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DEGRADATION PATTERN AND RISK ASSESSMENT OF IMIDACLOPRID IN COUNTRY BEAN USING GAS CHROMATOGRAPHY

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Abstract

The degradation pattern of Imidacloprid on country bean and its risk assessment for the safe consumption were studied. QuEChERS method was used for the extraction and cleanup of samples and the residues of Imidacloprid were estimated using Gas chromatography. The dissipation studies on country bean were carried out by application of Imidacloprid at five different dosages i.e. 100, 200, 300, 400, and 500 g a.i. ha^{-1}. Average initial deposits of Imidacloprid were 0.37, 0.73, 1.35, 2.15 and 3.52 $mg.kg^{-1}$, respectively. The residues reached below determination limit (BDL) of 0.01 mg kg^{-1} in nine days for recommended dose and 12 days for remaining higher doses . Half-life ($T_{1/2}$) of Imidacloprid on country bean was observed 2.47, 2.21, 1.90, 1.83 and 1.77 days, respectively, at 100, 200, 300, 400, and 500 g a.i. ha^{-1}. The chemodynamics study of Imidacloprid spray indicated that only 27.20 - 28.40% sprays were deposited to the target site (plant canopy) and 71.60-72.80% were lost to non-target site, such as soil and air. The drift of Imidacloprid to soil (39.47-40.20%) was higher than that of the air (32.13-32.60%). It was found that the theoretical maximum residue contribution (TMRC) values reached below maximum permissible intake (MPI) on 0 day (1 hour of treatment) in country bean samples treated with imidacloprid. This study, therefore, suggested that the use of Imidacloprid formulation at different dosages (up to five times than recommended doses) does not seem to pose any hazards to the consumers. The maximum Half-life ($T_{1/2}$) of Imidacloprid was found 2.47 days. Therefore, a waiting period of three days might be suggested to reduce the risk before consumption of country bean grown in Imidacloprid contaminated soil. These findings could provide guidance for the proper and safe use of this pesticide on country bean in Bangladesh.

Keywords: Degradation, GC, MPI, TMRC, half-life, chemodynamics.

Introduction

Country bean, *Lablab purpureus* (L) is a popular and widely grown vegetable in Bangladesh. It is a most important leguminous vegetable in Bangladesh and grown in a significant acreage next to the brinjal and tomato. The bean is commonly known as Seem in Bangladesh. The fresh pods and green seeds are eaten after boiling or used in curries. In Bangladesh, it is commercially cultivated in Jessore, Kushtia, Khulna, Bogra, Pabna districts, etc. The total land area under bean cultivation is 16,588 ha and yielding an average of 4.53 tons of fresh pods per ha and the total production is 88,582 tons. The area under this crop has been increasing day

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by day in the summer season in the south-western part of Bangladesh due to its more profit and high demand. There are some photo sensitive varieties which are grown in summer season, such as IPSA Seem I, IPSA Seem II, and BARI Seem III, etc. All these varieties can be grown year-round especially in the summer rainy season. In these contexts, the country bean having varieties suitable for production during off season can play a vital role to remove the vegetable deficiency (Alam *et al*., 2005).

In spite of being a prospective crop, high incidence of insect pests results in low yield and poor quality of country bean. Farmers of Bangladesh face significant yield loss of country bean every year due to severe attack of various insect pests. Although no regular statistical records are available, as per conservative estimate, the yield loss in country bean due to insect pests is reported to be about 12-30% (Hossain, 2000). Country bean is attacked by nine different insect species and one species of mite. The major pests attacking country bean are pod borer (*Maruca testulais)*, aphid (*Aphis craccivora*), bean bug, leaf weevil, hooded hopper, etc. (Alam, 2009 and Begum, 2003). Among the pests, bean pod borer is a serious insect pest of leguminous crops and legumes at vegetative to reproductive stage and cause substantial damage to flowers and fruit setting (Dina, 2009). Many authors reported aphid as the most common pest all over the world and lepidopterous larvae *Maruca testularis* as pests of bean causing damage by boring tender or mature pods.

As country been attacked by many pests and cause considerable damage, pest management is essential. Current management practices of insect pests are based almost entirely on chemical insecticides as they give quick result. As most of our people are illiterate, they use pesticides more than the recommended doses indiscriminately. In Jashore region, the farmer applies insecticides 84 to 140 times to country bean and 160 times to brinjal during a growing season (Anon., 2006). This overuse, misuse, and the way of using cause drifting loss to the nearest crop and in the atmosphere, which result in pest resurgence, stimulate the reproductive rate in certain pests, secondary pest outbreaks, mortality of beneficial insects, resistance of pest species and finally causes environment pollution (Alam *et al*., 2005). The improper use of pesticides may cause some residues to remain in the vegetables. Thus, fresh vegetables could be a potential source of toxic pesticides. Food safety has become a major public concern worldwide (Radwan and Salama, 2006). The irrational use of pesticides has created new pests that have never been a problem before (Haque *et al*., 2010). Therefore, the food matrices become unsafe for consumption.

