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Deepak S
Directorate of Plant Protection Quarantine and Storage, Faridabad, Haryana, India

Narasa Reddy
Directorate of Plant Protection Quarantine and Storage, Faridabad, Haryana, India

Gaikwad SM
Directorate of Plant Protection Quarantine and Storage, Faridabad, Haryana, India

Shashibhushan V
Department of Entomology, Professor Jayashankara Telangana Agricultural University, Hyderabad, Telangana, India

Correspondence
Deepak S
Directorate of Plant Protection Quarantine and Storage, Faridabad, Haryana, India

Bio-efficacy and dissipation of flubendiamide against shoot and fruit borer (*Earias vittella* Fab.) of okra

Deepak S, Narasa Reddy, Gaikwad SM and Shashibhushan V

Abstract
The bio-efficacy of selected insecticides *viz*., bifenthrin 10 EC at 80 g a. i. ha^{-1}, fipronil 5 SC at 500 g a. i. ha^{-1}, flubendiamide 480 SC at 60 g a. i. ha^{-1}, quinalphos 25 EC at 350 g a. i. ha^{-1}, profenofos 50 EC at 400 g a. i. ha^{-1} and beta-cyfluthrin 25 SC at 18.75 g a.i. ha^{-1} against shoot and fruit borer (*Earias vittella* Fab.) of okra was studied in the field conditions at Student's Farm of Agricultural College, Hyderabad during 2012. All the insecticidal treatments were superior over control after second spray where, Flubendiamide at 60 g a. i. ha^{-1} recorded lowest fruit borer infestation of 14.40 percent on number basis and 15.90 percent on weight basis. Further, dissipation pattern of Flubendiamide at 60 g a. i. ha^{-1} was studied by collecting 500 g of okra fruits from the field on zero (1 hr), one, three, five, seven and 10 and 15 days after second spray and analysed at laboratory of AINP on pesticide residues, Hyderabad. An initial deposit Flubendiamide was recorded to be 1.49 mg kg^{-1} dissipated to below detectable level on 10th day. The half life was 1.83 days with waiting period for safe harvest of okra fruit was 4.19 days.

Keywords: Okra, insecticides, fruit and shoot borer, flubendiamide, efficacy, dissipation and half-life

1. Introduction
Okra, *Abelomoschus esculentus* L. is a major economically important vegetable crop grown in India and alone accounts for 21 percent of total exchange earnings from vegetables export. It is grown on an area of 4.51 lakh ha with an annual production of 47.96 lakh tons and productivity of 10.62 ton per ha [1]. Okra crop is attacked by several insect pest right from germination to till harvest. Among them, the fruit and shoot borer (*E. vitella*) is important, as they bore into tender shoot and fruits, causes considerable loss in the yield of good fruits [4]. The loss in okra fruits due to the infestation of *E. vitella* ranges from 5.33 to 75.75 percent in field [15]. In order to reduce the loss caused due to *E. vitella* farmers are going for many sprays of chemical pesticide [12]. Such discriminate use of pesticides lead to problem of development of resistance, resurgence, environmental pollution and health hazards. Pesticide residue in fruits and vegetables has affected exports in recent years and should be strictly monitored owing to the high concern about the toxic properties of residues. Flubendiamide, N2- [1,1-Dimethyl-2-(methylsulfonyl) ethyl] -3- iodo-N1- {2-methyl-4- [1, 2, 2, 2-tetrafluoro-1-(trifluoromethyl) ethyl] phenyl} -1, 2- benzenedicarboxamide, is the first commercial member of a new, promising class of insecticides called 1,2-benzenedicarboxamides or phthalic acid diamides with exceptional activity against a broad spectrum of lepidopterous insects such as armyworms, bollworms, corn borers, cut worms, diamondback moths, fruit worms and loopers including resistant strains (17 and 5). In contrast to most commercially successful insecticides which act on the nervous system, flubendiamide disrupts the muscle function in insects and therefore, represents a unique mode of action. Hence, the current investigation was formulated to evaluate the efficacy of flubendiamide and to establish the dissipation pattern to fit in the pest management strategy.

2. Materials and methods
Investigations on bioefficacy of insecticides were studied at the Students' farm of Agricultural College, Hyderabad during September of 2012 and dissipation studies were done at the laboratory of AINP on Pesticide Residues, Hyderabad.

