EBAUB Journal Volume 1 January 2019

ISSN: 2617 - 8338

EBAUB Journal

An Academic Journal of
EXIM Bank Agricultural University Bangladesh

Cypermethrin Residue Analysis in Vegetables by Gas Chromatography with Electron Capture Detector (GC-ECD)

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ARTICLE INFO

Received date: Oct. 08, 2018
Accepted date: Jan. 10, 2019

ABSTRACT

Cypermethrin is a newly developed highly active insecticide of synthetic Pyrethroids that widely used in the agricultural crops mainly fruit and vegetables to protect from insect pests. Pyrethroid group of insecticide popularity is increasing because of its excellent effectiveness at low concentration, low environmental persistence and very low mammalian toxicity. This study was aimed to analyze Cypermethrin residue level using gas chromatography with electron capture detection method (GC-ECD) with three most popular vegetables Brinjal (*Solanum melongena*), Okra (*Abelmoschus esculentus*) and Country bean (*Lablab purpureus*). Cypermethrin was sprayed at 1ml/L and 2ml/L of concentrations in the field as per methodology and then residues were determined in different durations from 0 to 15 days after spraying (DAS). Cypermethrin residue was detected bellow maximum residue limits (MRL: 0.5 ppm) in Brinjal at 5 DAS, in Okra at 7 DAS and in Country bean at 10 DAS when 1.0 ml/L Cypermethrin applied. When applies as 2ml/L Cypermethrin, residue found immediate above MRLs at 7 DAS in Brinjal, Okra and Country bean at 0.511 ppm, 0.723 ppm and 0.562 ppm respectively. This study clarified that harvest of these three crops recommended at 5 DAS when low concentration Cypermethrin will apply.

Key words: Cypermethrin, GC-ECD, Insecticide, Pyrethroid, Residue, Vegetables

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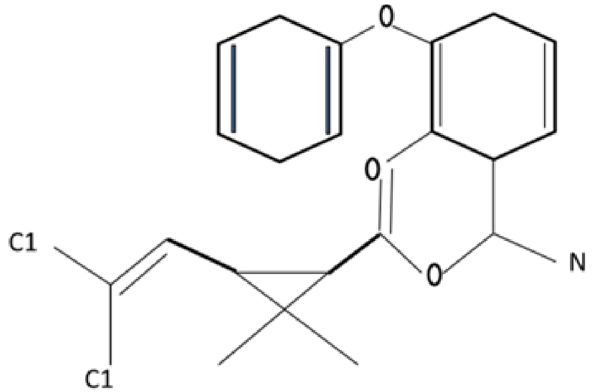
1. INTRODUCTION

Brinjal, Okra and Country bean are the most popular vegetables that grow more or less round the seasons in Bangladesh. Insect mainly borer pest is one of the major problems during production of these vegetables and farmers apply various types of chemicals for mitigating this problem. Cypermethrin [(±)-α-Cyano-(3-phenoxyphenyl)methyl(±)-cis/trans-3-(2,2dichlorovinyl)-2,2 dimethylcyclopropane carboxylate] is a synthetic Pyrethroid insecticide with

To Cite: Rahman, S., Sohel, M.H., Sayed, M,A., Ferdous, H.M & Prova, A. (2019). Cypermethrin residue analysis in vegetables by Gas Chromatography with Electron Capture Detector (GC-ECD). *EBAUB J*, 1, 123-129.

relatively lower mammalian toxicity, selective insecticide activity and lower environmental persistence.

