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Article

Pre-harvest interval profiles of selected insecticides in broccoli grown under supervised trials

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Abstract: Vegetables, including broccoli (*Brassica oleracea* var. *botrytis*), are important components of the human diet due to their high nutritional value. However, insect pests pose a major challenge to broccoli production, and pesticides are widely applied to maintain crop quality and yield. In Bangladesh, indiscriminate pesticide use often leads to residues in vegetables, posing risks to human health and the environment. This study aimed to determine the pre-harvest interval of commonly used insecticides in broccoli and compare residue levels with the maximum residue limits (MRLs) established by the European Union. Broccoli was sprayed with recommended doses of lambda-cyhalothrin, dimethoate, and chlorpyrifos plus cypermethrin at 1 mL/L, and acephate at 2 g/L, across four supervised field trials. Samples were collected at 0, 1, 3, 5, 7, 9, 11, 13, 15, and 17 days after spray (DAS), and residues were analyzed by gas chromatography using a flame thermionic detector for organophosphorus insecticides and an electron capture detector for pyrethroids. Residue levels exceeded the EU-MRL up to 11 DAS for lambda-cyhalothrin, 13 DAS for chlorpyrifos plus cypermethrin and acephate, and 15 DAS for dimethoate. The PHI was determined as 13 DAS for lambda-cyhalothrin, 15 DAS for chlorpyrifos plus cypermethrin and acephate, and 17 DAS for dimethoate. These findings provide valuable guidance for farmers on safe pesticide use and support the production of safe vegetables for consumers.

Keywords: vegetable safety; pesticide residues; food quality; crop protection; residue analysis

1. Introduction

Broccoli (*Brassica oleracea* var. *botrytis*) is a cole crop within the Brassicaceae (Cruciferae) family, and its consumption has risen steadily in recent years owing to its exceptional nutritional profile. Members of the Brassica group are rich in essential nutrients and bioactive compounds, including vitamins, carotenoids, dietary fiber, soluble sugars, minerals, glucosinolates, and diverse phenolic constituents (Quizhpe *et al*., 2024). Consumption of cruciferous vegetables, particularly broccoli, has been associated with a reduced risk of several malignancies, including lung, colorectal, breast, prostate, pancreatic, and gastric cancers (Tahata *et al*., 2018). Like many other vegetables, broccoli is vulnerable to a wide range of insect pests, including the diamondback moth, aphids, the imported cabbageworm, cabbage looper, and flea beetles (Moorthy *et al*., 2022). These pests can inflict substantial damage to the broccoli curds, adversely affecting both yield and quality. Globally, plant pests and diseases are responsible for reducing crop yields by an estimated 20–40% (Kourous, 2016).

Numerous studies have recommended the use of pesticides for controlling insect pests and diseases to enhance crop productivity (Islam *et al*., 2015; Rahman *et al*., 2019). Reports indicate that many farmers apply insecticides to vegetables indiscriminately, often selling the produce immediately after spraying or within 0–2 days after spray (DAS). To minimize crop loss and maintain broccoli quality, farmers frequently rely on such pesticide applications (Khatun *et al*., 2023). Improper or excessive use of pesticides during crop production frequently results in residual pesticide contamination in fruits and vegetables (Ahmed *et al*., 2016a; Ahmed *et al*., 2016b; Akter *et al*., 2017; Ahmed *et al*., 2018a; Ahmed *et al*., 2019; Islam *et al*., 2019; Ahmed *et al*., 2021b; Ahmed *et al*., 2024).

