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Flubendiamide: Residues and Risk Assessment in Tomato *Solanum lycopersicum*

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ABSTRACT

Flubendiamide belongs to a new chemical class, the phthalic acid diamides, widely used on tomato in India for the management of fruit borers. Flubendiamide is registered for use in India on tomato, but Maximum Residue Limits are not available as per Food Safety and Standards Authority of India. A research project was taken to study the dissipation pattern of flubendiamide on tomato cv. Nirupama in both open fields and poly-houses, when applied twice @ 48 g *a.i.* ha^{-1}, first spray was given 50 days after planting (fruit initiation) followed by the second spray at 10 days interval as per the farmers practice. Flubendiamide residues were quantified through regular sampling till the residues are below the determination level (BDL) of 0.05 mg kg^{-1} following the validated QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method. The qualitative and quantitative analysis of flubendiamide were performed on HPLC-PDA and LC-MS/MS. Initial deposits of 1.23 mg kg^{-1} were detected in the tomato samples collected from poly-house, which dissipated to BDL at the 10th day with half-life of 6.18 days. In open fields, deposits of 0.90 mg kg^{-1}dissipated to BDL at the 7th day with half-life of 6.07 days, and indicated that dissipation was slow in poly-house when compared to the open fields due to various factors. MRL of 3 mg kg^{-1}in poly-house tomato and 2 mg kg^{-1} in open field tomato is recommended based on the OECD (Organization for Economic Co-operation and Development) calculator and chronic hazard exposure assessment taking into consideration of average body weight, national per capita tomato consumption and acceptable daily intake (ADI) of flubendiamide. Among the various decontamination methods tested, veggy wash was found very effective in removing flubendiamide residues to the extent of 65.39 % which can be recommended as risk mitigation method for food safety, followed by 4%acetic acid solution (61.63%) and tap water wash was least effective (17.71%) in removing flubendiamide residues from tomato.

Highlights

- Maximum residue limits of 2 mg kg^{-1}flubendiamidecan be recommended on tomato based on risk analysis, and veggy wash removes flubendiamide residues on tomato up to 65%

Keywords: flubendiamide, tomato, poly-house, open fields, dissipation, risk analysis, decontamination methods, food safety

Tomato is one of the important and remunerative vegetable crops grown around the world for fresh market and processing. The production and productivity of the crop is greatly hampered by the insect pests and diseases. Among insect pests fruit borer, *Helicoverpa armigera* (Hübner)and tobacco caterpillar *Spodoptera litura* (Fabricius) pose major threat by feeding on leaves and fruits resulting in yield loss ranging from 20 to 60 percent (Tewari and Krishnamoorthy 1984; Lal and Lal 1996). The indiscriminate use of synthetic chemical pesticides to control the pests resulted in the development of resistance (Armes *et al.* 1994) and harmful pesticide residues in fruits. There is a need to replace ineffective ones with effective insecticides. In this direction, flubendiamide a novel insecticide, the first representative of a new chemical insecticide class - the diamides is found effective against lepidopteran insects. In contrast to the other insecticide classes targeting the insects' nervous system, flubendiamide acts at the receptor in the insects' muscle causing an immediate cessation of feeding and thus avoids crop damage. The unique mode of action makes the compound well suited as a tool in insect resistance management programmes. In India, CIB&RC recommended the usage of flubendiamide on tomato @ 48 g *a.i.* ha^{-1} with the waiting period of 5 days. It is available in the market as 20% WG and 39.35% SC formulations. With the changing food habits, tomato is being consumed as salad these days. Hence food safety issues are gaining importance. Further, with the intensive use of pesticides in poly-house crops, residues may be accumulated at levels higher than those permitted by the EU or the international maximum residue levels. The risk of pesticide residue in foods need to be addressed as per FSSAI (Food Safety and Standards Authority of India) and hence for the protection of consumer health and interests, household risk mitigation methods for the removal of pesticide residues in tomato are to be recommended based on the scientific evaluation, as the food habits are changing enormously. CAC MRLs of flubendiamide for tomato is 2 PPM and in India there are no specified MRLs of flubendiamide for tomato. Keeping these importantissues of concern, the present study was planned to study the dissipation dynamics of flubendiamide on tomato grown in poly-house and open field situations, to evaluate the decontamination methodologies for the removal of flubendiamide residues.