Although posing minimal threat to mammals and avian species, Pyrethroids are extremely toxic to aquatic organisms (Saha & Kaviraj, 2008). It is used to control many pests including lepidopterous pests of cotton, fruit, and vegetable crops and is available as an emulsifiable concentrate or wettable powder. Now-a-days it is very popular insecticide to control caterpillar pest of vegetables in Bangladesh (Rahman et al., 2014). About 98% of applied insecticide goes to the non-target species, air, water, soil and pollute the environment (Miller, 2004). Indiscriminate use of pesticide kills the natural enemy (Bacci et al., 2007) including predators and parasitoid (Ngowi et al., 2007), contaminate the soil, ground water and affect the human health depending on the toxicity (Anwar & Yunus, 2013; Sarwar, 2015). Residue of insecticide persists in the edible part of the vegetables and consumption of these vegetables causes hazards to human health through the food chain (Nafees & Jan, 2009). Many developed countries have set Maximum Residue Limits (MRLs) based on Potential Daily Intake (PDI) and Acceptable Daily Intake (ADI) for the safe consumption (Rahman, 2007). It is recorded that use of pesticides on the edible vegetables has raised risk of users with different acute and chronic diseases (Fatema et al., 2013). Researchers found that about 50-70% of the edible vegetables are contaminated with the toxic insecticidal residue (Karanth, 2002). No insecticide is found within the retention period less than three to five days (Rahman, 1999). A whimsical spray of insecticides and selling of vegetables after one to two days of spray application is assumed to be a normal practice because the lack of knowledge about pesticide residue among the farmers (Miah et al., 2014). In plants, the metabolic Cypermethrin degradation pathways have been studied in lettuce and cabbage plants. The primary metabolic reaction is ester cleavage which produces α-cyano-3phenoxybenzyl alcohol. Hydroxylation also occurs in various positions, but is a fairly minor pathway. Therefore, the present study was undertaken to analyze the residue status of Cypermethrin in vegetables and to find out the pre-harvest time of the vegetables for safe consumption.



Chemical structure of Cypermethrin.

2. MATERIALS AND METHODS

Fruits sample collection

Different concentrations (1ml/L and 2ml/L) of Cypermethrin (market name "Ripcord 10 EC", BASF Bangladesh Limited) in water were applied in the experimental Brinjal, Okra and Country bean fields (each plot 3m × 3m) considering Randomized Complete Block Design (RCBD) with three replications (blocks).Representative fruit samples of Brinjal, Okra and Country bean were collected randomly from each plot of the same treatment and make it one kg (one sample)at 0, 1, 3, 5, 7, 10, 12 and 15 days after spraying (DAS) to determine the residues present in the fruits. The collected samples were kept in deep freeze (-22^{0}C). The residue analysis was done at Insecticide Toxicology Laboratory, Department of Entomology, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh. Gas Chromatograph (GC)-17A and Electron Capture Detector (ECD) was used to quantify the residue level in fruit samples. The standard sample of Cypermethrin was obtained from Sigma-Aldrich Laborchemikalien, Germany. The formulated product of Cypermethrin (Ripcord 10 EC) was tested in the laboratory and confirmed 100% purity.

Extraction of Cypermethrin from fruit samples

The frozen fruit samples of Brinjal, Okra and Country bean were taken from the deep refrigerator and kept at room temperature for 5-6 hours for thawing. The experiment was conducted by the following procedure i.e., extraction, separation and cleanup of the samples. Around 250 g of each vegetable fruit sample was grounded thoroughly with the meat grinder (Hand Mixer M-122, Bamix, Switzerland). The 100 mL of hexane was added to grounded sample (20 g). Sodium sulfate (Na_2SO_4) was added to the sample until the water was removed. The mixture was macerated with mortar pestle and homogenized material poured into 250 mL conical flask and shacked (Orbital Shaking Incubator) for 6 hours. The slurry was filtered through a Buchner funnel with suction. The filtrate was dried to 5-7 ml by evaporate using a rotary vacuum evaporator. The concentrated filtrate transferred into 500 ml separatory funnel making 10 ml volume with hexane. Around 20 ml methanol was added as color removal with 10 ml filtrate and shaken vigorously for 3-5 minutes in a shaker. The clear solution was centrifuged at 12000 rpm for 5 min and supernatant was cleaned up by SPE cartridge. The final volume was adjusted to 10 ml and 2 µl was used.

