

Cypermethrin Chemodynamics in Okra Crop Agroecosystem in Bangladesh

Aaseif Uddin¹, Md. Shamim Hossain¹, Md. Mahbubar Rahman¹, Shahinor Rahman^{2*}, Md. Abdur Razzak Choudhury³

¹Department of Entomology, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Bangladesh

²Department of Entomology, EXIM Bank Agricultural University, Bangladesh

³Department of Entomology, Sylhet Agricultural University, Bangladesh

Abstract

Cypermethrin is a non-systemic insecticide widely used for the controlling okra shoot and fruit borer infestation in Bangladesh. At the time of cypermethrin application in the okra field by knapsack sprayer, it not only goes to the target site (plant) but also goes into the soil, air and other environmental components through drifting. Field research was carried out to study the dissipation pattern of cypermethrin in different growth ages of okra crop and laboratory research was directed to determine the residue of cypermethrin from fruit and soil. The highest deposition of cypermethrin (58.2%) was found in the target area (plant) at 105 days after sowing (DAS) and the lowest (47.3%) was at 45 DAS of okra crop. The drift loss of cypermethrin in air ranged from 4.65% to 7.85%. The drift loss was highest in soil recorded at 45 DAS (48.05%) and the lowest (36.25%) was at 120 DAS. Cypermethrin residues determined from fruit and soil samples sprayed at 1 ml/L in the field were above maximum residue limits (MRLs) up to three days after spraying (0.862 ppm) in fruit samples and up to five days after spraying in soil samples (0.554 ppm). In the case of spraying 2 ml/L of cypermethrin, fruit samples have residues above MRLs up to seven days after spraying (0.668 ppm) and soil samples have up to five days after spraying (0.653 ppm). So, okra may consume after three to seven days of spray application for the human health safety.

Keywords Agro-ecosystem, cypermethrin, dissipate, environment, residue.

Received June 26, 2016

Accepted September 10, 2016

Published December 15, 2016

*Corresponding author Shahinor Rahman E-mail shahinor_ent@yahoo.com Tel +8807815352529 Fax +88078153531



To cite this manuscript: Uddin A, Hossain SM, Rahman MM, Rahman S, Choudhury MAR. Cypermethrin chemodynamics in okra crop agroecosystem in Bangladesh. Sci Lett 2016; 4(3):185-189.

Introduction

Okra (*Abelmoschus esculentus*) is an important delicious vegetable crop in Bangladesh with higher nutritive value than that of tomato, brinjal and most of the cucurbits except bitter gourd [1]. But, this delicious vegetable is attacked by several insect pests, among them, okra shoot and fruit borer (OSFB) *Earias vittella* (Fabricius) is the most serious. Because of infestation of this pest, both quantitative and qualitative losses occur in every season in Bangladesh. So, farmers of Bangladesh use a variety of chemical insecticides in their okra field to protect mainly major okra pest besides other minor insect-pest for getting quick results [2]. Due to over-use, misuse and the way of using insecticides cause drifting loss to the soil and in the atmosphere which results in pest resurgence, secondary pest outbreaks, mortality of beneficial insects and microorganisms in soil, the resistance of pest species and finally environmental pollution [3,4,5].

The study of transport, conversion and dissipation of chemical materials in water, air, or soil with their deportment from one medium to another medium is referred as chemodynamics [6]. The indiscriminate

and haphazard use of pesticides practiced in a farmer's field [7] and contaminated agricultural products affect soil health, surface waters, aquifers, wildlife, foods, and feeds, also hazardous to human beings through the food chain [8-12]. 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 calculated that only about 0.1% of the applied pesticides goes the final destination [13]. It is to be marked that only 10-20% of the applied insecticides goes the plant canopy while the rest goes into the soil, air, water and various environmental components [14].

Many developed countries have set Maximum Residue Limits (MRLs) based on Potential Daily Intake (PDI) and Acceptable Daily Intake (ADI) for the safe consumption [15]. It is recorded that use of poisonous pesticides on the eating vegetables has raised the risk of oblivion of end users with different diseases [16]. Researchers found that about 50-70% of the eating vegetables are contaminated with the toxic insecticidal residue [17]. No insecticide is found with the retention period less than three to five days [18]. A whimsical spray of insecticides and selling of

vegetables after one to two days of spray application is assumed to be a normal practice because the lack of knowledge about pesticide residue among the farmer's [19].

