Asian Australas. J. Biosci. Biotechnol. 2016, 1 (2), 284-290

Asian-Australasian Journal of Bioscience and Biotechnology
ISSN 2414-1283 (Print) 2414-6293 (Online)
www.ebupress.com/journal/aajbb

Article

Quantification of residue degradation of fenvalerate and acephate in hyacinth bean and tomato under supervised field trial

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Received: 07 August 2016/Accepted: 24 August 2016/ Published: 31 August 2016

Abstract: The present study was undertaken to detect and quantify the left over residue of Fenvalerate and Acephate in bean and tomato and comparison between the detected residue levels with Maximum Residue Limit (MRL) set by FAO/WHO. Two supervised field trials (one for Fenvalerate and another for Acephate) were carried out sprayed with the field dose of Fenvalerate (1.0ml/L of water) and Acephate (1.0gm/L of water). Samples were collected at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 and 15 days after spray. The left over residue of Fenvalerate was detected up to 14 DAS, of which up to 3 DAS the quantities of residue were above MRL (1 ppm) in both the vegetables (bean, tomato). This insecticide remained 0.601-0.035 ppm residue in bean and 0.443-0.014 ppm in tomato which were below MRL at 4 to 14 DAS. The high amount of Acephate was detected for a longer period than Fenvalerate and it was found up to 14 DAS in which MRL values (0.5 ppm) were found above up to 9 DAS with 0.623 ppm in tomato and 0.928 ppm in bean. Samples of 10, 11, 12, 13 and 14 DAS of Acephate contained residues 0.289-0.032 ppm in bean and 0.465-0.029 ppm in tomato which were below MRL. At 15 DAS, no residue was detected. Therefore, bean and tomato can be harvested safely at 4 days after spray for Fenvalerate and 10 days after spray for Acephate.

Keywords: insecticides; residue; degradation; DAS; MRL; hyachinth bean; tomato

1. Introduction

Tomato and hyacinth bean are valuable, nutritious and very popular to consumers in Bangladesh. These vegetables are attacked by a variety of insect pests. Insecticides are one of the major components of plant protection for the farmers of Bangladesh because of its small quantity in packaging/bottling, storage ability and availability in the market. As crop production and insecticides are closely related and their left over residue might or might not persist in the environment that should be carefully examined and monitored. The pattern of insecticide usage in vegetables led to assume that major vegetable growing areas of Bangladesh should be overloaded with insecticide residue, since insecticides are being used by vegetable farmers irrationally, in some occasions whimsically. It was understood from farmers' interview that they use insecticides irrationally and indiscriminately (Anon., 2001; Ahmed *et al.*, 2005). A considerable number of farmers sell vegetables immediate after spray or at an interval of 0-2 days after spray (Anon., 2000). This led to assume that over-sprayed vegetable consumers might face health hazards and environment might be over loaded with insecticide residue. Pesticide being toxic can become a potential hazard to the manufacturers, the user, the public at large and the environment. Pesticide can produce negative impacts, both social and private (Antle and Pingali, 1994). Study on insecticide residue detection is considerably new; very little efforts have so far been made in Bangladesh to document residue load in our agro ecological condition. The detection and monitoring of pesticide residue particularly in vegetable and fruits is being done in regular basis in many countries (Krol *et al.*, 2000; Van der Schee, 2002; Kumar *et al.*, 2004; Rajeswaran *et al.*, 2004). Extensive deliberate use of pesticides

has resulted in contamination of our vital supplies, air, water and food. The risk to humans may be short term as well as long term depending on the persistence of the pesticide and the exposure period. Pesticide residue in food has become a consumer's safety issue and the consumers have the right to know how much pesticide get incorporated in the food they eat.

The detection, identification and quantification of pesticide in the food are becoming the public interest. But very little references are available on the presence of pesticides in vegetables in Bangladesh (Khatoon *et al.*, 2004). Considering these circumstances, the present study was undertaken to assess the amount and degradation rate of leftover residue of two frequently used insecticides in hyacinth bean and tomato.