Pesticide residues often persist in harvested crops when farmers do not adhere to the recommended pre-harvest interval. The presence of such residues in vegetables poses a significant concern for consumer health. To safeguard public health and meet the growing demand for safe food, pesticides should be applied in accordance with good agricultural practices (GAP). Regular monitoring of pesticide residues is essential to ensure compliance with GAP, as these residues can have harmful effects on humans, ecosystems, and the environment (Islam *et al*., 2021; Medina *et al*., 2021). Pesticide exposure has been associated with elevated risks of several adverse health outcomes, including various cancers, cardiovascular diseases such as heart disease and stroke, neurodegenerative conditions like Parkinson's disease, and a range of cognitive impairments (Ahmed *et al*., 2016c; Auyon *et al*., 2024). Studies also indicate that pesticide exposure can disrupt the gut microbiome, contributing to digestive disorders, and may further exert negative effects on bone health (Prodhan *et al*., 2018; Ahmed *et al*., 2018b). The nutritional value of fruits and vegetables is closely linked to enhanced digestive health, stronger bone development, improved vision, and a reduced risk of chronic conditions such as heart disease, stroke, diabetes, and several forms of cancer (Ahmed *et al*., 2019). Consumers have the right to access safe food, making cost-effective and sustainable crop production practices essential. To prevent the overuse of chemical pesticides, reduce financial burdens on farmers, and protect ecosystems, the adoption of bio-pesticide–based pest management strategies is increasingly important (Ahmed *et al*., 2020; Ahmed *et al*., 2021a). Some studies have examined the pre-harvest interval and safe pesticide use periods in vegetables, but such research remains limited in Bangladesh (Ahmed *et al*., 2021a; Ahmed *et al*., 2022a). Food products become safe for consumption only after the recommended pre-harvest interval has passed. This interval varies across pesticides and crops, influenced by plant growth characteristics, the physicochemical properties of the pesticide, and prevailing environmental conditions (O'Brien, 2014; Jacobsen *et al*., 2015).

Despite the widespread use of pesticides in broccoli cultivation in Bangladesh, limited scientific evidence exists regarding their dissipation patterns and pre-harvest intervals under local environmental conditions, creating a critical research gap in ensuring food safety. It was hypothesized that commonly used pesticides—lambda-cyhalothrin, chlorpyrifos plus cypermethrin, dimethoate, and acephate—persist in broccoli beyond the intervals typically assumed by farmers and may exceed established EU-MRLs for several days after application. This led to the central research question, how long do these pesticides remain above EU-MRLs in broccoli under supervised field conditions in Bangladesh? Accordingly, the study was conducted to quantify the dissipation of these pesticides in broccoli over time and determine scientifically validated pre-harvest intervals to support safe consumption and promote good agricultural practices. The study provides evidence-based pre-harvest intervals that can guide farmers, regulators, and consumers in ensuring safer broccoli production and reducing pesticide-related health risks.

2. Materials and Methods

2.1. Ethical approval

This study did not involve any animals or humans; therefore, ethical approval was not required.

2.2. Study area and period

Broccoli was cultivated across four supervised field trials in the experimental fields of the Entomology Division, Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh during the 2023–24 growing seasons. All standard intercultural practices, including irrigation, fertilization, and weeding, were carried out appropriately to ensure healthy crop growth. The collected samples were analyzed at the ISO/IEC 17025–accredited Pesticide Analytical Laboratory under the Entomology Division of BARI, Gazipur, Bangladesh (Figure 1).

2.3. Chemicals used in pesticide analysis

Certified Reference Materials (CRMs) of lambda-cyhalothrin, cypermethrin, chlorpyrifos, dimethoate, and acephate, each with a purity >99.99%, were obtained from Sigma Aldrich (St. Louis, MO, USA) via SF

Scientific, Dhaka, Bangladesh Limited. Analytical-grade acetonitrile (MeCN) and anhydrous magnesium sulfate ($MgSO_4$) were procured from Panreac (Barcelona, Spain), while sodium chloride (NaCl) was purchased from Chem-Lab (Zedelgem, Belgium). Primary Secondary Amine (PSA) was obtained from Agilent (Santa Clara, CA, USA) through SF Scientific, Dhaka, Bangladesh Limited.

