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Article

Determination of pre-harvest interval for fenvalerate and acephate in tomato and yard long bean using Gas Chromatography

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Abstract: The study was carried out to detect and quantify the left over residue of fenvalerate and acephate in tomato and yard long bean using Gas Chromatography coupled with Flame Thermionic Detector. Four supervised field trials were undertaken sprayed with the prescribed dose of fenvalerate at the rate of 1mL/L of water and acephate at the rate of 2g/L of water. The samples of tomato and yard long bean were collected at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 and 15 days after spray (DAS). The residue of acephate was detected up to 11 DAS in tomato and 14 DAS in yard long bean and all of the detected quantities were above Maximum Residue Limit (MRL) set by European Union. No residue was detected at 12 DAS in tomato and 15 DAS in yard long bean. The residue of fenvalerate was detected up to 12 DAS in tomato and 13 DAS in yard long bean, of which the quantities of residue of fenvalerate was above EU-MRL at 9 DAS in tomato and 11 DAS in yard long bean. Fenvalerate contained 0.076-0.008 mg/kg residue in tomato and 0.049-0.021 mg/kg residue in yard long bean which were below EU-MRL at 10 to 12 DAS in tomato and 12 to 13 in yard long bean which were below EU-MRL. No residue was detected at 13 DAS in tomato and 14 DAS in yard long bean. So, in case of acephate the pre-harvest interval (PHI) was determined at 12 DAS in tomato and 15 DAS in yard long bean and for fenvalerate it was 10 DAS in tomato and 12 DAS in yard long bean.

Keywords: fenvalerate; acephate, PHI; vegetables; MRL; gas chromatography

1. Introduction

The tomato (*Solanum lycopersicum* L.) commonly known as the tomato plant belongs to the Solanaceae family and is the second most important fruit or vegetable crop next to potato. Tomato is cultivated for fresh fruit usually eaten as salad and processed products. Tomatoes are a good source of vitamin C and the phytochemical lycopene (Gloria Lotha, 2019).Yard long bean (*Vigna unguiculata* L.) is known as borboti, asparagus bean, snake bean, chinese long bean, black-eye bean and long-podded cowpea (John and Lincoln, 2022) under the family Fabaceae, cultivated to be eaten as green pods. Both the vegetables are nutritious and commercial crop in Bangladesh. These crops are attacked by a variety of insect pests and diseases. Due to plant pests and diseases 20-40 percent of the crops yields are reduced globally (FAO, 2012). Many pesticides have been used for the management of insect pests in vegetables (Akter *et al.* 2008; Gupta *et al.* 2009; Singh *et al.* 2007). Fenvalerate and acephate are frequently used in vegetables for the protection of insect pests in Bangladesh. Pesticides are one of the major components of plant protection for the farmers of Bangladesh because of its small quantity in packaging/bottling, storage ability and availability in the market. It was reported from farmers' interview that they use insecticides irrationally and whimsically (Anon. 2001; Ahmed *et al.* 2005). Even many farmers sell vegetables at an interval of 0-2 days after spray (Anon. 2000) which is violation of judicial use of pesticides in vegetables. This led to assume that over-sprayed vegetable consumers might face health hazards and environment might be over loaded with pesticide residue (Antle and Pingali, 1994). The detection and monitoring of pesticide residue particularly in vegetable is being done in regular basis in many countries (Krol *et al.* 2000; Van der Schee, 2002; Kumar *et al.* 2004; Rajeswaran *et al.* 2004) and in some occasions in Bangladesh (Khatoon *et al.*, 2004; Kabir *et al*., 2008; 2008a; Ahmed *et al*., 2016a; 2016b; 2018; 2018a; 2019; 2021a; Islam *et al.,* 2019). Irrational use of pesticides has resulted in contamination of our food and the remaining residues of pesticides on harvested crop could have a deleterious effect on humans and the environment (McIntyre *et al.* 1989; Hajslova *et al.* 2003). The risk to humans may be short term as well as long term depending on the persistence of the pesticide and the exposure period.

Most of the farmers of Bangladesh do not follow the recommended dosages and use pesticides without taking into account of the waiting period. Every pesticide has a waiting period or pre-harvest interval (PHI), which is defined as the number of days required to lapse, between the date of final pesticide application and harvest, for residues to fall below the Maximum Residue levels (MRL) established for that crop. But very little references are available on the study of pre-harvest interval of vegetables in Bangladesh (Prodhan *et al*., 2018; Ahmed *et al*., 2020; 2021). Food products become safe for consumption only after pre-harvest interval has lapsed. The PHI differs from pesticide to pesticide and crop to crop. So, we have to re-set the pre harvest interval on the consideration of our environmental conditions. With these view, the present study was undertaken to re-set the PHI for fenvalerate and acephate in tomato and yard long bean in Bangladesh.

