Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

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Dissipation study and persistence of acephate insecticide in green chilli fruits

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Abstract
The dissipation patterns of acephate in green chilli fruits were studied after application of acephate at fruiting stage at recommended doses (RD) and double the recommended doses (DRD). Acephate was applied at the rate of 337.5 g *a.i*/ha and 675 g *a.i*/ha on chilli fruits and the fruit samples were collected at the 0, 1st, 3rd, 5th, 7th, 10th, 15th, 21st, 25th, and 30th day after spray. The initial deposits of acephate in chilli fruits from the two treatments were leads to 5.128 and 7.249 mg/kg with half life of 4.02 and 5.84 days at recommended and double the recommended dose, respectively. Based on the persistence studied waiting period of 20.12 and 34.08 days is suggested for acephate on chilli from consumer safety point of view.

Keywords: Chilli, dissipation pattern, acephate, recommended dose

1. Introduction
Chilli [*Capsicum annuum* L.] is one of the major spice crop in India. It is a member of Solanaceae family which represents a diverse plant group. The name is derived from Latin word Capsa that means hallow pod. Indian chillies have gained global demand due to high color value and low pungency (Mathur *et al.*, 2000) [1]. Chillies contain potent pungent substance capsaicin, with almost 80 per cent of the capsaicin being in its seeds and membranes. Besides, chillies are a good source of vitamins, minerals and beta-carotene. The capsaicin, an antioxidant is having medicinal properties.

The world production of chilli crop to around 7 million tonnes, which is cultivated on 1.5 million hectares of land. In India, Chilli was grown in an area of 136 (in ΄000 ha) and production of 3634 (in ΄000 MT) and the productivity, 11.5 (in ΄000 MT/ha) in 2016-17 (Anon., 2016-17)[2]. India is the world leader in chilli production followed by China and Pakistan. This shows that the bulk share of chilli production is held by the Asian countries, though it is produced throughout the world. A large demand for chilli comes from several chilli consuming countries such as India, China, Mexico, Thailand, USA, UK, Germany and Sweden. Indian share in global production ranges between 50 to 60 per cent. However, India is the only one source for hot chillies (Geetha and Selvarani, 2017) [3].

At present, Karnataka ranks second in area (132.20 thousand ha) and production of chilli (148.00 thousand tonnes) after Andhra Pradesh and is being extensively cultivated in Dharwad, Haveri, Belgaum, Gadag, Bellary, Gulbarga, Chikkamagalur and Raichur (Anon., 2009) [4]. Insecticides are repeatedly applied during the entire period of growth and sometimes even at the fruiting stage. Indiscriminate utilization of pesticides leads to accretion of residues in consumable brinjal fruits. The continuous ingestion of these residues, though in minute quantities, can result in their accumulation in the body causing adverse effects on human well-being. Thus, the knowledge of nature and concentrations of chemicals, which remain in food substrates at the time of their consumption, is very important from the point of view of public health.

In Karnataka, thrips, mites and white flies have been identified as key sucking pests of chilli of which leaf curl caused by mite and thrips is serious (Puttarudraiah, 1959) [5]. The yield loss due to chilli mite may go up to 96.39 per cent leading sometimes to complete failure of the crop itself (Kulkarni, 1922) [6]. Chilli thrips multiply appreciably at a faster rate during dry weather periods and causes yield loss of 30 to 50 per cent in South India (Vasundarajan, 1994) [7] and sometime more than 90 per cent yield reduction (Krishnakumar, 1995) [8]. Chilli leaf curl complex is one of the most destructive syndromes affecting chilli in India and is considered to be caused by thrips and mites. The crop is also vulnerable to fruit borer, *Helicoverpa armigera*. Shivaramu and Kulkarni (2001) [9] reported 20 to 30 per cent damage due to fruit

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borer, *H. armigera.* The persistence of acephate has been studied on various commodities like mango fruit (Mohapatra *et al.* 2011) [10], grapes (Reddy and Rao 2005) [11], olives and olive oil (Cabras *et al.,* 2000) [12], rice (Kong *et al.,* 2012) [13], cotton leaves (Battu *et al.,* 2007) [14], and brinjal (Iqbal *et al.,* 2007) [15]. An extensive study was done on dissipation of acephate in/on chilli revealed the persistence of acephate in chilli fruits. In order to recommend a safe waiting period for acephate, it is essential to determine the dissipation behavior of acephate.

2. Materials and Methods
2.1 Field Experimentation
Field experiment was carried out to evaluate the dissipation pattern of acephate 75 % SP on chilli during *kharif* 2017 at Agricultural Entomology experimental block, Main Agricultural Research Station, UAS, Raichur in a Randomised Block Design (RBD). A hybrid HPH 5531 was used for experiment wherein, 3 treatments of the recommended dose, double the recommended dose and untreated plots with 8 replications were maintained. The Application of acephate 75 % SP at 1 g/L (337.5 g *a.i*/ha) as a recommended dose and 2 g/L (675 g *a.i*/ha) as double the recommended dose was sprayed during fruit formation stage including untreated control plot. The green chilli fruit samples were drawn on 0 (2hr after spraying), 1, 3, 5, 7, 10, 15, 21, 25 and 30 days after spray. The collected samples were extracted and analysed through LC-MS/MS and calculated the residue of acephate at different days.