Quantification of Cypermethrin in samples

The concentrated extracts were subjected to analysis by GC-17A (Shimadzu) with Electron Capture Detector (ECD). The capillary column was AT-1, 30 m length, 0.25 mm ID and 0.25 µm film thickness. Nitrogen was used as a carrier and make up gas in ECD. The instrument parameters for GC-ECD to quantify the Cypermethrin residue were as follows: injection mode split; temperature 280^{o}C; flow control mode linear velocity and split ratio 10. The initial column temperature was 150^{o}C and then temperature increased to

160^{o}C and held for 1 min. Temperature further increased to 270^{o}C at the rate of 10^{o}C/min and held for 6 min. Finally the ECD temperature reached to 300^{o}C and total run time was confirmed by 18 min, where current supply: 1.00 pA and flow: 30 ml/min. were maintained. Prior to injection of the sample extract, standard solutions of Cypermethrin at different concentrations were prepared and injected with the above instrument parameters. The sample was calibrated (retention time, peak area, etc.) against four pointed calibrate ion curve of standard solution of concentrated insecticide. Each peak was characterized by its retention time. Sample results were expressed in ppm automatically by the GC software which represented the concentration of the final volume injected. From this value the amount of insecticide residue was determined with following formula:

Table 1 Quantity of Cypermethrin residues in Brinjal (*Solanum melongena*), Okra (*Abelmoschus esculentus*) and Country bean (*Lablab purpureus*).

Sample Collection (DAS)*	Concentrations of Cypermethrin residue (ppm) when 1.0 mL/Lcypermethrin apply			Concentrations of Cypermethrin residue (ppm) when 2.0 mL/Lcypermethrin apply			MRLs (ppm) *
	Brinjal	Okra	Country Bean	Brinjal	Okra	Country Bean	
0	2.791	2.816	2.723	3.217	3.310	3.018	0.5
1	2.032	2.120	2.252	2.608	2.714	2.561	0.5
3	1.065	1.095	1.125	1.463	1.562	1.338	0.5
5	0.042	0.525	0.469	1.061	1.074	1.043	0.5
7	0	0.065	0.051	0.511	0.723	0.562	0.5
10	0	0	0	0.031	0.085	0.040	0.5
12	0	0	0	0	0	0	0.5
15	0	0	0	0	0	0	0.5

*DAS: Days after spraying; MRLs: Maximum Residue Limits (recommended by FAO/WHO, 1993)

Concentration of injected sample (ppm) × Quantity of final volume (µl) / Amount of sample (kg).

Percentage (%) Degradation rate of Cypermethrin was calculated by using following formula:

100 – [Residue (ppm) at calculated day/Residue (ppm) at 0 Days after spraying] x 100.

3. RESULTS

Residues level of Cypermethrin in vegetable samples

The concentrated extracts of Brinjal, Okra and Country bean samples at different days after spraying (DAS, from 0 to 15 days) were analyzed by GC-2010to determine residues level of Cypermethrin. The results of Cypermethrin residues levels of three vegetables are summarized in Table 1.

Cypermethrin residues were detected (when applied at 1 ml/L) in the Brinjal fruit at 5 DAS (0.042 ppm) that immediate bellow to MRLs and at 3 DAS (1.065 ppm) that immediate above to MTLs. In the case of 2ml/L of Cypermethrin application, residue was detected at 10 DAS (0.031 ppm) and the quantities were above MRLs at 7 DAS (0.511 ppm). The absence of residue in the fruits found from 7 to 15 DAS when 1 mL/L Cypermethrin apply and it found from 12 to 15 DAS when 2 mL/L Cypermethrin apply.

Cypermethrin residues were found in Okra fruit at 7 DAS (0.065 ppm) and at 10 DAS (0.085 ppm) and quantities were above MRLs at 5 DAS (0.525 ppm) and at 7 DAS (0.723 ppm) when the Cypermethrin concentration was 1ml/L and 2ml/L, respectively. Free residual obtained in Okra fruit from 10 to 15 DAS when 1 ml/L Cypermethrin apply and it obtained from 12 to 15 DAS when 2 ml/L Cypermethrin apply.

