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Persistence and Dissipation of Flubendiamide and Des-iodo Flubendiamide in Cabbage (*Brassica oleracea Linne*) and Soil

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Abstract Flubendiamide belongs to a novel class of insecticide which controls lepidopteran pest complex of cabbage such as diamondback moth, cabbage white butterfly, cluster caterpillar etc. Being a newly introduced insecticide no information is available on its residue persistence in cabbage. A study was undertaken to evaluate the residue persistence of flubendiamide in cabbage and soil following 2 applications of flubendiamide 480 SC at the recommended and double the recommended dose of 24 and 48 g a.i. ha^{-1}. Initial residue deposits of flubendiamide in cabbage were 0.33 and 0.49 mg kg^{-1} respectively. The residues persisted for 10 days from the both the treatments and dissipated with the half-life of 3.9 and 4.45 days, respectively. Des-iodo flubendiamide, a metabolite of flubendiamide, was not detected in cabbage at any time during the study period. Soil sample collected from the treated field after 15 days was free from any residue of flubendiamide or its metabolite.

Keywords Cabbage · Des-iodo flubendiamide · Dissipation · Flubendiamide · Half-life

Cabbage (*Brassica oleracea Linne*) is a plant of the family Brassicaceae (or Cruciferae). It is the fourth most widely grown vegetable crop of India and the country ranks third in cabbage production in the world. In India, cabbage is an important cole crop grown in about 0.438 million hectares, producing about 6.335 million tonnes per annum (Mohan and Gujar 2003). It is one of the most popular vegetables in India, consumed both as raw and cooked form. Cabbage is grown throughout the year in India. The pest incidence in cabbage is generally more during February to September, though it is noticed throughout the year. Infestation of lepidopterous insects poses a great problem in cabbage cultivation. 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 and is recently registered for use against pest complex of cabbage. Flubendiamide represents a novel class of insecticides with extremely high activity against a broad spectrum of lepidopterous insects including diamondback moth, cabbage white butterfly, cluster caterpillar etc. in brassica vegetables (Tohnishi et al. 2005; Anonymous 2009). The efficacy of flubendiamide on important lepidopterous pests on cabbage was investigated in field trials conducted by the Japan Plant Protection Association (JPPA). This compound at a dose of 100 mg a.i. L^{-1} was superior to standard insecticides at recommended doses and achieved an all round protection (Hirooka et al. 2007b). Flubendiamide at 25 and 50 g a.i. ha^{-1} was extremely effective on diamondback moth (*Plutella xylostella*), normally resistant to conventional insecticides like spinosad, indoxacarb and fipronil. Flubendiamide did not show any phytotoxicity when applied even at a very high concentration of 400 mg L^{-1} (Hirooka et al. 2007a).

Latif et al. (2009) reported that flubendiamide is comparatively safe for natural enemies and might fit well into the integrated pest management (IPM) programs for brinjal. Shane (2006) reported that flubendiamide is an excellent fit in integrated pest management (IPM) program and

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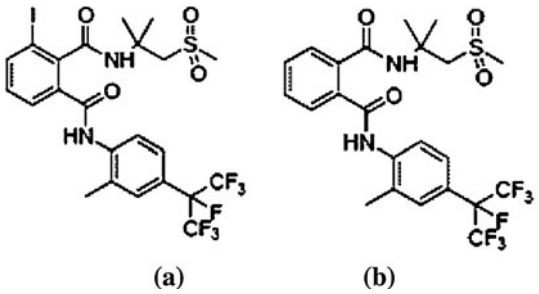

Fig. 1 Chemical structure of **a** flubendiamide, **b** des-iodo flubendiamide

insecticide resistance management (IRM) programs in a variety of crops because of the many favorable characteristics, including selective activity against a broad range of lepidopteran pests, a new mode of action, safety to pollinators, having a favorable environmental and ecological (low toxicity) profile, short pre-harvest interval (PHI), and low application rates leading to less environmental load. The present study was carried out to determine persistence of flubendiamide and its metabolite des-iodo flubendiamide residues in cabbage and soil following treatment at the dosage recommended for control of major pests of cabbage and also at double the recommended dose.

Materials and Methods

Flubendiamide (99.5% purity) its metabolite des-iodo flubendiamide (99.2% purity) (Fig. 1) and its formulation 480 SC were obtained from M/S Bayer Crop Science Limited (Mumbai, India). Standard solution of flubendiamide was prepared with gradient grade acetonitrile for HPLC and suitably diluted to obtain the working standards. All other reagents used in this study were of analytical grade.