2.2 Solvents and Reagents
Solvents and reagents used for extraction, ethyl acetate (≥ 99.8 GCMS grade) were obtained from Merck and Mumbai, India, methanol (≥ 99.9 LCMS grade) from JT Baker®, USA, Sodium sulphate, anhydrous magnesium sulphate from Himedia®, Mumbai, India and Primary secondary amine (PSA) from Agilent®, USA. Water was obtained from Make-Milli-Q®.

2.3 Instrumentation
The pesticide residues were analyzed by Liquid chromatography mass spectrometry (LC-MS/MS).

LC-MS/MS parameters

Model	SHIMADZU LC-MS 8040®
Column	Shimpack XR, ODS C18, 2mm id x 150mm
Flow rate	0.4 mL/min.
Heat block	400 ºC
N B gas flow	3 L/min.
Source	ESI +ve probe
Dissolution temperature	250 ºC
Drying gas flow (N$_2$)	15 L/min.
Injection volume	2 µL

2.4 Extraction and clean up procedure
The collected samples were extracted according to AOAC official method of analysis 2007.01, 20th edition of 2016 and chapter 10. About 10 g of grounded sample was weighed using analytical balance and transferred into 50 mL centrifuge tube and 5 mL of distilled water was added and further allowed to stand for 30 min. Later, 10 mL of ethyl acetate and 10 g sodium sulphate was added and vertex the mixture for one min. The sample mixture was then homogenized at 10000

to 13000 rpm for 3 min. The content was subjected to centrifugation at 5000 rpm for 5 min. at 10 °C.
After centrifugation, 7 mL of supernatant was transferred into 15 mL centrifuge tube containing 175 mg primary secondary amine (PSA) and 1.05 g magnesium sulphate and then vortex the mixture for 1 min. Centrifuge the supernatant with added reagents at 12000 rpm for 5 min. Then 3 mL each extractant was transferred into a test tube containing 300 µL of 10 % DEG (Diethylene glycol) in methanol and evaporated the content using nitrogen flash evaporator at 35 °C for 30 min. or near to dryness. Later reconstituted the residue with 1.5 mL of LC-MS/MS compatible solvent methanol. Sonicated the mixture in an ultrasonicator to dissolve residues completely. Then filtered the content using 0.22 µ PTFE nylon filter in LC vials.

2.5 Fortification and recovery studies
The recoveries of acephate in the untreated chilli matrix were carried out by spiking at LOQ level (0.01 mg/kg), 5 times of LOQ (0.05 mg/kg) and 10 times of LOQ (0.1 mg/kg) six with replications each and injected three times each. Fortification levels and the samples were extracted and cleaned up as per AOAC official method to validate the suitability of the method. Calculated the obtained concentration from the spiked sample and then calculated the per cent recovery by using formula.

$$\text{Residues (mg/kg)} = \frac{\text{Sample peak area} \times \text{Conc. of std. injected} \times \text{Std injected (µL)} \times \text{final volume of the sample (mL)}}{\text{Std peak area} \times \text{weight of sample analyzed} \times \text{sample injected (µL)}}$$

$$\text{Wt of the sample analyzed (g)} = \frac{\text{Sample weight (g)} \times \text{Aliquot taken (mL)}}{\text{Volume of extract (mL)}}$$

$$\text{Per cent recovery} = \frac{\text{Residue quantified in fortified sample}}{\text{Fortified level}} \times 100$$

$$\text{Dissipation percentage} = \frac{\text{Initial deposit (mg/kg) - Residues at given time (mg/kg)}}{\text{Initial deposit (mg/kg)}} \times 100$$

3. Results
Average recoveries from six different replications for acephate in chilli fortified at 0.01, 0.05 and 0.1 mg/kg were 89.30 %, 93.32 % and 84.08 %, respectively. The overall recovery was calculated to be 88.90 % (Table 1). The acceptance criteria for recovery were 70 to 120 %. However, the acephate recovery obtained in the present study was accepted as because it falls within the acceptable range as per the method validation guidelines according to SANTAE-2017.
The average initial deposits of acephate at recommended dose were 5.128, 3.837, 2.329, 0.969, 0.508, 0.467, 0.170, 0.131, 0.031 and 0.027 mg/kg at 0 (2 hours after spray), 1, 3, 5, 7, 10, 15, 21, 25 and 30 days after spray, respectively (Table 2). The residue gradually dissipated to 0.170 mg/kg on fifteenth day accounting to loss of 96.68 per cent however residue persisted till 30th day sample containing 0.027 mg/kg accounting loss of 99.47 per cent. The residue half-life values were 4.02 days at recommended dose. The degradation rate constant (k) was 0.0747 day^{-1} and the value of correlation coefficient was 0.9884 for recommended dosage with safe

waiting period (SWP) of 20.12 days.

