

## ORIGINAL ARTICLE

# Residues of pesticides acephate and methamidophos in capsicum grown in greenhouse and open field

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**Abstract**

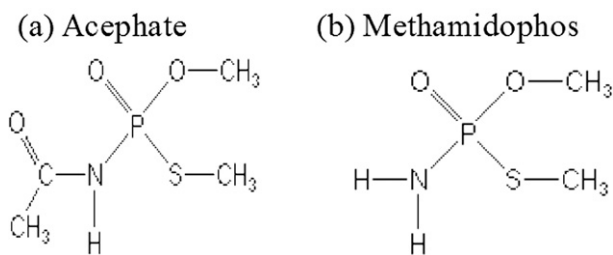
**Introduction** Capsicum grown in low-cost polyhouses (a protective shade made up of polythene used for growing high-value agricultural products) is often infested by thrips and acephate is an insecticide, which is frequently and repeatedly used for controlling this pest. **Objectives** Since a polyhouse microclimate is different from that of an open field, a study was carried out to compare the decline pattern of acephate residues in capsicum grown in polyhouse to that grown in open field. **Methods** Laboratory standardized methodologies using gas liquid chromatography was adopted to estimate residues of acephate and its metabolite, methamidophos in capsicum fruits. **Results and Conclusion** Higher initial residues (0.39 ppm) and persistence of acephate (pre-harvest interval = 16 days) and its methamidophos metabolite was detected in capsicum grown in polyhouse as compared to that grown in open field (pre-harvest interval = 12 days) in spite of higher rate of growth and consequent residue 'dilution' in polyhouse-grown capsicum.

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**Introduction**

Capsicum (sweet pepper) is a popular salad vegetable rich in vitamin A, C and minerals. Its cultivation is gaining popularity in peri-urban production systems of India because of easy access to urban markets. Capsicum yields in open-field cultivation ranges between 20 and 40 tonnes ha<sup>-1</sup>, where as in greenhouses, the yields obtained are 100–120 tonnes ha<sup>-1</sup>. In addition to much higher yield, superior quality fruits and off-season production make it an economic proposition to grow capsicum in naturally ventilated greenhouse or polyhouse (PH), which is essentially a greenhouse covered with simple transparent or translucent plastic sheet (Murthy *et al.*, 2009). In moderate climatic conditions of Bangalore, India, year round cultivation of capsicum is possible in a PH with the ambient temperature ranging from 12 °C during winter to 37 °C during summer. Capsicum is thus grown in polyhouses in more than 100 acres of land in Bangalore and nearby areas.

Prevalence of microclimate that is congenial for multiplication and spread of pests, high plant density, monocropping of susceptible genotypes and increased labour activities make PH grown plants prone to pests predominant among which are mites, thrips, whiteflies, powdery mildew and nematodes. Thrips (*Scirtothrips dorsalis* Hood) is an important pest of capsicum (Seal *et al.*, 2006), especially in the PH environment, and acephate (O, S-dimethyl acetylphosphoramidothioate) is an insecticide that is very commonly used to control it. Methamidophos (O,S-dimethyl phosphoramidothioate), is a primary metabolite of acephate (Figure 1), which is also used in many countries as an insecticide against chewing and sucking pests and is more toxic than acephate. The acute dermal toxicity of methamidophos DL<sub>50</sub> in rats is 130 mg kg<sup>-1</sup> and that of acephate is 2000 mg kg<sup>-1</sup> (Tomlin, 1994). Acephate and its methamidophos metabolite residues were evaluated at different intervals such as 1 h, 1 day, 3 days, etc., after the last spray, in capsicum grown under PH and compared to that, in



**Figure 1** Structural formula of (a) acephate, (b) methamidophos.

capsicum grown in open field (OF), in order to ascertain the effect of difference in environmental conditions on dissipation of residues in PH as compared to that in OF.