MATERIALS AND METHODS

Chemicals and Reagents

Certified Reference Materials (CRM) of flubendiamide (96.9% purity) were procured from M/S Sigma Aldrich, Germany, and the primary, intermediary and the working standards were prepared from the CRMs using GC PR grade acetone and hexane as solvents. Working standards were prepared in the range of 0.01 ppm to 0.5 ppm in 10 ml calibrated graduated volumetric flask using distilled n-hexane (Sigma- Aldrich) as solvent. Primary Secondary Amine (Agilent), magnesium sulfate anhydrous (Emsure grade of Merck), sodium sulfate anhydrous (Emparta ACS grade of Merck), acetonitrile (HPLC gradient grade of Merck), acetic acid glacial (HPLC grade of Merck), acetone (Emplure grade of Merck), and n-hexane (HPLC grade of Merck) were used in the study for sample preparation. Flubendiamide 20% WG (Takumi) was procured from the local market.

Analytical Instruments and Limits of Detection

The working standards of flubendiamide were injected in the Liquid Chromatograph with Photo Diode Array (PDA) Detector for estimating the lowest quantity of flubendiamide which can be detected under standard operating parameters. It was found that the LOD (limit of detection) for flubendiamide is 0.05 ng, and the linearity is in the range of 0.05 ng to 0.10 ng.

Method validation

Prior to field experiments, QuEChERS (Quick Easy Cheap Effective Rugged Safe) method for extraction and clean-up was validated as per SANCO/12571/2013 guidelines. Tomato fruits (5 kg) collected from control plots were homogenized with high volume homogenizer (Robot Coupe Blixer 7L) and 15g was taken into 50ml centrifuge tubes. The required quantity of flubendiamide intermediary standards is added to each 15g sample to get fortification levels of 0.05 mg kg^{-1}, 0.25 mg kg^{-1} and 0.5 mg kg^{-1} in three replications each. 30±0.1ml acetonitrile was added to the tube, and the sample was homogenized for 2-3 min using Heidolph silent crusher (low volume homogeniser). Then 3±0.1g sodium chloride was added to the tube and mixed by shaking gently, and was centrifuged for 3 min at 2500-3000 xg with Remi R-238 to separate the organic layer. The top organic layer of about 16 ml was taken into the 50 ml centrifuge tube to which 9±0.1 g anhydrous sodium sulphate was added to remove the moisture content. Eight millilitre of extract was taken in to 15 ml tube containing 0.4±0.01g PSA sorbent (for dispersive solid phase d-SPE clean up) and 1.2±0.01 g anhydrous magnesium sulphate, and the sample tube was vortexed for 30 sec followed by centrifugation for 5 min at 2500-3000 xg.2ml of extract was taken and filtered followed by the analysis of samples on HPLC. Tomato samples

Table 1: Recovery of flubendiamide residues from tomato

Replication	Fortified level (mg kg^{-1})					
	0.05 mg kg^{-1}		0.25 mg kg^{-1}		0.50 mg kg^{-1}	
	Residues recovered (mg kg^{-1})	Recovery %	Residues recovered (mg kg^{-1})	Recovery %	Residues recovered (mg kg^{-1})	Recovery %
R1	0.059	118.34	0.302	120.97	0.470	94.06
R2	0.056	111.22	0.262	104.99	0.521	104.27
R3	0.062	123.81	0.215	86.05	0.477	95.47
Mean		117.79		104.00		97.93
SD		6.314		17.479		5.533
RSD		5.360		16.806		5.650

