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# Persistence of acephate and cypermethrin in/on okra and cropped soil

**MU Mayannavar, CS Patil, BV Deore, SA Landge and PN Guru**

**Abstract**
Persistence of acephate and cypermethrin in/on okra was studied after application of insecticides at fruit initiation stage. Acephate @ 560 and 1120 g a.i. ha-1 and cypermethrin @ 50 and 100 g a.i. ha-1 were applied twice at an interval of 10 days. The initial residues dissipated with half life of 1.83 and 1.83 days in case of acephate and 2.38 and 2.00 days in case of cypermethrin at recommended and double the recommended dose, respectively. Considering the LOQ of 0.05 mg $kg^{-1}$, Pre-Harvest Interval (PHI) of seven days and five days can be suggested for acephate and cypermethrin for safe consumption of okra fruits.

**Keywords:** Acephate, cypermethrin, persistence, okra

## Introduction
Okra, *Abelmoschus esculentus* (L.) Moench known as 'Lady's finger in Western style or 'Bhendi' in Indian language, is considered to be major vegetable grown extensively in Indian subcontinent. It is commonly grown for its unripe fruits. In India, bhendi is commonly grown in Uttar Pradesh, Bihar, West Bengal, Andhra Pradesh, Madhya Pradesh, Karnataka, Gujarat, Rajasthan and Maharashtra. It is cultivated over an area of 530.80 thousand hectares with a production of 6350.30 thousand MT and productivity of 12 MT $ha^{-1}$. Andhra Pradesh (7.89 mha) having maximum area under okra cultivation with higher production. While, states like Bihar, Gujarat, Orissa, Jharkhand and Maharashtra also have considerable contribution in India's okra production. In Maharashtra, okra is cultivated over an area of 22 thousand hectares with production of 3.28 million tonnes and productivity of 15 MT $ha^{-1}$ (Anon., 2014) [2]. One of the major constraints in the production of okra in India is the incidence of insect pests. Nearly, 20 insect pests were reported to cause damage to okra crop (Butani and Verma, 1976) [3] which accounts for 65 to 100 percent loss.
Shoot and fruit borer, *Earias vittella* (Fabricius) is the most destructive insect pest of okra. The average fruit damage caused by shoot and fruit borer was estimated to be 35 to 76 percent (Narke and Suryawanshi, 1987) [11]. In order to control the damage caused by insect pests in okra, insecticides are being commonly used. Average pesticide usage in okra has been estimated to be 3.71 kg a.i. $ha^{-1}$ (Jayanthi and Kombairaju, 2005) [7].
Organophosphorous insecticides are widely used for the control of agricultural pests, thereby reducing the crop losses significantly. Indiscriminate use of pesticides in excess has resulted in occurrence of pesticide residues in agricultural commodities. The presence of pesticide residues in vegetable crops is a matter of great concern as vegetables are consumed fresh and directly. Farmers use substantial amount of pesticides throughout the period of growth and sometimes even at the fruiting stage in okra and do not observe 'safe waiting period' which results in accumulation of residues. Moreover the produce is harvested at short interval. Therefore, an insecticide should be effective and economic; but should not leave toxic residues.
Indiscriminate use of insecticides particularly at fruiting stage leads to accumulation of residues in okra. Recently, the residues of acephate, triazophos and cypermethrin etc., have been reported in okra. Though these insecticides were not recommended in okra in India, their residues have been recorded in market and farm gate samples of okra. Therefore, it is necessary to generate data on dissipation pattern of these non-recommended insecticides.
Among vegetables, brinjal, tomato, okra, cabbage and chilli need frequent application of pesticides during fruiting stage. While it is absolutely essential that any approved insecticide should remain active after its application to a crop to provide protection from target pests and yield economically viable produce, yet should dissipate to safe toxicological levels by the time man needed the produce from the treated crop for the consumption.

In view of this, it is essential to determine the time-bound dissipation behavior of insecticides.

## Material and Methods

### Test chemicals

The Certified Reference Materials of acephate and cypermethrin were made available by Pesticide Residue Laboratory, AINP on Pesticide Residues, MPKV, Rahuri whereas the formulated products (Sunphate 75 SP and Cymbush 25 EC) were made available by Modern Pesticide Testing Scheme, MPKV, Rahuri.