2. Materials and Methods

The study was conducted in the pesticide analytical laboratory and experimental field of Entomology Division, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur during 2011-12 seasons. The standard for Fenvalerate and Acephate were obtained from Sigma-Aldrich Laborchemikalien, Gmbh P O Box-100262 D-30918, Seelze, Germany via Bangladesh Scientific Pvt. ltd. Dhaka, Bangladesh. Standards of both insecticides contained 99.6% purity. Marketable size of hyacinth bean and tomato were collected from two different supervised field trials at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 and 15 days after spray (DAS) which were sprayed with Fenvalerate @1.0 ml/L of water and Acephate @1.0gm/L of water. The formulated products of those were Fenfen 20EC and Asataf 75SP. The purity of formulated insecticides were tested in the laboratory and found to be 100% pure.

2.1. Extraction and separation

Field collected samples (≥250g) were grounded thoroughly with the meat grinder (Handmixer M-122, Bamix, Switzerland). A sub sample of 20g was taken into a wide mouth jar then 100 ml of hexane was added to it. Sodium sulphate (Na_2SO_4) was also added with sample until water was removed from the sample. The mixture was then macerated with high-speed homogenizer (Ultraturax, IKA T18 basic, Germany) for 2 minutes. The homogenized material was then poured into 250 ml conical flask and placed into the shaker (Orbital Shaking Incubator, Rexmed, Sweden) for 12hrs continuous shaking. After shaking, the slurry was filtered through a Buchner funnel with suction. The flask and filter cakes were rinsed with 25 ml of hexane each. The filtrate was then transferred into 250 ml round bottom flask and was dried to 5 ml by evaporation using a rotary vacuum evaporator (Laborota-4001, Heidolph, Germany). The concentrated filtrate was then transferred into 500 ml separatory funnel making 10 ml in volume. Around 20 ml methanol was added with 10 ml filtrate and shaked vigorously for 5 minutes. After shaking, the separatory funnel was set on stand and kept undisturbed for 5 minutes. Then the clear part of the solution from the bottom of the separatory funnel was collected in a vial which was then centrifuged at 1200 rpm for 5 minutes (Laboratory Centrifuges, Sigma-3K30, Germany). After centrifuge, supernatant was collected for injection in GC.

2.2. Detection and Quantification of pesticide residue in samples

The concentrated extracts were subjected to analysis by GC-2010 (Shimadzu). For Acephate FTD (Flame Thirm ionized Detector) and for Fenvalerate ECD (Electron Capture Detector) were used. The capillary column used in FTD was ATTM-1, length 30m, Inner Diameter (ID) 0.25mm and film thickness 0.25µm and in case of ECD it was Optima 1 and length, ID and film thickness was same. Nitrogen was used as carrier and make up gas in ECD and in FTD it was Helium. The instrument parameters for detecting Acephate and Fenvalerate were as follow.

ACEPHATE

Equipment	: GC-2010
Detector	: FTD
[Injection Port SPL]	
Injection Mode	: Split
Temperature	: 250.0 C
Purge Flow	: 3.0 mL/min
Split Ratio	: 30.0
[Column Oven]	
Initial Temperature	: 150.0 C
Equilibration Time	: 1.0 min

Column Oven Temperature Program:

Total Program Time	: 10.00 min	
Rate (C/min)	Temperature (C)	Hold Time (min)
------	150.0	1.00
10.0	220.0	2.00

FENVALERATE

Equipment : GC-2010
Detector : ECD
[Injection Port SPL1]
Injection Mode : Split
Temperature : 280.0 C
Purge Flow : 3.0 mL/min
Split Ratio : 10.0
[Column Oven]
Initial Temperature: 160.0 C
Equilibration Time: 1.0 min