## Materials and methods

### Materials

Analytical-grade acephate and methamidophos standards, certified and 99.9% pure, were obtained from Riedel-de Haen, Germany. Ethyl acetate used was PR-grade quality from Spectrochem Pvt Ltd. (New Delhi, India) and anhydrous sodium sulphate was from Thomas Baker Pvt. Ltd. (Mumbai, India). The commercial pesticide used, Asataf (acephate 75% SP), was procured from Rallis (I) Pvt. Ltd. (Mumbai, India). Standard solution of acephate and methamidophos was prepared with acetone and suitably diluted with ethyl acetate to obtain working standards.

### Experimental design

Capsicum (cv. Indra) was grown in a low-cost PH and also in OF at the Indian Institute of Horticultural Research, Bangalore, during the summer season. The temperature and relative humidity inside the PH and in OF was recorded using Mastech MS 6505 thermo hygrometer (Shenzhen Mastech Industrial Co. Ltd., Hongkong, China), at 1400 h everyday. The experiments were conducted using a randomized block design, with three replicates for application of the insecticide acephate at 75 g a.i ha<sup>-1</sup> using 500 L ha<sup>-1</sup> spray solution. Three plots were treated as controls, which were sprayed only with water. The plots (3 m × 3 m) were separated by a 1-m distance from each other. Foliar spray of acephate was given at fruit formation stage of the crop on capsicum grown in PH as well as in OF. Four such applications were given at weekly intervals and analysis of capsicum fruits for acephate and methamidophos residues was carried out after the fourth spray of the insecticide at intervals of 0 (2 h), 1, 3, 5, 7, 10, 15 and 30 days.

### Sampling and sample preparation

Approximately 500 g capsicum fruits were collected from each replication, pooled together to make a sample size of

1.5 kg. Samples were extracted immediately after bringing from the field, the capsicum fruits were cut, quartered mixed together and representative 50 g of sample was drawn in triplicate. Each sample was extracted by blending in a high-speed Waring blender (Cole parmer India Pvt. Ltd., Mumbai, India), with 150 g anhydrous sodium sulphate and 150 mL of ethyl acetate. The extraction and clean-up of capsicum samples for residues of acephate and its metabolite methamidophos were carried out as per procedure reported by Hadjidemetriou *et al.* (1985). The sample extract obtained was filtered through anhydrous sodium sulphate. The filtration cake was re-extracted with 2 × 100 mL ethyl acetate and finally rinsed with 50 mL ethyl acetate. The combined ethyl acetate extracts were dried using anhydrous sodium sulphate and evaporated under vacuum at 40 °C to dryness. The extract was dissolved immediately in 5 mL of diethyl ether. A pre-washed silica gel adsorbent column was prepared by packing a glass column with 15 g of silica gel in ether. The residues were transferred quantitatively to the column using several 5 mL portions of diethyl ether. The column was washed with 100 mL of diethyl ether and 100 mL of 15% methanol in ether. The washings were discarded. Acephate and methamidophos residues were thereafter eluted with 10% methanol in ether. The eluate was evaporated to dryness using a rotary vacuum evaporator. The residues were dissolved in 5 mL of methyl isobutyl ketone for final quantitative analysis using a gas liquid chromatograph (GLC).

### GLC analysis of acephate and methamidophos

A GLC, Model-Varian 3800 (Varian Inc., Palo Alto, CA, USA), equipped with a thermionic sensitive detector fitted with a capillary column Varian CP Sil 8 CB (30 m × 0.53 mm, d.f. = 0.2 μ) was used. The temperature of the injector oven and detector were 280 °C and 300 °C, respectively. The column oven temperature was programmed at 130 °C (0.8 min) → (20 °C min<sup>-1</sup>) → 190 °C (5 min). The gas flow rates were 7.6 mL min<sup>-1</sup> (nitrogen), 4 mL min<sup>-1</sup> (hydrogen) and 175 mL min<sup>-1</sup> (zero air). All gases used were high-purity gases passed through moisture and hydrocarbon traps before passing through the GLC. The carrier gas nitrogen was also passed through an oxy trap in order to block traces of oxygen from entering the column. The thermionic sensitive detector bead current was set at 3.0 A. The retention time of acephate and its metabolite methamidophos at the above parameters were 5.2 min and 3.5 min, respectively. The limit of quantification (LOQ) was determined as the lowest concentration of analyte, which

