Biological Forum – An International Journal 15(4): 824-828(2023)

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Persistence and Dissipation of acephate residues in/on Bitter Guard under field conditions

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(Received: 09 February 2023; Revised: 16 March 2023; Accepted: 20 March 2023; Published: 20 April 2023)
(Published by Research Trend)

ABSTRACT: The dissipation patterns of acephate in bitter gourd fruits were studied after the application of acephate at the fruiting stage. Acephate was applied at the rate of 560 g a.i/ha on bitter gourd 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 bitter gourd fruits from the treatment (T_1) was 0.229 mg/kg with a half-life of 2.35 days. Based on the persistence studied waiting period of 7 days is suggested for acephate on bitter gourd from a consumer safety point of view.

Keywords: Bitter gourd, dissipation pattern, acephate, recommended dose GC-MS/MS, waiting period.

INTRODUCTION

The importance of pesticide-free bitter gourd fruits cannot be overstated, as they directly impact human health as well as the environment. The bitter gourd is also called bitter melon or *Momordica charantia* and is popular in many cuisines due to its unique flavour (Wang *et al*., 2022). Although it has a bitter taste, this vegetable is highly nutritious and has been used in traditional medicine for centuries to treat diabetes, heart disease, and digestion problems (Guo *et al*., 2018). Antioxidants, such as vitamins C and E, found in bitter gourd can contribute to the body's ability to combat oxidative stress and neutralize harmful free radicals. Antioxidants promote cellular health and slow down the aging process. Moreover, bitter gourd is known for its anti-inflammatory properties, which may help ease the symptoms of inflammatory conditions like arthritis by reducing inflammation (Kumari *et al*., 2017). The presence of various vitamins and minerals, such as vitamin C (Chen and Huang 2019) and zinc (Gayathry and John 2022), in bitter gourd strengthens the immune system (Aminah and Anna 2011). These nutrients support the production and function of immune cells, aiding the body's defense against infections, viruses, and diseases. However, the use of pesticides in farming practices can have detrimental effects on bitter gourd quality and pose risks to consumers.

Acephate is an organophosphosphate pesticide used to control insects and pests on plants. It has the chemical formula $C_4H_{10}NO_3PS$ and the IUPAC name N-(Methoxy-methylsulfanylphosphoryl) acetamide. An illustration of the molecular structure can be found in Fig. 1. This insecticide has been used as a systemic insecticide to control sucking and biting insects (Pohanish, 2014). When applied at recommended doses, Acephate is moderately persistent in the environment, lasting for 10–15 days. Specifically, it is used for the control of aphids and blackflies in vegetables and horticulture crops like bitter gourds (Mohapatra *et al*., 2011). In humans, acephate can inhibit cholinesterase (Miller *et al*., 2007), and overstimulate the nervous system, resulting in nausea, dizziness, confusion, and respiratory paralysis (Pesticides and Sheet 2010). However, it is still widely used in India for most of the cropping systems. It is therefore crucial to study toxic residue levels in bitter gourd fruits. Therefore, an attempt was made to study the toxic acephate residues in bitter gourd fruits. Keeping all this in view, this study aims to investigate the persistence pattern, dissipation behavior, and risk assessment of Acephate residues on bitter gourd fruits by applying Liquid-Liquid Extraction (LLE) method to sample preparation before estimating residues using gas chromatography based on the above facts.

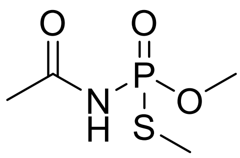

Fig. 1. Chemical Structure of Acephate.

MATERIAL METHOD

A. Chemicals and Reagents

The certified reference materials of Acephate with a purity of 96 % were acquired from Sigma Aldrich, Pvt, Limited. All the analytical organic solvents and reagents such as acetonitrile, acetone, sodium chloride, magnesium sulphate, and anhydrous sodium sulphate, were purchased from Merck (Darmstadt, Germany). Each of the chemicals used for the analysis was first subjected to glass distillation and then ran as a reagent blank.

B. Field Application and Sampling

The bitter gourd (*Momordica charantia* Mizo) variety "Pusa Domausmi" was raised following recommended agronomic practices at the Research Farm of Chaudhary Charan Singh Haryana Agricultural University, Hisar (29.14°N, 75.70°E). Acephate 25 EC formulation was sprayed only once at the time of 50% fruiting stage with a dosage of 250 g.a.i. ha^{-1} on selected experimental plots with the knapsack sprayer. Additionally, one of the experimental fields was left untreated to serve as a control. The samples in triplicate were collected randomly at 0 (2 h), 1, 3, 5, 7, 10, and 15 days after application (DAA). Samples were transported to the laboratory for residue analysis.