### Field experiment

Field experiment for residue studies was conducted during *Kharif* -2015 at the Instructional Farm, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar. Okra crop (Var. Mahyco hybrid 10) was raised by following recommended package of practices. Overall two sprays were given at an interval of 10 days, initiating the first spray at fruit initiation stage. According to residue studies protocol, prescribed by Central Insecticidal Board and Registration Committee (CIB & RC), two doses of acephate (recommended dose @ 560 g a.i. $ha^{-1}$ and double the recommended dose @ 1120 g a.i. $ha^{-1}$) and two doses of cypermethrin (recommended dose @ 50 g a.i. $ha^{-1}$ and double the recommended dose @ 100 g a.i. $ha^{-1}$) were evaluated for residues.

### Residue Analysis

### Standard Preparation

An accurately weighed 10 mg of an individual analytical grade insecticide standard was dissolved in 10 ml volumetric flask using suitable solvent to prepare the standard stock solution of 1000 mg $kg^{-1}$. Standard stock solution of each insecticide was further diluted to obtain immediate lower concentrations of 100 and 10 mg $kg^{-1}$. They were stored in a refrigerator at -20ºC. From intermediate standards, working standards of 0.50, 0.40, 0.25, 0.10 and 0.05 mg $kg^{-1}$ were prepared by suitably diluting the stock solution in n-hexane and used as standard check in residue determination, linearity and recovery studies.

### Method validation

Prior to analysis of samples, linearity of acephate and cypermethrin was established on GC-FPD and GC-MS, respectively. Accuracy and precision of the methods was determined by percent mean recovery and percent relative standard deviation.

### Limit of Detection and Limit of Quantification

The limit of detection of acephate and cypermethrin was determined by considering a signal-to-noise ratio of three with reference to the background noise obtained for the blank sample. The limit of quantification determined as 3 times of LOD.

### Linearity study

Five linear concentrations (0.05 mg $kg^{-1}$, 0.10 mg $kg^{-1}$, 0.25 mg $kg^{-1}$, 0.40 mg $kg^{-1}$, and 0.50 mg $kg^{-1}$) of each working standard i.e. acephate and cypermethrin were injected in triplicate. Linearity curve was established with concentrations of the standard and corresponding peak area.

### Recovery study

The analytical method for estimation of acephate and cypermethrin residues in okra and soil has been validated by conducting recovery studies using control samples of okra and soil. Ten gram each of control sample of blended okra fruits and soil was taken in 50 ml centrifuge tubes in three replicates each were spiked with acephate and cypermethrin separately at the required fortification levels *i.e.* LOQ, 5 x LOQ and 10 x LOQ, adding an appropriate volume of working standard of 10 mg $kg^{-1}$. This mixture was then shaken, in order to attain a proper homogeneity of pesticides in the samples. The extraction and cleanup procedures were followed as described hereunder. Percent recovery was calculated by using following formula.

$$\text{Percent recovery} = \frac{\text{Quantity of pesticide recovered}}{\text{Quantity of pesticide added}} \times 100$$

**Sampling:** The medium marketable size okra fruit samples (1 kg) were collected at random from each replicate of the treated and control plots separately at regular time interval of 0 (2 hr after spraying), 1, 3, 5, 7, 10 days till BDL after the second spray, whereas, soil samples from each treatment plot were collected at final harvest. The collected samples (okra and soil) were transferred immediately to the laboratory in an ice box. The samples were homogenized and kept at -20 °C in deep freezer until used for analysis.

**Extraction and clean up:** The okra fruits and soil samples were extracted and cleaned up using QuEChERS method (Anastassiades *et al*., 2003) [1].

**Okra fruits:** The entire laboratory sample (1kg) was crushed thoroughly in a grinder and approximately 10 g homogenized sample was weighed in a 50 ml polypropylene tube and tube was kept in deep freezer for 10 min. Homogenised sample was extracted with 10 ml ethyl acetate in presence of 10 g anhydrous $Na_2SO_4$ and centrifuged at 3500 rpm for 5 min. Transferred 2 ml supernatant to the 15 ml tube containing 50 mg PSA. The content was vortexed for 30 sec and then centrifuged for 2 min at 2500 rpm. The supernatant was filtered through 0.2 micron filter and instrumental analysis was carried out.

