Soil Environ. 40(2): 141-145, 2021
DOI:10.25252/SE/2021/222714
Online ISSN: 2075-1141
Print ISSN: 2074-9546

Short communication
Evaluation of cypermethrin dissipation rate in tomato, okra and cauliflower

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[Received:** October 05, 2021* ***Accepted:** November 8, 2021* ***Published Online:** December 04, 2021**]

Abstract

The behavior and dissipation of cypermethrin in some vegetable crops were studied for compliance with newly adopted maximum residue limits (MRL) of the Codex Alimentarius Commission. The insecticide was sprayed at recommended doses (60 g a.i.ha^{-1}) on tomato, okra, and cauliflower at the fruit initiation stage. The treated fruits of the vegetables were randomly sampled in triplicates at 0, 3, 5, 7, 10, and 15 day intervals after the insecticide treatment. The samples were exhaustively extracted in ethyl acetate, followed by florisil cleanup step for analysis of cypermethrin residues by high-performance liquid chromatography (HPLC). The cypermethrin dissipation conformed to pseudo–first–order kinetics. Cypermethrin degraded at an almost similar rate and the half-lives ranged between 3.1–3.3, 3.4–4.2, and 2.8–4.2 days in tomato, okra, and cauliflower, respectively. The pre-harvest intervals for compliance with MRLs were 14, 6, and 4 days for tomato, okra, and cauliflower, respectively.

Keywords: Cypermethrin, dissipation, pesticide residue, tomato, okra, cauliflower, HPLC

Cypermethrin or (RS)–∝–cyano–3–phenoxybenzyl (1RS, 3RS; 1RS, 3SR)–3–(2, 2–dichloro–vinyl)–2, 2–dimethyl cyclopropane carboxylate is a class II insecticide with digestive and contact mode of action against a wide range of insect pests, particularly those of Lepidoptera and Coleoptera in cotton, fruit, vegetables, vines, tobacco and other crops.(Macedo & Freire, 2011; Shilpakar & Karki, 2021). In Pakistan, class II cypermethrin insecticide is extensively used as a plant protection treatment. Despite a wide range of effectiveness, cypermethrin is not free from side effects, and direct and indirect human exposure appears in various signs and symptoms. Upon ingesting high doses, muscular tremors, ataxia, weakness of limbs, convulsions, coma, and death have been reported due to respiratory depression, while the dermal contact in the facial area may cause tingling or numbness (Ullah *et al.*, 2006). Cypermethrin has also been reported for carcinogenic and co–carcinogenic potential.(George & Shukla, 2011). It can also be metabolized to produce compounds with endocrine activities, such as 3–phenoxybenzoic acid (PBA) (Tyler *et al.*, 2000). The cypermethrin residues in crops usually result from direct application of cypermethrin in the field, and with rare instances of uptake from soil (Shah *et al.*, 2011) The cypermethrin applications as pre– or post-harvest treatment, however, leave residues on food products posing a potential risk to the consumer health (Jyot *et al.*, 2013). Although, considerable work has been done to study the cypermethrin residue dynamics and appropriate pre-harvest intervals for compliance with maximum residue limits (MRL) have been suggested for different crops and vegetables.(Tyler *et al.*, 2000; Ripley *et al.*, 2003; Gupta *et al.*, 2011; Horská *et al.*, 2020). WHO/FAO under Codex Alimentarius Commission has recently adopted modified guidelines of MRL. Because of this, an attempt has been made to study the residue dynamics of cypermethrin in okra, tomato and cauliflower. The study aims to investigate the risk potential of cypermethrin application, the residue dynamics and to determine safer ways of treatment as per newly adopted guidelines.

Certified, high purity (> 98%) reference standard of cypermethrin was purchased from Dr. Ehrenstorfer (Ausburg, Germany). Ethyl acetate, acetone, acetonitrile, methanol, and water of HPLC grade and n-hexane of analytical reagent grade were purchased from Merck. Anhydrous sodium sulphate, florisil (60–80 mesh), and charcoal of analytical reagent grade were also purchased from Merck (Darmstadt, Germany).

