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Dissipation study and persistence of acephate 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. In the state 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 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]. 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. 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).

Table: 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.

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

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

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

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

3. Results and Discussion

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.

Dissipation pattern showed a continuous decrease of residues from 1st day to 30th day for both the dosage of acephate as shown in Fig. 1 and 2 and chromatogram is given in Fig. 3 and 4. The acephate residues completely dissipated to 99.47 per cent on 30th day for recommended dose and 97.57 per cent on 30th day for double the recommended dose. The safe waiting period of 20.12 days for recommended dose and 34.08 days for double the recommended dose for acephate was suggested for safe consumption of green chilli.

4. 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.

5. Acknowledgements

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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

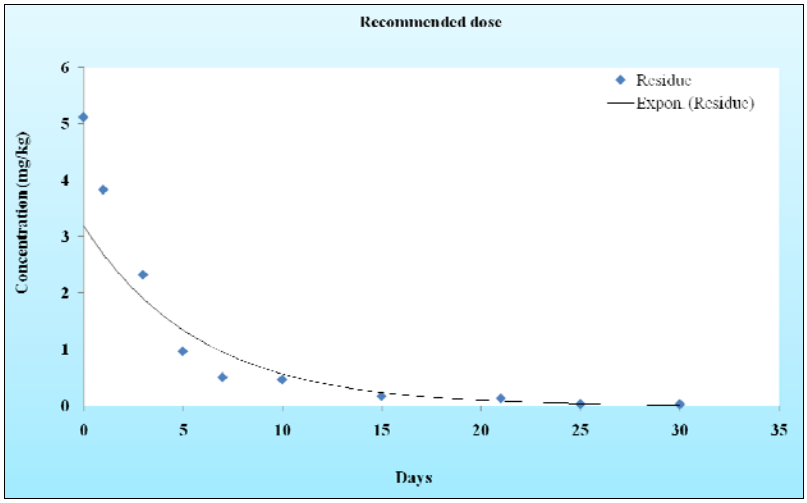


Fig 1: Dissipation curve for acephate 75 % SP sprayed at recommended dose in chilli

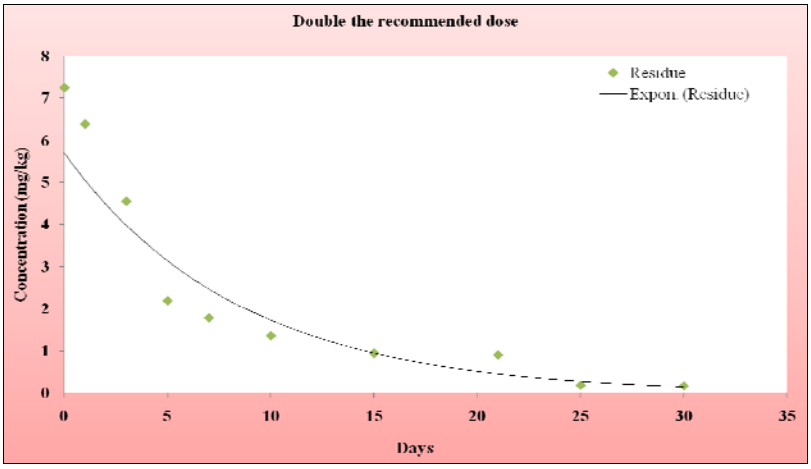
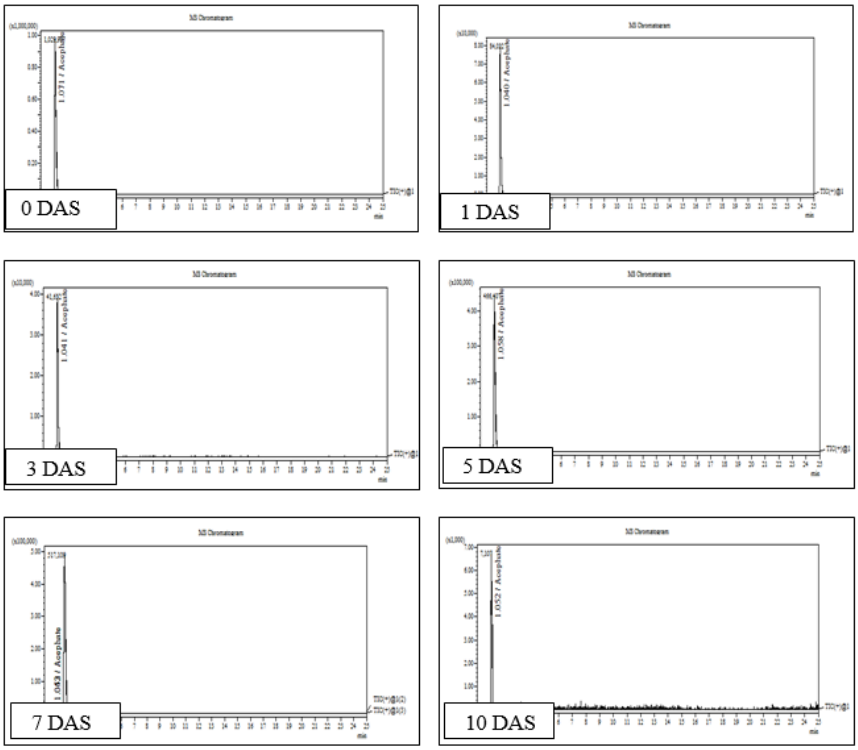