The residue study of flubendiamide on cabbage was carried out at the experimental farm of Indian Institute of Horticultural Research, Bangalore, India. The treatments were untreated control, recommended dose, 24 g a.i. ha^{-1} and double dose 48 g a.i. ha^{-1}. For each application 5 plots were taken. The spray application was given twice at head formation stage, by using a high pressure foot sprayer at an interval of 10 days. Untreated control plots were sprayed with water. Analysis of cabbage samples was carried out on 0, 1, 3, 5, 7, 10 and 15 days after the second application. Two cabbage heads were harvested from each plot, pooled together, packed in plastic bags and transported to the laboratory for processing. A total of 5 kg sample was collected from each treatment. The cabbage samples were cut into small pieces, mixed in a Waring blender and a representative 50 g macerated sample (3 replicates) was taken for analysis. Soil samples were collected after 15 days following the last application. From each of the five plots soil samples were collected from about 30 cm depth and 3–5 cm diameter using soil augur. From each plot 1 kg soil was collected, pooled together and mixed thoroughly. The soil sample thus obtained was air dried, passed through 2 mm sieve and a representative 100 g sample in triplicate was taken for analysis.

A 50 g portion of representative cabbage sample was homogenized with 100 mL of acetonitrile in a Waring blender and filtered under vacuum through a Buchner funnel. The container and the filter cakes were washed twice with 50 mL acetonitrile and the combined extracts were collected in a 500 mL flask. The combined extracts were evaporated in a rotary vacuum evaporator and transferred into a 1L separatory funnel. The aqueous phase was partitioned into 100 + 50 + 50 mL chloroform after adding 50 mL saturated sodium chloride solution. The combined chloroform fraction was dried over 25 g of anhydrous sodium sulphate and evaporated to 10 mL. Clean-up of flubendiamide and its metabolites from the co-extractives was carried out as per Battu et al. (2008). To the flask containing chloroform fraction 500 mg activated charcoal was added, mixed thoroughly and kept for 2 h with intermittent shaking. Further it was filtered through Whatman filter paper no 1, concentrated to near dryness and redissolved in gradient grade acetonitrile for analysis by High Performance Liquid Chromatography. A representative 100 g soil sample (3 replicates) was taken for analysis and processed as per the method described above without any charcoal clean-up. In order to estimate the efficiency of the method, a recovery experiment was conducted by fortifying untreated samples (cabbage head and soil) with analytical grade flubendiamide and its metabolite des-iodo flubendiamide at the rate of 0.01, 0.02, 0.05 and 0.1 mg kg^{-1} level in cabbage and soil and processed as per the analytical method described above to obtain the per cent recovery before carrying out field sample analysis.

Analysis of flubendiamide and des-iodo flubendiamide residues was carried out using a High Performance Liquid Chromatograph (HPLC) with the following parameters: Shimadzu, Prominence LC 20 AT HPLC with a photo diode array (PDA) detector and a MERCK, Purospher Star RP-18, 250–4 mm i.d. S.S., 5 μm, column. The mobile phase was acetonitrile: water (6:4, v/v), with a flow rate of 1 mL min^{-1}, wavelength, 235 nm and injection volume was 20 μL. With these operating parameters the retention time of the des-iodo flubendiamide was at 7.3 min and flubendiamide was 9.8 min (Fig. 2).

Results and Discussion

Recovery study carried out as per the method described above showed that recovery of flubendiamide in cabbage