The working stock solutions containing cypermethrin were prepared at 10, 1.0, and 0.1 µg mL^{-1}. The working

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Cite This Paper: Khan, B.A., W.U. Khan, F. Anjum, B. Jan, R.A. Khan and Zulqarnain. 2021. Evaluation of cypermethrin dissipation rate in tomato, okra and cauliflower. *Soil Environ.* 40(2): 141-145.

Table 1: Meteorological data during the experiments

	Crop	Min temp (ºC)	Max temp (ºC)	RH (%)	Rainfall (mm)
Year 1	Tomato (13th –28th May)	21.3 ± 1.38	39.9 ± 2.54	59.6 ± 4.65	3.6
	Okra (5th –20th May)	20.1 ± 4.30	40.0 ± 1.53	54.7 ± 6.50	6
	Cauliflower (2nd – 17th Nov)	14.1 ± 6.20	26.1 ± 1.68	65.7 ± 4.35	0
Year 2	Tomato (15th – 30th May)	20.8 ± 2.56	38.6 ± 2.37	59.2 ± 5.93	0
	Okra (3rd –18th May)	19.3 ± 3.25	39.3 ± 2.56	50.3 ± 11.9	2.8
	Cauliflower (2nd – 17th Nov)	9.9 ± 2.67	27.0 ± 1.73	60.4 ± 5.16	2.8

standard solutions were used for spiking blank samples and the preparation of matrix-matched calibration solutions.

The supervised trial was conducted in two consecutive years in district Peshawar, KPK, Pakistan. Two summer vegetables viz. okra, tomato, and one winter vegetable cauliflower planted with appropriate row and plant dimensions were used for the study. The weather condition during the study period on respective vegetables during the two years is given in Table 1.

The vegetables grown in three plots (each plot with 30 plants was considered as a replication) were sprayed with cypermethrin 20% EC at the concentration of 60 g ai ha^{-1} using a knapsack sprayer at the fruit initiation stage. Each treatment plot was separated by a buffered area in the same field. Plots without treatment served as control.

To investigate the dissipation of cypermethrin on different vegetables, fruit samples (1 kg) were harvested from tomato, okra, and cauliflower plants randomly at different sampling dates (0, 3, 5, 7, 10, and 15 days after insecticide application) from the treated and untreated plots. The samples were chopped and kept in the freezer at –20°C for 48 hours. Frozen vegetable samples were milled cryogenically with dry ice in Stephen Chopper. Subsamples (200 g) were stored at –4°C for further analysis.

The extraction of cypermethrin residues from samples was carried out using a modified procedure given by Kadenezki *et al.*(Kadenczki *et al.*, 1992). The sample (50 g) was extracted with 75 mL ethyl acetate and 25 g anhydrous sodium sulphate in a 250 mL Erlenmeyer flask on a horizontal shaker (Bremen, Germany) for 2 h at 80 cycle min^{-1} in three stages. After each extraction, the ethyl extract was pooled by passing through a Whatman (No. 4) filter paper. The pooled extract was concentrated by evaporating the excess solvent on a rotary evaporator (Rotavapor, Buchi, Switzerland) initially and then the extract was concentrated to near dryness under a gentle stream of nitrogen on a dry block at 30ºC. The extract was quantitatively transferred to a 2 ml volumetric flask and made up to the volume with ethyl acetate for the cleanup step.

The extracts were subjected to the cleanup step following the procedure of Florisil adsorption chromatography technique (Krynitsky *et al.*, 1988). Glass column (15 cm × 25 mm) fitted with a draw-off valve was used for the clean-up. The column was packed with glass wool at the bottom followed by 5 g Florisil pre-activated at 150ºC for 24 h followed by charcoal (1 g) and a 4 cm layer of anhydrous sodium sulphate on top. The packed column was flushed with ethyl acetate and the flow rate was adjusted to 5 mL min^{-1}. The column was loaded with 2 mL extract and ethyl acetate was used for elution. The eluate was collected in a 100 mL round–bottom flask and was concentrated to near dryness under a gentle stream of nitrogen on a dry block at 30ºC. The concentrated extract was quantitatively transferred to a 1 mL volumetric flask and made up to the volume with acetone for analysis using high–performance liquid chromatography (HPLC) technique.

