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Comparative persistence of flubendiamide residues in chilli following application as individual and combination formulation

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Abstract

Introduction Chilli, an important crop of India, is infested by various insect pests during its cultivation. A new insecticide flubendiamide is being registered in India individually as well as in combination with thiacloprid for use against lepidopteran and sucking pests of chilli. *Objectives* To study the dissipation pattern of flubendiamide following its application as flubendiamide alone and in combination with thiacloprid. *Methods* Standardized methodologies using high-performance liquid chromatography, were adopted to estimate residues of flubendiamide, its metabolite, des-iodo flubendiamide and thiacloprid in chilli. *Results* Average initial deposits of flubendiamide in chilli following its application as flubendiamide alone or as flubendiamide and thiacloprid combination were almost the same, i.e. 0.467 and 0.499 mg kg^{-1}, respectively, from treatment at recommended dose of 60 g a.i. ha^{-1}, 0.824 and 0.992 mg kg^{-1} from treatment at double the recommended dose. Residues dissipated to below detectable levels within 7–10 days, respectively. Des-iodo flubendiamide, a metabolite of flubendiamide was not detected in any of the chilli samples collected at different time intervals. Residues of thiacloprid also dissipated quickly and were below quantifiable level of 0.01 mg kg^{-1} at 7 and 10 days, respectively. *Conclusion* Flubendiamide residues behaved in almost identical manner when applied alone or in combination with thiacloprid.

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Introduction

Flubendiamide, N′-[1,1-dimethyl-2-(methylsulphonyl)ethyl]-3-iodo-N-{4-,2,2tetrafluoro-1-(trifluoromethyl) ethyl]-0-tolyl} phthalimide belongs to a new chemical class, the phthalic acid diamides, and is very effective against lepidopteran pests on a variety of crops (Tohnishi *et al.*, 2005). The novel biochemical mode of action of flubendiamide exhibits excellent larvicidal activity as an orally ingested toxicant by targeting and disrupting the Ca^{2+} balance. This results in rapid cessation of feeding and extended residual control, providing superior plant protection against a broad-range of economically important lepidopteran pests, including *Helicoverpa* spp., *Spodoptera* spp., *Plutella xylostella*, *Pseudoplusia* spp., *Trichoplusia* spp. and *Agrotis* spp. Flubendiamide has recently been introduced in India and is presently registered for use on crops such as rice, cotton, pigeon pea, cabbage and tomato. Thiacloprid, N-{3-[(6-Chloro-3-pyridinyl)methyl]-1,3-thiazolan-2-yliden} cyanamide, is a chloronicotinyl insecticide that is active against sucking insects pests such as aphids, whiteflies and some jassids, as well as against weevils, leaf miners and various species of beetles (Elbert *et al.*, 2000). A combined formulation of flubendiamide and thiacloprid is being registered in India by Bayer Crop Science for use in chilli, tomato, etc., for control of lepitopterous as well as sucking pests of these crops. Chilli (*Capsicum annum* Linnaeus) is an important cash crop of which India is the largest producer in the world. Chilli and chilli-based product export from India was valued at Rs. 10.8 billion during the year 2008–2009 (Spices Board India). However, scanty information is available on the persistence of flubendiamide and thiacloprid in chilli either as individual treatment or in the form of combined formulation of flubendiamide and thiacloprid. Thus, this study was taken up to compare residue persistence of flubendiamide in chilli following its foliar application with flubendiamide 480 SC and flubendiamide+thiacloprid 480 SC. The residue persistence of thiacloprid was also studied in chilli.

Materials and methods

Chemicals

The certified reference standards of flubendiamide (purity 99.5%) along with reference standard of des-iodo flubendiamide (purity 99.2%) and thiacloprid (purity 99.6%) were supplied by M/S Bayer Crop Sciences Limited (Mumbai, India). Acetonitrile used was of gradient grade for high-performance liquid chromatography (HPLC). All other reagents used were of analytical grade. The stock solutions of flubendiamide, des-iodo flubendiamide and thiacloprid were prepared at 1000 mg mL^{-1} in gradient grade acetonitrile.

