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Dissipation pattern of flubendiamide in/on okra *Abelmoschus esculentus* (L) moench fruits under climatic conditions of Western Tamil Nadu

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Abstract
A supervised field trial was conducted to study the dissipation pattern of flubendiamide in/on okra, *Abelmoschus esculentus* (L) Moench during kharif 2017, at Naraseepuram village, Coimbatore district of Tamil Nadu, The samples were collected up to 10 days after pesticide application along with control and processed by modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method. The final estimation of residues was carried out on Liquid Chromatography Mass Spectrometry (LCMS) with diode array detector. The mean initial deposit after two spraying of flubendiamide in/on okra fruit at recommended dose of 48 g a.i ha^{-1} was found to be 1.16 µg g^{-1}. More than 80 per cent of flubendiamide residues got dissipated on 5 days after treatment. The residues persisted up to 7 days after treatment and further dissipated to Below Detectable Limit (BDL < 0.05 µg g^{-1}) on 10 days after treatment. Dissipation of flubendiamide followed first order reaction kinetics and the calculated half life was 1.64 days. The safe waiting periods of 4.6 days was suggested based on the Codex Maximum Residual Limit (MRL) for fruiting vegetable.

Keywords: Okra, dissipation, flubendiamide, half-life, safe waiting period

1. Introduction
Okra *Abelmoschus esculentus* (L) Moench is an economically important vegetable crop grown in tropical and sub-tropical parts of the world. It is consumed as fresh or cooked and forms a major source of vitamins and minerals. Among various biotic stresses that constrain the successful cultivation of okra crop, the damage caused by insect pests is of significant importance. The pest complex in okra varies from region to region and the number of recorded species range from 13 to 72 depending on the agro-climatic conditions (Rao and Rajendran, 2003; Mandal *et al.,* 2006) [1, 2]. Among the pest complex, fruit and shoot borer *Earias* spp.*,* is the most serious as it take upper hand by causing direct damage to tender fruits and also shoot. First, the terminal portions of growing shoots are bored by the larvae by making tunnel, which results in wilting and premature dropping of developing fruits. In the reproductive stage of crop, the larvae bores into flower buds, small fruits, even mature pods and cause reduction in yield by damaging the seeds. The affected fruits show deformities in shape, remain stunted in growth and become unfit for human consumption (Acharya, 2010) [3]. Due to fruit damage caused by *Earias* spp., the marketable loss of 69 per cent (Rawat and Sahu, 1973) [4] and avoidable yield loss of 36 to 90 per cent was estimated (Misra *et al.,* 2002) [5]. When compared with healthy fruits, in okra fruits attacked by fruit and shoot borer, the normal seeds per fruit were reduced by 16.47 per cent with increase in stained seeds by 200 per cent and damaged seeds by 18.70 per cent (Sinha *et al.,* 1978) [6]. Flubendiamide N2-[1,1-dimethyl -2(methylsulfonyl)ethyl]-3-iodo-nl-[2-methyl-4-[1,2,2,2-tetrafluro(trifluoromethyl)ethyl}phenyl}-1,2 is a novel class of insecticide (Figure 1) with broad activity against lepidopteran insect pest like okra fruit and shoot borer. Its structure constitute of three parts: (1) a heptafluoroisopropyl group in the anilide moiety, (2) a sulfonylalkyl group in the aliphatic amide moiety and (3) an iodine atom at the three- position of the phthalic acid moiety (Tonishi *et al.,* 2005) [7]. It disturbs calcium balance in the muscles of the insects by acting on the rynodyne receptor, affecting muscle contraction (Ebbinghaus-Kintscher *et al.,* 2007) [8]. Hence, a detailed study was conducted on the dissipation behavior of flubendiamide in okra fruit and thereby to work out half life and safe waiting period for

flubendiamide in okra. Flubendiamide is registered since 2007 India on cotton and rice. Moreover, in okra ecosystem, greater use of flubendiamide against fruit and shoot borer is observed (Srinivasnaik *et al.,* 2015) [9]. As the information regarding persistence and dissipation of flubendiamide in/on okra is lacking, the present study was carried out to investigate the dissipation kinetics of flubendiamide residues in okra.

