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Persistence and Dissipation of the Insecticide Flubendiamide and its Metabolite Desiodo Flubendiamide Residues in Tomato Fruit and Soil

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Abstract A method for residue analysis of flubendiamide and its metabolite desiodo flubendiamide was developed using high performance liquid chromatography. This method was then used to evaluate the residual level and dissipation rate of flubendiamide and desiodo flubendiamide in the tomato fruit. The half-life of flubendiamide in tomato fruit was 1.64 and 1.98 days in recommended and double of the recommended dose, respectively. Tomato fruit and soil samples analyzed on the 10th day after the last spray revealed that flubendiamide and its metabolite desiodo flubendiamide residues at below determination level (0.01 μg g^{-1}) at either dose of application.

Keywords Flubendiamide · Desiodo flubendiamide · HPLC · Residues · Dissipation

Tomato (*Lycopersicon esculentum*) is one of the most important protective foods because of its special nutritive value and also because of its wide spread production. It is one of the most versatile vegetable with wide usage in Indian culinary tradition. Tomatoes are used for soup, salad, pickles, ketchup, puree, sauces and in many other ways it is also used as a salad vegetable. It is an economically important vegetable crop of India with an annual production of around 10 million tonnes. In India tomato occupies second position amongst the vegetable crops in terms of production. Fruit borer (*Helicoverpa armiegra*) is an important pest of tomato. It causes serious damage during the fruiting stage and the infested crop exhibits damaged fruits, shoots and leaves.

Flubendiamide (Fig. 1) belongs to a chemical family of benzenedicarboxamides or phthalic acid diamides with insecticidal activity through the activation of the ryanodine-sensitive intracellular calcium release channels, leading to the cessation of feeding immediately after ingestion of the compound. The compound shows extremely strong insecticidal activity especially against lepidopterous pests including resistant strains (Tohnishi et al. 2005; Ebbinghaus et al. 2007).

Being a new compound not much information is available on its method of analysis on flubendiamide and its metabolite desiodo flubendiamide in/on tomato fruit and soil in tropical Indian condition. In this study, a simple and rapid method for residue analysis of flubendiamide and its metabolite desiodo flubendiamide tomato fruit and soil was developed. The dissipation dynamics of flubendiamide and desiodo flubendiamide were investigated in tomato crop under the field conditions. This simple residue analytical method would provide a new tool to evaluate the safe application rate of flubendiamide for tomato crops.

Materials and Methods

The flubendiamide (96.7% purity) and desiodo flubendiamide (99.3% purity) standards and flubendiamide

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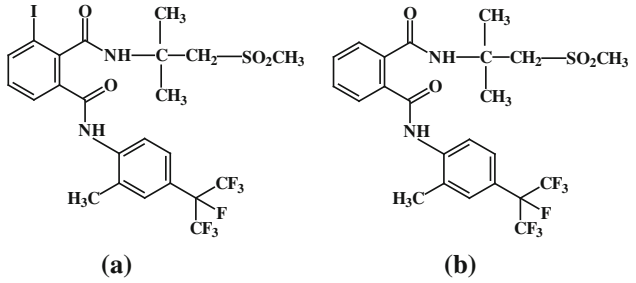

Fig. 1 The chemical structures of flubendiamide (**a**) and desiodo flubendiamide (**b**)

formulation (20 WG) were obtained from Rallis India Ltd (Bangalore, India). All the other chemicals and solvents were from J.T. Baker, Mumbai (India) and were of analytical grade. Primary secondary amine (PSA, 40 μm, Bondesil) were purchased from Varian (Palo Alto, CA, USA). Analytical-grade sodium chloride and anhydrous magnesium sulphate was purchased from Merck Company (Mumbai, India). Stock solutions (1,000 μg mL^{-1}) were prepared by dissolving reference standards in acetonitrile.