**Soil:** A representative 10 gm soil sample was taken in 50 ml polypropylene tube. Added 20 ml acetonitrile and shaked vigorously for 1 minute. To this, 4 gm magnesium sulphate and 1 gm sodium chloride was added and then centrifuged at 3300 rpm for 5 minutes. Transferred 10 ml supernatant to 15 ml tube, containing 1.5 gm $MgSO_4$ and 0.250 g primary secondary amine (PSA). It was shaked for few minutes, sonicated for 1 minute and then centrifuged again for 10 minute at 4400 rpm. From this 2 ml aliquot of supernatant was taken and evaporated to dryness using nitrogen concentrator at 40 °C (water bath temperature). Reconstituted the dry residues in 2 ml of ethyl acetate and filtered through 0.2 micron filter in vials and instrumental analysis was performed.

### Determination

### GC-MS parameters

Residue estimation of cypermethrin was performed using GC-MS. Identification of pesticide residues was accomplished by retention time and compared with known standard at the same conditions.

| Column Type | VF-5MS, 30m × 0.25 µm × 0.25 mm |
|---|---|
| Oven Programming | 80 $^0$C……. 1 min hold<br>@ 11 $^0$C/min 140 $^0$C …… 3 min hold<br>@ 5 $^0$C/min 225 $^0$C ……. 5 min hold<br>@ 8 $^0$C/min 280 $^0$C ……. 7 min hold |
| Detector | Interface temperature – 285 $^0$C |
| | Ion source temperature -250 $^0$C |
| | Mass range (M/Z) 40 – 400 |
| Injector | Injector temperature- 250 $^0$C |
| | Injection Volume - 1µl |
| | Injection Mode- split less |
| Column flow | 1.48 ml min$^{-1}$ |
| Carrier gas | Helium (99.99%) |
| Retention time | 18.5 min |

**GC-FPD Parameter**

Analysis of acephate was carried out by Gas Chromatography equipped with Flame Photometric Detector and automatic injection system (Shimadzu-GC-2010). GC solution software was used as the data analysis system. Typical GC conditions used for analysis are as below.

| Column type | DB-1,30 m X 0.25 µm X 0.25 mm |
|---|---|
| Column temperature | 170 °C.....3 min hold<br>@ 6.5 °C min$^{-1}$ 220 °C.....2min hold<br>@ 10 °C min$^{-1}$ 280 °C.....6min hold |
| Injector temperature | 250 °C |
| Column temperature | 170 °C |
| Detector temperature | 300 °C |
| Injection volume | 1µl |
| Column flow | 0.99 ml min$^{-1}$ |
| Hydrogen flow | 90 ml min$^{-1}$ |
| Air flow | 120 ml min$^{-1}$ |
| Retention time | 3.93 min |

**Statistical Analysis-**

The simple statistical analysis was carried out in the Microsoft Excel programme with the help of computer. The mean residues, standard deviation, regression equation, $R^2$ value and half life were calculated in excel programme.

**Results and Discussion**

**Limit of Detection and Limit of Quantification**

The limit of detection of the tested insecticides was 0.01 mg kg$^{-1}$ resulted by considering a signal-to-noise ratio of compound with reference to the background noise (3:1) obtained for the blank sample. The limits of quantification determined as the lowest concentration in okra of a given compound giving a response that could be quantified with RSD lower than 20 percent, was 0.05 mg kg$^{-1}$ for all tested pesticides.

**Linearity study**

Five linear concentrations (0.05, 0.10, 0.25, 0.40 and 0.50 mg kg-1) of each working standard i.e., acephate and cypermethrin were injected in triplicate and the linearity lines were drawn. The response was linear over the range tested and $R^2$ values were 0.996 and 0.998 for acephate and cypermethrin, respectively as given in Fig.1 and Fig. 2. These results indicated that the GC-FPD and GC-MS analysis method is valid for residue determination of the tested insecticides in okra fruits and soil.

**Recovery studies**

Accuracy of the analytical method was determined by recovery studies. Mean recovery obtained from the studies reflected the accuracy of method. Precision of the method was reflected by the relative standard deviation. Recovery experiments were conducted on untreated okra fruits and soil fortified with three concentrations i.e., 0.05, 0.25 and 0.50 mg kg$^{-1}$ of individual pesticide. The mean of recovery of acephate carried out at the levels of 0.05, 0.25 and 0.50 mg kg$^{-1}$ on okra fruits and soil (Table 1) were 92.03, 94.65, 97.95 percent and 86.11, 90.94, 93.31 percent, respectively. For cypermethrin, mean recovery at the levels of 0.05, 0.25 and 0.50 mg kg$^{-1}$ on okra fruits and soil (Table 1) were 83.76, 93.36, 83.66 percent and 78.40, 92.93, 82.20 percent, respectively. These results indicated that the analytical method employed for the extraction and clean up of okra fruits and soil samples was found accurate and precise as mean recovery percentages and relative standard deviation were within the limits prescribed by SANCO (2011).

