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Persistence of Acephate and Cypermethrin on Cotton Leaves, Cottonseed, Lint and Soil

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Abstract Following foliar applications of combination formulation (cypermethrin 5% + acephate 45% DF) at 850 and 1,700 g ha^{-1}, resulting in active application of acephate at 382.5 and 765 g a.i. ha^{-1} whereas active application of cypermethrin at 42.5 and 85 g a.i. ha^{-1}, the average initial deposits of acephate on cotton leaves were found to be 13.45 and 27.73 mg kg^{-1}, at single and double the doses of application, respectively. Residues of acephate declined below detectable level of 0.02 mg kg^{-1} after 15 days of applications at application rates with $t_{½}$ values of 1.56 and 0.68 days, respectively. Similarly, the average initial deposits of cypermethrin were found to be 22.31 and 32.45 mg kg^{-1}, respectively. Cypermethrin residues reached below its detectable level of 0.02 mg kg^{-1} after 21 days of its application at both the dosages of application. The half-life values for cypermethrin were observed to be 0.71 and 0.69 days, corresponding to single and double the dose of application, respectively. Interestingly, none of the samples of cottonseed, lint and soil showed presence of acephate or cypermethrin at the detection limit of 0.02 mg kg^{-1} at first pick of the harvest time of the crop.

Keywords Acephate · Cypermethrin · Dissipation · Half-life · Residues

Cotton (*Gossypium hirsutum* L.) is an important cash crop of the Punjab state, India. It was grown on 557,000 ha in 2005–2006. The total production was 2,395 thousand bales with average lint yield of 731 kg ha^{-1} for the Punjab state (Anonymous 2007). During the last few years, the level of production in Punjab was on the decline, which has recently been picked up due to introduction of Bt cotton. Among various factors responsible for its low yield are due to losses caused by a large number of insect pests particularly bollworms and sucking pests on cotton. Cypermethrin, synthetic pyrethroids have been recommended for control of bollworm complex of cotton whereas, acephate have been introduced for the control of grown up larvae of American bollworm of cotton (Anonymous 2007). Acephate applied at 146 or 292 g ha^{-1} gave good reduction of *A. devastans*, protected the bolls against bollworms (Gupta and Kavadia 1984). Acephate and its metabolite, methamidophos were toxic to *Heliothis* spp (Frank et al. 1984). Keeping in view the bioefficacy of acephate and cypermethrin in cotton, the present studies were undertaken to study the persistence of acephate and cypermethrin on cotton leaves, cottonseed, lint and soil.

Materials and Methods

All the solvents used were of laboratory grade. These were redistilled in all glass apparatus and suitability of the solvents and other chemicals was ensured by running reagent blanks along with actual analysis.

The technical material of cypermethrin (99.0% purity) and acephate (98.6% purity) and formulation (acephate 45% + cypermethrin 5% DF) were supplied by M/s United Phosphorus Ltd. Mumbai.

The field experiment was conducted at Entomological Research Farm, PAU, Ludhiana. Cotton (var. F 1586) was raised according to good agronomic practices in

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randomized block design (R.B.D.) and the size of each plot was 100 m^2. There were three replications for each treatment i.e. control (T_1), single dose acephate 45% + cypermethrin 5% DF at 850 g ha^{-1} (T_2) and double dose at 1,700 g ha^{-1} (T_3). The characteristics of the field soil were: sand (78.0%); silt (10.2%); clay (17.8%); organic carbon (0.30%); EC 0.30 dsm^{-1} and pH (8.0).

The ready-mix formulation containing acephate 45% + cypermethrin 5% DF, was applied on cotton crop at flowering stage at 850 and 1,700 g ha^{-1}, as high volume spray by manually operated Aspee Knapsack sprayer equipped with triple action nozzle using about 500 L of spray fluid per hectares.

Samples of cotton leaves (200 g) were collected from control and treated plots of each treatment 0, 1, 3, 5, 7, 10, 15 and 21 days after application of the insecticide whereas cotton seed, lint and soil samples were collected at 1st pick at harvest.

