)	BDL (100)
Regression equation	-	Y = 3.2009 – 0.0972X	Y = 3.4566 – 0.0995X	Y = 3.2989 – 0.1022X	Y = 3.5202 – 0.0921X
Half-life (days)	-	3.10	3.04	2.95	3.27

[1] Recommended dose.
[2] Double the recommended dose.
BDL = below detectable level (<0.05 µg/g); g.a.i. = gram active ingredient; ND = non detectable.

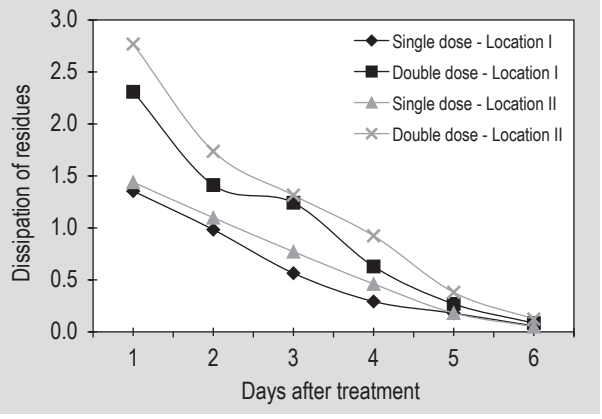

Figure 1. Dissipation of residue percentage from location I and II.

the quantification limit of 0.05 µg/g, 20 days after spraying for both doses and locations. The dissipation pattern of flubendiamide in green cardamom pod followed first order kinetics and half-life values were calculated using regression and was found to be 2.95 to 3.27 days. The dissipation kinetics of flubendiamide has already been studied in okra and tomato fruits under different edaphoclimatic conditions. The half-life of flubendiamide in tomato has been reported to be 4 days and for okra it was 5 days (Das *et al.*, 2012; Mohapatra *et al.*, 2011). The dissipation of the pesticide residues in/on crops depends on various factor including environmental conditions, formulation type, application manner, plant nature, dose rate and interval between applications etc. (Farag and Hend, 2012). The dissipation of the flubendiamide from the both the locations were slightly variable, irrespective of environmental conditions, plant type, experimental locations and doses.

The flubendiamide residue in dried cardamom and black seed obtained from green cardamom collected at 20th day was below the detectable limit of 0.05 µg/g at recommended and double the recommended doses from both the locations. Desiodo flubendiamide was not detected at 0.05 µg/g level in green cardamom, dried cardamom and black seed samples collected at different time intervals from the two locations. The residues of flubendiamide and desiodo flubendiamide estimated on soil after the third spray on 20th day were below detectable level at recommended and double the recommended doses. Mohapatra *et al.* (2010) stated that flubendiamide dissipates from soil surfaces through the process of photodegradation which explains its absence in the soil. Based on the results of this study, it is suggested that the application of flubendiamide (Fame 480 SC) on cardamom it is recommended that 20 days withdrawal period for the consumers to minimise any health hazard to consumers.

4. Conclusions

The adopted QuEChERS method for extraction of flubendiamide in cardamom gave more than 85% recovery. The method is comparatively simple and selective to determine residues of flubendiamide and desiodo flubendiamide in cardamom. Based on the residue study of flubendiamide (Fame 480 SC) on cardamom it

is recommended that 20 days withdrawal period may be allowed for safe consumption of cardamom.

Acknowledgements

We would like to thank Bayer CropScience Ltd. for their financial support to carry out the field experiments.

Conflict of interest

M. Deepa, S.A. Jayaprakash, M. Paramasivam, D. Eswar, C. Selvi and S. Chandrasekaran declare that they have no conflict of interest.

References

Das, S.K., Mukherjee, I. and Das, S.K., 2012. Dissipation of flubendiamide in/on okra (*Abelmoschus esculenta* (L.) Moench) fruits. Bulletin of Environmental Contamination and Toxicology 88: 381-384.

Doyeli, S., Anita, R. and Samsul, A., 2009. A multi-residue method for the analysis of organophosphorus residues in cooked and polished rice using accelerated solvent extraction and dispersive-solid phase extraction (d-SPE) technique and uncertainty measurement. Journal of Environmental Science and Health 44: 706-716.

Farag, M.M. and Hend A,M., 2012. Dissipation and residues of mandipropamid in grape using QuEChERS methodology and HPLC-DAD. Analytical Chemistry: 267596.

Gahukar, R.T., 2011. Use of indigenous plant products for management of pests and diseases of spices and condiments: Indian perspective. Journal of Spices and Aromatic Crops 20: 1-8.

Hoskins, W.M., 1961. Mathematical treatment of the rate of loss of pesticide residues. FAO Plant Protection Bulletin 9: 163-168.

Lehotay, S., 2004. Quick, easy, cheap, effective, rugged and safe (QuEChERS) approach for determining pesticide residues. In: Vidal Martinez, J.L. and Garrido Frenich, A. (eds.). Pesticide analysis, methods in biotechnology. Humana Press, New York, NY, USA.

Masaki, T., Yasokawa, N., Tohnishi, M., Nishimatsu, T., Tsubata, K., Inoue, K., Motoba, K. and Hirooka, T., 2006. Flubendiamide, a novel Ca^{2+} channel modulator, reveals evidence for functional cooperation between Ca^{2+} pumps and Ca^{2+} release. Molecular Pharmacology 69: 1733-1739.

Mohapatra, S., Ahuja, A.K., Deepa, M., Jagadish, G.K., Rashmi, N. and Sharma, D., 2011. Development of an analytical method for analysis of flubendiamide, desiodo flubendiamide and study of their residue persistence in tomato and soil. Journal of Environmental Science and Health 46: 264-271.

Mohapatra, S., Ahuja, A.K., Deepa, M., Sharma, D., Jagadish, G.K. and Rashmi, N., 2010. Persistence and dissipation of flubendiamide and desiodo flubendiamide in cabbage (*Brassica oleracea* Linne) and soil. Bulletin of Environmental Contamination and Toxicology 85: 352-356.

Murugan, M., Shetty, P.K., Ravi, R., Subbiah, A. and Hiremath., M.B., 2011. Environmental impacts of intensive cardamom (small) cultivation in Indian cardamom hills: the need for sustainable and efficient practices. Recent Research in Science and Technology 3: 09-15.

Paramasivam, M. and Banerjee, H., 2011. Simultaneous determination of flubendiamide its metabolite desiodo flubendiamide residues in cabbage, tomato and pigeon pea by HPLC. Bulletin of Environmental Contamination and Toxicology 87: 452-456.

Paramasivam, M. and Banerjee, H., 2012. Persistence and dissipation of the insecticide flubendiamide and its metabolite desiodo flubendiamide residues in tomato fruit and soil. Bulletin of Environmental Contamination and Toxicology 88: 344-348.

Sahoo, S.K., Sharma, R.K., Battu, R.S. and Singh, B., 2009. Dissipation kinetics of flubendiamide on chilli and soil. Bulletin of Environmental Contamination and Toxicology 83: 384-387.

Takkar, R., Sahoo, S.K., Singh, G., Battu, R. S. and Singh, B., 2012. Dissipation pattern of flubendiamide in/on brinjal. Environmental Monitoring Assessment 184: 5077-5083.

Tohnishi, M., Nakao, H., Furuya, T., Seo, A., Kodama, H., Tsubata, K., Fujioka, S., Kodama, H., Hirooka, T. and Nishimatsu, T., 2005. Flubendiamide, a novel insecticide highly active against lepidopterous insect pests. Journal of Pesticide Science 30: 354-360.