Column Oven Temperature Program:
Total Program Time: 29.00 min

Rate (C/min)	Temperature (C)	Hold Time (min)
------	160.0	1.00
10.0	230.0	6.00
2.0	260.0	0.00

Prior to the injection of the sample extract, standard solutions of different concentrations of both pesticide groups were prepared and injected with the above instrument parameters. The samples were calibrated (retention time, peak area etc.) against three to four pointed calibration curve of standard solution of concerned pesticide. Each peak was characterized by its retention time. Sample results were expressed in ppm automatically by the GC software which represented the concentration of the final volume injected. From this value the actual amount of pesticide residue present in the sample was determined by using the following formula.

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

3. Results

The results of the analysis of Fenvalerate and Acephate residue in hyacinth bean and tomato sample are summarized in the Table 1-4.

Residue of Fenvalerate was detected up to 14 DAS and the quantities were above MRL up to 3 DAS and these were 2.061 ppm, 1.950 ppm, 1.640 ppm, 1.030 ppm at 0, 1, 2, and 3 DAS, respectively. This insecticide remained 0.035 to 0.601 ppm residues which were below MRL at 4 to 14 DAS in hyacinth bean. No residue was detected at 15 DAS.

Table 1. Quantity of residue of Fenvalerate (Fenfen 20EC) estimated from hyacinth bean.

Days after spraying (DAS)	Sample weight (g)	Total volume prepared (ml)	Injected volume (µl)	Concentration obtained in final volume (ppm)	Residue of Fenvalerate left (ppm)
0	20	10	2	4.122	2.061
1	20	10	2	3.900	1.950
2	20	10	2	3.280	1.640
3	20	10	2	2.06	1.030
4	20	10	2	1.202	0.601
5	20	10	2	1.142	0.571
6	20	10	2	0.716	0.358
7	20	10	2	0.636	0.318
8	20	10	2	0.598	0.299
9	20	10	2	0.526	0.263
10	20	10	2	0.442	0.221
11	20	10	2	0.392	0.196
12	20	10	2	0.282	0.141
13	20	10	2	0.200	0.100
14	20	10	2	0.070	0.035
15	20	10	2	ND	ND

* Maximum Residue Limit (MRL) of Fenvalerate in bean: 1.0 ppm; ND= Not detected

Table 2. Quantity of residue of Fenvalerate (Fenfen 20EC) estimated from tomato.

Days after spraying	Sample weight (g)	Total volume prepared (ml)	Injected volume (µl)	Concentration obtained in final volume (ppm)	Residue of Fenvalerate left (ppm)
0	20	10	2	3.876	1.938
1	20	10	2	3.028	1.514
2	20	10	2	2.520	1.260
3	20	10	2	2.020	1.010
4	20	10	2	0.886	0.443
5	20	10	2	0.718	0.359
6	20	10	2	0.566	0.283
7	20	10	2	0.508	0.254
8	20	10	2	0.446	0.223
9	20	10	2	0.396	0.198
10	20	10	2	0.360	0.180
11	20	10	2	0.304	0.152
12	20	10	2	0.214	0.107
13	20	10	2	0.146	0.073
14	20	10	2	0.084	0.042
15	20	10	2	ND	ND

*MRL of Fenvalerate in tomato: 1.0 ppm; ND= Not detected

The results revealed that residue of Fenvalerate could be detected up to 14 DAS. The quantities of residue were above MRL up to 3 DAS and these were 1.938 ppm, 1.514 ppm, 1.260 ppm and 1.010 ppm at 0, 1, 2 and 3 DAS, respectively. While samples of 4 to 14 DAS contained 0.443 to 0.042 ppm residue which were below MRL set by FAO-WHO (1993). No residue was detected at 15 DAS.

Table 3. Quantity of residue of Acephate (Asataf 75SP) estimated from hyacinth bean.