C. Sample Preparation

Using LLE, a representative 15 g bitter gourd sample was mixed with 100 mL of acetone in conical flasks and mechanically shaken for an hour. The extracted sample was filtered through a nylon filter of 0.22 mm in separate reagent bottles and concentrated to a volume of 10 mL using a rotary evaporator. The samples were cleaned by liquid-liquid partitioning with 600 mL of 10% NaCl brine solution, followed by vigorous shaking for 1 min with dichloromethane (DCM) and hexane (100, 50, 50 mL) thrice to remove non-emulsifying contaminants. After passing through anhydrous Na_2SO_4, the organic layer was collected to ensure that all moisture had been removed. The extracts were concentrated and reconstituted with 3 mL n-hexane for analysis.

D. GC-MS/MS Analysis

Pesticide analytes in samples were determined by GC-MS/MS (Shimadzu GC-MS TQ 8040) equipped with a capillary column (SH-Rxi-Sil MS column of 0.25 µm thick film having 30 m length and 0.25 mm internal diameter) using helium gas as the carrier gas at a constant flow rate of 1.5 mL min^{-1}. Samples were injected (1 µl) with an autosampler (20iAOC) in splitless injection mode. The temperature of the injection port was 250°C and programming of the oven temperature was done to optimize the working conditions. The oven temperature programming began at 80^0 C and remained at this temperature for 2 min, then start to increase up to 180 °C at 20 °C/min ramp rate and attain the temperature of 300 °C, at rate of 5 °C/min and remains for 10 min. Pesticide residues could be confirmed and quantified by using GC-MS/MS in Multiple Reaction Monitoring (MRM) with a ESI(+) source of ionization throughout a scanning mass range of 40-1000 m/z. Peaks in the total ion chromatogram of the sample recorded in MRM mode were detected based on their particular retention time (R_T) and their characteristic ion peaks in the mass chromatogram. The retention time on of Acephate was found to be 3.142 min (Fig. 2). The analysis was carried out in a completely air-conditioned laboratory with a temperature of less than 22°C and a relative humidity of less than 60%.

E. Dissipation Studies

The data on the residues over days were analysed using first-order kinetics with the equation (1) as follows:

$$C_t = C_o e^{-K_1 t} \quad (1)$$

Where C_0 represents the initial concentration (mg kg^{-1}); C_t concentration of the pesticide residue (mg kg^{-1}) at time t (in days), and K_1 denotes the rate constant (day^{-1}). A regression coefficient (R^2) was used to depict the link between residue data and time by plotting the log[residues (mg kg^{-1}) x 10^3] on the y-axis and days after application on the x-axis. The half-life ($t_{1/2}$) of residues was calculated according to Hoskin's formula (Hoskins, 1961).

F. Data Analysis

Data are represented as mean ± S.D (Standard deviation). For each parameter involved in the dissipation and decontamination processes, analysis of variance (ANOVA) was performed to analyze the interactions among different treatments, and days after the application. Differences in means were determined to be statistically significant at a p-value of 0.05. The software Origin Pro 9.0 (Origin Lab Corporation, Northampton, MA, USA) was used to create all of the figures.

RESULTS AND DISCUSSION

Results were satisfactory for all validation parameters studied according to SANTE (2021) for acephate analysis on bitter gourd. The recovery of the compound from bitter gourd is presented in Table 1. It was found that the recoveries of acephate ranged from 76.63-97.33%, which was within the acceptable range of 70-120%. Because chromatographic peaks did not interfere with matrix peaks, the method was highly selective. Since the analysis was conducted with GC-MS/MS, matrix effects were almost negligible. The LOD and LOQ for acephate were 0.01 and 0.05 mg/kg, respectively.

The residue data and percent dissipation of Acephate (25 EC) are shown in Table 2 (Fig. 3) indicating that the foliar application of recommended (560 ga.i. ha^{-1}(T_1)), of Acephate (25 EC) on Bitter gourd fruits in field conditions shown an initial residue of 0.229 mg/kg at respective dose. The insecticide dissipated to about 26.20 percent after 1 day of application. After then, progressive degradation in the concentration of residues deposited due to T_1 application in bitter gourd fruits was observed with dissipation rates of 67.69 and 72.93 for 3 and 5 days after the application (Fig. 4). Further, it was noted that the residues reached below the limit of quantification i.e. 0.05 mg/kg on the 7^{th} day. Thus, the dissipation can be considered to be rapid and almost complete. The factor which appeared to have played a role in the dissipation of Acephate is the high temperature. The dissipation of Acephate followed first-order kinetics with a half-life value to be 2.35days. this faster dissipation trend of acephate was also reported by several publications describing the half-lives of 1–2 days in brinjal, cotton seeds, and green mustard under foliar spray treatment (Battu *et al.,* 2009; Chai *et al.,* 2009; Kaur *et al.,* 2015).

