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Research Article

Determination of Pre-harvest Interval of Cypermethrin and Chlorpyrifos in Tomato and Yard Long Bean

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Abstract

In agriculture, a wide range of pesticides have been used to improve crop quality and productivity. Farmers in Bangladesh are using pesticides in vegetables following no rules and regulations as a result it has been a potential risk to the environment and human health. For this reason, an experiment was conducted to determine the pre-harvest interval of cypermethrin and chlorpyrifos in tomatoes (*Solanum lycopersicum*) and yard-long beans (*Vigna unguiculata*) considering the maximum residue limit (MRL) set by the European Union. The crops were sprayed with the recommended dose of chlorpyrifos (2 ml/L) and cypermethrin (1 ml/L) in four supervised field trials. Samples were collected at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 days after spray (DAS). Gas chromatography was used for a simple and effective multiple pesticide residue analysis where Flame Thermo ionized Detector for chlorpyrifos, and Electron Capture Detector for cypermethrin were used for the determination of the pesticide residues. The quantities were above MRL up to 3 DAS for cypermethrin and 7 DAS for chlorpyrifos in tomatoes. The detected residues were above MRL in yard-long beans up to 4 DAS for cypermethrin and 9 DAS for chlorpyrifos. The pre-harvest interval was determined in tomatoes at 4 DAS for cypermethrin, 8 DAS for chlorpyrifos, and in the case of yard-long beans, it was 5 DAS for cypermethrin and 10 DAS for chlorpyrifos.

Introduction

Vegetables are an essential component of a balanced diet. They are low in calories and fat and high in vitamins and minerals, dietary fiber, and phytochemicals (Karmakar et al., 2016, Keatinge et al., 2011). Tomatoes and yard-long beans are widely recognized as two of the most popular and consumed vegetables in the world, because of their nutritional value and health benefits (Bhandari et al., 2021, Awika et al., 2017). A large volume of yard-long beans and tomatoes are being spoiled every year due to pest attacks. Several insect pests including fruit borer, aphids, fruit flies, and fungal and viral diseases are found to attack tomatoes (Depenbusch et al., 2023). The main pests of yard-long beans are aphids, pod borers, thrips, red mites, leaf miners, leaf beetles, green sting-bug, jute hairy caterpillars, hooded hoppers, and semi looper (Uddin et al., 2013). It is assessed that the pest may cause a loss of up to 80%-100% in tomato crop production in both greenhouses and fields. Plant pests and diseases cause a 20 to 40% crop yield reduction; in fact, the world will need to produce 60% more food for the increasing world population by 2050 (Kourous, 2016).

As a result, farmers are intensively applying pesticides in their fields to control pests and diseases to increase production and profits (VSS et al., 2020). Like many other developing countries, farmers of Bangladesh are using pesticides extensively to increase the crop yield per acre (Chowdhury et al., 2012, Islam et al., 2015). The extensive application of chemical pesticides leads to a high residue in fruits and vegetables that results in adverse effects on human health directly or indirectly (Medina et al. 2021; Tari and Patil 2014; VSS et al. 2020) Organophosphorus, organochlorines, carbamates, and synthetic pyrethroids are the most commonly used pesticides in developing nations (Mohammed et al., 2019). Although insecticides come in a variety of classes, the most common ones for treating plants are those that contain phosphorus and chlorine. Because of their strong bioaccumulation effect, organophosphorus

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pesticides (OPPs) are gradually displacing organochlorines (Suryono et al., 2019). OPPs are amides, esters, or thiols that are derived from phosphoric or phosphonic acid. Because they are readily hydrolyzed, they do not survive in the environment for extended periods (Sidhu et al., 2019; Bala et al., 2022). However, their many poisonous properties (they inhibit the acetylcholinesterase enzyme) and the possibility of accumulation in the food chain can pose a threat to human health (Boneva et al., 2021). When pyrethroids were introduced in the 1970s, they presented a viable substitute that was both effective and less harmful to the environment than its predecessors (Chrustek et al., 2018). Although pyrethroids and other pesticides are unquestionably beneficial in reducing agricultural losses and guaranteeing food security, their extensive use has sparked worries about their detrimental effects on the environment and human health (Mahmood et al., 2016; Peshin et al., 2009).