Days after spraying	Sample weight (g)	Total volume prepared (ml)	Injected volume (µl)	Concentration obtained in final volume (ppm)	Residue of Acephate left (ppm)
0	20	10	2	9.876	4.938
1	20	10	2	8.178	4.089
2	20	10	2	6.502	3.251
3	20	10	2	5.472	2.736
4	20	10	2	4.510	2.255
5	20	10	2	3.510	1.755
6	20	10	2	2.936	1.468
7	20	10	2	2.568	1.284
8	20	10	2	2.348	1.174
9	20	10	2	1.856	0.928
10	20	10	2	0.578	0.289
11	20	10	2	0.352	0.176
12	20	10	2	0.164	0.082
13	20	10	2	0.130	0.065
14	20	10	2	0.064	0.032
15	20	10	2	ND	ND

Maximum Residue Limit of Acephate in bean: 0.5 ppm; ND= Not detected

The left over residue of Acephate in the hyacinth bean sample had been detected up to 14 DAS, of which up to 9 DAS the quantity of residue were above MRL. At the 0 DAS the residue was 4.938 ppm and they were 4.089 ppm, 3.251 ppm, 2.736 ppm, 2.255 ppm, 1.755 ppm, 1.468 ppm, 1.284 ppm, 1.174 ppm and 0.928 ppm at 1, 2, 3, 4, 5, 6, 7, 8, DAS and 9 DAS, respectively. All these quantities were above MRL. The quantity decreased down to 0.289 ppm at 10 DAS, 0.176 ppm at 11 DAS, 0.082 ppm at 12 DAS, 0.065 ppm at 13 DAS and 0.032 ppm at 14 DAS, respectively. All these amount which were below MRL. No residue was detected at 15 DAS.

Table 4. Quantity of residue of Acephate (Asataf 75SP) estimated from tomato.

Days after spraying	Sample weight (g)	Total volume prepared (ml)	Injected volume (µl)	Concentration obtained in final volume (ppm)	Residue of Acephate left (ppm)
0	20	10	2	5.560	2.780
1	20	10	2	5.046	2.523
2	20	10	2	3.008	1.504
3	20	10	2	2.676	1.338
4	20	10	2	2.590	1.295
5	20	10	2	1.922	0.961
6	20	10	2	1.780	0.890
7	20	10	2	1.648	0.824
8	20	10	2	1.524	0.762
9	20	10	2	1.246	0.623
10	20	10	2	0.930	0.465
11	20	10	2	0.788	0.394
12	20	10	2	0.578	0.289
13	20	10	2	0.202	0.101
14	20	10	2	0.058	0.029
15	20	10	2	ND	ND

Maximum Residue Limit of Acephate in tomato: 0.5 ppm; ND= Not detected

From the table, it was observed that Acephate residue was detected in the tomato sample up to 14 DAS and the quantities were over MRL up to 9 DAS and these were 2.780 ppm, 2.523 ppm, 1.504 ppm, 1.338 ppm, 1.295 ppm, 0.961 ppm, 0.890 ppm, 0.824 ppm, 0.762 ppm and 0.623 ppm at 0, 1, 2, 3, 4, 5, 6, 7, 8 and 9 DAS, respectively. The quantity decrease down to 0.465 to 0.029 ppm at 10 to 14 DAS which were below MRL. At 15 DAS, no residue was detected.

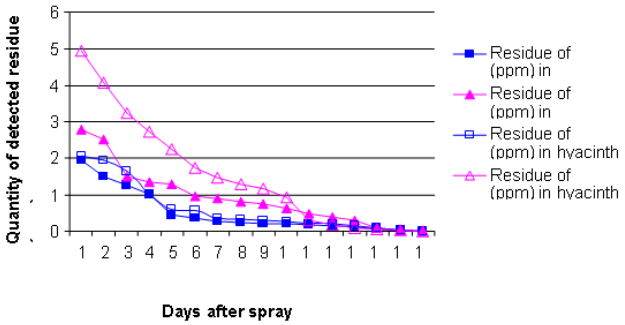

Figure 1. The trend of degradation of detected residue of Fenvalerate and Acephate in hyacinth bean and tomato over time.

