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Article

Determination of pre harvest interval for selected pesticides in hyacinth bean in the agro-climatic conditions of Bangladesh

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Abstract: The present study was conducted to determine the pre harvest interval of chlorpyrifos and quinalphos in hyacinth bean in the agro-climatic condition of Bangladesh to ensure safe food for the consumers. A supervised field trial was undertaken in the experimental field of Entomology Division of BARI, Gazipur and the selected pesticides (chlorpyrifos and quinalphos) were sprayed with the recommended dose (1.5 ml/L of water). The samples were collected at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 12 days after spray (DAS). The collected samples were extracted and cleaned up using modified Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) extraction technique and the residues were determined by Gas Chromatography (GC) coupled with Flame Thermionic Detector (FTD). The residue of chlorpyrifos was detected upto 8 DAS (0.027 mg/Kg) at a level above the EU-MRL, and at 9 DAS, no residue was detected. So, the PHI for chlorpyrifos was set at 9 DAS. On the other hand, the residue of quinalphos was detected upto 9 DAS, of which the level of residue was above EU-MRL upto 8 DAS (0.02 mg/kg). At 9 DAS, the quinalphos residue remained 0.009 mg/Kg, which was below EU-MRL. So, the PHI for quinalphos was set at 9 DAS.

Keywords: hyacinth bean, pre harvest interval; organophosphorus pesticide residues; QuEChERS extraction; gas chromatography; food safety

1. Introduction

The Hyacinth bean, *Dolichos lablab* L. is an important winter vegetable crop in Bangladesh. It belongs to the family Fabaceae. Though it is commonly known as country bean in Bangladesh, but it has different names at different regions of the country like Sheem, Uri, Ushi, Deshi Sheem etc. Internationally the crop has also different names e.g. Hyacinth bean, Bonavist bean, Dolichos bean, Indian bean, Egyotian kidney bean, Lima bean, Faba bean etc. (Jadhav *et al*., 1987). It is remarkable that bean crops show a broad range of adaptation to the most varied climatic conditions within a wide range of geographic area that extends from around 50^{o} north latitude to 32^{o} south latitude.

Hyacinth bean is a big source of essential vitamins and generally cultivated in rabi seasons usually around the homestead by trailing its vine either on trees or by providing different kinds of supports. Although beans are assessed as the major group of vegetables grown intensively in rabi seasons, some varieties of hyacinth bean can

be grown year round including kharif seasons. The importance of hyacinth bean has thus highly significance from growing season point of view (Hossain and Awrangzeb, 1992).

In spite of being a prospective crop, high rate of insect pests infestation is responsible to its low yield and poor quality. Farmers face significant yield loss of beans every year due to severe attack of various insect pests in our country. Generally, insect pests cause enormous quantity of yield losses in every season. Though, regular statistical records are not kept, as per conservative estimate the yield loss in hyacinth bean due to insect pests is reported to be about 12-30% (Hossain, 1990). The insect pests infestation depends on the biochemical constituents of the fruits (Prodhan *et al.,* 2006), chlorophyll content of the leaf (Prodhan *et al.,* 2006a), and anatomical characteristics of the fruits (Prodhan *et al.,* 2007). Alam (1969) observed that hyacinth bean is attacked by nine different insect species and one species of mite. Dina (1979) and Bakar *et al*. (1980) found *M. testulalis* G. is a serious insect pest of leguminous vegetables. Butani and Jotwani (1984) found aphid as the most common pests all over the world and lepidopterous larvae *M. testulalis* (Geyer) as pests of bean causing damage by boring tender of mature pods. Bean pod borer is able to establish itself on legumes from vegetative to reproductive stage. Aphid is responsible for feeding damage and transmission of plant virus (Kennedy, 1976).

Due to plant pests and diseases 20 to 40 percent of the crop yields are reduced globally (FAO, 2012). To overcome these situations farmers are using pesticides. Pesticides play a key role to control the insect pests and diseases and hence protect and promote production (Prodhan *et al*., 2015). On the other hand, pesticides create several adverse effects on human health and the environment (Fenik *et al.,* 2011; McIntyre, 1989; Prodhan *et al.,* 2015a). These negative impacts of pesticides are increasing day by day in order to increase uses of pesticides. 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.

