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Residual Evaluation of Chromafenozide by using HPLC in Soil and Cotton

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Abstract

Infestation of *Spodoptera litura* in cotton has been frequently reported in Haryana, India. Recommendation of chromafenozide insecticide at 200 g/ha was generated against this pest during 2010 in Haryana. Before recommending it for growers use, its residual studies after cotton harvest were undertaken in soil, cotton lint and cotton seeds by using HPLC, which indicated that there were no residues of chromafenozide in soil, cotton lint and cotton seeds.

Key words : Residual evaluation, Chromafenozide, Soil, Cotton.

The cotton leafworm *Spodoptera littoralis* Boisd. (Lepidoptera : Noctuidae) is one of the most important economic pests attacking cotton in Egypt (1). Recently attack of *Spodoptera litura* in cotton has been frequently reported in Haryana, India. Chromafenozide 80% WP (PII401) insecticide at 200g/ha was recommended in cotton against *Spodoptera litura* in Haryana during 2010. Chromafenozide was discovered in Japan in 2000 which is potent against various lepidopteran larvae (2, 3) with low toxicity profile towards mammals, birds, fishes and arthropods (insect pollinators, predators and parasitoids). Mixture of cloropyriphos with chromafenozide can be integrated in an IPM program against this insect (4). Before generating any sound recommendation, it is imperative to know its residual behavior in soil and crop commodities. Therefore, residual studies were undertaken in soil, cotton lint and cotton seeds by using HPLC after cotton harvest. The samples of soil, cotton lint and seeds each from treated as well as untreated check plots were collected at harvest and stored at temperature -20 ± 2 C for residual analysis. The residual analysis of soil, cotton lint and seed samples were done during 2010 by using HPLC as per prescribed method sent by PI Industries Ltd., Gurgaon.

Methods

Apparatus used in the study were as follows : Water's HPLC, equipped with PDA (Photodiode Detector Array) detector, mechanical shaker, rotary vacuum evaporator, manifold evaporator, required glass wares. Reagents use were dichloromethane (GR), acetonitrile (GR), water (HPLC grade), anhydrous sodium sulfate ($NaSO_4$), N-hexane (GR), florisil (60 × 100 mesh), activated charcoal, acetonitrile (HPLC grade), sodium chloride (GR), ethyl acetate (GR).

Sample Processing for HPLC

Extraction from Soil. The soil samples taken from treated and untreated checks were homogenized and passed through 2 mm sieve ; 50 g representative soil sample from each were taken in different conical flasks. 200 ml of acetonitrile : water (8 : 2 vol/vol) as an extraction mixture was added in each sample. These flasks were kept on mechanical shaker for 60 minutes for complete extraction of the chemical. This soil extract was filtered through Whatman filter paper no. 1 and repeated the extraction process of each sample by taken 100 ml of acetonitrile as an extraction mixture for 60 minutes. Again this soil extract was filtered through Whatman filter paper No. 1. These above filtrates were combined and reduced the volume up to 20 ml with rotary flash evaporator under reduced pressure and at temperature below 40 C.

Extraction from Cotton Lint. The cotton lint samples taken from treated and untreated checks were finely minced and prepared the representative samples of each. Placed 25 g of each representative sample in different beaker. To this added 300 ml acetonitrile: water (8 : 2 vol/vol) mixture as an extraction solvent and blended in warring blender for 5 minutes. The

Table 1. Residues of chromafenozide in soil, cotton lint and cotton seeds. ND : Not detected.

	Commodity	Untreated	Residues (ppm)
1	Soil	R_1	ND
		R_2	ND
		R_3	ND
		Treated	
		R_1	ND
		R_2	ND
		R_3	ND
2	Cotton lint	Untreated	
		R_1	ND
		R_2	ND
		R_3	ND
		Treated	
		R_1	ND
		R_2	ND
		R_3	ND
3	Cotton seeds	Untreated	
		R_1	ND
		R_2	ND
		R_3	ND
		Treated	
		R_1	ND
		R_2	ND
		R_3	ND

above extract filtered through Whatman filter paper no. 1 and the left sample material in beaker were rinsed thrice with 50 ml of fresh acetonitrile solvent and filtered. The combined filtrates were concentrated by rotary vacuum evaporator under reduced pressure and temperature below 40 C. to remove acetonitrile completely.

Extraction from Cotton Seeds. Each sample of 50g was taken from grinded representative cotton seeds (from treated and untreated check) in separate beaker. The extraction process were completed by adding 300 ml extraction mixture i.e. acetonitrile : water (8

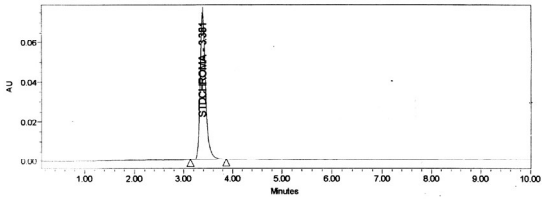

Figure 1. Chromatographic peak of chromafenozide standard identified based on retention time (RT).