Figure 1 shows the trend of degradation of detected residue of Fenvalerate and Acephate in the sample of hyacinth bean and tomato over time. From the figure it was clearly observed that the degradation rate of Acephate was slow in both the vegetables though it was above MRL at 9 DAS and after that the residue decreased very quickly. The rate of degradation of Fenvalerate was faster than Acephate in both the vegetables and it was above MRL at 3 DAS.

4. Discussion

The left over residue of Fenvalerate was detected up to 14 DAS and the quantities of residue were above MRL up to 3 DAS in both the vegetables (bean, tomato). The residue decrease down 0.601-0.035 ppm in bean and 0.443-0.014 ppm in tomato which were below MRL at 4 to 14 DAS. The high amount of Acephate residue was detected for a longer period than Fenvalerate and the MRL values were found above up to 9 DAS with 0.623 ppm in tomato and 0.928 ppm in bean. Samples of 10 to 14 DAS of Acephate contained residues 0.289-0.032 ppm in bean and 0.465-0.029 ppm in tomato which were below MRL. The rate of degradation of the tested insecticides was varied. The plant behavior might be related to physico-chemical properties of pesticides for example uptake by plants, metabolism, etc. (O' Brein, 1967; Virgina and Bajet, 1996). Tejada *et al.* (1983) showed that the disappearance of residue in and on plants is the effect of the interaction of environmental conditions such as the wind, rain, sun, humidity and temperature and chemical and physical factors such as volatilization and growth of the plant. Geigy (1956-67) observed the Diazinon residue levels after spraying the field dose were <0.1 ppm in cabbage at 7 DAS, 0.4 ppm in cauliflower at 5 DAS and < 0.1 ppm in cucumber at 7 DAS. But it differed from Kabir *et al*. (2008) who reported the residue of Diazinon up to 6 DAS with recommended dose in brinjal and the quantities were above MRL up to 3 DAS and Quinalphos residue level was above MRL up to 4 DAS in yard long bean. Adnan *et al.* (2006) found Diazinon residue above MRL up to 8 DAS in sweet pepper grown in green house. Kumar *et al.* (2004) found the presence of Quinalphos residue in farmgate vegetables in Delhi though it was below the MRL. Ahmed *et al.* (2011) reported that Acephate was detected up to 15 DAS which was the longer period of degradation than that of other insecticides (Cypermethrin, Malathion, Diazinon, Quinalphos and Fenitrothion) but its quantity were above MRL at 7 DAS in brinjal; 5 DAS in cauliflower and bean. Cypermethrin was detected above MRL up to 5 DAS in cauliflower, bean and brinjal. Singh and Kalra (1992), Agnihotri *et al.* (1990) and Singh and Kalra (1996) found detectable level of residue of Cypermethrin and Fenvalerate up to 7 days of spraying of recommended dose. The results of the present work agreed in some cases with the works of the above authors although the methods of residue analysis were different.

5. Conclusions

The degradation rate of Acephate was found slower in tomato and hyacinth bean and faster in both the vegetables for Fenvalerate. Therefore, Fenvalerate was likely to be the most suitable insecticide for the vegetables which would be harvested at shortest period of time having withholding period of 4 DAS but in case of Acephate it was 10 DAS. Acephate having higher waiting period with 10 DAS which could not ensure the safety use in short time harvested vegetables. The findings of the study will help farmers, researchers, consumers and stakeholders to undertake activity for safe food production and processing.

Acknowledgements
The authors are grateful to pesticide analytical laboratory, Division of Entomology, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur for financial support and successful completion of this study.

Conflict of interest
None to declare.