The average initial deposits of acephate at double the recommended dose were 7.249, 6.393, 4.560, 2.202, 1.787, 1.365, 0.953, 0.913, 0.193 and 0.176 mg/kg at 0 (2 hours after spray), 1, 3, 5, 7, 10, 15, 21, 25 and 30 days after spray, respectively (Table 1). The residue gradually dissipated to 0.953 mg/kg at fifteenth day accounting to loss of 86.85 per cent, however, residue persisted till 30th day sample containing 0.176 mg/kg accounting loss of 97.57 per cent. The residue half-life value was 5.84 days at double the recommended dose. The degradation rate constant (k) was 0.0514 day^{-1} and the value of correlation coefficient was 0.9599 for double the recommended dosage with safe waiting period was 34.08 days.

4. Discussion
4.1 Initial deposits for acephate
In our study, initial deposits of a pesticide depends upon a number of factors like concentration, formulation, weather conditions, substrate characteristics, type of sprayer used, distance between the nozzle and plant surface and properties of carrier etc. Besides these factors, Ebling (1963) [18] also reported that factors like plant type (erect or prostrate), shape of plant parts (broad, narrow or linear) and growth of plant parts (slow or fast) influence the persistence of residues on plant surface. Keeping most of these factors constant the results pertaining to initial deposits have been discussed. The initial deposits of acephate on chilli fruits were found to be 5.128 μg/g at single dose and were 1.4 times lower than that obtained from double dose *i.e.* 7.249 μg/g. The results of present findings showed a close similarity to those obtained by Battu *et al.* (2007) [19] who reported an increase of about 2 fold deposits for double dose over single dose on cotton crop. However, Mohapatra *et al.* (2011) [20] observed 1.6 fold increases in deposits due to higher dose over lower dose. Kumar *et al.* (2000) [21] studied the dissipation of acephate (1500 g *a.i.*/ha) in chilli fruits revealed an initial deposits of 0.19 mg/kg, dissipated to an extent of 0.11 mg/kg and below detectable level by 15 days. Singh (2013)[22] studied the dissipation of acephate @ 560 and 1120 g *a.i.*/ha on capsicum revealed initial deposits of 0.540 and 1.036 mg/kg at single and double doses, respectively.

4.2 Dissipation pattern
The dissipation of acephate depend upon crop factors like stage of crop, rind of fruit, internal material, weather factors like moisture, temperature, rain and surface characteristics to hold deposits. The residue data of acephate on chilli fruits indicated that initially there was a rapid loss of insecticide residues followed by slower rate of dissipation. The residue gradually dissipated in fifteenth day samples contained 0.170 and 0.953 mg/kg residues accounting to loss of 96.68 and 86.85 per cent for single dose and double dose, respectively as shown in Fig. 1 and 3 and chromatogram is given in Fig. 2 and 4. The results are in accordance with findings of Kumar *et al.* (2000) [21] studied the dissipation kinetics of acephate in chilli showed that acephate was dissipated below detectable level in the range of 7 to 10 days. Likewise the results were also inline with Chuanjiang *et al.* (2010) [23], who reported acephate persistence up to 7 days and initial deposits became half in 1.36 and 1.07 days from tomatoes and pakchoi, respectively. Battu *et al.* (2007) [19] studied residues of acephate in cotton, declined below detectable level of 0.02 mg/g after 15 and 21 days of applications at single and double

doses of 500 and 1000 g *a.i.*/ha. Chai *et al.* (2009) [24] applied acephate @ 1 g/L, four times at weekly interval on green mustard, an exponential dissipation was observed with 30 to 45 per cent decrease in its concentration from day 0 to 2. At day 4 residues were between 3.50 and 4.47 mg/kg.

4.3 Residue half life values (RL50)
The acephate deposits dissipated to half at single dose in 4.02 days as compared to 5.84 days at double dose from chilli fruits, indicating imminence in dissipation behavior of the insecticide at both doses. The results obtained in the present study showed that the acephate dissipated to half in about 4.02 days and 5.84 days at single dose and double dose respectively and but took more than 30 days to reach below the determination level due to ecological factors *viz.,* low temperature, relative humidity, physical and chemical properties of acephate, but the obtained results were not in similarity with other reports given by many other workers with finding of Trevizan *et al.* (2005) [25] and Kong *et al.* (2012) [26], who showed that acephate dissipated to half in about 2 days and reached below determination level on 7 and 10 days at single and double dose, respectively. The half life of acephate has been reported as 1.9 to 2.0 days in cotton (Battu *et al.*, 2007) [19] and 1.4 days in pakchoi (Chuanjiang *et al.*, 2010) [23]. Brar (2013) [27] reported that half life value of acephate was 1.8 to 2.2 days at single and double doses, respectively. Singh (2013) [28] studied the dissipation of acephate with half life value of 1.9 and 1.8 days at single and double doses, respectively. Mayannavar *et al.* (2017) [29] reported the persistence of acephate in/on okra. The initial residues dissipated with half life of 1.83 and 1.83 days at recommended and double the recommended dose, respectively.