Several studies in Bangladesh have confirmed the presence of organophosphorus and pyrethroid residues in fruits and vegetables collected from the market (Islam et al., 2021; Hasan and Rahman, 2019; Ahmed et al., 2018; Tasnim et al., 2022), but very little information exists on determining their pre-harvest interval (PHI) conducting field trials. The present study is conducted to determine the pre-harvest interval (PHI) of chlorpyrifos and cypermethrin in tomato and yard-long beans by assessing the maximum pesticide residue level (MRL) on different days after spraying in supervised field trials. It is expected that the result of this study will add some vital information on pesticide residue data and degradation rate to ensure the safety of the general people who are consuming the vegetables.

Materials and Methods

The study was conducted at the Entomological Research Field, Bangladesh Agricultural Research Institute (BARI), Gazipur, and the chemical analysis was conducted at the Pesticide Analytical Laboratory (PAL), Division of Entomology.

Sample collection and storage

Tomato and yard-long beans were cultivated in four supervised field trials from November 2018 to May 2019. The vegetables were grown in 2.5m×2.5m plots according to the fertilizer recommendation guide provided by the Bangladesh Agricultural Research Council (BARC) (Ahmmed et al., 2018) and irrigated as required. Cypermethrin (Relothrin 10 EC @1 ml/L of water) and chlorpyrifos (Dursban 20 EC @2 ml/L of water) were sprayed evenly at the recommended dose. Fresh marketable-size of yard long beans and tomatoes were collected from four supervised field trials at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 days after spray (DASs). The samples were collected in polythene bags with appropriate tagging of each sample and brought to the laboratory at the quickest time from the field by using a cool box to prevent any cross-contamination. The samples were then preserved in a refrigerator at -20°C until analysis.

Extraction, separation, and clean up

The modified QuEChERS extraction technique was used for the extraction and clean-up of the vegetable samples (Prodhan et al., 2015). The chopped samples were ground thoroughly with the fruit blender. A representative 10 g portion of a thoroughly homogenized sample was weighted in a 50 mL polypropylene centrifuge tube. Then 10 mL of acetonitrile (ACN) was added to the centrifuge tube. The centrifuge tube was closed properly and shaken vigorously for 30 seconds using a vortex mixer. Then, 4 g of anhydrous $MgSO_4$ and 1 g of NaCl were added into the centrifuge tube, and it was shaken immediately by the vortex mixer for 1 min to prevent the formation of magnesium sulfate aggregates. Afterward, the extract was centrifuged for 5 min at 5000 rpm. An aliquot of 3 mL of the ACN layer was transferred into a 15 mL micro centrifuge tube containing 600 mg anhydrous $MgSO_4$, and 120 mg Primary Secondary Amine (PSA). Then it was thoroughly mixed by vortex for 30 s and centrifuged for 5 min at 4000 rpm (Laboratory Centrifuges. Sigma-3K30, Germany). After centrifuge, a 1 mL supernatant was filtered by a 0.2 µm PTFE filter, and then it was taken in a clean GC vial for injection.

Detection and Quantification of pesticide residue in samples

The concentrated extracts were subjected to analysis by GC-2010 (Shimadzu). Flame Thermo ionized Detector (FTD) was used for organophosphorus (chlorpyrifos) insecticide. The capillary column used in FTD was AT^{TM}-1, length 30m, Inner Diameter (ID) 0.25mm, and film thickness 0.25um. Pyrethroid (cypermethrin) sample extracts were detected by an Electron Capture Detector (ECD). The capillary column used in ECD was Rtx-CL Pesticides 2, length 30 m, ID 0.32 mm, and film thickness 0.25 µm. Helium was used as a carrier and makeup gas in FTD; in case of ECD, it was nitrogen (Table 1).

Table 1. The instrument parameters for the detection and quantification of chlorpyrifos and cypermethrin

Instrument parameters	Chlorpyrifos	Cypermethrin
Instrument	GC-2010	GC-2010
[Injection Port SPL]		
Injector (Auto)	AOC 20i	AOC 20i
Injection Mode	Split	Split
Temperature	260 °C	280 °C
Carrier Gas	He	N_2
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	20.0	10.0
Injection Volume	1.0 µL	1.0 µL
[Column Oven]		
Column Oven Temp. Program:		
Initial Temperature	160 °C	160 °C
Final Temperature	240 °C	270 °C
Equilibrium Time	1.0 min, Hold time: 1	1.0 min, Hold time: 8
Total Program Time	12.0 min	20.0 min
[Column Information]		
Column Name	AT^{TM}-1	Rtx-CLPesticides2
Column Length	30.0 m	30.0 m
Film Thickness	0.25 µm	0.25 µm
Inner Diameter	0.25 mm	0.32 mm
[Detector Channel 1]		
Detector	FTD	ECD
Temperature	280 °C	300 °C
Stop Time	12.0 min	20.0 min
Current	0.1 pA	1.0 pA
Makeup Gas	He	N2
Makeup Flow	30.0 mL/min	30.0 mL/min
H_2 Flow	1.5 mL/min	-
Air Flow	145.0 mL/min	-

