Persistence of chlorfluazuron in cabbage under different agro-climatic conditions of India and its risk assessment

PRITAM GANGULY,[a,*] SUHRID RANJAN BARIK,[a] SANDIP PATRA,[b] SANKHAJIT ROY,[a] and ANJAN BHATTACHARYYA[a]

[a] Bidhan Chandra Krishi Viswavidyalaya, Nadia, West Bengal, India [b] Indian Council of Agricultural Research for North Eastern Hill Region, Shillong, Meghalaya, India

* Address correspondence to pritam0410@gmail.com

Abstract

A multilocational field trial was conducted at 4 locations in India—Rajasthan, Gujarat, Madhya Pradesh, and West Bengal—to determine the persistence in cabbage of chlorfluazuron applied twice at 75 and 150 g active ingredient ha^{-1}. Cabbage head samples were collected from each replicated plot on 0 (2 h after spraying), 1, 3, 5, 7, 10, and 15 d after final insecticide application, including an untreated control. Chlorfluazuron residue in cabbage and field soil was estimated by high-performance liquid chromatography using a photo diode array detector. The limit of determination and limit of quantification of the method were recorded as 0.05 and 0.10 µg g^{-1}, respectively. Results revealed that chlorfluazuron dissipated linearly with progress of time, following first-order kinetics. The mean (± standard deviation) half-life value of chlorfluazuron in cabbage was found to be 7.18 ± 0.71 d, considering different locations and treatments. The residue was below the level of quantification in the harvested cabbage and soil samples. Harvesting cabbage in the experimental location, at least on day 7, after 2 applications of chlorfluazuron at the recommended dose, may not pose any ill effect for Indian adults.

Keywords: Chlorfluazuron, Cabbage, Persistence, Risk Assessment

INTRODUCTION

Cabbage (*Brassica oleracea* var. *capitata*) is a very popular vegetable, in high demand because of its high nutritional and medicinal value [1] and consumer preference. Its use against various diseases related to the stomach and digestion has been documented in the literature [2, 3]. This vegetable has an excellent anticancer property, and the presence of indole-3-carbinol in cabbage may protect the body against bowel cancer [4]. Cabbage prefers a cool and moist climate for growth and can also tolerate frost injury. In India, it is grown mainly during the winter season and in a few places during the rainy season. This vegetable can be cultivated in different types of loamy soil having neutral pH. India is ranked second in cabbage production after China, with an average yield of approximately 22.9 MT ha^{-1} [5]. Recently, however, the crop yield has shown a decreasing trend as a result of serious pest attacks in the field [6]. The diamondback moth (*Plutella xylostella*), one of the most important insect pests of cruciferous crops, has

been doing considerable damage to cabbage production in India [7, 8]. Previously, this pest was managed successfully by use of generic insecticides, such as malathion, quinalphos, and endosulfan; but subsequently it has built up resistance to different classes of insecticides, including synthetic pyrethroids [9].

Chlorfluazuron (N-[[[3,5-dichloro-4-[[3-chloro-5-(trifluoromethyl)-2-pyridinyl]oxy]phenyl]amino]carbonyl]-2,6-difluorobenzamide) (Figure 1), a member of the benzophenyl urea group, is a potent chitin synthesis inhibitor and controls several lepidopteran caterpillars, including the diamondback moth. It can enter insect body parts through contact and ingestion and create toxic effects. The compound does not have systemic and translaminar activity in plants [10]. This molecule was introduced by Ishihara Sangyo Kaisha [11] in the late 1980s for control of insecticide-resistant lepidopteran pests on vegetables and fruits, especially in Australia, Hungary, Japan, the Philippines, and Vietnam. In India, it was recently registered for use in cabbage and cotton to protect against several insect pests, such as the diamondback moth, the tobacco leaf–eating caterpillar, and the American bollworm [12]. Reports on negative impacts of chlorfluazuron on natural enemies and pollinating insects are very rare. It can be easily adopted in integrated pest-management schedules. The compound exhibits ovicidal activity against the common cutworm (*Spodoptera litura*) and the adult German cockroach (*Blattella germanica*) [13, 14]. Chlorfluazuron also has been found to effectively control flies in nonagricultural application [15] and termites by using bait matrix [16, 17], which may extend the spectrum of its use.