It can be revealed that Cypermethrin residues were detected (when applied at 2 ml/L) in the Country bean fruit sample at 10 DAS (0.040 ppm) and the quantity was above MRLs at 7 DAS (0.562 ppm). When Cypermethrin applied 1ml/L in the Country bean sample, residues were found at 7 DAS (0.051 ppm) and the quantity was above MRLs at 3 DAS (1.125 ppm). No residual found in Country bean fruit from 10 to 15 DAS when 1 ml/L Cypermethrin apply and it found from 12 to 15 DAS when 2 ml/L Cypermethrin apply. Cypermethrin residues were detected up to 7 DAS and up to 10 DAS at 1ml/L and 2ml/L, respectively among all of the three vegetables (Brinjal, Okra and Country Bean).

Degradation pattern of residues in vegetable samples

Cypermethrin residue degradation in three vegetables (Brinjal, Okra and Country bean) samples is presented in Fig. 1, examined at 1, 3, 5, 7 and 10 DAS.

In Brinjal, 27.19% Cypermethrin residue was degraded at 1 DAS, which gradually increased to 61.84% at 3 DAS and 98.49% at 5 DAS. At 7 and 10 DAS degradation rates were 100% i.e. no residue was found, when sprayed 1mL/L of Cypermethrin. In case of the concentration 2ml/L, degradation rates were 18.94%, 54.53%, 67.02%, 84.11% and 99.03%those took1, 3, 5, 7 and 10 DAS. Hundred percentage degradation of Cypermethrin obtained at 10 DAS in Okra sample when 1 ml/L concentration sprayed. From 1 to 7 DAS, Cypermethrin gradually degraded with increasing of duration. The degradation rates were found 24.72%, 61.11%, 81.35% and 92.70% at 1, 3, 5 and 7 DAS, respectively. The similar observation in degradation rate of Cypermethrin was found in case of 2 ml/L concentration application but 100% reduction was not found in lieu of this it was 97.43% at 10 DAS. The rates of 18.01%, 62.81%, 67.55% and 78.16% degradation seems to observe at 1, 3, 5 and 7 DAS. For the Country bean sample, residue of Cypermethrin degradation 98.08% (7 DAS) and 98.67% (10 DAS) for 1ml/L and 2ml/L Cypermethrin respectively, were highest levels. The lowest rates were found 17.30% and 15.15% at 1 DAS for both concentrations i.e. 1 ml/L and 2 ml/L, respectively. The reduction rates were 58.69% and 55.67%, 82.77 and 65.44%, 98.08% and 81.38% at 3, 5 and 7 DAS for 1 ml/L and 2 ml/L concentrations of Cypermethrin, respectively.

4. DISCUSSION

Cypermethrin acts mainly on the nervous system of vertebrates and invertebrates act as both stomach poison and a contact poison (Jin & Webster, 1998).These long-lasting trains can cause hundreds to thousands of repetitive nerve impulses in the sense organs. This repetitive activity is induced by Pyrethroid damage to the voltage-dependent sodium channel inhibiting ATPase enzymes, causing sodium channels to stay open much longer than normal (Vijverberg & van den Bercken, 1990).

In plants, the metabolic Cypermethrin degradation pathways have been studied in lettuce and cabbage plants. The primary metabolic reaction is ester cleavage which produces α-cyano-3phenoxybenzyl alcohol. Hydroxylation also occurs in various positions, but is a fairly minor pathway. Studies were conducted under field and greenhouse conditions. Degradation occurred more rapidly under field conditions with the greenhouse half-life reported to be approximately 8 days (Leahey, 1985). Cypermethrin occurs as a mixture of both the cis- and trans- isomers. The cis-/trans- ratio in technical grade Cypermethrin is 1:1 (Kidd & James, 1991). Furuzawa et al. (1981) reported different half lives for cis- and trans-cypermethrin isomers in cabbage. The initial degradation half-lives on and in cabbage leaves were 4-5 and 7-8 days for trans- and cis- isomers, respectively. Therefore, cis- isomers remain active longer and degrade more slowly than trans isomers.