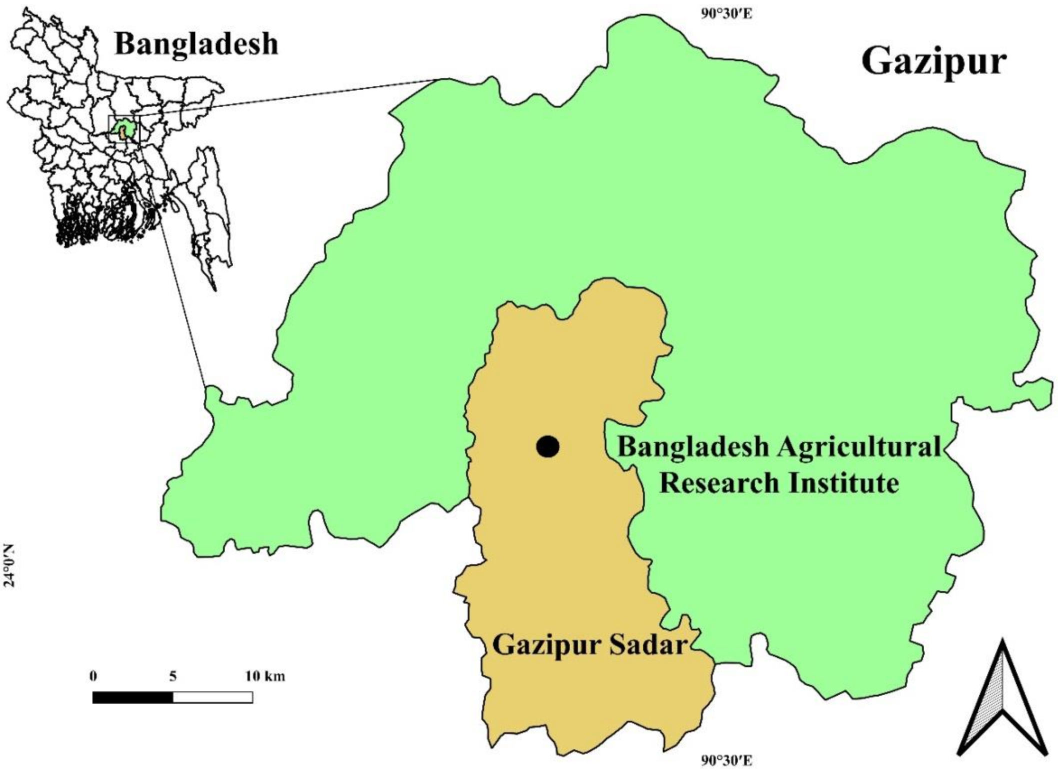

Figure 1. Broccoli samples collected from the experimental fields of BARI, Gazipur, Bangladesh.

2.4. Collection of broccoli samples from supervised field trial

Marketable broccoli samples were collected from four supervised field trials at 0, 1, 3, 5, 7, 9, 11, 13, 15, and 17 DAS. The crops were treated with the recommended doses: 1 mL/L of water for lambda-cyhalothrin, chlorpyrifos plus cypermethrin, and dimethoate, and 2 g/L of water for acephate at the experimental field of the Entomology Division, BARI, Gazipur. The formulated products used were Karate 2.5EC (lambda-cyhalothrin), Nitro 505EC (chlorpyrifos plus cypermethrin), Asataf 75SP (acephate), and Tafgor 40EC (dimethoate). The purity of all formulated insecticides was confirmed as 100% in the Pesticide Analytical Laboratory. Broccoli samples (1 kg each) were transported to the laboratory in cooled boxes for extraction, separation, and clean-up procedures and stored at –20 °C until analysis.

2.5. Extraction, separation and clean up

Sample extraction, separation, and cleanup were carried out using the modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method as described by Prodhan *et al*. (2015) and later referenced by Ahmed *et al*. (2016a).

2.6. Detection and quantification of pesticide residue in samples

The final concentrated extracts were analyzed using a Shimadzu GC-2010 (Japan) equipped with a flame thermionic detector (FTD) and an AT-1 capillary column for the detection of organophosphorus pesticides (acephate, chlorpyrifos, and dimethoate). For the detection of pyrethroid pesticides (lambda-cyhalothrin and cypermethrin), the extracts were analyzed using an electron capture detector (ECD) with an Rtx-CLPesticide 2 capillary column, following the method described by Ahmed *et al*. (2021a).

2.7. Analysis of pesticides residue data and pre-harvest interval

In this study, pesticide residue data were analyzed using the GC-2010 software integrated with the GC instrument. Prior to sample injection, matrix-matched standard solutions of the selected pesticides at different concentrations were prepared and injected following the instrument parameters described by Ahmed *et al*. (2021a). The samples were quantified using a four-point calibration curve prepared with the corresponding matrix-matched standard solutions. Each chromatographic peak was identified based on its retention time. The GC-2010 software automatically calculated the concentrations in mg/kg, representing the amount of pesticide in the final injected volume. The actual residue in the sample was then determined using the following formula,

$$\text{Residue in sample (mg/ka)} = \frac{\text{Conc.obtained in injected volume (mg/ka) x Quantity of final volume (L)}}{\text{Amount of sample taken (kg)}}$$

Residue levels of the four insecticides in all collected broccoli samples were calculated following these procedures, and the first sampling day on which the residue fell below the established MRL was identified and designated as the PHI.

2.8. Statistical analysis

The pesticide residue data were analyzed using the GC-2010 software integrated with the Shimadzu gas chromatograph, which automatically calculated concentrations in mg/kg based on the matrix-matched calibration curves. Statistical analyses, including calculation of mean residues and dissipation patterns, were performed using Microsoft Excel 2016. The study site map was prepared using the QGIS version 3.44.6.