References
Adnan I, Al-Samariee, AMS Khloud and AAB Mabrouk, 2006. Residue levels of three organophosphorus insecticides in sweet pepper grown in commercial green houses. Pesticide Sci., 22: 189- 194.

Agnihotri NP, SN Sinha and AK Chakrabatri, 1990. Bioefficacy of some synthetic pyrethroid insecticides against *Leucinodes orbonalis* Guen. and their residues in brinjal fruit. Indian J. Ent., 52:373-378.

Ahmed MS, MA Sardar, M Ahmad and KH Kabir, 2011. Qualitative and quantitative analysis of selected insecticide and detection of their residues from vegetables. Ph.D. Thesis: Department of Entomology, Bangladesh Agricultural University, Mymensingh. 102 pp.

Ahmed MS, MA Sardar, MA Haque and KH Kabir, 2005. A survey on the pattern of insecticidal usage for the protection of brinjal (*Solanum melongena)* from the attack of insect pests in Jessore. Bangladesh J. Zool., 33: 57-63.

Anonymous. 1993. Codex alimentarius, pesticide residues in food. Joint FAO/WHO Standards Program, FAO, Rome, Italy, Vol. 2, pp. 86-109.

Annonymous. 2000. Annual report 1999-2000, Entomology Division, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur, Bangladesh

Anonymous. 2001. Coordinated research on insecticide residue and resistance in major vegetables grown in Bangladesh. Report on Contact Research Project, BARC, BARI, Joydebpur, Gazipur, 102 p.

Antle JM and PL Pingali, 1994. Pesticides, Productivity and Farmer Health: a Philippine case study. American Journal of Agril. Economics, 76: 418-430.

Geigy Chemical Company. 1956-67. Unpublished data and methods of analysis in pesticide petitions, submitted to the US Food and Drug Administration.

Krol WJ, TL Arsenault, HM Pylypiu and MJ Mattina, 2000. Reduction of pesticide residues on produce by rinsing. J. Agric. Food Chem., 48: 46-70.

Van der Schee HA, 2002. Report of pesticide residue monitoring results of Netherlands. 2001. Inspectorate of Health Protection and Veterinary Public Health, Dee Haag, Amsterdam, Netherlands.

Rajeswaran J, I Merlin Kamal, S Chandrasekaran, R Jayakumar and S Kuttalam, 2004. Harvest time residue of carbosulfan in brinjal fruits. J. Food, Agriculture and Environment, 2: 276-277.

Khatoon JA, MS Islam, NM Talukder and MA Hossain, 2004. Monitoring the residue level of three selected pesticides in Red Amaranth. J. Biol. Sci., 4: 474- 479.

Kabir, KH, MA Rahman, MS Ahmed, MDH Prodhan and MW Akon, 2008. Quantitative analysis of some common insecticides used against vegetable insect pests. Bangladesh J. Agriculturist, 1: 259-264.

Kumar B, VK Madan, J Singh and TS Kathpal, 2004. Monitoring of pesticidal contamination of farm gate vegetables. J. Earth Environ. Sci. Springer Netherlands, 90: 65-71.

O' Brien RD, 1967. Insecticides Action and Metabolism. AP New York, 332 p.

Singh IP and RL Kalra, 1996. Persistence and degradation of Fenvalerate on brinjal. Indian J. Ent., 58: 27-31.

Tejada AW, ED Magallona and EB Lakan-Ilaw, 1983. Insecticide residues in vegetables: Application of the modified approach to organophosphate insecticide residues in string bean (*Vigna sesquipedalis* Fruw.) Phil. Agr. U. P. Los Banos, 66: 405-416.

Virgina RO and CM Bajet, 1996. Pesticides in the Philippine environment. In: Proceedings. Anniversary and Annual Scientific Meeting, (Dizon TD, Eusebio JE, Duenas JN, Palis FV and Mabbayad MO eds.). Pest Management Council of the Philippine, Davao City, pp. 61-77.