Analytical quality control

Gas chromatography equipped with ECD and FTD were checked for linearity. The instrumental detection limit for GC-FTD and ECD was 0.001 mg/Kg for organophosphorus and pyrethroid insecticides. An untreated tomato and yard-long bean sample were collected as blank and treated exactly as the sample including exposure to all glassware, equipment solvents, and reagents used in the sample matrix. No analytic peak was detected in the laboratory reagent blank. An aliquot of fortified sample matrix was prepared to which known quantities of the pesticides were added in the laboratory in the mg/Kg range. This laboratory-fortified matrix was analyzed exactly like the sample. Extraction and clean-up were done as mentioned and the recoveries from untreated control samples of tomato and yard-long bean fortified with the analyzed compounds at a level of 0.5 mg/Kg was 90-100% for organophosphorus mix. Before the injection of the first sample solution, a standard solution was injected at least three times to check the opening conditions, and the constancy of the detector signal was checked by injecting serial dilution of organophosphorus and pyrethroid mix.

Before injecting the sample extract, standard solutions of different concentrations of two insecticides were prepared and injected with the above instrument parameters. The samples were calibrated (retention time, peak area, etc.) against a five-pointed calibration curve of the standard solution of the concerned insecticide. 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.

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

The Calibration Curve of chlorpyrifos was made using the peaks of the chromatogram of 50.000, 100.000, 200.000, 300.000, 500.000 conc. standard solution (Fig 1.). The Calibration Curve of cypermethrin was made using the peaks of the chromatogram of 0.200, 0.500, 1.000, 2.000 conc. standard solution (Fig. 2).

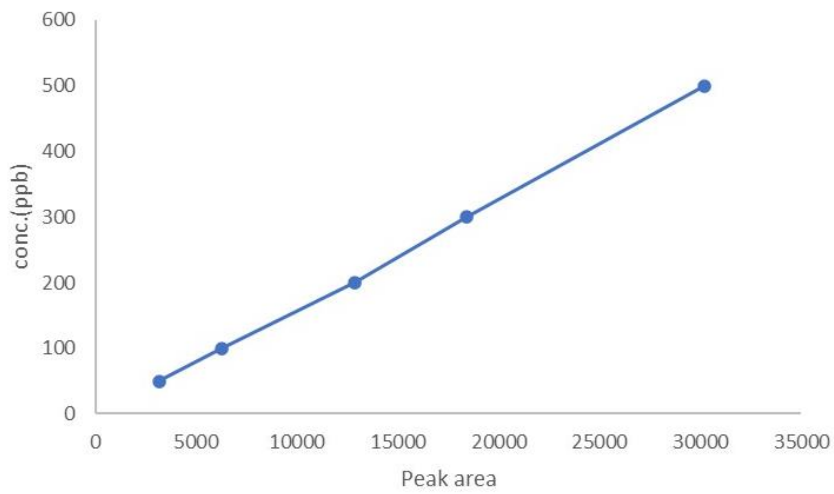

Figure 1. Calibration curve made with five different concentrations of chlorpyrifos standard