2. Materials and Methods

The standard for acephate and fenvalerate were obtained from Sigma-Aldrich Laborchemikalien, Seelze, Germany via S. F. Scientific Pvt. ltd. Dhaka, Bangladesh. Standards of both insecticides contained 99.6% purity. Marketable size of tomato and yard long bean were collected from supervised field trials at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 and 15 days after spray (DAS) which were sprayed with fenvalerate at the rate of 1mL/L of water and acephate at the rate of 2g/L of water. The formulated product of fenvalerate was Iback 20EC and acephate was Asataf 75SP. The purity of formulated insecticides were tested in the pesticide analytical laboratory and found to be 100% pure. Methanol, acetone, gradient grade acetonitrile, sodium chloride (NaCl), anhydrous magnesium sulphate ($MgSO_4$) and Primary Secondary Amine (PSA) were also purchased from Bangladesh Scientific Pvt. ltd. Dhaka, Bangladesh.

2.1. Extraction, separation and clean up

The QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) extraction technique was used for the extraction and clean-up of samples which was slightly modified and adopted. Collected field samples (≥ 250 g) were chopped with a sharp knife and grounded thoroughly with the fruit blender. A representative 10g portion of thoroughly homogenized sample was weighted in a 50 mL polypropylene centrifuge tube. Then 10 mL of acetonitrile (MeCN) was added into the centrifuge tube. The centrifuge tube was closed properly and shaken vigorously for 30s by the use of 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 minute to prevent the formation of magnesium sulfate aggregates. Afterwards, the extract was centrifuged for 5 min at 5000 rpm. An aliquot of 3 mL of the MeCN 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 30s and centrifuged for 5 minutes 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 vial for injection in Gas Chromatography (GC).

2.2. Operating condition of GC- FTD and GC-ECD

The concentrated extracts were analyzed by GC-2010 (Shimadzu). For tested organophosphorus insecticide (acephate) Flame Thermionic Detector (FTD) was used. The capillary column used in FTD was ATTM-1, length 30m, Inner Diameter (ID) 0.25mm and film thickness 0.25µm. The instrument parameters for GC-FTD were as follows: Injection port temperature was 260^0C, Purge Flow was 3mL/min and Split ratio was 20. Column oven initial temperature was 160^0C which went up to 240^0C following 8 min incremental time. Detector temperature was 280^0C, Stop time: 12 min, Current: 0.5 pA, Makeup flow: 30 mL/min, H_2 Flow: 1.5 mL/min and Air flow: 145 mL/min. For detection of pyrethroid (fenvalerate) sample extract was again analyzed with Electron Capture Detector (ECD). The capillary column used in ECD was AT-1, length 30m, ID 0.25 mm and film thickness 0.25 µm. Helium was used as carrier and make up gas in FTD and in case of ECD, it was Nitrogen. The instrument parameters for detecting pyrethroid (fenvalerate) were as follows: Injection port temperature was 280^{0}C, Flow control mode: linear velocity, Split ratio: 10, Column oven initial temperature was 160^{0}C which went up to 270^{0}C following 6 min incremental time. Detector temperature was 300^{0}C, Stop time: 18 min, Current: 1.0 pA and Makeup flow was 30 mL/min.

Before the injection of the sample extract, matrix-matched standard solutions of different concentrations of the selected pesticides were prepared and injected with the above instrument parameters. The samples were analyzed against four point calibration curve prepared with matrix-matched standard solution of concern 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 pesticide residue present in the sample was determined.

2.3. Determination of Pre Harvest Interval

The quantity of residues in all of the collected samples for both the pesticide and every vegetable were calculated following the described procedures. Then the sampling day which was next following MRL was selected. That selected day was chosen as PHI, since the level of residue on that day was below MRL.

3. Results

The residue data pertaining to acephate and fenvalerate in yard long bean and tomato have been presented in Table 1-4.

3.1. Acephate

The yard long bean and tomato samples containing residues were analyzed using the GC-FTD with developed parameters. The results obtained from this analysis have been summarized in Tables 1 and 2.

Table 1. Quantity of residue of acephate (Asataf 75SP) estimated from yard long bean.