2.1 Bio-efficacy
Okra crop was raised in a randomized block design (RBD) with seven treatments replicated thrice using "Arka Anamika" variety at spacing of 45×15 cm and rest of the agronomic practices were followed as per the Acharya NG Ranga Agricultural University package of practices. The insecticide treatments used were bifenthrin 10 EC at 80 g a.i. ha^{-1}, fipronil 5 SC at 500 g a.i. ha^{-1}, flubendiamide 480 SC at 60 g a.i. ha^{-1}, quinalphos 25 EC at 350 g a.i. ha^{-1}, profenofos 50 EC at 400 g a.i. ha^{-1} and beta-cyfluthrin 25 SC at 18.75 g a.i. ha^{-1}. The above mentioned concentrations were prepared in water and sprayed on okra plants at 50 percent flowering stage and thereafter, repeated at 15 days interval. Spraying was done using knapsack sprayer (20 litres). While spraying, care was taken to attain complete spray coverage of the plants and to avoid any drift among the treated plots. The untreated control plots were left unsprayed.

In each treatment, five plants were selected at random and tagged. Healthy and infested fruits from those tagged plants were harvested after five days after each spray and recorded percent infestation on number and weight basis separately. Then data was analyzed with arc sine values obtained from the conversion of percent of infestation of fruits [6].

2.2 Residue Analysis
2.2.1 Chemicals and Sampling
Okra fruits were drawn at zero (one h), one, three, five, seven, ten and fifteen days after the last spray. The samples of 500 g fruit from each replication were collected at random and brought to the laboratory for residue analysis. Samples were then chopped and prepared for residue determination.

The certified reference standard of flubendiamide (purity 99.5%) was supplied by M/s. Bayer Crop Science Limited (Mumbai, India). The solvents used in this study were analytical grade and were redistilled in glass apparatus and also their suitability was ensured by running them in reagent blanks along with actual analysis. Acetonitrile and water were of HPLC grade. The stock solution of flubendiamide was prepared at the concentration of 1,000 ug mL^{-1}.

2.2.2 Extraction and Column Clean-up
Representative sample of 50 g okra fruits was extracted with 50 ml acetonitrile twice by using mechanical shaker for 30 minutes. The extraction mixture was filtered and evaporated to near dryness by vacuum rotary evaporator and the contents were re-dissolved in 40 ml of acetonitrile. A glass column was packed with 5 g of alumina using hexane as solvent and drained the excess of solvent. Then, sample was transferred in to the column and eluted with 100 ml of 10:1 hexane: ethyl acetate solvent mixture. Discarded the first 10 ml fraction and collected the elute over anhydrous sodium sulphate, the process was repeated thrice. Approximately 300 ml of elute was collected. The elute was completely dried or drained and evaporated to near dryness. The residues were recovered in five ml of acetonitrile for High Pressure Liquid Chromatograph (HPLC) analysis.

2.2.3 HPLC Analysis
Analysis of flubendiamide residues was carried out using a High Performance Liquid Chromatograph (HPLC) with the following parameters: Shimadzu, LC 20 at HPLC with a photo diode array (PDA) detector and a SPD-M 20A, RP-18e, Chromolith 100 x 4.6 mm i.d, column. The mobile phase was acetonitrile: water (6:4, v/v), with a flow rate of 0.5 ml min^{-1}, wavelength, 230 nm and injection volume was 20 µL. With these operating parameters the retention time of the flubendiamide was 8.9 min.

2.2.4 Rate of Recovery
The average of recovery percentages of flubendiamide from okra fruit samples were 88 percent at 0.01 ppm and 87 percent at 0.1 ppm level, respectively.

2.2.5 Kinetic Study
In order to calculate the rate of degradation, waiting period and half-life of Flubendiamide on okra fruits, Hoskin's (1961) linear regression equation was followed.

The period to be allowed to expect the residues to reach below the tolerance limit after treatment for safe use of the treated okra fruits was calculated by using the formula (Gunther and Blinn, 1955).