RSD: Relative Standard Deviation

fortified with flubendiamide at 0.05 mg kg^{-1}, 0.25 mg kg^{-1} and 0.5 mg kg^{1} were analyzed and the mean recovery of the residues were calculated for applying the recovery factor while calculating the residues in the samples. Fortification and recovery test results are presented in (Table 1) and the method followed for the qualitative and quantitative estimation of flubendiamide is suitable up to 0.05 mg kg^{-1}levels as the recoveries obtained are 117.79%, 104.00% and 97.93% respectively, at 0.05, 0.25 and 0.50 mg kg^{-1} fortification level. The residues detected below 0.05 mg kg^{-1} were mentioned as levels Below Determination Level (BDL) in all cases.

Field experiments and sample collections

Tomato cv. Nirupama was raised in both poly-house and open field laid out in the Randomized Block Design at the spacing of 60 cm ×45 cm with each plot size of 20 m^2 and all Good Agricultural Practices (GAPs) recommended by the University to raise tomato crop were followed. Flubendiamide 20% WG procured from the local market was sprayed @ 48 g a.i. ha^{-1} twice; first spray at the fruit initiation stage followed by the second spray at 10 days after the first spray, using high volume knapsack sprayer with a spray solution of 500 L ha^{-1}. Pest damage free and crack free tomato fruits of 5 kg were collected from each plot in separate polythene bags and brought to the laboratory. Samples were collected at regular intervals i.e. 0, 1, 3, 5, 7, 10, 15, 20 days after the last spray for dissipation studies. For the evaluation of risk mitigation or decontamination methods, zero day samples were collected separately in large quantities and made into 6 sets, each in 4 replications. One set of sample is analyzed for initial deposits of flubendiamide. The remaining sets of samples were subjected to various decontamination methods separately and the residues were calculated to know the efficiency of the various decontamination methods in the removal of pesticide residue from the tomato samples. The decontamination or risk mitigation methods selected for the evaluation of efficiency in the removal of pesticide residues from tomato were presented in (Table 2). After decontamination treatments, the samples were shade dried for 10 min by placing on clean blotting papers and analyzed for the residues remaining on tomato.

Calculation Methods

Half-life was calculated as per Hoskins (1966) from the first-order dissipation kinetics. OECD (Organization for Economic Cooperation and Development) MRL calculator was used for the calculation of MRL and chronic hazard risk analysis was performed using TMDI (Theoretical Maximum Daily Intake) for arriving at MRL for the recommendation taking in to consideration the national per capita tomato consumption, average body weight and ADI of flubendiamide. In case of decontamination studies, per cent removal of flubendiamide was calculated.

RESULTS AND DISCUSSION

Tomato fruits collected at regular intervals from flubendiamide sprayed research plots of open field and poly-house were analyzed and the data are presented in Table 3. In poly-house experiments, initial deposits of 1.23 mg kg^{-1} flubendiamide were detected at 2 hours after the last spray, which

Table 2: Decontamination Methods for removal of flubendiamide residues from tomato

Sl. No.	Treatment	Details of treatment
T_1	Tap water wash	4 L of tap water was taken into a plastic tub of 7 L capacity and 2 Kg of tomato fruits were dipped in the tub for 10 min, followed by the tap water wash for 10 sec.
T_2	Soaking in 2% salt solution	4 L of 2 % salt solution was prepared by mixing 80 g of table salt in 4 L of water in a plastic tub of 7 L capacity and 2 Kg tomato fruits were dipped in the tub for 10 min, followed by the tap water wash for 10 sec.
T_3	Dipping in 0.1% baking soda	4 L of 0.1% baking soda solution was prepared by mixing 4 g of baking soda in 4 L of water in a plastic tub of 7 L capacity and 2 Kg tomato fruits were dipped in the tub for 10 min, followed by the tap water wash for 10 sec.
T_4	Soaking in 4% acetic acid	4 L of 4% acetic acid solution was prepared by mixing 160 ml of acetic acid glacial 100% in 4 L of water in a plastic tub of 7 L capacity, the mixture was kept for 1 min and 2 Kg of tomato fruits were dipped in the tub for 10 min, followed by the tap water wash for 10 sec.
T_5	Veggy wash	4 L of veggy wash was prepared by mixing 160 ml of acetic acid glacial 100%, 4 g of baking soda and lemon juice with 4 lemons in 4 L of water in a plastic tub of 7 L capacity, the mixture was kept for 1 min and 2 Kg tomato fruits were dipped in the tub for 10 min, followed by the tap water wash for 10 sec.

