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Dissipation of Flubendiamide in/on Okra [*Abelmoschus esculenta* (L.) Moench] Fruits

Shaon Kumar Das · Irani Mukherjee · Suvo Kumar Das

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Abstract A field experiment was undertaken at Indian Agricultural Research Institute, New Delhi during kharif (rainy season) in the year 2010 to evaluate the residue persistence of flubendiamide in/on okra fruits following foliar application of Belt 39.35% SC formulation at 24 (standard dose) and 48 (double dose) g a.i. ha^{-1}. After HPLC analysis study revealed that residues of flubendiamide in/on okra persisted till 5th and 7th day after the last spray at standard and double dose, respectively. The residues of flubendiamide were reported as parent compound, and des-iodo flubendiamide, a metabolite (photo product) of flubendiamide, was not detected in/on okra at any time during the study period. The initial deposits of 0.28 and 0.53 μg g^{-1} in/on okra fruits reached below determination level of 0.01 μg g^{-1} on the 7th and 10th day at standard and double dose, respectively. The half life of flubendiamide in/on okra fruits ranged from 4.7 to 5.1 days at standard and double dose, respectively. Soil sample collected from the treated field on the 15th day after the last spray revealed residues of flubendiamide or its metabolite below determination level (0.01 μg g^{-1}) at single and double dose.

Keywords Okra · Flubendiamide · Des-iodo flubendiamide · Half life · Recovery · Dissipation

S. K. Das (✉) · I. Mukherjee
Division of Agricultural Chemicals, Indian Agricultural Research Institute, New Delhi 110012, India
e-mail: shaon.iari@gmail.com

S. K. Das
Department of Agricultural Chemistry and Soil Science, BCKV, Mohanpur 741256, India

Okra [*Abelmoschus esculentus* (L.) Moench], also known as ladies finger, belongs to the family malvaceae and genus *Abelmoschus* is one of the important vegetable crops grown during spring-summer and rainy season in India. It is also one of the important dietary requirements for Indians containing several nutritional values and locally known as bhindi. This crop is subjected to ravage by over 37 insect-pests throughout its growth period from germination till harvest time (Nayyar et al. 1976). Among various insect-pest in okra, leafhopper, *Amrasca biguttula biguttula* (Ishida) and shoot and fruit borer, *Earias* spp. is a major concern and cause havoc damage. Leafhopper alone had caused 32.06%–40.84% (Singh and Brar 1994) and shoot and fruit borer caused 50% reduction in fruit yield (Brar et al. 1994). Larvae of fruit and shoot borer bore into shoots during the vegetative growth stage and later in flowers and fruits, rendering fruit unfit for human consumption.

Increasing awareness of the potential impact of persistent crop protection agents has led to the development of ecofriendly new molecules to ensure minimum risk to man and environment. Flubendiamide N^2-[1,1-dimethyl-2(methylsulfonyl)ethyl]-3-iodo-N^1-[2-methyl-4-[1,2,2,2-tetrafluoro-1-(trifluoromethyl)ethyl]phenyl]-1,2 benzenedicarboxamide (Fig. 1) is introduced newly in India by Bayer Crop Science. It represents a novel class of insecticides with extremely high activity against a broad spectrum of lepidopteron insect-pest like fruit and shoot borer including resistant strain. It is the representative of a class of chemicals, benzenedicarboxamides or phthalic acid diamides. In contrast to most other commercially available pesticides which act on nervous system, flubendiamide disrupts the calcium balance in the muscles of the insects by acting on the ryanodine receptor, affecting the muscle contraction (Ebbinghaus-Kintscher et al. 2007). The pesticide is registered by EPA for use on corn, cotton, pome

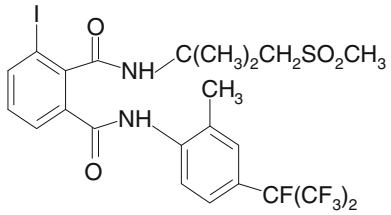

Fig. 1 Structure of flubendiamide

and stone fruit, tree nut crops, grapes and vegetable crops (including cucurbit vegetables, fruiting vegetables and okra) (USEPA 2008). Flubendiamide is registered in India since 2009 on cotton and rice and cabbage.

As the information regarding persistence and dissipation of flubendiamide in/on okra is lacking, the present study was carried out to investigate the persistence and dissipation kinetics of flubendiamide residues in/on okra and soil.

Materials and Methods

Chemicals

Formulation of flubendiamide Belt 39.35% SC was obtained from M/S Bayer Crop Science Limited (New Delhi, India). Double distilled water and HPLC grade acetonitrile was used for HPLC analysis. HPLC grade solvent was procured from Merck India Ltd. These were filtered and de-gassed prior to use. All the glassware were soaked in chromic acid solution and washed thoroughly with water. These were rinsed with acetone and air-dried before use. All other reagents used during recovery experiment in this study were of analytical grade. Formulation was used in field trials after dilution in tap water.