Indiscriminate use of these chemicals, particularly at fruiting stage, leads to its accumulation in the vegetables which consequently cause hazards to human beings through food chain. These results serious contamination of different component of environment (surface water, aquifers, soil, etc.) including human, wildlife and other organisms. The consequence of such frequent and poor application of pesticide cause serious contamination of environment through chemodynamics of pesticide. It is to be noted that only 10-20% of the applied pesticides reach the target site while the rest enter into

various environmental component (Gill *et al.,* 2008). Different kinds of pesticides are used and its consumption of Bangladesh reached 54000 metric tons of formulated product having 14700 active ingredients.

Imidacloprid (I-[(6-chloro-3-pyridinyl)-methyl]-N-nitro-2-imidazolidinimine), a neonicotinoid insecticide is frequently used to control chewing and sucking insects. The insecticide is extremely potent against wide range of arthropods, including aphids, scale insects, white flies, some heteroptera, coleoptera, and lepidoptera species (Decourtye *et al*., 2004). It is a soil or plant-applied insecticide used in a wide variety of crops. It has a new mode of action, low toxicity to warm-blooded animals, good systemic properties with a long-lasting action (Frenich *et al*., 2000). The development, activity, mode of action and effectiveness have been described by Leicht (1993) and its physical, chemical and toxicological properties have been summarized in the pesticide manual (Tomlin, 1994). The compound was introduced in Europe by Bayer (Leverkusen, Germany). The parent and its main metabolite (6-chloronicotinic acid) are polar compounds with high solubility in water.

In mammals, the primary effects of Imidacloprid are mortality, transient cholinergic and transient growth retardation. Exposure to high doses may be associated with degenerative changes in the testes, thymus, bone marrow, and pancreas. Cardiovascular and hematological effects have also been observed at higher doses. The primary effects of Imidacloprid in longer term, but lower-dose exposure is on the liver and thyroid (Anatra-Cordone and Durkin, 2005). Recent research suggests that widespread agricultural use of Imidacloprid may be contributing to honey bee colony collapse disorder results the decline of honey bee colonies (Chensheng *et et al*., 2012 and Whitehorn *et al*., 2012). As a result, many countries have restricted the use of Imidacloprid and other neonicotinoids (Carrington, 2012). In January 2013, the European Food Safety Authority (EFSA) stated that Imidacloprid as a neonicotinoid pose an unacceptably high risk to honeybees (EFSA, 2013) and impairs its memory and brain metabolism (Decourtye *et al*., 2004). Due to lack of knowledge about pesticide, the farmers are severely affected by these toxic pesticides as well as toxify the environmental components. Residues of Imidacloprid persist in soil and edible plant parts which consequently cause hazards to human being through food chain. However, now-a-days the mass media is creating awareness about the risk involved in the use of pesticide in agriculture. This has created interest among the people regarding the accumulation of residues of the toxic chemicals in daily food.

Liu *et al*. (2006) reported that Imidacloprid was stable in water at neutral and acidic pH and slowly degraded in alkaline conditions. Vegetation also increased the dissipation rate of . Imidacloprid as well as its half-lives (Anhalt *et al*., 2007). The breakdown of principle component may be caused by harsh environmental condition, chemical interaction or photolytic reaction (Segura *et al*., 2008). The degradation pattern and risk assessment studies of Imidacloprid for a given crop in open field conditions of each growing area are necessary to investigate. Till now, no investigation has been made on this aspect in Bangladesh. Therefore, the present piece

of work has been conducted to determine the degradation pattern and risk assessment of Imidacloprid in country bean agroecosystem.

Materials and Methods

The study was conducted to assess the risk and to observe the degradation pattern of Imidacloprid residues in country bean agroecosystem of Bangladesh during October 2016 to February 2017. Field experiment was conducted in the field of Department of Entomology and laboratory analysis was done in the pesticide and environmental toxicology laboratory of Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU). The experimental site was located at Madhupur Tract (24°09' N latitude and 90°26' E longitude) having an elevation of 8.2 m from sea level. The soil type of the experimental field belongs to the sub-tropical climatic zone characterized by shallow red brown terrace was under Salna series of Madhupur Tract of Agro Ecological Zone (AEZ 28).

Sampling and data collection

The whole area of experimental field was divided into five blocks and each block was again divided into 36-unit plots. *S*ize of each plot was 1.5 m^2. After seven days of plot preparation, five bean seeds were sown per plot and followed by a light irrigation to ensure soil moisture for germination. Imidacloprid (Admire 20 SL) was applied during flowering and fruiting stage at the rate of 100 g a.i. ha^{-1} (in 1^{st} block) 200 g a.i. ha^{-1}(2^{nd} block), 300 g a.i. ha^{-1}(3^{rd} block), 400 g a.i. ha^{-1} (4^{th} block) and 500 g a.i. ha^{-1}, (5^{th} block), which were considered as a treatment of the experiment. Pesticide was sprayed as foliar application with the help of a Knapsack sprayer fitted with hollow cone nozzle. About 250 g country bean pod sample was collected randomly at 0 (2 h), 1, 3, 6, 9, and 12 days after the application of the sprays. The country bean leaf samples were collected from each plot separately, packed in polyethylene bags and brought to the laboratory for processing. Samples were extracted and cleaned up immediately after sampling.