It is obvious that modern insecticides are highly toxic to every organism of the animal kingdom including human beings. Even in a well-managed condition spillover of spray materials on soil, water and other abiotic materials is highly likely to cause pollution. Transformation and volatilization of such materials cause residue load in the environment (Spencer *et al*., 1973). It is evident that the more persistent pesticide, the greater is the danger to the environment. Although it is not well documented but insecticide residues hamper microbial activities in soil, destroy aquatic lives and non-target invertebrates and cause enormous damage to higher vertebrates (Ware and Roan, 1970; Edwards, 1974).

As a safety measure for the consumers, many developed countries have set Maximum Residue Limit (MRL) based on the Acceptable Daily Intake (ADI) and Potential Daily Intake (PDI). In Bangladesh, since harvesting and selling of vegetables are done without perplexing for the pre-harvest interval, pesticide residue levels in such hyacinth bean would undoubtedly be above Maximum Residue Limit. In Bangladesh, most of the hyacinth bean growers are illiterate and they are not able to read and understand what is written on the label of pesticides. They mostly depend on ill motive pesticide dealers/retailers of their respective locations who have no clear idea about insect pests and pesticides but usually recommend insecticides that create serious problems for public health and the environment.

Pesticide residue in food has become a consumer safety issue and the consumer has the right to know how much pesticide get incorporated in the food he eats. The detection, identification and quantification of pesticide in the food we eat are a problem of increasing public interest. But still a few references are available on pesticide residues present in vegetables and also the withholding period or pre-harvest interval (PHI) of major vegetables grown in Bangladesh. Every pesticide has a withholding period, waiting period, lapse 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 tolerance level established for that crop or for a similar food type. The PHI differs from pesticide to pesticide and crop to crop. Food products become safe for consumption only after withholding period has lapsed. By this time, the pesticide residues get dissipated. However, 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.,* 1999). Due to lack of education, the farmers of our country do not follow the prescribed dosages and use pesticides at any stage of the crop without any awareness of the residues and their ill effects on human health. The treated fruits and vegetables are picked/ harvested without taking into account the withholding period.

Now a days, the quick, easy, cheap, effective, rugged and safe (QuECHERS) extraction and cleanup method is widely used for extraction and clean up of pesticide residues in a wide variety of matrices (Prodhan *et al.,* 2018; 2016; 2016a). This method is gaining popularity day by day compared to the other existing methods like liquid liquid extraction, super critical fluid extraction etc. (Prodhan *et al*., 2017). In Bangladesh, Gas Chromatography

coupled with flame thermionic detector and electron capture detector is widely used for the quantification of pesticide residues in vegetables and fruits (Rahman *et al.,* 2021; Ahmed *et al.,* 2021; 2021a; Parvin *et al.,* 2021; Islam *et al.,* 2021; 2019; 2019a; 2019b; Hasan *et al.,* 2017; Islam *et al.,* 2014; Kabir *et al.,* 2008; 2008a) as they are very much sensitive for the quantification of organophosphorus pesticides and synthetic pyrethroid pesticides. But, the application of LC-MS/MS are very limited (Prodhan *et al.,* 2022; 2022a). The gas chromatography is widely used as it is comparatively cheap and easy to maintain. On the other hand, the sensitivity is quite good for the selective pesticides. Based on the above fact, the gas chromatography coupled with flame thermionic detector was used in this study to determine the pre harvest interval of selected pesticides (chlorpyrifos and quinalphos) in hyacinth bean to ensure the supply of safe food for the consumers.

2. Materials and Methods

2.1. Ethical approval

This study did not require ethical approval.

2.2. Chemicals and reagents

The chlorpyrifos and quinalphos standard were obtained from Sigma-Aldrich Laborchemikalien (St Louis, MO, USA) via Bangladesh Scientific Pvt. ltd. Dhaka, Bangladesh. The necessary solvents like methanol, acetone, acetonitrile, sodium chloride, anhydrous magnesium sulphate and Primary Secondary Amine (PSA) were obtained from Bangladesh Scientific Pvt. ltd. Dhaka, Bangladesh.