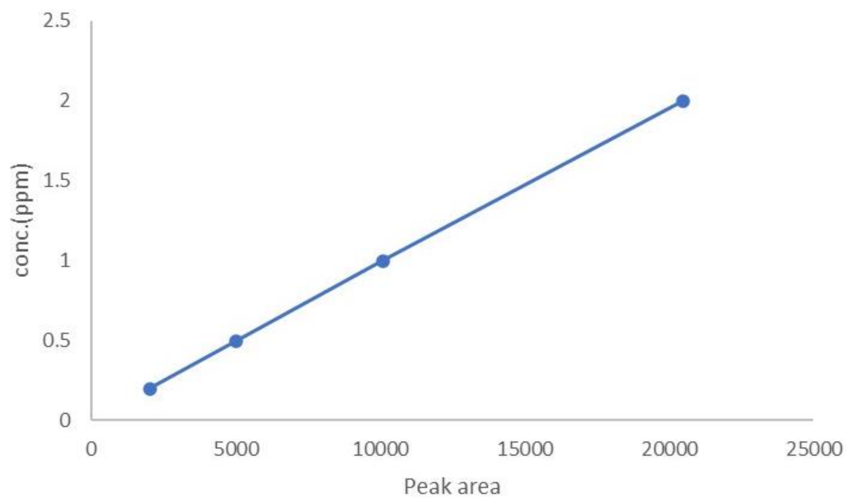

Figure 2. Calibration curve made with four different concentrations of cypermethrin standard

Determination of Pre-Harvest Interval (PHI)

At first the residues of the collected samples for both the insecticides and vegetables were calculated following the described procedures. Then the sampling day which was next following the maximum residue level (MRL) was selected. That selected day was chosen as PHI since the level of residue on that day was below MRL.

Results and Discussion

Degradation of chlorpyrifos in tomato and yard-long bean

The residue of chlorpyrifos in tomato was detected up to 8 DAS and detected quantities of residue were 5.619 mg/Kg, 4.251 mg/Kg, 3.046 mg/Kg, 2.450 mg/Kg, 1.362 mg/Kg, 0.584 mg/Kg, 0.262 mg/Kg, 0.124 mg/Kg and 0.056 mg/Kg, at 0, 1, 2, 3, 4, 5, 6, 7 and 8 DAS, respectively (Table 2.). The quantities were above MRL up to 7 DAS set by the European Union Pesticides database (2015). Samples of 8 DAS contained 0.056 mg/Kg which was below MRL. No residue was detected at 9 DAS. So, the PHI of chlorpyrifos for tomato can be selected at 8 DAS.

Table 2. Quantity of residue of chlorpyrifos (Dursban 20 EC) estimated from tomato using GC-FTD

Days after spraying	Sample weight (g)	Injected volume (μL)	Amount of residue (mg/Kg)	EU MRL (mg/Kg)
0	10	1	5.619	0.1
1	10	1	4.251	
2	10	1	3.046	
3	10	1	2.450	
4	10	1	1.362	
5	10	1	0.584	
6	10	1	0.262	
7	10	1	0.124	
8	10	1	0.056	
9	10	1	ND	

ND= Not Detected; EU=European Union; MRL=Maximum Residue Limit

The results revealed that residue of chlorpyrifos was detected in up to 9 DAS in yard-long beans. The quantities of residues were 6.985 mg/Kg, 5.294 mg/Kg, 3.985 mg/Kg, 2.561 mg/Kg, 1.405 mg/Kg, 0.864 mg/Kg, 0.620 mg/Kg, 0.415 mg/Kg, 0.172 mg/Kg, and 0.064 mg/Kg at 0, 1, 2, 3, 4, 5, 6, 7, 8 and 9 DAS respectively (Table 3.). All the tested samples showed residues above the MRL set by the European Union Pesticides database (2015). No residue was detected at 10 DAS. So, the PHI of chlorpyrifos for yard-long beans can be selected at 10 DAS. A similar experiment was conducted by Khanom et al. (2023), where they determined the pre-harvest interval for chlorpyrifos in Hyacinth bean (*Dolichos lablabat*) at 9 DAS.

Table 3. Quantity of residue of chlorpyrifos (Dursban 20 EC) estimated from yard-long bean using GC-FTD

Days after spraying	Sample weight (g)	Injected volume (μL)	Amount of residue (mg/Kg)	EU MRL (mg/Kg)
0	10	1	6.985	0.01
1	10	1	5.294	
2	10	1	3.985	
3	10	1	2.561	
4	10	1	1.405	
5	10	1	0.864	
6	10	1	0.620	
7	10	1	0.415	
8	10	1	0.172	
9	10	1	0.064	
10	10	1	ND	

ND= Not Detected; EU=European Union; MRL=Maximum Residue Limit

The persistence of chlorpyrifos in both recommended and double doses was described by Gupta et al. (2011), in both cases chlorpyrifos residues persisted beyond 7 days in tomato, which agreed with the present study. A study conducted with tomatoes using high-performance liquid chromatography and QuEChERS methodology stated that tomatoes could safely be consumed after 15 days of application at recommended rates for chlorpyrifos (Ramadan et al., 2016). On the other hand, an experiment in the greenhouse using gas chromatography to find the residual behavior of chlorpyrifos in different vegetables (Lu et al., 2014). Although they used excessive amounts of pesticide, they found 0.03 mg/Kg and 0.01 mg/Kg of chlorpyrifos residues in pepper and eggplant fruits respectively at PHI 7 d. These results also agreed with the present experiment (Lu et al., 2014).