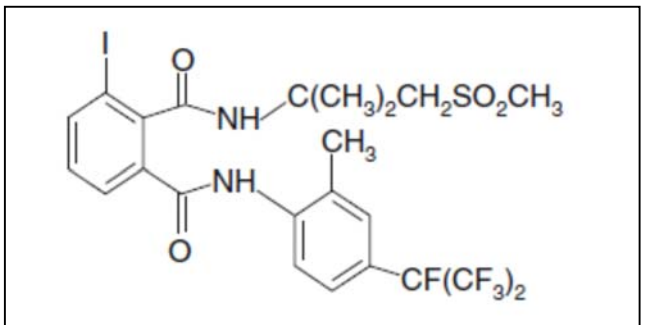

Fig 1: Structure of Flubendiamide

2. Materials and methods

2.1. Chemicals and reagents

The reference standard of flubendiamide (98.1% purity) was purchased from M/S Sigma Aldrich, Bangalore, India. Acetonitrile of HPLC grade, sodium chloride and anhydrous magnesium sulphate of analytical grade were purchased from Merck (Mumbai, India). Primary Secondary Amine (PSA) (Bondesil 40 µm) and Graphitized Carbon Black (GCB) were purchased from M/S Agilent technologies, USA.

2.2. Preparation of standard solution

Primary stock solutions of flubendiamide (400 µg mL^{-1}) standards was prepared by dissolving 10.70 mg of analyte in 25 mL HPLC grade acetonitrile in a volumetric flask. An intermediate stock solution of 100 µg mL^{-1} was prepared from primary stock solution and from this another intermediate stock solution of 10 µg mL^{-1} was prepared. Working standard solutions of flubendiamide (0.025 to 1 µg mL^{-1}) was prepared by diluting intermediate stock. It was used to find the retention time of flubendiamide and for quantitative determination of residues in samples. All the stock and working standard solutions were stored in the refrigerator at -20 ˚C.

2.3. Efficacy of analytical method

2.3.1 Linearity

The linearity study was conducted by injecting five different concentrations of standard solution following three replications of flubendiamide.

2.3.2 Determination of LOD and LOQ

The limit of detection (LOD) was calculated by considering signal-to-noise ratio of three with reference to the background noise obtained from blank sample and the limit of quantification (LOQ) by considering a signal to noise ratio of ten.

2.3.3 Recovery experiment

Recovery studies were conducted to assess the validity of the present method. The homogenized untreated okra fruit samples (10g) were spiked at three different concentrations *viz.,* 0.05, 0.25 and 0.5 µg g^{-1} of separately using analytical standard solution of flubendiamide. Each treatment was replicated three times with untreated control. The spiked samples were equilibrated for one hour and residues were extracted and estimated as per the method mentioned above. The control okra fruit samples were analysed and the result indicated that blank sample did not contribute any interference with the target compounds. The percentage recovery was calculated by comparing the peak area of the spiked standards with those of the pure standards (Figure 2) by using the below formula.

$$\text{Per cent recovery} = \frac{\text{Residue quantified in fortified sample}}{\text{Fortified level}} \text{ X } 100$$