In our experiment we found Cypermethrin residue up to 5, 7 and 10 DAS and among them above MRLs up to 3, 5 and 5 DAS on Brinjal, Okra and Country bean, respectively with concentration 1ml/L. In 2ml/L concentration, we found residues up to 10 DAS and among them above MRLs at 7 DAS, at three vegetables experimented. To degrade 100% Cypermethrin, it required at least 5 to 10 DAS depending upon concentration used.

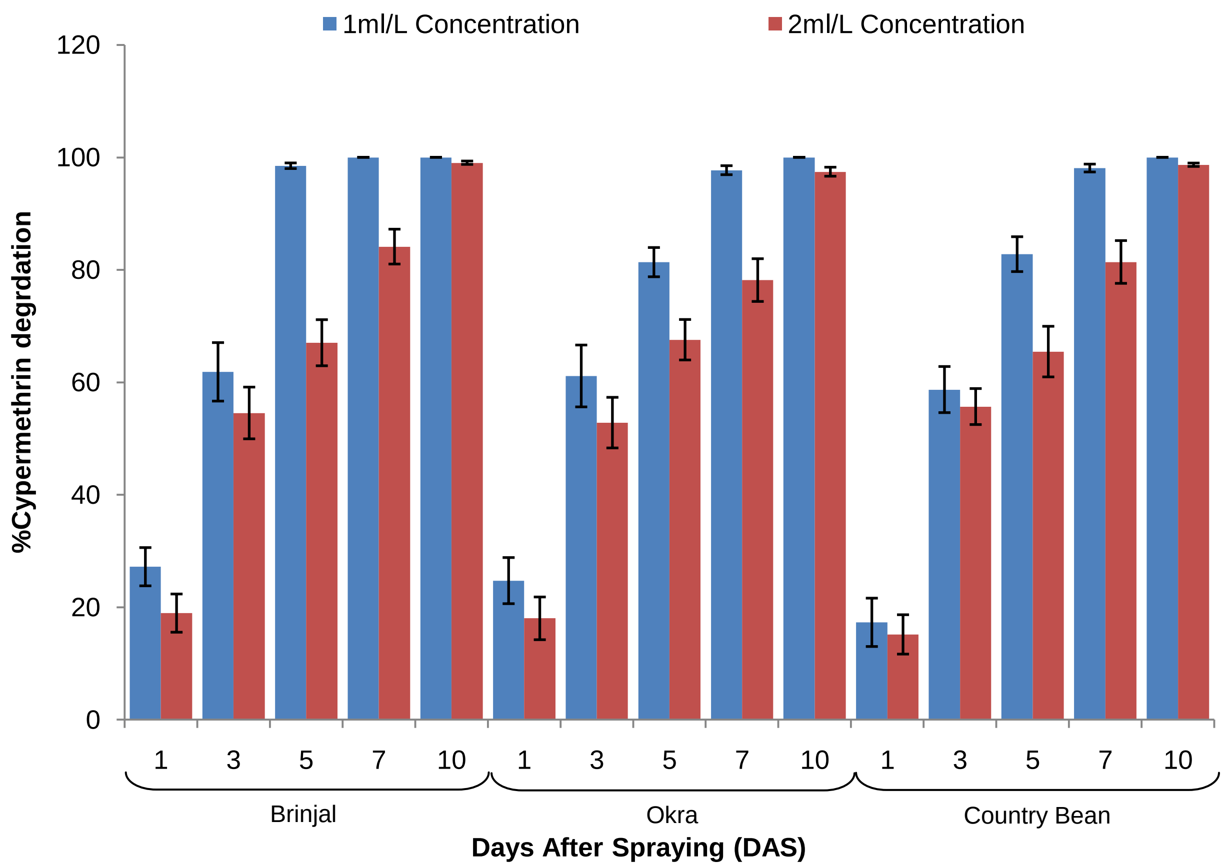


Fig. 1 Degradation pattern of Cypermethrin residues in three vegetables (Brinjal, Okra and Country Bean).