Chemodynamics of imidacloprid in country bean agroecosystem

For determining insecticide application loss in soil, a polythene sheet (2.25 m^2) was initially placed randomly in the 1.5 m^2 plot. Then the foam sheet (1.25 cm thickness) of the same size (weighed) was placed on the polythene sheet to avoid loss of applied insecticide to the ground. Immediately after application of insecticide, the foam was weighed by electric balance. Insecticide application loss in soil was determined by subtracting the initial weight from the final weight of the foam sheet. Application loss in air was measured by weighing the polythene sheet before and after the insecticide application. Insecticide retained on target canopy was determined by subtracting the quantity of insecticide lost in the soil and the air, from the total quantity of insecticide applied.

Chemicals and reagents

The technical grade analytical standard of Imidacloprid (purity 99.1%) was procured from M/S Sigma Aldrich, Germany. The formulation Admire 20 SL (AP-486)) was obtained from Bayer Crop Science Limited Bangladesh. Analysis of acetonitrile (GC grade) extract of the formulation showed only Imidacloprid and none of its metabolic products and was found to be accurate with respect to its active ingredient. Solvents like

ethyl acetate, acetonitrile (GC grade), water (distill), sodium chloride (ASC reagent grade 99.9%) and analytical grade activated anhydrous $MgSO_4$ were obtained from Merck, Darmstadt, Germany. Sodium sulfate anhydrous (AR grade), Primary Secondary Amine (PSA) Sorbent and activated graphitic carbon black (GCB, 400 mesh) were procured from registered chemical importer of Bangladesh. All common solvents were redistilled in all-glass apparatus before use. The suitability of the solvents and other chemicals was ensured by running reagent blanks before actual analysis.

Preparation of standard solution

A standard stock solution of imidacloprid (1 mg. L^{-1}) was prepared in GC grade acetonitrile. The standard solution was required for constructing a calibration curve (10, 20, 30 ngL^{-1}). It was prepared from stock solution by serial dilutions with GC grade acetonitrile. All standard solutions were stored at 4°C before use.

Extraction and cleanup

A standardized analytical method with slight modification was followed for extraction of Imidacloprid residues as reported by Sharma (2013). The country bean fruit samples were prepared following QuEChERS method for the determination of Imidacloprid residues. At first, country bean pod was cutting into small pieces. A sub sample of 15 g of country bean was weighed and blended. After that, it was dispersed into a 50 ml centrifuge tube and then 15 ml acetonitrile (1% acetate acid) was dispensed into it. The sample was homogenized using high speed homogenizer (Heidolph Silent Crusher-M®) for 2-3 min at 1400-1500 rpm. 1.5 g Sodium Chloride (NaCl) and 6 g $MgS0_4$ was added and kept the sample overnight by covering the test tube with parafilm to homogenize the sample for phase separation. The contents were centrifuged at 2500-3000 rpm for 10 min. An aliquot of 10 ml ethyl acetate layer was transferred in a test tube. The acetonitrile extract was then subjected to cleanup by dispersive solid phase extraction (DSPE), an aliquot of 6 ml acetonitrile was transferred into a separatory funnel containing 0.15 ± 0.01 g PSA sorbent and n-hexane was added to the aliquot and vigorously shaken for 1 min. The layers were allowed to separate. Then the acetonitrile was drained out and n-hexane remained in the separatory funnel. Now sulfuric acid was added to hexane so that microorganisms were killed. After 1-1.5 min, sulfuric acid drained out of the separatory funnel and distilled water was added to the hexane to remove sulfuric acid from the aliquot. The aliquot was drained out through the bed of anhydrous sodium sulphate with a filter paper.

Instruments

The quantification of Imidacloprid residues was done by using Gas Chromatograph (GC) (Table 1). The Gas Chromatograph (Model: GC 17) equipped with Electron Capture Detector, was supplied by Shimadzu, Australia. The sample injector was equipped with auto injector (AOC, 20i). For instrument control, data acquisition and processing, GC solution software was used.

The experimental conditions are presented in the Table 1. An aliquot was injected into the GC with auto injector. Tentative identification of the suspected insecticide was carried out in relation to retention times of the pure analytical standard. An injection volume of 2 µL was used in all the studies.

Table 1. Details of gas chromatographic conditions used in this study

Parameter	:	Condition			
Column	:	Column name: Rtx-CL pesticide (Sl# 726625) Column Length: 30 m Inner Diameter: 0.32 mm Column Max Temp: 340 °C			
Column temperature program	:	Rate °C/min	Temperature (°C)	Hold time (min)	Total Program
		---	220	1.0	9 min
		5	235	4.0	
Oven temperature	:	300 ºC			
Injector temperature	:	280 ºC			
Detector temperature	:	240 ºC			
Gas flow rate	:	Nitrogen as carrier, 30 mL min-1			
Injection volume	:	1 µL			

Residues of imidacloprid (Fig.1) were quantified by comparison of peak height per peak area of standards with that of unknown or spiked samples run under identical conditions. Under these operating conditions the retention time of imidacloprid was found 8.295 min.

Data analysis

Data were collected, coded, analyzed, and tabulated using the Statistic 10 and Microsoft Excel 2016 program. Residual data were analyzed by GC solution software version 2.3.