So, it is crying need to examine the dissipation pattern of insecticide at field level and find out the actual retention period of insecticide in fruits and soil that environmental and health specialist took necessary action for the construction of law, rules, and regulation in the use of insecticide in respect of Bangladesh. So, the present experiment was conducted to measure the dissipation pattern of cypermethrin at different growth stages of okra crop and the cypermethrin residues were analyzed from the edible part (fruit) and the soil for the consumer's safety and help to initiate rules and regulations on the use of insecticide in farmers' field.

Materials and methods

Location and design of experiment

Field trial and laboratory studies were conducted in the Department of Entomology, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur, Bangladesh during March 2014 to September 2014. Firstly, the experimental field was divided into 18 equal plots (4×3 m) considering three replications. The seeds of okra were collected from the Gazipur market. Manure and fertilizer application, thinning and gap filling, trailing, mulching and weeding were done properly. Ripcord 10 EC (cypermethrin) at 1 ml/L and 2 ml/L of cypermethrin was sprayed at every 15 days interval after observing 5% level of pod infestation.

Determination of application loss of insecticide

Spray material (water) with insecticide (Ripcord 10 EC) was weighed by electric balance and applied in the field by knapsack sprayer. For measuring the loss of applied insecticide through the soil, a thin foam sheet of the plot size (weighed) was placed on the experimental plot for averting the applied insecticide to the soil. Straight away after application, the foam sheet was finally weighed by an electric balance. Application loss of insecticide through the soil was calculated by deducting the utmost weight from the preliminary weight of the thin foam sheet. The experimental plots were covered by the thin plastic material (polythene sheet). Instantly after insecticide spray, the ultimate weight of the plastic sheet was measured by an electric scale and by subtracting between the ultimate and initial weights of polythene sheet to find application loss of insecticide through the air. Insecticide retained on the

target plant canopy was measured by deducting the quantity of insecticide lost through soil + quantity lost in the air from the total quantity of insecticide applied. This methodology reported by Rahman et al. [20] was adopted with necessary modifications.

Collection of fruit and soil samples

The representative samples (fruits and soil) from the each plot were taken randomly at 0, 1, 3, 5, 7, 10, 12 and 15 days after spraying (DAS). Immediately after collection, samples were kept in the chilled box and finally stored in the deep freezer for the determination of residual cypermethrin.

Determination of residual cypermethrin

Gas chromatograph GC-2010 (Shimadzu, Kyoto, Japan) with electron capture detector (ECD) was used to analyze the residues of cypermethrin in samples. The standard of cypermethrin tested in the laboratory and found >99.6% purity. The purity of the insecticides Ripcord 10 EC (cypermethrin) was tested in the laboratory and found to be 100% pure. The frozen samples were taken from the deep freeze and kept at normal room temperature for 5-6 hour. The methodology suggested by Horwitz and Latimer [21] was adopted for separation, extraction, and clean-up of the samples. A total of 20 g fruit sample was transferred into a mortar and Na₂SO₄ (5 g or as much as required to absorb water) was added. Later, grind to prepare a paste and 50 ml solvent hexene/methanol/acetone (1:1:1) was added and homogenized by ultraturax (2 min). The mixture was transferred to a conical flask and placed in a shaker. After 12 h, the mixture was filtered through Whatman paper 40, dried using a rotary vacuum evaporator and make volume 10 ml for GC-2010 analysis.

For GC analysis, 10 µl samples were injected in the injector. The capillary column used was AT-1, length 30 m, ID 0.25 mm and film thickness 0.25 µm. Nitrogen was used as a carrier with the flow rate of 30 ml/min. The initial temperature was 160°C held for 1 min and then ramped up to 270°C at the rate of 10°C per min and held for 6 min. A standard calibration curve was made by injecting different concentration of standard cypermethrin. The injected volume was 1 µl. Each peak was characterized by its retention time. Sample results were quantitated in ppm automatically by the GC software. The actual amount of insecticide residues present in the sample was determined using the following formula:

$$\text{Cypermethrin (ppm)} = \frac{\text{Cypermethrin in injected volume (ppm)} \times \text{quantity of final volume (L)}}{\text{amount of sample (kg)}}$$

Results

Dissipation pattern of cypermethrin at different growth stages of okra crop

The highest deposition of cypermethrin at the target site (Okra crop) was found at 105 DAS (58.2%) and the lowest at 45 DAS (47.3%). The deposition was increased with time up to 105 DAS and decreased thereafter (Table 1).

