# Chemical Residues of some Pyrethroid Insecticides in Egg plant and Okra Fruits: Effect of Processing and Chemical Solutions

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(Received on 26th September 2011, accepted in revised form 13th December 2011)

**Summary**: We investigated residual levels of cypermethrin [(*RS*)-*α*-cyano-3-phenoxybenzyl (1*RS*,3*RS*;1*RS*,3*SR*)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate],deltamethrin [(*S*)-*α*-cyano-3-phenoxybenzyl(1*R*,3*R*)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane car- boxylate] and lambda cyhalothrin [(*RS*)-*α*-cyano-3-phenoxybenzyl (*Z*)-(1*RS*,3*RS*)-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate] in eggplant and okra fruit and their dissipation patterns during different processing operations *viz* washing, peeling, cooking and blanching, and dipping in acidic and alkaline solutions. The samples were extracted in ethyl acetate and cleaned up with activated charcoal. After concentration on rotary evaporator the samples were analysed on High Performance Liquid Chromatograph (HPLC). Mobile phase was methanol:water (85:15) and separation of insecticides was achieved on C-18 cloumn at 202 nm with UV-VIS detector. The samples of eggplant and okra fruit were collected from supervised fields as well as from farmer's field. In the supervised field trials, the higher levels of residues of each of the three insecticides were found in the raw samples of eggplant and okra fruit. In raw eggplant fruit the residues were in the order of cyhalothrin (1.40±0.15 mg $kg^{-1}$) > cypermethrin (1.13±0.01 mg $kg^{-1}$) > deltamethrin (0.65±0.1 mg $kg^{-1}$), whereas in raw okra fruit these were in the order of cypermethrin (1.17±0.1 mg $kg^{-1}$) > deltamethrin (1.08±0.12 mg $kg^{-1}$) > cyhalothrin (0.48±0.03 mg $kg^{-1}$). Among all the processing operations, cooking was found the most effective for reduction of the insecticide residues followed by blanching, peeling, washing and dipping in the tap water in both vegetables. Treatments with acidic solutions were more effective than with alkaline solutions for reduction of residues of insecticides in these vegetables. These results have implications for assessing dietary exposure limits of these insecticides.

Keywords*:* dissipation, cypermethrin, deltamethrin, cyhalothrin, washing, peeling, cooking, Okra, Egg Plant.

## Introduction

Food production in the world, particularly in the third world countries, has to keep pace with the rapid increase in population and this would be impossible without the use of pesticides. Out of various methods recommended from time to time, the chemical method at present is considered the most effective to combat the insect pests of crops and vegetables effectively and economically. Synthethic prethroid insecticides are now increasingly used on many crops because of their some interesting properties, such as greater photostability, effective-ness at low concentrations, low environmental persistence, and easy breakdown as compared to organochlorine and organophosphorus insecticides [1, 2]. They constitute approximately 25% of worldwide insecticide market for plant protection and have been in use for more than 40 years [3].

Among vegetables, brinjal (*Solanum melongena* L.) and Okra (*Abelmoschus esculentus* L.) are common and important staple vegetable crops of Pakistan. However, these are badly affected by a number of insect-pests attack during their growing periods. These include fruit and shoot borer, fruitworm and cutworm [4]. The brinjal suffers badly at fruiting stage due to attack of shoot and fruit borer causing up to 70% damage to the crop making it totally unfit for human consumption [5]. To overcome these losses, use of pyrethroid insecticides *viz* cypermethrin [(*RS*)-*α*-cyano-3-phenoxy-benzyl(1*RS*,3*RS*;1*RS*,3*SR*)-3-(2,2-dichlorovinyl)-2,2-dimethyl cyclo propane carboxylate], deltamethrin [(*S*)-*α*-cyano-3-phenoxy -benzyl (1*R*,3*R*)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxy-late] and cyhalothrin [(*RS*)-*α*-cyano-3-phenoxy-benzyl(*Z*)-(1*RS*,3*RS*)-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-di-methylcyclopro-pane-carboxylate] is very common in this region and they are repeatedly applied, even at the fruiting stage. However, indiscriminate and haphazard use of these chemicals, particularly at fruiting stage, leads to their accumulation in the vegetables which consequently cause hazards to human beings through food chain [6]. However, there is no regular testing of food

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residues in Pakistan. However, the mass media has created awareness about the risks involved in the use of pesticides in agriculture. This has created a havoc regarding the accumulation of residues of these toxic chemicals in daily food.