3. Results

3.1. Residue levels and pre-harvest interval (PHI) of lambda-cyhalothrin in broccoli

The residual levels of lambda-cyhalothrin in broccoli were detectable up to 11 DAS, with concentrations of 3.986, 2.469, 1.682, 0.873, 0.461, 0.154, and 0.012 mg/kg at 0, 1, 3, 5, 7, 9, and 11 DAS, respectively. All detected residues exceeded the European Union maximum residue limit (EU-MRL) of 0.010 mg/kg. No residues were detected at 13 DAS, indicating that the PHI for lambda-cyhalothrin in broccoli can be set at 13 DAS (Table 1).

Table 1. Residue levels of lambda-cyhalothrin (Karate 2.5EC) in broccoli.

Days after spraying	Sample weight (g)	Injected volume (µL)	Amount of residue (mg/kg)	EU-MRL (mg/kg)
0	10	1	3.986	0.010
1	10	1	2.469	
3	10	1	1.682	
5	10	1	0.873	
7	10	1	0.461	
9	10	1	0.154	
11	10	1	0.012	
13	10	1	ND	

ND=not detected; EU-MRL=European Union maximum residue limit.

3.2. Residue levels and pre-harvest interval of chlorpyrifos plus cypermethrin in broccoli

The residues of chlorpyrifos plus cypermethrin (Nitro 505EC) in broccoli were detectable up to 13 DAS. Chlorpyrifos residues ranged from 4.502 mg/kg at 0 DAS to 0.066 mg/kg at 13 DAS, consistently exceeding the EU-MRL (0.010 mg/kg) throughout this period. Cypermethrin residues were detectable up to 7 DAS, with concentrations of 3.469, 2.604, 1.456, and 0.514 mg/kg at 0, 1, 3, and 5 DAS, respectively, exceeding the EU-MRL of 0.50 mg/kg. At 7 DAS, cypermethrin residue dropped to 0.048 mg/kg, below the EU-MRL. No chlorpyrifos residues were detected at 15 DAS, indicating that the PHI for chlorpyrifos plus cypermethrin in broccoli can be set at 15 DAS (Table 2).

Table 2. Residue levels of chlorpyrifos plus cypermethrin (Nitro 505EC) in broccoli.

Days after spraying	Sample weight (g)	Injected volume (µL)	Amount of residue (mg/kg)	
			Chlorpyrifos	Cypermethrin
0	10	1	4.502	3.469
1	10	1	2.968	2.604
3	10	1	1.634	1.456

Table 2. Contd.

Days after spraying	Sample weight (g)	Injected volume (μL)	Amount of residue (mg/kg)	
			Chlorpyrifos	Cypermethrin
5	10	1	0.927	0.514
7	10	1	0.682	0.048
9	10	1	0.456	ND
11	10	1	0.235	
13	10	1	0.066	
15	10	1	ND	

ND= not detected; EU-MRL of chlorpyrifos: 0.010 mg/kg; EU-MRL of cypermethrin: 0.50 mg/kg.

3.3. Residue levels and pre-harvest interval of acephate in broccoli

Acephate residues (Asataf 75SP) in broccoli were detectable up to 13 DAS. The residue concentrations ranged from 4.356 mg/kg at 0 DAS to 0.041 mg/kg at 13 DAS, all exceeding the EU-MRL (0.010 mg/kg). No acephate residues were detected at 15 DAS, indicating that the PHI for acephate in broccoli can be set at 15 DAS (Table 3).

Table 3. Residue levels of acephate (Asataf 75SP) in broccoli.

Days after spraying	Sample weight (g)	Injected volume (μL)	Amount of residue (mg/kg)	EU MRL (mg/kg)
0	10	1	4.356	
1	10	1	3.502	
3	10	1	2.046	
5	10	1	1.005	0.010
7	10	1	0.454	
9	10	1	0.295	
11	10	1	0.136	
13	10	1	0.041	
15	10	1	ND	

ND= not detected; EU-MRL= European Union maximum residue limit.

3.4. Residue levels and pre-harvest interval of dimethoate in broccoli

Dimethoate residues (Tafgor 40EC) in broccoli were detectable up to 15 DAS. The residue concentrations ranged from 3.485 mg/kg at 0 DAS to 0.014 mg/kg at 15 DAS, all exceeding the EU-MRL (0.010 mg/kg). No residues were detected at 17 DAS, indicating that the PHI for dimethoate in broccoli can be set at 17 DAS (Table 4).

