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Acetamiprid residues in cotton lint, seed, oil and soil

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Abstract
The present investigation on estimation of acetamiprid residues in cotton lint, seed, oil and soil was conducted at Department of Agricultural Entomology, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani (MS) India. The results of harvest time residues of acetamiprid evidenced that cotton lint samples contained higher amount of acetamiprid residues as compared to cotton seed and oil. None of the treatments showed residues in cotton lint and seed below MRL (0.1 mg/kg). However, in cotton oil residues were detected below MRL except in oil samples collected from acetamiprid + cypermethrin (20+100) g, acetamiprid + quinalphos (20+1000) g and acetamiprid + chlorpyrifos (20+1000) g a.i.ha^{-1} treated plots. The residue levels of acetamiprid in soil samples were below MRL in all the treatments. The overall results concluded that these newer insecticidal combinations (acetamiprid + cypermethrin, acetamiprid + chlorpyrifos and acetamiprid + quinalphos) can be used as effective tools in management of cotton insect-pests with a word of caution that they carry residues much above MRL. As such this schedule needs to be recasted using lower concentrations for harvest time residues.

Keywords: acetamiprid, residue, cotton, lint, seed, oil, soil

Introduction
Cotton is most important commercial crop known as “king of natural fibers” and world over commonly referred as “white gold” which belongs to family Malvaceae and genus *Gossypium.* It is leading plant fibre crop and is grown commercially in temperate and tropical regions of more than 50 countries with a total coverage of 34.1 million hectares. India ranked first in terms of cultivated area (12.7 million ha), occupying over a quarter of the world cotton area, followed by China, USA, and Pakistan. India accounts for about 37 per cent of the global cotton area and contributes to 26 per cent of the global cotton produce, currently ranking first in the world. During 2014-15, the total area under cotton was 126.55 lakh ha with production of 400 lakh bales of 170 kg per bale and average productivity of 537 kg per ha in India (Anonymous, 2015). At national level Maharashtra ranked first in area, second in production and eleventh in productivity (CAB, 2015).
Several factors are responsible for low productivity of cotton. Menace caused by the insect-pests is a major one. Cotton crop is subjected to damage by 162 species right from emergence till the final picking. In Maharashtra about 25 pests are reported to cause damage to cotton crop at different growth stages (Thakare *et al*., 1983). Introduction of *Bt* cotton technology has solved the bollworm problem but continuous cultivation of *Bt* cotton has at some places led to increased incidence of sucking and other pests in the recent years (Nagrare *et al.*, 2009). The important sucking insect-pests attacking *Bt* cotton are jassid (*Amrasca biguttula biguttula* Ishida), thrips (*Scirtothrips dorsalis* Hood), aphid (*Aphis gossypii* Glover.), whitefly (*Bemisia tabaci* Gennadius) and mealy bug (*Phenacoccus solenopsis* Tinsley).
Several new groups of insecticides have been recommended against sucking pest complex of cotton. Neonicotinoid insecticides are highly selective agonists of insect nicotinic acetylcholine receptors and provide farmers with invaluable, highly effective tools against sucking pests such as leafhopper, aphid, thrips and whitefly, world's most destructive crop pests. Today this class of insecticides comprises at least seven major compounds with a market share of more than 25 per cent of total global insecticide sales (Bassa *et al*., 2015). Acetamiprid is a novel neonicotinoid insecticide (Fig. 1), has high systemic and translaminar activity and hence gives excellent efficacy against sucking pest complex including strains resistant to other chemistries. However, the indiscriminate and injudicious use of single insecticide leads to residues in food stuff. To evaluate the deleterious effects of acetamiprid in crops and to ensure the consumers safety, residue dynamics of acetamiprid in environment such as in soil (Gupta *et al*., 2008 and Junxue We *et al*., 2012)), ground water (Pritam *et al*., 2013) and in crops okra (Singh and Kulshrestha 2005), gram (Gupta *et al*., 2005), mustard plant (Pramanik *et al*., 2006), chilli (Sanyal *et al*., 2008), tea (Gupta and Shanker 2009),

zucchini (Park *et al*., 2010b), water melon (Junxue We *et al*., 2012), egg plant (Romeh and Hendawai, 2013), fruits (Bakırcı *et al*., 2014) and cardamom (Pratheeshkumar and Chandran 2015) have been investigated. However, the residue dynamic of acetamiprid on/in cotton lint, seed, oil and soil has never been investigated. In view of this, the present investigation was undertaken to study the harvest time residues of acetamiprid in cotton lint, seed, oil and soil.