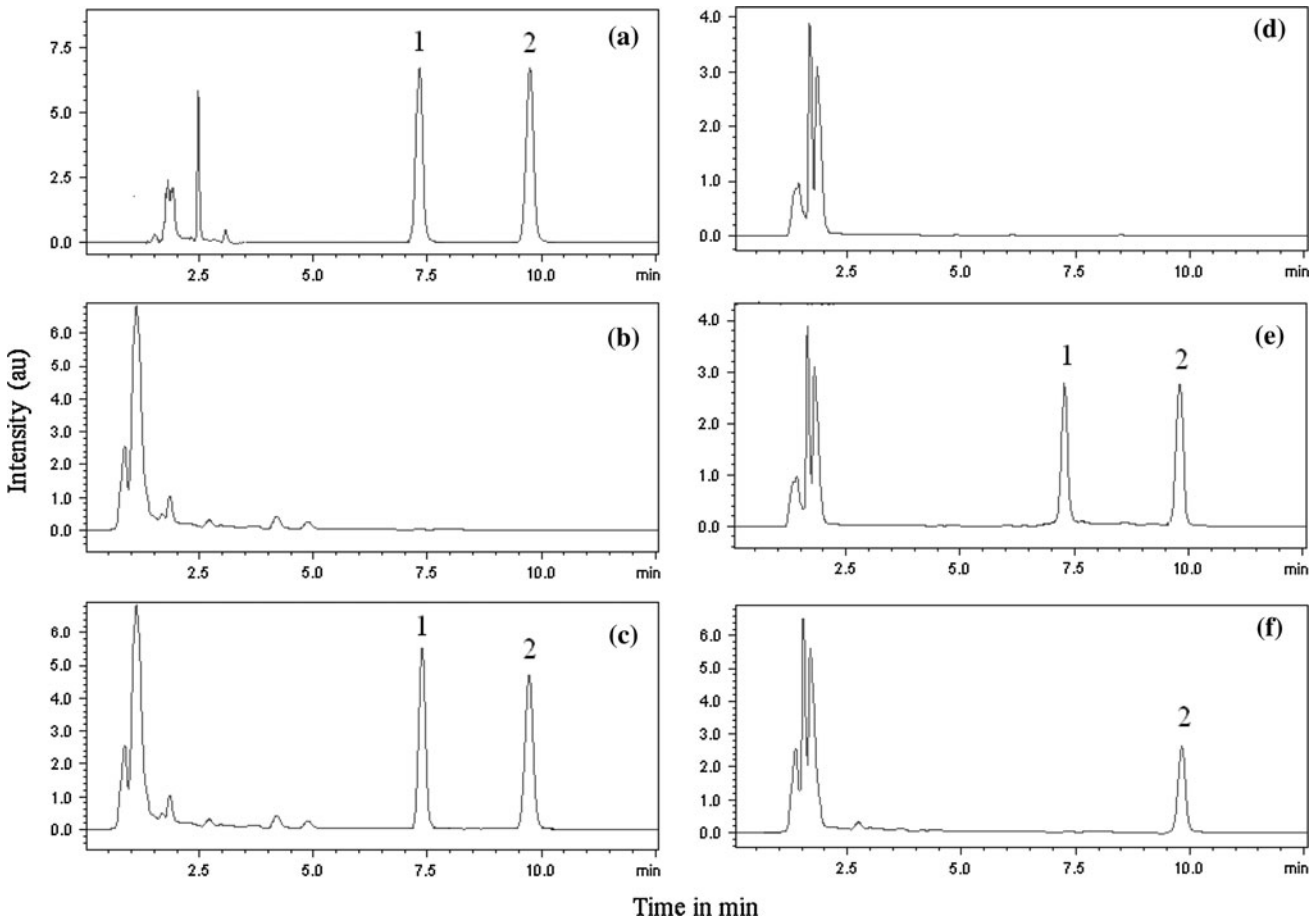

Fig. 2 GLC chromatogram of **a** reference standards, **b** soil untreated control, **c** soil fortified, **d** cabbage untreated control, **e** cabbage fortified, **f** field treated cabbage sample (*1*. des-iodo flubendiamide; *2*. flubendiamide)

was in the range of 87.25–89.06% and that of des-iodo flubendiamide was, 87.64–88.76%. Recovery of flubendiamide and des-iodo flubendiamide from soil was much higher, being 98.08–99.90% for flubendiamide and 97.05–98.65% for its metabolite (Table 1). Limit of quantification of the method was 0.01 mg kg^{-1}.

Residue deposit of flubendiamide on cabbage was very low from treatment at the recommended dose. Two applications of flubendiamide 480 SC @ 24 g.a.i. ha^{-1} resulted in the initial residue deposit of 0.33 mg kg^{-1} in cabbage. The same formulation when applied in a similar manner @ 48 g a.i. ha^{-1}, the initial residues of flubendiamide detected was 0.49 mg kg^{-1}. Dissipation of flubendiamide residues from cabbage head was slow. It persisted for 10 days from both the treatments and reached below detectable level (<0.01 mg kg^{-1}) by the 15th day. Flubendiamide residues dissipated with half-lives of 3.9 and 4.45 days from treatment at 24 and 48 g a.i. ha^{-1}, respectively