Table 4. Residue levels of dimethoate (Tafgor 40EC) in broccoli.

Days after spraying	Sample weight (g)	Injected volume (μL)	Amount of residue (mg/kg)	EU MRL (mg/kg)
0	10	1	3.485	
1	10	1	2.574	
3	10	1	1.151	
5	10	1	0.943	
7	10	1	0.524	0.010
9	10	1	0.340	
11	10	1	0.152	
13	10	1	0.064	
15	10	1	0.014	
17	10	1	ND	

ND= not detected; EU-MRL= European Union maximum residue limit.

4. Discussion

The residue dynamics of lambda-cyhalothrin in broccoli showed a clear declining trend over time, with residues detectable up to 11 DAS and ranging from 3.986 mg/kg at the day of application to 0.012 mg/kg at 11 DAS. All of the quantities were above EU-MRL set by the EU pesticides database (2021). Despite this steady reduction, all measurable concentrations remained above the EU-MRL of 0.010 mg/kg, highlighting the persistence of lambda-cyhalothrin under field conditions. The complete dissipation of the insecticide by 13 DAS indicates that residue levels fall below the safety threshold only after this period, thereby establishing the PHI at 13 DAS. Ahmed *et al.* (2019) reported lambda-cyhalothrin residue detected up to 10 DAS in yard long bean and the residue was above EU-MRL up to 9 DAS (0.086 mg/kg) and the PHI of lambda-cyhalothrin for yard long bean had been selected at 10 DAS. This finding aligns with previous reports showing that synthetic pyrethroids, including lambda-cyhalothrin, exhibit moderate environmental persistence due to their lipophilic properties and affinity for plant tissues (Ahmed *et al*., 2021a; Ahmed *et al*., 2022a). The pattern of decline observed in this study is consistent with first-order dissipation kinetics commonly reported for pyrethroid insecticides in vegetables (Chau *et al*., 2020). From a food safety perspective, the results emphasize the importance of adhering to the recommended PHI to prevent exposure to unsafe residue levels, particularly in crops consumed fresh or with minimal processing (Munir *et al*., 2024).

The dissipation behavior of the combined formulation of chlorpyrifos plus cypermethrin in broccoli demonstrated distinct degradation patterns for the two active ingredients. Chlorpyrifos showed relatively slow dissipation, with detectable residues persisting up to 13 DAS and concentrations consistently exceeding the EU-MRL (0.010 mg/kg). Even at 13 DAS, its residue level remained at 0.066 mg/kg, indicating a prolonged persistence in broccoli tissues. Complete degradation was observed only by 15 DAS, establishing the PHI for the formulation at 15 days. In contrast, cypermethrin degraded more rapidly, with residues declining from 3.469 mg/kg at application to below the EU-MRL (0.50 mg/kg) by 7 DAS, and becoming undetectable at 9 DAS. Prodhan *et al*. (2018) reported that cypermethrin residues in yard-long bean and tomato were detectable up to 7 DAS, with concentrations remaining above the FAO/WHO MRL until 4 DAS. Based on this dissipation pattern, the authors recommended a pre-harvest interval of 5 days for both crops to ensure residue levels fall below the established safety threshold. This faster dissipation rate is consistent with the physicochemical characteristics of cypermethrin, a pyrethroid known to degrade more readily under field conditions compared to organophosphates like chlorpyrifos (Chai *et al*., 2009). Ahmed *et al*. (2021a) reported a PHI of 5 DAS for cypermethrin in both brinjal and tomato, while the recommended PHI for chlorpyrifos was 8 DAS in brinjal and 10 DAS in cauliflower. Similarly, Ramadan *et al*. (2016) observed a longer PHI of 15 DAS for chlorpyrifos in tomato. These findings are consistent with the outcomes of the present study. Khanom *et al*. (2023) documented a PHI of 9 DAS for chlorpyrifos in hyacinth bean, and Auyon *et al*. (2024) noted detectable chlorpyrifos residues up to 9 DAS in yard-long bean and 8 DAS in tomato. The contrasting dissipation patterns of the two insecticides highlight the influence of chemical structure on environmental persistence. Chlorpyrifos, being more stable and lipophilic, binds strongly to plant matrices and breaks down more slowly, whereas cypermethrin undergoes faster photolytic and metabolic degradation (Gupta *et al*., 2011). From a food safety standpoint, the results underscore the need for growers to adhere to the longer PHI determined by the more persistent component—in this case, chlorpyrifos—to ensure that harvested broccoli meets international residue standards (Jallow *et al*., 2017).

