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Article

Degradation of selected organophosphorus pesticide residues and their pre harvest interval determination in hyacinth bean grown commercially in Bangladesh

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Abstract: The consumers are very much concerned about food safety. To ensure safe food for the consumers, it is essential to follow the pre harvest interval (PHI) of pesticides. Therefore, in order to ascertain the pre-harvest interval of fenitrothion and diazinon in hyacinth bean in the environmental conditions of Bangladesh, this study was started. The chosen organophosphorus insecticides (fenitrothion and diazinon) were sprayed with the recommended dose (1.5 ml/L of water) in a field trial at the Entomology Division of BARI, Gazipur. At 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 12 days after spray (DAS), samples were taken. The Gas chromatography (GC) with a Flame Thermionic Detector (FTD) was used to quantify the residues after the samples were prepared using a modified Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) extraction method. Fenitrothion residue was found up to 8 DAS, and at 7 DAS (0.018 mg/Kg), the level of residue was above the MRL. At 8 DAS, the fenitrothion residue was 0.007 mg/kg, which was below MRL. Pre-harvest interval (PHI) for fenitrothion was determined at 8 DAS, and no residue was found after 9 DAS. Diazinon residue was found up to 9 DAS, with the level of residue above the MRL at 8 DAS (0.016 mg/Kg). At 9 DAS, the diazinon residue was 0.007 mg/kg, which was below MRL. Pre-harvest interval (PHI) was determined for diazinon at 9 DAS, and as of 10 DAS, no residue was found.

Keywords: hyacinth bean; degradation; pre harvest interval; fenitrothion and diazinon residues; QuEChERS extraction; GC-FTD

1. Introduction

Hyacinth bean is a potential source of many vitamins and are typically grown in rabi seasons, usually close to the farm house. Almost every household grows country beans in their homestead (Singha *et al.*, 2018). Although it is believed that beans make up the majority of crops cultivated extensively during the rabi seasons, certain types are grown all year. As a result, from the perspective of the growing season, hyacinth beans are quite significant. Its low output is caused by the infestation of insect pests. Because of the severe attacks of insect pests, farmers experience a large yield loss each year (Mollah *et al*., 2017). The insect pests infestation depends on the biochemical constituents of fruits (Prodhan *et al.*, 2006), chlorophyll content of leaf (Prodhan *et al.*, 2006a), and also the anatomical characteristics of the fruits (Prodhan *et al.*, 2007). Hyacinth bean is attacked by 55 species of insect pests and mites (Chopkar *et al*., 2020).

According to Prodhan *et al.* (2015), pesticides are essential for preventing diseases and controlling insect pests in order to increase productivity. In addition, pesticides have a number of negative consequences on the environment and human health (Fenik *et al.*, 2011; Prodhan *et al.*, 2015a). In order to boost the usage of pesticides, these harmful effects are getting worse every day. Besides, the farmers may have the option to use some indigenous plant extracts and botanical pesticides (Rahaman *et al.*, 2008; Shah *et al.,* 2008) instead of toxic chemical pesticides for controlling the insect pests of different crops. But the farmers are not interested to use these control measures as these control mechanism are not sustainable.

To ensure that customers are eating safe food, developed nations have established Maximum Residue Limits (MRL) based on Acceptable Daily Intake (ADI) and Potential Daily Intake (PDI). Vegetables are harvested and sold in Bangladesh without observing the pre-harvest interval, there is little question that pesticide residue levels in such hyacinth beans will exceed the Maximum Residue Limit. The majority of hyacinth bean farmers in Bangladesh lack basic literacy skills, making it difficult for them to read and comprehend pesticide labels. They rely primarily on local dealers and retailers who are ill-equipped to choose the appropriate insecticides. Pesticide to pesticide and crop to crop, the PHI varies. The PHI is the number of days between the final pesticide application date and harvest that must pass in order for residue levels to drop below the tolerance limit determined for that crop or for a comparable food type. The only time a food product is safe to eat is after the withholding period has passed. The pesticide residues had vanished by this point.