**Dissipation of acephate in/on okra fruits**

Dissipation of residues in plant depends on climatic conditions, type of application, dosage and intervals between application and time of harvest. The results revealed reduction in residue levels of these tested pesticides in okra fruits with time.

In case of acephate 75 SP @ 560 and 1120 g a.i.ha$^{-1}$, initial residues were recorded as 1.94 and 3.47 mg kg$^{-1}$. The initial residues reached to below detection limit on 10$^{th}$ and 15$^{th}$ day at recommended and double the recommended dose, respectively. The half-life ($RL_{50}$) values of acephate for okra were 1.83 and 1.83 days, for both the dosages, respectively (Table 2). Similar studies were conducted by Prasad *et al*. (1993) [12], where the residues of acephate dissipated rapidly and reached below detectable limit after 10-15 days with dissipation rate of 44 to 55 percent within a day. Eijaza *et al*. (2015) [5] reported half-life ($RL_{50}$) of 2.5 days with dissipation of 94.96 percent after seven days and 99.34 percent after 30 days of acephate treatment in okra. In okra fruits, residues of acephate reached BDL within seven days as reported by Gupta *et al*. (2008) [6]. These reports support the present finding.

**Dissipation of cypermethrin in/on okra fruits**

As regards cypermethrin 25 EC @ 50 and 100 g a.i. ha-, initial deposits were recorded as 0.32 and 0.75 mg kg$^{-1}$. The cypermethrin residues were below detectable limit (BDL) on seventh and tenth day. The residual half-life ($RL^{50}$) values recorded were 2.38 and 2.00 days, respectively (Table 2). The residue level of cypermethrin reached BDL on 10$^{th}$ day as reported by Prasad *et al*. (1993) [12]. Dissipation of cypermethrin residues degraded from 55.7 to 73.5 percent within seven days. Similar behavior of these insecticides in ready mix (Spark and Polytrin C) formulations on okra fruit was observed by Shah *et al*. (1999) [15]. Khan *et al*. (1999) [9] reported half-life ($RL_{50}$) of 2.25 days for cypermethrin in okra fruits. These reports lend support to the present findings. However, in contrast to this, the half-life ($RL_{50}$) values of cypermethrin on chickpea green pods were 8.36 and 9.40 days following application of cypermethrin @ 60 and 90 g a.i. ha$^{-1}$, respectively as reported by Kumar *et al*., 1998 [10].

**Dissipation of acephate and cypermethrin in soil**

Analysis of soil samples for the residues of acephate and cypermethrin was carried out on the 30$^{th}$ day after second application. No residues were detected in untreated samples. The residues of acephate and cypermethrin in soil at harvest were found to be below detection limit (BDL) at both the doses. Samriti and BeenaKumari (2011) [13] showed that there

was no persistence of cypermethrin in soil and residues dissipated completely in soil with a half-life ($RL_{50}$) of 1.9 days on okra crop. Jyot *et al*. (2013) [8] reported that the residues of cypermethrin dissipated to more than 70 percent within 7 days. These reports also lend support the present finding.

Studies on dissipation of pesticides revealed that acephate persisted up to seven and ten days on okra at recommended and double the recommended doses, respectively. While, persistence of cypermethrin was for five and seven days, respectively. Half-life ($RL_{50}$) values calculated far acephate on okra were 1.83 and 1.83 days, for both doses, respectively. As there are no MRL's available for acephate in okra, LOQ of 0.05mg $kg^{-1}$ is taken as default MRL. Considering this, Pre-Harvest Interval (PHI) of seven days can be suggested for acephate for safe consumption of okra fruits. Half-life ($RL_{50}$) recorded for cypermethrin in/on okra were 2.38 and 2.00 days for recommended and double the recommended dose, respectively. As there is no MRL available for cypermethrin in okra, 0.05 mg $kg^{-1}$ may be taken as a default MRL. On the basis of this, Pre-Harvest Interval (PHI) of five days can be suggested for cypermethrin.