2.3. Sample collection and sample preparation

The hyacinth bean samples were collected from the supervised field trial after the application of selected pesticides (chlorpyrifos and quinalphos) with a recommended dose (1.5 ml/L of water). The samples were collected at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 12 days after spray (DAS). The amount of each sample was 1 Kg. Sample was collected in individual polyethylene bag to prevent the contamination. The collected samples were taken to the Pesticide Analytical Laboratory, Entomology Division, Bangladesh Agricultural Research Institute (BARI) on the same sampling day. The whole unit of each sample cut into small pieces and mixed properly. Clean air tight polythene bags were used to store chopped sample in a refrigerator at -20°C until extraction and cleanup process.

2.4. Extraction and clean up

The QuEChERS extraction method was used for the extraction and clean-up of samples which was modified by Prodhan *et al*. 2015. The chopped samples were grounded thoroughly with the fruit blender. A representative 10-g properly homogenized sample was weighted in a 50 mL polypropylene centrifuge tube. Then, 10 mL of acetonitrile (ACN) was added into the centrifuge tube. The centrifuge tube was closed properly and shaken vigorously for 30 s by vortex mixer. Then, 4 g of anhydrous $MgSO_4$ and 1 g of NaCl were added into the centrifuge tube, and it was shaken by the vortex mixer for 1 minute. Afterwards, the extract was centrifuged for 5 min at 5000 rpm. An aliquot of 3 mL of the upper 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 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 GC vial for injection.

2.5. Instrumental analysis

A Gas Chromatograph (GC-2010 Shimadzu) coupled to Flame Thermionic Detector (FTD) was used for the quantification of selected organophosphorus pesticides (chlorpyrifos, and quinalphos). The separation was done by Rtx-OPPesticide2 capillary column (30 m long, 0.32 mm i.d and 0.32 µm film thicknesses), helium was used as carrier and make up gas as well. The column flow rate was 1.5 mL/min. The temperature for the injector and the detector was set to 250 °C and 280 °C, respectively, and for the column oven, the temperature was programmed, which was started from 150 °C (1 min hold) and went up to 220 °C with an incremental rate of 10 °C (2 min hold). Spit mode was used for the injection of samples (1 µL) and the split ratio was 10.0. The total run time was 10 min. Identification was performed by comparing the retention time of the matrix matched calibration standard and the quantification was done using the calibration curve prepared with matrix matched calibration standard.

3. Results and Discussion

3.1. Chlorpyrifos residue in hyacinth bean

The hyacinth bean sample containing chlorpyrifos residue was analyzed using Gas Chromatography coupled with FTD. The results obtained from this analysis are summarized in Table 1.

Table 1. The level of chlorpyrifos residue (mg/kg) estimated from the samples of hyacinth bean.

Days after spraying	Total volume prepared	Injected volume (µL)	Level of residues (mg/kg)	EU-MRL (mg/kg)
0	10	1	0.532	0.01
1	10	1	0.409	
2	10	1	0.324	
3	10	1	0.213	
4	10	1	0.143	
5	10	1	0.118	
6	10	1	0.087	
7	10	1	0.044	
8	10	1	0.027	
9	10	1	ND	
10	10	1	ND	
12	10	1	ND	

The residue of chlorpyrifos in hyacinth bean was detected up to 8 DAS and the level of residues were above EU-MRL (European commission 2015) up to 8 DAS and these were 0.532 mg/kg, 0.409 mg/kg, 0.324 mg/kg, 0.213 mg/kg, 0.143 mg/kg, 0.118 mg/kg, 0.087 mg/kg, o.044 mg/kg and 0.027 mg/kg at 0, 1, 2, 3, 4, 5, 6, 7 and 8 DAS, respectively. No residue was detected at 9, 10 and 12 DAS. So, the PHI of chlorpyrifos for hyacinth bean can be selected at 9 DAS.

