Asian Australas. J. Biosci. Biotechnol. 2021, 6 (2), 60-67; doi: 10.3329/aajbb.v6i2.56141

Asian-Australasian Journal of Bioscience and Biotechnology

ISSN 2414-1283 (Print) 2414-6293 (Online) www.ebupress.com/journal/aajbb

Article

Estimation of residue degradation of cypermethrin and chlorpyrifos in brinjal, tomato and cauliflower under supervised field trial

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Received: 16 June 2021/Accepted: 10 August 2020/ Published: 31 August 2021

Abstract: A study was carried out to detect and estimate the residue of cypermethrin and chlorpyrifos in brinjal, tomato and cauliflower using Gas Chromatography (GC) coupled with Electron Capture Detector (ECD) and Flame Thermionic Detector (FTD). Three supervised field trials were conducted and sprayed with the recommended dose of cypermethrin (1 ml/L of water) and chlorpyrifos (3 ml/L of water) in three vegetables by knapsack sprayer. Samples were collected at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and 11 days after spray (DAS). The quantity of cypermethrin residue was above EU-MRLs (Maximum Residue Limit set by European Union) up to 4 DAS with 0.538 mg/kg in cauliflower; 3 DAS with 0.508 mg/kg in brinjal and 0.695 mg/kg in tomato. The quantity decreased down 0.328-0.019 mg/kg in cauliflower (4-9 DAS); 0.098-0.012 mg/kg (4-7 DAS) in brinjal and 0.458-0.022 mg/kg (4-7 DAS) in tomato which were below EU-MRLs (0.5 mg/kg). The quantity of chlorpyrifos residue was above EU-MRLs up to 9 DAS with 0.012 mg/kg in cauliflower but in brinjal and tomato, the detected quantities were above EU-MRLs up to 7 DAS with 0.029 mg/kg and 0.017 mg/kg residue. No residue was detected from brinjal and tomato samples collected at 8 DAS. But in cauliflower, no residue was detected at 10 days after spray. So, brinjal, tomato and cauliflower can be harvested safely at 4 DAS and 5 DAS for cypermethrin and in case of chlorpyrifos it was 8 DAS in brinjal and tomato and 10 DAS in cauliflower.

Keywords: residue; degradation; chlorpyrifos; cypermethrin; MRL; gas chromatography

1. Introduction

Cauliflower, brinjal and tomato are rich in vitamins and minarals and consumers of Bangladesh are well-liked. At present, quality and safe food production is a public demand in Bangladesh. But vegetable productions are seriously hampered by the attack of insect pests. Brinjal, cauliflower and tomato are attacked by a variety of insect pests (Nayar *et al.*, 1994; Rekha and Mallapur, 2007; Riley and Sparks, 2008). The farmers of Bangladesh spray pesticides as a major component of plant protection because of its small quantity in packaging, storage facility at nomal temperature and availability in the market. The pattern of insecticide usage in vegetables led to assume that major vegetable growing areas of Bangladesh should be overloaded with pesticide residue, since pesticides are being used by farmers whimsically. It was understood from farmers' interview that they use insecticides indiscriminately. Most of the commercial farmers sell vegetables at an interval of 0-2 days after spray (Anon, 2001; Ahmed *et al.*, 2005). It was reported that over-sprayed vegetable consumers might face health hazards and environment might be over loaded with pesticide residue. Synthetic toxic pesticide can become hazard to the public and the environment. Pesticide can produce negative impacts, both social and private (Antle and Pingali, 1994). Some of the acute and chronic human illnesses have now emerged as a consequence of intake of polluted water, air or food (Gill and Garg, 2014). There is a link between pesticides

exposure and the incidences of human chronic diseases affecting nervous, reproductive, renal, cardiovascular, and respiratory systems (Mostafalou and Abdollahi, 2012).

In Bangladesh, most of the vegetable growers do not follow the prescribed dosages and use pesticides at any stage of the crop without maintaining waiting period. By this time, the pesticide residues get dissipated of its abuse. It is noted that food products become safe for consumption only after withholding period has lapsed. The monitoring of pesticide residue mainly in vegetables was done in regular basis in many countries (Van der Schee, 2002; Kumar *et al.*, 2004; Rajeshwaran *et al.*, 2004, Ahmed *et al.*, 2016a; Ahmed *et al.*, 2018a; Ahmed *et al.*, 2019; Ahmed *et al.*, 2021). The extent and rate of dissipation depends on the nature of the pesticide, crop, cultural practices and various environmental conditions under which the crop is grown or a treated commodity is stored (Handa *et al.*, 2018b, Prodhan *et al.*, 2018; Ahmed *et al.*, 2020). Considering these, the present study was carried out to estimate the amount and degradation rate of residue of two commonly used insecticides in brinjal, tomato and cauliflower.