The drift loss of cypermethrin in air ranged from 4.65% to 7.85%. Air drifting was the highest at 135 DAS which was followed by 90 DAS (7.82%), 60 DAS (7.05%), 75 DAS (6.28%), 120 DAS (5.95%) and 105 DAS (4.85%). The minimum loss was recorded at 45 DAS (4.65%). The loss was sporadic; no pattern was found (Table 1).

The loss of cypermethrin in soil ranged from 36.25% to 48.05%. The observed application loss of cypermethrin in soil was maximum at 45 DAS and it was decreased with the advancement of days. The loss was reduced as the canopy increased with the time, but slightly reduced at the last harvest (Table 1).

Table 1 Fate of cypermethrin application in okra field sprayed at 15 days interval at the rate of 1ml/L starting from 45 DAS till the last harvest (135 DAS).

Days after sowing	Cypermethrin deposited		Cypermethrin lost	
	Plant (%)	Air (%)	Soil (%)	
45	47.3±2.3	4.65±0.8	48.05±2.7	
60	48.1±2.5	7.05±0.9	44.85±2.5	
75	50.27±1.6	6.28±1.2	43.45±1.2	
90	52.83±4.0	7.82±1.2	39.35±3.2	
105	58.2±3.6	4.85±0.4	36.95±3.1	
120	57.8±0.6	5.95±0.3	36.25±0.7	
135	55.5±0.8	7.85±0.4	36.65±0.3	

Determination of cypermethrin residue from okra fruit and soil samples

The concentrated extracts of okra fruit and the soil samples at different DAS were analyzed by GC-2010. The results of cypermethrin residues in okra fruit and soil samples are summarized in Table 2 and Table 3. Table 2 showed that cypermethrin residues were detected (when applied at 1 ml/L) in the okra fruit up to 7 DAS (0.085 ppm) and the quantities were above MRLs up to 3 DAS (0.862 ppm), recommended by FAO/WHO [22]. In the case of soil samples, residues were detected up to 5 DAS (0.554 ppm) and all values were above MRLs.

From Table 3, it can be revealed that cypermethrin residues were detected (when applied at 2 ml/L) in the okra fruit sample up to 10 DAS (0.236 ppm) and the quantities were above MRLs up to 7 DAS (0.668 ppm). In soil samples, residues were detected up to 7

DAS (0.326 ppm) and up to 5 DAS (0.653 ppm) all values were above MRLs.

Table 2 Quantity of cypermethrin residues in okra fruit and soil samples (1ml/L).

Sample collection (DAS)	Fruit (ppm)	Soil (ppm)	MRLs (ppm)
0	2.775	1.992	0.50
1	2.061	1.439	0.50
3	0.862	1.130	0.50
5	0.281	0.554	0.50
7	0.085	0	0.50
10	0	0	0.50
12	0	0	0.50
15	0	0	0.50

DAS = Days after spraying; MRL = FAO/WHO recommended Maximum Residue Limit

Table 3 Quantity of cypermethrin residue in okra fruit and soil samples (2 ml/L).

Sample collection (DAS)	Fruit (ppm)	Soil (ppm)	MRLs (ppm)
0	3.007	2.827	0.50
1	2.511	2.166	0.50
3	1.98	0.99	0.50
5	1.281	0.653	0.50
7	0.668	0.326	0.50
10	0.236	0	0.50
12	0	0	0.50
15	0	0	0.50

DAS = Days after spraying; MRL = FAO/WHO recommended Maximum Residue Limit

Trends of cypermethrin residue degradation

Cypermethrin residue degradation in okra fruit and soil samples is presented in Fig. 1 and Fig. 2. Fig. 1 showed cypermethrin residue degradation in okra fruits sprayed at 1 ml/L of cypermethrin. There was 25.7% cypermethrin residue degradation at 1 DAS which increased up to 68.9% at 3 DAS, 89.8% at 5 DAS and 96.9% at 7 DAS. At 10 DAS no residue was detected. Cypermethrin residue degradation in soil of the okra field sprayed at 1 ml/L of water. It is clear that 27.7% cypermethrin residue was degraded at 1 DAS which gradually increased to 43.2% at 3 DAS and 72.1% at 5 DAS. At 7 DAS no residue was found.

Fig. 2 represents cypermethrin residue degradation in okra fruit and soil samples sprayed at 2 ml/L of cypermethrin. In the fruit samples, it can be received that 16.4% cypermethrin residue were degraded at 1 DAS which increased gradually to 34.1% at 3 DAS, 57.4% at 5 DAS and 77.7% at 7 DAS. At 10 DAS 92.1% residues were degraded and at 12 DAS no residue was obtained. In the case of soil samples, residue degraded 23.3% at 1 DAS, which increased up to 64.9% at 3 DAS, 76.9% at 5 DAS and 88.4% at 7 DAS. No residue was detected at 10 DAS.

