

Article

Validation and efficacy of various decontamination solutions in eliminating acephate residues from okra

Komal Rawat*, Anjana Srivastava, Shishir Tandon, Gajanpal Singh

Department of Chemistry, College of Basic Science and Humanities, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar 263145, Uttarakhand, India

* Corresponding author: Komal Rawat, komalrawat0911@gmail.com

CITATION

Rawat K, Srivastava A, Tandon S, Singh G. Validation and efficacy of various decontamination solutions in eliminating acephate residues from okra. Trends in Horticulture. 2024; 7(2): 4507.
<https://doi.org/10.24294/th.v7i2.4507>

ARTICLE INFO

Received: 1 February 2024

Accepted: 19 April 2024

Available online: 24 May 2024

COPYRIGHT



Copyright © 2024 by author(s).

Trends in Horticulture is published by EnPress Publisher, LLC. This work is licensed under the Creative Commons Attribution (CC BY) license.

<https://creativecommons.org/licenses/by/4.0/>

Abstract: The experiments were carried out to validate an analytical method and to examine the impact of various decontaminating solutions on the removal of acephate residues from okra. Acephate analysis was performed using HPLC-UV, and sample extraction was done using the QuEChERS method. Method validation encompassed assessing specificity, linearity, precision, accuracy, as well as limits of detection (LOD) and quantification (LOQ). The method exhibited excellent linearity with R^2 values ≥ 0.99 . LOD and LOQ were determined at $0.5 \mu\text{g mL}^{-1}$ and $2 \mu\text{g mL}^{-1}$, respectively. The results indicated average recoveries ranging from 80.2% to 83.3% with a % RSD below 5%. The decontamination procedures include rinsing with running tap water, soaking in lukewarm water, 2% CH_3COOH , 1% NaCl , 5% NaHCO_3 , 0.01% KMnO_4 , and in commercially available decontamination products such as nimwash, veggie clean, and arka herbiwash for a duration 10 minutes. Among all the treatments, soaking in nimwash solution showed remarkable effectiveness (96.75% removal), followed by veggie clean (94.97% removal) and arka herbiwash (95.80% removal). Washing okra samples in running tap water was found to be the least effective compared to other treatments.

Keywords: validation; vegetables; decontamination; HPLC-UV; acephate

1. Introduction

Okra, scientifically identified as *Abelmoschus esculentus* L., is an annual herb and a crucial vegetable crop extensively cultivated in tropical regions globally. It spans 2.5 million hectares of cultivation, producing an annual yield of 10.5 million tonnes [1]. India, being the primary global producer of okra, cultivates this significant vegetable crop year-round. The extensive cultivation in India contributes to 60.4% of the worldwide production. Okra serves as a rich source of vitamins, calcium, potassium, and various other minerals. Its cultivation occurs in the rainy and spring-summer. However, during the fruiting stage, the crop is susceptible to substantial harm inflicted by fruit and shoot borers [2]. To address the threat of insect-pest infestation, farmers often engage in indiscriminate pesticide application on vegetable crops, particularly during the fruiting stage, aiming to protect the crops from damage. Unfortunately, this practice leads to the presence of toxic residues on both the fruits and the soil beneath the crops. The residues persist during harvest, accumulating and increasing in concentration as they move up the food chain through a process known as bio-magnification. The consumption of vegetables with high pesticide contamination, whether in raw or processed form, poses a significant risk of serious health hazards for consumers [3].