Experimental details

Chilli (variety, Arka Lohit) crop was raised during *Rabi* (a major crop growing season in India, starting November), 2009, according to recommended agronomic practices at Indian Institute of Horticultural Research, Bangalore, India, using a randomized block design. A 50 m^2 plot was divided into 5 m^2 sub-plots each acting as a treatment replication. The treatments were (i) untreated control, (ii) flubendiamide alone as flubendiamide 480 SC formulation at 60 and 120 g a.i. ha^{-1}, twice at 10-day interval and (iii) flubendiamide+thiacloprid applied as a combination formulation, flubendiamide 24%+thiacloprid 24% (480 SC) at 60+60 and 120+120 g a.i. ha^{-1} thrice at 10-day interval. All foliar applications were made using a knapsack sprayer starting at fruit formation stage. In control plots, only water was sprayed. About 50 g of green chillies were collected from each replication plot to make 500 g of representative sample for each treatment starting about 2 h after the last spray application, i.e., on 0, 1, 3, 5, 7, 10, 15 and 21 days. The green chillies ripened to red chillies in about 30 days time. Red chilli samples were collected at harvest, i.e. on the 30th day after the last spray. Soil samples were collected from 0 to 15 cm depth at 21 days after the last application was given. Samples were extracted immediately after bringing from the field.

Estimation of flubendiamide and des-iodo flubendiamide residues

The extraction and clean-up of chilli and soil samples for residues of flubendiamide and its metabolite des-iodo flubendiamide were carried out as per procedure reported by Battu *et al.* (2008). A representative 50 g sample of chopped and macerated green/red chilli in triplicate was dipped overnight in 100 mL acetonitrile in an Erlenmayer flask. The extract was filtered under vacuum through Whatman filter paper using Celite as filtration aid, into a 1 L separatory funnel along with rinsing of acetonitrile. The filtrate in the separatory funnel was diluted with 600 mL brine solution and the contents were partitioned three times with 100, 50 and 50 mL with chloroform. The chloroform fractions were combined, dried over anhydrous sodium sulfate and treated with 500 mg activated charcoal powder for about 2–3 h at room temperature. The clear extract so obtained was filtered through Whatman filter paper No. 1, concentrated to near dryness and again redissolved in about 20 mL gradient grade acetonitrile, which was concentrated using rotary vacuum evaporator at 40 °C. The process was repeated to completely evaporate chloroform and the final volume was reconstituted to 5 mL using gradient grade acetonitrile. Soil sample (100 g) in triplicate were also analyzed in a manner similar to that described for chilli samples.

Estimation of thiacloprid residues

A representative 50 g chilli sample of chopped green/red chilli in triplicate was extracted by blending with 150 mL acetonitrile using a Waring blender. The extract was filtered and rinsing along with acetonitrile under vacuum and evaporated using rotary vacuum evaporator at 50 °C. The aqueous remainder was washed with 3 × 50 mL hexane after adding 50 mL of saturated sodium chloride solution followed by 100 mL of hexane:ethyl acetate (98:2 v/v) and the washings were discarded. The lower aqueous phase was then partitioned against 3 × 50 mL dichloromethane and the organic layers combined after passing through anhydrous sodium sulfate. The combined dichloromethane extract was concentrated to near dryness in a rotary vacuum evaporator and the residues redissolved in 2 mL ethyl acetate. The concentrated ethyl acetate extract was passed through a glass chromatography column (50 × 1.5 cm) containing florisil deactivated with 5% water and using ethyl acetate as solvent. The column was eluted with 20 mL ethyl acetate, the eluate was concentrated and residues redissolved into 5 mL gradient grade acetonitrile for analysis by HPLC.

HPLC analysis

A Shimadzu analytical HPLC with Prominence 20AT pump and photo diode array detector (Shimadzu, Japan) was used with a stainless steel, 300 × 4.6 mm, Lichrospher RP 18 column (E. Merck, Germany). The residues of flubendiamide and its metabolite des-iodo flubendiamide were estimated using a mobile phase of acetonitrile:water, 60:40 v/v, at a flow rate 1 mL min^{-1}. Residues were estimated at a wavelength (λ_{max}) of 235 nm. The residues of thiacloprid were estimated using the same HPLC and column operated with a mobile phase of gradient grade acetonitrile:water, 30:70 v/v, at a flow rate of 1 mL min^{-1}. The wavelength of detection was 270 nm. Under the above operating conditions the retention times of flubendiamide, des-iodo flubendiamide and thiacloprid were found to be 12.17, 10.13 and 9.49 min, respectively. The limits of quantification (LOQ) determined at the S/N ratio of 3:1, of flubendiamide, des-iodo flubendiamide and thiacloprid were 0.05, 0.05 and 0.01 mg kg^{-1}, respectively.