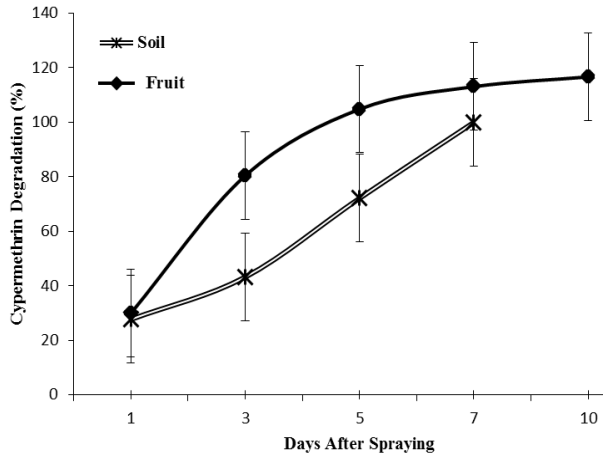


Fig. 1 Trend of cypermethrin residue degradation in okra fruit and soil samples sprayed at 1ml/L at different days after spraying.

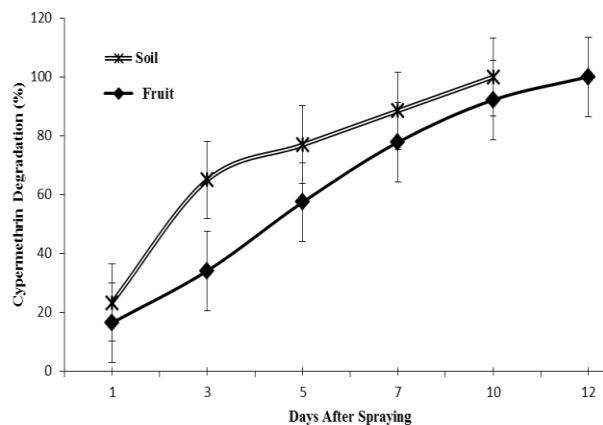


Fig. 2 Trend of cypermethrin residue degradation in okra fruit and soil samples sprayed at 2ml/L at different days after spraying.

Discussion

In the present study, the loss of cypermethrin through drifting in air ranged from 4.65 to 7.85% and through the soil, it ranged from 48.05 to 36.65% after different DAS. Rahman et al. [20] reported that, with the progress of plant growth stages [(45 days after transplanting (DAT) to 143 DAT], the rate of cypermethrin deposition (17.6% to 48.5%) in the target site (eggplant) increased significantly. Consequently, the loss of insecticide during the initial stage of plant progress was maximum and decreased with the advancement of the growth of the eggplant. Rahman et al. [20] also reported that in the eggplant agroecosystem cypermethrin loss through soil ranged from 77.2% to 45.9% with the corresponding 45 DAT to 143 DAT and loss through drifting in air ranged from 5.2% to 5.5% with different plant progress stages. The application drifts loss of cypermethrin ranged from 3.9% to 7.1% in air and in soil ranged

from 35.9% to 47.3% in the summer country bean ecosystem [23]. During the application of a pesticide, 10-20% loss was recorded in the vine type plants [24]. Depending on spraying system, pesticide loss may occur due to the height of the plant, canopy coverage, size, shape, slope etc. and spray loss may be 14-45% [25]. During a typical spray up to 90% spray losses of pesticide was recorded through drifting [26]. Wind velocity, temperature, relative humidity and different types of meteorological factors hamper the spray during the application of pesticide [27].