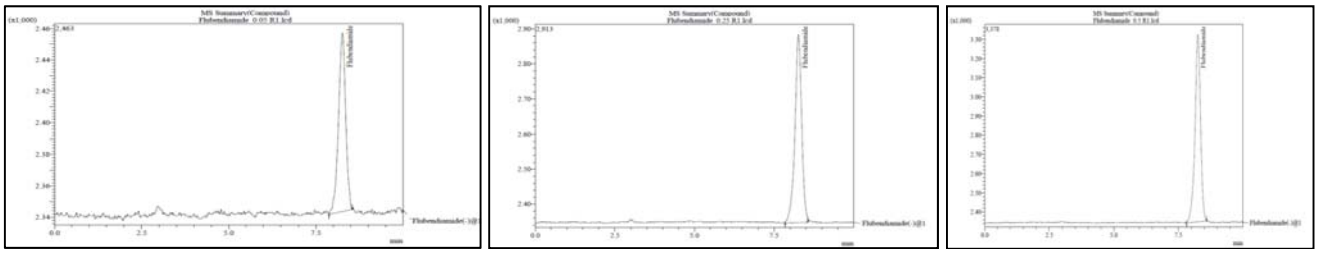

Fig 2: LCMS chromatogram of flubendiamide standard

2.4. Extraction and clean-up

The spiked samples were processed by adopting modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method (Anastassiades *et al.,* 2003) [10]. A representative sample of 10 g was transferred into a 50 mL centrifuge tube and mixed using a vortexer for one minute after adding 20 mL of acetonitrile. About 4 g of anhydrous magnesium sulphate ($MgSO_4$) and 1 g of sodium chloride (NaCl) were subsequently added, shaken well by vortexer and then centrifuged at 6000 rpm for 10 minutes. After centrifuging, 9 mL of supernatant was transferred to test tube containing anhydrous sodium sulphate (Na_2SO_4) and 6 mL of supernatant aliquot was transferred into a 15 mL centrifuge tube containing 100 mg Primary Secondary Amine (PSA), 600 mg anhydrous magnesium sulphate ($MgSO_4$) and 10 mg Graphitised Carbon Black (GCB). The mixture was vortexed for 1 minute and then centrifuged for 10 minutes at 3000 rpm. The upper extract (4 mL) was transferred into a turbovap tube and concentrated to dryness under a gentle stream of nitrogen in a turbovap LV at 40˚ C. 1mL of HPLC grade acetonitrile was added to test tube, shaken well reconstituted 1 mL was transferred into a 1.5 mL glass auto sampler vial for analysis. The residues of flubendiamide were estimated by Liquid Chromatography Mass Spectrometry (LCMS).

2.5. Field experiment

A supervised field trial was conducted to study the dissipation of flubendiamide at Naraseepuram village, Thondamuthur, Coimbatore, on the okra hybrid CO-4, during kharif 2017.

The experiment was laid out in randomized block design in a plot size of 20m^2 and replicated thrice, including untreated control. The okra plots were sprayed with flubendiamide 480 SC (48 g a.i. ha^{-1}) twice i.e., first spraying at 50% flowering stage and subsequent spraying at 10 days interval using hand operated knapsack sprayer. It was ensured that the insecticide under investigation had not been used earlier in the experimental plot.

2.6. Sampling

One kilogram of fruit sample was collected randomly at 0 (one hour after spraying), 1, 3, 5, 7 and 10 days after spraying from flubendiamide treated field along with untreated control separately. The collected samples were transported to the laboratory and processed immediately. The fruits were chopped into small pieces, from which a sub sample of 500 g was taken and homogenized with a mixer grinder. The residues were extracted by following the above mentioned extraction and clean-up process.

2.7. LCMS parameters

The estimation of flubendiamide residues were performed by LCMS (Shimadzu, series 2020) equipped with diode array detector (SPD-M20A), Chromatographic separation was achieved with reverse phase - C18 (Agilent) column, 250 mm length x 4.6 mm id x 3 µ particle size in a column oven, at 40˚C. The low pressure gradient condition employed with a mobile phase of acetonitrile and water with 5 mM ammonium acetate (70:50) with a flow rate of 0.8 ml minute^{-1} and the injection volume was 20 µL. Nitrogen gas was used as nebulizer gas. The drying gas flow rate was 15 L minute^{-1} and nebulizing gas flow rate was 1.5 L minute^{-1}. The Desolvation Line (DL) temperature was 250 ˚C and heat block temperature was 200˚C. The ions were monitored at negative SIM (Single Ion Monitoring) mode with an ESI (Electrospray Ionization) interface. The mass ratio (m/z) and retention time for flubendiamide were 682 g mol^{-1} and 8.26 minutes, respectively.

2.8. Quantification of pesticide residues

The final quantification was worked out using the following formula with the parameters from chromatogram as

$$\text{Residues (ppm)} = \frac{As}{Astd} \times \frac{Wstd}{Ws} \times \frac{Vs}{Asj}$$

As : Peak area of the sample
Astd : Peak area of the standard
Wstd : Weight of the standard in ng
Ws : Weight of the sample in g
Vs : Volume of the sample (final extract in mL)
Asj : Aliquot of the sample injected in mL

2.9. Data analysis

The insecticide degradation pattern was analysed by applying seven transformation functions as suggested by Hoskins (1961) [11] and Timme *et al.* (1986) [12]. The half-life was calculated using Pesticide Residue Half Life Calculator software developed by Department of Soil Science, Tamil Nadu Agricultural University, Coimbatore based on Regupathy and Dhamu (2001) [13] and best fit degradation model was determined. The safe waiting period was worked out as per the formula given by Handa *et al.* (1999) [14] using Codex Maximum Residual Limit (MRL)

$$\text{Safe waiting period (TMRL)} = \frac{\log K2 - \log (MRL/\text{ tolerance})}{\log K1}$$

3. Results and discussion

The linearity of the calibration curves was established in the range of 0.025 to 0.5 µg g^{-1} and the correlation coefficient (R^2) obtained was 0.999 (Figure 3).