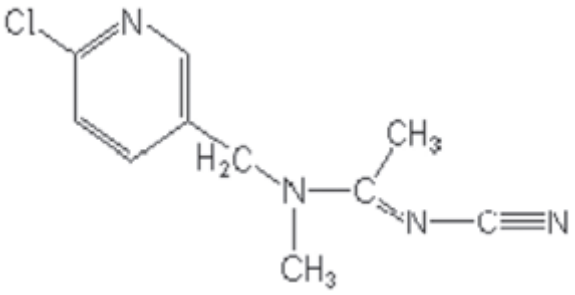

Fig 1: Chemical structure of acetamiprid

Materials and Methods

The present research work was conducted at Department of Agricultural Entomology, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani (MS) India. The field experiment was laid in randomized block design with three replications and 7 treatments. Popular hybrid cotton variety Ganga (PHH 316) was sown at a spacing of 90 x 60 cm. Recommended package of practices except insect-pest control measures were followed to raise the crop. A total of four sprayings were applied with high volume knapsack sprayer. Insecticides were applied on the crop depending on economic threshold level. At the time of harvest, samples of cotton lint, seed, oil and soil were collected and harvest time residues of acetamiprid were estimated from cotton lint, seed, oil and soil with standard procedure of sampling, extraction, partitioning, cleanup and estimation. The residue analysis work was conducted in the Pesticide Testing Laboratory of Dept of Agril. Entomology, VNMKV, Parbhani and in the laboratory of All India Network Project on Pesticide Residue (AINPPR) of Dept. of Agril. Entomology, MPKV, Rahuri (MS). Insecticidal residue analysis was done by following different steps as given under.

2.1. Sampling or sample preparation

2.1.1. Cotton lint and cotton seed

At harvest, samples of seed cotton were collected at random from each treatment per replication and placed in clean and well labelled polythene bags with due care to avoid any contamination and immediately brought in the laboratory. The samples collected from three replications for each treatment were pooled together and they were made into two replicates for residue analysis. The seed cotton samples were air-dried and delinted by mechanical ginning to get cotton seed and lint to analyze them separately. Finally samples of cotton lint and cotton seed were sub sampled to 250 g and 500 g, respectively.

2.1.2. Cotton oil

Oil extraction was done by solvent extraction method. Treatment wise cotton seeds were ground to powder by using mechanical grinder. Thereafter sample powder was filled in paper thimbles and its open end was sealed with cotton swab. When pockets made from filter paper were used, both the open ends were tied with cotton thread. Later on thimbles / pockets were transferred to glass jar for washing with solvent (hexane). The solvent pulled the oil and the solvent / oil mixture was then decanted off and kept open for solvent evaporation. The solvent used for this process was food grade hexane, which has the advantage of removing virtually all oil from the seed powder with minimal wastage, and yet it was easily removed from the oil afterward.

2.1.3. Soil

At the time of harvest soil samples were collected from each treatment per replication at random and packed in clean polythene bags. Well labelled samples were immediately brought in the laboratory. The samples collected from 3 replications were air-dried, sieved and homogenized to uniform size. Finally soil samples were sub sampled to 250 g by cone and quartering method.

2.2. Extraction

2.2.1. Cotton lint and cotton seed

Weighed samples of cotton lint (25 g) and cotton seed (50 g) were soaked and extracted with 100 ml methanol using over end mechanical shaker for about 30 minutes. Then the sample extract was filtered through Buchner funnel over laid with Whatman No. 1 filter paper. The process of sample extraction was repeated twice with 100 ml methanol at each time. The combined methanol extract or filtrate was concentrated to 50 ml at 40^{o}C using vacuum rotary evaporator.

2.2.2. Cotton oil

For extracting insecticidal residues, 25 ml of cotton oil was mixed well with 25 ml of hexane. It was then extracted with 100 ml of acetonitrile saturated with hexane, using end over end mechanical shaker for about 30 minutes. Thereafter the sample extract was filtered through Buchner funnel over laid with Whatman No. 1 filter paper. The process of sample extraction was repeated twice with 25 ml of acetonitrile at each time. The complete extraction was ensured by washing the filter paper with 25 ml of additional acetonitrile. Finally filtrate was collected and concentrated to 50 ml at 40^{o}C using vacuum rotary evaporator.

2.2.3. Soil

Soil samples (50 g) were extracted with 150 ml of acetone in an end over end mechanical shaker for about 2 hours. Thereafter, the sample extract was filtered through Buchner Funnel over laid with Whatman No. 1 filter paper. The process of re-extraction of the soil sample was performed twice with 75 ml acetone for about one hour at each time. The complete extraction was ensured by washing the filter with 50 ml additional acetone. The combined extract was concentrated to 30 ml using rotary vacuum evaporator.