Days after spraying	Sample weight (g)	Injected volume (µL)	Amount of Residue (mg/kg)	EU MRL (mg/kg)
0	10	2	6.117	0.01
1	10	2	4.181	
2	10	2	3.793	
3	10	2	2.648	
4	10	2	1.956	
5	10	2	1.425	
6	10	2	1.270	
7	10	2	1.011	
8	10	2	0.952	
9	10	2	0.748	
10	10	2	0.565	
11	10	2	0.384	
12	10	2	0.138	
13	10	2	0.026	
14	10	2	0.012	
15	10	2	ND	

The left over residue of acephate in the yard long bean sample had been detected up to 14 DAS. All of the detected quantities of residue were above EU-MRL and these were 6.117 mg/kg, 4.181 mg/kg, 3.793 mg/kg, 2.648 mg/kg, 1.956 mg/kg, 1.425 mg/kg, 1.270 mg/kg, 1.011 mg/kg, 0.952 mg/kg, 0.748 mg/kg and 0.565 mg/kg 0.384mg/kg, 0.138mg/kg, 0.026mg/kg and 0.012mg/kg at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 11, 12, 13 and 14 DAS, respectively. No residue was detected at 15 DAS. So the PHI can be selected at 15 DAS.

Table 2. Quantity of residue of acephate (Asataf 75SP) estimated from tomato.

Days after spraying	Sample weight (g)	Injected volume (µL)	Amount of Residue (mg/kg)	EU MRL (mg/kg)
0	10	2	4.875	0.01
1	10	2	3.609	
2	10	2	2.985	
3	10	2	1.652	
4	10	2	1.213	
5	10	2	0.985	
6	10	2	0.524	
7	10	2	0.387	
8	10	2	0.251	
9	10	2	0.124	
10	10	2	0.041	
11	10	2	0.015	
12	10	2	ND	

The left over residue of acephate in the tomato sample had been detected up to 11 DAS. All of the detected quantities of residue were above EU-MRL and these were 4.875 mg/kg, 3.609 mg/kg, 2.985 mg/kg, 1.652 mg/kg, 1.213 mg/kg, 0.985 mg/kg, 0.524 mg/kg, 0.387 mg/kg, 0.251 mg/kg, 0.124 mg/kg and 0.041 mg/kg and 0.015 mg/kg at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11 DAS, respectively. At 12 DAS no residue was detected. So, the PHI can be selected at 12 DAS.

3.2. Fenvalerate

The tomato and yard long bean samples containing residues were analyzed using the GC-ECD with set parameters. The results obtained from this analysis are summarized in Tables 3 and 4.

Table 3. Quantity of residue of fenvalerate (Iback 20EC) estimated from tomato.

Days after spraying	Sample weight (g)	Injected volume (µL)	Amount of Residue (mg/kg)	EU MRL (mg/kg)
0	10	1	5.613	0.1
1	10	1	4.321	
2	10	1	2.658	
3	10	1	1.927	
4	10	1	1.343	
5	10	1	0.828	
6	10	1	0.527	
7	10	1	0.434	
8	10	1	0.287	
9	10	1	0.149	
10	10	1	0.076	
11	10	1	0.029	
12	10	1	0.008	
13	10	1	ND	

Residue of fenvalerate in tomato was detected up to 12 DAS and the quantities were above MRL up to 9 DAS and these ware 5.613 mg/kg, 4.321 mg/kg, 2.658 mg/kg, 1.927 mg/kg, 1.343 mg/kg, 0.828 mg/kg, 0.527 mg/kg, 0.434 mg/kg, 0.287 mg/kg and 0.149 mg/kg at 0, 1, 2, 3, 4, 5, 6, 7, 8 and 9 DAS, respectively. While sample of 10, 11 and 12 DAS contained 0.076 mg/kg, 0.029 mg/kg and 0.008 mg/kg residue which were below EU-MRL. No residue was detected at 13 DAS. So, the PHI can be selected at 10 DAS.

Table 4. Quantity of residue of fenvalerate (Iback 20EC) estimated from yard long bean.

Days after spraying	Sample weight (g)	Injected Volume (µL)	Amount of Residue (mg/kg)	EU MRL (mg/kg)
0	10	1	7.958	0.1
1	10	1	6.235	
2	10	1	5.391	
3	10	1	4.965	
4	10	1	3.890	
5	10	1	2.018	
6	10	1	1.706	
7	10	1	0.854	
8	10	1	0.595	
9	10	1	0.426	
10	10	1	0.230	
11	10	1	0.108	
12	10	1	0.049	
13	10	1	0.021	
14	10	1	ND	

From the Table 4, it was observed that fenvalerate residue was detected in the yard long bean sample up to 13 DAS and the quantity were above EU-MRL up to 11 DAS and these were 7.958 mg/kg, 6.235 mg/kg, 5.391 mg/kg, 4.965 mg/kg, 3.890 mg/kg, 2.018 mg/kg, 1.706 mg/kg, 0.854 mg/kg, 0.595 mg/kg, 0.426 mg/kg, 0.230 mg/kg and 0.108 mg/kg at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11 DAS, respectively. The quantity decreased down to 0.049 mg/kg at 12 DAS and 0.021 mg/kg at 13 DAS which were below EU- MRL. At 14 DAS, no residue was detected. So, the PHI can be selected at 12 DAS.