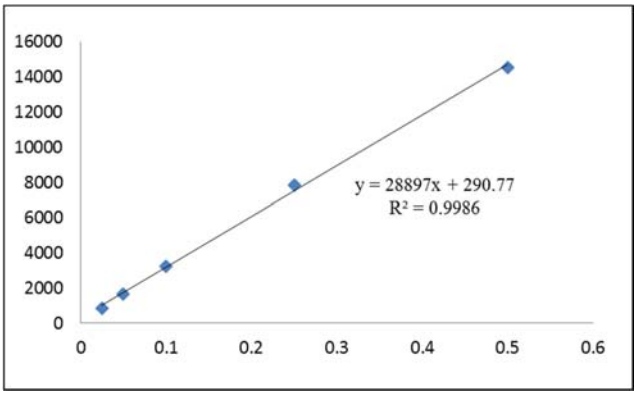

Fig 3: Linearity calibration curve of flubendiamide – LCMS

The LOQ and LOD values [1] for flubendiamide were 0.015 and 0.05 µg g$^-$ in LCMS. The results of the recovery study of flubendiamide carried out in okra fruits (Table 1) revealed that the mean per cent recoveries of flubendiamide were 90.09, 81.95 and 87.00 in okra fruits with Relative Standard Deviation (RSD) percentage of 5.00, 2.05 and 4.78, when samples were spiked at 0.05, 0.25 and 0.5 µg g^{-1}, respectively. The mean per cent recoveries of flubendiamide residues in okra ranged from 80 to 120 per cent and RSD were below 20 per cent. According to Sanco (2009) [15] (Document No.SANCO/10684/2009), any recovery range of 60 to 140 per cent is acceptable for method validation. Based on the recovery study, the suitability of modified QuCHERS method for residue analysis of flubendiamide in okra thus confirmed.

Table 1: Recovery percentage of flubendiamide in okra fruit

Spiking level (µg g $^{-1}$)	Recovery %			Mean*± SD	RSD
	R1	R2	R3		
0.05	89.96	94.66	85.65	90.09 ± 4.51	5.00
0.25	83.89	80.93	81.03	81.95 ± 1.68	2.05
0.5	88.61	82.28	90.12	87.00 ± 1.16	4.78

*Mean of three replicates, SD- Standard Deviation, RSD- Relative Standard Deviation

The persistence and dissipation of flubendiamide 480 SC in/on okra fruits sprayed @ 48 g a.i ha^{-1} (Table 2) revealed that the average initial deposits (1 hour after spraying) of flubendiamide was 1.16 µg g^{-1}. Within first 24 hours after spraying, 53.32 per cent loss in flubendiamide residue was recorded. Mohapatra *et al.* (2011) [16] reported the similar mean initial deposits (0.83 µg g^{-1}) of flubendiamide @ 48 g a.i. ha^{-1} in tomato. Sharma *et al*. (2011) [17] reported the average initial deposits of flubendiamide @ 60 and 120 g a.i. ha^{-1} in chilli were 0.467 and 0.824 µg g^{-1}, respectively. In brinjal, Takkar *et al.* (2011) [18] and Chawla *et al.* (2011) [19] reported the average initial deposit of 0.33 and 0.17 µg g^{-1} of flubendiamide (480 SC) @ 90 g a.i. ha^{-1}, respectively. The variation in the rate of dissipation of flubendiamide in okra, chillies and brinjal and may be due to changes in the crop matrix and dosage. In the present study, the residue level of flubendiamide was 0.54, 0.23, 0.12 and 0.05 µg g^{-1} on 1, 3, 5 and 7 days after spraying, respectively. On 3 days after treatment, loss of 79.93 per cent was observed s and on 5 days after treatment more 80 per cent loss of residue was observed.

The residue persisted up to 7 days after treatment with loss of 95.39 per cent. This result goes in line with Vemuri *et al.* (2014) [20] who reported that the mean initial deposit of flubendiamide @ 60 g a.i. ha^{-1} was 0.84 µg g^{-1}, which persisted upto 7 days after treatment with 98.80 per cent loss. Then the residues of flubendiamide gradually declined and reached BDL of 0.05 µg g^{-1} on 10 days after treatment (Figure 4). This is in accordance with Das *et al.* (2012) [21] who reported that the flubendiamide residues in/on okra reached BDL (0.01 µg g^{-1}) on 10 days after treatment in okra @ 48 g a.i. ha^{-1}.

The dissipation pattern of flubendiamide was computed following seven transformations and based on the coefficient of determination; the best fit observed was first order reaction for recommended dose (Table 3). Das *et al.* (2012) [21] also reported that the rate of degradation of flubendiamide in/on okra fruits followed first-order kinetics. The statistical parameters like intercept (a), slope of regression lines (b) and half-life with confidence limits are presented in Table 4. The half-life value of 1.64 days for flubendiamide in okra was found. The present result was in accordance with the findings of Paramasivam and Banerjee (2011) [22] who reported that the half-life of flubendiamide (48 g a.i. ha^{-1}) in tomato fruit was 1.64 days. Vemuri *et al.* (2014) [20] reported the similar half-life of 1.84 days for flubendiamide @ 60 g a.i. ha^{-1} in okra fruit. Since, Food Safety Authority of India's (FSSAI) MRL for flubendiamide on okra is not available; Codex MRL for fruiting vegetables (0.2 µg g^{-1}) was used for calculating safe waiting period of 4.2 days.