Acephate, classified as a highly potent yet less toxic organophosphorus insecticide, finds widespread application in safeguarding crops like rice, wheat, cotton,

tea, tobacco, and vegetables from pests. Despite its efficacy in controlling insect infestations, its usage raises concerns for both human health and the environment [4]. The presence of acephate residues in vegetables has garnered significant attention, primarily due to the potential health risks associated with its metabolite, methamidophos [5]. The likelihood of elevated pesticide residue levels in vegetables during harvest is notably high, given the short interval between application and harvest [6]. Consequently, the monitoring and removal of pesticides become crucial elements to ensure the safety of vegetables for consumption. Researchers worldwide are actively exploring innovative techniques for effectively eliminating residue of pesticides. This includes utilizing common household chemicals as decontaminants to newer non-thermal techniques [7]. Numerous global monitoring studies have documented pesticide residues in fruits and vegetables [8–12]. In recent years, the issue of pesticide residues in harvested okra has gained significance, posing not only various health challenges but also acting as a significant impediment to international trade [13]. Considering these circumstances, a study was initiated to develop and validate a method for determining pesticide residues in okra. Additionally, the study aimed to compare the effectiveness of different decontamination procedures in removing acephate residues from the okra crop.

2. Materials and methods

2.1. Chemicals and reagents

The analytical grade acephate standard was sourced from Sigma Aldrich, India, while the acephate formulation (Acemain 95% SG) was acquired from the local market.

2.2. Method validation

Method validation ensures that the analytical method utilized for a specific test is appropriate for its intended purpose. Linearity, specificity, limit of detection (LOD), limit of quantification (LOQ), accuracy (% recovery) and precision (% RSD) parameters were assessed accordance the European Union guidelines [14].

2.2.1. Linearity

A solution of acephate at a concentration of $100 \mu\text{g mL}^{-1}$ was prepared by dissolving the insecticide in acetonitrile to form a stock solution. Following this, $10 \mu\text{g mL}^{-1}$ of working solution was prepared from this stock solution. Further dilutions ranging from 2 to $30 \mu\text{g mL}^{-1}$ were made from this working solution using the same diluent. The working standard of acephate was injected three times at five linear concentrations, and the calibration curve was generated by correlating each peak area with its corresponding concentration.

2.2.2. Limit of Detection (LOD) and Limit of Quantification (LOQ)

The values for limit of detection (LOD) and limit of quantification (LOQ) were established utilizing following formula:

$$LOD = 3.3 \times \sigma/S \text{ and } LOQ = 10 \times \sigma/S$$

where σ = Standard deviation of the intercept and S = Slope of calibration curve.

The pesticide concentrations were determined using a calibration curve and regression equation derived from the linearity graph.

2.2.3. Specificity

To assess specificity, the okra sample and reagent blank were spiked with acephate working standards at a concentration of $2 \mu\text{g mL}^{-1}$. These samples were then analyzed using the established chromatographic conditions on the HPLC equipment.

2.2.4. Recovery studies

To establish the reliability of the analytical methods, recovery experiments were conducted. The analytical method for determining acephate residues in okra was validated by performing recovery studies using control okra samples. For each control sample of okra, 10 g was placed in separate 50 mL centrifuge tubes in triplicate. Each sample was then spiked separately with acephate at the required fortification levels, i.e., the limit of quantification (LOQ), 5 times the LOQ, and 10 times the LOQ. The mixture was thoroughly shaken to ensure proper homogeneity of the insecticide in the samples. Subsequent extraction and clean-up procedures were carried out following the QuEChERS method as described below. The percent recovery was calculated using the given formula.

$$\text{Recovery (\%)} = [\text{Recovered concentration/Actual concentration}] \times 100$$

Accuracy was determined by performing five replicates at each recovery level, and the percent relative standard deviation (RSD) was then calculated.