The determination step was carried out using a Shimadzu Chromatograph comprising of LC–10 AS pumps, 20–ll Reodyne injector, SPD–10A UV/VIS detector operating at 230 nm for cypermethrin. Chromatographic separation was performed using reverse-phase C-18 analytical column (Supelco, 25 cm × 4.6 mm (i.d)). The working condition of HPLC was binary gradient with acetonitrile: water (70:30) mobile phase adjusted at a flow rate of 1 mL min^{-1}. The mobile phases were pre-cleaned by passing through a 0.45 µm filter and degassed for 10 min to remove dissolved gasses. The injection volume was 20 µL.

The performance of the analytical procedure (HPLC–UV/VIS) was assessed by obtaining calibration curves for different matrix-matched calibration solution concentrations plotted against response. Instrument response was linear (r^2 = 0.998) over a wide range of concentrations 0.5 – 200 µg mL^{-1} = 0.01 – 4.0 mg kg^{-1}. The lower limits of detection (LOD) and quantification (LOQ) for cypermethrin were determined experimentally as the average of chromatographic noise for blank soil extracts measured at the retention time of cypermethrin over 2 different days. The LOD recorded as 3 times the baseline noise and LOQ as 10 times the baseline noise was 0.02 and 0.06 mg kg^{-1}, respectively (Sánchez-Brunete *et al.*, 2004).

Table 2: Concentration of cypermethrin residues in different vegetables grown in two years

	Year 1			Year 2		
Days After Spray	**Tomato**	**Okra**	**Cauliflower**	**Tomato**	**Okra**	**Cauliflower**
0	1.86 ± 0.007	2.19 ± 0.089	2.06 ± 0.111	2.99 ± 0.175	1.55 ± 0.089	3.74 ± 0.187
1	1.61 ± 0.005	1.29 ± 0.087	1.61 ± 0.089	2.67 ± 0.281	1.03 ± 0.139	1.04 ± 0.087
3	1.56 ± 0.071	0.77 ± 0.089	1.32 ± 0.089	2.63 ± 0.342	0.62 ± 0.078	0.73 ± 0.089
5	1.35 ± 0.018	0.49 ± 0.019	0.78 ± 0.089	1.59 ± 0.037	0.42 ± 0.033	0.35 ± 0.023
7	0.86 ± 0.006	0.27 ± 0.017	0.58 ± 0.087	0.53 ± 0.024	0.31 ± 0.028	0.17 ± 0.028
10	0.15 ± 0.006	0.19 ± 0.017	0.39 ± 0.038	0.51 ± 0.048	0.23 ± 0.039	0.12 ± 0.015
15	0.09 ± 0.004	0.12 ± 0.014	0.18 ± 0.025	0.15 ± 0.027	0.12 ± 0.019	0.07 ± 0.013

Table 3: The pseudo-first–order kinetic equations of cypermethrin residual dynamics on different vegetables grown in two year

		Kinetic Equation	r^2	Half life (days)	Pre–harvest Interval (days)
Year 1	Tomato	$C_t = 2.53e^{-0.222\,t}$	0.96	3.11	11
	Okra	$C_t = 1.54e^{-0.200\,t}$	0.95	3.45	6
	Cauliflower	$C_t = 1.94e^{-0.161\,t}$	0.99	4.28	4
Year 2	Tomato	$C_t = 3.54e^{-0.208\,t}$	0.95	3.31	14
	Okra	$C_t = 1.15e^{-0.162\,t}$	0.95	4.26	5
	Cauliflower	$C_t = 1.63e^{-0.248\,t}$	0.97	2.78	2

The performance of the extraction and cleanup procedure described earlier was evaluated by using blank tomato, okra and cauliflower samples spiked at 0.05 and 10 mg kg^{-1} concentrations. Each fortification level was used in five replicates. The average recoveries for 0.05 and 10 mg kg^{-1} fortification levels were 90 and 98% for tomato, 85 and 87% for okra, and 93 and 94% for cauliflower, respectively. The precision of the method was calculated for each vegetable matrix as relative standard deviation (%RSD). The % RSD values for cypermethrin in different vegetable matrices were generally below 20%. Therefore, the method has shown desirable accuracy and precision for different vegetables.