Table 3: Dissipation of flubendiamide residues in open fields and poly-house situations

Days after treatment	Residues in Poly-house (mg kg^{-1})						Residues in Open field(mg kg^{-1})					
	R1	R2	R3	R4	Mean	% dissipation	R1	R2	R3	R4	Mean	% dissipation
0	1.23	1.23	1.23	1.23	1.23	0	0.93	0.87	0.92	0.87	0.90	0
1	0.77	0.74	0.76	0.77	0.76	38.21	0.47	0.48	0.50	0.49	0.49	45.55
3	0.35	0.30	0.34	0.35	0.34	72.35	0.28	0.28	0.30	0.29	0.29	67.77
5	0.15	0.14	0.14	0.15	0.15	87.80	0.14	0.13	0.12	0.14	0.13	85.55
7	0.08	0.09	0.07	0.08	0.08	93.49	BDL	BDL	BDL	BDL	BDL	100.00
10	BDL	BDL	BDL	BDL	BDL	100.00	BDL	BDL	BDL	BDL	BDL	100.00
15	BDL	BDL	BDL	BDL	BDL	100.00	BDL	BDL	BDL	BDL	BDL	100.00
20	BDL	BDL	BDL	BDL	BDL	100.00	BDL	BDL	BDL	BDL	BDL	100.00
Regression equation	Y = 0.912 + (-0.112) X						Y = 0.727 + (-0.114) X					
R^2	0.782						0.876					
Half-life	6.18 days						6.07 days					

Below Determination Level (< 0.05 mg kg^{-1})

dissipated to BDL of 0.05 mg kg^{-1} by the 10th day after the last spraying on tomato. The initial deposits were dissipated to 0.76, 0.34, 0.15 and 0.08 mg kg^{-1} by 1, 3, 5 and 7 days after the last spray, respectively. The dissipation pattern showed the decrease of residues from the first day to the 7th day. The residues dissipated by 38.21, 72.35, 87.80 and 93.49% at 1, 3, 5 and 7 days, respectively. In open field situations Initial deposits of 0.90 mg kg^{-1} flubendiamide were detected at 2 hours after the last spray, which dissipated to BDL of 0.05 mg kg^{-1} by the 7th day. The initial deposits were dissipated to 0.49, 0.29 and 0.93 mg kg^{-1} at 1, 3 and 5 days after the last spray, respectively. The dissipation pattern showed the decrease of residues from the first day to the 5th day. The residues dissipated by 45.55, 67.77 and 85.55% at 1, 3 and 5 days, respectively. Maximum Residue Limits are not fixed for flubendiamide on tomato as per FSSAI, while Codex Alimentarius Commission (CAC) set it as 2 mg kg^{-1}. It is evident that there is a clear difference in the dissipation pattern of flubendiamide in poly-house and open fields. Initial deposit of 1.23 mg kg^{-1} was recoded in poly-house, where as in open fields it was 0.90 mg kg^{-1}, reaching Below Determination Level (BDL) of 0.05 mg kg^{-1} by the 10th day and the 7th day, respectively. As per CIBRC and Insecticide Act, 1968, flubendiamide is recommended for the use on tomato @ 48 g a.i. ha^{-1} for controlling fruit borer. The same dosage was used in the present study to know the dissipation dynamics in two situations and it is evident that 0.90 mg kg^{-1} initial deposits dissipated to BDL level by the 7^{th} day in open field situation, and the findings of present investigation are in agreement with the results of Sharma *et al.* (2013) who reported that flubendiamide persisted up to 5 days after the application on tomato @ 48 g a.i. ha^{-1}. Similar results were reported by Kooner *et al.* (2010) who reported that flubendiamide persisted up to 5 days after the application on tomato @ 48 g *a.i.* ha^{1}.