Results and Discussion

Country bean pod samples were analyzed for imidacloprid insecticide residues employing analysis by Gas Chromatograph. All the pod samples showed the presence of residues of imidacloprid up to nine days after treatment (DAT) except 100 g a.i. ha^{-1} (i.e., recommended dose). Complete degradation of Imidacloprid was found after 12 DAT. It means that there needs a risk assessment of Imidacloprid for safety consumption of country bean.

Protocol development and validation

In the present investigations, recovery experiments were carried out at different levels to establish the reliability and validity of analytical method and to know the efficacy of the procedures. Pods of country bean were spiked with Imidacloprid at different levels and analyzed separately as per the methodology described above. The control samples of country bean from untreated plot and reagent blanks were also processed in the same way so as to find out the interferences due to substrate and reagents separately. The average recoveries values were found to be more than 85% (Table 2). The results had been presented as such without converting by any correction factor. The precision of the method was determined by repeatability studies of the method and expressed by RSD (relative standard deviation) values.

The quantification was accomplished by calibration curve prepared by diluting the stock solution. The assessment of linearity

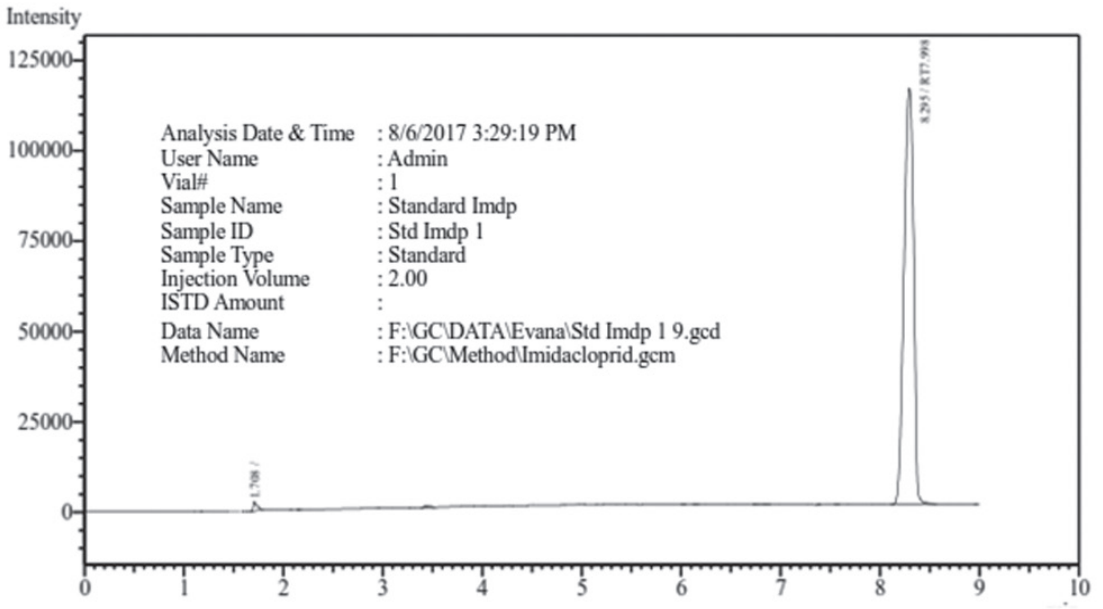

Fig.1. Chromatogram of Imidacloprid insecticide standard

Table 2. Recovery and repeatability (RSD) of Imidacloprid on country bean at different levels

Insecticide	Level of fortification (mg. kg^{-1})	Mean[a] recovery (%)	SD	RSD
	0.10	90.57	1.57	1.63
Imidacloprid	0.05	85.27	1.48	1.72
	0.01	87.38	1.55	1.75

was done by statistical data obtained with correlation coefficient of 0.9989. Limit of quantification (LOQ) was found to be 0.01 mg kg^{-1} and limit of detection (LOD) being 0.003 mg kg-^{1}. The cleaned-up procedure for this methodology was found efficient since no significant matrix effect was observed.

Persistence of Imidacloprid on country bean pods

Average initial deposits of Imidacloprid were found 0.37, 0.73, 1.35, 2.15 and 3.52 mg.kg^{-1}, (Table 3) following application @ 100, 200, 300, 400, and 500 g a.i. ha-^{1}, respectively. For 100 g a.i. ha^{-1}, residues were observed as 0.19 (Fig. 2),0.04,0.03 and dissipation were 47%, 88%, 92% for 1st, 3rd, 6th days after application. It remained below determination limit at 9th days. The residues reached below determination limit of 0.01 mg kg^{-1} at nine days following application of Imidacloprid @ 100 g a.i. ha^{-1}. Imidacloprid residues were also found below the determination limit at 12 days following application @ 200 and 300 g a.i. ha^{-1}. At higher dosages of 400 and 500 g a.i ha-1, these residues reached below the determination limit of 0.01 mg kg^{-1} in 12 days and thereby showing 100% loss following

Table 3. Residues and Dissipation of Imidacloprid (mg. kg^{-1}) on country bean at different intervals and doses

