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Determination of the harvest time residues of acetamiprid 20 SP in cotton lint, seed, oil and soil

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Abstract
Cotton (*Gossypium hirsutum* Linn.) is an important agricultural crop cultivated in India for more than 5000 years. It plays a pivotal role in our national economy, by providing livelihood for 60 million people. It suffers from the ravages of several insect and mite pests, right from germination to harvest, resulting in about 15 - 40 per cent yield loss. One of the major causes for low yield is the damage caused by sucking insect pests *viz.*, aphid (*Aphis gossypii* Glover), leafhopper (*Amrasca biguttula biguttula* Ishida), thrips (*Thrips tabaci* Lind.) and whitefly, (*Bemisia tabaci* Genn.) in the early stages of crop growth. Acetamiprid is a novel, neonicotinoid insecticide having N-cyano acetamidine compound that provides excellent control of sucking pests. The compound has excellent systemic and translaminar activities and used against the control of sucking pests of cotton. Two field experiments - one each in summer and winter season were conducted at Tamil Nadu Agricultural University, Coimbatore to determine the harvest time residues of acetamiprid 20 SP. The different treatments included acetamiprid 20 SP @ 10, 20, 40 and 80 g a.i./ha and Pride 20 SP at 20 g a.i./ha were sprayed to determine the residues level in cotton lint, seed and oil and also in soil samples. The results revealed that acetamiprid 20 SP applied @ 10, 20, 40 and 80 g a.i./ha and Pride 20 SP at 20 g a.i./ha left residues at below detectable level in cotton lint, seed and oil at first, third and fifth harvest as well as in the soil sample collected at fifth harvest period in both the seasons. The interval between last spray and first harvest was 80 and 98 days in the field experiment I and II, respectively.

Keywords: Acetamiprid, neonicotinoid, lint, seed, oil, residues, soil

Introduction
Cotton has been part of the fabric of human existence for thousands of years. Cotton is the most important natural textile fibre, as well as cellulosic textile fiber, in the world, used to produce apparel, home furnishings, and industrial products. Cotton has always been a major part of the textile industry and today provides almost 38% of the world textile consumption. Among various cardinal factors responsible for poor yield of cotton, damage caused by the insect pests is one of the major causes for reduced yield. In the early stage, sucking pests like cotton aphid, *Aphis gossypii* (Glover), leafhopper, *Amrasca biguttula biguttula* (Ishida), whitefly, *Bemisia tabaci* (Gennadius) and thrips, *Thrips tabaci* Lind. and in the late stage, bollworm complex cause significant damage to the crop. The yield loss in cotton due to sucking pests alone was 46.5 per cent (Panchabhavi *et al*., 1990) [6] and the bollworm complex accounted for 44.5 per cent (Dhawan *et al*., 1988) [1].

The neonicotinoids are the newest major group of insecticides, which includes acetamiprid, imidacloprid, clothianidin, dinotefuran, nitenpyram, thiacloprid, and thiamethoxam (Tomizawa and Casida, 2005) [9]. Neonicotinoids have proved to be ideal alternatives to organophosphates and carbamates (Elbert *et al*., 1995) [2] with much lower rate of application as compared to traditionally used insecticides (Schmuck, 2001) [7]. In the present study, acetamiprid is selected which is used against sucking insects, such as aphids and leafhoppers in cotton. Once entered in the body it attacks on the central nervous system of insect by binding of acetylcholine, the major excitatory neurotransmitter in insects to the nAChRs, that further cause excitation and paralysis, followed by the death of the insect.

Recently, highly efficacious insecticides with novel mode of action are available which are becoming increasingly important in agriculture as a component of integrated pest management and resistance management strategies. These insecticides are required only in few grams in comparison to older class of compounds which are required in few hundred grams and are perceived to carry higher safety/ environmental risks (Wing *et al*., 2000) [13].

One of the major properties of acetamiprid is its persistence. Normally chloronicotinyl compounds persist for more than a month, which keeps plants free from insects. Acetamiprid is particularly well adapted for orchard protection. Due to its rapid shock action and persistence, good control of aphids on pome fruits, stone fruits and citrus is regularly obtained

with acetamiprid which is equal to or better than the standard products in this group of insecticides (Lacombe, 1999) [5].