Table 4: Chronic hazard exposure assessment for recommending flubendiamide MRLs on tomato

OECD MRL calculator Data sets	Poly-house	Open field
Total number of data (n)	6	5
Percentage of censored data (%)	17	20
Number of non-censored data	5	4
Lowest residue	0.050	0.050
Highest residue	1.230	0.900
Median residue	0.245	0.290
Mean	0.435	0.372
Standard deviation (SD)	0.469	0.340
Correction factor for censoring (CF)	0.889	0.867
Proposed MRL estimate	**Poly-house**	**Open field**
Highest residue	1.230	0.900
Mean + 4 SD	2.313	1.731
CF X 3 Mean	1.160	0.967
Unrounded MRL	2.313	1.731
Rounded MRL	3.000	2.000
Risk Analysis	**Poly-house**	**Open field**
Average human body weight (kg)	55	
National per capita intake of tomato	806 g $month^{-1}$	
Daily intake of crop (C) = kg $person^{-1}$	0.027	
Consumption of crop C($F_{C)}$ = kg bw^{-1}	0.00049	
ADI for flubendiamide (mg kg bw^{-1})	0.02	
TMDI = Fc × MRL (from OECD calculator)	0.00147	0.00098
TMDI v/s ADI	TMDI < ADI	TMDI < ADI
Proposed MRL (mg kg^{-1})	3.00	2.00
Codex MRL (mg kg^{-1})	2.00	
FSSAI (India) MRL (mg kg^{-1})	Not available	
EU MRL (mg kg^{-1})	0.2	

The dissipation of flubendiamide is very slow in case of flubendiamide when compared to the other pesticides tested in the study in both poly-house and open field situations. The flubendiamide in poly-house (controlled environment) dissipated at very slow rate and it is degraded to BDL by the 10^{th} day. Similar trends are also reported by many workers who studied on other vegetables as well. Sahoo *et al.* (2009) reported that flubendiamide sprayed on chilli @ 60 g a.i. ha^{-1} resulted in the initial deposit of 1.06 mg kg^{-1}which then dissipated to BDL in 7 days and at a higher dose, it took 10 days. Jyothsna *et al.* (2012) reported that flubendiamide sprayed on gherkin @ 48 g *a.i.* ha^{-1} reported the initial deposit of 0.92 mg kg^{-1} and the residues reached BDL by the 7^{th} day. The research findings of various workers such as Paramasivam (2013), shows that flubendiamide spray on cabbage @ 25 g *a.i.* ha^{-1} resulted in 0.31 mg kg^{-1} deposits but persisted up to 15 days to reach BDL, indicating very slow dissipation rates on cabbage. Similar results were reported by Mohapatra *et al.* (2010) where flubendiamide spray on cabbage @ 48 g a.i. ha^{-1} resulting 0.49 mg kg^{-1} deposits took 15 days for the dissipation to BDL.