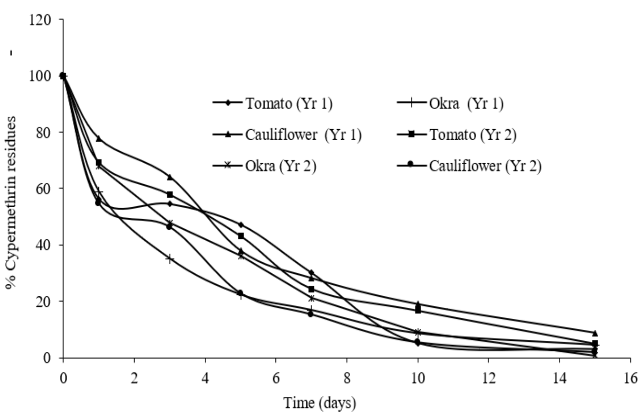

Figure 1: Degradation of cypermethrin on different vegetables over time

Cypermethrin degradation dynamic on the three vegetables grown under field condition was studied assuming a simple non–linear pseudo–first-order kinetics model $C_t = C_0 e^{-kt}$ (Ntow *et al.*, 2007; Wang *et al.*, 2016), where C_t is the concentration (mg kg^{-1}) of the analyte at time t (days) after cypermethrin application; C_o is the initial concentration (mg kg^{-1}) immediately after the treatment, and k is the first-order rate constant (day^{-1}). The goodness of fit test showed the model appropriate for the data interpretation ($r^2 > 95\%$) in all the cases. Half-life ($T_{1/2}$ = -1/k (LN 0.5) and the pre-harvest interval ((*PHI = (LN intercept – LN tolerance) / k*) for cypermethrin residues on each vegetable were also calculated using the rate constant (*k*).

The concentration of cypermethrin residues decreased over time, but the decrease was more rapid initially and slowed in the later stages (Figure 1). During 15 days, the decrease of cypermethrin residues was over 90% in all the vegetables in both the years (Table 2). The degradation pattern of cypermethrin residues in all the cases followed the pseudo–first–order kinetics (Figure 1; Table 3). The dissipation dynamics of cypermethrin on different vegetables in different years are given in table 3.

Dissipation date of cypermethrin, fipronil and tebuconazole in vegetables were studied and first-order kinetic model was employed. These pesticides were proven to be persistent in the crops that were grown in protected plots than those in natural plots (Chau *et al.*, 2020).

The residue levels of cypermethrin at different treatment intervals in tomatoes are shown in Table 2. The initial deposit declined from 1.86 to 0.09 mg kg^{-1} (98%) and from 2.99 to 0.15 mg kg^{-1} (94%) in 15 days after treatment during the two years. The rate of cypermethrin disappearance was 0.222 and 0.208 day^{-1} during the two years. Based on the dissipation rate, half-life values were 3.11 and 3.31, and the pre-harvest interval for tomatoes was calculated as 11 and 14 days in the respective years (Table 3).

The residue levels of cypermethrin at different treatment intervals in okra are given in Table 2. The cypermethrin residues in okra dissipated to 98%, where the initial cypermethrin deposit declined from 2.19 to 0.1 mg kg^{-1} in 15 days in the first year of study. In the second year, the initial cypermethrin residues declined from 1.55 to 0.12 mg kg^{-1} that corresponds to 92 % dissipation in 15 days. The dissipation rate was 0.2 and 0.162 day^{-1} in the two years. The half-life of cypermethrin residues in okra was calculated as 3.45 and 4.26 days and the pre-harvest interval was 5 to 6 days in the two years (Table 3).

