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Chemodynamics of Cypermethrin Insecticide in Summer Country Bean Ecosystem in Bangladesh

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ABSTRACT

Cypermethrin insecticide was used in summer country bean for controlling pod borer infestation. During application this toxic insecticide not goes only target site but also goes other non target site including soil and environment. Chemodynamical field research was conducted to measure the application loss of cypermethrin insecticide in summer country bean ecosystem and residue was measured from fruit and soil sample to seek of consumer's safety and environmental health. During cypermethrin application by knapsack sprayer, the highest deposition was recorded in target site (Plant) at 105 Days After Sowing (DAS) (59.7±3.4%) and the lowest at 45 DAS (48.8±2.6%) of summer country bean. The drift loss of cypermethrin through air ranged from 3.9±0.3 to 7.1±0.1%. The highest drift loss through soil found at 45 DAS (47.3±2.6%) and lowest at 120 DAS (35.5±0.1%) aged crop. Cypermethrin residue was determined from fruit and soil sample sprayed at 1 mL L^{-1} of water in the field and residue obtained (0.170 ppm) up to 7 Days After Spraying (DAS) and (0.501 ppm) up to 5 DAS, respectively and the quantities were above the Maximum Residue Limits (MRLs) up to 5 DAS (0.531 ppm) in fruit sample and in soil samples all the values were above MRL.

Key words: Insecticide drift loss, pod borer, environment, MRLs, canopy, cypermethrin

INTRODUCTION

As country bean is 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 standard requirement indiscriminately (Zafar *et al*., 2012). For example, farmers of Bangladesh particularly of intensive vegetable growing areas like Jessore apply insecticides 84 to 140 times in a growing season (Anonymous, 2003). This over-use, misuse and the way of using which cause drifting loss to the nearest crop and in the atmosphere which results in pest resurgence, stimulation of the reproductive rate in certain pests, secondary pest outbreaks, mortality of beneficial insects, resistance of pest species and finally environmental pollution (Alam *et al*., 2005; Meyers and Bull, 2002; Cothran *et al*., 2013).

Chemodynamics means the study of the transport, conversion and fate of chemical substances in air, water or soil including their movement from one medium to another (Thibodeaux, 1996). The indiscriminate use of pesticides in rural and urban area of Bangladesh has contaminated

agricultural produces, affects soil health (Handa *et al*., 1999; Dutta *et al*., 2010; Sofo *et al*., 2012) surface waters, aquifers, wildlife, foods and feeds all over the country. Indiscriminate and haphazard 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 (Nafees *et al*., 2009; Mostafalou and Abdollahi, 2012). The consequences of such frequent and poor application method of pesticide cause serious contamination of environment through chemodynamics of pesticide. In general, it has been estimated that only about 0.1% of the pesticides reach the target organisms (Carriger *et al*., 2006). It is to be noted that only 10-20% of the applied pesticides reach the target site while the rest enter into the various environmental component including soil, air and water (Gill and Garg, 2014).

To assure safety of the consumers, many of the develop countries have set Maximum Residue Limits (MRLs) based on Acceptable Daily Intake (ADI) and Potential Daily Intake (PDI) that should not be exceeded for a food items to be consumed safe for consumption (Rahman, 2007). It is assumed that use of toxic pesticides on vegetables has raised the risk of intoxication of consumers along with diseases (Fatema *et al*., 2013). In Bangladesh, farmers have no idea about the pesticide residue level in the food as well as their affect and their level is above MRLs. About 50-70% of the vegetables are contaminated with the residues of pesticides (Karanth, 2002). No insecticide is available, whose retention period which is less than three to five days (Rahman, 1999). Whimsical spray of insecticides and selling of vegetables after one to two days of spray application are assumed to be a normal practice because lack of knowledge about pesticide residue among the farmer's in Bangladesh (Miah *et al*., 2014).

So, it's need to be examining the actual loss of insecticide in field level and find out the actual retention period of insecticide in fruits and soil thus the food safety and environmental specialist took necessary action for construction of law and regulation in use of insecticide in respect of Bangladesh. Therefore, the present study was undertaken to measure the application loss of cypermethrin insecticide at different growth stages of summer country bean ecosystem and the residue was analyzed from fruit and soil sample to seek of consumer's safety and environmental health.