Days after treatment	100 g a.i. ha^{-1}	200 g a.i. ha^{-1}	300 g a.i. ha^{-1}	400 g a.i. ha^{-1}	500 g a.i. ha^{-1}
0 (1 hour after spray)	0.37±.14	0.73±0.22	1.35±0.09	2.15±0.08	3.52±0.17
1	0.19±0.71 (47.65)	0.36±0.11 (51.43)	0.58±0.04 (56.73)	0.89±0.03 (58.22)	1.43±0.07 (59.35)
3	0.04±0.02 (88.51)	0.08±0.02 (88.71)	0.15±0.01 (88.99)	0.25±0.01 (88.57)	0.40±0.02 (88.56)
6	0.03±0.02 (92.90)	0.05±0.02 (92.68)	0.10±0.01 (88.99)	0.18±0.01 (91.86)	0.31±0.02 (91.34)
9	BDL (100.00)	0.24±0.002 (96.71)	0.05±0.01 (96.46)	0.06±0.01 (97.42)	0.07±0.01 (98.11)
10	BDL (100.00)	BDL (100.00)	BDL (100.00)	BDL (100.00)	BDL (100.00)

BDL = Below determination limit (<0.01 mg kg^{-1})

application of Imidacloprid at all the doses (Fig 3). The findings revealed that higher rate of application of Imidacloprid resulted in higher initial deposits. As with other insecticides, the residues of Imidacloprid on country bean declined with time and fairly high rate of dissipation was observed.

The results were in agreement with those of Zhang *et al*. (2012) who reported the fate of imidacloprid in rice field ecosystems after application @ 300 mL a.i. ha^{-1}. The residues of Imidacloprid on rice straw were 3.16, 1.59 and 1.20 mg kg^{-1} after 2 h, 8 h and 24 h, respectively. In rice straw, 0.008 mg kg^{-1} Imidacloprid was found even after 20 days of the application of pesticide.

Similarly, Malhat *et al*. (2012) reported that the Imidacloprid residues on tomato after application of Imidacloprid 20% SC @ 60 ml feddan^{-1} (1 feddan = 4200 m^2).

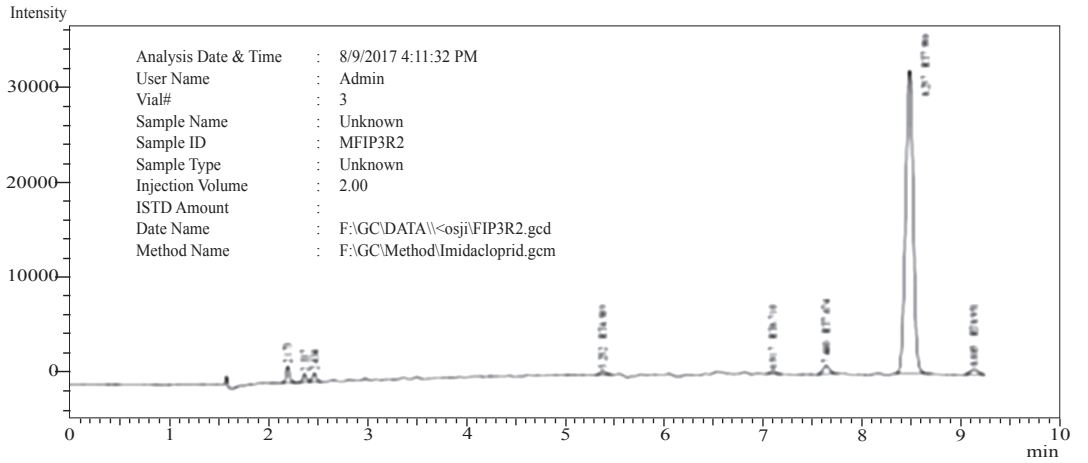

Fig. 2. Chromatogram of dissipation kinetics of Imidacloprid on country bean after 1 DAT.

The concentration of Imidacloprid 2 h after treatment was 2.308 mg kg^{-1}. These residues were below determination limit of 0.01 mg kg^{-1} at 11 days after the application of pesticide. Malhat *et al*. (2012) also reported the residues of Imidacloprid on grapes after application of Imidacloprid formulation at same dose. But, the initial deposit (1 h after application) of Imidacloprid was found 2.829 mg. kg^{-1}. The residue of Imidacloprid dissipated to 99.09% at 11 days after the application of pesticide. These residues were dissipated in grapes to undetectable limits in 12 days after last treatment. Kar *et al.* (2013) studied the environmental fate of Imidacloprid residues on cauliflower curds following 3rd application of Imidacloprid (Coragen 18.5 SC) @ 9.25 and 18.50 g a.i. ha^{-1}. The mean initial deposits of Imidacloprid were 0.18 and 0.29 mg kg-^{1} on the curds at recommended and double the recommended dosages, respectively. These residues reached below the determination limit of 0.01 mg kg^{-1} at 3 and 5 days, respectively.