2. Materials and Methods

The present study was conducted at Pesticide Analytical Laboratory and field of Entomology Division, Bangladesh Agricultural Research Institute Gazipur (BARI). The insecticide standard for chlorpyrifos and cypermethrin were obtained from Sigma-Aldrich Laborchemikalien, Gmbh P O Box-100262 D-30918, Seelze, Germany via Bangladesh Scientific Pvt. ltd. Dhaka, Bangladesh. Standards of chlorpyrifos contained 99.3% purity and cypermethrin had 98% purity. Marketable size of brinjal, tomato and cauliflower were collected from three different supervised field trials at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11 days after spray (DAS) which were sprayed with chlorpyrifos @ 3 mL per litre of water and cypermethrin @ 1 ml/L of water by knapsack sprayer. The formulated products of those were Dursban 20EC and Cymbush 10EC. The purity of formulated brand of both the insecticides were tested in the Pesticide Analytical Laboratory and found 100% pure.

2.1. Sample extraction, separation and clean up

The sample extraction technique was used prescribed by William and George (2005) that was adopted with little modifications. Collected samples (\geq 250g) were chapped and grounded thoroughly with the meat grinder (Handmixer M-122, Bamix, Switzerland). A sub sample of 20 g was taken into a wide mouth jar then 100 mL of hexane was added to it. Sodium sulphate (Na₂SO₄) was also added with sample until water was removed from the sample. The mixture was then macerated with high-speed homogenizer (Ultraturax, IKA T18 basic, Germany) for 2 minutes. The homogenized material was then poured into 250 mL conical flask and placed into the shaker (Orbital Shaking Incubator, Rexmed, Sweden) for 12 hrs continuous shaking. After shaking, the slurry was filtered through a Buchner funnel with suction. The flask and filter cakes were rinsed with 25 mL of hexane each. The filtrate was then transferred into 250 mL round bottom flask and was dried to 5 mL by evaporation using a rotary vacuum evaporator (Laborota-4001, Heidolph, Germany). The concentrated filtrate was then transferred into 500 mL separatory funnel making 10 mL in volume. Around 20 mL methanol was added with 10 mL filtrate and shaked vigorously for 5 minutes. After shaking, the separatory funnel was set on stand and kept undisturbed for 5 minutes. Then the clear part of the solution from the bottom of the separatory funnel was collected in a vial which was then centrifuged at 1200 rpm for 5 minutes (Laboratory Centrifuges, Sigma-3K30, Germany). After centrifuge, supernatant was collected for injection in Gas Chromatography.

2.2. Detection and estimation of pesticide residue in samples

The concentrated extracts were subjected to analysis by GC-2010 (Shimadzu). Flame Therm ionic Detector (FTD) was used for the detection of chlorpyrifos and the Electron Capture Detector (ECD) was used for the detection of cypermethrin. The capillary column used in FTD was ATTM-1, length 30m, Inner Diameter (ID) 0.25mm and film thickness 0.25µm and in case of ECD it was Optima 1 and length, ID and film thickness was same. Nitrogen was used as carrier and make up gas in ECD and in FTD it was Helium. The instrument parameters for detecting chlorpyrifos and cypermethrin were in Table 1.

Table 1.	The	instrument	narameters	for	GC-FTD	and	GC-ECD
Lanc L.	Inc	msti ument	parameters	101	GC-FID	anu	GC-ECD.