Field Experiment and Sampling

A field experiment was conducted in/on okra crop (Variety: Parbhawa) at the research fields of Division of Entomology, Indian Agricultural Research Institute, New Delhi, during kharif (rainy season) of the year 2010 following recommended agronomic practices. Plot size was 3.0×3.0 m^2 with spacing 60×30 cm (rows $\times$ plants). The experiment was conducted in a randomized block design using three replicates for each treatment. Three sprays of flubendiamide (39.35% SC) were applied at 24 and 48 g a.i. ha^{-1} at an interval of 7 days on okra plants using Knapsack sprayer. The first spray was made at the initiation of fruiting whereas the last spray at fruiting stage. Water was sprayed in the control plot. Okra fruits were drawn at 0 (1 h), 1, 3, 5, 7, 10 and 15 days after the last spray and were stored in deep freezer (−20°C) till analysis. Soil samples were analysed on 15th day from the last spray.

Extraction and Cleanup

Okra were harvested, pooled together, packed in plastic bags and transported to the laboratory for processing. Flubendiamide and its des-iodo metabolite residues were extracted from okra and soil as per the method of Fenoll et al. (2009) with slight modification. Fresh and healthy 1,000 g of okra fruits from each replication were drawn and immediately chopped and homogenized. From this, a representative subsample of 10 g was transferred to 100-mL capacity polypropylene tube followed by addition of 50 mL of acetonitrile. In case of soil, 10 g of sub-soil and 100 mL of acetonitrile:water (7:3, *v/v)* were used. The polypropylene tubes of okra fruit and soil were subjected to sonication for 30 min. This was followed by salting out by addition of 2 g NaCl, shaking for 5 min and centrifugation at 2,500 rpm for 15 min. Known amount of supernatant was drawn, evaporated to dryness in turbovap under nitrogen and final volume was made up to 2 mL with acetonitrile–water (7:3,*v/v*).

HPLC Analysis

Residues for flubendiamide and des-iodo flubendiamide were estimated by HPLC system (Merck-Hitachi)—Consisting of a L-7100 (computer operated dual pump), a L-7400 (UV detector) and a L-7200 (Auto sampler), HPLC column (30 cm)—Lichrospher, RP-18 (5 µm). A mixture of acetonitrile–water (70: 30, *v/v*) was used as the mobile phase, with a flow rate of 0.5 mL min^{-1}. The injection volume was 10 µL and the wavelength was set at 210 nm (λ_{max}, determined by using spectrophotometer. At this setting with 210 nm wavelength, the retention time for des-iodo flubendiamide and flubendiamide was 8.6 and 10.1 min, respectively. The residues were calculated by comparing the peak areas of the samples with that of matching standards run under same HPLC conditions. DT_{50} was calculated by using Hoskins formula (Hoskins 1961). In order to study the performance of the method, recovery study was carried out at 0.01, 0.05, 0.10 µg g^{-1},

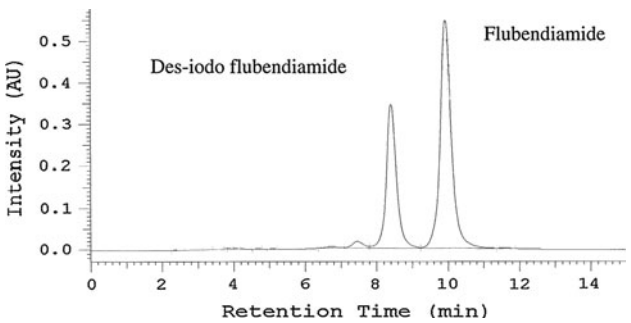

Fig. 2 Standard chromatograms of flubendiamide and des-iodo flubendiamide

Table 1 Recovery of flubendiamide and desiodo residues from okra fruit and soil at different fortification level

Fortification level (μg g^{-1})	Recovery %*			
	Flubendiamide		Des-iodo flubendiamide	
	Okra	Soil	Okra	Soil
0.01	85.27 ± 2.28	93.16 ± 1.45	84.43 ± 1.98	95.07 ± 3.45
0.05	86.12 ± 1.97	95.45 ± 3.47	85.27 ± 2.43	97.25 ± 1.37
0.10	88.14 ± 3.12	97.18 ± 2.75	87.24 ± 2.02	99.42 ± 2.81

* Mean ± SD of three replicates determinations

for flubendiamide and desiodo. Limit of determination or quantitation for either compound was 0.01 μg g^{-1}. Standard chromatogram of flubendiamide and des-iodo flubendiamide is shown in Fig. 2.