4. Discussion

It was found from the study that residue of acephate in yard long bean and tomato had been detected up to 14 DAS and in case of tomato it was 11 DAS. The levels of residues were 4.181-0.012 mg/kg in yard long bean at 1-14 DAS and 3.609-0.015 mg/kg in tomato at 1-11 DAS. All of the detected amounts were above MRL set by European Union (2021). No residue was detected at 15 DAS in yard long bean and 12 DAS in tomato. The PHI can be selected for acephate at 15 DAS in yard long bean and 12 DAS in tomato. The residue of fenvalerate was detected up to 13 DAS in yard long bean 12 DAS in tomato. The amount of residues ranged from 6.235-0.108 mg/kg at 1-11 DAS in yard long bean and 4.321-0.149 mg/kg at 1-9 DAS in tomato which were above MRL set by European Union. While samples of 12 to 13 DAS contained 0.049-0.021mg/kg residue in yard long bean which were below EU-MRL. But the samples of tomato at 10 to 12 DAS had 0.076-0.008 mg/kg residue which were below EU-MRL. The PHI can be selected for fenvalerate at 12 DAS in yard long bean and 10 DAS in tomato. The PHI differed from pesticide to pesticide and crop to crop because of the plant behavior might be related to physio-chemical properties of pesticide and the effect of the interaction of environmental conditions such as wind, rain, sun, humidity, temperature and chemical and physical factor such as volatilization and growth of the plant (O' Brein, 1967; Virgina *et al.*, 1996; Jacobsen *et al.*, 2015).

Adnan *et al.* (2006) found diazinon residue above MRL up to 8 DAS in sweet pepper grown in green house. Ahmed *et al.* (2016) found the residue of fenvalerate was detected up to 14 DAS in hyacinth bean and tomato and the quantities were above MRL up to 3 DAS set by FAO/WHO (1993) and the selected PHI for fenvalerate was 4 DAS in both the vegetables because that period the MRL value was higher than EU-MRL (2021). Ahmed *et al.* (2020) reported that dimethoate residue was detected up to 13 DAS in cauliflower, 12 DAS in hyacinth bean and 11 DAS in eggplant. Ahmed *et al.* (2021) also reported cypermethrin residue was detected up to 9 DAS in cauliflower, 7 DAS in brinjal and tomato and the residue was above MRL at 4 DAS in cauliflower and 3 DAS in brinjal and tomato and the selected PHI was 5 DAS in cauliflower; 4 DAS in brinjal and tomato. But in case of chlorpyrifos the selected PHI was 8 DAS in brinjal and 10 DAS in cauliflower. Ahmed *et al.* (2022) reported that waiting period for dimethoate was 10 DAS in tomato and 11 DAS in yard long bean. But for fenitrothion it was 9 DAS in tomato and 10 DAS in yard long bean at prescribed dose. Duara *et al.* (2003) found the level of residue of cypermethrin and fenvalerate below MRL at 7 DAS of recommended dose in brinjal. Prodhan *et al.* (2018) found diazinon, quinalphos and malathion residue in yard long bean was detected up to 10 DAS and the quantities of residue was above MRL at 5 DAS and the PHI for tested pesticides in yard long bean was 7 DAS. They also found cypermethrin residue in yard long bean and tomato detected up to7 DAS and the selected PHI was 5 DAS in both the vegetables. The findings of the study agreed with the observation of the above authors.

5. Conclusions

The present study determined the pre-harvest interval (PHI) for acephate and fenvalerate in two popular vegetables in Bangladesh. The determined PHI for acephate was 12 DAS in tomato and 15 DAS in yard long bean. In case of fenvalerate the selected PHI was 10 DAS in tomato and 12 DAS in yard long bean. The findings of the study will help the farmers and other stakeholders to take proper initiative for the production of safe vegetables for consumers.

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Data availability

All the data presented in this study are contained in the manuscript.

Conflict of interest

None to declare.

Authors' contribution

Conceptualization and execution of study: Md. Sultan Ahmed; Methodology: Mohammad Dalower Hossain Prodhan, Md. Sultan Ahmed; Assisted in the laboratory works: Afroza begum and Marina Afroze; Writing-original draft preparation: Md. Sultan Ahmed; Review and editing: Mohammad Dalower Hossain Prodhan, Chandrima Emtia, Nirmal Kumar Dutta and Debasish Sarker. All authors have read and approved the final manuscript.

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