The aim of present study was to evaluate residual levels of three commonly used pyrethroid insecticides, cypermethrin, deltamethrin and cyhalothrin, in eggplant and okra fruits and to assess the effect of different chemicals and household processes: such as washing, peeling, blanching and cooking on the fate of residues of these pyrethroids.

## Results and Discussion

*Effect of Processing on Pyrethroid Residues in Eggplant and Okra Fruits*

The data given in Table-1 and 2 show that the residues of the insecticides were significantly high in unprocessed (raw) eggplant and okra fruits. They ranged between 1.13 mg $kg^{-1}$ to 1.17 mg $kg^{-1}$ for cypermethrin, 0.65 mg $kg^{-1}$ to 1.08 mg $kg^{-1}$ for deltamethrin and 0.48 mg $kg^{-1}$ to 1.40 mg $kg^{-1}$ for cyhalothrin in eggplant and okra fruit, respectively. However, the residues significantly reduced after they were washed, peeled, blanched or cooked (Fig. 1 and 2). Among all the processing techniques cooking was found the most effective in the elimination of residues of the three insecticides.

The results showed that washing under tap water dislodged pyrethroid residues in eggplant and okra fruit significantly. As the pyrethroids are non-systemic in nature, therefore most of the residues remain as microparticles on the surface and are easily removed by the mechanical stirring with water during washing. Thus washing had a significant effect on the removal of residues in eggplant and okra fruit because of less/no penetration of the chemical into the cuticle layer of the plant surface and resulting in deposits removable by washing. These results agree with those obtained by several researchers [7-10] who reported 50-60% removal of the residues by the washing operation. A reduction of 10-30% of alphamethrin residues in tomato and eggplant have been reported [11].

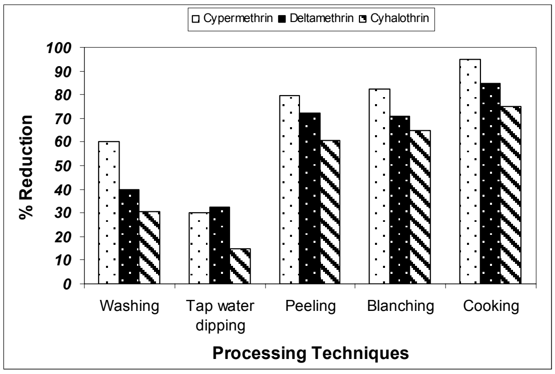


Fig. 1: Percent reduction of pyrethroid residues in eggplant fruit after processing.

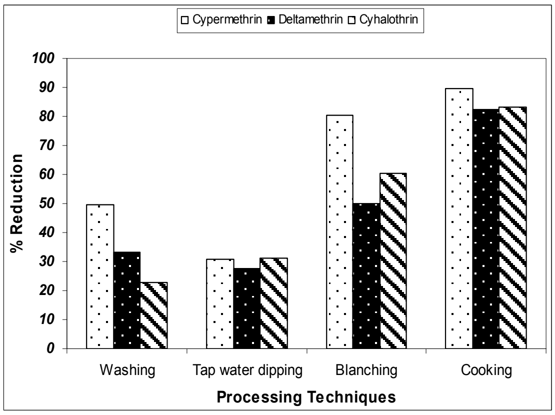


Fig. 2: Percent reduction of pyrethroid residues in okra fruit after processing.