could be determined in the matrix at a signal to noise ratio of 4:1. LOQ of acephate and methamidophos residues in capsicum was 0.01 mg kg<sup>-1</sup>. Recovery studies were carried out in order to validate LOQ and establish the reliability of the analytical method for the present study, by fortifying capsicum fruits with acephate and methamidophos at the level of 0.01 mg kg<sup>-1</sup>, i.e. the LOQ, 0.05 mg kg<sup>-1</sup> (five times LOQ) and 0.1 mg kg<sup>-1</sup> (10 times LOQ). The mean recoveries of acephate was 77.9% to 85.7% and that of methamidophos was 81.2% to 91.3% in capsicum (Table 1). To ensure that the analysis of samples at different time intervals were not

subjected to bias due to sample degradation and/or instrument sensitivity, standards of acephate and methamidophos were analysed on each day of analysis period. The response peaks were comparable with the difference in means from at least five replicate measurements not more than ± 5%. Example chromatograms of a ‘blank’ capsicum, ‘spiked’ capsicum and an actual treated sample are provided in Fig. 2.

## Results and discussion

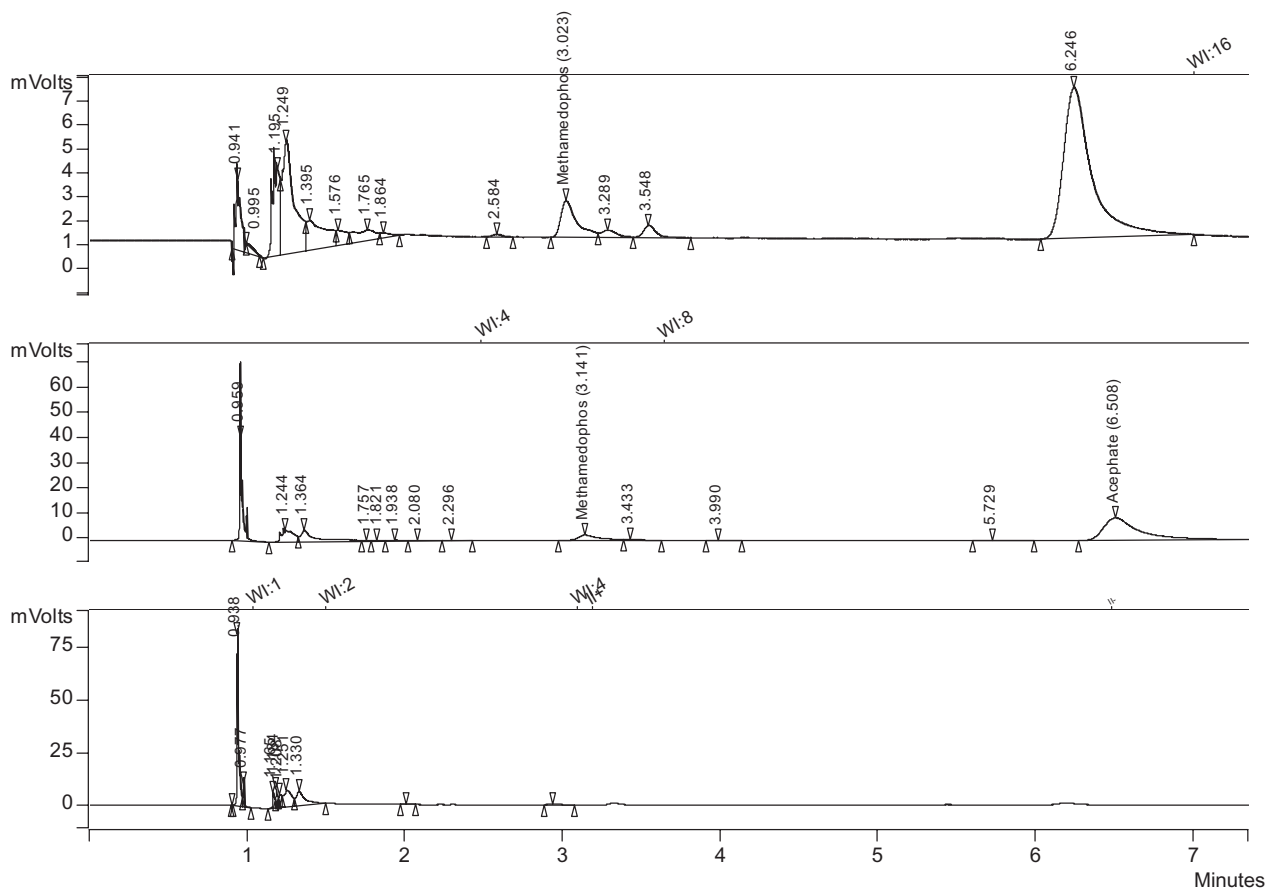