4.4 Waiting period
The waiting period of acephate on chilli was found to be 20.12 and 34.08 days at single and double doses, respectively. But these values were not closer to those results obtained by Reddy and Rao (2005) [30], who found 5.13 days waiting period for harvesting of acephate treated grape berries. However, Brar (2013) [27] suggested 7.9 and 9.4 days waiting period for acephate treated brinjal fruits at single and double dose, respectively. Mayannavar *et al.* (2017) [29] reported the persistence of acephate in/on okra by considering the LOQ of 0.05 mg/kg, Pre-Harvest Interval (PHI) of seven days can be suggested. Singh (2013) [22] studied the dissipation of acephate on capsicum revealed that safe waiting periods were calculated at respective dosages were 6.8 and 7.8 days. A safe pre-harvest interval of 30 days is recommended for consumption of mango fruits following treatment of acephate at the recommended dose (0.75 kg *a.i.*/ha), Mohapatra *et al.*(2011) [20].

5. Conclusion
This research study clearly shows that, the dissipation pattern showed a continuous decrease of residues from 1st day to 30th day for acephate and the residues were below detectable limit (BDL) on 30th days for both the doses. The safe waiting period of acephate is more because it's mainly depends on chemical properties, nature of the chemical and substrate, climatic conditions, type of application, plant species and dosage. It could be concluded that due to slower dissipation of acephate, this insecticide needs to be applied with caution with adequate time gap before harvest to avoid detection of its residues at harvest. So the acephate could be applied at the

standard application dose considering faster dissipation compared to the double dose. The SWP for this insecticide will be useful to the farmers to ensure safe consumption.

Table 1: Recovery study and per cent recovery of acephate at different spiked fortification level

Insecticide	Spiking level (mg/kg)	Standard area	Sample area*	Residue concentration (mg/kg)*	Recovery %*	Overall Average	SD	% RSD
Acephate	0.01	27,117	48,434.50 (39,979.3-53,472)	0.0089 (0.0074-0.0096)	89.30 (95.57-98.59)	88.90	7.4980	8.4340
	0.05	166,384	310,551.33 (294,691-323,513)	0.0467 (0.0443-0.0486)	93.32 (88.56-97.22)			
	0.1	371,891	625,385.17 (607,387-643,634)	0.0841 (0.0817-0.0865)	84.08 (81.66-86.54)			

* Figures in parentheses indicate the range values for recovery of acephate and diafenthiuron

Table 2: Acephate residues (mg/kg) in chilli at different days after treatment of recommended and double the recommended dose

Insecticide	Treatments	Residue level	Residue level (mg/kg) Days after treatment									
			0	1	3	5	7	10	15	21	25	30
Acephate	Recommended dose	Mean ± SD	5.128± 0.881	3.837± 0.600	2.329± 0.343	0.969± 0.064	0.508± 0.014	0.467± 0.023	0.170± 0.016	0.131± 0.026	0.031± 0.003	0.027± 0.006
		% Dissipation	--	25.17	54.58	81.10	90.09	90.89	96.68	97.44	99.39	99.47
	Double the recommended dose	Mean ± SD	7.249± 0.531	6.393± 0.583	4.560± 0.723	2.202± 0.194	1.787± 0.152	1.365± 0.065	0.953± 0.024	0.913± 0.010	0.193± 0.040	0.176± 0.004
		% Dissipation	--	11.80	37.09	69.62	75.34	81.16	86.85	87.40	97.33	97.57