Table-1: Effect of processing on the residue levels of the pesticides applied to egg plant fruit.

| Pesticides | Raw mg $kg^{-1}$ ± SD | Washing mg $kg^{-1}$ ±SD | Tap water dipping mg $kg^{-1}$ ±SD | Peeling mg $kg^{-1}$ ± SD | Blanching mg $kg^{-1}$ ± SD | Cooking mg $kg^{-1}$ ± SD |
|---|---|---|---|---|---|---|
| Cypermethrin | 1.13±0.01 | 0.45±0.01 | 0.79±0.01 | 0.23±0.002 | 0.20±0.02 | 0.06±0.005 |
| Deltamethrin | 0.65±0.1 | 0.39±0.06 | 0.44±0.07 | 0.18±0.03 | 0.19±0.03 | 0.10±0.14 |
| Cyhalothrin | 1.40±0.15 | 0.97±0.09 | 1.19±0.11 | 0.55±0.05 | 0.49±0.05 | 0.35±0.06 |

SD= Standard deviation of the mean

Table-2: Effect of processing on residue levels of the pesticides applied to okra fruit.

| Pesticides | Raw mg $kg^{-1}$ ± SD | Washing mg $kg^{-1}$ ± SD | Tap water dipping mg $kg^{-1}$ ± SD | Blanching mg $kg^{-1}$ ± SD | Cooking mg $kg^{-1}$ ± SD |
|---|---|---|---|---|---|
| Cypermethrin | 1.17±0.13 | 0.59±0.07 | 0.81±0.09 | 0.23±0.03 | 0.12±0.01 |
| Deltamethrin | 1.08±0.12 | 0.72±0.08 | 0.78±0.09 | 0.54±0.06 | 0.19±0.01 |
| Cyhalothrin | 0.48±0.03 | 0.37±0.03 | 0.33±0.03 | 0.19±0.07 | 0.08±0.02 |

SD= Standard deviation of the mean

Peeling with a knife is common household practice for most fruits and vegetables. Eggplant is eaten normally without their peel. Pyrethroid insecticides are applied directly to the crops and show very limited movement or penetration into the cuticle. Therefore, the residues of these materials are confined to the outer surfaces where they are amenable to removal by peeling, hulling or trimming operations. Cypermethrin, deltamethrin and cyhalothrin are non-systemic insecticides. Therefore, when peel was removed, great fractions of insecticide residues were also removed. Peeling reduced 80% residues of cypermethrin, 66% of cyhalothrin and 70% of deltamethrin in eggplant in the current study. Other studies carried out [12] also showed that most of the residue concentration was located in or on peel. Awasthi [12] reported that peeling off the mango fruit skin completely removed the residues of various pesticides including cypermethrin, reflecting the accumulation of residues in the fruit pericarp only and no further transfer of pesticide into the fruit pulp. In another study, 73% reduction in chlorpyrifos, 82% reduction in lindane level, 75% reduction in cypermethrin residues and 77% reduction in ethylenebisdithiocarbamates after peeling of asparagus was reported by Chavarri *et al.*, [7]. Thus, removal of the peel may reduce a large fraction of residues, leaving little in the edible portions. This is especially important for fruits which are not eaten with their peels. However, the peel from commercial peeling processes is sometimes used as animal feed or for the production of essential oils or pectin (citrus, apple etc.). For such industrial processes, it is imperative to consider that non-systemic surface residues are often concentrated in the peel.

Tap water dipping was found relatively less effective in the reduction of residues as compared to washing and peeling with reduction in the residues ranging from 27 to 31%. However, the results are in line with those reported for mango fruit, where the reduction in residues of fenvelerate and cypermethrin ranged between 21 and 27%, 66 and 68% for dimethoate and fenthion, rspectively [12].

Cooking/boiling was found the most effective among all processing operations. Cooking eliminated 95%, 86% and 78% residues of cypermethrin, deltamethrin and cyhalothrin, respectively in eggplant, where as for okra fruit these were 92%, 82% and 85%, respectively. Processes involving heat can enhance volatilization of the chemicals and their hydrolysis thus reducing the residue levels [13]. Hotellier [14] reported that deltamethrin residues reduced appreciably by cooking. However, Kumari [15] found 37, 40 and 42% reduction in synthetic pyrethroids in eggplant, cauliflower and okra fruits, respectively. Similarly 19 to 40% reduction in deltamethrin residues was observed in vegetables in a study by Randhawa *et al.*, [10].