Half-life of Imidacloprid on country bean leaves

The degradation kinetics of the Imidacloprid in country bean pods was determined by plotting residue concentration against time, and the maximum squares of correlation coefficients found were used to determine the equations of best fit curves (Fig. 3). The persistence of this insecticide is generally expressed in terms of half-life ($T_{1/2}$) or DT_{50} i.e., time for disappearance of pesticide to 50% of its initial concentration. The dissipation of Imidacloprid on country bean follow first order kinetics. The Half-life value ($T_{1/2}$) is the time required for half of the given quantity of material to dissipate (Gunther and Blinn, 1955). Half-life ($T_{1/2}$) of Imidacloprid was calculated by fitting first order kinetics as per Hoskins (1961) as:

$$C = C_{0.}\, e^{-kt} \text{ ---------- (1)}$$

$$T_{1/2} = ln\ 2.k^{-1} \text{ ----------(2)}$$

Where C is the chemical concentration (mg.kg^{-1}) at

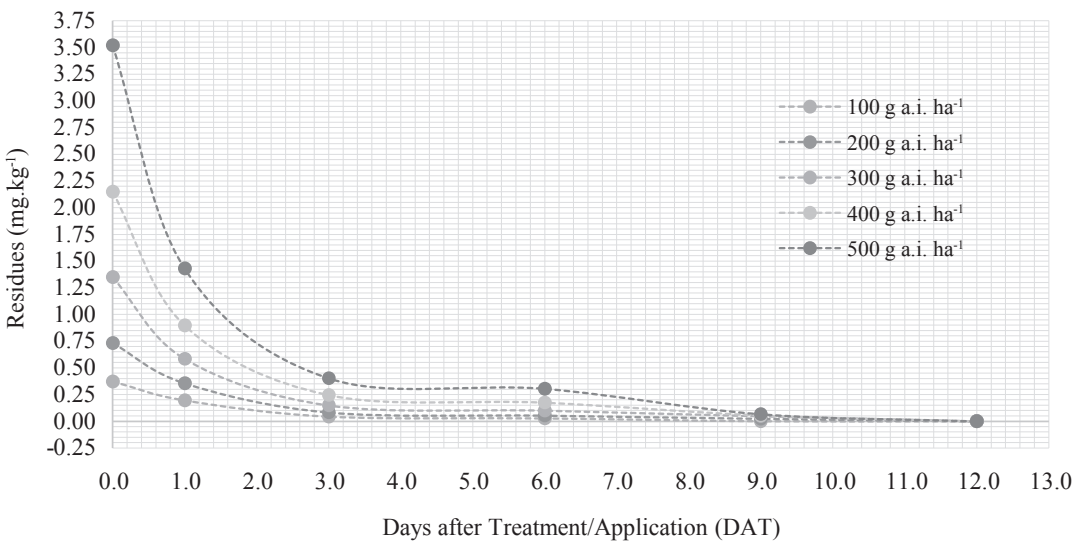

Fig. 3. Semi-logarithm graph showing dissipation kinetics of Imidacloprid on country bean.

time t (days) and C_0 is the initial concentration ($mg.kg^{-1}$), K is the first order kinetic constant day^{-1} independent of C and C_0. The Half-life value ($T_{1/2}$) was calculated using equation (2) with the obtained kinetics (K) from equation (1). The calculated Half-life value ($T_{1/2}$) were found 2.47, 2.21, 1.90, 1.83 and 1.77 days, respectively when applied @ 100, 200, 300, 400, and 500 g a.i. ha^{-1} (Table 4). These results also revealed that increase of doses (concentration) correspondingly decrease the half-life of Imidacloprid.

Half-life ($T_{1/2}$) of Imidacloprid on cauliflower curds worked out as 1.36 and 1.25 days, respectively, when applied @ 9.25 and 18.50 g a.i. ha-' (Kar *et al*., 2013). The dissipation rate of Imidacloprid on tomato fruits followed first order kinetics.

The half-life of Imidacloprid was found 3.30 days in tomato 2.70 days in grapes after application @ 60 ml per feddan (Malhat *et al*., 2012). The half-life of Imidacloprid in rice straw was 3.50 days following application of Imidacloprid @ 300 mL a.i. hm^{-2}. The kinetics of Imidacloprid was described by the equation $C=1.171e^{-o.198t}$ with R=0.902 (Zhang *et al*., 2012).

Chemodynamics of Imidacloprid spray in country bean agro-ecosystem

Fate of Imidacloprid application on country bean sprayed @ 1.0 ml. L^{-1} and 2.0 ml. L-1 (Table 5) of water at 120 DAT is presented in. Results revealed that deposition of Imidacloprid spray volume on plants at different doses did not vary significantly. During application, Imidacloprid was not only received by the plants but also it was drifted to other non-target sites, mainly in the air and soil. The percent loss of Imidacloprid in soil was much higher than that of air.

Risk assessment of Imidacloprid on country bean

The indiscriminate use of any pesticides on vegetable leads to unwanted residues, which may constitute barriers to exporters and domestic consumers when they exceed MRLs (Chahil *et al.*, 2015). However, MRLs for Imidacloprid on country bean pods are

Table 4. Regression analysis and half-life for the dissipation of imidacloprid

Dose (g a.i. ha^{-1})	Regression equation (Y)	Half-life (days)	Correlation coefficient (R^2)
100	-0.0253x + 0.2369	2.47	0.6366
200	-0.048x + 0.4568	2.21	0.6149
300	-0.0854x + 0.8131	1.90	0.5905
400	-0.1355x + 1.2876	1.83	0.5899
500	-0.2212x + 2.0974	1.77	0.5870

Table 5. Fate of Imidacloprid spray volume in country bean agroecosystem

Doses (ml/L/Plant)	Imidacloprid deposited on plant (%)	Imidacloprid lost (%)	
		Air	Soil
1.00	27.20±1.21	32.60±0.95	40.20±0.36
2.00	28.40±2.03	32.13±1.03	39.47±1.15

not available; hence, the risk assessment was calculated. Theoretical maximum residues contribution (TMRC) were calculated and compared with maximum permissible intake (MPI) to evaluate the risk of the Imidacloprid to country bean consumers (Table 6).

