INTERNATIONAL JOURNAL OF ADVANCE RESEARCH, IDEAS AND INNOVATIONS IN TECHNOLOGY

ISSN: 2454-132X
Impact factor: 4.295
(Volume 5, Issue 6)
Available online at: www.ijariit.com

Analytical method validation of acetamiprid+ bifenthrin formulation by Reverse Phase High Performance Liquid Chromatography (R-HPLC)

Dr. K. Ayyavoo
ayyavoo_vasanth@yahoo.com
Bioscience Research Foundation,
Chennai, Tamil Nadu

Dr. C. Tamilselvan
drselvan@brfchennai.com
Bioscience Research Foundation,
Chennai, Tamil Nadu

ABSTRACT

Many types of combination of pesticide are being used effectively as an insecticide, weedicide, growth regulators and may other applications successfully. To control termites and insecticide these combinations of acetamiprid and bifenthrin are being used effectively in the plant kingdom. Acetamiprid is an organic compound basically and the bifenthrin is and pyrethroid molecule. These molecules are being used as an insecticide; even though chemically different in nature. These molecules in different concentrations and combinations are being used over 100 countries; it is very important to detect and quantify with lesser time and cost-effective. This HPLC method is a simple method to quantify these two molecules within 17 minutes. The simple HPLC analytical method is a reversed-phase chromatography principle by using acetonitrile and 1% Ortho Phosphoric Acid (OPA) in HPLC grade water 80:20 ratio. In this method Shimadzu C18 column is used with the dimension as 250mm length; 4.6 mm dimension and 5µm silica particle size packed. The detection was carried out at 225nm, with the Shimadzu detector supported with LC solution software. In this method, these acetamiprid was eluted at 3.6 minutes and the bifenthrin was eluted at 2.7 minutes. This analytical method was complying all the validation parameters of SANCO 3029; version 4 and ICH guideline.

Keywords— *Acetamiprid and Bifenthrin, RP-HPLC, SANCO 3030/99 Rev.4, ICH Guideline*

1. INTRODUCTION

Acetamiprid is an organic chemical and this molecule consist a main pyridine, chloride, N-methyl and emine system along with a cynide residue. This is a very fast degrading molecule in the soil (9 days for 90%) and the environment (14 days 50%) The pyridine molecule substituted at 1, 4 positions by chloride and a side chain consist of N-methyl residue with cyanide system. The well-known pyrethroid permethrin, cypermethrin like molecular structure has this bifenthrin; whereas this bifenthrin consists of trifluoride atoms instead of chloride and in the biphenyl system has a methyl in the ortho position of the chain attachment. The fluoride system so effectively controls the termites in the soil applications. These molecules are being used as an insecticide; even though chemically different in nature. These molecules in different concentrations and combinations are being used over 100 countries; it is very important to detect and quantify with lesser time and cost-effective. This HPLC method is a simple method to quantify these two molecules within 17 minutes. The simple HPLC analytical method is a reversed-phase chromatography principle by using acetonitrile and 1% Ortho Phosphoric Acid (OPA) in HPLC grade water in the ratio of 80:20 in the analytical system.

2. MATERIALS AND METHOD

2.1 Reagents and chemicals used

All the analytical grade solvents and water were used in this analytical method development. A class 'A' glass wear used in this analytical method development processes.

2.2 Instrument

In this experiment used HPLC was periodically calibrated and well maintained to develop this analytical method effectively. This HPLC used to separation and quantification of acetamiprid and bifenthrin in the Wettable Powder (WP) formulation. The analytical instrument details *viz;* HPLC - Shimadzu, Model - LC 2030; Detector - UV-Vis.; Absorption at 225 nm; Column used, Shimadzu C18 (250 x 4.6, 5µ); mobile phase used Acetonitrile and Water (1% OPA); ratio of 80:20 (v/v) with flow rate 1 ml/min. With this HPLC conditions, the acetamiprid and bifenthrin molecules are separated. The elution of acetamiprid and bifenthrin were observed at 3.6 minutes and 12.7 minutes respectively.

2.3 Preparation of Mobile phase

A volume of 80% Acetonitrile and 20% were programmed through the LC solution software and used for dmethod development and quantification analysis.