MATERIALS AND METHODS

The field trial was carried out at the experimental farm of Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur, Bangladesh during March 2014 to August 2014. Laboratory studies were conducted in the Pesticide Toxicology Laboratory, Department of Entomology, BSMRAU. The soil was well prepared and good tilth was ensured for commercial crop production. The target land was divided into 18 equal plots (4×3m) with plant to plant distance of 1.0 m. The land of the experimental field was ploughed with drawn disc plough followed by laddering to make the soil suitable for seed sowing. The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications. The whole area of experimental field was divided into 3 blocks and each block was again divided into 6 unit plots. The seeds of IPSA Seem-2 were collected from the Department of Horticulture, BSMRAU. Manure and fertilizer application, thinning and gap filling, trailing, mulching and weeding were done properly. After sowing, a light irrigation was given. Subsequent irrigation was applied at the interval of 10-15 days inspecting the soil moisture level in all the plots. Ripcord 10 EC (cypermethrin) at 1 mL L^{-1} of water sprayed after observing 5% level of pod infestation and repetition of the same at 15 days interval. The following

parameters were considered for measuring the application loss of insecticides and study of chemodynamics of cypermethrin:

- Weight of insecticide spray fluid on foam sheet placed on ground (Application loss in soil)
- Weight of insecticide spray fluid on polythene sheet placed over the plant canopy (Application loss in air)
- Weight of insecticide spray fluid on plant = {Total weight of spray volume-(1+2)}
- Residue analysis of cypermethrin in pods
- Residue analysis of cypermethrin in soil

Spray material (water) with insecticide (Ripcord 10 EC) was weighed by electric balance and applied in the field by knapsack sprayer. For determining insecticide application loss in soil, a polythene sheet was initially placed to cover the ground of (4×3 m) 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 finally by electric balance. Insecticide application loss in soil was determined by subtracting the initial weight from the final weight of the foam sheet. The plots (4×3 m) were housed by the polythene sheet. Underside of the roof of the house, foam sheet was placed to cover the ground of the plots. Immediately after application of insecticide, final weight of the polythene sheet was taken by electric balance and loss of insecticide in the air was measured from the difference between final and initial weight of polythene sheet. How much insecticide retained on target canopy was determined by subtracting the quantity of insecticide lost in the soil + quantity lost in the air from the total quantity of insecticide applied.

Representative samples (fruits and soil) were taken randomly at 0, 1, 3, 5, 7, 10, 12 and 15 DAS for determining of residue. The collected samples were kept in deep freeze. GC-2010 was used to analyses the residue level in samples. The Standard Cypermethrin was obtained from Sigma-Aldrich Laborchemikalien Gmbh, P.O. Box 100262, D-30918, Seelze, Germany via Bangladesh Scientific Pvt. Ltd. Dhaka, Bangladesh. Standards of all pesticides contained >99.6% purity. The purity of the selected formulated insecticides Ripcord 10 EC (Cypermethrin) was tested in the laboratory and found to be 100% pure. The frozen samples were taken from deep freeze and kept in room temperature for 5-6 h for thawing. The methodology prescribed by Horwitz and Latimer Jr. (2005) with necessary modification was adopted for extraction, separation and clean-up of the sample.

Around ≥250 g sample was grounded thoroughly with the meat grinder (Handmixer M-122, Bamix, Switzerland). From this, 20 g sub sample was taken into a wide mouth jar (250 mL). Then 100 mL of hexane was added to it. Sodium sulphate (Na_2SO_4) was also added with the sample until water was removed. The mixture was then macerated with mortar pastle. The homogenized material was then poured into 250 mL conical flask and placed on a shaker (Orbital shaking incubator) for 6 h continuous shaking. After shaking, the slurry was filtered through a Buchner funnel with suction. The flask and filter cakes were rinsed with 8-10 mL of hexane each.

The filtrate was then transferred into 250 mL round bottom flask and was dried to 5-7 mL by evaporation using a rotary vacuum evaporator. The concentrated filtrate was then transferred into 500 mL separatory funnel making 10 mL volume with hexane. For color removal, around 20 mL

methanol was added with 10 mL filtrate and shaked vigorously for 3-5 min in a shaker. After shaking, the separatory funnel was set on a stand and kept undisturbed for 3-5 min. Then the clear part of the solution from the bottom of the separatory funnel was collected in vial which was then centrifuged at 12000 rpm for 5 min (Laboratory Centrifuges, SCR 18B, Japan). After centrifuge, the supernatant was cleaned up by SPE cartridge. Then the final volume was adjusted to 10 mL and this was used for injection.