In this study, we found residues up to 7 DAS and 10 DAS in fruit samples and 5 DAS and 7 DAS in soil samples when it was treated with 1 ml/L and 2 ml/L cypermethrin, respectively. Rahman et al. [28] found that cypermethrin residues were detected in the eggplant fruit samples up to 5 DAS and the quantities were above MRLs up to 3 DAS and when sprayed at 1ml/L. In the case of 2ml/L, residues obtained in fruit samples up to 10 DAS and the quantities were above MRLs up to 7 DAS. In respect of soil samples residue obtained up to 5 DAS (0.608 ppm) and up to 7 DAS (0.351 ppm) when sprayed at 1 ml/L and 2 ml/L respectively. Hossen [29] reported that cypermethrin residues were detected in tomato up to 5 DAS and the quantities were over MRLs up to 3 DAS. Whereas Prodhan et al. [30] observed cypermethrin residues in the yard-long bean which were above the MRLs up to 5 DAS. Our results showed that at 10 DAS no residue was detected in fruit samples. In the case of soil samples, no residue was found at 7 DAS. Paul et al. [23] reported similar trends of cypermethrin residue degradation. Keith and Walker [31] reported that the cypermethrin has a moderate persistence in soils. They also reported that microorganisms play an important role in the cypermethrin degradation. It has been reported that cypermethrin residue degrades more slowly in waterlogged and anaerobic conditions. Cypermethrin is usually non-persistent in soils, but it is recorded that cypermethrin persists for 2-4 weeks in the case of sandy soils. Persistence of cypermethrin was increased in the case of soil with high clay content, high organic matter, the absence of microbial activities and anaerobic conditions [32].

Conclusions

The above results clearly showed the dissipation pattern of cypermethrin in okra crop agroecosystem. Cypermethrin residues present in fruit and soil are harmful to human health, microorganisms, beneficial insects and as well as the environmental components including water and air. These results suggest to the farmers not to use insecticide indiscriminately but use

insecticide at recommended rate when the plant canopy spread at a maximum level approximately 75 days after sowing (age) or at least 5% fruit infestation level. Okra may be consumed at least three and seven days after spraying of cypermethrin if it was treated with 1 ml/L and 2 ml/L, respectively.

Conflict of interest

The authors declare that they have no conflict of interest.