However, there are some reports different from our results showing slower dissipation with half-life with 5 days of acephate residue in apple samples under the same application of direct foliar spray (Sanz-Asensio *et al*., 1999). These results also showed that acephate residue decreased below MRL at 20 days after application on apple, which was comparable to the findings in this study. These findings are different from our results, because Fresh fruits have higher water content than that vegetables, leading to easier translocation and higher accumulation of the pesticide with strong water solubility such as acephate in the commodities (Matthews and Shackel 2005). This could explain the slower dissipation rates of acephate in apple fruits compared to the dissipation trends in vegetable samples.

Table 1: Recovery for Acephate in spiked bitter gourd Examples at different levels.

Substrates	**Level of fortification (mg/kg)**	**% Recovery* (Mean ± S.D)**
Bitter gourd	0.05	81.22 ± 2.26
	0.01	83.45 ± 1.23

*Mean ± S.D of three replicates

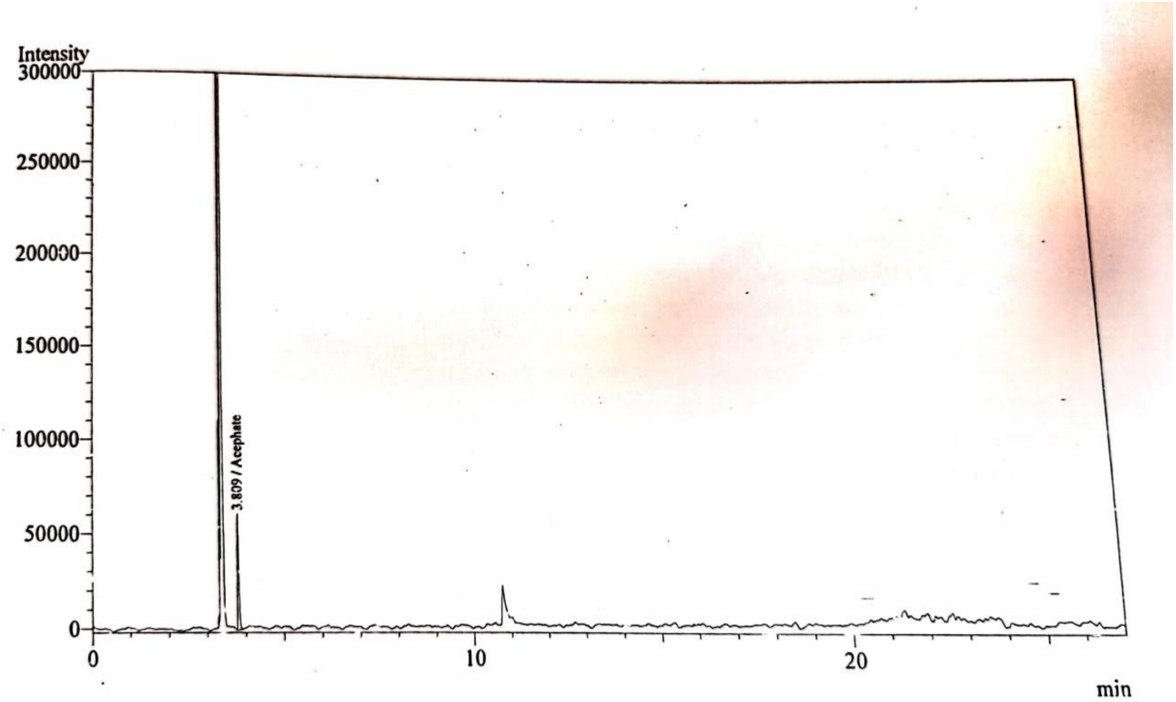

Fig. 2. Chromatogram of GC-MS/MS showing the peak of acephate at 3.199 retention time.

Table 2: Residues of Acephate (mg/kg) in bitter guard after the application of T_1 dose

Days after the treatment	**Dose (T_1= 560 g a.i.ha^{-1})**				
	R_1	**R_2**	**R_3**	**Average residues*±SD (mg/kg)**	**% Dissipation**
0 (2h)	0.257	0.201	0.230	0.229 ± 0.028	-
1	0.155	0.166	0.186	0.169 ± 0.016	18.78
3	0.074	0.074	0.075	0.074 ± 0.001	67.25
5	0.062	0.61	0.060	0.062 ± 0.001	73.80
7	<LOQ	<LOQ	<LOQ	-	-
10	<LOQ	<LOQ	<LOQ	-	-
15	<LOQ	<LOQ	<LOQ	-	-