The dissipation profile of acephate in broccoli showed a gradual decline in residue concentrations over time, with detectable levels persisting up to 13 DAS. Initial residues were relatively high (4.356 mg/kg at 0 DAS) and decreased steadily to 0.041 mg/kg at 13 DAS; however, all measured values remained above the EU-MRL of 0.010 mg/kg during this period. Complete degradation occurred by 15 DAS, at which point no acephate residues were detected, establishing the PHI for acephate in broccoli at 15 days. Ahmed *et al*. (2016c) reported that acephate residues persisted up to 14 DAS in hyacinth bean and 15 DAS in tomato, with a recommended PHI of 10 DAS for both crops. In a subsequent study, Ahmed *et al*. (2022b) found that acephate residues were detectable up to 11 DAS in tomato and 14 DAS in yard-long bean, and all measured concentrations exceeded the European Union MRL throughout the detectable period. The relatively slow degradation rate of acephate observed in this study is consistent with its systemic nature and high water solubility, which facilitate absorption into plant tissues and reduce the rate of dissipation (Lin *et al*., 2020). Such persistence in cruciferous vegetables has been reported in other field studies, highlighting the tendency of acephate to remain in edible plant parts for extended periods, especially under field conditions where factors such as canopy structure and microclimate may slow chemical degradation (Kong *et al*., 2012). The residue pattern emphasizes the importance of adhering to the calculated PHI to minimize consumer exposure and ensure regulatory compliance. Since acephate residues stayed above the EU-MRL until 13 DAS, premature harvesting could pose potential food safety risks (Beyuo *et al*., 2024).

Dimethoate residues in broccoli declined progressively from 3.485 mg/kg at 0 DAS to 0.014 mg/kg at 15 DAS, showing a consistent dissipation trend characteristic of organophosphate insecticides. Although the concentration approached the regulatory threshold by 15 DAS, all detectable residues up to this point remained above the EU-MRL of 0.010 mg/kg, indicating a slower degradation rate in broccoli tissues under the supervised field conditions. The absence of detectable residues at 17 DAS demonstrates that dimethoate requires a longer degradation period compared with many other short-persistence insecticides, likely due to its physicochemical properties and systemic movement within plant tissues (Kariyanna *et al*., 2024). A similar study by Ahmed *et al*. (2020) reported that dimethoate residues persisted up to 11 DAS in cauliflower, 10 DAS in hyacinth bean, and 9 DAS in eggplant. The corresponding PHIs were determined as 12 DAS for cauliflower, 11 DAS for hyacinth bean, and 10 DAS for eggplant. The differences between their findings and the present study can be attributed to variations in crop type. Jacobsen *et al*. (2015) noted that pre-harvest intervals vary between pesticides and crops due to differences in plant physiology, the physicochemical properties of the pesticides, and environmental factors such as wind, rainfall, sunlight, humidity, and temperature. These findings highlight the importance of adhering to an extended pre-harvest interval of at least 17 days to ensure compliance with international residue standards and safeguard consumer safety (Baffoe *et al*., 2024). The observed dissipation pattern also reinforces the need for crop- and compound-specific PHI determination rather than relying on generalized guidelines.

5. Conclusions

The PHI was determined to be 13 DAS for lambda-cyhalothrin (Karate 2.5EC), 15 DAS for chlorpyrifos plus cypermethrin (Nitro 505EC) and acephate (Asataf 75SP) in broccoli, while for dimethoate (Tafgor 40EC), it was 17 DAS. These findings provide a scientific basis for ensuring the safe harvest of vegetables for consumers. Additionally, the results can guide researchers, policymakers, and other stakeholders in implementing measures to guarantee the production of safe food. Future studies focusing on monitoring pesticide residues, particularly pre-harvest intervals for various pesticides and crops, are essential to strengthen food safety.

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Data availability

All relevant data are within the manuscript.

Conflict of interest

None to declare.

Authors’ contribution

Md. Sultan Ahmed: conceptualization, execution of study, methodology, sample collection, laboratory works, writing-first draft, review and editing; Mohammad Dalower Hossain Prodhan: methodology, writing-review and editing; Afroza Begum: execution of study, writing-review and editing; Marina Afroze: sample collection, writing-review and editing; Nirmal Kumar Dutta: field execution of study, writing-review and editing. All authors have read and approved the final manuscript.

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