### *Effect of Chemical Treatment on Pesticide Residues in Eggplant and Okra Fruit*

The levels of insecticide residues in eggplant and okra fruit after chemical treatments are given in Table-3 and 4. The residues of each three of pyrethroids significantly decreased as compared to those in raw samples when the vegetables were treated with 4%, 8%, 12% each of NaOH, and acetic acid solutions. From these results it is clear that these washing solutions alone are highly effective in reducing the insecticide residues. Solution of 4% NaOH decreased 27 and 40% in insecticide residues in eggplant and okra fruit, respectively; 8% NaOH resulted in a reduction of 55 and 60%, respectively; where as 12% NaOH showed maximum reduction of 75% in residues in the vegetables (Fig. 3 and 4).

Table-3: Effect of chemical solutions on pesticide residues in eggplant fruit (mg $kg^{-1}$ ±SD).

| Pesticide | Raw | NaOH solution | | | Acetic acid solution | | |
|---|---|---|---|---|---|---|---|
| | | 4% | 8% | 12% | 4% | 8% | 12% |
| **Cypermethrin** | **1.13±0.01** | **0.67±0.01** | **0.51± 0.01** | **0.39± 0.01** | **0.83±0.01** | **0.53±0.01** | **0.02±0.01** |
| **Deltamethrin** | **0.65±0.10** | **0.53±0.08** | **0.37±0.06** | **0.23±0.03** | **0.49±0.07** | **0.40±0.06** | **0.26±0.04** |
| **Cyhalothrin** | **1.4± 0.15** | **1.34± 0.14** | **1.05±0.1** | **0.79± 0.07** | **1.26± 0.12** | **0.65± 0.06** | **0.36±0.03** |

SD= Standard deviation of the mean

Table-4: Effect of chemical solutions on pesticide residues in okra fruit (mg $kg^{-1}$ ±SD).

| Pesticides | Raw | NaOH solution | | | Acetic Acid solution | | |
|---|---|---|---|---|---|---|---|
| | | 4% | 8% | 12% | 4% | 8% | 12% |
| **Cypermethrin** | **1.17±0.13** | **0.59±0.06** | **0.47±0.05** | **0.29±0.03** | **0.86±0.10** | **0.55±0.07** | **0.04±0.04** |
| **Deltamethrin** | **1.08±0.12** | **0.89±0.10** | **0.75±0.08** | **0.45±0.05** | **0.82±0.09** | **0.59±0.06** | **0.24±0.02** |
| **Cyhalothrin** | **0.48±0.04** | **0.39±0.03** | **0.27±0.02** | **0.21±0.02** | **0.39±0.03** | **0.30±0.02** | **0.14±0.02** |

SD= Standard deviation of the mean

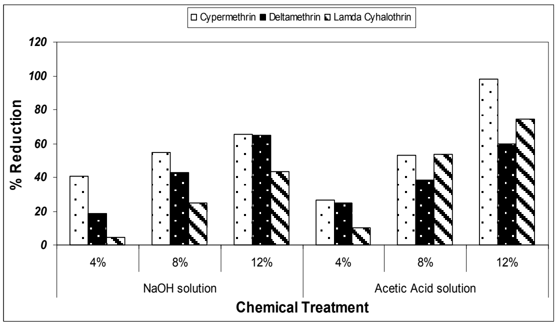


Fig. 3: Percent reduction of pyrethroid residues in eggplant fruit by chemical treatment.