Recovery studies

Recovery studies were carried out in order to establish the reliability of the analytical method for the present study by fortifying chilli fruits and soil with flubendiamide and des-iodo flubendiamide at the level of 0.05 mg kg^{-1}, i.e., the LOQ level, 0.25 mg kg^{-1} (five times LOQ level) and 0.5 mg kg^{-1} (10 times LOQ level). Recovery studies were also carried out by fortifying chilli fruits and soil with thiacloprid at the level of 0.01 mg kg^{-1} (LOQ level), 0.05 mg kg^{-1} (five times LOQ level) and 0.1 mg kg^{-1} (10 times LOQ level) separately with analytical standard of thiacloprid. The mean recoveries (five replicates) of flubendiamide was 77.8–89.6% in chilli, 81.2–91.1% in soil and that of des-iodo flubendiamide was 81.2–91.3% in chilli and 82.3–94.9% in soil. The mean recovery for thiacloprid was 84.6–92.6% in chilli and 86.4–91.4% in soil (Table 1).

Table 1 Fortification and recovery of flubendiamide, its metabolite des-iodo flubendiamide and thiacloprid in chilli and soil

Level of fortification (mg kg^{-1})	Percent mean recovery ± SD*			
	Flubendiamide		Des-iodo flubendiamide	
	Chilli	Soil	Chilli	Soil
0.05	77.8 ± 1.98	81.2 ± 0.87	81.4 ± 0.83	82.3 ± 1.74
0.25	80.7 ± 0.55	91.1 ± 2.49	81.2 ± 2.82	83.0 ± 2.28
0.5	89.6 ± 0.60	84.7 ± 2.21	91.3 ± 0.91	94.9 ± 2.59
			Thiacloprid	
			Chilli	Soil
0.01			84.6 ± 1.48	86.4 ± 2.73
0.05			89.6 ± 0.65	88.1 ± 0.56
0.1			92.6 ± 2.30	91.4 ± 1.10

*Average of three replicate analyses ± SD.

Results and discussion

The results of dissipation of flubendiamide in chilli following its application as flubendiamide alone and as combination formulation of flubendiamide and thiacloprid are presented in Table 2, Figure 1. The average initial deposits of flubendiamide in chilli following application of flubendiamide 480 SC and flubendiamide (24%) + thiacloprid (24%), 480 SC were almost the same, i.e. 0.467 and 0.499 mg kg^{-1}, respectively, from recommended dose of application of 60 g a.i. ha^{-1}. The same were 0.824 and 0.992 mg kg^{-1}, respectively, when double the recommended dose of application (120 g a.i. ha^{-1}) was given. Flubendiamide residues dissipated with half-lives of 1.9–2.8 days to below detectable level of 0.05 mg kg^{-1} within 7 and 10 days following application at recommended and double the recommended doses, respectively, irrespective of the type of formulation used. No significant difference in flubendiamide residue persistence was observed following application as flubendiamide 480 SC as compared with application as flubendiamide (24%) + thiacloprid (24%), 480 SC. Red chilli samples harvested at 30 days after the last application did not contain any residues of flubendiamide. Des-iodo flubendiamide, a metabolite of flubendiamide was not detected in any of the green/red chilli samples collected at different time intervals. No residues of flubendiamide and/or des-iodo flubendiamide were detected in soil sampled at 21 days after the last spray.

Sahoo and Battu (2009), have studied the behavior of flubendiamide residues in chilli following a similar dose of application in a very different agro climatic zone of India (Trans Gangetic plains region) having high day temperatures. They reported more than 80% dissipation of flubendiamide residues within 3 days of the last application at both the dosages with half-life of flubendiamide residues being $<$ 1 day. The somewhat longer persistence of flubendiamide in the present study (half-life of flubendiamide = 2–2.7 days) could be due to difference in agro climatic conditions, as the present study was carried out in Bangalore, which falls in Southern Plateau and Hills Region of India having moderate day temperatures. The average temperature varied between 19.9 and 33.4 °C, whereas relative humidity and total rainfall were 53.3–70.7% and 75 mm, respectively, during the period of study. However, in both the studies, the residues

Table 2 Residues of flubendiamide in chilli following application of flubendiamide alone and combination formulation of flubendiamide and thiacloprid

	Residues of flubendiamide (mg kg^{-1}) in chilli ± SD*			
	From individual formulation		From combination formulation	
DALT	60 g a.i. ha^{-1}	120 g a.i. ha^{-1}	60+60 g a.i. ha^{-1}	120+120 g a.i. ha^{-1}
0	0.467 ± 0.02	0.824 ± 0.02	0.499 ± 0.01	0.992 ± 0.01
1	0.361 ± 0.02	0.623 ± 0.01	0.442 ± 0.01	0.654 ± 0.01
3	0.206 ± 0.01	0.309 ± 0.01	0.209 ± 0.00	0.367 ± 0.00
5	0.135 ± 0.01	0.182 ± 0.00	0.140 ± 0.00	0.172 ± 0.00
7	BDL	0.087 ± 0.02	BDL	0.081 ± 0.00
10	BDL	BDL	BDL	BDL
15	BDL	BDL	BDL	BDL
21	BDL	BDL	BDL	BDL
Red chilli	BDL	BDL	BDL	BDL

*Average residues ± SD from analyses of triplicate laboratory samples taken from a composite field sample.
DALT, days after last treatment; BDL, below detection limit (0.05 mg kg^{-1}).