$Y = a + b X$

where,

Y - Log of tolerance limit
a - Log of initial deposit
b - Slope of the regression line

Waiting period is the minimum number of days to lapse before the insecticide reaches the tolerance limit. The waiting periods were calculated by the following formula.

$$T_{tol} = \frac{[a - \text{Log tol}]}{B}$$

where,

T_{tol} - Minimum time required for the pesticide residue to reach below the tolerance limit.
tol - Tolerance limit of the insecticide
a - Apparent initial deposits obtained in the regression equation
b - Slope of the regression line

Half-life (RL_{50}) is the time in days required to reduce the pesticide residues to half of its initial deposits.

Log 2 0.301
RL_{50} = …… = …….
K_1 K_1

where,

K_1 = Slope of regression line[9]

3. Results and Discussion
3.1 Bio-efficacy
All the insecticidal treatments were superior over control at both sprays. On number basis, cumulative efficacy of insecticides after second spray revealed that Flubendiamide at 60 g a.i. ha^{-1} was the most effective treatment with least infestation of 14.40 percent. Next best treatments were beta-cyfluthrin at 18.75 g a.i. ha^{-1}, profenofos at 400 g $a.i^{-1}$ and quinalphos at 350 g a.i. ha^{-1} being at par recorded 23.40, 24.20 and 25.30 percent of fruit borer infestation. A next best treatment was bifenthrin at 80 g $a.i^{-1}$ with 29.29 percent followed by fipronil at 500 g $a.i^{-1}$ with 31.40 percent of fruit borer infestation. The highest fruit borer infestation was recorded in control.

On weight basis also Flubendiamide at 60 g $a.i^{-1}$ was the most promising treatment with least fruit borer infestation of 15.90 percent. Next to follow were beta-cyfluthrin at 18.75 g $a.i^{-1}$, profenofos at 80 g $a.i^{-1}$ and bifenthrin at 80 g $a.i^{-1}$ were being at par recorded 20.40, 23.12, 24.47 percent fruit borer infestation. Quinalphos at 350 g $a.i^{-1}$ and Fipronil at 500 g $a.i^{-1}$ with 27.60 and 31.69 percent of fruit borer infestation,

respectively proved to be least effective as compared to other insecticidal treatment, but were significantly superior to untreated control.

On comparative basis of different insecticide treatments, flubendiamide at 60 g a. i. ha^{-1} was the most effective treatment (Table-1) in reducing *E. vitella* infestation in okra and the current findings were in conformity with the research findings [14] that flubendiamide is a new chemical option for control of multi-resistant noctuid pests and an excellent choice in resistant management strategies for lepidopteran pests in general. These results were also in accordance with the findings of (8, 17, 10 and 13).

3.2 Dissipation

The initial deposit and subsequent residues of flubendiamide at 60 g a. i. ha^{-1} in okra fruits at an interval of zero (1 hr), three, five, seven, 10 and 15 days after last spray were presented in Table-2 and Figure-1. An initial deposit of 1.49 mg kg^{-1} was gradually dissipated to 0.84, 0.47, 0.10 and 0.01 mg kg^{-1} with the percent dissipation was 43.30, 68.08, 93.14 and 98.88 on one, three, five and seven days, respectively. The residues fell below maximum residue limit (MRL) of 0.2 mg kg^{-1} in 4.19 days (T_{tol}) after the treatment. The half-life (RL_{50}) of flubendiamide was worked to be 1.83 days.

The present findings are in agreement with research reports that [16] an initial deposit of 1.06 and 2.00 mg kg^{-1} following application of flubendiamide 480 SC at 60 and 120 g a.i. ha^{-1} on chilli and which were dissipated to below detectable level of 0.01 mg kg^{-1} in seven and 10 days, respectively. In okra the initial deposits of 0.28 and 0.53 µg g^{-1} was reached below determination level (BDL) of 0.01 µg g^{-1} on the seventh and 10th day with half-life 4.7-5.1 days when flubendiamide 39.35 SC sprayed at 24 and 48 g a.i. ha^{-1}, respectively [3]. Furthermore, research reports reveals that residues of flubendiamide were reached below determination level on fifth and 10th day after application at 90 and 180 g a.i. ha^{-1}, respectively on brinjal [2]. This variation in the rate of dissipation in chilli, okra and brinjal fruits may be due to changes in the crop matrix. In addition, the dissipation of pesticide residues in/on crops depends on climatic conditions, type of application, plant species, dosage, interval between application and time of harvest [11].