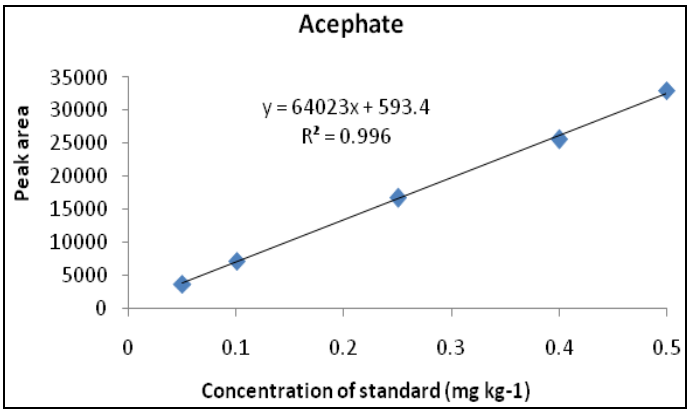


**Fig 1:** Linearity of acephate standard

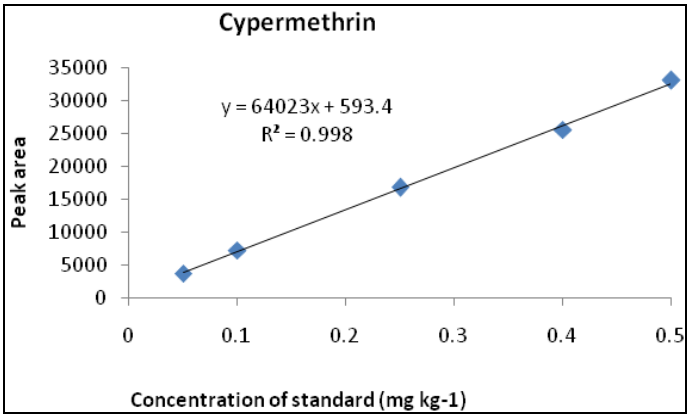


**Fig 2:** Linearity of cypermethrin standard

**Table 1:** Recovery of acephate and cypermethrin in/on okra fruits and soil

| Substrate | Fortification Level (mg $kg^{-1}$) | Recovery (%) | |
|---|---|---|---|
| | | Acephate | Cypermethrin |
| | | Mean ± SD | Mean ± SD |
| Okra fruits | 0.05 | 92.03 ± 3.34 | 83.76 ± 5.37 |
| | 0.25 | 94.65 ± 8.00 | 93.36 ± 0.66 |
| | 0.5 | 97.95 ± 6.88 | 83.66 ± 1.10 |
| Soil | 0.05 | 86.11 ± 2.85 | 78.40 ± 1.82 |
| | 0.25 | 90.94 ± 3.40 | 92.93 ± 1.70 |
| | 0.5 | 93.31 ± 4.18 | 82.20 ± 1.35 |

**Table 2:** Residues of acephate and cypermethrin in/on okra fruits and soil

| **Interval between last spray and sampling** | **Control** | **Residues (mg $kg^{-1}$)** | | | |
|---|---|---|---|---|---|
| | | **Acephate** | | **Cypermethrin** | |
| | | @ 560 g a.i. $ha^{-1}$ | @ 1120 g a.i. $ha^{-1}$ | @ 50 g a.i. $ha^{-1}$ | @ 100 g a.i. $ha^{-1}$ |
| 0 day (2 hr) | ND | 1.94 (±0.06) | 3.47 (±0.05) | 0.32 (±0.05) | 0.75 (±0.01) |
| 1 day | ND | 1.17 (±0.02) | 2.10 (±0.05 | 0.24 (±0.03) | 0.61 (±0.01) |
| 3 day | ND | 0.76 (±0.03) | 1.13 (±0.03) | 0.17 (±0.03) | 0.29 (±0.00) |
| 5 day | ND | 0.40 (±0.01) | 0.64 (±0.05) | 0.07 (±0.01) | 0.14 (±0.00) |
| 7 day | ND | 0.11 (±0.01) | 0.26 (±0.05) | BDL | 0.07 (±0.02) |
| 10 day | ND | BDL | 0.07 (±0.01) | BDL | BDL |
| 15 day | ND | BDL | BDL | BDL | BDL |
| $RL_{50(days)}$ | -- | 1.83 | 1.83 | 2.38 | 2.00 |
| Soil (at harvest) | ND | BDL | BDL | BDL | BDL |
| ND-Not detected BDL-Below Detection Limit | | | | | |

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