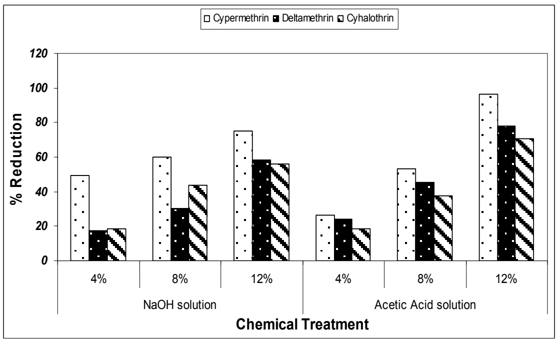


Fig. 4: Percent reduction of pyrethroid residues in okra fruit by chemical treatment.

Acetic acid solution was found more effective than NaOH in the dissipation of cypermethrin, deltamethrin and cyhalothrin residues. Reduction in the pyrethroid residues increased with increase in concentration of the washing solution. There are some reports which show efficacy of chemical washing to dislodge the pesticides from crops. Pesticides treated tomato samples on washing with different levels of acetic acid solution gave, 47.0%, 33.7%, 91.5%, 86.0% and 93.7% loss in lindane, DDT, dimethoate, profenofos and pirimip-hosmethyl, respectively. The pesticide residues reduction increased with increase in concentration of solutions [16, 17]. The chemical solution causes dissipation of the pesticides through pH change. Diping treatment of fruits in water, NaCl solution, HCl, acetic acid, NaOH solutions and potassium permanganate removed 50 to 60% of surface residues of pyrethroids compared to 40 to 50% removal by hydrolytic degradation with NaOH and a detergent solution removed 50 to 60% residues [18].

## Experimental

*Material*

All solvents and other reagents used were of purity compatible with pesticide residue analysis. Analytical grade reference insecticide standards of cypermethrin, deltamethrin and lambda cyhalothrin (>95% purity) were purchased from Dr. Ehrenstorfer GmbH, Germany. Chemical structures of the insecticides are given in Fig. 5. Commercial formulation of insecticides was purchased from open market. Acetic acid, sodium hydroxide, anhydrous sodium sulphate, sodium chloride, acetone, activated charcoal and glass wool were purchased from Merck, Pakistan, where as HPLC grade chemicals/solvents like water, methanol and ethyl acetate were from Sigma, Pakistan.

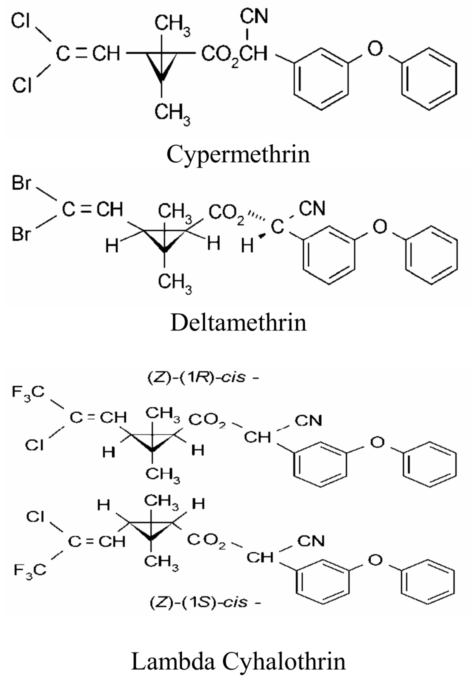


Fig. 5: Chemical structures of the pesticides.

*Sample Collection*

A survey was conducted at three different locations in the adjoining villages of Rawalpindi and Islamabad regarding the use of pesticides by the local farmers. Eggplant and okra fruit samples were collected from ten different fields. The farmers were allowed to use their traditional methods of pesticide application on these vegetables. At maturity the samples of eggplant and okra fruit were collected from the farmer's fields and brought to the laboratory and stored at -4°C. In the supervised field trial the okra and eggplant fields were selected and sprayed with cypermethrin (Arrivo 10 EC) @ 250 mL $Acre^{-1}$, lambda cyhalothrin (Karate 2.5 EC) @ 300 mL/acre and deltamethrin (Decis 2.5 EC) @ 250 mL $Acre^{-1}$ twice at the insect pest infestation. Each insecticide was dissolved in 100 liter water and sprayed with

Knap-sack sprayer. Both the vegetable crops received normal agronomic practices.