Methodology for acetamiprid residue analysis

Vidal *et al*. (2002) [12] described two methods for determining the highly polar pesticide, acetamiprid in vegetables. Residues are extracted with ethyl acetate and co-extractives removed with a graphite carbon-based packing ENVI-carb cartridge. Analysis was performed by HPLC with post-column photoderivatization, fluorescence detection, and HPLC-electrospray ionization mass spectrometry. The clean-up step is not necessary in the last case. The limits of detection were 6µg/l for HPLC – fluorescence detection using matrix matched standards and 1.5 µg/l for HPLC-ES-MS detection. Recovery data were obtained by fortifying vegetable matrix at 0.01, 0.1 and 0.5 mg/kg, with recoveries between 65 and 75 per cent for HPLC fluorescence and between 72 and 77 per cent for HPLC ES-MS.

A study was conducted on the degradation and residue of methidation, diflubenzuron, abamectin and acetamiprid after application in citrus by Zhen *et al*. (2000) [14]. Their dissipation followed first-kinetic order, and their half life values were 30.3, 6.2, 24.4 and 12.7 days in citrus and 5.4, 9.0, 34.1 and 9.2 days in soil, respectively. Acetamiprid and its metabolites were extracted in methanol and derivatized to methyl-6-chloronicotinate through alkali hydrolysis, potassium permanganate oxidation and then esterification of diazomethane, followed by column chromatography clean-up and GC determination. The limit of detection was 0.01 ppm and the recovery of fortified samples ranged from 74 to 92 per cent (Tokieda *et al*., 1997a) [10]. A gas chromatographic (GC) method for the determination of residues of acetamiprid in various fruits and vegetables (including potatoes, apples, aubergines and cabbages) was described by Tokieda *et al*. (1997b) [11]. The limit of detection was 0.005 ppm when 20g of sample was used and 2 microlitre was injected into the GC from the final solution (5 ml). The use of pesticides has become an integral and economically essential part of modern agriculture. Pesticides are often applied several times during one crop season and a part always reaches the soil. Although acetamiprid has been reported to be effective against many insect pests, detailed studies on the residues of acetamiprid in cotton crop is wanting. Hence, Investigations were carried out to determine the harvest time residues of acetamiprid 20 SP in cotton lint, seed, oil and soil.

Materials and Methods

Two field experiments were conducted to determine the residues of acetamiprid 20 SP on cotton cultivars SVPR-3 (Summer season) and MCU -12 (Winter season). The field experiments were conducted at Eastern Block, Tamil Nadu Agricultural University, Coimbatore-3. The experiments were laid out as described under. Sampling of cotton kapas was done during first, third and fifth pickings. The samples were ginned to analyse residues in the seed, lint and oil.

Experimental details

The insecticides used in the present investigation and their dosages were as follows.

T_1 – Acetamiprid 20 SP @ 10 g a.i./ha
T_2 – Acetamiprid 20 SP @ 20 g a.i./ha
T_3 – Acetamiprid 20 SP @ 40 g a.i./ha
T_4 – Acetamiprid 20 SP @ 80 g a.i./ha
T_5 – Pride 20 SP @ 20 g a.i./ha
T_6 – Untreated check.

The experiments were laid out in randomized block design with four replications and the plot size was 5 x 4m ($20m^2$). Regular agronomic practices were followed as per the Tamil Nadu Agricultural University (TNAU) Crop Production Guide. In the experiment I, during April – October 2003 (Summer cotton) cotton cultivar, SVPR-3 was grown and, single spraying was given on 55 days after sowing (DAS) with a pneumatic knapsack sprayer using 500 litres of spray fluid per hectare. In the experiment II, during September-2003 - March-2004 (Winter cotton) with the cotton cultivar, MCU-12, two rounds of spraying were given, one on 60 DAS and the other on 88 DAS.

Residues of Acetamiprid 20 SP

Preparation of standards

Acetamiprid technical material was obtained from Mahamaya Life Sciences Private Ltd., New Delhi. The stock solution of 1000 ppm was prepared by dissolving 103.41 mg of technical material (96.7 per cent purity) in 100 ml of HPLC grade acetone. From this stock, intermediate stock solutions of 100 ppm and 10 ppm were prepared. Using 10 ppm stock, working standards of 0.5, 1, 2, 3, 5 and 10 ppm were prepared in acetone.

Recovery studies

Samples were fortified with working standards at 0.5, 1 and 2 ppm level to find out the recovery of acetamiprid. The recovery factor worked out was taken for final calculation.