Cypermethrin has also been used to control the elm bark beetle and, according to Jin and Webster (1998), acts more rapidly than chlorpyrifos. Cypermethrin was applied at the basal 1m of the trunks of elm trees. Elm bark, cambium and wood tissue were collected separately to determineits ability to penetrate, and separate bark samples were taken to determine Cypermethrin's persistence. The experimental results indicated that Cypermethrin does not penetrate the bark and yet it is still 100% effective in killing bark beetles after 60 days from the application (Jin & Webster, 1998).

Rahman et al. (2015) reported that in eggplant Cypermethrin residue was found above MRLs up to 3 and 7 DAS when it was treated at the rate of concentration of 1ml and 2ml/L of water. Similar results were found in case of

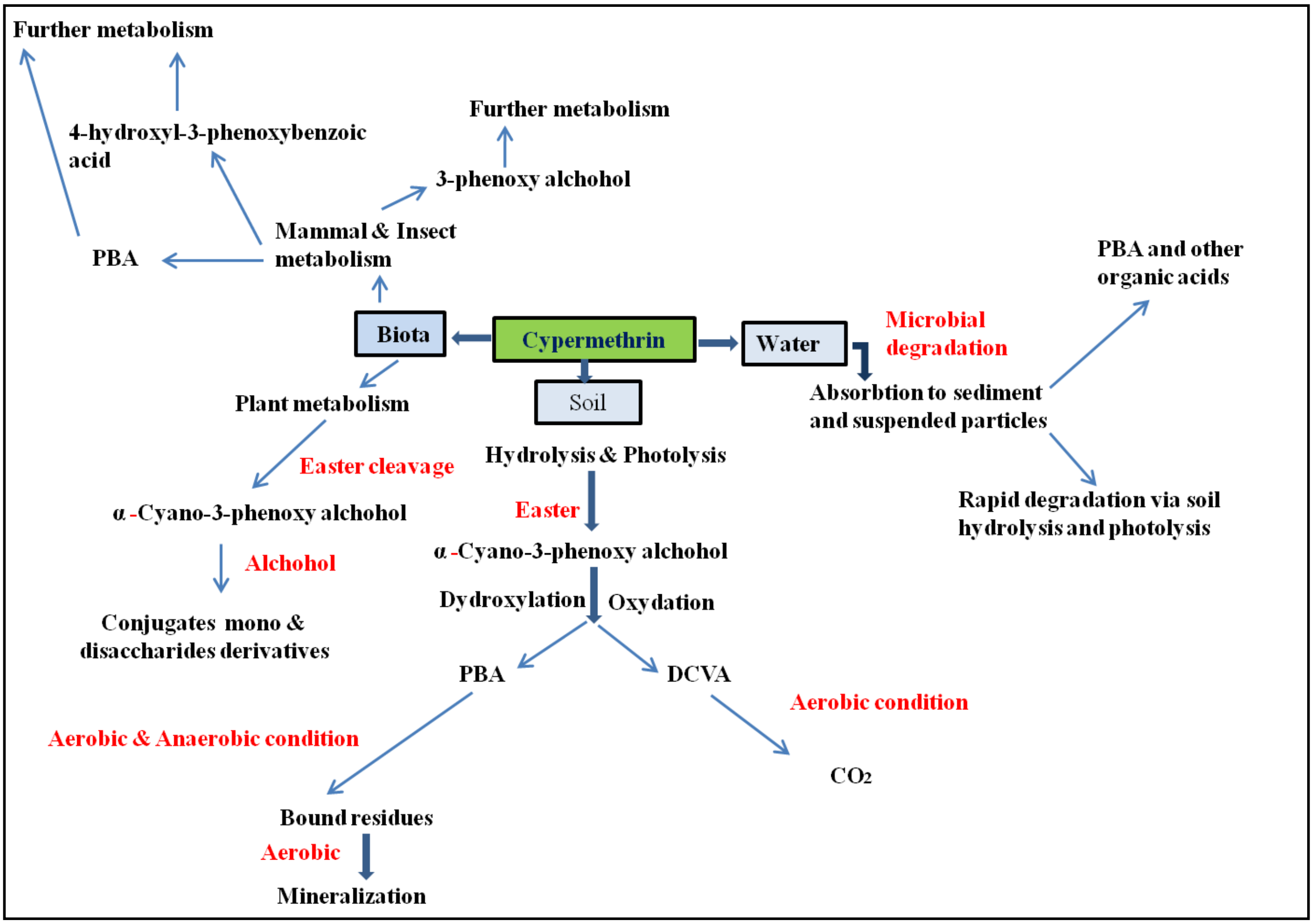


Fig. 2 The pathway of Cypermethrin degradation process in biota, soil and water.

Okra vegetables reported by Uddin et al.(2016). Whereas Cypermethrin residues in Yard-long bean were found above the MRLs up to 5 DAS, reported by Prodhan et al. (2010). Lozowicka et al. (2015) showed that, in the tomatoes, the levels of three Pyrethroid residues (10 samples, 17.8 %) were 0.02–0.25 mg kg^{-1} for lambda-cyhalothrin, 0.03 mg kg^{-1} for cyfluthrin and 0.1–0.09 mg kg^{-1} for Cypermethrin. Sah et al., (2018) analyzed ten samples of Brinjal, collected from market and found 7 samples contained pesticide residues. 3 samples of brinjal were found contaminated with endosulphan (0.276- 0.840 mg kg-1), two with quinalphos (0.076-0.156 mg kg-1), and one with fenvalerate (0.385 mg kg-1) which were below MRL. However, one sample of Brinjal contained Cypermethrin residues (0.407 mg kg-1) above MRL. The highest contaminated sample is Brinjal followed by cauliflower, cabbage and tomato reported by Charan et al. (2010) in farm gate vegetables.