*Treatment of Contaminated Vegetables*

To determine the effects of household processes, the collected samples were divided into lots. They were subjected to washing under tap water (~25 ºC), peeling, blanching and cooking till softness as described elsewhere [19]. Moreover, the contaminated samples were soaked for 10 min in tap water, acetic acid solutions at concentrations of 4, 8 and 12% and aqueous solutions of sodium hydroxide at concentrations of 4, 8 and 12% prior to the extraction, cleanup and analysis of each sample.

*Extraction and Cleanup*

The insecticide residues in the vegetable samples were extracted and cleaned up following the method of [20] with some modification. Briefly, 1 kg of the vegetable was chopped into small pieces and blended in a Warring Blender (Anex, Germany) to make a fine paste. A representative sample of 50 g was taken and extracted with 50 mL ethyl acetate, 20g anhydrous sodium sulphate and 10 g NaCl on mechanical shaker (IKA, UK) for 1 h at 150 rpm. The extract was filtered through Whatman No. 4 filter paper. Glass wool was placed at the bottom of column used for cleanup. The column was then filled with activated charcoal that has been previously activated at 300 ºC for 3 h with a top layer of anhydrous sodium sulphate. The column was washed with acetone before loading the sample to the column. The cleaned up extract was collected in 100 mL flask and was then concentrated using rotary evaporator (Eyela, USA) at 60 ºC. The concentrated sample was transferred to sampling vials and the solvent was evaporated by gentle stream of nitrogen to just dryness. The dried samples were dissolved in 500 µL methanol prior to analysis and filtered through 0.45 µm nylon membrane filter (Millipore, Germany) before injection to High Performance Liquid Chromatography (HPLC) in order to remove any particulate material present in the sample.

*Instrumental Analysis and Method Validation*

The cleaned extracts were analyzed on a HPLC (Shimadzu Class-VP V6.13 SPI) equipped with variable-wavelength ultraviolet detector. The column used was reverse phase C-18 (25 mm x 4.6 mm id). Mobile phase was methanol and water (85:15) at a flow rate of 1 mL min$^{-1}$. The injection volume was 20 µL and the detector was set at 220 nm and run time was 15 minutes. Retention time obtained for different insecticides are shown in Table-5. Three metabolites of cypermethrin were identified and residues of cypermethrin are expressed as sum of the metabolites. A chromatogram of unknown okra sample is shown in Fig 6a. A high degree of resolution for separation of different components of sample (insecticides) was achieved under the conditions used in the study. When the same sample of okra was spiked with known quantities of each of the three insecticides the same degree of resolution was achieved as shown in Fig 6b. The spiked samples confirmed the presence of insecticides in the okra sample.

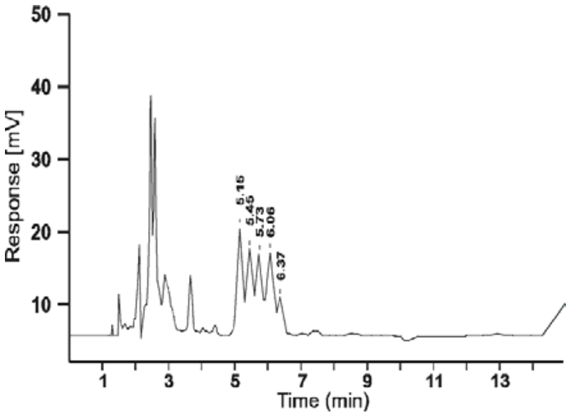


Fig. 6a: Chromatogram of okra sample showing residues of lambda cyhalothrin, deltamethrin and cypermethrin.