Lint

Ten gram of cotton lint was soaked in n-hexane –acetone (1:1 v/v) overnight. Then the samples were filtered through Buchner funnel with repeated washings. The extract was concentrated to 10 ml and passed through a glass column packed with anhydrous sodium sulphate (2g) + Florisil® (2g) + anhydrous sodium sulphate (2g). The column was pre-washed with 20 ml of hexane + acetone (9:1 v/v) and the insecticide was eluted with a mixture of hexane + acetone (9:1 v/v). The elutant was concentrated to near dryness, the residue was dissolved in acetonitrile (HPLC grade) and fed into HPLC.

Seed

a) Extraction

Twenty gram cotton seed was soaked in 50 ml of acetonitrile overnight, homogenized and filtered through Buchner funnel with repeated washings. The pooled acetonitrile extract was evaporated to near dryness.

b) Clean up

(i) Liquid – liquid clean up

The aqueous remainder was treated with 50 ml of saturated sodium chloride and 150 ml of hexane (three 50 ml portions) in a 500 ml separating funnel. After shaking well, the lower aqueous phase was collected and to which 100 ml of hexane: ethyl acetone (98:2 v/v) was added and shaken well. Once again, the lower aqueous phase was collected and partitioned with three 50 ml portions of dichloromethane. The pooled dichloromethane extract was passed through anhydrous sodium sulphate. The extract was evaporated to near dryness and the aqueous remainder was dissolved in ethyl acetate.

(ii) Solid – liquid clean up

For column chromatography, 1.5 cm (id) x 50 cm (length) glass columns were used. Florisil® deactivated with 5 per cent water was used as an adsorbent @ 4.5 g per sample. The drip tip of the chromatographic column was plugged with cotton wool. The Florisil® was slurried with 20 ml ethyl acetate and applied quantitatively into the column. This was sandwiched with two cm layers of anhydrous sodium sulphate. The column was prewashed with 20 ml ethyl acetate. The dry residue dissolved in small amount of ethyl acetate was poured on top of the column by means of a pipette and allowed to percolate. The active ingredient was eluted with 20 ml portions of acetonitrile (HPLC grade). The elutant was concentrated to near dryness, the residue was dissolved in acetonitrile and fed into HPLC.

Oil

Fifty gram cotton seed was blended, tumbled and placed in Soxhlet apparatus and allowed to run for 6-8 h in hexane to extract the oil. The hexane portion was collected, condensed and the oil content weighed. To this 5 ml of acetonitrile and 50 ml sodium chloride (3%) was added. Lower aqueous phase was collected and to this 100 ml of hexane: ethyl acetate (98:2 v/v) was added, partitioned and cleaned up as mentioned above.

Soil

a) Sampling

Soil samples were collected from all the treated soils of the two field experiments, by using a hand held auger driven to a plough depth of 15 cm. A minimum of 20 cores were taken across the field and bulked together from which a single representative sample of 500 g was taken by quartering technique. Quartering was done by dividing the thoroughly mixed samples into four equal parts. The two opposite quarters were discarded and the remaining two quarters were remixed and the process was repeated until the desired sample size of 500 g was obtained. From this, a sub sample of 10 g of soil was taken for residue analysis.

b) Extraction and clean up

Ten gram of soil sample collected the field was soaked with acetonitrile (50 ml) overnight and filtered through Buchner funnel with repeated washings. The pooled acetonitrile extract was concentrated to 10 ml. Partitioning was done with dichloromethane (50 ml) 50 ml of saturated sodium chloride solution. Partitioning was repeated twice with 25 ml of portions of dicholromethane. Dichloromethane extract was collected and passed through anhydrous sodium sulphate. The extract was condensed to 10 ml.

Glass chromatographic column (50 cm length and 1.5 cm diameter) was used in the column clean up. The drip tip of the glass column was plugged with cotton wool and packed air tight using the silica gel, sandwiched with anhydrous sodium sulphate layers. The column was pre washed with hexane initially and eluted with 100 ml of acetone. The collected elutant was condensed to near dryness and reconstituted with acetonitrile (HPLC grade) to 10 ml and then fed into HPLC.

Final determination

End analysis was done by High Performance Liquid Chromatography (HPLC), Hitachi model L 6200 with the following operating parameters.