Table 1: Bio-efficacy of insecticides against shoot and fruit borer (*E. vittella*)

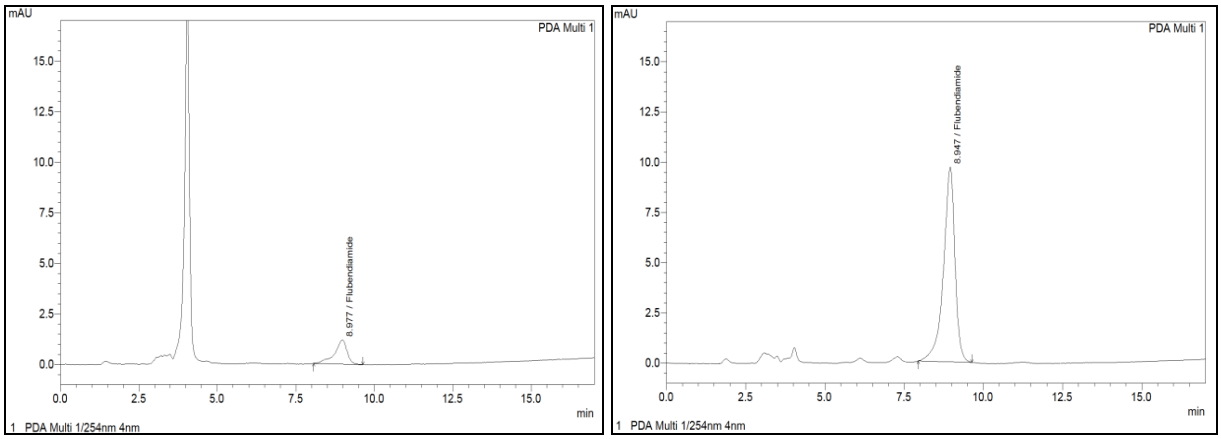
Treatments	Dosage (g a.i. ha^{-1})	Fruit Damage (%)			
		Number basis		Weight basis	
		I Spray	II Spray	I Spray	II Spray
T_1 Bifenthrin	80	14.50^{ab} (22.40)*	29.29^{cd} (32.70)*	13.10^{b} (21.19)	24.47^{bc} (29.60)
T_2 Fipronil	500	15.40^{b} (23.10)	31.40^{d} (34.10)	19.70^{d} (26.30)	31.69^{de} (34.20)
T_3 Flubendiamide	60	11.07^{a} (19.40)	14.40^{a} (22.30)	9.20^{a} (17.56)	15.90^{a} (23.40)
T_4 Quinalphos	350	16.60^{b} (24.00)	25.30^{bc} (30.20)	17.60^{cd} (24.76)	27.60^{cd} (31.70)
T_5 Profenofos	400	13.69^{ab} (21.70)	24.20^{b} (29.40)	14.20^{bc} (22.11)	23.12^{bc} (28.70)
T_6 Beta-cyfluthrin	18.75	12.80^{ab} (21.00)	23.40^{b} (28.90)	13.10^{b} (21.20)	20.40^{b} (26.80)
T_7 Control	-	39.15^{c} (38.70)	41.28^{e} (40.00)	32.93^{e} (34.99)	34.86^{e} (35.60)
S.Em.±	-	1.02	0.94	1.00	1.09
C.D at 5%	-	3.15	2.89	3.08	3.28
C.V.(%)	-	7.28	5.23	7.21	6.26

*Figures in the parentheses are arc sine transformed values.

