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Dissipation pattern of flubendiamide 480% SC in Dolichos bean

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Abstract
Field and laboratory experiments were conducted during *kharif,* 2015 to study the dissipation pattern of Flubendiamide 480% SC @ 60 g a.i ha^{-1} by spraying thrice on field bean collecting samples at regular intervals *i.e.* 0, 1, 3, 5, 7, 10 and 15days after last spray and analyzed. The results of flubendiamide residues showed that, the initial deposits of 1.79 mg kg^{-1} were detected on field bean pods. The residues recorded at 1, 3, 5 and 7^{th} day after third spraying were found to be 0.90, 0.51, 0.13 and 0.06 mg kg^{-1}, respectively and showing a dissipation per cent of 49.72, 71.51, 92.74 and 96.65 per cent, respectively. The residues were below detectable level (BDL) after ten days showing hundred per cent dissipation.

Keywords: initial deposit, dissipation, BDL waiting periods

Introduction
Dolichos bean belongs to the family Leguminosae, is an important pulse cum vegetable crop in India and is cultivated extensively for its fresh tender pods, leaves and seeds and as cattle feed. In India this is grown mostly in Andhra Pradesh, Karnataka, Tamil Nadu, Kerala and Assam. The fresh and dried seeds constitute major vegetarian source of proteins in the human diet of Indians. The field bean fresh pods are acceptable and liked by all, especially during winter season under South Indian conditions are rich in nutritive value and are rich source of carbohydrates, minerals, vitamins, such as vitamin A, vitamin C, fat and fiber. The protein content of field bean is quite high varying from 20.0 to 28.0 per cent [1]. However, the primary cause attributed for lower yields of field bean is due to the heavy infestation of an array of pest complex. As many as 55 species of insects were recorded [2] and a species of mite feeding on the crop from seedling stage to the harvest of the crop in Karnataka, of which, the pod borers were considered to be most important as they regularly caused crop loss to the tune of 80-100 per cent [3]. Pod borers were the key impediments for the low productivity in India to a loss up to nearly 54 per cent in field beans and the major yield loss was inflicted by the pod feeders which include both the pod borers and pod bugs [4]. Pesticide use has increased rapidly over the last two decades at the rate of 12 per cent per year [5]. The wide spread use of pesticides resulted in the presence of their toxic residues in various environmental components/commodities [6, 7, 8].
Indiscriminate and improper use of pesticides on vegetables and negligence to follow proper waiting periods make marketed vegetables very often contaminated with pesticides [9]. Many farm gate vegetable samples showed presence of insecticide residues (Singh *et al*. 1999) [10]. Literature reveals that vegetables which contain the residues of pesticides above their respective maximum residue limit MRL may pose health hazards to consumers [11, 12]. Thus, contamination of vegetable crops is sometimes more than the prescribed tolerance limits. The extensive and irrational use of pesticides resulted in the presence of residues of insecticides on different edible plant parts used for human consumption resulting in various public health problems beside environmental ill effects. The increasing amount of pesticide residues in vegetables has been a major concern to the consumers, as some of these insecticides leave residues on pods which may persist up to harvest. Presence of pesticide residues in the harvested beans was posing problem at the time of export and in recent times importing countries have rejected few consignments. Hence, great significance has to be given to estimate pesticide residues in beans and their dissipation pattern to fix waiting periods for safe consumption.

Materials and Methods

The experiment was laid out in a Randomized Block Design (RBD) utilizing flubendiamide and untreated control replicated thrice with a plot size of 20 m^2 (5mx4 m) by spraying thrice on field bean @ 60 g a.i ha^{-1}, first at 50% flowering and the second and third spray ten days later to evaluate the dissipation pattern by collecting samples at regular intervals *i.e.* 0, 1, 3, 5, 7, 10 and 15 days after last spray in polythene bags and brought to the laboratory immediately for further sample processing in the laboratory as detailed here under.