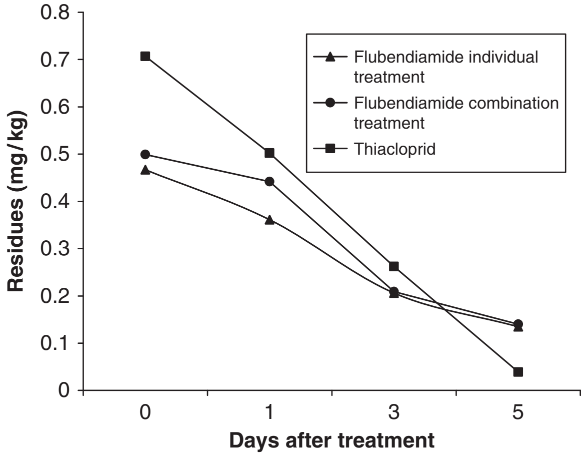

Figure 1 Dissipation of residues of flubendiamide and thiacloprid at recommended dose of application with time.

dissipated to below detectable levels within 7 and 10 days from treatment at recommended dose and double the recommended dose, respectively. Thus, although the initial rate of dissipation may vary depending on the climatic conditions during the period of study, the residues of flubendiamide degrade to below detectable level within a week of last application, if applied at recommended dose.

Integrated Pest Management (IPM) is a tool that uses multiple pest management tactics to efficiently produce crops, while minimizing undesirable environmental and health risks. This study showed that flubendiamide is a pesticide with high dissipation rate in chilli, which can be safely integrated into an IPM package for chilli. In an earlier study conducted to evaluate the efficacy of flubendiamide as an IPM component for the management of brinjal shoot and fruit borer, it was seen that that application of flubendiamide at 5% level of fruit infestation in combination with other pest-control methods could be used for the management of this pest (Latif *et al.*, 2009). Flubendiamide is also known to degrade within 3–5 days in tomato (Kooner *et al.*, 2009) and result in very low, viz. $< 0.07\%$, of applied insecticide in edible parts (head) of cabbage (Anonymous, 2009). Also, unchanged parent compound, i.e. flubendiamide was the only residue detected in all the above studies including the present one and no residue of metabolite, viz. des-iodo flubendiamide was detected. It has been reported earlier that des-iodo metabolite residues of flubendiamide have not been found to be significant in tomato or apple metabolism studies with radiolabelled flubendiamide (Anonymous, 2009).

In case of thiacloprid, the initial residues following application as combination formulation with flubendiamide at recommended and double the recommended doses were 0.71 and 1.01 mg kg^{-1}, respectively (Table 3, Figure 1). The residues quickly dissipated and were below quantifiable level of 0.01 mg kg^{-1} at 7 and 10 days, respectively, after the last application. The residues dissipated with half-life of 1.2–2.1 days. The combination formulation of flubendiamide and thiacloprid can therefore safely be used in chilli for control of lepidopteran as well as sucking pests of this crop at the recommended dose of application. The European Union maximum residue limit for flubendiamide including des-iodo flubendiamide in chilli peppers is 0.2 mg kg^{-1}. Considering the above safe residue level the waiting period required for harvest of safe produce would be 4 days for

Table 3 Residues of thiacloprid following its application as combination formulation of flubendiamide and thiacloprid

	Residues of thiacloprid (mg kg^{-1}) in chilli	
DALT	60+60 g a.i. ha^{-1}	120+120 g a.i. ha^{-1}
0	0.707 ± 0.01	1.011 ± 0.03
1	0.502 ± 0.02	0.658 ± 0.03
3	0.262 ± 0.01	0.400 ± 0.02
5	0.039 ± 0.00	0.219 ± 0.01
7	BDL	0.097 ± 0.00
10	BDL	BDL
15	BDL	BDL
21	BDL	BDL
Red chilli	BDL	BDL

DALT, days after last treatment; BDL, below detection limit (0.01 mg kg^{-1}).

chillies irrespective of whether individual or combined formulation of flubendiamide was sprayed on the crop. The European Union maximum residue limit of thiacloprid in chilli peppers is 1.0 mg kg^{-1} and therefore only 1-day waiting period is required for safe harvest of chillies following its application as combined formulation with flubendiamide. Also, residues of flubendiamide as well as thiacloprid would be below detectable levels in chillies at harvest if application at recommended dose is stopped only a week before harvest.

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