$$\% \text{ RSD} = (\text{Standard Deviation/Mean}) \times 100$$

2.3. Extraction and analysis

Acephate residues were extracted from okra samples using the QuEChERS extraction method. Each 10 g portion of chopped vegetable sample was placed in triplicate into a 50 mL centrifuge tube along with 5 mL of distilled water and 5 mL of acetonitrile. After vortexing for 2–3 min, the mixture was allowed to stand for 10 minutes. Then, 3 g of anhydrous magnesium sulfate and 1 g of sodium chloride were added, followed by shaking for an additional minute. The tubes were vortexed again and centrifuged at 3000 rpm for 5 min. For cleanup, dispersive solid-phase extraction (dSPE) was utilized. This involved adding 1 g of MgSO_4 , 150 mg of PSA (primary secondary amine) reagent, and graphitized carbon black to the mixture, which was then centrifuged at 3000 rpm for another 5 min. The top supernatant acetonitrile layer was extracted using a pipette and passed through a dispersive solid-phase extraction (SPE) cartridge for further cleanup. The resulting solution was filtered through a $0.45 \mu\text{m}$ Polytetrafluoroethylene (PTFE) disc filter and transferred to a sample vial for analysis using HPLC–UV.

2.4. Optimization of chromatographic conditions

The initial criterion for selecting the mobile phase solvent was based on the solubility of the analyte in methanol and acetonitrile. Since both solvents showed adequate solubility. Subsequently, various ratios of acetonitrile: water and methanol: water were tested to optimize separation and resolution. Different flow rates were also explored to minimize retention time (R_t) while maintaining good resolution. Additionally, different UV wavelengths were tested to minimize interference and

achieve optimal chromatographic results. Following selection, the mobile phase composition underwent filtration using a 0.45 µm membrane filter and was subsequently sonicated prior to analysis.

Quantitative analysis of the insecticide via HPLC utilized the Dionex Ultimate 3000 system, comprising an RP-C18 column (250 × 4.6 mm) with a particle size of 5 µm, a 20 µl injector loop, UV-VIS detector, and dual pump. Details regarding the HPLC parameters, such as the mobile phase, wavelength, flow rate, and retention time during analysis, are provided in **Table 1**.

Table 1. Operated HPLC conditions.

Pesticide	Mobile Phase	Wavelength (nm)	Flowrate (ml min ⁻¹)	Retention time
Acephate	ACN/Water (60:40)	214	0.8	3.4

2.5. Experimental details of decontamination studies

The experiment was carried out from July 2022 to September 2022 at the Vegetable Research Centre in Pantnagar, Uttarakhand, India. Okra crop variety *Kasi pragati* was planted in plots measuring 5 m × 5 m, with plants spaced at 60 cm × 45 cm. All the recommended agronomic practices were followed for raising the crop. The mature okra was sprayed with acemain 95% SG at the rate 1000 g a.i/ha. The control plots were not subjected to any spraying, and vegetable samples weighing approximately 250–300 g were harvested at the 50%–60% fruiting stage for recovery studies. In order to study the effect of different culinary processes on reduction of pesticidal residues, a representative sample of okra fruits was collected 48 h after the application and subjected to different decontamination processes.

The okra sample was divided into eleven equal portions, each subjected to different culinary processes to assess their effects. These processes included washing with running tap water, soaking in lukewarm water, soaking in a 1% NaCl solution, soaking in a 5% NaHCO₃ aqueous solution, soaking in a 2% CH₃COOH solution, soaking in a 0.01% KMnO₄ solution, and dipping in commercially available decontaminant formulations (such as Veggi Clean, Nimwash, and Arka Herbiwash) for 10 min. Each treatment was replicated three times. Samples of okra fruits without any process were also analyzed for the residues along with the processed samples.

3. Results

3.1. Method validation

The method was validated for various parameters such as linearity, specificity, limit of detection, quantification accuracy and precision. For the tested analyte within a concentration range of 2 to 30 µg mL⁻¹, the linear equation and correlation coefficient (R^2) obtained from regression analysis demonstrated good linearity, with R^2 values ≥ 0.99 . The chromatogram peak exhibited no matrix peaks that could hinder the analysis of the target insecticides. Additionally, the retention times for acephate remained consistently at 3.4 min, indicating the purity of the analyte peaks and confirming the method's specificity under the selected chromatographic conditions.