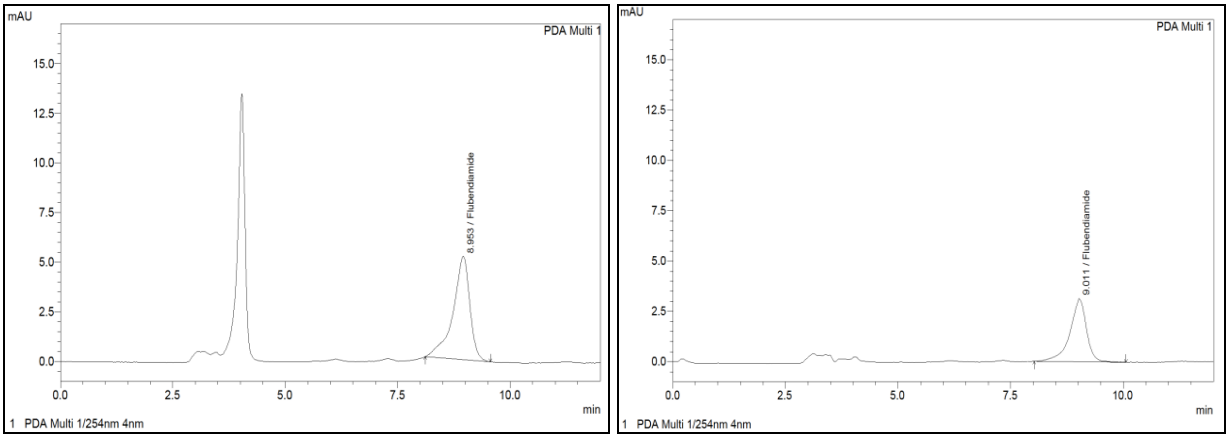
Table 2: Dissipation of flubendiamide (60 g a.i. ha^{-1}) in okra

Days	Residue (mg kg^{-1})				Dissipation %
	R1	R2	R3	Average	
0	1.49	1.51	1.48	1.49	--
1	0.86	0.82	0.86	0.84	43.30
3	0.48	0.46	0.49	0.47	68.08
5	0.10	0.10	0.10	0.10	93.14
7	0.02	0.02	0.01	0.01	98.88
10	BDL	BDL	BDL	BDL	100
15	BDL	BDL	BDL	BDL	100

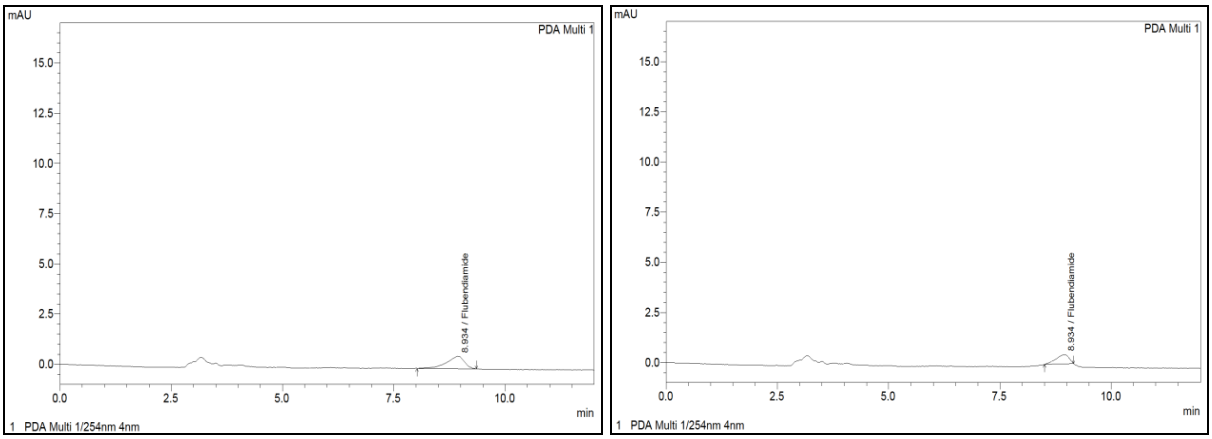
BDL – Below Detectable Level



Flubendiamide Standard (1ppm) Flubendiamide (0 DAS)



Flubendiamide (1 DAS) Flubendiamide (3 DAS)



Flubendiamide (5 DAS) Flubendiamide (7 DAS)

Fig 1: Flubendiamide (60 g a.i. ha^{-1}) chromatograms

4. Conclusion

The information generated in the present study clearly shows effectiveness of flubendiamide against *E. vitella* infestation. As the flubendiamide is representative of new chemical insecticide class- the diamide, acts on insect muscles receptors causing an immediate cessation of feeding and thus avoided the shoot and fruit borer infestation in fruit. Flubendiamide has shown faster rate dissipation rate, where their residue reached below detectable level within five days (MRL in India on okra is 0.2 mg kg^{-1}). These input could be utilized in formulating the spray schedule for managing okra pests and thereby reducing the ill effects of insecticide on environment and human health.

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