All analyses were conducted with an Agilent 1200 series HPLC equipped with UV–Vis detector. A reverse-phase BDS, Hypersil C-18 (5 mm), 150 mm long, 4.6 mm i.d, was used as the column and maintained at room temperature. The mobile phase consisted of acetontirile/water (60/40, v/v), with a flow rate of 1.0 mL min^{-1}. The retention time (RT) was 9.77 and 7.66 min for flubendiamide and desiodo flubendiamide, respectively. The residues were calculated by comparing the peak areas of the samples with that of matching standards run under same HPLC conditions. Blank analyses were performed in order to check interference from the matrix.

Recovery studies were carried out in order to establish the reliability of the analytical method and to know the efficiency of extraction and clean up steps employed for the present study, by fortifying the tomato fruit and soil samples with different levels of 0.01, 0.05 and 0.10 μg g^{-1} with analytical standard solution of flubendiamide and desiodo flubendiamide. The samples were analyzed following the described procedure. Results of recovery study are shown in Table 1.

The residue study of flubendiamide on tomato was carried out at the experimental seed research farm of Kalyani, West Bengal, India, following the good agricultural practices of the region. Tomato crop (ARCH-128) was raised in the field in a randomized block design with plot size of 30 m^2 each replicate. Flubendiamide 20% WG foliar application was given to tomato crop using a knapsack sprayer first time at fruiting stage and again after 15 days. The applications were standard dose 50 g a.i. ha^{-1} and double dose 100 g a.i. ha^{-1}. Untreated control plots were sprayed with water. The spray volume was 500 L ha^{-1}. The maximum and minimum temperature during tomato crop was 31.5 and 23.1°C, respectively, with average relative humidity of 85.55%. The rainfall recorded during the period was 6.0 mm.

Residue analysis of tomato fruits were carried out after the second spray, over a period of 10 days, i.e. on the 0 (2 h), 1, 3, 5, 7 and 10th day. Tomato fruit (500 g) samples were collected from 5 to 7 places randomly in each treatment plots replication wise on each date of sampling. Samples from untreated control plots were also collected separately in the same way. From each plot 500 g (approximately) tomato samples were harvested and samples from all replicates were pooled together. From each treatment 2.5 kg samples were collected and the fruit samples were comminuted with a grinder. Soil samples were collected after 10 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 20 g sample in triplicate was taken for analysis.

A representative (10 g) from each replication of each treatment homogenized tomato fruit sample was taken in a 50 mL centrifuge tube and then 10 mL of HPLC grade acetonitrile was added. The sample was kept undisturbed for 30 min and then polypropylene tubes were vortexed for 2 min. This was followed by salting out by addition of 1 g NaCl, and 4.0 g $MgSO_4$ were added, and the vortexing process was repeated for 2 min. Then the sample was centrifuged for 10 min at 10,000 rpm. From it 6 mL clear upper

Table 1 Recoveries of flubendiamide and desiodo flubendiamide from tomato and soil

Fortified concentration (μg g^{-1})	Mean recovery (%)[a] ± SD			
	Flubendiamide		Desiodo flubendiamide	
	Tomato fruit	Soil	Tomato fruit	Soil
0.01	97.33 ± 1.16	86.77 ± 1.82	97.47 ± 0.51	89.02 ± 1.17
0.05	97.48 ± 1.86	92.87 ± 2.70	98.16 ± 1.22	94.01 ± 3.91
0.1	98.33 ± 1.37	90.74 ± 1.54	98.00 ± 0.48	92.18 ± 1.91

[a] Average of three replicates

Table 2 Residues of flubendiamide in tomato fruit and soil

Days after treatment	Residues recovered ± SD (μg g^{-1})							
	Standard dose @ 50 g a.i. ha^{-1}				Double dose @ 100 g a.i. ha^{-1}			
	R1	R2	R3	Mean[a] ± SD	R1	R2	R3	Mean[a] ± SD
0	0.24	0.29	0.27	0.27 ± 0.03 (−)	0.44	0.49	0.46	0.46 ± 0.03 (−)
1	0.12	0.17	0.15	0.15 ± 0.03 (44.4)	0.22	0.25	0.31	0.26 ± 0.05 (43.5)
3	0.06	0.10	0.08	0.08 ± 0.02 (70.4)	0.09	0.11	0.14	0.11 ± 0.03 (76.1)
5	0.03	0.04	0.03	0.03 ± 0.01 (88.9)	0.07	0.08	0.10	0.08 ± 0.02 (82.6)
7	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
10	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Soil (10th day)	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL

Figures in parenthesis dissipation percentage

BDL below detection limit, *SD* standard deviation

[a] Mean of three replicates

layer was transferred into a 10 mL centrifuge tube prefilled with 25 mg PSA and 150 mg anhydrous magnesium sulfate. The mixture was then placed in vortex for 2 min and again centrifuged for 5 min at 5,000 rpm. Then 2 mL supernatant liquid was collected and transferred to turbovap tube and evaporated to dryness under a gentle stream of nitrogen by using the Turbovap LV (Caliper Life Sciences, Russelsheim, Germany) set at 40°C and 7.5 psi Nitrogen flow. The residue was then reconstituted in 0.5 mL of mobile phase and filtered through a 0.2 mm filter prior to HPLC analysis.

A representative homogenized soil sample (20 g each) was taken in a conical flask (250 mL) and shaken for half an hour using a mechanical shaker with 100 mL acetonitrile and the extract was filtered through filter paper (Whatman No. 42.) mounted on a buchner funnel. The pooled filtrate was concentrated to about 50 mL using a rotary evaporator with a water bath at 40°C and transferred to separatory funnel. The sample was partitioned thrice with 100 mL hexane (saturated with acetonitrile) and the upper hexane layer was discarded each time. The lower acetonitrile layer was partitioned against dichloromethane (3 × 100 mL) by addition of 4% saturated NaCl solution. The combined acetonitrile layer was collected and concentrated to dryness on a rotary evaporator (40°C). The residue was made up with acetonitrile: water (60:40, v/v) for estimation by HPLC.

Results and Discussion

The method evaluation was carried out to determine the fortified recoveries, precision and limits of detection of the analytical method. The standard solutions of flubendiamide and desiodo flubendiamide were added to the untreated tomato fruit at levels of 0.01, 0.05 and 0.10 μg g^{-1}. The fortified samples were analyzed using the procedure described with three repetitions. The results of the recovery study of flubendiamide and desioido flubendiamide carried out at the levels of 0.01, 0.05 and 0.10 μg g^{-1} in tomato fruit and soil are presented in Table 1. Flubendiamide and desioido flubendiamide recoveries in tomato fruits ranged from 97.33–98.33% to 97.47–98.16%, respectively and standard deviation of 1.16–1.86%. The corresponding recoveries for soil were 86.77–92.87% and 89.02–94.01%. As the recovery percentage is more than 85% for all the substrates, hence the method can be adopted for residue and dissipation study for both flubendiamide and desiodo flubendiamide in tomato fruit and soil samples. Standard calibration curve of flubendiamide and desiodo flubendiamide was constructed by plotting concentration against peak area. Good linearity was achieved, limit of detection (LOD) and limit of quantification (LOQ) considered when signal to noise ratio of 3:1 and 10:1, respectively. LOD and LOQ were determined as 0.003 and 0.01 μg g^{-1}, respectively.

When the dissipation of flubendiamide in/on tomato fruit was studied, it was observed that flubendiamide and desiodo flubendiamide residues were detected. The insecticide persisted for 5 days on the fruits at standard and double dose of application, respectively (Table 2). The initial deposits of 0.27 and 0.46 μg g^{-1} immediately after application in/on tomato fruits at standard and double dose resulted in 44.4% and 43.5% loss within first 24 h, respectively. On the 3rd day, loss of 70.4–76.1% was observed in the flubendiamide residues in standard and double dose, respectively. The residues gradually declined thereafter and reached below determination level on the 7th day at both the doses. The results showed that flubendiamide dissipated rapidly after