Instrument parameters	Chlorpyrifos	Cypermethrin
Instrument	GC-2010	GC-2010
[Injection Port SPL]		
Injector (Auto)	AOC 20i	AOC 20i
Injection Mode	Split	Split
Temperature	250^{0} C	280^{0} C
Flow Control Mode	Linear velocity	Linear velocity
Linear Velocity	40.0 cm/sec	40.0 cm/sec
Purge Flow	3.0 ml/min	3.0 ml/min
Split Ratio	30.0	10.0
Injection Volume	2.0 µl	2.0 μl
[Column Oven]		
Column Oven Temperature Program:		
Initial Temperature	$150^{0}C$	$160^{\circ}C$
Final Temperature	220^{0} C	270^{0} C
Equilibrium Time	1.0 min, Hold time: 2	1.0 min, Hold time: 6
Total Program Time	10.0 min	18.0 min
[Column Information]		
Column Name	ATTM-1	Optima-1
Column Length	30.0 m	30.0 m
Film Thickness	0.25 μm	0.25 μm
Inner Diameter	0.25 mm	0.25 mm
[Detector Channel 1]		
Detector	FTD	ECD
Temperature	280^{0} C	300^{0} C
Stop Time	10.0 min	18.0 min
Current	1.0 pA	1.0 pA
Makeup Flow	30.0 ml/min	30.0 ml/min
H ₂ Flow	1.5 ml/min	-
Air Flow	145.0 ml/min	-

Prior to the injection of the sample extract, standard solutions of different concentrations of both insecticides were prepared and injected with the above instrument parameters. The samples were calibrated (retention time, peak area etc.) against four pointed calibration curve of standard solution of concerned pesticide. Each peak was characterized by its retention time. Sample results were expressed in mg/kg automatically by the GC software which represented the concentration of the final volume injected. From this value the actual amount of insecticide residue present in the sample was determined by using the following formula.

Conc. obtained in injected volume (mg/kg) X Quantity of final volume (L)

Residue in sample (mg/kg) = -----

Amount of sample taken (kg)

3. Results

The brinjal, cauliflower and tomato samples containing chlorpyrifos residues were analyzed using GC-FTD with set parameters and for the detection of cypermethrin residue, it was GC-ECD. The results obtained from this analysis have been summarized in Table 2-7.

Days after spraying	Sample weight (g)	Total volume prepared (ml)	Injected volume (µl)	Concentration obtained in final volume (mg/kg)	Amount of residue (mg/kg)	EU MRL (mg/kg)
0	20	10	2	3.144	1.572	0.5
1	20	10	2	1.646	0.823	
2	20	10	2	1.308	0.654	
3	20	10	2	1.016	0.508	
4	20	10	2	0.196	0.098	
5	20	10	2	0.114	0.057	
6	20	10	2	0.050	0.025	
7	20	10	2	0.024	0.012	
8	20	10	2	ND	ND	

Table 2. Quantity of residue of cypermethrin (Cymbush 10EC) estimated from brinjal.

Residue of cypermethrin in brinjal was detected up to 7 DAS and the quantities were above EU-MRL up to 3 DAS and these were 1.572 mg/kg, 0.823 mg/kg, 0.654 mg/kg and 0.508 mg/kg at 0, 1, 2 and 3 DAS, respectively. Samples of 4 to 7 DAS contained 0.098-0.012 mg/kg residues which are below MRL set by European Union. No residue was detected at 8 DAS.

	Table 3.	Quantity of	residue of	cypermethrin	(Cymbush	10EC)	estimated	from caulif	lower
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Days after spraying	Sample weight (g)	Total volume prepared (ml)	Injected volume (µl)	Concentration obtained in final volume (mg/kg)	Amount of residue (mg/kg)	EU MRL (mg/kg)
0	20	10	2	5.604	2.802	0.5
1	20	10	2	2.610	1.305	
2	20	10	2	1.884	0.942	
3	20	10	2	1.468	0.734	
4	20	10	2	1.076	0.538	
5	20	10	2	0.656	0.328	
6	20	10	2	0.402	0.201	
7	20	10	2	0.274	0.137	
8	20	10	2	0.102	0.051	
9	20	10	2	0.038	0.019	
10	20	10	2	ND	ND	

Residue of cypermethrin was detected up to 9 DAS in cauliflower. The detected quantities were above EU-MRL up to 4 DAS and these were 2.802 mg/kg, 1.305 mg/kg, 0.942 mg/kg 0.734 mg/kg and 0.538 mg/kg at 0, 1, 2, 3 and 4 DAS, respectively. While samples of 5 to 9 DAS contained residues 0.328-0.019 mg/kg which were below MRL set by European Union. No residue was detected at 10 DAS.