2.3. Clean-up

This step involved quantitative isolation of insecticide residue from co-extractives obtained during the process of extraction.

2.3.1. Partition

The concentrated extract of each sample (cotton lint, seed, oil and soil) was subjected to liquid-liquid partioning. The sample extract was diluted with 50 ml (10 %) NaCl solution by pouring in a separatory funnel along with 50 ml hexane and vigorously shaken for 3 minutes (aqueous solution of sodium chloride was added to separatory funnel, to get clean partitioning of liquid). Layer of hexane was discarded and aqueous phase was extracted twice with 50 ml

dichloromethane. Then combined organic layer was passed / filtered through glass funnel containing a layer of anhydrous sodium sulphate. The complete extraction was ensured by washing filter bed with 50 ml additional dichloromethane. This extract was concentrated to dryness using rotary evaporator and finally residues were dissolved in 5 ml hexane for the column chromatography.

2.3.2 Column clean up

Sintered disc glass column (2 cm i.d x 40 cm height) was packed with 10 g florisil. The column was pre-washed with 50 ml mixture of acetone: hexane 20: 80 and discarded the elute. Then dried extract was rinsed thrice with 5 ml of hexane at each time to transfer simultaneously on the separate pre-washed column for each treatment sample. Finally the column was eluted with a mixture of 120 ml acetone : hexane 50:50 (at the rate of 2-3 ml min^{-1}). The elute was concentrated to near dryness and added with 5 ml hexane and transferred in glass stoppered test tubes (10 ml) for the estimation of residues by Gas Liquid Chromatograph (GLC).

2.4. Estimation

Residues of acetamiprid were estimated by Gas Liquid Chromatograph (GLC) equipped with high temperature electron capture detector (ECD) provided with Ni-63 radio isotopic source. The standard solution of 1 ppm of acetamiprid was prepared and injected in GLC to obtain retention time, by setting temperatures of injection port, oven and detector. This retention time was then considered for detection of acetamiprid residues in treated samples. Thereafter, treatment sample extracts were injected simultaneously followed by standard solutions of acetamiprid compound (each injection ranged between1 to 5 µl depending on the response peak of acetamiprid residues in the sample extract). Quantity of acetamiprid was computed on the basis of height count by comparing with height count of closely matching chromatograph of known injected standard. The quantity of sample extract of each sample was diluted to 5 ml and used for injection which was equivalent to 25 g of cotton lint, 50 g of cotton seed, 25 ml of cotton oil and 50 g of soil. Rest of details of GLC are given below.

1.	Model of GLC	:	Tracor 540 (U.S.A.)
2.	GLC detector	:	Electron Capture Detector (Ni-63)
3.	Liquid phase column	:	3 % OV 101 on (chromosorb WHP) (80/100 mesh) loaded in stainless steel (TCB 204) Length : 1 meterDiameter : 1/8 inches
4.	Nitrogen gas flow	:	40 ml min^{-1}
5.	Hydrogen gas flow	:	---
6.	Zero air flow	:	---
7.	Working temperature		
a.	Injector port	:	250°C
b.	Column oven	:	210°C
c.	Detector	:	310°C
8.	Retention time (minutes)	:	2.22 (± 0.05)
9.	Minimum detectable	:	0.5 ηg quantity
10.	Sensitivity	:	0.05 ppm

Method of calculation

$$\text{Acetamiprid residue (mg/kg)} = \frac{FA \times Vend \times WSt}{FSt \times Vi \times G}$$

G = Weight of analytical sample in g
Vend = Final volume of sample solution
Vi = Aliquot volume of Vend injected into GC in µl
FA = Peak area per height of the analytical sample solution obtained from Vi
FSt = Peak area per height for the standard solution obtained from WSt
WSt = Amount of the reference standard substance injected with the standard solution in ng

Results and Discussion

The overall results revealed that in general, lint samples contained higher amount of acetamiprid residues as compared to cotton seed and oil. None of the treatments showed residues in cotton lint below minimum quantification limit of 0.1 mg kg^{-1} (ppm). It is evidenced that acetamiprid left terminal residues of 1.6814, 1.7677, 1.9060, 2.5446, 2.1000 and 2.3136 ppm in lint samples when applied in insecticidal combinations of acetamiprid + cypermethrin (10+50) and (20+100) g, acetamiprid + quinalphos (10+500) and (20+1000) g and acetamiprid + chlorpyrifos (10+500) and (20+1000) g $a.i.ha^{-1}$, respectively. However, acetamiprid residues were not detected in untreated control. The data of these studies could not be compared as no such study has so far been done in India. The present findings are parallel to the results of Blossom and Singh (2004); and Battu *et al*. (2003) who reported residues of conventional insecticides in cotton lint. In contrast, Mukherjee and Gopal (2001) clearly showed that imidacloprid residues did not persist till harvest in cotton lint when used as seed dresser.

