Journal of Spices and Aromatic Crops
Vol. 24 (1) : 07–11 (2015)
www.indianspicesociety.in/josac/index.php/josac

Indian Society for Spices

Dissipation of flubendiamide (480 SC) in cardamom [*Elettaria cardamomum* (L.) Maton]

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Received 29 November 2012; Revised 13 March 2014; Accepted 30 June 2014

Abstract

Insecticide flubendiamide was sprayed thrice at 21 days interval between December-February on cardamom at two concentrations, i.e. 0.72 g a.i. 10 L^{-1} (X), and 1.44 g a.i. 10 L^{-1} (2X). Samples of capsules were collected at regular intervals for 15 days after application of the insecticide for residue analysis. The initial deposit of flubendiamide in capsules was 0.42 and 0.60 mg kg^{-1} for X and 2X treatments, respectively that dissipated with a half-life of 1.25 and 2.53 days, respectively. No residue of des-iodo flubendiamide (the metabolite of flubendiamide) was detected in any of the samples up to 15 days. The limit of quantification (LOQ) of the method was 0.05 mg kg^{-1}, for both flubendiamide and des-iodo flubendiamide.

Keywords: cardamom, des-iodo flubendiamide, flubendiamide, pesticide residue

Introduction

Cardamom, the 'queen of spices' is one of the most important commercial crop of Kerala and the best known spice of the world. Even though India accounts for largest area under cardamom cultivation, the productivity is low mainly due to attack by diverse pests at all stages of crop, necessitating frequent application of pesticides for their control (Kumaresan 2008). The residues of pesticides deposited during plant protection operations are a major concern throughout the world. Pesticide residue in spices has affected our exports in the past few years (George *et al*. 2013) and should be strictly monitored owing to the high concern about the toxic properties of residues. Flubendiamide, N2-[1,1-Dimethyl-2-(methylsulfonyl) ethyl] -3-iodo-N1- {2-methyl-4- [1, 2, 2, 2-tetrafluoro-1-(trifluoromethyl) ethyl] phenyl} -1, 2-benzenedicarboxamide, is the first commercial member of a new, promising class of insecticides called 1,2-benzenedicarboxamides or phthalic acid diamides with exceptional activity against a broad spectrum of lepidopterous insects such as armyworms, bollworms, corn borers, cut worms, diamondback moths, fruit worms and loopers including resistant strains (Tohnishi *et al*. 2005; Ebbinghaus *et al*. 2007). In contrast to most commercially successful insecticides which act on the nervous system, flubendiamide disrupts the muscle function in insects and, therefore, represents a unique mode of action. The present study was undertaken to estimate the residue dissipation of flubendiamide (Fame, 480 SC) applied at two doses during the bearing stage of cardamom, so as to assess its safety for use in cardamom.

Materials and methods

Chemicals

The analytical grade reference standards (99% pure) of flubendiamide and des-iodo flubendiamide were supplied by M/s Bayer Crop Science India Ltd. All solvents used were of HPLC grade and further purified by distillation and the suitability of solvents was ensured by running reagent blank along with actual analysis. All the glass wares were pre washed with chromic acid solution and rinsed with acetone in order to avoid contamination. The mobile phase solvents were degassed through 0.45µm pore size filter before use. Other reagents / adsorbents were activated before use.

Preparation of Standard Solutions

A stock standared solution of 1000 µg mL^{-1} standard for each pesticide was prepared by dissolving the required amount of Certified Reference Material (CRM) in acetonitrile. An intermediate stock solution of 100 µg mL^{-1} was prepared by suitably diluting the standard stock solution from which working standard solutions of 10.0, 5.0, 1.0, 0.5, 0.1, 0.05, 0.01, and 0.005 µg mL^{-1} were prepared. The suitability of solvent was ensured by running the reagent blank along with actual analysis.

Linearity range and Limit of Detection

Standard solutions containing 0.005, 0.01, 0.05, 0.1, 0.5, 1.0, 5.0 and 10 µg mL^{-1} of the individual pesticide were injected into HPLC (Shimadzu 20AT) equipped with PDA detector. The minimum concentration of pesticide giving response upto three times that of noise level was considered as instrument detection limit (LOD). The peek area of pesticides was plotted against concentration and linearity range of each pesticide was determined.