The limit of detection (LOD) and limit of quantification (LOQ) values for acephate was determined to be 0.5 and 2 $\mu\text{g mL}^{-1}$, respectively, as indicated in **Table 2**.

The recovery rates for fortified okra samples with acephate at concentrations of 2, 10, and 20 $\mu\text{g mL}^{-1}$ ranged from 80.2% to 83.3%, with relative standard deviation (RSD) values consistently below 5%. All assessed validation parameters were observed to be within acceptable ranges.

Table 2. Validation data of standardized analytical method.

Pesticide	Variation coefficient	LOD ($\mu\text{g mL}^{-1}$)	LOQ ($\mu\text{g mL}^{-1}$)	Recovery (%) with RSD ($\mu\text{g mL}^{-1}$)		
				2	10	20
Acephate	0.9951	0.5	2	80.2 \pm 2.3	82.6 \pm 3.1	83.3 \pm 1.7

3.2. Decontamination studies

The residues of acephate in okra sample have led to a significant reduction in acephate residues in okra samples. The reduction percentages and residue levels are detailed in **Table 3**.

Table 3. Residues and % reduction of acephate in okra after different decontamination treatments.

Decontamination Treatments	Residues ($\mu\text{g mL}^{-1}$)	% Reduction
	Acephate (95%SG)	
Control (Without pesticide)	-	-
T-0 (Without any decontamination)	68.04	-
T-1 (Washing with Running Tap water for 1min.)	15.43	77.33
T-2 (Soaking in water for 10 min.)	15.42	77.34
T-3 (Soaking in Lukewarm water for 10 min.)	6.56	90.37
T-4 (1% NaCl for 10 min.)	6.03	91.14
T-5 (5%NaHCO ₃ for 10 min.)	4.21	93.81
T-6 (2% Acetic acid for 10 min.)	10.08	85.18
T-7 (0.01% KMnO ₄ for 10 min.)	5.74	91.58
T-8 (Veggie clean for 10 min.)	3.42	94.96
T-9 (Nimwash for 10 min.)	2.21	96.76
T-10 (Arka Herbiwash for 10 min.)	2.86	95.79

As indicated in **Table 3**, the levels of acephate residue in okra treated with various washing solutions were consistently lower compared to the control (untreated) samples, where the initial deposits of acephate were the highest. Soaking okra in various decontaminant solutions, including tap water, lukewarm water, 1% NaCl, 2% CH₃COOH, 0.01% KMnO₄, and commercial formulations, resulted in varying degrees of reduction in acephate residues. The effectiveness of pesticide decontamination is influenced by the interactions between the pesticide and the physiochemical properties of the applied decontaminants. In our investigations, soaking okra in all solutions demonstrated the ability to reduce acephate residues by more than 50%. The elimination rate of acephate using a salt solution exceeded 50%. This can be elucidated

by the fact that the acephate exhibit higher solubility in a saline environment, making it easier for them to access the salt solution when vegetable samples are immersed in it. Similarly, our experiment demonstrated that both acetic acid and sodium chloride displayed significant efficacy in removing acephate. This phenomenon could be attributed to the components of acid and salt present in these solutions.

Regarding commercially available decontaminants such as veggie clean, nimwash, and arka herbiwash, all three products proved to be quite effective in removing acephate residues. Among these, nimwash was found to be the most effective, followed by Arka Herbiwash and veggie clean. Soaking okra samples in a 5% NaHCO₃ solution for 10 minutes proved to be highly efficient in eliminating acephate residues. In line with this, another study demonstrated that soaking okra in a mixture of 5% NaHCO₃ and 0.01% KMnO₄ resulted in acephate residue decontamination percentages of up to 55.63% and 79%, respectively [7]. Similar results were showed by Srivastava et al. [15] in which dipping of chilli in 5% NaHCO₃ for 10 minutes were found quite operative in removing acephate along with its metabolite methamidophos. In our studies, KMnO₄ and 1% NaCl solution could eliminate acephate to an extent >50%.