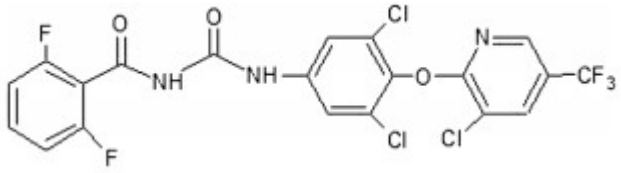

Figure 1

Structure of chlorfluazuron.

In addition to having an insect-controlling ability, chlorfluazuron may persist in the environment and pose a toxicity threat to humans. It has a partition coefficient (n-octanol/water, at pH 7, 20 °C) of 5.8, which indicates that chlorfluazuron has the potential to bioaccumulate [18]. This compound may be accumulated in the body through the food chain. Thus, the present field study was designed to understand the persistent nature of chlorfluazuron in cabbage and to assess safety risks associated with its use. To the best of our knowledge, there was a dearth of information about dissipation kinetics of chlorfluazuron in field cabbage in India before the present experiment was planned. Hence, efforts were made to evaluate the half-life of this compound in cabbage at different locations in India to determine safe consumption of cabbage after chlorfluazuron application.

MATERIALS AND METHODS

Chemicals and reagents

Chlorfluazuron 5.4% emulsifiable concentrate formulation and chlorfluazuron analytical standard (United Phosphorus Limited) were used in the experiment. All solvents and reagents used were of analytical grade. A stock standard solution of 100 ppm chlorfluazuron was prepared (100 mg L^{-1}) by dissolving 100 mg analytical standard of chlorfluazuron in 1000 mL methanol. The working standard solutions were made from the above stock standard through a serial dilution technique as required.

Field experiment

The persistence of chlorfluazuron in cabbage was studied at 4 different locations in India: Rajasthan, Gujarat, Madhya Pradesh, and West Bengal. Popular varieties of cabbage were raised in 20-m^2 plots in each location (Table 1). Chlorfluazuron (5.4% emulsifiable concentrate) was sprayed twice at 75.0 g active ingredient (a.i.) ha^{-1} (T_1) and 150.0 g a.i. ha^{-1}(T_2) using a knapsack sprayer [19] at a 15-d interval in the field with 500 L water ha^{-1} as the spray liquid. The insecticide was applied at the vegetative stage of cabbage after initial infestation of diamondback moth. To ensure a uniform spray, the sprayer was calibrated prior to application regarding nozzle flow rate, spray width, and walking speed of the applicator. The experiment was conducted in randomized block design. Control plots were also maintained, in which only water spray, and no chlorfluazuron, was applied (T_3).

Table 1. Variety, climatic condition, and soil types of 4 different locations

Parameters	Location I (Durgapura, Rajasthan)	Location II (Anand, Gujarat)	Location III (Indore, Madhya Pradesh)	Location IV (Kalyani, West Bengal)
Variety	Kranti	Sutton Express	Varun	Pusa Drumhead
Average maximum temperature (°C)	32.00	32.65	34.13	28.33
Average minimum temperature (°C)	22.30	15.34	23.87	14.83
Average relative humidity I (%)	74.00	73.58	69.19	96.08
Average relative humidity II (%)	34.00	30.60	25.22	63.42
Average rainfall (mm)	2.00	0.00	1.82	0.00
Soil texture	Sandy loam	Clayey loam	Sandy loam	Sandy loam
Soil pH	6.91	7.90	7.86	6.85
Soil organic carbon (%)	0.85	0.44	1.41	0.76

Sampling

Cabbage head samples (approximately 500 g) were collected from each replicated plot on 0 (2 h after spraying), 1, 3, 5, 7, 10, and 15 d after final insecticide application, including the untreated control plot. Harvest samples of cabbage head and field soils (at harvest, from 15 cm depth) were also collected from each replicated plot for analysis.

Sample preparation

Samples were processed immediately by the following methods so as to avoid loss of chlorfluazuron residues as a result of any kind of degradation during storage.