3.2. Quinalphos residue in hyacinth bean

The hyacinth bean sample containing quinalphos residue was analyzed using Gas Chromatography coupled with FTD. The results obtained from this analysis are summarized in Table 2.

Table 2. The level of quinalphos residue (mg/kg) estimated from the samples of hyacinth bean.

Days after spraying	Total volume prepared	Injected volume (µL)	Level of residue (mg/kg)	EU-MRL (mg/kg)
0	10	1	0.562	0.01
1	10	1	0.426	
2	10	1	0.352	
3	10	1	0.240	
4	10	1	0.181	
5	10	1	0.146	
6	10	1	0.101	
7	10	1	0.041	
8	10	1	0.02	
9	10	1	0.009	
10	10	1	ND	
12	10	1	ND	

The residue of quinalphos in hyacinth bean was detected up to 9 DAS and the levels of residues were above EU-MRL (European commission 2015) up to 8 DAS. The detected residues were 0.562 mg/kg, 0.426 mg/kg, 0.352 mg/kg, 0.240 mg/kg, 0.181 mg/kg, 0.146 mg/kg, 0.101 mg/kg, 0.041 mg/kg and 0.02 mg/kg at 0, 1, 2, 3, 4, 5, 6, 7 and 8 DAS, respectively. The sample of 9 DAS contained 0.009 mg/kg, which was below the EU-MRL. No residues were detected at 10 and 12 DAS. So, the PHI of quinalphos for hyacinth bean can be selected at 9 DAS.

3.3. Trend of residue degradation

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

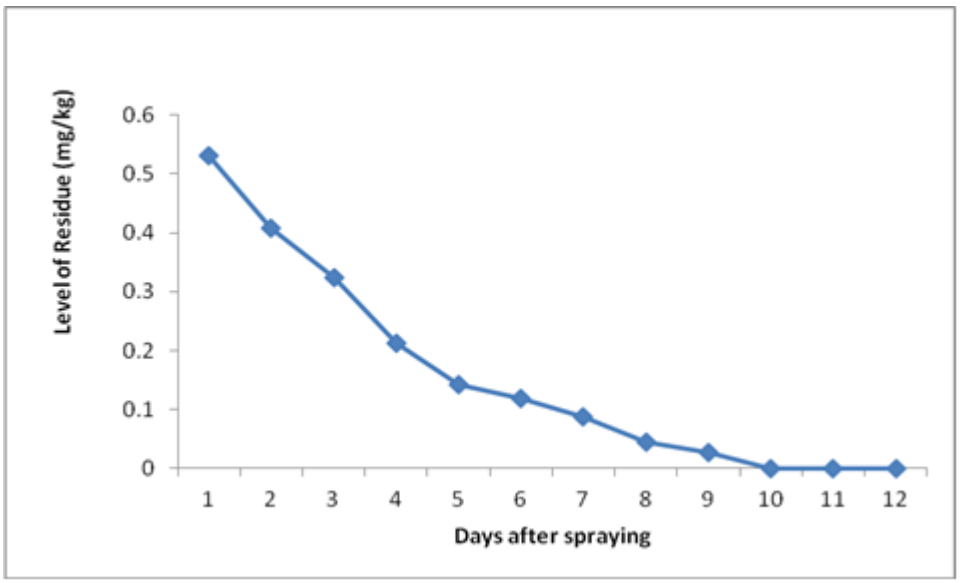

Figure 1. The trend of chlorpyrifos residue (mg/kg) degradation over time.