Results and Discussion

Results of the recovery study of flubendiamide and des-iodo flubendiamide carried out at 0.01, 0.05 and 0.10 μg g^{-1} in okra fruit and soil are presented in Table 1. Recovery study carried out as per the method described above showed that recovery of flubendiamide in okra was in the range of 85.27%–88.14% and that of des-iodo flubendiamide was, 84.43%–87.24%. Recovery of flubendiamide and des-iodo flubendiamide from soil was much higher, being 93.16%–97.18% for flubendiamide and 95.07%–99.42% for its metabolite (Table 1). Limit of quantification of the method was 0.01 μg g^{-1}. According to Sanco 2009 (Document No.SANCO/10684/2009), any recovery range of 60%–140% is acceptable for method validation. Thus, based on the recovery study, the performance of the method adopted in this experiment could be considered satisfactory for both flubendiamide and des-iodo flubendiamide.

When the dissipation of flubendiamide in/on okra fruit was studied, it was observed that flubendiamide residues were detected as parent compound only, and no des-iodo flubendiamide was detected above its LOQ of 0.01 μg g^{-1}. The insecticide persisted for 5 and 7 days on the fruits at standard and double dose of application, respectively (Table 2). The initial deposits of 0.28 and 0.53 μg g^{-1} immediately after application in/on okra fruits at standard and double dose resulted in 32.14% and 22.64% loss within first 24 h, respectively. On the 3rd day, loss of 46.43% and 43.40% was observed in the flubendiamide residues in standard and double dose, respectively. The residues gradually declined thereafter and reached below determination level (0.01 μg g^{-1}) on the 7th day at standard dose. At double dose, there was a decline by 60.37% and 86.79% in the residue at 5th and 7th day, respectively. Thereafter, the residues were below detection limit from 10th day onwards at double dose. The soil sample Taken on the 15th day after the last application showed flubendiamide residues below determination level. This may be due to the fact that photodegradation on soil surfaces is a route of dissipation of flubendiamide in the environment.

Table 2 Residues of flubendiamide and des-iodo flubendiamide in/on okra and soil

Days after application	Average residues recovered (μg g^{-1})* ± SD	
	Standard dose @ 24 g a.i. ha^{-1}	Double dose @ 48 g a.i. ha^{-1}
Okra		
0	0.28 ± 0.01	0.53 ± 0.01
1	0.19 ± 0.03 (32.14)	0.41 ± 0.03 (22.64)
3	0.15 ± 0.01 (46.43)	0.30 ± 0.02 (43.40)
5	0.09 ± 0.02 (67.86)	0.21 ± 0.05 (60.37)
7	BDL	0.07 ± 0.04 (86.79)
10	BDL	BDL
15	BDL	BDL
Soil		
15	BDL	BDL

* Average of three replicates; Figures in parenthesis shows % dissipation

BDL below determination level i.e., <0.01 μg g^{-1}

The rate of degradation of flubendiamide in/on okra fruits followed first-order kinetics in present study and based on the rate kinetic equation i.e., $Ct = C_0.e^{-kt}$ (where Ct = residues at time t, C_0 = initial concentration and k = rate constant), the half life of flubendiamide was worked out to be 4.80 and 5.10 days for standard and double dose, respectively (Table 3; Fig. 3).

Conclusion

The information generated in the present study clearly shows slightly slow dissipation of flubendiamide in/on okra fruits. It persisted for 5 days from standard dose treatments and reached below detectable level (<0.01 μg g^{-1}) by the

Table 3 Regression equation and half-life for first order dissipation of flubendiamide in okra

Treatment	Dissipation type	Regression equation	*K* values	r^2	DT_{50} (days)
Standard dose	Single phase	$y = -0.0626x + 1.695$	0.1443	0.9431	4.8
Double dose	Single phase	$y = -0.0589x + 1.046$	0.1358	0.9524	5.1

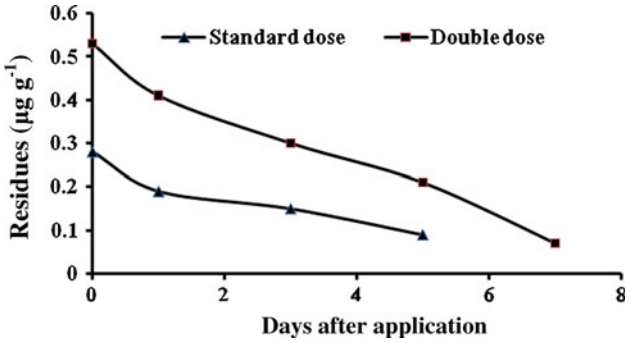

Fig. 3 Persistence of flubendiamide in/on okra at standard and double dose

7th days. For double dose it persisted for 7 days and reached below detectable level by 10th days. Maximum residue limit (MRL) for flubendiamide in India for cotton and rice is fixed at 0.1 ppm and based on this, it may be conclude that the waiting period for okra should be considered 5 days. This information could be utilized for planning the spray schedule, better formulation for slightly faster degradation in/on okra fruits and safety evaluation.

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