Furthermore, studies revealed that solutions of 5% NaHCO₃ and 0.01% KMnO₄ were effective in dislodging residues from okra while it has been demonstrated that application of sodium bicarbonate, effectively eliminates specific pesticide residues such as thiabendazole and phosmet from apples [16]. The saline 1% NaCl solution served to be a good decontaminant for acephate. The strong electrolytic property of salt solutions possessing a net charge are capable of interacting with the pesticides and creating an attractive force to make certain, their elimination from vegetable matrix [17]. Hence, washing with salt (sodium chloride) solution is a convenient way of lowering of pesticidal contaminants from fruits and vegetables. 2% acetic acid also removed residues up to 80%. Reports indicate that incorporating reagents such as CH₃COOH, NaCl, and H₂O₂ into the washing water can significantly reduce pesticide residues by disrupting the chemical bonds between the pesticides and the surface of the crop [18]. The effectiveness of pesticide mitigation through washing with lukewarm or hot water is influenced by the physical and chemical properties of the pesticides. Previous studies have reported that boiling vegetables is more efficient in removing pesticide residues compared to simple washing [19,20]. However, washing under running tap water and soaking in water for 10 min. found to least effective in removal of residues from okra samples as compared to other solutions.

4. Conclusions

The validated HPLC-UV method for assessing acephate residues in okra have been proven to be accurate, sensitive, and easy to use. Nimwash has demonstrated superior efficacy as a cleansing solution for removing acephate residues from okra, surpassing two other commercial formulations. Given the often-elevated pesticide levels in vegetables sold in the market, it is imperative to monitor and decontaminate them. The decontamination treatments incur minimal costs, utilizing readily available household solutions. However, it is crucial to use these treatment solutions only once, as their reuse may compromise the efficiency of pesticide removal. Commercial

decontaminants are also cost-effective when adhering to the guidelines on their labels. While the current methods safeguard consumers from excessive pesticide use in okra, the adoption of combination technologies could address challenges associated with the diminished efficacy of individual treatments, ensuring the availability of a safe supply of fresh fruits and vegetables.

Author contributions: Conceptualization and methodology, AS and ST; execution of fields/lab experiments, AS, KR, GS and ST; data curation, KR and GS; analysis of data and interpretation, KR and GS; original draft preparation, KR; review, AS and ST. All authors have read and agreed to the published version of the manuscript.

Funding: The authors are thankful for the financial assistance provided by Coordinator, All India Network Project on Pesticide Residue Analysis, IARI, New Delhi, vide grant no. AINP/23 dated 04/06/2021.

Acknowledgments: The authors are thankful for the financial assistance provided by Coordinator, All India Network Project (AINP) on Pesticide Residue Analysis, Indian Agricultural Research Institute (IARI), New Delhi for carrying out these studies.

Conflict of interest: The authors declare no conflict of interest.