Recommended dose: Correlation Coefficient r = 0.9884
Regression equation y = 1.5032 - 0.0747x
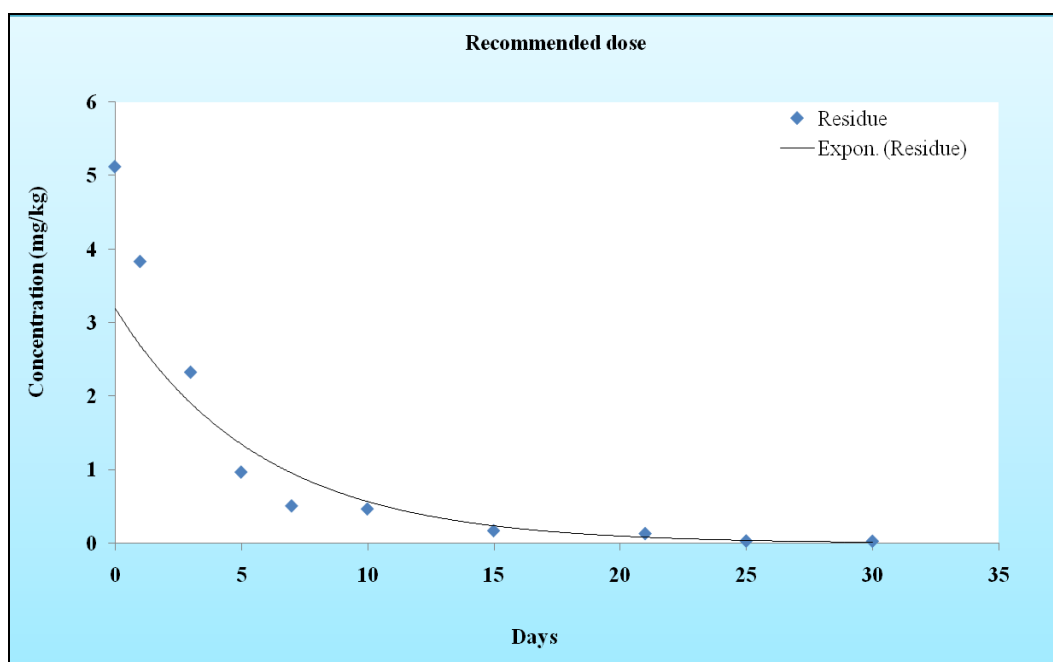$t_{1/2}$ = 4.02 d
K = -0.0747
SWP = 20.12 d
Double the recommended dose: Correlation Coefficient r = 0.9599
Regression equation y =1.7549-0.0514x
$t_{1/2}$ = 5.84 d
K = -0.0514
SWP = 34.08 d