After 15 days of cypermethrin application on cauliflower, the residue level declined from 2.06 to 0.18 mg kg^{-1} (98%) in the first year and from 3.74 to 0.07 mg kg^{-1} (98%) in the second year. In cauliflower, the cypermethrin dissipated at the rate of 0.161 and 0.248 day^{-1}. Based on the dissipation rate, the half-life of cypermethrin on cauliflower were 2.76 and 4.28 days, and 2 and 4 days were estimated as the pre-harvest interval for cauliflower in the two crop seasons (Table 3). In a study conducted by Chau and his colleagues showed that the half-lives of cypermethrin was longer than those of fipronil (Chau *et al.*, 2020).

The initial deposit of cypermethrin residue varied among the vegetables. Where in both the year's initial deposit on tomato was higher than the other two vegetables. This might be due to variation in the plant architecture and fruit orientation in different vegetables (Ebeling, 1963). The cypermethrin dissipation rate and half-lives for the three vegetables in both the year were statistically non–significant ($\alpha = 0.05$) in one–way ANOVA. The weather data for the two years show little variation especially for rainfall in different vegetable seasons as well as in different years. These variations did not seem to affect the rate of cypermethrin dissipation in this study. Although, the initial cypermethrin deposits varied in the vegetables, yet the almost similar rate of degradation further qualifies the pseudo–first–order kinetic i.e., the rate constant is independent of initial residue concentration (Anal, 1997; Metwally *et al*., 1997). The average half-lives of 3.21 days for tomato, 3.85 days for okra, and 3.5 days for cauliflower are somewhat following some previous studies. Gupta *et al*. (2011) described a similar pattern of cypermethrin dissipation in tomatoes and reported the half-life between 2.9–4.8 days. Similarly, Zhang *et al*. (2007) reported 2.6 days half-life on spring cabbage (Gupta *et al.*, 2011).

FAO/WHO Codex Alimentarius Commission has modified the cypermethrin maximum residue limits (MRL) for tomato (0.2 mg kg^{-1}), okra (0.5 mg kg^{-1}) and cauliflower (1.0 mg kg^{-1}) (Authority, 2011). The results of the study were used to devise pre-harvest intervals for safer application of cypermethrin using the newly adopted MRLs (Table 3). Due to higher initial cypermethrin deposit and lower MRLs for tomato, 11 to 14 days were estimated as the pre-harvest interval for the residues to fall below MRL in tomato. For the same amount of cypermethrin dosage, 5 to 6 days were sufficient for the residues to fall under MRL in okra. However, due to higher MRL for cauliflower crops, only 2 to 4 days were estimated as safer pre-harvest intervals for cypermethrin application.

Dissipation of cypermethrin residues in vegetables followed pseudo–first–order kinetics. The rate of dissipation was almost the same amongst the vegetables as well as over different years. The variation in the half-lives was also non-significant among the vegetables in different years. The pre-harvest interval for the residues to drop below tolerance limits was highly dependent on the magnitude of the initial residue possibly due to differences in the plant architecture as well as the prescribed residue limits. Pre–harvest intervals of 14, 6, and 4 days were estimated for cypermethrin application on tomato, okra, and cauliflower, respectively. It can be concluded that such trials are necessary from time to time for all the vegetable crops especially where regulatory modifications are adopted, to ensure compliance with the new tolerance limits to ensure food safety.

Acknowledgment

We are thankful to the Agriculture Research System Khyber Pakhtunkhwa for their financial and technical support.

References

Anal, J.T. 1997. Bibliography of analytical, nutritional and clinical methods. *Food Chemistry* 60(2): 273-275.

Authority, E.F.S. 2011. Modification of the existing MRLs for cypermethrin in various crops. *EFSA Journal* 9(6): 2280.

Chau, N.D.G., L.S. Le and N. Van Hop. 2020. Dissipation of the pesticides fipronil, cypermethrin, and tebuconazole in vegetables: A case study in Thua

Thien-Hue province, Central Vietnam. *Journal of Pesticide Science* 45(4): 245-252.

Ebeling, W. 1963. Analysis of the basic processes involved in the deposition, degradation, persistence, and effectiveness of pesticides. p. 35-163. In: Residue Reviews/Rückstands-Berichte, Springer.