Fresh beans (5kg) were homogenized with robot coupe blixer and homogenized

↓

15±0.1g sample was taken in 50 ml centrifuge tube

↓

Required quantity of standard (CRM) added to get desired fortification level

↓

30±0.1 ml acetonitrile was added to the tube

↓

The sample was homogenized at 14000-15000 rpm for 2-3 min using Heidolph silent crusher

↓

3±0.1g sodium chloride was added to tube and mixed by shaking gently

↓

Centrifuged for 3 min at 2500-3000 rpm to separate the organic layer

↓

The top organic layer of about 16 ml was taken into the 50 ml centrifuge tube

↓

9±0.1 g anhydrous sodium sulphate was added to remove the moisture content

↓

8 ml of extract was taken in to 15 ml tube containing 0.4±0.01g PSA sorbent (for dispersive solid phase d-SPE cleanup) and 1.2±0.01 gr anhydrous magnesium sulphate

↓

The sample tube was vertexed for 30 sec followed by centrifugation for 5 min at 2500-3000 rpm

↓

The extract of about 2ml was transferred into test tubes and evaporated to dryness using turbovap with nitrogen gas and reconstituted with 1ml n-Hexane: Acetone (9:1) for GC analysis with ECD for analysis. (Table-1)

Extraction and Clean –Up

Certified Reference Materials (CRMs) of were procured from Dr. Erhenstorfer, Germany. Primary, intermediary and working standards were prepared from these CRMs using acetone and *n*-hexane as solvents.

Working standards of the pesticide were prepared in the range of 0.01 ppm to 0.5 ppm in 10 ml calibrated graduated volumetric flask using distilled *n*-hexane as solvent. All the standards were stored in deep freezer maintained at -20^{0}C.

Based on the response of the Mass Spectrometer to different quantities (ng) of CRM standards injected under the HPLC operational parameters given in table 1, it was found that the LOD (limit of detection) for flubendamide was 0.05 ng, and the linearity was in the range of 0.05 ng to 0.10 ng, as given in fig 1.

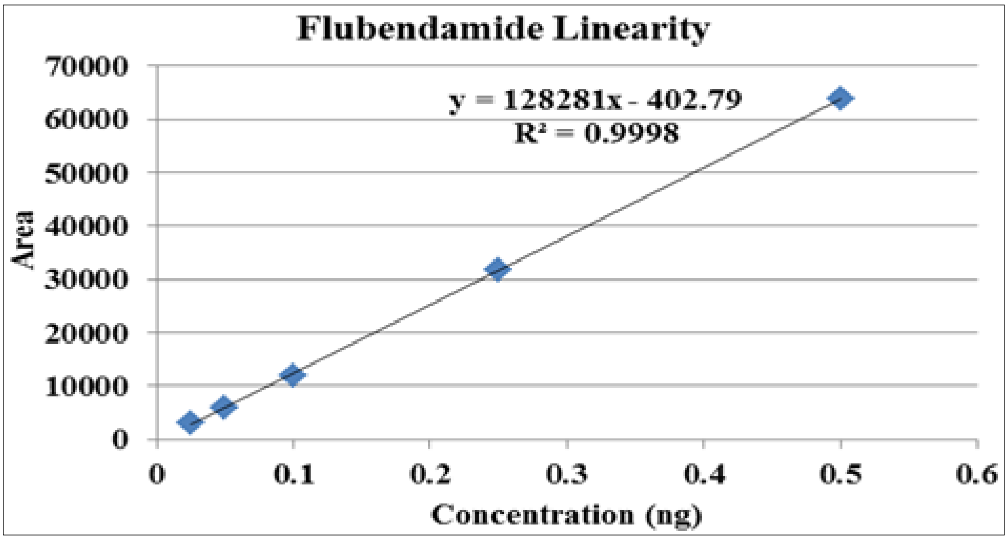

Fig 1: Calibration curve for flubendiamide

Table 1: Recovery of flubendiamide from fortified field bean samples

Details	Recoveries of flubendiamide from fortified field bean samples					
	Fortified level					
	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	0.051	101.43	0.228	91.10	0.493	98.66
R2	0.051	101.18	0.234	93.54	0.493	98.57
R3	0.052	103.27	0.228	91.33	0.517	103.43
Mean		101.96		91.99		100.22
SD		1.138		1.350		2.779
RSD		1.116		1.467		2.773