Fig 1: Dissipation curve for acephate 75 % SP sprayed at recommended dose in chilli
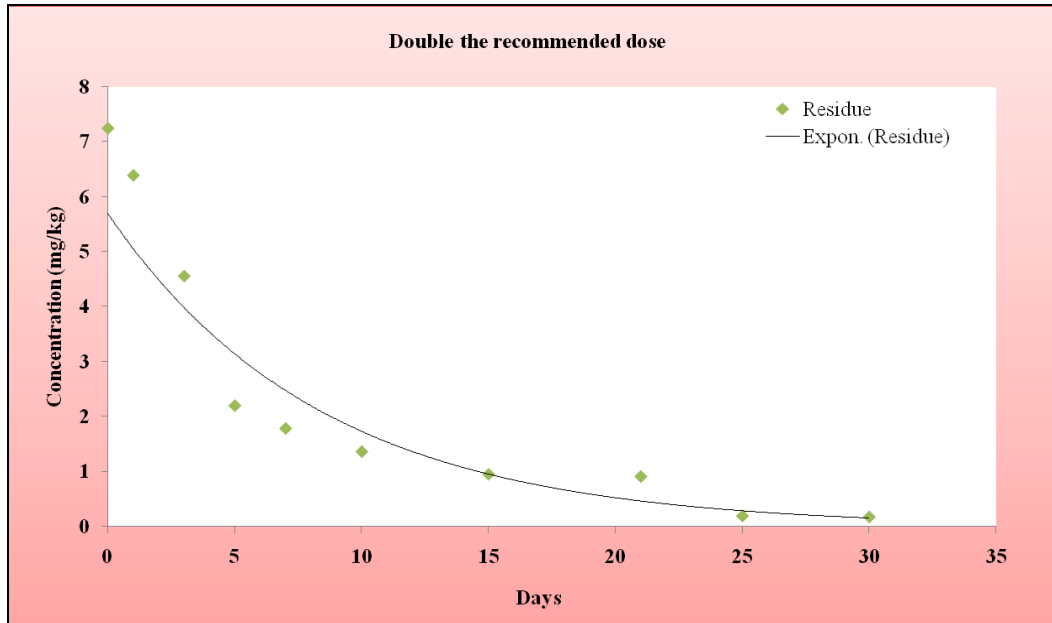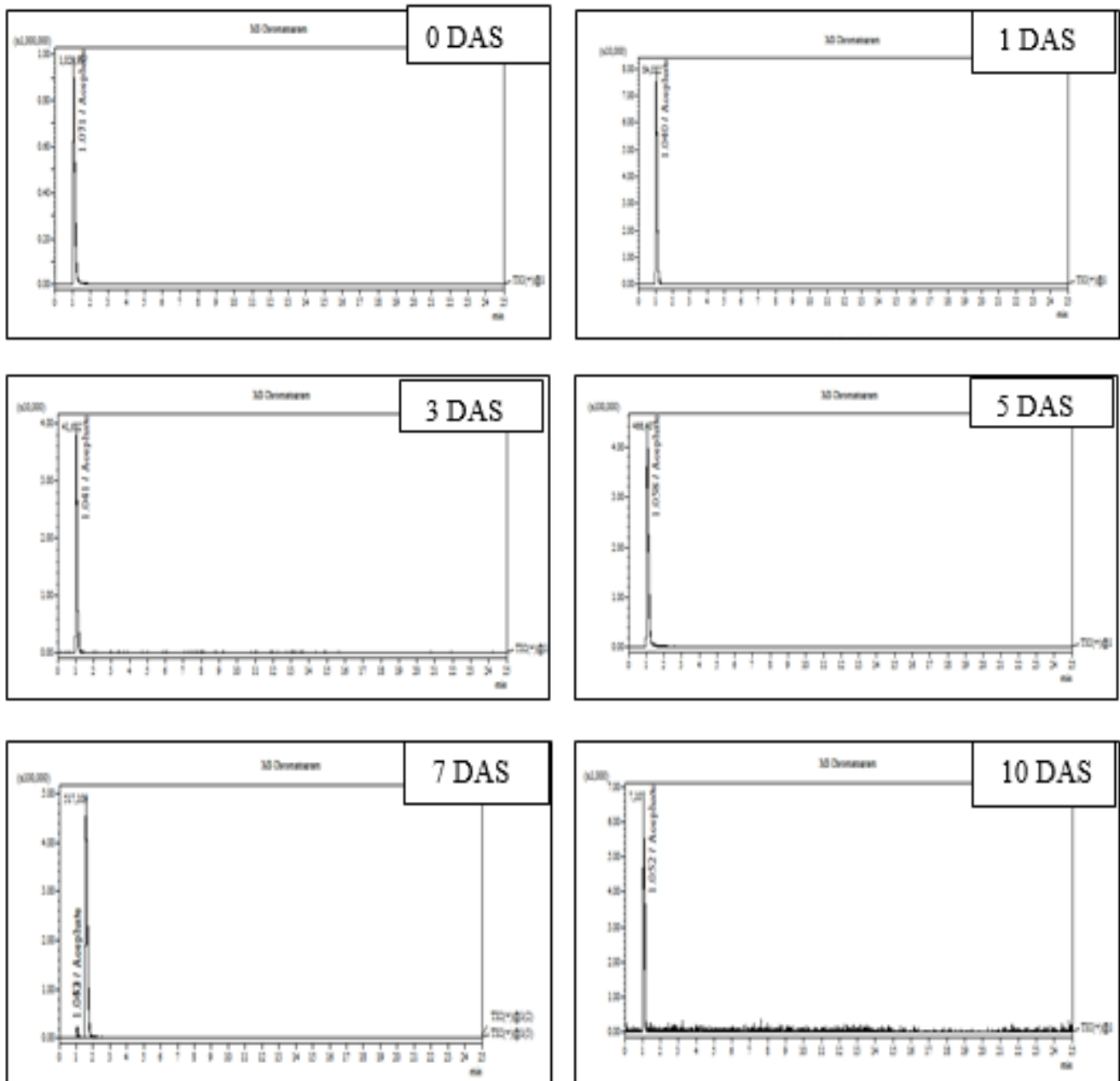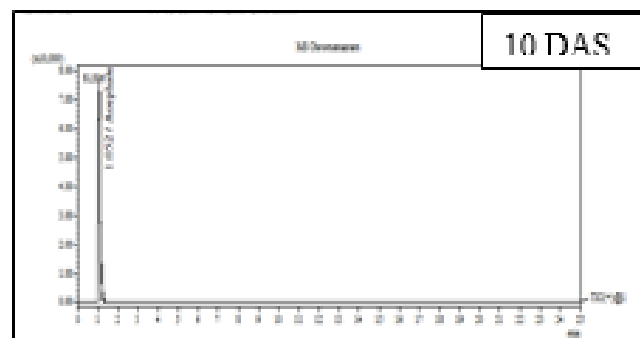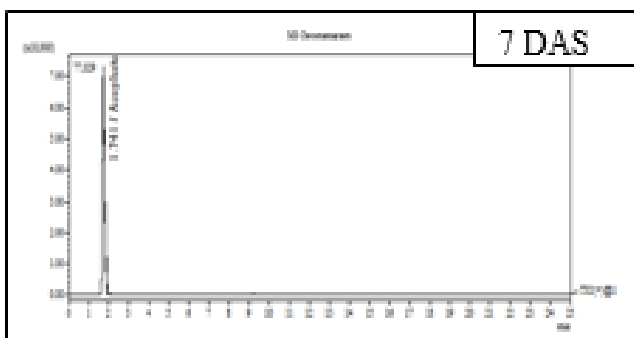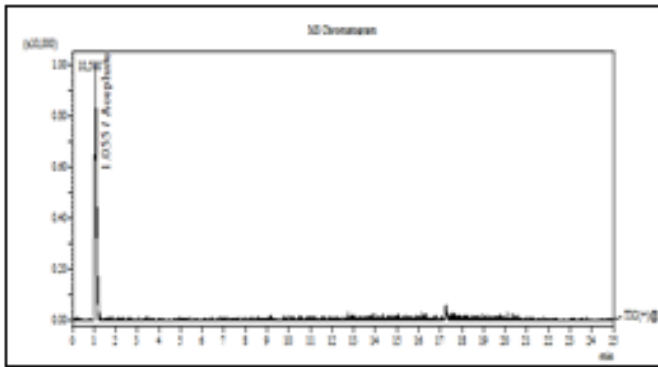