In India, as per Food Safety and Standards Authority of India (FSSAI) MRLs are not fixed for flubendiamide on tomato. Hence, risk analysis is necessary for setting MRLs based on the supervised field trials. Based on the present studies in poly-house, as per OECD calculator, MRL of 3 mg kg^{-1} can be suggested since the TMDI calculated based on OECD, MRL is not more than the ADI of 0.02 mg kg body weight. Hence, MRL of 3 mg kg^{-1} is suggested based on the risk analysis. Similarly, in open field studies, OECD calculator suggests MRL of 2 mg kg^{-1} for flubendiamide on tomato which is in line with the Codex MRL of 2 mg kg^{-1} on tomato. Further this MRL can be taken as the proposed MRL for the risk analysis studies indicates that TMDI is lower than the ADI. The efficiency of various risk mitigation methods for the removal of flubendiamide residues from tomato is presented in Table 5. The percentage removal of flubendiamide residues from tomato when subjected to different decontamination solutions at 2 hours after spraying showed that dipping in veggy wash solution for 10 min followed by tap water wash for 30 sec was the most effective treatment than the rest. In this treatment residues were reduced up to 65.39%. Next

Table 5: Removal of flubendiamide residues from tomato fruits with different decontamination methods

Treatments	Mean of flubendiamide detected (mg kg-1)*	Amount removed (mg kg-1) **	Percent removed
Tap water wash	0.74 ± 0.052	0.16 ± 0.046	17.71 ± 1.85
2% salt solution	0.54 ± 0.040	0.36 ± 0.058	39.75 ± 0.95
0.1% Baking soda solution	0.49 ± 0.043	0.41 ± 0.076	45.30 ± 0.81
4% Acetic acid solution	0.34 ± 0.059	0.55 ± 0.090	61.63 ± 0.55
Veggy wash	0.31 ± 0.020	0.59 ± 0.104	65.39 ± 1.15

*C. D. at 5% = 1.55; Initial deposit = 0.90 mg kg^{-1}; * Mean of three replications; ** Amount removed = Initial deposit-Mean of replicates of each treatments.*

promising treatment was 4% acetic acid solution (61.63%) followed by baking soda solution (45.30 %), 2% salt solution (39.75%) and tap water wash (17.71%).

Based on the percentage removal of residues, it was statistically proven that there is significant difference in the efficiency of decontaminating solutions in removing residues of the above mentioned pesticides. Many workers (Radwan *et al.* 2004; Jayakrishnan *et al.* 2005; Zhang *et al.* 2006; Klinhom *et al.* 2008; Liang *et al.* 2012) suggested that washing with 1% acetic acid solution, and 0.1% $NaHCO_3$ solution removes various pesticide residues in different vegetables, and the extent of removal varies from the type of pesticide and vegetable. Research conducted by Abou-Arab, 1999 showed that washing of tomato fruits with 10% salt solution removed 90.80% and 82.40% of dimethoate and profenophos residues and tap water wash was the least effective treatment.

Washing tomato fruits with water removed dimethoate and profenophos residues up to 18.80 and 22.17% respectively. Studies conducted by Rao*et al.* (2014) and Vemuri et *al*. (2014) revealed that washing of brinjal and tomato with 2% salt solution is effective in removing various pesticides. Based on the test reports, it can be concluded that flubendiamide can be removed from tomato for food safety with simple house processing methods, and out of all methods, washing with AINP formulation i.e. veggy wash proved to be the best, and also economical. So, this result can be propagated and popularized among the home makers for the removal of pesticides from tomato when used as fresh vegetable salad. It can also create confidence that they are consuming safe food without any pesticide residues.

CONCLUSION

Dissipation pattern of flubendiamide varied from the open field situation to the poly-house conditions when sprayed as per the farmers practice. The risk analysis that depends on MRLs was calculated using OECD calculator, ADI of CAC, per capita tomato consumption in India and the average body weight indicated that MRLs of 3.0 mg kg^{-1} and 2.0 mg kg^{-1} can be suggested for poly house and open field grown tomato, respectively, as the TMDI did not exceed ADI. Flubendiamide application @ 48 g *a.i.* ha^{-1} twice can be included in GAPs of Tomato in India with MRL of 2.0 mg kg^{-1}under open field cultivation. Washing of market tomatoes with veggy wash or 4% acetic acid solution or 2% salt solution can be followed for the removal of flubendiamide residues.

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