Summarized the above procedure of sample extraction for residue determination are as follows: 1 kg field sample, representative sample (500 g), cut into small pieces and take working sample (20 g), transfer into morter, addition of Na_2SO_4 (5 g or as much as required to absorb water), making of pest and add solvent (Hexene/Methanol/Acetone 50 mL), homoegenization by ultraturax (2 min), transfer into conical flask and place into shake for 12 h, filter through whatman paper 40, drying by RVP, make 10 mL volume (which represents 20 g sample), This extract is ready for GC/GCMS/HPLC injection (1-10 μL).

Detection and quantification of cypermethrin residue in samples: The concentrated extracts were subjected to analysis by GC-17A (Shimadzu) with Electron Capture Detector (ECD). The capillary column used was AT-1, length 30 cm, ID 0.25 mm and film thickness 0.25 μm. Nitrogen was used as carrier and make up gas in ECD.

Instrument parameters for GC-ECD to quantify the cypermethrin residue were as follows:

- Injection port SPL
  - Injection mode: Split
  - Temperature: 280°C
  - Flow control mode: Linear velocity
  - Split ratio: 10
- Column oven
  - Initial temperature: 160°C
  - Column oven temperature program as shown in Table 1
  - Total program time: 18.00 min
- Detector channel 1 ECD
  - Temperature: 300°C
  - Stop time: 18 min
  - Current: 1.00 pA
  - Makeup flow: 30 mL min^{-1}

The injected volume of supernatant was 1 μL. Each peak was characterized by its retention time. Sample results were quantitated in ppm automatically by the GC software, which represented the concentration of the final volume injected and from this, the actual amount of pesticide residue present in the sample was determined by using the following formula:

$$\text{Residue in sample (ppm)} = \frac{\text{Concentration obtained in injected volume (ppm)} \times \text{Quantity of final volume (L)}}{\text{Amount of sample taken (kg)}}$$

Table 1: Column oven temperature program

Rate (°C min^{-1})	Temperature (°C)	Hold time (min)
---	160.0	1.00
10.0	270.0	6.0