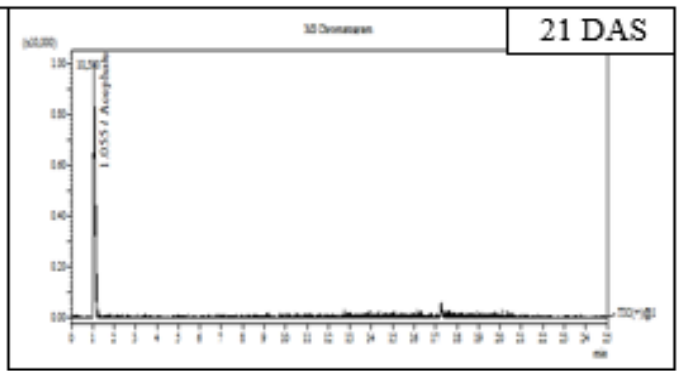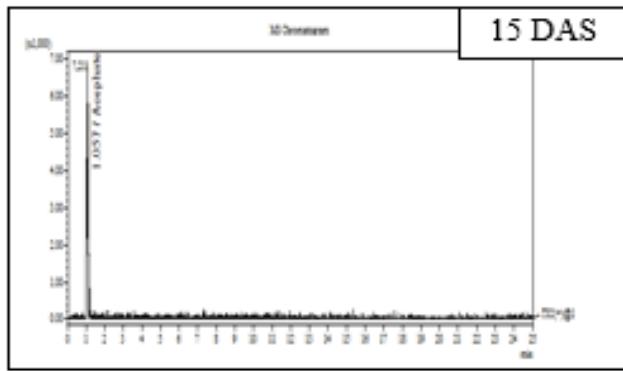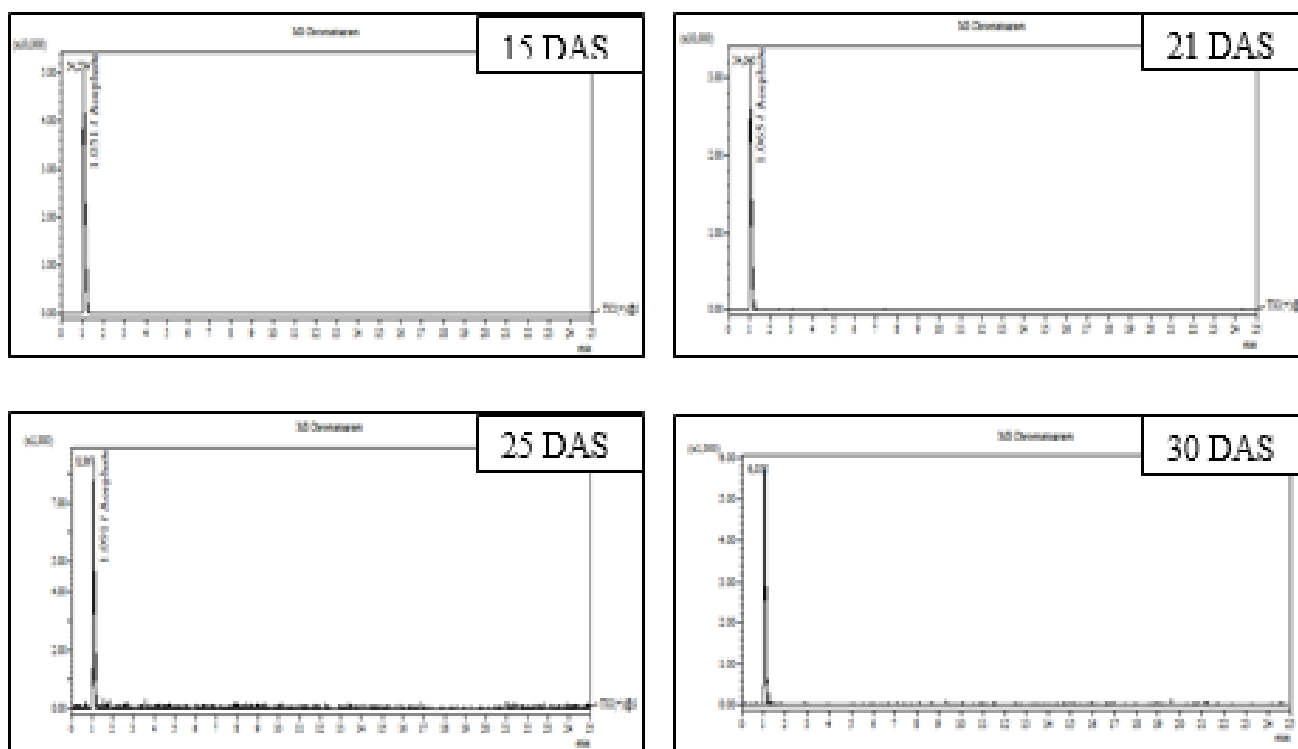
Fig 2: Dissipation curve for acephate 75 % SP sprayed at double the recommended dose in chill
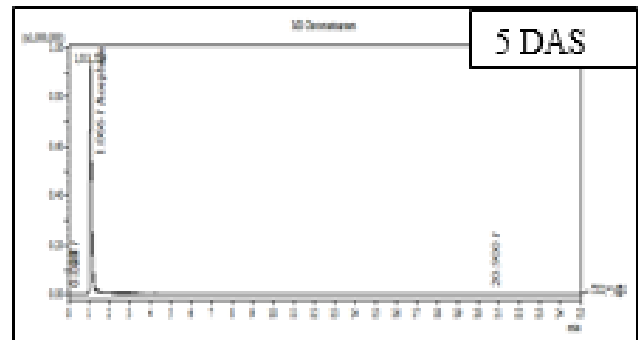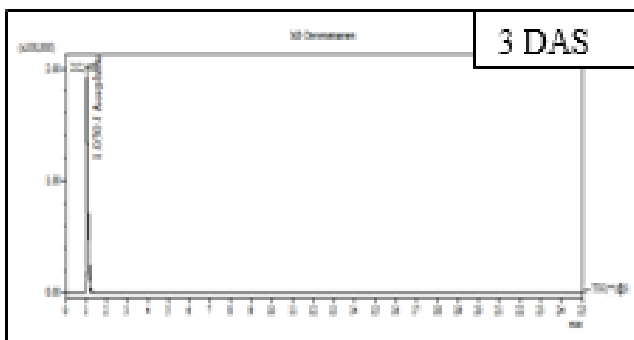