In a range of matrices, pesticide residues have recently been extracted from and cleaned up using the QuECHERS extraction and cleanup technique (Prodhan *et al.*, 2016, 2016a, 2018). Compared to other currently used methods such liquid liquid extraction, ultra critical fluid extraction, etc., this method is becoming more and more popular every day (Prodhan *et al*., 2017). The use of GC-MS/MS and LC-MS/MS for determining pesticide residues in Bangladesh is relatively limited (Prodhan *et al.*, 2022, 2022a, 2023). To quantify pesticide residues in vegetables and fruits, gas chromatography in combination with flame thermionic detector and electron capture detector is widely employed (Kabir *et al.*, 2008; Islam *et al.*, 2014; Hasan *et al.*, 2017, 2019, 2019a, 2019b; Rahman *et al.*, 2021; Parvin *et al.*, 2021; Islam *et al.*, 2021) because they are sensitive for the detection of synthetic pyrethroid pesticides and organophosphorus pesticides. With this view, the pre-harvest interval of two specific organophosphorus pesticides (fenitrothion and diazinon) in hyacinth bean was determined in this study using gas chromatography equipped with a flame thermionic detector.

2. Materials and Methods

2.1. Ethical approval

Ethical approval was not required for this study.

2.2. Chemicals and reagents

The analytical standards for fenitrothion and diazinon were purchased from Sigma-Aldrich Laborchemikalien in Dhaka, Bangladesh, via Bangladesh Scientific Pvt. Ltd. Bangladesh Scientific Pvt. Ltd. in Dhaka, Bangladesh and the necessary solvents, including methanol, acetone, acetonitrile, sodium chloride, anhydrous magnesium sulphate, and Primary Secondary Amine (PSA) were also provided by the same company.

2.3. Sample collection and sample preparation

Samples were taken from the controlled field experiment after the recommended dose of fenitrothion and diazinon (1.5 ml/L of water) had been applied. At 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 12 days after spray (DAS), samples were taken. Each sample was 1 kg in weight. To avoid contamination, each plastic bag used to collect the sample was sealed. On the same sampling day, the obtained samples were delivered to the Pesticide Analytical Laboratory, Entomology Division, Bangladesh Agricultural Research Institute (BARI). Each sample's entire unit was divided into smaller parts and thoroughly mixed. Chopped samples were kept in sterile, airtight polythene bags in a freezer at -20°C until extraction and cleanup.

2.4. Extraction and clean up

The obtained samples were prepared using Prodhan *et al.* 2015's modified QuEChERS extraction and cleanup procedure. Using a standard fruit blender, the chopped samples were suitably homogenized. In a 50 mL centrifuge tube, a typical 10-g homogenized sample was weighed. Acetonitrile (ACN), 10 mL, was then poured into the centrifuge tube. After correctly sealing the centrifuge tube, a vortex mixer was used to vigorously agitate it for 30 s. The centrifuge tube was then filled with 1 g of NaCl and 4 g of anhydrous MgSO4 before being agitated for a minute by the vortex mixer. The extract was then centrifuged at 5000 rpm for 5 minutes. A 15 mL micro centrifuge tube containing 600 mg anhydrous MgSO4 and 120 mg Primary Secondary Amine (PSA) was filled with a 3 mL supernatant from the upper ACN layer. The mixture was then completely combined using a vortex for 30 seconds, followed by a 5-minute centrifugation at 4000 rpm (Laboratory Centrifuges, Sigma-3K30, Germany). A 1 mL supernatant was centrifuged, filtered through a 0.2 m PTFE filter, and then transferred to a clean GC vial for injection.

2.5. Instrumental analysis

The measurement of a few organophosphorus pesticides, including fenitrothion and diazinon, was done using a gas chromatograph (GC-2010 Shimadzu) with a Flame Thermionic Detector (FTD). Helium was employed as a carrier and make-up gas for the separation, which was carried out using a Rtx-OPPesticide2 capillary column (30 m length, 0.32 mm i.d., and 0.32 m film thicknesses). The flow across the column was 1.5 mL/min. The temperature for the column oven was designed to start at 150 °C (1 minute hold) and rise to 220 °C with an incremental rate of 10 °C (2 minutes hold). The temperature for the injector and detector were set to 250 °C and 280 °C, respectively. The split ratio employed for the sample injection (1 L) was in spit mode. The 10-minute runtime was the total. Quantification was carried out using the calibration curve created by the matrix matched calibration standard, and identification was based on the retention time of the matrix matched calibration standard.

3. Results and Discussion

3.1. Fenitrothion residue in hyacinth bean

The sample containing fenitrothion residue was analyzed by Gas Chromatography coupled with FTD. The results found from this study are summarized in Table 1.

Table 1. The fenitrothion residue level (mg/kg) found in the hyacinth bean samples.