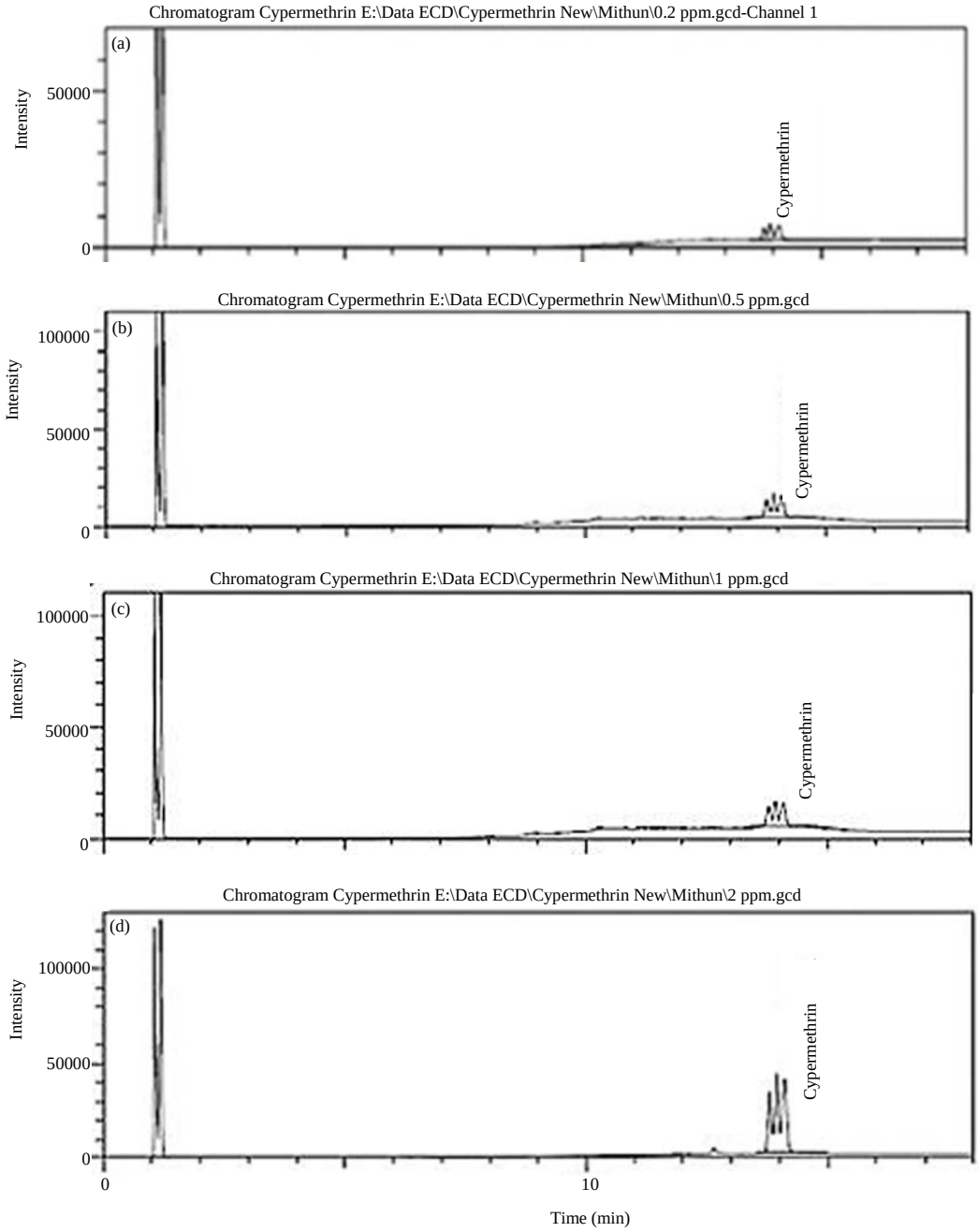

Fig. 1(a-d): Chromatogram of (a) 0.20 ppm, (b) 0.50 ppm, (c) 1.00 ppm and (d) 2.00 ppm standard solution of cypermethrin

The calibration curve along with the chromatograms of standard solution of 0.2, 0.5, 1.00 and 2.00 ppm concentrations of cypermethrin are shown in Fig. 1a-d, respectively.

RESULTS

Fate of cypermethrin under field condition at different growth stages of summer country bean (IPSA seem 2): The highest deposition of cypermethrin in target site (Plant) was found at 105 DAS (59.7±3.4%) and the lowest was at 45 DAS (48.8±2.6%). The deposition of

cypermethrin on plant was also observed at 60 DAS was 49.6±2.7, at 75 DAS was 51.77±1.0, at 90 DAS was 54.33±4.3, at 120 DAS was 59.3±0.3 and at 135 DAS was 57±0.1. The deposition rate increased with time up to 105 DAS and then it reduced (Table 2).

The drift loss of cypermethrin ranged from 3.9±0.3 to 7.1±0.1%. The observed drift loss at 45 DAS was 3.9±0.3, at 60 DAS was 6.3±0.7, at 75 DAS was 5.53±0.1, at 90 DAS was 7.07±1.2, at 105 DAS was 4.1±0.1, at 120 DAS was 5.2±0.3 and at 135 DAS was 7.1±0.1. The loss was sporadic, no pattern was found. The highest drift loss was found at 135 DAS (7.1±0.1%) and lowest was at 45 DAS (3.9±0.3%) (Table 2).

The loss of cypermethrin in soil ranged from 35.9±0.0 to 47.3±2.6%. The observed loss of cypermethrin in soil at 45 DAS was 47.3±2.6, at 60 DAS was 44.1±2.8, at 75 DAS was 42.7±1.1, at 90 DAS was 38.6±3.1, at 105 DAS was 36.2±3.5, at 120 DAS was 35.5±0.1 and at 135 DAS was 35.9±0.0. The loss reduced day by day as the canopy increased with the time but slightly reduced at last harvest. The highest loss in soil was found at 45 DAS (47.3±2.6%) and lowest was at 135 DAS (35.9±0.0%) (Table 2).

Determination of residue from fruit and soil samples: The concentrated extracts of country bean and the soil sample from the ground of the plot at different DAS were subjected to analysis by GC-2010 with pre-set parameters. The results of the analysis of cypermethrin residue in country bean sample are summarized in Table 3.