Table 4. (Duantity	of residue	of cype	rmethrin	(C ¹	vmbush	10EC)	estimated	from	tomato.
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Days after spraying	Sample weight (g)	Total volume prepared (ml)	Injected volume (µl)	Concentration obtained in final volume (mg/kg)	Amount of residue (mg/kg)	EU MRL (mg/kg)
0	20	10	2	5.964	2.982	0.50
1	20	10	2	3.452	1.726	
2	20	10	2	1.934	0.967	
3	20	10	2	1.390	0.695	
4	20	10	2	0.916	0.458	
5	20	10	2	0.178	0.089	
6	20	10	2	0.094	0.047	
7	20	10	2	0.044	0.022	
8	20	10	2	ND	ND	

The results revealed that residue of cypermethrin could be detected up to7 DAS in tomato. The quantities of residue were above EU-MRL up to 3 DAS and these were 2.982 mg/kg, 1.726 mg/kg, 0.967 mg/kg and 0.695 mg/kg at 0, 1, 2 and 3 DAS, respectively. While sample of 4 DAS to 7 DAS contained 0.458 mg/kg to 0.022 mg/kg cypermithrin residue which were below MRL set by European Union. No residue was detected at 8 DAS.

Days after spraying	Sample weight (g)	Total volume prepared (ml)	Injected volume (µl)	Concentration obtained in final volume (mg/kg)	Amount of residue (mg/kg)	EU MRL (mg/kg)
0	20	10	2	2.784	1.392	0.01
1	20	10	2	1.462	0.731	
2	20	10	2	1.034	0.517	
3	20	10	2	0.784	0.392	
4	20	10	2	0.422	0.211	
5	20	10	2	0.234	0.117	
6	20	10	2	0.134	0.067	
7	20	10	2	0.058	0.029	
8	20	10	2	ND	ND	

Table 5. Quantity of residue of chlorpyrifos (Dursban 20EC) estimated from brinjal.

The left over residue of chlorpyrifos in brinjal had been detected up to 7 DAS. The quantities of residues were 1.392 mg/kg, 0.731 mg/kg, 0.517 mg/kg, 0.392 mg/kg and 0.211 mg/kg 0.117 mg/kg, 0.067 mg/kg and 0.029 mg/kg at 0, 1, 2, 3, 4, 5, 6 and 7 DAS, respectively. All of the detected quantities (1.392- 0.029 mg/kg) of residues were above MRL set by European Union. No residue was detected at 8 DAS.

Table 6. Quantity of residue of chlorpyrifos (Dursban 20EC) estimated from cauliflower.

Days after spraying	Sample weight (g)	Total volume prepared (ml)	Injected volume (µl)	Concentration obtained in final volume (mg/kg)	Amount of residue (mg/kg)	EU MRL (mg/kg)
0	20	10	2	3.062	1.531	0.01
1	20	10	2	1.874	0.937	
2	20	10	2	1.622	0.811	
3	20	10	2	1.264	0.632	
4	20	10	2	0.956	0.478	
5	20	10	2	0.602	0.301	
6	20	10	2	0.394	0.197	
7	20	10	2	0.098	0.049	
8	20	10	2	0.054	0.027	
9	20	10	2	0.024	0.012	
10	20	10	2	ND	ND	

Form the Table 6, it was observed that chlorpyrifos residue was detected in the cauliflower sample up to 9 DAS. The quantities of residues were 1.531 mg/kg, 0.937 mg/kg, 0.811 mg/kg, 0.632 mg/kg, 0.478 mg/kg, 0.301 mg/kg, 0.197 mg/kg, 0.049 mg/kg, 0.027 mg/kg and 0.012 mg/kg at 0, 1, 2, 3, 4, 5, 6, 7, 8 and 9 DAS, respectively. All of the tested samples showed residues (1.531-0.012 mg/kg) which were above MRL set by European Union. At 10 DAS, no residue was detected.

Days after spraying	Sample weight (g)	Total volume prepared (ml)	Injected volume (µl)	Concentration obtained in final volume (mg/kg)	Amount of residue (mg/kg)	EU MRL (mg/kg)
0	20	10	2	2.172	1.086	0.01
1	20	10	2	1.474	0.737	
2	20	10	2	1.284	0.642	
3	20	10	2	1.026	0.513	
4	20	10	2	0.958	0.479	
5	20	10	2	0.266	0.133	
6	20	10	2	0.134	0.067	
7	20	10	2	0.034	0.017	
8	20	10	2	ND	ND	

Table 7. Quantity of residue of chlorpyrifos (Dursban 20EC) estimated from tomato.