The cabbage sample was cut into very small pieces and put in a homogenizer vessel (PT 3100; Polytron). The sample was homogenized for 4

to 5 min. After that, a representative sample (5 g) was put in a 50-mL centrifuge tube from the vessel, and 20 mL methanol was added into the tube. The tube was then kept undisturbed for 2 h. A mixture of ChemElut (1 g; Agilent) and anhydrous Na_2SO_4 (1 g) was added into the sample. The sample was then mixed thoroughly by vortexing for 1 min and centrifuged (Avanti J-30I Centrifuge; Beckman Coulter) for 10 min at 5000 rpm. An aliquot of 1 mL of the supernatant was collected from the tube. It was then filtered through a syringe filter using a 0.22-µm nylon filter and diluted with methanol. Finally, the sample was analyzed by high-performance liquid chromatography (HPLC) equipped with a photo diode array detector.

A representative soil sample (50 g) was taken in a conical flask (250 mL) and added to a 100-mL mixture of acetone and water (8:2, v/v). It was soaked overnight, followed by shaking in a mechanical shaker for 30 min the next day. After that, the sample was filtered with filter paper (Whatman 42) using 100 mL of the same solvent for washing and concentrated in a rotary vacuum evaporator. The concentrated extract was partitioned thrice with ethyl acetate (100 + 50 + 50 mL). A combined ethyl acetate fraction was concentrated to 2 mL in a rotary vacuum evaporator. A chromatographic glass column was packed with a mixture of silica gel (5 g) and florisil (5 g) in between 2 layers of sodium sulfate (2 g). Hexane was used as the packing solvent. The concentrated extract was poured into the column. Then the column was eluted with 100 mL of hexane followed by 100 mL of methanol. A methanol fraction was collected and evaporated to dryness in a rotary vacuum evaporator at 40 °C, and the volume was reconstituted in methanol (HPLC grade) for HPLC analysis.

HPLC operating parameters

The Shimadzu HPLC system equipped with a photo diode array detector was used for quantitative analysis of chlorfluazuron. A Chromatopak C_{18} column (250 × 4.6 mm; Peerless Basic) was used for analyte separation. The solvent mixture of methanol and water (95:5, v/v) was flowed at 1 mL min^{-1} as the mobile phase. In these conditions, chlorfluazuron was detected (at $\lambda_{max} = 255$ nm) at a retention time of 5.9 ± 0.20 min (Figure 2).

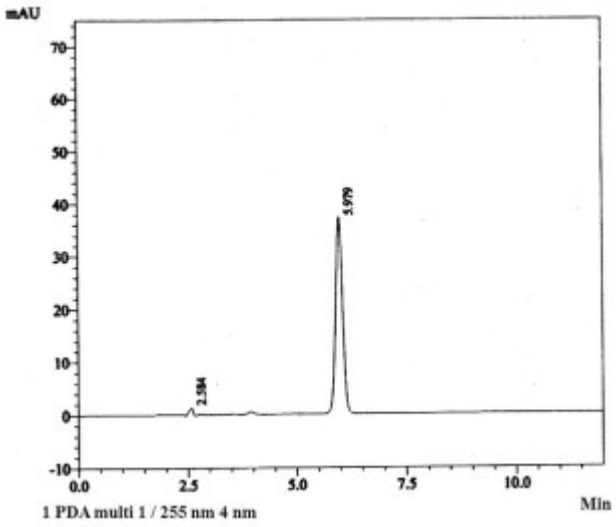

Figure 2

Chromatogram of analytical standard of chlorfluazuron under high-performance liquid chromatography operating conditions. mAU = milli-absorption units; PDA = photo diode array.

Recovery study

A recovery study was performed to judge the reliability of the analytical method described. Untreated cabbage head and soil sample were spiked at 3 levels with 0.10, 0.50, and 1.00 µg g^{-1} of chlorfluazuron. Using the same methodology for both cabbage and soil as described previously in the *Methods* section, samples were processed and analyzed. Cabbage and soil samples were also analyzed before spiking, and no detectable residue of chlorfluazuron was found. The precision of the method in terms of repeatability was checked on the basis of relative standard deviation (RSD %).