From Table 3, it can be revealed that cypermethrin residue was detected in the country bean sample up to 7 DAS and the quantities were above MRLs up to 5 DAS. At 0 DAS the residue in the sample was 2.975 ppm which degraded to 2.161 ppm at 1 DAS and 0.962 ppm at 3 DAS. At 5 DAS it was 0.531 ppm which was still above the MRLs set by FAO/WHO (1993). At 7 DAS the residue was found 0.170, which was below the MRLs.

Table 3 also indicated that cypermethrin residue detected in the soil sample up to 5 DAS. At 0 DAS the residue in the sample was 1.977 ppm which degraded to 1.339 ppm at 1 DAS and 1.120 ppm at 3 DAS. At 5 DAS it was 0.501 ppm and all the value was above the MRLs.

Table 2: Fate of cypermethrin application in country bean sprayed at 15 days interval starting from 45 DAS till the last harvest (i.e., 135 DAS)

		Cypermethrin loss in	
DAS	Cypermethrin deposited on plant (%) ±SE*	Air (%) ±SE	Soil (%) ±SE
45	48.8±2.6	3.9±0.3	47.3±2.6
60	49.6±2.7	6.3±0.7	44.1±2.8
75	51.77±1.0	5.53±0.1	42.7±1.1
90	54.33±4.3	7.07±1.2	38.6±3.1
105	59.7±3.4	4.1±0.1	36.2±3.5
120	59.3±0.3	5.2±0.3	35.5±0.1
135	57.0±0.1	7.1±0.1	35.9±0.0

*SE: Standard error, DAS: Days after sowing

Table 3: Quantity of cypermethrin residue in country bean (pod) and soil samples in the plot when applied at 1 mL L^{-1} of water, compared to FAO/WHO recommended MRLs

Sample collection (DAS)	Sample weight (g)	Injected volume (μL)	Level of insecticide residue in fruit (ppm)	Level of insecticide residue in soil (ppm)	FAO/WHO recommended MRLs (ppm)
0	20	2	2.975	1.977	0.50
1	20	2	2.161	1.339	0.50
3	20	2	0.962	1.120	0.50
5	20	2	0.531	0.501	0.50
7	20	2	0.170	0.000	0.50
10	20	2	0.000	0.000	0.50
12	20	2	0.000	0.000	0.50
15	20	2	0.000	0.000	0.50

DAS: Days after sowing

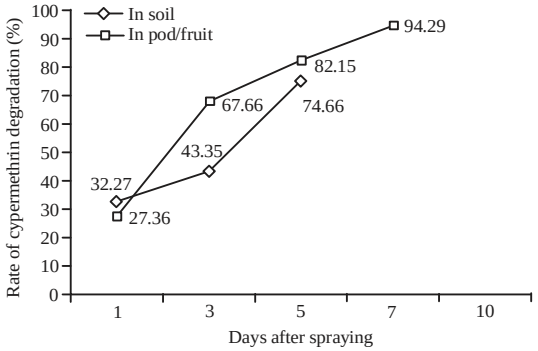

Fig. 2: Trend of cypermethrin residue degradation in pod and in soil samples of the country bean plot sprayed at 1mL L^{-1} of water at different days after spraying

Trend of residue degradation: Trend of degradation of cypermethrin residue in pod and soil sample with 1 mL L^{-1} of water is shown in the Fig. 2.

Figure 2 showed that cypermethrin residue degradation in country bean sprayed at 1 mL L^{-1} of water. It can be received that 27.36% cypermethrin residue degraded at 1 DAS which increased up to 67.66% at 3 DAS, 82.15% at 5 DAS and 94.29% at 7 DAS. At 10 DAS no residue was detected. Cypermethrin residue degradation in soil of the country bean field plot sprayed at 1 mL L^{-1} of water. It is clear that 32.27% cypermethrin residue degraded at 1 DAS which gradually increased to 43.35% at 3 DAS and 74.66% at 5 DAS. At 7 DAS no residue was found.