However, acceptable daily intake (ADI) for Imidacloprid had been observed to be 0.06 mg kg^{-1} body weight day^{-1} (European Food Safety Authority (EFSA), 2013). Maximum permissible intake (MPI) was obtained by multiplying the ADI with the weight of an average Bangladeshi person (54 kg) (Mukherjee and Gopal, 2000). Maximum permissible intake was calculated to be 3240 μg $person^{-1}$ day^{-1} without any appreciable risk of life. An average Bangladeshi consumes 5.0 g of country bean for a balanced diet (*Kassam - Khamis*, 2000).

The TMRC values on 0 (1 hour after treatment) day was found 1.86, µg.$person^{-1}day^{-1}$ at recommended dose (100 g a.i. ha^{-1}). The TMRC values were 3.67, 6.75, 10.75 and 17.61 μg $person^{-1}$ day^{-1} at 200, 300, 400 and 500 g a.i. ha^{-1}, respectively. All the values were found much lower as compared to MPI; hence, the insecticide Imidacloprid was well below the acceptable risk level at the time of consumption. These studies, therefore, suggested that the use of Imidacloprid formulation at recommended and five times more than recommended doses does not seem to pose any hazards to the consumers. However, a waiting period of two days might be suggested to reduce the risk before consumption of country bean pods.

Table 6. Theoretical maximum residue contribution (TMRC) in country bean

Days after treatment (DAT)	Maximum permissible intake (MPI) (µg.$person^{-1}$.day^{-1})	100 g a.i. ha^{-1}		200 g a.i. ha^{-1}		300 g a.i. ha^{-1}		400 g a.i. ha^{-1}		500 g a.i. ha^{-1}	
		Mean residue ($mg.kg^{-1}$)	TMRC (µg.$person^{-1}$.day^{-1})	Mean residue ($mg.kg^{-1}$)	TMRC (µg.$person^{-1}$.day^{-1})	Mean residue ($mg.kg^{-1}$)	TMRC (µg.$person^{-1}$.day^{-1})	Mean residue ($mg.kg^{-1}$)	TMRC (µg.$person^{-1}$.day^{-1})	Mean residue ($mg.kg^{-1}$)	TMRC (µg.$person^{-1}$.day^{-1})
0	3240	0.37	1.86	0.73	3.67	1.35	6.75	2.15	10.75	3.52	17.61
1	3240	0.19	0.97	0.36	1.78	0.58	2.92	0.90	4.49	1.43	7.16
3	3240	0.04	0.21	0.08	0.41	0.15	0.74	0.25	1.23	0.40	2.01
6	3240	0.03	0.13	0.05	0.27	0.10	0.50	0.18	0.88	0.30	1.52
9	3240	BDL	-	0.02	0.12	0.05	0.24	0.06	0.28	0.07	0.33
12	3240	BDL	-	BDL	-	BDL	-	BDL	-	BDL	-

Conclusion

The residual behavior of Imidacloprid in country bean clearly shows its degradation pattern, fate, half-life and the risk associated with public health. The reliability, validity and precision of the method was done for percent recovery, LOD & LOQ and RSD. Imidacloprid residues were found to be below determination at nine days following application at recommended dose (100 g a.i. ha^{-1}) and 12 days @ 200, 300, 400, and 500 g a.i. ha^{-1}, respectively, in country bean samples. The higher rate of application of Imidacloprid resulted in higher initial deposits. The residues of Imidacloprid in country bean declined with time and fairly high rate of dissipation was observed. The dissipation of Imidacloprid on country bean follow first order kinetics. The Half-life of Imidacloprid ($T_{1/2}$) were found 2.47, 2.21, 1.90, 1.83, and 1.77 days, respectively, when Imidacloprid was applied @ 100, 200, 300, 400, and 500 g a.i. ha^{-1}. The chemodynamics study of Imidacloprid spray indicated that only 27.20 -28.40% sprays were deposited to the target site and others lost to non-target site, such as soil and air. Maximum Permissible Intake (MPI) was calculated to be 3240 μg. $person^{-1}$. day^{-1} without any appreciable risk of life. The calculated TMRC values found much below the MPI in country bean samples. Hence the studied Imidacloprid residue does not pose any threat to public health.

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References

Alam, M. Z. 2009. Insect pests of vegetables and their control in Pakistan. The Agricultural Information Service. Department of Agriculture.

Alam, M. Z., F. M. A. Rauf, A. K. Rahman and A. Cork. 2005. Determination of the status of different borers pest complex of country bean. Annual Report, Entomology Division, BARI, Gazipur. Pp. 32-37.

Anatra-Cordone, M. and P. Durkin. 2005. Imidacloprid in human health risk. *US Department of Agriculture, Arlington, VA*. Pp. 112-128.