Field Experiment

A field experiment was conducted to investigate the persistence of flubendiamide (Fame 480 SC) in cardamom (Variety- 'Njallani') at Cardamom Research Station, Pampadumpara of Kerala Agricultural University during December 2010 to February 2011 in a randomized block design with three treatments and three replications for each treatment. The experimental site was located at an altitude of 1,068 m above mean sea level at 9° 47' 27" N latitude, and 77° 09' 28" E longitudes with humid tropical climate. A plot size of 3 × 3 m (9 m^2) containing 4 plants in full bearing stage was selected as individual replicate for each treatment, including control. For one treatment, a total of 12 plants were sprayed using the respective concentrations. Sufficient spacing was provided between treatments and replicates so as to minimize the chances of cross contamination while spraying. Three applications of flubendiamide each @ 0.72 g a.i. 10 L^{-1} (X) and its double dose @ 1.44 g a.i. 10 L^{-1} (2X) were given at 21 days interval on 8 year old cardamom plants and 1.0 L insecticide solution was applied per clump using hand operated knapsack sprayer, when sufficient number of capsules was available for sampling. Untreated control plots were sprayed with water. Samples of cardamom capsules were randomly collected from all the four plants corresponding to each replication on 0, 1, 3, 5, 7, 10 and 15 days after the third spray.

Sampling Procedure

Samples of cardamom capsules (100 g) from each replication were collected randomly in properly labeled polythene bags. The samples were immediately brought to the laboratory for sub-sampling (50 g), extraction and clean up.

Recovery experiment

Being a new compound, the literature on the method of analysis on flubendiamide and its metabolite des-iodo flubendiamide on cardamom is scanty and hence a recovery experiment was conducted to assess the extraction efficiency of different pesticides spiked in cardamom. The multiresidue estimation procedure recommended for fruits and vegetables as per AOAC (2007) was tried for flubendiamide residue estimation. Samples of cardamom with no history of pesticide application were used for spiking flubendiamide and des-iodo flubendiamide each at three different levels. A 12.5 g finely ground sample taken in a 50 mL centrifuge tube was spiked separately with flubendiamide and des-iodo flubendiamide at 0.05, 0.25 and 0.5 µg g^{-1} levels.

The sample was extracted using 25 mL acetonitrile after the addition of 4 g NaCl by shaking for 3 minutes in a vortex shaker. The sample tubes were centrifuged at 4000 rpm for 5 minutes. About 12 mL of the supernatant was collected in another 50 mL centrifuge tube and 5 g anhydrous Na_2SO_4 was added, stirred well and kept for 5 minutes. The extract was then cleaned by adding 0.125 g PSA (Primary Secondary Amine) and centrifuged at 2500 rpm for 4 minutes from which 4 mL supernatant was collected. The extract was then evaporated to dryness in a turbovap at 45°C, by repeated addition of HPLC grade acetonitrile. The residues were re- dissolved in HPLC grade acetonitrile and made up to 1 mL and analysed using HPLC (Shimadzu LC 20AT) equipped with PDA detector. The separation was performed on a 250 × 4.6 mm i.d. C18 column (Phenomenex Luna 5 µ RP-C18 100A) with water and acetonitrile as mobile phase with a gradient run in the ratio of 4:6 for 20 minutes. The flow rate was 1 mL per minute and the injected volume was 20 µL. The retention time (min) of flubendiamide was 11.09 and that of des-iodo flubendiamide was 9.33. Accordingly, the minimum amount of the pesticide that can be quantified by the above procedure was calculated as per the standard procedure. The recovery of each of the spiked insecticide was assessed by comparing the peak area with standards of known concentration (Sharma 2007). The residues were estimated using the formula,

Residues ($\mu g\ g^{-1}$) = [Sample area × Conc. of std. ($\mu g\ g^{-1}$)] × [Final vol. of extract (mL)] / [Standard area × Weight of sample (g)]

Estimation of residues was performed as per the method described in the recovery experiment.

Results and discussion

An analysis of the data on the recovery of flubendiamide fortified at three different levels in cardamom indicated a mean recovery of 98.42% (Table 1). The recovery ranged from 93.05-101.96%. The standard deviation of recoveries ranged from 1.65-4.25 and all the RSD values were lower than 4.57. The data on the recovery of des-iodo flubendiamide fortified at three different levels indicated a mean recovery of 99.77% (Table 2). The recovery ranged from 92.96-107.63%. The standard deviation of recoveries ranged from 1.34-1.8 and all the RSD values were lower than 1.82. Considering a satisfactory recovery (70-110%) and RSD values (< 20), the method can be considered appropriate for the estimation of flubendiamide and des-iodo flubendiamide residues from cardamom.