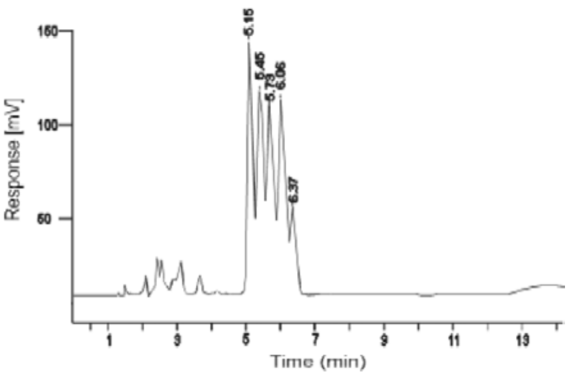


Fig. 6b: Spiked chromatogram of okra sample showing residues of lambda cyhalothrin, deltamethrin and cypermethrin

Table-5: Linear range, recovery and retention time of insecticides.

| Insecticides | Retention time (min) | Recovery (%) | Spike level (µg) | Linear range (µg) | $R^2$ |
|---|---|---|---|---|---|
| Lambda Cyhalothrin | 5.15 | 97 | 30 | 1.11 to 71 | 0.999 |
| Deltamethrin | 5.45 | 98 | 30 | 0.91 to 58 | 0.999 |
| Cypermethrin (sum of metabolites) | 5.73, 6.06, 6.37 | 95 | 30 | 1.17 to 75 | 0.998 |

The method used for determination of pyrethriod insecticide residues was validated before analysis of actual samples. For this purpose 30 µg each of three insecticides were added in the 50 g blended okra (from supervised field where no spray was done) and extracted as described above. Cleanup and concentration was also performed by following the same set of conditions. Reproducible recoveries of > 94% were obtained for the insecticides used in this study (Table-5). Linear range of determination for each of the insecticide was constructed by using step wise dilution analysis of each insecticide on HPLC. The standard curve showed that linear range was found to be 1.17 to 75 µg, 0.91 to 58 µg and 1.11 to 71 µg for cypermethrin, deltamethrin and lambda cyhalothrin, respectively. The analytical method was found to be highly sensitive to fulfill quality criterion for insecticide residue analysis. The $R^2$ value for standard curve of each insecticide is also shown in Table 5. Then concentration of unknown sample was quantified by using the same standard curves, respectively for each of the insecticide (Fig 7).

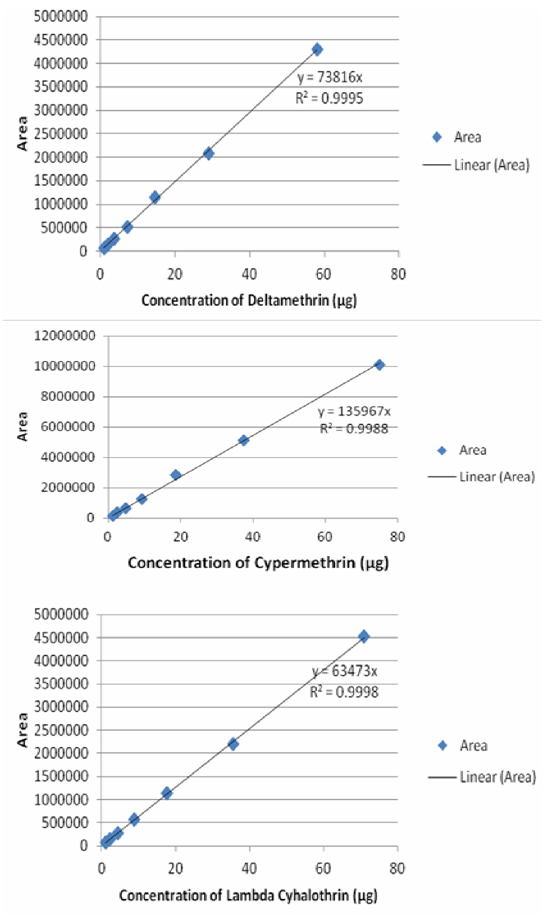


Fig.7: Standard curves for deltamethrin, cypermethrin and lambda cyhalothrin.