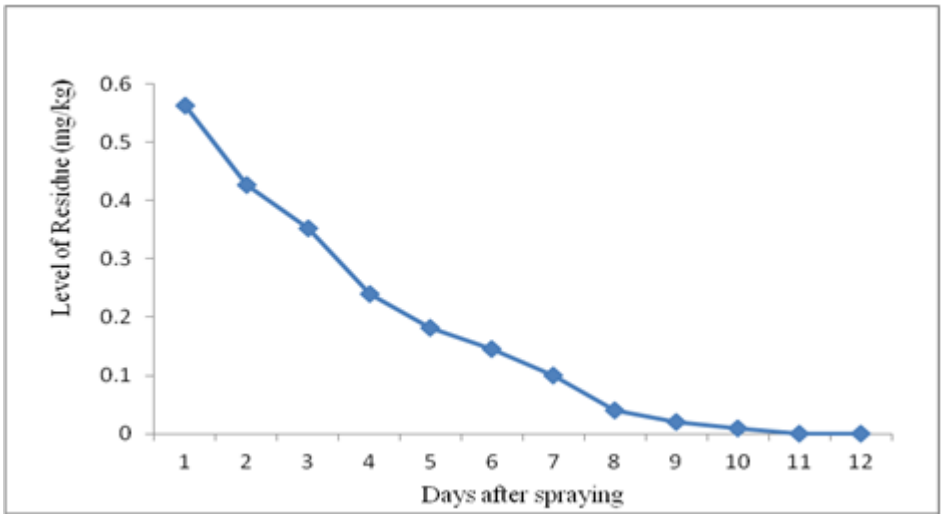

Figure 2. The trend of quinalphos residue (mg/kg) degradation over time.

From the Figures 1 and 2, it was observed that the degradation rate of both insecticides is more or less similar because the physico-chemical properties for both of them are quite similar. But the level of detected residues and the degradation rate were not same for all of the selected insecticides at different DAS. The level of detected residues at 0 DAS were 0.532 mg/kg, 0.562 mg/kg for chlorpyrifos and quinalphos, respectively. The residues were detected upto 8 DAS for chlorpyrifos and 9 DAS for quinalphos.

Up until now, a number of research works have been conducted for the determination of pesticide residues in fruits (Prodhan *et al*., 2021), vegetables (Alam *et al*., 2023; 2022; Tasnim *et al*., 2022; 2022a; Parven *et al*., 2021; Nisha *et al.*, 2021; Hasan *et al.*, 2021; Habib *et al.*, 2021; Nahar *et al.*, 2020; Prodhan *et al.*,2018a; Aktar *et al.*, 2017), sugarcane (Kabir *et al.*, 2007), betel leaf (Prodhan *et al.*, 2023), water (Prodhan *et al.*,2021a) and others matrices (Hoque *et al.*, 2021; Prodhan *et al.*, 2018b; 2010; 2009). Most of the research works mentioned above have been conducted on the monitoring of pesticide residues in Bangladesh with the marketed samples. However, to determine pre harvest intervals of different pesticides in different fruits and vegetables are limited. Based on the available research works on pre harvest intervals, the results of the present study can be compared nicely.

The results of the present study are supported by the findings of Prodhan *et al*. (2018c). They conducted a study to determine the pre harvest interval of some selected pesticides including quinalphos in yard long bean, eggplant and cabbage 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. The findings of this study are in a good agreement with the findings found by Hossain *et al*. (2014). They have been conducted a study to determine the PHI of acephate and cypermethrin in yard long bean and found that the PHI for acephate and cypermethrin was 7 DAS. Therefore, the findings of the present study will help the consumers to ensure the safe food, as well as to provide a good indication for the policy planners and the relevant stakeholders to take necessary initiatives for ensuring the safe food for the consumers.

4. Conclusions

Hyacinth bean, the delicious winter vegetables in Bangladesh, is more intensively and commercially grown vegetable. In order to manage the insect pests, the farmers using toxic chemical pesticides to control these insect pests. Due to this reason, residues of commonly used pesticides remain in the harvested crops which create a lot of adverse effect in the human health and the environment as well. In this study, two popular pesticides (chlorpyrifos and quinalphos) were sprayed in hyacinth bean to determine the PHI of these pesticides in hyacinth bean ensuring safe hyacinth bean and found that the determined PHI for both of the selected pesticides were 9 DAS. It means that if the hyacinth bean is harvested after 9 days of chlorpyrifos and quinalphos spray, it will not cause any harm for the consumers.

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Data availability

All relevant data are within the manuscript.

Conflict of interest

None to declare.

Authors' contribution

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

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