Days after spraying	**Total volume prepared**	**Injected volume (μL)**	**Level of Residue (mg/kg)**	**MRL (mg/kg)**
0	10.0	1.0	0.4880	0.01
1	10.0	1.0	0.3550	
2	10.0	1.0	0.2990	
3	10.0	1.0	0.2010	
4	10.0	1.0	0.1290	
5	10.0	1.0	0.1240	
6	10.0	1.0	0.0650	
7	10.0	1.0	0.0180	
8	10.0	1.0	0.0070	
9	10.0	1.0	ND	
10	10.0	1.0	ND	
12	10.0	1.0	ND	

The amount of fenitrothion residues was above MRL up to 7 DAS and was detected up to 8 DAS. The level of residues were 0.4880 mg/kg, 0.3550 mg/kg, 0.2990 mg/kg, 0.2010 mg/kg, 0.1290 mg/kg, 0.1240 mg/kg 0.0640 mg/kg and 0.0180 mg/kg at 0, 1, 2, 3, 4, 5, 6 and 7 DAS, respectively. Sample of 8 DAS contained 0.0070 mg/kg fenitrothion residue which was below EU-MRL set by European Union. No residue was detected at 9, 10 and 12 DAS. Therefore, 8 DAS might be chosen as the PHI of fenitrothion for hyacinth bean.

3.2. Diazinon residue in hyacinth bean

The hyacinth bean sample containing diazinon residue was analyzed using Gas Chromatography coupled with FTD. The results found in this study are summarized in Table 2.

Table 2. The diazinon residue level (mg/kg) found in the hyacinth bean samples.

Days after spraying	**Total volume prepared**	**Injected volume (μL)**	**Level of Residue (mg/kg)**	**MRL (mg/kg)**
0	10.0	1.0	0.3390	0.01
1	10.0	1.0	0.2790	

Table 2. Contd.

Days after spraying	Total volume prepared	Injected volume (µL)	Level of Residue (mg/kg)	MRL (mg/kg)
2	10.0	1.0	0.2090	
3	10.0	1.0	0.1530	
4	10.0	1.0	0.1150	
5	10.0	1.0	0.0990	
6	10.0	1.0	0.0590	
7	10.0	1.0	0.0310	
8	10.0	1.0	0.0160	
9	10.0	1.0	0.0070	
10	10.0	1.0	ND	
12	10.0	1.0	ND	

The amount of diazinon residues was above MRL up to 8 DAS and was detected up to 9 DAS. The level of residues were 0.3390 mg/kg, 0.2790 mg/kg, 0.2090 mg/kg, 0.1530 mg/kg, 0.1150 mg/kg, 0.0990 mg/kg 0.0590 mg/kg, 0.0310 mg/kg and 0.0160 mg/kg at 0, 1, 2, 3, 4, 5, 6, 7 and 8 DAS, respectively. Sample of 9 DAS contained 0.0070 mg/kg diazinon residue which was below EU-MRL set by European commission. No residue was detected at 10 and 12 DAS. Therefore, 9 DAS might be chosen as the PHI of diazinon for hyacinth bean.

3.3. Trend of residue degradation

The trend of degradation of fenitrothion and diazinon residues in the hyacinth bean samples over time is shown in Figures 1 and 2.

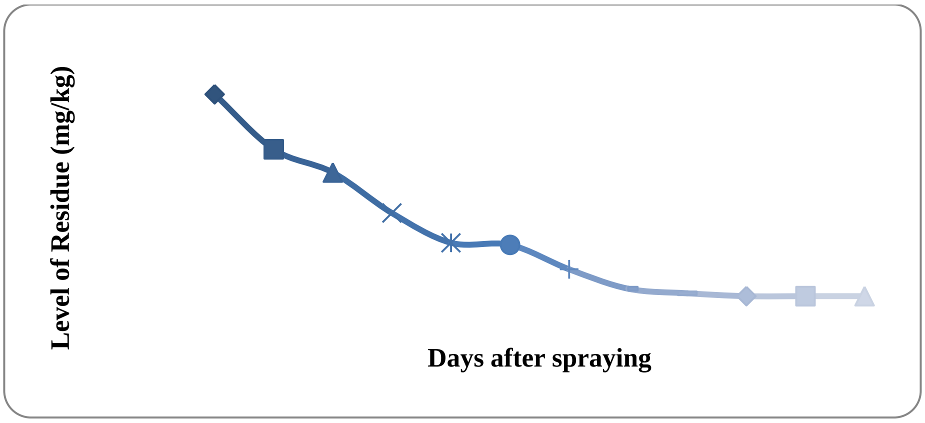

Figure 1. The trend of fenitrothion residue (mg/kg) degradation over time.