The data on extent of harvest time acetamiprid residues in/ on cotton seed indicated that cotton seed samples collected from all the newer insecticidal combinations treated plots detected acetamiprid residue above MRL (0.1 mg kg^{-1}). Application of acetamiprid + cypermethrin (10+50) and (20+100) g, acetamiprid + quinalphos (10+500) and (20+1000) g and acetamiprid + chlorpyrifos (10+500) and (20+1000) g $a.i.ha^{-1}$ left residues of 0.3032, 0.4467, 0.3838, 0.5689, 0.4767 and 0.6789 ppm in cotton seed samples at harvest. The present findings confirm the results of Blossom and Singh (2004); and Battu *et al.* (2003) who reported residues of conventional insecticides in seed. In contrast, Mukherjee and Gopal (2001) clearly showed that imidacloprid residues did not persist till harvest in cotton seed when used as seed dresser.

Analogously, Gupta *et al*. (2005) could not detected acetamiprid residues in gram seed and fodder at harvest.

The data on harvest time residues of acetamiprid in cotton oil evidenced that application of the newer insecticidal combinations like acetamiprid + cypermethrin (20+100) g, acetamiprid + quinalphos (20+1000) g and acetamiprid + chlorpyrifos (20+1000) g a.i.ha^{-1} left terminal residues of 0.1169, 0.1109 and 0.1234 ppm. Whereas, the residue levels of acetamiprid were below MRL (0.1 mg kg^{-1}) in the treatments acetamiprid + cypermethrin (10+50) g a.i.ha^{-1} (0.0923 ppm), acetamiprid + quinalphos (10+500) g a.i.ha^{-1} (0.0868 ppm) and acetamiprid + chlorpyrifos (10+500) g a.i.ha^{-1} (0.0722 ppm). Residues may get dried on the surface, adsorbed to waxy material in the outer portion of the fruit or vegetable, or translocated into the inner tissues of the plant with reduction in the removal of the active principle (Ripley and Edgington, 1983)

The results on harvest time residues of acetamiprid in soil revealed that the application of newer insecticidal combinations like acetamiprid + cypermethrin (10+50) and (20+100), acetamiprid + quinalphos (10+500) and (20+1000) g and acetamiprid + chlorpyrifos (10+500) and (20+1000) g a.i.ha^{-1} left terminal residues of 0.0385, 0.0562, 0.0489, 0.0595, 0.0469 and 0.0595 ppm. Considering the MRL of 0.1 ppm for acetamiprid in cotton, the residues were well below the tolerance limit. Similar tread of results were observed by Junxue We *et al*. (2012) who detected terminal residue of acetamiprid below MRL in soil. This might be because, neonicotinoid compounds were unstable to light, especially the UV light. Effect of light on dissipation was more pronounced in case of acetamiprid residues in soil (Gupta *et al*., 2008). Pesticide dissipation depends on physical and chemical factors, including environmental conditions, mode of application, plant species and growth rate, dosage, interval between applications and time of harvest (Khay and Abd El-Aty, 2008).

Conclusion

Thus, the overall results concluded that these newer insecticidal combinations (acetamiprid + cypermethrin, acetamiprid + chlorpyrifos and acetamiprid + quinalphos) can be used as effective tools in management of cotton insect-pests with a word of caution that they carry residues much above MRL. As such this schedule needs to be recasted using lower concentrations for harvest time residues.

Table 1: Residues of acetamiprid in cotton lint, seed, oil and soil

Sr. No.	Treatments	Dose g a.i.ha^{-1}	Acetamiprid residues (mg kg^{-1})			
			Lint	Seed	Oil	Soil
1	Acetamiprid 0.4% + Cypermethrin 2% EC	10+50	1.6814	0.3032	0.0923	0.0385
2	Acetamiprid 0.4% + Cypermethrin 2% EC	20+100	1.7677	0.4467	0.1169	0.0562
3	Acetamiprid 0.4% + Quinalphos 20 % EC	10+500	1.9060	0.3838	0.0868	0.0489
4	Acetamiprid 0.4% + Quinalphos 20 % EC	20+1000	2.5446	0.5689	0.1109	0.0595
5	Acetamiprid 0.4% + Chlorpyrifos 20 % EC	10+500	2.1000	0.4767	0.0722	0.0469
6	Acetamiprid 0.4% + Chlorpyrifos 20 % EC	20+1000	2.3136	0.6789	0.1234	0.0595
7	Untreated control	---	ND	ND	ND	ND

MRL for Acetamiprid is 0.1 ppm

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