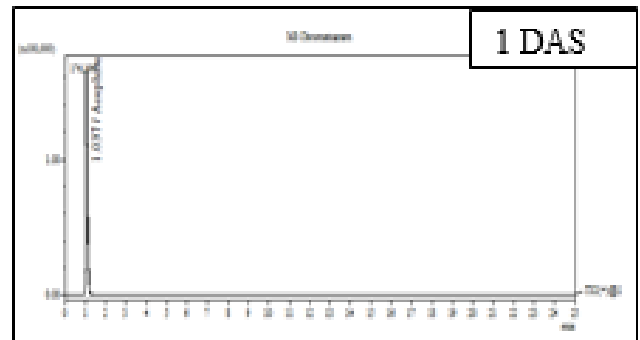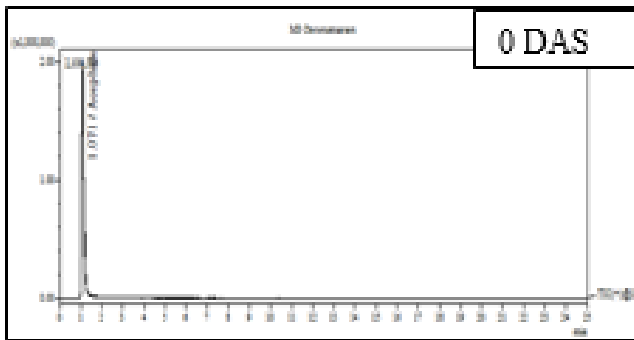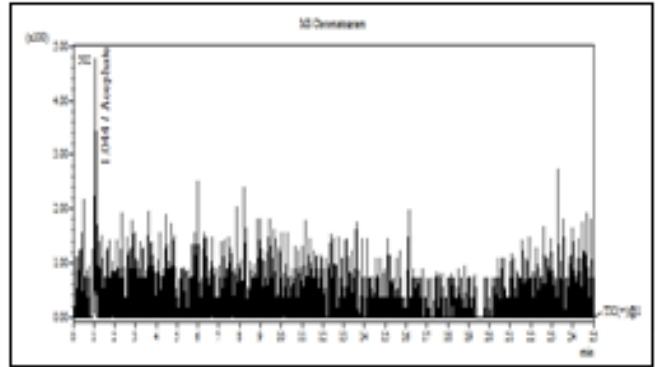
Fig 3: LC-MS/MS chromatograms for dissipation studies of acephate 75 % SP sprayed at double the recommended dose in chilli

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