Degradation of cypermethrin in tomato and yard-long bean

Residue of cypermethrin in tomatoes was detected up to 6 DAS and the quantities were above MRL up to 3 DAS. The levels of residues were 2.986 mg/Kg, 1.854 mg/Kg, 0.978 mg/Kg, and 0.653 mg/Kg at 0, 1, 2 and 3 DAS, respectively (Table 4.). The samples of 4, 5, and 6 DAS contained 0.365 mg/Kg, 0.146 mg/Kg, and 0.059 mg/Kg, which were below MRL set by the European Union Pesticides database (2015) (Table 4.). No residue was detected at 7 DAS. So, the PHI of cypermethrin for tomato can be selected at 4 DAS.

Table 4. Quantity of residue of cypermethrin (Relothrin 10 EC) estimated from tomato using GC-ECD

Days after spraying	Sample weight (g)	Injected volume (µL)	Amount of residue (mg/Kg)	EU MRL (mg/Kg)
0	10	1	2.986	0.5
1	10	1	1.854	
2	10	1	0.978	
3	10	1	0.653	
4	10	1	0.365	
5	10	1	0.146	
6	10	1	0.059	
7	10	1	ND	

ND= Not Detected; EU=European Union; MRL=Maximum Residue Limit

The left-over residue of cypermethrin in yard-long bean samples had been detected up to 8 DAS and the quantities were above MRL up to 4 DAS and these were 4.567 mg/Kg, 3.452 mg/Kg, 2.380 mg/Kg, 1.146 mg/Kg and 0.754 mg/Kg at 0, 1, 2, 3 and 4 DAS respectively (Table 5.). The level of residues was 0.438 mg/Kg, 0.251 mg/Kg, 0.143 mg/Kg, and 0.052 mg/Kg at 5, 6, 7, and 8 DAS (Table 5) which were below MRL set by the European Union Pesticides database (2015). No residue was detected at 9 DAS. So, the PHI of cypermethrin for yard-long beans can be selected at 5 DAS. A similar experiment was conducted in Thessaloniki, Greece (Prodhan et al., 2018), where they determined PHI 5 DAS both for yard-long beans and tomatoes for cypermethrin, using the Maximum Residue Limit (MRL) set by FAO/ WHO. The PHI of cypermethrin for the yard-long bean is 5 DAS is similar to this experiment. But in case of tomatoes, the result is varied. Several scientists in Bangladesh found that cypermethrin residues were detected up to 5 DAS in yard-long beans and 3 DAS in tomatoes. They also found cypermethrin residues above MRL up to 3 DAS in country beans, brinjal, cabbage, and cauliflower. However, they detected residues up to 10 DAS in cauliflower, 8 DAS in cabbage, and 7 DAS in country beans and brinjal (Anonymous, 2012). The present study agreed with the results of said scientists.

Table 5. Quantity of residue of cypermethrin (Relothrin 10 EC) estimated from yard-long bean using GC-ECD

Days after spraying	Sample weight (g)	Injected volume (µL)	Amount of residue (mg/Kg)	EU MRL (mg/Kg)
0	10	1	4.567	0.7
1	10	1	3.452	
2	10	1	2.380	
3	10	1	1.146	
4	10	1	0.754	
5	10	1	0.438	
6	10	1	0.251	
7	10	1	0.143	
8	10	1	0.052	
9	10	1	ND	

ND= Not Detected; EU=European Union; MRL=Maximum Residue Limit

Degradation rate of insecticide residues in tomato and yard-long bean

Chlorpyrifos degradation rate in yard-long beans and tomatoes are almost the same which was detected up to 9 DAS in yard-long beans and 8 DAS in tomatoes (Fig 3.). But cypermethrin degrades slowly in yard-long beans as compared to tomatoes (Fig 3.). The plant behavior might be related to the physico-chemical properties of pesticides such as the uptake of chemical solution by the plants and their different metabolism (Virgina and Bajet, 1996). The decrease in pesticide residues is due to the physical and chemical properties and the natural capability of the plant itself to break down the pesticide in the environment (Abo-El-Seoud et al., 1995). Moreover, the degradation and residual behaviors of insecticides after their application may be affected by many factors such as plant species, insecticide chemical structure, type of formulation, volatilization, application method, climate, and photodegradation (Garau et al., 2002).