## Conclusion

Residual effect of cypermethrin, deltamethrin and lambda cyhalothrin in eggplant and okra fruits and their dissipation patterns during different processing operations *viz* washing, peeling, cooking and blanching and dipping in chemical solutions was studied. The results revealed that processing and chemical treatments were effective in reduction of the insecticide residues (cypermethin, deltamethrin and lambda cyhalothrin) in eggplant and okra fruits. Though washing with water is important to decrease the concentrations of insecticide residues, cooking of vegetables helps greatly to eliminate major fractions of the pesticide residues. Simple dipping of vegetables in acidic or alkaline solutions can also reduce the insecticide residues efficiently. The acceptable maximum residue limits established by the FAO/WHO Codex Alimentarius Commission, or other international bodies refer to the whole vegetables and therefore are appropriate for assessing compliance with Good Agricultural Practices. However, these limits may be of limited significance for assessing dietary exposure to pesticides from the vegetables such as eggplant and okra, which are consumed after household processing.

## Acknowledgements

The authors are grateful to the National Institute of Food Science and Technology University of Agriculture, Faisalabad, for providing facilities to carry out a part of the research project.

## References

1. G. F. Pang, C. L. Fan and Y. Z. Chao, *Journal Association of Official Analytical Chemists International*, **77**, 738 (1994).
2. G. F. Pang, C. L. Fan, Y. Z. Chao and T. S. Zhao, *Journal of Chromatography*, **67**, 348 (1994).
3. T. J. Shafer, D. A. Meyer and K. M. Crofton, *Environmental Health Perspectives*, **113**, 123 (2005).
4. A. Saleem and Ibrar-ul-Haq, Pesticides standardized in Punjab - dose(s), crop(s) and target pest(s). Directorate of Agricultural Information Government of the Punjab, Agriculture Department, Lahore (2003).
5. B. Duara, A. Baruah, S. C. Deka and N. Burman, *Pesticide Research Journal*, **15**, 43 (2003).
6. M. Nafees, M. R. Jan, *Soil and Environment*, **28**, 113 (2009).
7. M. J. Chavarri, A. Herrera and A. Arino, *International Journal of Food Science and Technology*, **40**, 205 (2005).

8. S. Jayakrishnan, A. K. Dikshit, J. P. Singh and D. C. Pachauri, *Bulletin of Environmental Contamination and Toxicology*, **75**, 324 (2005).
9. M. A. Randhawa, F. M. Anjum, A. Ahmed and M. S. Randhawa, *Food Chemistry*, **103**, 1016 (2007).
10. M. A. Randhawa, F. M. Anjum, M. S. Randhawa, A. Ahmed, U. Farooq, M. Ibrar and M. A. Randhawa, *Journal of Chemical Society of Pakistan*, **30**, 227 (2008).
11. K. Gill, B. Kumari and T. S. Kathpal, *Journal of Food Science and Technology*, **38**, 43 (2001).
12. M. D. Awasthi, *Journal of Food Science and Technology*, **30**, 132 (1993).
13. P. T. Holland, B. Hamilton, B. Ohlin and M. W. Skidmore, *Pure and Applied Chemistry*, **66**, 335 (1994).
14. M. L. Hotellier, *Deltamethrin Momograph Roussel Vcalf.*, Pp**.** 285-315 (1982).
15. B. Kumari, *ARPN Journal of Agriculture and Biological Sciences*, **3**, 46 (2008).
16. S. M. Ismail, A. Ali and R. A. Habiba, *Journal of Agricultural and Food Chemistry*, **41**, 610 (1993).
17. A. A. K. Abou-Arab, *Food Chemistry*, **65**, 509 (1999).
18. M. D. Awasthi and A. Lalitha, *Journal of Entomology Research*, **7**, 139 (1986).
19. M. A. Randhawa, F. M. Anjum, M. R. Asi, M. S. Butt, A. Ahmed and M. S. Randhawa, *Journal of Scientific and Industrial Research*, **66**, 849 (2007).
20. L. Kadenezki, Z. Arpad, I. Gardi, A. Ambrus, L. Gyorfi and G. Reese, *Journal Association of Official Analytical Chemists International*, **75**, 53 (1992).