References

1. Food and Agriculture Organization of the United Nations. FAOSTAT. Rome, FAO; 2020.
2. Awata BG, Dwlyya VP, Rajput KG. Field evaluation of some newer insecticide for the control of Jassids (*Amrasca bigutulla bigutulla* Ishida) on Bendi. *Pestology*. 1984; 8(7): 14-15.
3. Kumari B, Kumar R, Madan VK, et al. Magnitude of pesticidal contamination in winter vegetables from Hisar, Haryana. *Environmental monitoring and assessment*. 2003; 87: 311-318. doi: 10.1023/A:1024869505573
4. Chuanjiang T, Dahui L, Xinzhong Z, et al. Residue analysis of acephate and its metabolite methamidophos in open field and greenhouse pakchoi (*Brassica campestris* L.) by gas chromatography–tandem mass spectrometry. *Environmental Monitoring and Assessment*. 2009; 165(1-4): 685-692. doi: 10.1007/s10661-009-0979-5
5. Fen Z, Li J. Progressing on toxicity and residual analysis of methamidophos. *Journal of Traditional Chinese Veterinary Medicine*. 2003; 21(5): 40-42.
6. Rawat K, Srivastava A, Tandon S, et al. Method validation for simultaneous determination of four neonicotinoids in vegetables by liquid chromatography. *Analytical Sciences*. 2022; 39(4): 431-439. doi: 10.1007/s44211-022-00227-y
7. Srivastava A, Singh GP, Srivastava PC. Method validation for determination of nine pesticides in okra and their mitigation using different solutions. *PLOS ONE*. 2021; 16(12): e0260851. doi: 10.1371/journal.pone.0260851
8. Essumang DK, Doodoo DK, Adokoh CK, et al. Analysis of Some Pesticide Residues in Tomatoes in Ghana. *Human and Ecological Risk Assessment: An International Journal*. 2008; 14(4): 796-806. doi: 10.1080/10807030802235243
9. Zawiyah S, Che Man YB, Nazimah SAH, et al. Determination of organochlorine and pyrethroid pesticides in fruit and vegetables using SAX/PSA clean-up column. *Food Chemistry*. 2007; 102(1): 98-103. doi: 10.1016/j.foodchem.2006.05.003
10. Kumari B, Madan VK, Kathpal TS. Monitoring of Pesticide Residues in Fruits. *Environmental Monitoring and Assessment*. 2006; 123(1-3): 407-412. doi: 10.1007/s10661-006-1493-7
11. Kumari S, Chauhan R, Ramprakash, Kumari B. Persistence and decontamination of bifenthrin residues in okra fruits. *African Journal of Agriculture Research*. 2013; 8(38): 4833-4838.
12. Parmar KD, Korat DM, Shah PG, Singh S. Dissipation and decontamination of some pesticides in/on okra. *Pesticide Research Journal*. 2012; 24(1): 42-46.
13. Meenambigai C, Bhuvanawari K, Mohan K, Sangavi R. Pesticides usage pattern of okra, *Abelmoschus esculentus* (L) Moench in Tamil Nadu. *Journal of Entomology and Zoology Studies*. 2017; 5(6): 1760-1765.
14. European Commission. Guidance document on analytical quality control and method validation procedures for pesticide residues and analysis in food and feed. SANTE/11813/2017. European Commission; 2017.

15. Srivastava A, Rahul, Singh GP, et al. Determination of pesticides residues and evaluation of decontamination treatments in chili. *Indian Journal of Horticulture*. 2022; 79(2): 249-255. doi: 10.5958/0974-0112.2022.00034.2
16. Yang T, Doherty J, Zhao B, et al. Effectiveness of Commercial and Homemade Washing Agents in Removing Pesticide Residues on and in Apples. *Journal of Agricultural and Food Chemistry*. 2017; 65(44): 9744-9752. doi: 10.1021/acs.jafc.7b03118
17. Rasolonjatovo MA, Cemek M, Cengiz MF, et al. Reduction of methomyl and acetamiprid residues from tomatoes after various household washing solutions. *International Journal of Food Properties*. 2017; 20(11): 2748-2759. doi: 10.1080/10942912.2016.1250099
18. Cengiz MF, Certel M. Decontamination techniques of pesticide residues before food processing or consumption. *Akademik Gıda*. 2012; 10(2): 69-74.
19. Satpathy G, Tyagi YK, Gupta RK. Removal of Organophosphorus (OP) Pesticide Residues from Vegetables Using Washing Solutions and Boiling. *Journal of Agricultural Science*. 2012; 4(2). doi: 10.5539/jas.v4n2p69
20. Vemuri SB, Rao CS, Swarupa S, et al. Simple decontamination methods for removal of pesticide residues in brinjal. *Scholars Journal of Agriculture and Veterinary Sciences*. 2015; 2(1A): 27-30.