LOQ = 0.05 mg/kg; LOD = 0.01 mg/kg, *Average residues of three replicates; SD = Standard Deviation

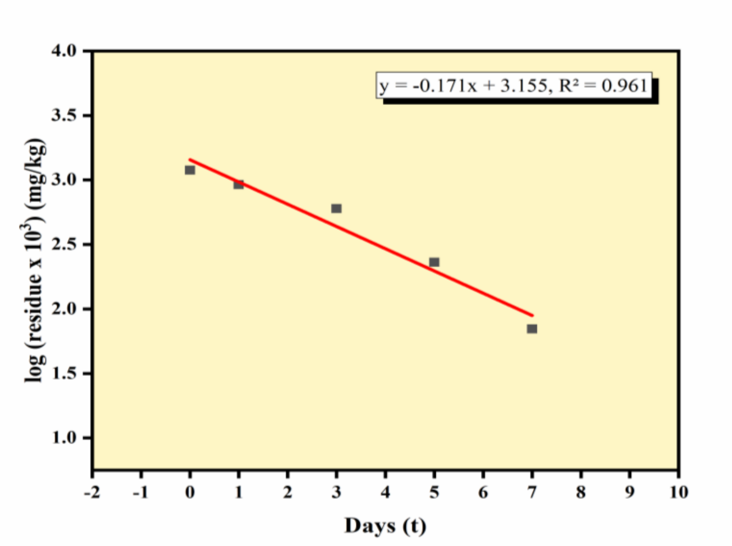

Fig. 3. Plot of log[residues (mg/g) x 10^3] of Acephate in Bitter gourd fruits v/s days.

Table 2: Dissipation parameters of Fenpropathrin residues in bitter gourd.

Dissipation parameters	**Dose (T_1= 560 g a.i.ha^{-1})**
Regression equation	y = -0.119x + 2.330
R^2	0.932
K_1	0.294
C_o	0.229
$t_{1/2}$	2.35

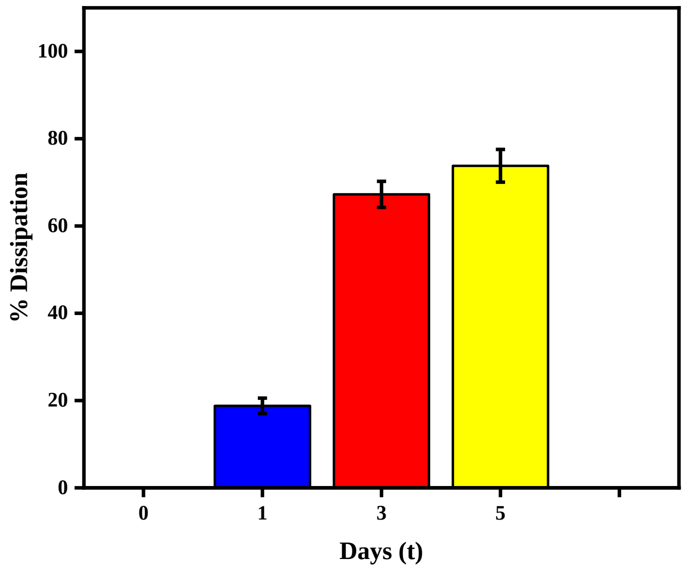

Fig. 4. Chart showing the dissipation pattern of Acephate in bitter gourd at T_1 dose.

CONCLUSION

The dissipation of Acephate in bitter gourd fruits followed first-order kinetics. Half-life values for Acephate on bitter gourd at the recommended dosage were observed to be 2.35 days. The residues of Acephate were found to be eliminated after 7 days. Therefore, it is recommended to wait for 7 days before harvesting and consuming bitter gourd after the application of Acephate.

FUTURE AND SCOPE

The future and scope of research on the dissipation of acephate in the environment are critical for addressing the environmental and human health challenges associated with pesticide use. As concerns over acephate residues and their long-term effects grow, scientists are actively studying the fate, behavior, and impacts of pesticides in ecosystems

Acknowledgement. The authors appreciate the assistance received from the head of the Department of Entomology and Chemistry at CCS, Haryana Agricultural University, Hisar, and the Department of Chemistry at Guru Jambeshwar University of Science and Technology, Hisar, for their contributions to this study by providing the necessary laboratory equipment and staff.

Conflict of Interest. The authors state that they have no known competing financial interests or personal ties that might seem to have influenced the research reported in this paper.

Declaration of Funding Information. During this investigation, no grant was supplied by an external funding source.

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How to cite this article: Pooja, Sushil Ahlawat and Ashu (2023). Persistence and Dissipation of acephate residues in/on Bitter Guard under field conditions. *Biological Forum – An International Journal, 15*(4): 824-828.