Table 3 Results of statistical interpretation of dissipation data of flubendiamide in tomato fruit

Treatment	Regression equation	R^2	$T_{(1/2)}$ days
50 g a.i.ha^{-1}	y = 2.409 − 0.1831x	0.99	1.64
100 g a.i.ha^{-1}	y = 2.598 − 0.1522x	0.95	1.98

application. A straight line was found when the log of residue was plotted against time and values of coefficient of determination (R^2) in tomato fruit samples establishing that first order reaction kinetics was involved in the dissipation process. The significant correlation co-efficient (r^2 = 0.95–0.99) indicated statistical conformity of the dissipation data to first order kinetics. The half-life of flubendiamide in tomato fruit in lower dose was 1.64 and higher dose was 1.98 days (Table 3).

With the degradation of the parent compound, the dissipation trend of the metabolite desiodo flubendiamide should be increased at the beginning and then declined regularly (Table 4). However, with the decline of flubendiamide, the metabolite desiodo flubendiamide in tomato fruit was declined regularly in lower and higher dose. From the Table 4, it was revealed that in the standard and double dose, the metabolite desiodo flubendiamide could not be detected after 2 h of spraying which might be due to insignificant formation of quantifiable amount. The significant amount of metabolite formation was observed on first day and persisted up to third day in case of standard dose and fifth day in double dose with the maximum amount of 0.08 and 0.15 μg g^{-1} on first day after last application. The desiodo flubendiamide formation in tomato crop was well agreement with the earlier studies conducted in tomato (Justus et al. 2007). Photodegradation on tomato fruit samples is a route of dissipation of flubendiamide in the environment. Therefore the tropical climatic conditions of India might have contributed to the disappearance of flubendiamide from tomato fruit samples.

Rates of flubendiamide and its degradation product desiodo flubendiamide, suggesting that degradation of these pesticides affected by environmental climate. When applied at both the standard dosage and at double times this, no detectable residues of either flubendiamide or desiodo flubendiamide were found in soil or tomato at harvest. Although flubendiamide can easily degrade into desiodo flubendiamide, the observed low residual levels suggest that flubendiamide is safe when applied at the recommended dosage.

Harvest soil samples collected at 10 days after last spray of flubendiamide did not show the presence of either flubendiamide or desiodo metabolite at detection limit of 0.01 μg g^{-1} irrespective of treatments. This indicates that flubendiamide application is quiet safe for succeeding crop.

After the application of flubendiamide (20 WDG) in standard and double dosage, the tomato fruit and soil were taken during the harvest time from the treated plots. The concentration level of flubendiamide and desiodo flubendiamide in these samples was determined. The results showed that the concentration flubendiamide and desiodo flubendiamide in tomato fruit, and soil were all below the LOQ. Maximum residue limit for flubendiamide in India for cotton and rice is fixed at 0.1 μg g^{-1}, and based on this, the waiting period for tomato could be considered 3.62 days. This input could be utilized in formulating the spray schedule and safety evaluation on the insecticide in tomato.

Table 4 Residues of desiodo flubendiamide in tomato fruit and soil

Days after treatment	Residues recovered ± SD (μg g^{-1})							
	Standard dose @ 50 g a.i.ha^{-1}				Double dose @ 100 g a.i. ha^{-1}			
	R1	R2	R3	Mean[a] ± SD	R1	R2	R3	Mean[a] ± SD
0	ND	ND	ND	ND	ND	ND	ND	ND
1	0.08	0.07	0.10	0.08 ± 0.02	0.18	0.12	0.15	0.15 ± 0.03
3	0.06	0.04	0.05	0.05 ± 0.01	0.08	0.10	0.07	0.08 ± 0.02
5	BDL	BDL	BDL	BDL	0.06	0.07	0.05	0.06 ± 0.01
7	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
10	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Soil (10th day)	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL

ND not detected, *BDL* below detection limit, *SD* standard deviation

[a] Mean of three replicates

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