Anhalt, J. C., T. B., Moorman and W. C. Koskinen. 2007. Biodegradation of imidacloprid by an isolated soil microorganism. *Environ Sci. Health Part B*. 42: 509-514.

Anonymous. .2006. Country Paper Bangladesh, SAARC Workshop on Pesticide Management. Department of Agricultural Extension (DAE), Environment pollution Concern, Khamarbari, Dhaka, Bangladesh. 19 P.

Begum, R. A. 2003. Techniques of growing legume vegetable. In: Intensive vegetable growing and its utilization. A compilation of lecture materials of training course held in BARI, Gazipur, Bangladesh. 22-25 November. 94 P.

Carrington, D. 2012. Pesticides linked to honeybee decline. The Guardian. UK. Retrieved April 7, 2012.

Chahil, G. S., K. Mandal, S. K. Sahoo, and B. Singh. 2015. Risk assessment of mixture formulation of spirotetramat and imidacloprid in chilli fruits. *Environ. Monit. Assess.* 187: 4105.

Chensheng, L. U., K. M. Warchol and R. A. Callahan. 2012. *In situ* replication of honey bee colony collapse disorder. *Bull. of Insectology* 65: 78-112.

Decourtye, A., C., Armengaud, M. Renou, J. Devillers, S. Cluzeau, M. Gauthier and M. H. Pham Delègue. 2004. Imidacloprid

impairs memory and brain metabolism in the honeybee (*Apis mellifera* L.). *Pesti. Biochemi. and Physiol.* 78: 83-92.

Dina, S. O. 2009. *Maruca testularis* G., a pest of soybean in Nigeria. Tropical Grain Legume. Bull. 11: 28-30.

EFSA (European Food Safety Authority). 2013. Conclusion on the peer review of the pesticide risk assessment for bees for the active substance clothianidin. *EFSA J.* 11: 3066.

Frenich, A. G., F. E. González, J. M. Vidal, P. P. Vázquez and M. M. Sánchez. 2000. Determination of imidacloprid and its metabolite 6-chloronicotinic acid in greenhouse air by high-performance liquid chromatography with diode-array detection. *J. Chromatogr. A*. 869(1): 497-504.

Gill, Y., C. Sinfort., S. Guillaum., Y. Brunet and B. Palagos. 2008. Influence of micrometeorological factors on pesticide loss to the air during vine spraying: Data analysis with statistical and fuzzy inference models.

Gunther, F. A. and R. C. Blinn. 1955. *Analysis of insecticides and acaricides*, Vol. 80, No. 2, 163 P. LWW.

Haque, U., R. J. Magalhaes, H. L. Reid, A. C. Clements, S. M. Ahmed, A. Islam, T. Yamamoto, R. Hague Glass, G.E. 2010. Spatial prediction of malaria prevalence in an endemic area of Bangladesh. *Malar. J.* 9: 120.

Hoskins, W. M. 1961. Mathematical treatment of the rate of loss of pesticide residues. FAO *Plant Protec. Bull.* 9 (163168): 214-215.

Hossain, Q. T. 2000. Status and Management of Vegetables pests in Bangladesh. 28P .

Kar, A., K. Mandal and B. Singh. 2013. Environmental fate of chlorantraniliprole residues on cauliflower using QuEChERS technique. *Environ. Monit. Assess.* 185: 1255-1263.

Kassam-Khamis, T., P. A., Judd, and J. E. Thomas. 2000. Frequency of consumption and nutrient composition of composite dishes commonly consumed in the UK by South Asian Muslims originating from Bangladesh, Pakistan and East Africa (Ismailis). *J. Hum. Nutr. Diet.* 13: 185-196.

Leicht, W. 1993. Pflanzenschutz Nachr. Bayer 46:109.

Liu, W., W. Zheng, Y. Ma and K. K. Liu. 2006. Sorption and degradation of Imidacloprid in soil and water. *Journal Environ. Sci. Health Part B*, 41: 623-634.

Malhat, F. M., L. Abdallah and L. Hegazy. 2012. Dissipation of Imidacloprid ill tomato fruits and soil. *Bull. Environ. Contain. Toxicol.* 88: 349-351.

Mukherjee, I., and M. Gopal. 2003. Pesticide residues in vegetable. *In Proceedings of Symposium on risk assessment of pesticide residues in water and food*, Pp. A1-8.

Segura, C., C. Zaror, H. D. Mansilla and M. A. Mondaca. 2008. Imidacloprid oxidation by Photo-Fenton reaction. *J. Hazard. Mater.* 150: 679-686.

Sharma, K. K. 2013. Pesticide residue analysis manual. Directorate of Information and Publications of Agriculture, Indian Council of Agricultural Research. Pp. 112-118.

Tomlin, C. 1994. The Pesticide Manual Incorporating the Agrochemical Handbook. *British Crop Protection, Surrey, England*.

Whitehorn, P. R., S. O'connor, F. L. Wackers and D. Goulson. 2012. Neonicotinoid pesticide reduces bumble bee colony growth and queen production. *Science* 1215025.

Zhang, S., X. Yang, X. Yin, C. Wang, and Z. Wang. 2012. Dispersive liquid–liquid microextraction combined with sweeping micellar electrokinetic chromatography for the determination of some neonicotinoid insecticides in cucumber samples. *Food Chem.* 133: 544-550.