Data analysis

The dissipation behavior of chlorfluazuron in cabbage under field conditions followed first-order kinetics. The logarithm of pesticide residual concentration versus time interval gives a straight line, which shows that the dissipation process follows first-order kinetics. The first-order kinetics is expressed as [20]

$$C_t = C_0 e^{-kt}$$

where C_0 is the initial concentration, C_t is the concentration of the pesticide residues at time t, and k is the degradation rate constant.

The residual half-life (RL50) can be termed as the time in days required for the concentration of pesticide residue to reduce to one-half of its original deposit [20]. The residual half-life of chlorfluazuron was recorded using the following equation

$$RL50 = (\ln 2)/k$$

Safety assessment

The safety risk of chlorfluazuron in cabbage was assessed by comparing the theoretical maximum residue contribution with the maximum permissible intake of chlorfluazuron. The theoretical maximum residue contribution is recorded by multiplying daily consumption of the food commodity by the amount of chlorfluazuron residue (in parts per million), which is expressed in milligrams per day [21]. Maximum permissible intake is estimated by multiplying the acceptable daily intake of chlorfluazuron by the average body weight of a human being. Because acceptable daily intake is deduced as the safe intake limit for a healthy adult, maximum permissible intake is also calculated considering an adult's body weight [22]. The average body weight of an Indian person is 55 kg [23].

RESULTS AND DISCUSSION

Method validation

The analytical method was validated on the basis of linearity, reproducibility, and precision. Chlorfluazuron showed good linearity for 6 different concentrations (thrice replicated), ranging from 0.05 to 2.0 $\mu g\, g^{-1}$. The limit of determination (signal to noise ratio 3:1) and limit of quantification (signal to noise ratio 6:1) of the method were determined as 0.05 and 0.10 $\mu g\, g^{-1}$, respectively (Figure 3). The average recovery percentage of chlorfluazuron from cabbage head and soil samples is presented in Table 2, along with its corresponding standard deviation (SD) and RSD values. The recovery of chlorfluazuron was found in the range of 86 to 96% for both cabbage and soil. The RSD values ranged from 1.10 to 6.82 in cabbage and from 1.10 to 6.59 in soil at different spiking levels. This was well in agreement with the acceptance limit of method validation [24]. Hence, the analytical method was found to be efficient and adopted for the persistence study of chlorfluazuron in cabbage.

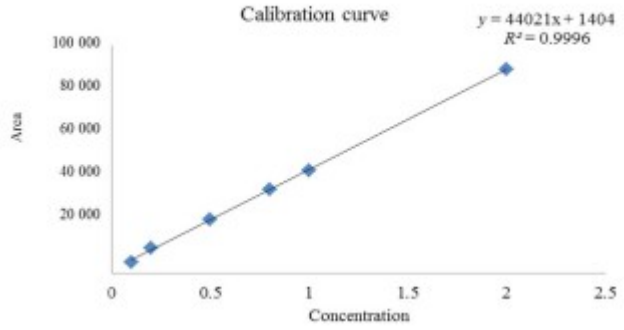

Figure 3

Calibration curve of areas corresponding to different concentrations of analytical standard of chlorfluazuron.

Table 2. Recovery of chlorfluazuron at different fortification levels in cabbage and soil

Location	Fortification level (μg g^{-1})	Mean[a] ± SD		Average recovery (%)		RSD (%)	
		Cabbage	Soil	Cabbage	Soil	Cabbage	Soil
Location I (Durgapura, Rajasthan)							
	0.10	0.086 ± 0.002	0.090 ± 0.004	86.00	90.00	2.33	4.01
	0.50	0.460 ± 0.030	0.470 ± 0.017	92.00	94.00	6.52	3.69
	1.00	0.910 ± 0.010	0.910 ± 0.010	91.00	91.00	1.10	1.10
Location II (Anand, Gujarat)							
	0.10	0.088 ± 0.001	0.086 ± 0.004	88.00	86.00	1.14	5.07
	0.50	0.460 ± 0.017	0.450 ± 0.010	92.00	90.00	3.77	2.22
	1.00	0.930 ± 0.020	0.910 ± 0.020	93.00	91.00	2.15	2.20
Location III (Indore, Madhya Pradesh)							
	0.10	0.091 ± 0.006	0.091 ± 0.006	91.00	91.00	6.59	6.59
	0.50	0.440 ± 0.030	0.480 ± 0.020	88.00	96.00	6.82	4.17
	1.00	0.890 ± 0.010	0.870 ± 0.035	89.00	87.00	1.12	3.98
Location IV (Kalyani, West Bengal)							
	0.10	0.087 ± 0.004	0.089 ± 0.005	87.00	89.00	4.60	5.95
	0.50	0.480 ± 0.010	0.440 ± 0.026	96.00	88.00	2.08	6.01
	1.00	0.880 ± 0.030	0.890 ± 0.046	88.00	89.00	3.41	5.15

[a]Values are average of 3 replicates.
SD = standard deviation; RSD = relative standard deviation.