The results revealed that residue of chlorpyrifos could be detected up to 7 DAS in tomato. The detected quantities were 1.086 mg/kg, 0.737 mg/kg, 0.642 mg/kg, 0.513 mg/kg, 0.479 mg/kg 0.133 mg/kg, 0.067 mg/kg and 0.017 mg/kg at 0, 1, 2, 3, 4, 5, 6 and 7 DAS, respectively. All of the detected quantities (1.086- 0.017 mg/kg) of residues in tomato were above EU-MRL. No residue was detected at 8 DAS.



Figure 1. Degradation of cypermethrin and chlorpyrifos in brinjal, cauliflower and tomato over time.

The trends of degradation of detected residue of cypermethrin and chlorpyrifos in the samples of three different vegetables viz. brinjal, cauliflower and tomato over time are shown in Figure 1. From the figure it was observed that the degradation rate of chlorpyrifos was slow in all of the analyzed vegetables although their residue levels were above MRL up to 7 DAS in brinjal and tomato and 9 DAS in cauliflower. The rate of degradation of cypermethrin was faster than chlorpyrifos in all of the vegetables and the levels of their residues were below MRL up to 3 DAS in brinjal and tomato and 4 DAS in cauliflower.

4. Discussion

The results revealed that residue of cypermethrin was detected up to 7 DAS and the quantities were above MRL up to 3 DAS in brinjal and tomato. While for at 4 to 7 DAS the residue decreased down 0.098-0.012 mg/kg in brinjal and 0.458-0.022 mg/kg in tomato that were below MRL. But in cauliflower the residue of cypermethrin was detected up to 9 DAS and the quantities were above MRL up to 4 DAS. Samples of 5 to 9 DAS contained 0.328-0.019 mg/kg residues which were below MRL. Residue of chlorpyrifos was detected up to 7 DAS in brinjal and tomato and in case of cauliflower it was 9 DAS. All of the detected quantities of chlorpyrifos residues were above MRL in vegetables. The rate of degradation of the tested insecticides was varied among the three tested vegetables. The plant behavior might be related to physico-chemical properties of pesticides for example uptake by plants, metabolism, etc. (O' Brein, 1967; Virgina and Bajet, 1996). Tejada *et al.* (1983) reported that the disappearance of residue in and on plants is the effect of the interaction of environmental

conditions such as the wind, rain, sun, humidity and temperature and chemical and physical factors such as volatilization and growth of the plant.

Agnihotri *et al.* (1990) and Singh and Kalra (1996) found detectable level of residue of cypermethrin and fenvalerate up to 7 days of spraying of recommended dose. Ahmed *et al.* (2016b) reported that acephate was detected up to 14 DAS in hyacinth bean and tomato which was the longer period of degradation than fenvalerate but their quantity were above MRL at 9 DAS in both the vegetables. For fenvalerate, the detected residue level was above MRL up to 3 DAS in hyacinth bean and tomato. Ahmed *et al.* (2020) found dimethoate residue was detected up to 11 DAS in cauliflower; 10 DAS in hyacinth bean and 9 DAS in eggplant which were longer period of degradation than quinalphos. The residue of quinalphos detected up to 9 DAS in cauliflower; 7 DAS in hyacinth bean and 8 DAS in eggplant and all of the detected quantities were above MRL in three vegetables. According to Prodhan *et al.* (2018) residue of quinalphos was detected up to 15 DAS in cauliflower; 12 DAS in eggplant; 10 DAS in yard long bean which was longer period of degradation than cypermethrin and the detected residue levels were above MRL up to 10 DAS in cabbage; 7 DAS in eggplant and 5 DAS in yard long bean. The residue of cypermethri was detected up to 7 DAS in yard long bean and tomato and the quantities of residues were above MRL up to 3 DAS in both the vegetables. The results of the present study agreed with the works of the earlier authors.

5. Conclusions

The degradation rate of cypermethrin was found faster in brinjal, tomato and cauliflower and slower in all the analyzed vegetables for chlorpyrifos. So, cypermethrin might be the most suitable insecticide for the vegetables which would be harvested at shortest period of time having waiting period of 4 DAS in brinjal and tomato and 5 DAS in cauliflower but in case of chlorpyrifos, vegetables could be harvested at 8 DAS in brinjal and tomato and 10 DAS in cauliflower. The findings of the study will help farmers, consumers and other stakeholders to undertake proper activity for the production of safe vegetables.

Conflict of interest

None to declare.

Acknowledgements

The authors are thankful to Pesticide Analytical Laboratory, Division of Entomology, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur for financial assistance to carry out this study.

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