References

- [1] Thampi SK, Indira V. Nutritive value and organoleptic evaluation of thamaravenda genotypes (*Abelmoschus caillei* L.). *J Trop Agric* 2000; 38:38-40.
- [2] Basu AN. *Bemisia tabaci* Gennadius-crop pest and principal whitefly vector of plant viruses. Oxford & IBH Pub. Co. Pvt. Ltd. New Delh., 1995.
- [3] Alam MZ, Rouf FMA, Rahman AK, Cork A. Determination of the status of different borers pest complex of country bean. Annual Report, Entomology Division, Bangladesh Agricultural Research Institute (BARI), Gazipur; 2005, p. 32-37.
- [4] Meyers LA, Bull JJ. Fighting change with change: Adaptive variation in an uncertain world. *Trends Ecol Evol* 2002; 17:551-557.
- [5] Cothran RD, Brown JM, Relyea RA. Proximity to agriculture is correlated with pesticide tolerance: Evidence for the evolution of amphibian resistance to modern pesticides. *Evolut Applic* 2013; 6:832-841
- [6] Thibodeaux LJ. Environmental chemodynamics: movement of chemicals in air, water and soil. 2nd Ed., Willey Publications, Chichester, UK; 1996, p. 624.
- [7] Zafar S, Ahmed A, Ahmad R, Randhawa MA, Gulfranz M, Ahmad A, Siddique F. Chemical residues of some pyrethroid insecticides in eggplant and okra fruits: Effect of processing and chemical solutions. *J Chem Soc Pak* 2012; 34:1169-1175.
- [8] Handa SK, Agnihotri NP, Kulshrestha G. Effect of pesticide on soil fertility. In: Pesticide residues: significance, management and analysis, Handa SK, Agnihotri NP, Kulshrestha G, (Eds.). Research Periodicals & Book Publishing House, India; 1999, p. 184-198.
- [9] Dutta M, Sardar D, Pal R, Kole RK. Effect of chlorpyrifos on microbial biomass and activities in tropical clay loam soil. *Environ Monit Assess* 2010; 160:385-391.
- [10] Sofo A, Scopa A, Dumontet S, Mazzatura A, Pasquale V. Toxic effects of four sulphonylureas herbicides on soil microbial biomass. *J Environ Sci Health B* 2012; 47:653-659.
- [11] Nafees M, Jan MR, Khan H, Rashid N, Khan F. Soil contamination in Swat valley caused by cadmium and copper Sarhad J Agric 2009; 25:37-43.
- [12] Mostafalou S, Abdollahi M. Concerns of environmental persistence of pesticides and human chronic diseases. *Clin Exp Pharmacol* 2012; S5:e002.
- [13] Carriger JF, Rand GM, Gardinali PR, Perry WB, Tompkins MS, Fernandez AM. Pesticides of potential ecological concern in sediment from South Florida canals: An ecological risk prioritization for aquatic arthropods. *Soil Sediment Contam* 2006; 15:21-45.
- [14] Gill HK, Garg H. Pesticides: Environmental impacts and management strategies. In: Pesticides-toxic aspects, Larramendy ML, Soloneski S, (Eds.). In Tech Publisher, Rijeka, Croatia; 2014, p. 187-230.
- [15] Rahman MM. Maximum residue limits of pesticides in agricultural commodities and foods: Bangladesh perspective in national and international contest. A key note paper. Proceedings of the National Workshop on Maximum Residue Limits of Pesticides in Agricultural Commodities, Bangladesh Agricultural Research Council (BARC) under USAID Fund, Dhaka, Bangladesh. April 11, 2007.
- [16] Fatema M, Rahman MM, Kabir KH, Mahmudunnabi M, Akter MA. Residues of insecticide in farm and market samples of Eggplant in Bangladesh. *J Entomol Zool Stud* 2013; 1:147-150.
- [17] Karanth NGK. Challenges of limiting pesticide residues in fresh vegetables: The Indian Experience. In: Food Safety Management in Developing Countries, Hanak E, Boutrif E, Fabre P, Pineiro M, (Eds.). CIRAD-FAO, Montpellier, France; 2002, p. 1-13.
- [18] Rahman AM. Effect and residues of two selected insecticides applied for control of brinjal shoot and fruit borer in eggplant. M.S. Thesis, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh, 1999.
- [19] Miah SJ, Hoque A, Paul A, Rahman A. Unsafe use of pesticide and its impact on health of farmers: A case study in Burichong Upazila, Bangladesh. *IOSR J Environ Sci Toxicol Food Technol* 2014; 8:57-67.
- [20] Rahman S, Rahman MM, Alam MZ. Chemodynamics of cypermethrin in eggplant agroecosystem in Bangladesh. *Int J Agron Agric Res* 2015a; 6:22-28.
- [21] Horwitz W, Latimer Jr. GW. Official Methods of Analysis of AOAC International. 18th Ed., Chapter 10, AOAC International, Gaithersburg, MD, USA; 2005, p. 41.
- [22] FAO/WHO. Pesticide residues in food-1993: Part I-residues. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Expert Group on Pesticide Residues, Geneva, September 20-29, 1993, p. 257-287.
- [23] Paul M, Hossain MS, Rahman MM, Khaliq QA, Rahman S. Chemodynamics of cypermethrin insecticide in summer country bean ecosystem in Bangladesh. *Res J Environ Toxicol* 2016; 10:50-59.
- [24] Gil Y, Sinfrot C, Guillaume S, Brunet Y, Palagos B. Influence of micrometeorological factors on pesticide loss to the air during vine spraying: Data analysis with statistical and fuzzy inference models. *Biosyst Eng* 2008; 100:184-197.
- [25] De Rudnicki V, Ruelle B, Scheyer L, Coustilières A. Embedded NICT* tools and traceability to control phytochemical treatments. Proceedings of the International Conference on Agricultural Engineering: Towards Environmental Technologies, Clermont-Ferrand, France, September 6-8, 2010, p. 1-10.
- [26] Bedos C, Cellier P, Calvet R, Barriuso E, Gabrielle B. Mass transfer of pesticides into the atmosphere by volatilization from soils and plants: Overview. *Agronomie* 2002; 22:21-33.
- [27] Hewitt AJ, Maber J, Praat JP. Drift management using modeling and GIS systems. Proceedings of the World Congress of Computers in Agriculture and Natural Resources, Iguacu Falls, Brazil, March 13-15, 2002, p. 290-296.
- [28] Rahman S, Rahman MM, Hossain MS. Cypermethrin residue analysis of fruit and soil samples in eggplant ecosystem in Bangladesh. *Sci Lett* 2015b; 3:138-141.
- [29] Hossen KJ. Development of management practices against tomato fruit borer and quantification of residue. M.S. Thesis, Department of Entomology, Sher-e-Bangla Agricultural University, Bangladesh. 2008, p. 24.
- [30] Prodan MDH, Rahman MA, Akon MW, Ahmed MS, Kabir KH. Determination of pre harvest interval for quinalphos, malathion, cypermethrin and diazinon in major vegetables. Annual Report 2009-10, Division of Entomology, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur, 2010, p. 146-158.
- [31] Keith LH, Walker MH. EPA's Pesticide Fact Sheet Database. Lewis Publishers, Chelsea, MI, USA; 1992, p. 32.
- [32] Chapman RA, Harris CR. Persistence of four pyrethroid insecticides in a mineral and an organic soil. *J Environ Sci Health B* 1981; 16:605-615.