Fig 2: Dissipation curve for acephate 75 % SP sprayed at double the recommended dose in chill



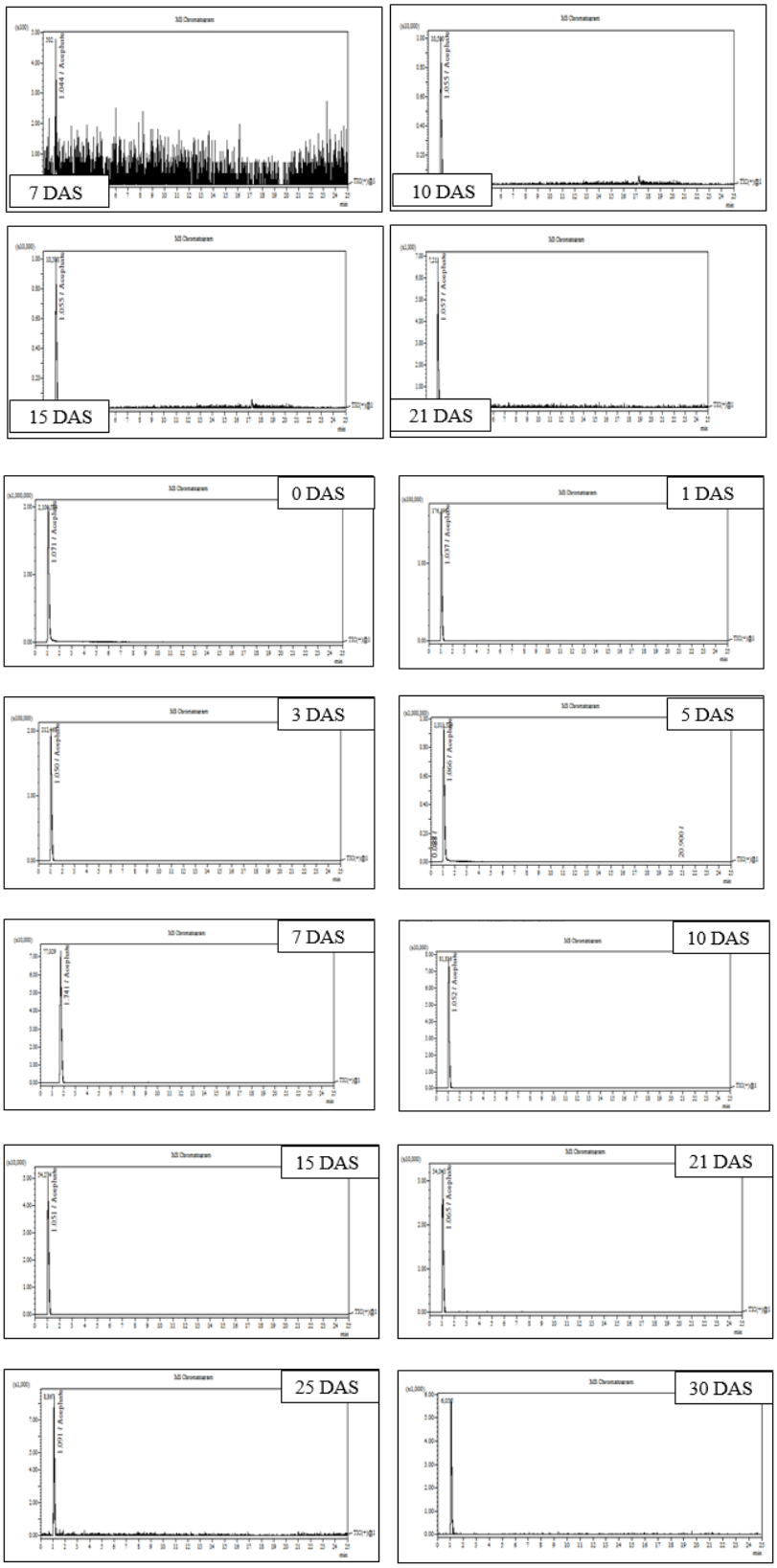


Fig 4: LC-MS/MS chromatograms for dissipation studies of acephate 75 % SP sprayed at double the recommended dose in chilli

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