DISCUSSIONS

Rahman *et al*. (2015) reported that deposition of cypermethrin in brinjal plants at different Days After Transplanting (DAT) showed significant difference ($F_{14,30}$ = 69.9, $p<0.001$). The highest percentage (49.4±0.2%) of cypermethrin was received by the plant (target site) at 129 DAT which was statistically similar to that of 101 DAT to 143 DAT and the lowest (17.6±2.6%) was received at 45 DAT which was statistically similar to 66 DAT. The results indicated that with the progress of growth stages the rate of cypermethrin deposit in the target site increased significantly. Consequently the loss of insecticide during initial stage of plant growth was maximum and decreased with the advancement of growth stage of the plant.

Rahman *et al*. (2015) also reported significant loss of applied cypermethrin through air at different DAT. Drifting in the air i.e., insecticide loss through air drifting was the highest at 143 and 122 DAT (5.5%) which was statistically similar to that of 136 DAT (5.3±0.2%), 129 DAT (5.3±.02%), 115 DAT (5.4±0.2%), 108 DAT (4.8±0.1%), 101 DAT (4.5±0.1%), 52 DAT (4.4±0.7%) and 45 DAT (5.2±0.3%). Minimum loss was recorded at 80 DAT (3.6±0.3%) which was statistically similar to that of 52 to 108 DAT (3.7-4.8%). Loss of applied cypermethrin through drifting to the soil at different growth stages of eggplant showed significant difference ($F_{14,30}$= 64.6, $p<0.001$). Loss of sprayed cypermethrin in soil through drifting was the highest at 45-73 DAT and it ranged from 72.6±3.5 to 77.2±2.6% and no significant difference was noticed among them.

According to Gil *et al*. (2008), the pesticide losses in the air applied on the vine type of plant was ranged from 10-20% but the loss of insecticide in air of the present study was little more than 7%. According to De Rudnicki *et al*. (2010), pesticide loss depends upon the canopy coverage, shape, slope and the height of the plant and spray loss may be 14-45% in field depending on different spraying system. Up to 90% spray losses of pesticide were commonly seen during a typical spray in the air and soil through drifting (Bedos *et al*., 2002). Different micro meteorological factors such

as temperature, wind velocity, relative humidity etc., are also found to hamper the spray during application and causes the loss of pesticide (Hewitt *et al*., 2002).

Hossen (2008) reported that cypermethrin residue was detected in tomato sample up to 5 DAS and the quantities were over MRLs up to 3 DAS and his finding is quite comparable to the present study. Whereas Prodhan *et al*. (2010) observed cypermethrin residue in yard-long bean which was above the MRLs up to 5 DAS.

Rahman (2013) found that cypermethrin residue was detected in the eggplant fruit sample up to 5 DAS and the quantities were above MRLs up to 3 DAS. At 0 DAS the residue in the sample was 2.575 ppm which degraded to 1.961 ppm at 1 DAS and 0.762 ppm at 3 DAS. At 5 DAS it was 0.031 ppm which was below the MRLs set by FAO/WHO (1993).

Rahman (2013) reported that cypermethrin residue was detected in the soil sample up to 5 DAS. At 0 DAS the residue in the sample was 2.007 ppm which degraded to 1.539 ppm at 1 DAS and 1.140 ppm at 3 DAS. At 5 DAS it was 0.608 ppm and all the value was above the MRLs, but here in this study our results showed little different it may be because of different crop and other micro metrological factors.

Keith and Walker (1992) reported that the cypermethrin has a moderate persistence in soils. He also opined that microbes play a significant role in the degradation of cypermethrin. It has been reported that cypermethrin degrades more slowly under anaerobic and waterlogged conditions. Cypermethrin is relatively non-persistent in soils, in sandy soils it persists for 2-4 weeks. Increased cypermethrin persistence was observed in soil with high organic matter, high clay content, reduced microbial activity and anaerobic conditions (Chapman and Harris, 1981).

The above results and discussion clearly highlights the application loss of insecticides in farmer's level in summer country bean ecosystem and residue presents in fruit and soil of the experimental plot that is harmful for human health and as well pollutes the environmental component. The persistent nature of pesticides has impacted our ecosystem to such an extent that pesticides have entered into various food chains and into the higher trophic levels such as that of humans and other large mammals. Some of the acute and chronic human illnesses have now emerged as a consequence of intake of polluted water, air or food. As a result it might be a suggestion for the farmers not to use insecticide indiscriminately but the use of insecticide at minimum level when plant canopy spread at a maximum level approximately 90 days after sowing or at least 5% pod infestation level and pod may be consumed at least 5 days after spraying of insecticide.

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