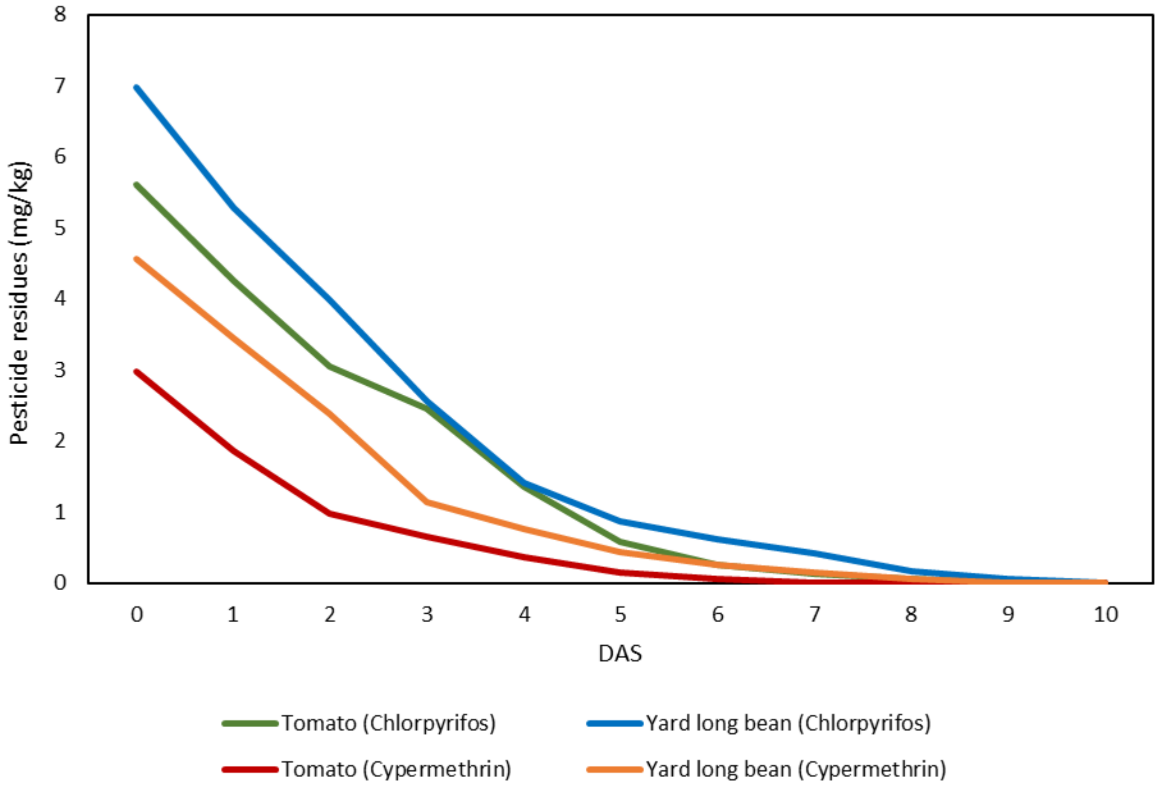

Figure 3. Degradation rate of insecticide residues in tomato and yard-long bean

Conclusion

The acquired results defined that; the pre-harvest interval (PHI) of chlorpyrifos for tomatoes can be selected at 8 DAS and for yard-long beans at 10 DAS. For cypermethrin, the PHI can be selected at 5 DAS for yard-long beans and 4 DAS for tomatoes. Yard-long beans showed a slower degradation rate than tomatoes for both pesticides. The degradation rate of chlorpyrifos is slower than that of cypermethrin. So, it can be concluded that it is better to use cypermethrin than chlorpyrifos as the degradation rate of cypermethrin is lower than that of chlorpyrifos. Food products become safe for consumption only after PHI is maintained when pesticides are applied to crops. So, the experiment could be effective in raising awareness about pesticide use and providing safe harvest time to reduce pesticide contamination as well as environmental pollution. The findings of the study will help consumers, policy planners, growers, decision-makers, and researchers to undertake activities for getting safe food. So, a regular monitoring program for pesticide residue analysis especially emphasis on PHI of different pesticides and crops is needed for safe food production.

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