Persistence in cabbage

The residue data of chlorfluazuron in cabbage head samples for different locations and different day's interval are given in Table 3. Dissipation of chlorfluazuron followed first-order kinetics (Figure 4). Residues were found below the level of quantification of 0.10 μg g^{-1} in all untreated control samples (T_3) throughout the study.

Table 3. Dissipation of chlorfluazuron in cabbage at different locations[a]

Days	Residues (μg g^{-1})							
	Location I (Durgapura, Rajasthan)		Location II (Anand, Gujarat)		Location III (Indore, Madhya Pradesh)		Location IV (Kalyani, West Bengal)	
	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2
0	2.51 ± 0.07 (–)	5.45 ± 0.06 (–)	2.13 ± 0.06 (–)	4.06 ± 0.04 (–)	1.97 ± 0.05 (–)	3.87 ± 0.07 (–)	2.49 ± 0.13 (–)	5.14 ± 0.07 (–)
3	1.13 ± 0.05 (55.11)	2.02 ± 0.04 (62.87)	1.11 ± 0.05 (47.89)	1.85 ± 0.05 (54.52)	1.09 ± 0.05 (44.50)	1.77 ± 0.04 (54.35)	1.73 ± 0.09 (30.52)	3.67 ± 0.12 (28.53)
5	0.67 ± 0.05 (73.31)	1.54 ± 0.05 (71.74)	0.67 ± 0.04 (68.39)	1.32 ± 0.03 (67.41)	0.67 ± 0.05 (65.99)	1.35 ± 0.05 (65.20)	1.15 ± 0.13 (53.82)	2.32 ± 0.08 (54.93)
7	BLOQ	1.12 ± 0.04 (79.39)	BLOQ	0.90 ± 0.07 (77.75)	BLOQ	0.92 ± 0.06 (76.23)	BLOQ	1.24 ± 0.10 (75.94)
10	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ
15	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ
Regression equation	Y = 3.3992 – 0.1149X	Y = 3.6836 – 0.0969X	Y = 3.3323 – 0.0994X	Y = 3.5848 – 0.0925X	Y = 3.3018 – 0.0932X	Y = 3.5608 – 0.0877X	Y = 3.3763 – 0.1085X	Y = 3.6685 – 0.0856X

[a]Chlorfluazuron (5.4% emulsifiable concentrate) was sprayed twice at 75.0 g active ingredient (a.i.) ha^{-1} (T_1) and 150.0 g a.i. ha^{-1} (T_2).
BLOQ = below level of quantification of 0.10 μg g^{-1}.