The leaves were cut into small pieces, macerated in a blender and representative 10 g sample was dipped in 100 mL methanol for 24 h. Filtered the contents and dried the extract over anhydrous sodium sulphate and concentrated to 5 mL under vacuum at <30°C. The extract was cleaned up using prewashed and activated 20 g silica gel (60–120 mesh) mixed with 1 g activated charcoal packed in a glass column (60 cm long × 1 cm dia) supported on cotton plug and a layer of anhydrous sodium sulphate. The column was eluted with 100 mL mixture of methanol and acetone (1:1, v/v). Concentrated the cleaned up extract under vacuum at <30°C to about 10 mL and stored in a 15 mL stoppered centrifuge tube for further estimation on gas chromatograph using Flame thermionic detector (FTD). The operating conditions of the instrument were: column temperature 160°C, injector 200°C, detector 300°C. The flow rate of inert gas air, nitrogen and hydrogen were 145, 30 and 2 mL min^{-1}, respectively. Under these conditions the retention time of acephate was found to be 3.12 min (Battu et al. 2007).

The leaves were cut into small pieces, macerated in a blender and a representative 10 g sample was dipped in 100 mL acetone for 24 h. Filtered the contents into 1 L separatory funnel and added 500 mL brine solution. Partitioned into 100 and 50 mL portion of hexane. Again partitioned the aqueous phase into 100 and 50 mL portions of dichloromethane. Combined both the hexane and dichloromethane fractions and dried over anhydrous sodium sulphate and treated with activated charcoal powder fro 2–3 h. Filtered the clear extract through Whatman filter paper no. 1 and concentrated to 5 mL under vacuum at <30°C and estimated on gas chromatograph equipped with electron capture detector (ECD). The operating conditions of the instrument were; column temperature 240°C, injection port 260°C, detector 280°C with nitrogen flow rate at 40 mL min^{-1}. Under these conditions, the retention time of cypermethrin was found to be 7.10 min.

Dipped a representative 5 g lint sample into 200 mL acetone + hexane (1:1 v/v) mixture for 24 h. Filtered the contents into 1 L separatory funnel along with rinsings of acetone. Diluted with 600 mL brine solution and partitioned into dichloromethane (2 × 75 mL) and hexane (2 × 75 mL) each time. Combined both the dichloromethane and hexane fractions, dried over anhydrous sodium sulphate and concentrated under vacuum at <30°C to about 5 mL. The residues of cypermethrin and acephate were estimated using GLC equipped with ECD/NPD.

A representative 5 g cotton seed sample was grounded with 10 g anhydrous sodium sulphate. Dipped into 150 mL mixture of methanol and chloroform (2:1, v/v) for 24 h. Filtered the contents. Concentrated to near dryness and dissolved the resulting fat into 50 mL hexane. Partitioned using equal volume of acetonitrile saturated with hexane thrice (3 × 50 mL). Collected the acetonitrile fraction into 1 L separatory funnel and added about 600 mL of brine solution. Partitioned into dichloromethane (2 × 75 mL) and hexane (2 × 75 mL). Combined both the dichloromethane and hexane fractions, dried over anhydrous sodium sulphate under vacuum at <30°C to 5 mL. Estimated residues of cypermethrin and acephate using GLC equipped with ECD/NPD.

A representative 20 g soil sample was dipped into 150 mL mixture of methanol and water (2:1, v/v) for 24 h. Filtered through Whatman filter paper no. 1 in 1 L separatory funnel along with rinsings of the solvent mixture. Diluted with 600 mL brine solution. Partitioned twice with dichloromethane (2 × 75 mL) and twice with hexane (2 × 75 mL). Combined both the dichloromethane and hexane fractions and dried over anhydrous sodium sulphate. Concentrated to about 5 mL under vacuum at <30°C. The residues of cypermethrin and acephate were estimated using GLC equipped with ECD/NPD.

Cotton leaves, cotton seed, lint and soil samples were spiked with acephate and cypermethrin at different levels and analyzed as per the methodology described above. Percent recovery of acephate and cypermethrin in cotton leaves, cotton seed, lint and soil was found to be consistent and more than 80% (Table 1).

Results and Discussion

Following foliar applications of combination formulation (cypermethrin 5% + acephate 45% DF) at 850 and 1,700 g ha^{-1}, resulting in active application of acephate at 382.5 and 765 g a.i. ha^{-1}, the average initial deposits of acephate on cotton leaves were found to be 13.45 and

Table 1 Percent recovery of acephate and cypermethrin from cotton leaves, cotton seed, lint and soil samples

Substrate	Insecticide (s)	Level of fortification (mg kg^{-1})	Percent recovery Mean ± SD[a]
Cotton leaves	Acephate	0.1	96.7 ± 5.4
		0.2	97.0 ± 4.0
	Cypermethrin	0.1	84.7 ± 5.0
		0.2	87.6 ± 2.5
Cotton seed	Acephate	0.1	81.0 ± 2.3
		0.2	83.7 ± 3.6
	Cypermethrin	0.1	92.3 ± 4.6
		0.2	92.2 ± 3.8
Lint	Acephate	0.1	87.3 ± 2.6
		0.2	84.7 ± 2.2
	Cypermethrin	0.1	98.0 ± 2.0
		0.2	98.5 ± 2.5
Soil	Acephate	0.1	100.0 ± 2.3
		0.2	94.9 ± 3.6
	Cypermethrin	0.1	90.1 ± 2.3
		0.2	98.7 ± 1.3