Mobile phase	:	Acetonitrile (HPLC grade) : Double distilled water (35:65 v/v)
Column	:	Lichro sphere ®60- RP. Select B (5 µm) 250-4 (ID)
Flow rate	:	1 ml/ minute
Detector	:	UV Spectro photometer detector at 254 nm
Quantity injected	:	20 µl (fixed loop)
Recorder	:	Hitachi Integrator D 2500

The amount of residue was determined by comparing the sample response with the response of standard by using the formula,

$$\text{Residues in ppm} = \frac{H_s}{H_{std}} \times \frac{W_{std}}{W_s} \times \frac{V_{ex}}{V_s} \times \frac{A_s}{A_{std}}$$

Where,

H_s	-	Peak height of the sample
H_{std}	-	Peak height of the standard
W_{std}	-	Weight of the standard injected in ng
W_s	-	Weight of the sample in g
V_{ex}	-	Volume of the final extract in ml
V_s	-	Quantity of the sample injected in µl
A_s	-	Attenuation of the sample
A_{std}	-	Attenuation of the standard

Results

The standard chromatogram of acetamiprid is illustrated in Fig.1. The mean recovery of acetamiprid was 88.5 per cent from fortified lint samples, 89.8 per cent from seed, 87.2 per cent from oil and 97.5 per cent from soil samples at 0.5, 1 and 2 ppm level. The minimum detection limit of the instrument was 0.5 ng and the determinability level in lint and soil was 0.1 µg/g considering 10 g weight of the sample and final volume of the extract as 2 ml. The determinability level in seed and oil was 0.05 µg/g considering the weight of the sample was 20 g and 50 g, respectively and final volume of the extract as 2 - 5 ml. The level of residues of acetamiprid 20 SP at 10, 20, 40 and 80 g a.i./ha as foliar spray were at below detectable level in lint, seed and oil samples of first, third as well as fifth pickings and soil samples collected during that period in both field experiments I (summer) and II (winter) (Table 1). The interval between last spray and first harvest was 80 and 98 days in the field experiment I and II, respectively.

Table 1: Harvest time residues of acetamiprid 20 SP in cotton lint, seed, oil and soil (µg/g) – Field experiment I and II (Mean of two observations)

S. No.	Treatments	Dose g a.i./ha	Residues in µg/g at harvest									
			Lint			Seed			Oil			Soil
			I picking	III picking	V picking	I picking	III picking	V picking	I picking	III picking	V picking	V picking
1.	Acetamiprid 20 SP	10	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
2.	Acetamiprid 20 SP	20	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
3.	Acetamiprid 20 SP	40	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
4.	Acetamiprid 20 SP	80	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
5.	Untreated check	-	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL

BDL – Below detectable level

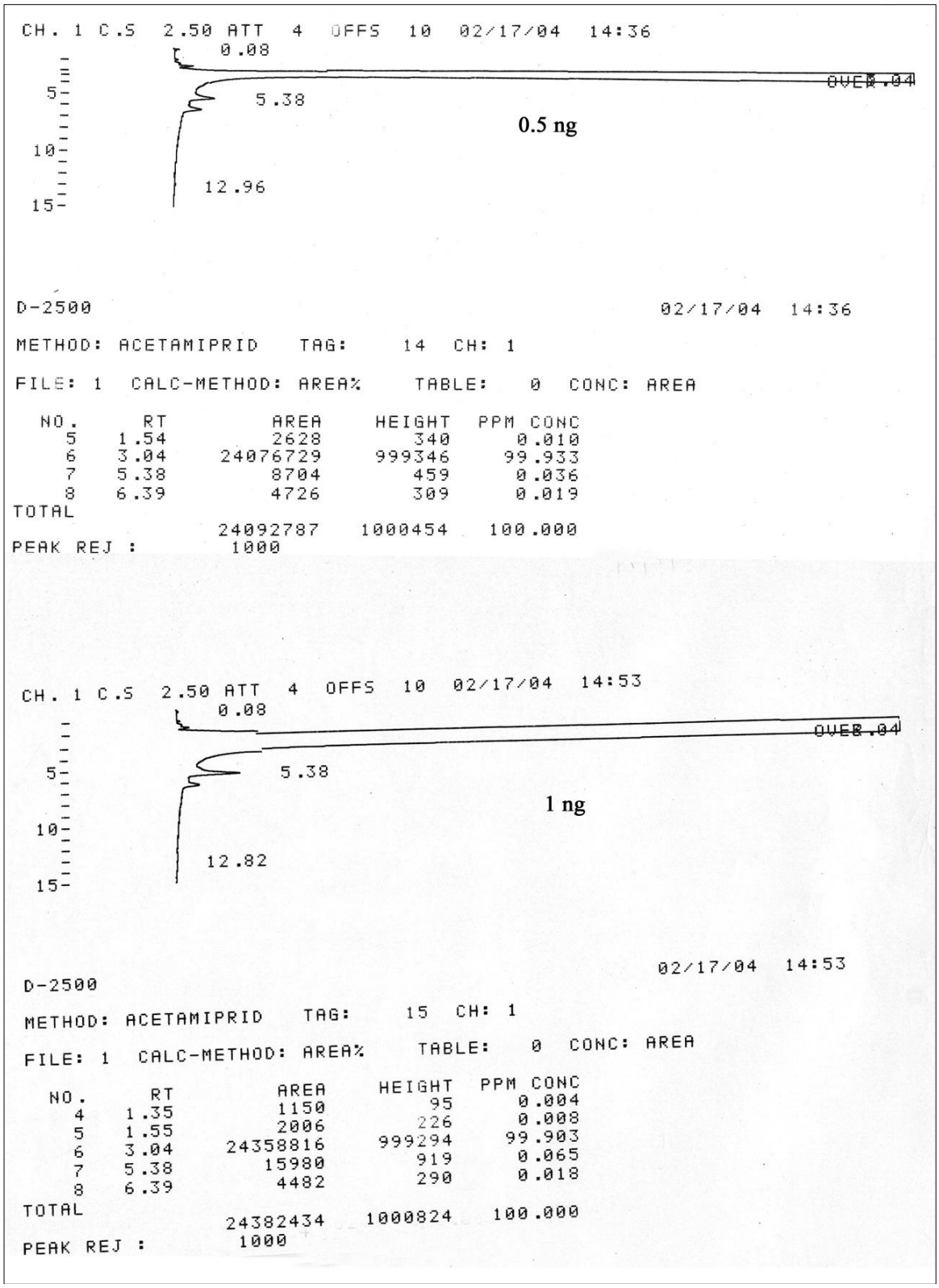

Fig 1: standard chromatogram of acetamiprid

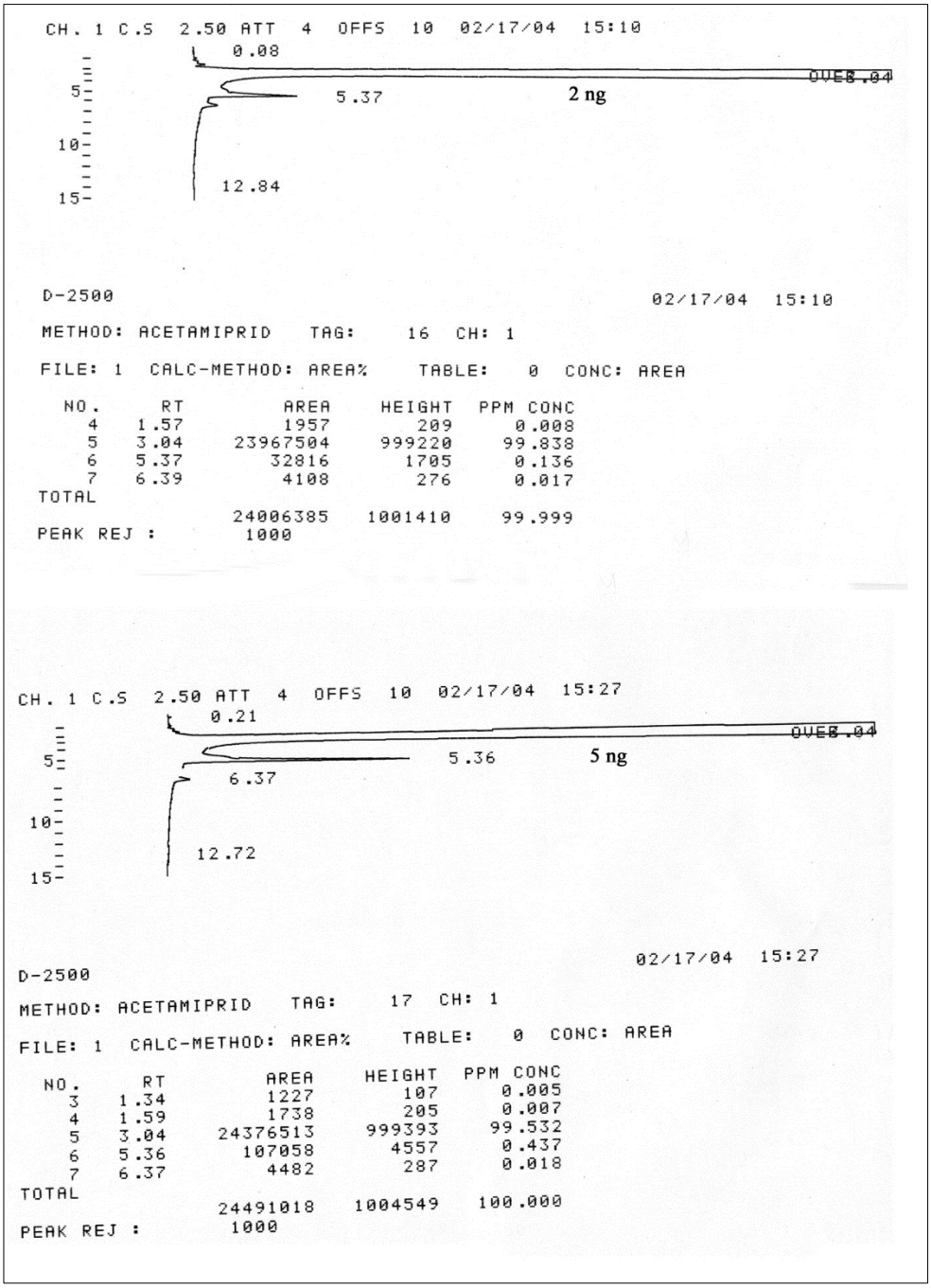

Fig 1: standard chromatogram of acetamiprid (contd.,)

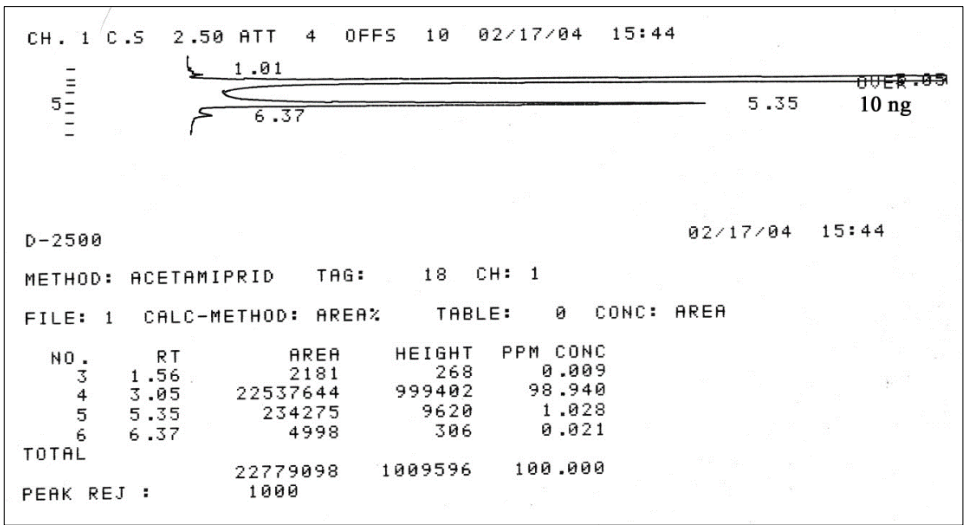

Fig 1: standard chromatogram of acetamiprid (contd.,)

Discussion

Acetamiprid 20 SP applied @ 10, 20, 40, 80 g a.i./ha and Pride 20 SP left residues at below detectable levels in cotton lint, seed and oil at first, third and fifth harvest and fifth harvest day in soil sample. The interval between last spray and first harvest was 80 and 98 days in the field experiment I and II, respectively. This below detectable level of residues is in line with the findings of Kumar (1998) [4] and Suganthy (2003) [8] who found that the residues of imidacloprid in cotton lint, seed, oil and soil were at below detectable levels. These results agree with the findings of Gupta *et al*. (1998) [3] who reported that no residues of seed treated imidacloprid was detected in samples of lint and seed.

Conclusion

Acetamiprid 20 SP applied @ 10, 20, 40, 80 g a.i./ha and Pride 20 SP had residues at below detectable levels, in cotton lint, seed and oil at first, third and fifth harvest and in soil at fifth harvest day in both the seasons. The interval between last spray and first harvest was 80 and 98 days in the field experiment I and II, respectively.

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