George, J. and Y. Shukla. 2011. Pesticides and cancer: Insights into toxicoproteomic-based findings. *Journal of Proteomics* 74(12): 2713-2722.

Gupta, S., V.T. Gajbhiye, R.K. Sharma and R.K. Gupta. 2011. Dissipation of cypermethrin, chlorpyriphos, and profenofos in tomato fruits and soil following application of pre-mix formulations. *Environmental Monitoring and Assessment* 174(1): 337-345.

Horská, T., F. Kocourek, J. Stará, K. Holý, P. Mráz, F. Krátký, V. Kocourek and J. Hajšlová. 2020. Evaluation of pesticide residue dynamics in lettuce, onion, leek, carrot and parsley. *Foods* 9(5): 680.

Jyot, G., K. Mandal, R. Battu and B. Singh. 2013. Estimation of chlorpyriphos and cypermethrin residues in chilli (*Capsicum annuum* L.) by gas–liquid chromatography. *Environmental Monitoring and Assessment* 185(7): 5703-5714.

Kadenczki, L., Z. Arpad, I. Gardi, A. Ambrus, L. Gyorfi, G. Reese and W. Ebing. 1992. Column extraction of residues of several pesticides from fruits and vegetables: A simple multiresidue analysis method. *Journal of AOAC International* 75(1): 53-61.

Krynitsky, A.J., C.J. Stafford and S.N. Wiemeyer. 1988. Combined extraction-cleanup column chromatographic procedure for determination of dicofol in avian eggs. *Journal of the Association of Official Analytical Chemists* 71(3): 539-542.

Macedo, M.R. and M.D.G.M. Freire. 2011. Insect digestive enzymes as a target for pest control. *Invertebrate Survival Journal* 8(2): 190-198.

Ntow, W.J., J. Ameyibor, P. Kelderman, P. Drechsel and H.J. Gijzen. 2007. Dissipation of endosulfan in field-grown tomato (*Lycopersicon esculentum*) and cropped soil at Akumadan, Ghana. *Journal of Agricultural and Food Chemistry* 55(26): 10864-10871.

Ripley, B.D., G.M. Ritcey, C.R. Harris, M.A. Denommé and L.I. Lissemore. 2003. Comparative persistence of pesticides on selected cultivars of specialty vegetables. *Journal of Agricultural and Food Chemistry* 51(5): 1328-1335.

Sánchez-Brunete, C., B. Albero and J.L. Tadeo. 2004. Multiresidue determination of pesticides in soil by gas chromatography− mass spectrometry detection. *Journal of Agricultural and Food Chemistry* 52(6): 1445-1451.

Shah, M.M., A. Hassan, Q. Mahmood, K. Akbar and M.S. Khan. 2011. Assessment of pesticide residues on selected vegetables of Pakistan. *Journal of the Chemical Society of Pakistan* 33(6): 816-821.

Shilpakar, O. and B. Karki. 2021. Cypermethrin poisoning manifesting with prolonged bradycardia: A case report. *Toxicology Reports* 8: 10-12.

Tyler, C.R., N. Beresford, M. Van Der Woning, J.P. Sumpter and K. Tchorpe. 2000. Metabolism and environmental degradation of pyrethroid insecticides produce compounds with endocrine activities. *Environmental Toxicology and Chemistry: An International Journal* 19(4): 801-809.

Ullah, M., M. Ahmad, N. Ahmad, M. Khan and I. Ahmad. 2006. Toxic effects of cypermethrin in female rabbits. *Pakistan Veterinary Journal* 26(4): 193.

Wang, P., M. Rashid, J. Liu, M. Hu and G. Zhong. 2016. Identification of multi-insecticide residues using GC-NPD and the degradation kinetics of chlorpyrifos in sweet corn and soils. *Food Chemistry* 212: 420-426.

Zhang, Z.-Y., X.-J. Liu, X.-Y. Yu, C.-Z. Zhang and X.Y. Hong. 2007. Pesticide residues in the spring cabbage (*Brassica oleracea* L. var. capitata) grown in open field. *Food Control* 18(6): 723-730.