[a] Mean ± S.D of three replicate determinations

27.73 mg kg^{-1}, respectively, at lower and higher dosages. One day after application, the residues of acephate dissipated to almost 46% and 65% in single and double dose applications, respectively. Thereafter, the residues of acephate declined slowly and after one week, percent dissipation was observed to be about 86% at single and 89% at double dose. Corresponding to single and double doses applied, the half-life values ($t_{½}$) were found to be 1.56 and 0.68 days, respectively (Table 2). When acephate was applied at 382.5 and 765 g a.i. ha^{-1}, the residues of acephate in cotton seed, lint and soil were observed to be below its limit of detection of 0.02 mg kg^{-1}, at both single and double dosages (Table 4).

Following foliar application of Lancer Gold at 1,000 and 2,000 g ha^{-1}, the effective rate of acephate was 500 and 1,000 g a.i. ha^{-1} with an average initial deposit of 14.2 and 31.4 mg kg^{-1}, respectively. Thereafter, the residues of acephate declined slowly and after one week, dissipation was observed to be about 89% at single and 93% at double dose (Battu et al. 2007).

Acephate is rapidly absorbed into the leaf tissue of cotton plants when applied foliarly, with nearly 40% of the applied acephate present in the internal extract and 25%

Table 2 Residues of acephate (mg kg^{-1}) on cotton leaves

Days after treatment[a]	382.5 g a.i. ha^{-1}			765 g a.i. ha^{-1}		
	Replication(s)	Mean ± SD	Dissipation (%)	Replication(s)	Mean ± SD	Dissipation (%)
0	13.88	13.45 ± 0.53		29.57	27.73 ± 2.93	
	14.87			28.25		
	11.60			25.37		
1	7.02	7.24 ± 0.49	46.17	8.58	9.87 ± 1.61	64.47
	6.90			10.83		
	7.80			10.21		
3	3.48	3.08 ± 0.36	73.10	3.89	4.60 ± 0.72	83.41
	2.76			4.56		
	3.02			5.34		
5	2.61	2.53 ± 0.24	81.19	3.29	3.63 ± 0.37	86.91
	2.73			3.58		
	2.26			4.03		
7	1.95	1.91 ± 0.16	85.80	3.28	3.03 ± 0.27	89.07
	2.05			2.74		
	1.73			3.06		
10	0.32	0.31 ± 0.01	97.70	0.80	0.80 ± 0.21	97.11
	0.30			0.83		
	0.30			0.77		
15	BDL	BDL	100.0	BDL	BDL	100.0
	BDL			BDL		
	BDL			BDL		

BDL = Below detectable level of 0.02 mg kg^{-1}

[a] Cotton leaf samples from untreated control plots did not show the presence of acephate at 0.02 mg kg^{-1}

Table 3 Residues of cypermethrin (mg kg^{-1}) on cotton leaves

Days after treatment[a]	42.5 g a.i. ha^{-1}			85 g a.i. ha^{-1}		
	Replication	Mean ± SD	Dissipation (%)	Replication	Mean ± SD	Dissipation (%)
0	21.46	22.31		34.96	32.45 ± 2.48	
	23.46			30.00		
	22.00			32.40		
1	10.78	10.62 ± 0.20	52.40	14.52	16.06 ± 2.15	50.51
	10.70			18.52		
	10.39			15.09		
3	2.05	3.02 ± 0.21	86.46	8.81	8.23 ± 0.64	74.64
	3.25			7.54		
	2.96			8.34		
5	2.23	2.23 ± 0.03	90.0	5.68	5.02 ± 0.77	84.53
	2.19			5.20		
	2.26			4.17		
7	1.59	1.56 ± 0.34	93.01	3.98	3.14 ± 0.75	90.32
	1.21			2.92		
	1.88			2.52		
10	0.25	0.24 ± 0.03	98.96	0.52	0.61 ± 0.08	98.12
	0.25			0.63		
	0.21			0.67		
15	0.20	0.21 ± 0.03	99.06	0.39	0.34 ± 0.04	98.95
	0.24			0.34		
	0.19			0.30		
21	BDL	BDL	100.0	BDL	BDL	100.0
	BDL			BDL		
	BDL			BDL		