Table 1 Recovery study of flubendiamide and des-iodo flubendiamide residues on cabbage and soil at various fortification levels

Fortified concentration (mg kg^{-1})	Mean recovery (%)* ± SD			
	Flubendiamide		Des-iodo flubendiamide	
	Cabbage	Soil	Cabbage	Soil
0.01	88.55 ± 1.532	98.08 ± 1.011	87.64 ± 1.844	97.05 ± 2.346
0.02	87.25 ± 1.124	99.04 ± 1.006	88.32 ± 1.098	97.86 ± 1.593
0.05	88.56 ± 2.075	99.80 ± 2.005	88.76 ± 1.225	97.92 ± 1.218
0.10	89.06 ± 2.683	99.90 ± 1.024	88.46 ± 2.592	98.65 ± 1.815

* Average of three replicates

Table 2 Residues of flubendiamide and des-iodo flubendiamide in cabbage and soil

Days after treatment	Untreated control	Average residues recovered ± SD (mg kg^{-1})									
		Standard dose @ 24 g a.i.ha^{-1}					Double dose @ 48 g a.i.ha^{-1}				
		Flubendiamide				Des-iodo flubendiamide	Flubendiamide				Des-iodo flubendiamide
		R1	R2	R3	Mean ± SD		R1	R2	R3	Mean ± SD	
0	ND	0.324	0.337	0.330	0.330 ± 0.0006	BDL	0.496	0.467	0.507	0.490 ± 0.0207	BDL
1	ND	0.286	0.263	0.291	0.280 ± 0.0146	BDL	0.411	0.400	0.423	0.411 ± 0.0115	BDL
3	ND	0.212	0.233	0.220	0.222 ± 0.0106	BDL	0.332	0.340	0.331	0.334 ± 0.0049	BDL
5	ND	0.192	0.175	0.189	0.185 ± 0.0091	BDL	0.263	0.240	0.241	0.248 ± 0.0130	BDL
7	ND	0.123	0.128	0.132	0.128 ± 0.0045	BDL	0.192	0.201	0.182	0.192 ± 0.0095	BDL
10	ND	0.051	0.049	0.048	0.049 ± 0.0015	BDL	0.102	0.092	0.095	0.096 ± 0.0051	BDL
15	ND	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Soil (at 15th day)	ND	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL

Limit of Quantification (LOQ)−0.01 mg kg $^{-1}$

Below detectable limit <0.01 mg kg $^{-1}$

ND Not detected

(Table 2). Des-iodo flubendiamide, a metabolite of flubendiamide was not detected at any time during the sampling period. Soil sample collected 15 days after the last spray was free from residues of both flubendiamide and its metabolite des-iodo flubendiamide.

Six residue trials on cabbage with flubendiamide 240 WG or 480 SC formulation @ 48–108 g a.i. ha^{-1} have been carried out in Australia. Residues of flubendiamide after a 3 day withholding period were <0.045, 0.190, 0.27, 0.76 and 2.72 mg kg^{-1} following three foliar applications @ 48 g a.i. ha^{-1} made at weekly intervals (Anonymous 2009). A similar study was carried out on broccoli and the residues of flubendiamide were 0.128, 0.218 and 0.25 mg kg^{-1} after a 3 day withholding period following three foliar applications @ 48 g a.i. ha^{-1} (Anonymous 2009). In the present study 2 applications of flubendiamide 480 SC @ 48 g a.i after 3 days were 0.333 mg kg^{-1} and with half this dosage residues were 0.22 mg kg^{-1} which are well within the reported results. As reported in the present study absence of metabolite, des-iodo flubendiamide has been reported from metabolism studies carried out earlier. The metabolism of flubendiamide was investigated in cabbage and tomato using flubendiamide labelled with ^{14}C in either the phthalic or aniline ring. In cabbage leaves, parent compound accounted for approximately 90% of the total radioactive residues 3 weeks after treatment with radiolabelled flubendiamide. Similar levels of parent compound were also observed after 6 weeks with the residues of des-iodo flubendiamide being <0.01 mg kg^{-1}. In tomato unchanged parent compound accounted for 99% of the total radioactivity (Anonymous 2009). In soil after 15 days, residues of both flubendiamide and des-iodo flubendiamide were absent. This may be due to the fact that photodegradation on soil surfaces is a route of dissipation of flubendiamide in the environment. These results suggest that flubendiamide can be used on cabbage safely with a pre-harvest interval of 10 days.

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