Table 2: Persistence and dissipation of flubendiamide 480 SC residues in/on okra fruit

Days after application	Flubendiamide 480 SC @ 48 g a.i. ha^{-1}				
	Residues (µg g^{-1})				Dissipation %
	R1	R2	R3	Mean*± SD	
Control	ND	ND	ND	ND	-
0 (1hr)	1.16	1.18	1.13	1.16 ± 0.02	-
1	0.54	0.52	0.56	0.54 ± 0.02	53.32
3	0.22	0.25	0.23	0.23 ± 0.01	79.93
5	0.11	0.13	0.12	0.12 ± 0.01	89.45
7	0.05	BDL	0.06	0.05 ± 0.01	95.39
10	BDL	BDL	BDL	BDL	100.00

* Mean of three replicates, ND – Not Detected, BDL – Below Detectable Level (< 0.05 µg g ^{1})

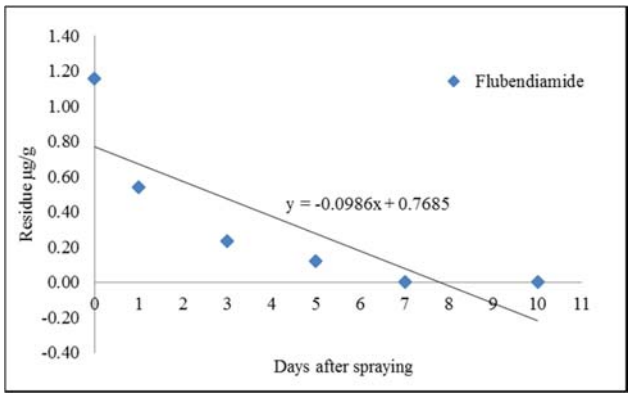

Fig 4: Dissipation of flubendiamide residues in/on okra fruit

Table 3: Correlation coefficient for flubendiamide 480 SC residues on okra by different methods of transformation of residue data

Function	r	Test of significance	r ^{2}	Modi r^2
First order	-0.9939	**	0.9878	0.95
1.5th order	0.9947	*	0.9894	0.96
2nd order	0.9686	*	0.9382	-831.54
RF first order	-0.9793	*	0.959	0.84
RF 1.5 first order	0.9782	*	0.9569	0.86
RF 2nd order	0.9265	NS	0.8584	-9.26
Inverse P L	0.9889	*	0.9779	0.99

RF- Root function, Inverse PL- Inverse Power Law, r- Correlation coefficient, Modi r sq Modified 'r' square, *Significant at 5 % level, **Significant at 1 % level

Table 4: Dissipation pattern for flubendiamide 480 SC (x dose) on okra with statistical parameters

Function	a	UCL	LCL	b	UCL	LCL	T Half	UCL	LCL
First order	0.0181	0.6755	-0.6393	-0.4219	-0.2618	-0.5820	1.6428	2.2660	1.0196
1.5th order	0.7563	1.1127	0.3999	0.4583	0.5452	0.3714	0.6835	1.0307	0.3363
2nd order	-1.1018	3.3094	-5.5130	2.2770	3.3516	1.2024	-0.4839	-2.4347	1.4669
RF first order	1.0143	2.0014	0.0272	-1.4352	-1.1431	-1.7273	0.232	0.3281	0.1383
RF 1.5 first order	-0.2456	0.8239	-1.3151	1.5108	2.0995	0.9221	-0.0279	-0.2719	0.2161
RF 2nd order	-5.7382	4.1581	-15.6345	7.3011	12.7487	1.8535	0.6177	2.9392	-1.7038
Inverse P L	-0.0335	0.4839	-0.5509	1.0477	1.3373	0.7581	1.9379	2.2922	1.5836

RF- Root Function; Inverse PL- Inverse Power Law; UCL-Upper Confidence Limit; LCL-Lower Confidence Limit; T Half- Half life

4. Conclusion

The study indicated that the residues of flubendiamide @ 48 g a.i ha^{-1} in okra dissipated BDL (< 0.05 µg g^{-1}) on 10 days after treatment with calculated half-life of 1.64 days. The present study provided adequate information to the farmers for safe harvesting period (4.6 days) for okra sprayed with flubendimide under agroclimatic conditions.

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6. References

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