### Analysis and residue dissipation

Application of acephate resulted in persistence of its residues in capsicum fruits (Table 2). Initial residues of acephate were higher in capsicum grown in polyhouse (0.396 mg kg<sup>-1</sup>) than in capsicum grown in OF (0.182 kg<sup>-1</sup>), but the same became comparable within 10 days time (0.021 and 0.025 mg kg<sup>-1</sup> respectively) probably due to higher rate of growth of capsicum in PH as compared to that in OF

**Table 1** Percent recovery of acephate and methamidophos residues

Analyte	Mean recovery (%)* ±SD at fortification level		
	0.01	0.05	0.10
Acephate	77.9 ± 1.8	82.6 ± 1.2	85.7 ± 1.0
Methamidophos	81.2 ± 2.5	86.3 ± 2.1	91.3 ± 0.4

\*Average of three replicates



**Figure 2** Chromatograms of ‘blank’ capsicum (bottom), capsicum fortified with 0.05 ppm acephate and methamidophos (middle), 5<sup>th</sup> day capsicum sample from polyhouse (top).

**Table 2** Residues of acephate and methamidophos in open field- and polyhouse-grown capsicum

Days after last treatment	Acephate residues (mg kg <sup>-1</sup> )		Methamidophos residues (mg kg <sup>-1</sup> )	
	Open field	Polyhouse	Open field	Polyhouse
0	0.182	0.396	<0.01	0.010
1	0.173	0.333	<0.01	0.012
2	0.154	0.269	0.015	0.010
3	0.053	0.204	0.052	0.016
5	0.036	0.102	0.010	0.020
7	0.029	0.069	<0.01	0.031
10	0.021	0.025	<0.01	0.015
15	0.016	0.020	<0.01	0.010
21	<0.01	0.013	<0.01	<0.01
25	<0.01	<0.01	<0.01	<0.01

Note: Concentrations are based on wet weight of capsicum.