Results and Discussion

Flubendiamide 480% SC @ 60 g a.i. ha^{-1} was sprayed three times with an interval of 10 days and the spray was initiated when the field bean crop was attained 50 percent flowering. The field bean green pod samples were collected at regular intervals of zero
(2 hours after spray), 1, 3, 5, 7, 10 and 15 days after last spray. The collected pod samples were processed and estimated for residues of flubendiamide on High Performance Liquid Chromotography (HPLC). Dissipation pattern of flubendiamide is presented in table 2 and depicted in figure 2. The results of flubendiamide residues showed that, the initial deposits of 1.79 mg kg^{-1} were detected on field bean pods. The residues recorded at 1, 3, 5 and 7th day after third spraying were found to be 0.90, 0.51, 0.13 and 0.06 mg kg^{-1}, respectively and showing a dissipation per cent of 49.72, 71.51, 92.74 and 96.65 per cent, respectively. The residues were below detectable level (BDL) after ten days showing hundred per cent dissipation. The regression equation was Y= 1.3949 + (-0.2240) X with R^2 value of 0.8246 (Table-2 and Fig.2).
The MRL values were not available for flubendiamide in field bean by either Codex Alimentarius Commission (CAC) or by Food Safety and Standards Authority of India (FSSAI). Hence the day at which the residues reached below detectable level
(10 days) was considered as waiting period.
Since sufficient literature was not available on the dissipation pattern of flubendamide in field bean crop, the present investigation results were compared with available literature on other crops.
The results are in agreement with the findings of Sahoo *et al.* (2009) [13] who found the initial deposits of flubendiamide on chilli as 1.06 and 2.00 mg kg^{-1}, respectively, at two applications of flubendiamide 480 SC @ 60 and 120 g a.i. ha^{-1} at 10 days interval. Residues of flubendiamide dissipated below detectable level (BDL) of 0.01 mg kg^{-1} in 7 and 10 days at single and double dosages, respectively while Mohapatra *et al.* (2010) [14] observed the initial deposits of flubendiamide in cabbage as 0.33 and 0.49 mg kg^{-1} respectively @ 24 and 48 ga.i.ha^{-1} which persisted for 10 days in both the treatments. The variation of initial deposits in the present investigation may be due to change in the crop [15].
Das *et al.* (2011) [16] reported that the initial deposits of flubendiamide 39.35 SC 0.28 and 0.53 mg kg^{-1} in/on okra fruits reached below determination level of 0.01 mg g^{-1} on the 7th and 10th day at 24 g a.i. ha^{-1} and 48 g a.i. ha^{-1} doses, respectively.
Mohapatra *et al.* (2011) [9] also recorded the initial deposits of flubendiamide on field treated tomato from treatments @ 48 and 96 g a.i. ha^{-1} as 0.83 and 1.68 mg kg^{-1}, respectively and persisted for 15 days in both the treatments. Sahoo *et al.* (2009) [13] also found that when three applications of flubendiamide 480 SC @ 48 and 96 g a.i. ha^{-1} at
7 day intervals showed initial deposits of 0.68 and 1.17 mg kg^{-1}, respectively in chickpea pods. The variation in intial deposits in the present investigation compared to those of Sahoo *et al.* (2009) [13], Das *et al.* (2011) [16] and Mohapatra *et al.* (2011) [17] may be due to the variation in dosages of application in field bean, okra and tomato and chick pea and the variation may be due to matrix variations.
The present results show similarity with the findings of Reddy *et al.* (2013) [18] who studied the dissipation of flubendiamide 480 SC @ 60 g a.i.ha^{-1} applied twice as foliar spray, on okra wherein the initial deposit of 1.49 mg kg^{-1} dissipated by 98.88 per cent by seventh day and reached below detectable level (BDL) by tenth days.

Shashi bushan *et al*. (2014) [19] reported that the initial deposits of flubendiamide at 60 ga.i. ha^{-1} sprayed at 50 per cent flowering and repeated 15 days after first spray recorded 1.49 mg kg^{-1} which dissipated to below detectable level (BDL) on 10th day.