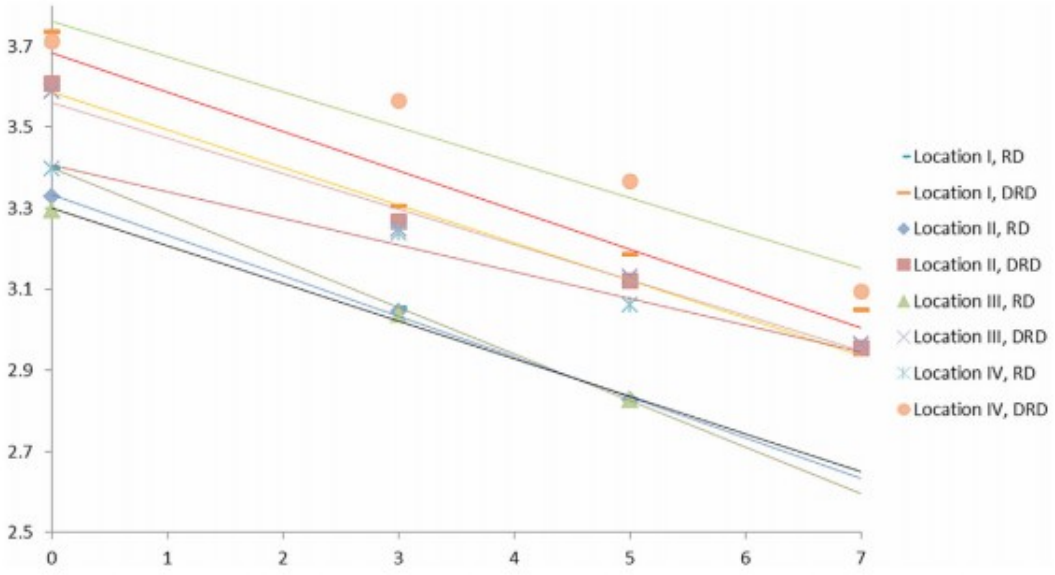

Figure 4

Dissipation of chlorfluazuron in cabbage at different locations. RD = recommended dose; DRD = double the recommended dose.

At location I, the initial deposits of chlorfluazuron in the cabbage head were found to be in the range of 2.51 to 5.45 μg g^{-1}. The calculated half-life values were 6.03 and 7.15 d for $T_1$and T_2, respectively. More than 70% of initial deposits dissipated within 5 d for both doses.

At location II, chlorfluazuron residues were initially found in the range of 2.13 to 4.06 μg g^{-1}, and the half-life values were 6.97 and 7.49 d, respectively, for T_1 and T_2. Approximately 68% of initial deposits were dissipated within 5 d, irrespective of doses.

Chlorfluazuron was initially deposited between 1.97 and 3.87 μg g^{-1} in cabbage at location III, and the half-life values ranged between 7.44 and 7.90 d for T_1 and T_2, respectively. The compound dissipated to approximately 65% within 5 d for both doses.

At location IV, the initial deposits of chlorfluazuron ranged from 2.49 to 5.14 μg g^{-1}, and the calculated half-life values were 6.39 and 8.10 d for T_1 and T_2, respectively. More than 50% of initial deposits dissipated within 3 d, irrespective of dose rates.

The mean half-life value of chlorfluazuron in cabbage was found to be 7.18 ± 0.71 (SD) d, considering different locations and treatments. The residues were below the level of quantification on days 7 and 10 after application for T_1 and T_2, respectively, at all locations. Because the compound itself is not readily taken up by plants through leaves or roots [10], it is obvious that the residues remaining on the plant surface shall be dissipated rapidly through various weathering processes [25]. This fact is supported by the findings of Shim et al. [26], who reported that certain external factors, such as UV radiation, heat decomposition, volatilization, and other complex conditions,

were primarily responsible for chlorfluazuron dissipation in pears. The residue was found below the level of quantification in harvested samples of cabbage and field soil, irrespective of treatments and locations.

Safety risk assessment

The recommended dietary allowance of vegetables of an Indian adult is considered to be 300 g d^{-1} [27]. The theoretical maximum residue contribution value at day 5 after final application of chlorfluazuron (recommended dose) in cabbage was found to be 0.201 mg $d^{-1}$for the first 3 locations (i.e., locations I–III), which was less than the maximum permissible intake value (0.275 mg chlorfluazuron d^{-1}). At location IV, the theoretical maximum residue contribution value at day 7 after application of chlorfluazuron (recommended dose) was less than the maximum permissible intake value, as the residue was below the level of quantification of 0.10 µg g^{-1}. When the dose was double the recommended dose, the theoretical maximum residue contribution value was less than the maximum permissible intake value at day 10 after application, irrespective of location. Different agro-climatic conditions were considered for selection of locations to judge the influence of distinct environmental exposure on persistence of chlorfluazuron in cabbage. It was thus evident from the result that harvesting cabbage at least on day 7 after 2 applications of chlorfluazuron (recommended dose) in all the locations could be safe for human consumption (for adults).