Cypermethrin is relatively non-persistent in soil, and in sandy soil, it persists for 2-4 weeks. Increased Cypermethrin persistence was observed in soil with high organic matter, high clay content, reduced microbial activity and anaerobic conditions (Meyer et al., 2013). Ahn et al., (2011) has shown that Diazinonand Chlopyrifos residues were stable up to 30months.Cypermethrin poses a substantial threat to fish and other aquatic organisms and is highly toxic to honeybees (Lozowicka et al.,2013).The indiscriminate use of pesticides in rural and urban area of Bangladesh has contaminated agricultural producers, which affected soil health, surface water, aquifers, wildlife, food and feeds all over the country

(Handa et al., 1999; Dutta et al., 2010; Sofo et al., 2012). Cypermethrin may cause DNA damage and reduces mitotic and nuclear divisions in human (Kocaman &Topaktas, 2009). Its dermal exposure caused repetitive firing of sensory nerve endings leading to systemic signs characterized by dizziness, headache and disturbances in consciousness, muscular fasciculation, convulsive attacks and coma (Perger & Szadkowski, 1994; Luty et al., 1998).

CONCLUSION

Cypermethrin is a synthetic, pyrethroid insecticide that is extremely effective against a wide range of insect pests. In this study, the Cypermethrin residues were detected around 10 days after spraying and those were above the maximum residue limit level up to 7 days after spraying when the concentration of Cypermethrin was 2 ml/L. Whereas, when the concentration of Cypermethrin was 1 ml/L, the residues were detected up to 5 to 7 days after spraying and those were above the maximum residue limit level up to 3 to 5 days after spraying. As a result, it might be a suggestion to the farmers to use insecticide at a minimum rate to keep the environment safe.

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