resulting in residue 'dilution'. In 10 days for example, fruit weight increased from 52 g to 187 g in PH while the fruit weight increase was from 51 g to 87 g only, in OF, during the same period. Also, initial residues in OF was lesser probably due to wind drift. In a PH, the entire spray application settles down on the plant as there is no wind drift and very little volatilization losses during or immediately after the application and the crop density is also higher. Ninety-one percent of the original residues dissipated within 15 days after final application of acephate in OF while in PH, 94% of the original residues had dissipated by the 15<sup>th</sup> day and residues dissipated to below detectable limits (0.01 mg kg<sup>-1</sup>) within 21 days in OF and within 25 days in PH. The calculated half-life ( $t_{1/2}$ ) of acephate residues in capsicum was 3.93 days in OF and 4.66 days in PH. Residues of methamidophos, a toxic metabolite of acephate was found to be present in very low amounts, viz. 0.01–0.05 mg kg<sup>-1</sup> in capsicum harvested between 2<sup>nd</sup> and 5<sup>th</sup> day from OF. In PH, however, methamidophos residues were found in all samples except in the 21<sup>st</sup> day sample, in the range of 0.01 to 0.03 mg kg<sup>-1</sup>. Residues levels of acephate as well as methamidophos were higher in capsicum grown in PH, during the first 15 days of sampling. The pre-harvest interval for acephate residues in OF-grown capsicum was determined to be 12 days considering the European Union maximum residue limit of 0.02 mg kg<sup>-1</sup>, while that in PH-grown capsicum was 16 days. Thus, in spite of higher residue 'dilution' due to faster rate of growth, residues persisted for a longer time in PH and therefore, frequency of acephate application may be reduced at near harvest stages in PH.

Studies by Chuanjiang *et al.* (2009) to analyse the dynamic degradation and final residues of acephate and its metabolite methamidophos in pakchoi (*Brassica campestris* L.) in OF

**Table 3** Temperature and relative humidity parameters in polyhouse and open field

S. No.	Location	Mean temperature (°C)	Mean % relative humidity
1.	Polyhouse	34.7	45.0
2.	Open field	36.2	34.5

and greenhouse has shown that residue levels of acephate and methamidophos in pakchoi was lesser in OF-grown pakchoi at 7, 11, 14 or 18 days after the last application of acephate than in PH-grown pakchoi. This is in conformation to the results obtained in the present study. Acephate and methamidophos residues were also evaluated in greenhouse-grown tomatoes and compared to an OF tomato crop (Trevisan *et al.*, 2005). The metabolism of acephate into methamidophos was found to be very low in tomato fruits but important in leaves and not well characterized in soil. The acephate and methamidophos residues were, in general, higher in the protected cultivation conditions, especially, in the leaves and soil, than in the OF, which is also in agreement with results obtained in the present study. However, greenhouses vary greatly in the temperature and relative humidity conditions inside them mainly due to the variety of material used, structural differences, use of additional shade net, etc. In case of the present study, additional shade nets were used and at midday, the mean temperature inside the PH, under canopy area at the height of fruits, was about 1.5 °C lower than in OF while the average percent relative humidity was 10.5% higher in PH (Table 3). In the OF during the period under study, there was no rainfall, and average wind speed was 6.63 km h<sup>-1</sup>, and therefore, these factors may not have influenced lesser persistence of residues in OF. Thus, differences in environmental conditions in the PH, i.e. lesser temperature, higher relative humidity and lesser incident ultraviolet radiation resulted in increased persistence of acephate and methamidophos residues in PH-grown capsicum in spite of greater residue 'dilution' due to faster rate of crop growth in PH. The influence of rainfall (except wash off by watering) and wind speed are considered to be negligible on the dissipation of pesticide residues in greenhouse (indoors) conditions (Brouwer *et al.*, 1997).

There are many documented reports of high residues of pesticides in crops grown in greenhouse, viz. high residues of lufenuron in Chinese cabbage (Khay *et al.*, 2008), fenpropathrin in tomatoes and green beans (Martínez Galera *et al.*, 1997), fungicides, thiram, cymoxanil, benomyl, vinclozolin and benzimidazolecarbamate methyl ester in greenhouse lettuce (Meloni *et al.*, 1981). The results obtained in

the present study indicated high persistence of acephate and its methamidophos metabolite in PH as compared to that in OF. Separate pesticide application protocols therefore have to be developed for assuring safety of capsicum grown in greenhouses for harvesting such crop with pesticide residues within permissible levels.

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