CONCLUSION

The present study shows that the half-life values of chlorfluazuron range between 6.03 and 8.10 d considering the 4 different experimental locations. Similar values were obtained by Liang et al. [28], who reported that the half-life of chlorfluazuron was 4 to 6 d in Chinese cabbage. Lee et al. [29] recorded a 5.5 d half-life of chlorfluazuron residue in perilla leaves. Moreover, the residue was found below the level of quantification in harvested samples of cabbage and soil, irrespective of location and variety. From this finding, it can be understood that there may not be any residual impact of chlorfluazuron on the next crop to be grown. Dissipation of chlorfluazuron can be studied further in different cabbage-growing locations that fall outside the range used in the present study (i.e., areas that receive more rainfall, cooler temperatures, or even frost). The analytical method can be improved further by adopting mechanized insecticide application, as well as using more sophisticated instruments, such as liquid chromatography–mass spectrometry, for lowering detection limits. From a safety point of view, the present experiment reveals that cabbage heads may be safely consumed by Indian adults on day 7 after 2 applications of chlorfluazuron at the recommended dose. Shim et al. [26] mentioned that chlorfluazuron can be safely applied to protect pears when used 2 to 3 times at 14 d before harvest. Thus, it can be concluded that chlorfluazuron has relatively low persistence when applied in cabbage at the recommended dose and may not pose any ill effect if consumed on day 7 after 2 applications.

Acknowledgment

We are very thankful to M/S United Phosphorus Limited, Mumbai, for sponsoring this experiment.

Data availability

Readers may contact the first author (pritam0410@gmail.com) for any information or details pertaining to this article.

References

1. Samec D, Pavlovi c I, Salopek-Sondi B. 2017. White cabbage (Brassica oleracea var. capitata f. alba): Botanical, phytochemical and pharmacological overview. Phytochem Rev 16:117–135. 2. Cheney G. 1949. Rapid healing of peptic ulcers in patients receiving fresh cabbage juice. California Medicine 70:10–15. 3. Komatsu Y, Aiba Y, Nakano Y, Koga Y. 2016. Probiotics, prebiotics, and biogenics for the stomach. In Venketeshwer R, Leticia GR, eds, Prebiotics and Probiotics in Human Nutrition and Health. InTech, Rijeka, Croatia, pp 363–381. 4. Singh R, Sharma RS. 2005. Cabbage. In Thamburaj S, Singh N, eds, Textbook of Vegetables, Tubercrops and Spices. Directorate of Information and Publications of Agriculture, Indian Council of Agricultural Research, New Delhi, India, pp 97–119. 5. Gupta S, Sharma RK, Gajbhiye VT, Gupta RK. 2015. Residue behavior of combination formulations of insecticides in/on cabbage and their efficacy against aphids and diamondback moth. Environ Monit Assess 187:4076. 6. Singh KI, Debbarma A, Singh HR. 2015. Field efficacy of certain microbial insecticides against Plutella xylostella Linnaeus and Pieris brassicae Linnaeus under cabbage-crop-ecosystem of Manipur. Journal of Biological Control 29:194–202. 7. Chelliah S, Srinivasan K. 1986. Bioecology and management of diamondback moth in India. Proceedings, First International Workshop, Tainan, Taiwan, March 11–15, 1985, pp 63–76. 8. Mondal S, Chatterjee ML. 2007. Management in diamond back moth (Plutella xylostella L.) in cabbage with new biopesticide spinosad 2.5 SC. Proceedings, National Workshop on Organic Horticulture—Its Production, Processing, Marketing and Export for Sustainability, Nadia, West Bengal, India, June 8–10, 2007, pp 204–206. 9. Mehrotra KN. 1993. Status of insecticide resistance in insect pests. In Dhaliwal B, Singh B, eds, Pesticides—Their Ecological Impact in Developing Countries. Commonwealth, New Delhi, India, pp 32–35. 10. Farzana P. 2012. Chlorfluazuron as reproductive inhibitor. In Farzana P, ed, Insecticides—Pest Engineering. InTech, Rijeka, Croatia, pp 23–62. 11. Omatsu M, Yoshida K, Toki T. 1991. Development of malformed larvae induced by a benzoylphenyl urea insecticide, chlorfluazuron, in the common cutworm Spodoptera litura Fabricius. J Pestic Sci 16:189–194. 12. Central Insecticide Boards and Registration Committee. 2016. Major uses of pesticides. Directorate of Plant Protection, Quarantine and Storage, Department of Agriculture, Cooperation and Farmers Welfare, Ministry of Agriculture and Farmers Welfare, Government of India, Faridabad, Haryana, India. [cited 2016 September 9]. Available from: http://www.cibrc.nic.in/mup.htm 13. Perveen F, Miyata T.