Diwan *et al*. (2015) [20] reported that the initial deposits of flubendiamide in tomato were 0.90 and 1.82 μg g^{-1} following three applications @ 48 and 96 g a.i.ha^{-1} at 10 days interval, respectively which reached BDL of 0.01 μg g^{-1} on seventh and tenth day at both the doses, respectively. Buddidathi *et al.* (2016) [21] evaluated the dissipation pattern of flubendiamide in capsicum fruits under poly house and open field after giving spray applications at the recommended and double doses of 48 g a.i. ha^{-1} and 96 g a.i. ha^{-1}. Initial residue deposits of flubendiamide on capsicum fruits grown under poly house conditions were (0.977 and 1.834 mg kg^{-1}) higher than that grown in the field (0.665 and 1.545 mg kg^{-1}). Flubendiamide residues persisted for 15 days in field-grown and for 25 days in poly-house-grown capsicum fruits.

The present findings are in conformity of the results reports of Diwan *et al*. (2015) [20] and Buddidathi *et al.* (2016) [21] especially in case of initial deposits at 96 g a.i. ha^{-1} of flubendiamide in both tomato and capsicum fruits, respectively.

Conclusion

Flubendiamide has shown faster rate dissipation rate, where their residue reached below detectable level within seven days. These input could be utilized in formulating the MRL values for Indian bean as well as schedule for managing pod borers of Indian bean and thereby reducing the ill effects of insecticide on environment and human health.

Table 2: Dissipation of flubendiamide 480% SC (60 g a.i. ha^{-1}) in field bean after three sprays

Days after last spray	Residues of flubendamide (mg kg^{-1})				Dissipation %
	R1	R2	R3	Average	
0	1.78	1.83	1.76	1.79	--
1	0.91	0.88	0.9	0.90	49.72
3	0.53	0.49	0.51	0.51	71.51
5	0.14	0.12	0.14	0.13	92.74
7	0.08	0.05	0.06	0.06	96.65
10	BDL	BDL	BDL	BDL	100
15	BDL	BDL	BDL	BDL	100
Regression equation	Y= 1.3949+ (-0.2232) X				
R^2	0.8226				
MRL	NA				
Safe waiting period	10 days				
BDL- Below Determination Level NA- Not Available					

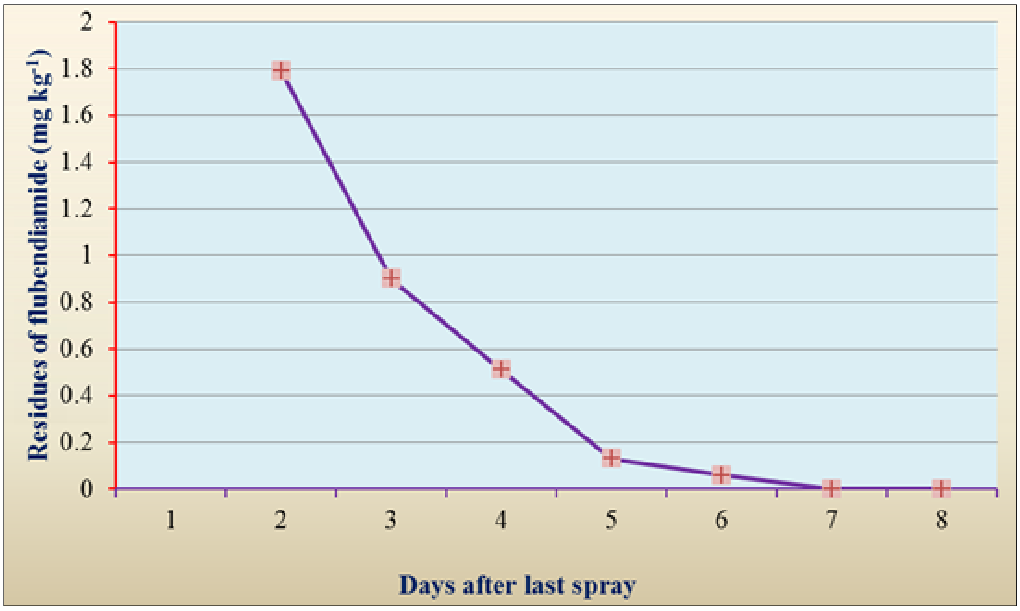

Fig 2: Dissipation of flubendiamide 480% SC in field bean after three sprays

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