BDL = Below detectable level of 0.02 mg kg^{-1}

[a] Cotton leaf samples from untreated control plots did not show the presence of cypermethrin at 0.02 mg kg^{-1}

remaining on the leaf surface 24 h after application which was very much clear from the Table 2, that one day after application, the residues of acephate dissipated to almost 50% and 60% in single and double dose applications. The unrecovered acephate probably was translocated from leaves or bound in unextractable form inleaf tissue. The low vapour pressure of acephate probably indicates that loss due to volatilisation would be negligible which also depicted from results. Little to no degradation of acephate to methamidophos occurred on the leaf surface (Bouchard and Lavy 1982).

Behavior assessment and ground water pollution potential models were used to assess the potential of acephate and its metabolite methamidophos to contaminate groundwater. It was observed that dissipation rate of acephate increased with increasing soil temperature and moisture content in both silty and silty clay loam soils and it followed first order kinetics (Jui Hung et al. 2000).

Persistence of acephate was studied in 45 L PVC tubes filled with water and sandy loam soil exposed to atmosphere during Oct. 1988 after acephate application rate of 250 and 500 g a.i. ha^{-1}. It was observed that low levels of acephate persisted up to 30 days with no leaching of acephate residues (Agnihotri et al. 1989).

Acephate was applied at 300 g a.i. ha^{-1} to cotton against bollworms at flowering stage and residues were determined at harvest time in lint and cottonseed. The residues of acephate were found to be below its detectable limit (Agnihotri et al. 1986). Acephate applied at 146 or 292 g ha^{-1} gave good reduction of *A. devastans*, protected the bolls against bollworms and left only negligible residues and considered safe. Application of acephate up to 1,160 g ha^{-1} had no phytotoxic effects (Gupta and Kavadia 1984).

A single foliar application of ^{14}C labelled acephate was absorbed rapidly by cotton leaves (>50%) and unabsorbed residues were essentially depleted in 48 h (Bull 1979). The absorbed acephate was found to get metabolised to methamidophos to the extent of about 9%.

Distribution and fate of ^{14}C-acephate was studied in tomato plants (3−8 weeks old) planted in soil inside a PVC tube and insecticide was applied to the soil. The ^{14}C-acephate residues and their metabolites in soil and plant leaves were determined. The results revealed residues in soil to

Table 4 Residues of acephate and cypermethrin (mg kg^{-1}) on cotton seed, lint and soil

Substrate	Acephate		Cypermethrin	
	382.5 g a.i. ha^{-1} Mean ± SD[a]	765 g a.i. ha^{-1} Mean ± SD	42.5 g a.i. ha^{-1} Mean ± SD	85 g a.i. ha^{-1} Mean ± SD
Cotton seed	BDL	BDL	BDL	BDL
Lint	BDL	BDL	BDL	BDL
Soil	BDL	BDL	BDL	BDL

BDL = Below detectable level of 0.02 mg kg^{-1}

[a] Mean ± S.D. of three replicate determinations

the extent of about 59%, 24%, 17% and 3% on day 1, 4, 7 and 14, respectively (Tungguldihardjo and Anwar 1997). TLC analysis showed that acephate and methamidophos, respectively, in leaves were 3.9% and 0.8% on day 1; 4.5% and 1.3% on day 4; 7.1% and 7.5% on day 7 and 5.5% and 10.3% on day 14.

Following foliar applications of combination formulation (cypermethrin 5% + acephate 45% DF) at 850 and 1,700 g a.i. ha^{-1}, containing cypermethrin at 42.5 and 85 g a.i. ha^{-1}, the average initial deposits of cypermethrin were found to be 22.31 and 32.45 mg kg^{-1}, respectively. Cypermethrin residues dissipated by 50%–52% in 1 day. Half-life values of cypermethrin on cotton leaves were found to be 0.71 and 0.69 days at single and double dose application rate, respectively. Residues of cypermethrin reached below detectable level of 0.02 mg kg^{-1} in 21 days, in both the treatments, respectively (Table 3). When cypermethrin was applied at 42.5 and 85 g a.i. ha^{-1}, the residues of cypermethrin in cottonseed, lint and soil were observed to be below its limit of detection of 0.02 mg kg^{-1}, at both single and double dosages (Table 4).

Similarly, residues of cypermethrin on cottonseed and lint was below detectable limit at harvest when six applications of cypermethrin at 50 and 100 g a.i. ha^{-1} was given starting at 50% flowering stage followed by 10 days intervals for subsequent applications (Singh et al. 2001).

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