2000. Effects of sublethal dose of chlorfluazuron on ovarian development and oogenesis in the common cutworm. Spodoptera litura (Lepidoptera: Noctuidae). Ann Entomol Soc Am 93:1131–1137. 14. Demark JJ, Bennett GW. 1990. Ovicidal activity of chitin synthesis inhibitors when fed to adult German cockroaches (Dictyoptera: Blattellidae). J Med Entomol 27:551–555. 15. Quesada BL, Montoya-Lerma J. 1994. Laboratory evaluation of chlorfluazuron against larval phlebotomine sand flies (Diptera: Psychodidae). J Econ Entomol 87:1129–1132. 16. Peters BC, Fitzgerald CJ. 2003. Field evaluation of the bait toxicant chlorfluazuron in eliminating Coptotermes acinaciformis (Froggatt) (Isoptera: Rhinotermitidae). J Econ Entomol 96:1828–1831. 17. Rojas MG, Morales-Ramos JA. 2004. Disruption of reproductive activity of Coptotermes formosanus (Isoptera: Rhinotermitidae) primary reproductives by chitin synthesis inhibitors. J Econ Entomol 97:2015–2020. 18. Lewis KA, Tzilivakis J, Warner D, Green A. 2016. An international database for pesticide risk assessments and management. Hum Ecol Risk Assess 22:1050–1064. 19. Department of Agriculture and Cooperation, Ministry of Agriculture, Government of India. 2015. AESA based IPM—Cruciferous vegetables. AESA based IPM Package 19. New Delhi, India. [cited 2017 January 24]. Available from: http://farmer.gov.in/imagedefault/ipm/AESA%20based %20IPM%20Cabbage %20and%20Cauliflower%20(final%2025-02- 2014).pdf 20. Lu MX, Jiang WW, Wang JL, Jian Q, Shen Y, Liu XJ, Yu XY. 2014. Persistence and dissipation of chlorpyrifos in Brassica chinensis, lettuce, celery, asparagus lettuce, eggplant, and pepper in a greenhouse. PLoS One 9:e100556. 21. Paramasivam M, Selvi C, Chandrasekaran S. 2014. Persistence and dissipation of flubendiamide and its risk assessment on gherkin (Cucumis anguria L.). Environ Monit Assess 186:4881–4887. 22. Renwick AG. 1998. Toxicokinetics in infants and children in relation to the acceptable daily intake and TDI. Food Addit Contam 15:17–35. 23. Mukherjee I, Gopal M. 2000. Environmental behavior and translocation of imidacloprid in eggplant, cabbage and mustard.Pest Manag Sci 56:932–936. 24. Directorate General Health and Consumer Protection. 2010. Guidance document on pesticide residue analytical methods. SANCO/825/00/ rev. 8.1/16/11/2010. European Commission, Brussels, Belgium. 25. Edwards CA. 1975. Factors that affect the persistence of pesticides in plants and soils. Pure Appl Chem 42:39–56. 26. Shim JH, Abd El-Aty AM, Choi JH, Choi YS. 2007. Post-harvest HPLC determination of chlorfluazuron residues in pears treated with different programs. Biomed Chromatogr 21:695–700. 27. Krishnaswamy K. 2011. Dietary Guidelines for Indians—A Manual, 2nd ed. National Institute of Nutrition, Indian Council of Medical Research, Hyderabad, India. 28. Liang DX, Shang HL, Qian CF. Ming JX. 1992. The degradation and residues of chlorfluazuron in Chinese cabbage. Acta Phytophylacica Sinica 19:187–191. 29. Lee MH, Kim SH, Park YG, Shin BG, Kim JH, Kwon CH, Sohn JK, Kim JE. 2007. Residual pattern of pesticide, chlorfluazuron in perilla leaves under plastic house. Korean Journal of Pesticide Science 11:106–116.