Table 1. Recovery of flubendiamide in cardamom capsules

Level of spiking ($\mu g\ g^{-1}$)	Recovery (%)			Mean Recovery (%)	RSD* (%)
	R1	R2	R3		
0.05	99.81	102.44	103.63	101.96 ± 1.95	1.91
0.25	99.62	98.99	102.11	100.24 ± 1.65	1.65
0.50	92.98	94.57	91.60	93.05 ± 4.25	4.57

*RSD=Relative standard deviation

Table 2. Recovery of des-iodo flubendiamide in cardamom capsules

Level of spiking ($\mu g\ g^{-1}$)	Recovery (%)			Mean Recovery (%)	RSD* (%)
	R1	R2	R3		
0.05	106.55	107.01	109.33	107.63 ±1.49	1.38
0.25	96.99	98.58	100.59	98.72 ±1.80	1.82
0.50	91.42	93.58	93.88	92.96 ±1.34	1.44

*RSD=Relative standard deviation

Flubendiamide when applied at the full bearing stage at a dose of 0.72 g a.i. 10 L^{-1} (X) resulted in a mean initial deposit of 0.42 mg kg^{-1} which dissipated to 0.08 mg kg^{-1} on 3rd day after application. At this dose, the half life of flubendiamide in cardamom was 1.25 days (Table 3). No residues could be detected in samples collected 5 days after application. The mean initial residues of flubendiamide when applied at the dose of 1.44 g a.i. 10 L^{-1} (2X) was 0.60 mg kg^{-1} and the residue got dissipated to 0.07 mg kg^{-1} on 7th day after application. No residues were detected on the 10th day. The half life of flubendiamide in cardamom at this dose was 2.53 days (Table 3). Residues of desiodo flubendiamide, the metabolite of flubendiamide were detected in 5th and 7th day samples of 2X treatment, at levels below the LOQ.

cabbage ranged from 3.9-4.45 days following two applications at the recommended and double the recommended dose of 24 and 48 g a.i. ha^{-1}, where initial residue deposits of flubendiamide in cabbage were 0.33 and 0.49 mg kg^{-1}, respectively. In both the treatments, inspite of a faster degradation of the chemical, no residues of the metabolite was detected in the samples, presumably due to the higher polarity of the desiodo flubendiamide with high water solubility which would have resulted in a faster elimination of the compound either through cell sap or formation of conjugates with different glycone moieties in the system.

The result of the study indicated the toxicological safety of flubendiamide when applied for pest control in cardamom. The compound persisted only up to 7 days after

Table 3. Residues of flubendiamide in cardamom after application of third spray

Days after application	Residues of flubendiamide (mg kg^{-1})											
	T0 - Untreated (Control)				T1- Single dose (0.72 g a.i. 10 L^{-1})				T2 - Double dose (1.44 g a.i. 10 L^{-1})			
	R1	R2	R3	Mean	R1	R2	R3	Mean	R1	R2	R3	Mean
0	BDL	BDL	BDL	BDL	0.36	0.52	0.39	0.42±0.08	0.82	0.43	0.56	0.60±0.19
1	BDL	BDL	BDL	BDL	0.29	0.20	0.24	0.24±0.04	0.48	0.48	0.43	0.47±0.02
3	BDL	BDL	BDL	BDL	0.09	0.09	0.08	0.08±0.05	0.46	0.40	0.35	0.43±0.05
5	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.22	0.25	0.31	0.26±0.04
7	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.08	0.06	0.08	0.07±0.01
10	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
15	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Half Life								1.25 days				2.53 days

BDL=Below detectable level (0.05 mg kg^{-1})

A faster dissipation of flubendiamide was observed at the lower rate of application of 0.72 g a.i. 10 L^{-1}, presumably due to the exposure of a lower concentration of the chemical to various biotic and abiotic factors. Persistence of flubendiamide was more when applied at a higher rate, presumably due to a higher concentration of the chemical, resulting in a slower degradation. This is similar to the results of Mohapatra *et al*. (2010) who reported that the half life values of flubendiamide in

application, thereby offering a toxicologically safe and residue free produce in the marketable produce. The application of flubendiamide can be considered to be safe from the point of view of contamination of the produce.

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