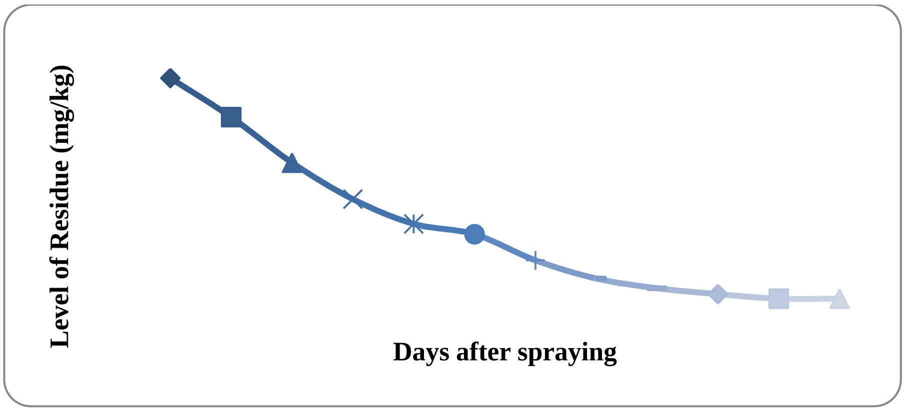

Figure 2. The trend of diazinon residue (mg/kg) degradation over time.

Because of their similar physico-chemical characteristics, it can be shown from Figures 1 and 2 that both pesticides degrade at roughly the same rate. However, for all of the chosen pesticides at different DAS, the level of observed residues and the rate of degradation were not the same. For fenitrothion and diazinon, the levels of identified residues at 0 DAS were 0.4880 mg/kg and 0.3390 mg/kg, respectively. The residues were detected upto 8 DAS for fenitrothion and 9 DAS for diazinon, respectively.

Numerous studies have been conducted so far to determine the pesticide residues in fruits (Prodhan *et al*., 2021), vegetables (Aktar *et al.,* 2017; Prodhan *et al.,*2018a; Nahar *et al.,* 2020; Parven *et al*., 2021; Nisha *et al.,* 2021; Hasan *et al*., 2021; Habib *et al.*, 2021; Tasnim *et al*., 2022, 2022a; Alam *et al*., 2022, 2023), sugarcane (Kabir *et al.,* 2007), betel leaf (Prodhan *et al.*, 2023a), water (Prodhan *et al.,*2021a) and others matrices (Prodhan *et al.*, 2009, 2010, 2018b; Hoque *et al.*, 2021). Among them, most of the research works cited above have been conducted to monitor pesticide residues in Bangladesh with the marketed samples. On the other hand, for the determination of pre harvest intervals of pesticides in different fruits and vegetables are limited. Based on the available research works on pre harvest intervals, the results of the present study are in a good agreement.

Hossain *et al.* (2014) found that the PHI for acephate and cypermethrin was 7 DAS. In our previous study, we have found that the PHI of chlorpyrifos and quinalphos in hyacinth bean was 9 DAS (Khanom *et al.*, 2023). The similar findings of the present study was found by Prodhan *et al*. (2018c). They conducted a study for the determination of pre harvest interval of few selected pesticides and found that the pre harvest interval of quinalphos was 7 DAS in yard long bean, 10 DAS in eggplant and 12 DAS in cabbage. Therefore, the findings of this study will help the the policy planners and the relevant stakeholders to take necessary actions to ensure safe food for the consumers.

4. Conclusions

In this study, two widely used organophosphorus pesticides (fenitrothion and diazinon) were applied in hyacinth bean for the determination of PHI of the selected pesticides to ensure the supply of safe hyacinth bean and it was found that the PHI for fenitrothion was 8 DAS, and for diazinon it was 9 DAS. From the findings of the present study, it can be recommended that the farmers have to wait until 8 DAS in case of fenitrothion application and for diazinon application, the farmers have to wait until 9 DAS.

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Data availability

The tables, figures and texts in this article contain the data that support the findings of this study.

Conflict of interest

None to declare.

Authors' contribution

Conceptualization: [Mohammad Dalower Hossain Prodhan]; Methodology: [Rozina Khanom], [Mohammad Dalower Hossain Prodhan], [S M Mizanur Rahman]; Formal analysis and investigation: [Rozina Khanom], [Mohammad Dalower Hossain Prodhan]; Writing - original draft preparation: [Rozina Khanom], [Md. Safiqul Islam], [Mohammad Dalower Hossain Prodhan]; Writing - review and editing: [Mohammad Dalower Hossain Prodhan], [S M Mizanur Rahman], [Md. Safiqul Islam]; Supervision: [S M Mizanur Rahman], [Mohammad Dalower Hossain Prodhan]. All authors have read and approved the final manuscript.

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