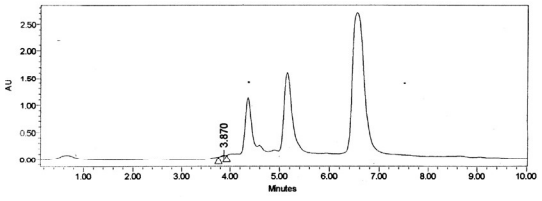

Figure 2. Chromatographic peak of chromafenozide treated soil identified based on retention time (RT).

: 2, vol/vol) and blending with wearing blender for 5 minutes. The extract was filtered through Whatman filter paper no. 1 and the left material in beaker was rinsed thrice with 50 ml of fresh acetonitrile solvent. The combined filtrates concentrated till the complete removal of the acetonitrile by rotary vacuum evaporator under reduced pressure and temperature below 40 C.

Clean up

The above concentrated extract was diluted with 50 ml of 5% NaCI aqueous solution,transferred into separating funnel and subsequently partitioned with 100 ml dichloromethane. The dichloromethane layer was collected by passing through 10 g anhydrous Na_2SO_4 to remove the water and repeated the partition process of aqueous layer with 50 ml alone of dichloromethane and collected the dichloromethane layer by passing through 10 g Na_2SO_4. The aforesaid organic layer was concentrated by using rotary vacuum evaporator under reduced pressure and temperature below 40 C. The residual concentrate was dissolved about in 7 ml of n-hexane prior to use in column chromatography. The chromatographic column was sandwiched with florisil (60 × 100 mesh)

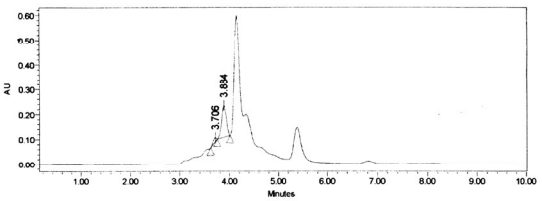

Figure 3. Chromatographic peak of chromafenoizide treated cotton lint identified based on retention time (RT).

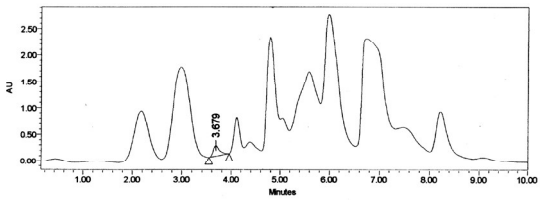

Figure 4. Chromatographic peak of chromafenozide treated cotton seeds identified based on retention time (RT).

between two layers of anhydrous Na_2SO_4. The column was first washed with 10 ml of n-hexane followed by 20 ml of n-hexane/ethyl acetate mixture solution (17 : 3, vol/vol) and discarded the eluate. Placed the residual concentrate at the top of the column and elute the chromafenzide residues from the column with 70 ml dichloromethane. Reduced the volume with rotary flash evaporator under reduced pressure and temperature below 40 C and dried these samples with manifold evaporator. Finally the volume was made with HPLC grade acetonitrile and subjected to HPLC analysis coupled with PDA detector under mention HPLC condition.

All the sample extracts from soil, cotton lint and seeds were cleaned and prepared for HPLC analysis by following the above clean up method.

Procedure for HPLC Analysis

Prepared the stock reference standard solution of chromafenazide i.e. of 100 ppm in acetonitrile (HPLC grade) and from this stock solution, prepared the standard solutions of different concentrations. Inject the 20 µl of each standard solution into the HPLC column and run at 1 ml/min flow with selected mobile phase under mentioned conditions to obtain the chromatogram (Fig.1) and draw calibration curve for integrations of unknown samples. After standardization of the method, inject the aforesaid processed and clean samples of all commodities (from treated and untreated check) one by one under same mentioned conditions for HPLC and conclude the results.

HPLC Operating Parameters for Analysis of Chromafenozide

HPLC model	:	Water's
HPLC column	:	X-Terra RP 18 5µm 4.6 × 250 mm
Detector	:	Photodiode Array (PDA 996)
Volume injected in column	:	20 µl
Mobile phase	:	Acetonitrile (HPLC grade)
Flow rate	:	1 ml/min
Adsorption maxima (λ_{max})	:	202 nm
Retention time (R_t)	:	3.3 minute

Results and Discussion

The HPLC estimation of soil, cotton lint and seeds indicated that there were no residues of chromafenozide in soil, cotton lint and cotton seeds (Table 1 and Figs. 2, 3 and 4).

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