3. ANALYTICAL METHOD VALIDATION

3.1 Specificity

3.1.1 Preparation of standard stock solutions: An amount of 10.03 mg Acetamiprid reference standard with purity 99.72% and 10.03 mg Bifenthrin reference standard with purity 99.71% were weighed accurately into clean and dry 10 mL volumetric flasks separately, dissolved with mobile phase and made up to the mark with the mobile phase. This solution was equivalent to 1000.19 mg/L and 1000.09 mg/L respectively. From this, an aliquot of each 2.5 ml solution was mixed in a 25 mL volumetric flask, diluted with the mobile phase. This solution was equivalent to 100 mg/L

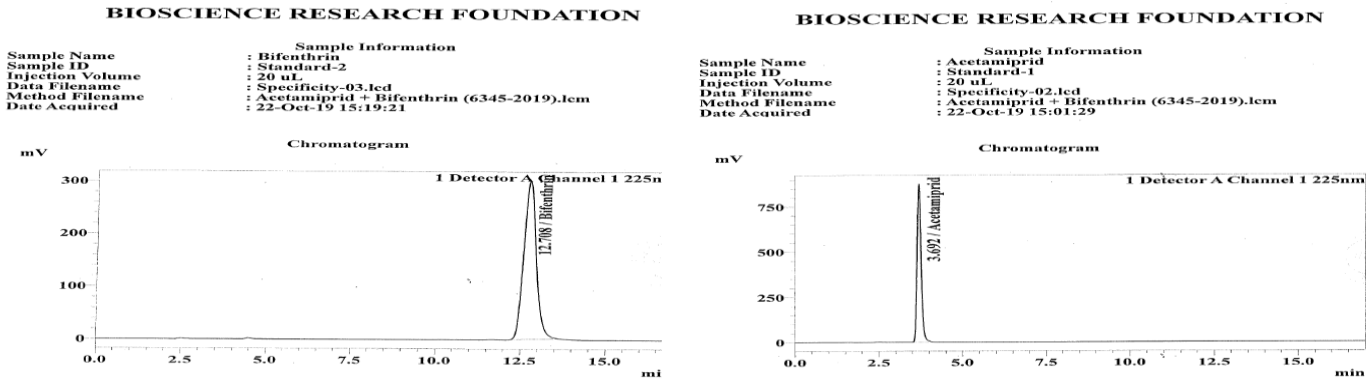

Fig. 1: Typical Chromatogram for Acetamiprid and Bifenthrin

3.1.2 Preparation of Sample Solution: An amount of 10.0 mg test substance was weighed accurately into a clean and dry 100 mL volumetric flask, dissolved in the mobile phase and made up to the mark with the mobile phase. This solution was equivalent to 100 mg/L and used for determination of Specificity.

The Specificity of HPLC method for Acetamiprid + Bifenthrin was determined by injecting the Standard and Sample solutions along with blank (mobile phase) and observed that there was no interference found with the main peak of interest. Hence, this method was considered to be specific for the analysis of the test substance

3.2 Linearity

3.2.1 Preparation of Standard Stock Solution and working standard: The Specificity standard solution (100 mg/L) was used for the determination of Linearity. From the Specificity Standard solution (100 mg/L), the serial dilutions were made by using mobile phase, to prepare further concentrations such as 0.1, 1, 5, 10, 15 and 20 mg/L respectively.

The prepared standard solutions were injected by an autosampler into the HPLC system and a linear curve was plotted for the concentration of standard versus observed peak area and the correlation coefficient was determined respectively. The results are presented in Table 1.

Table 1: Linearity of Acetamiprid & Bifenthrin Reference Standard.

Std. Code	Concentration (mg/L)	Std. Area (mV) Acetamiprid	Std. Area (mV) Bifenthrin
L-Std-1	0.1	7290	7425
L-Std-2	1	72635	74998
L-Std-3	5	371563	376147
L-Std-4	10	749165	762219
L-Std-5	15	1109865	1144957
L-Std-6	20	1501232	1551079
Intercept		-2013.3	-5650.8
Slope		74830	77314
Correlation Coefficient (r)		1.0000	0.9999

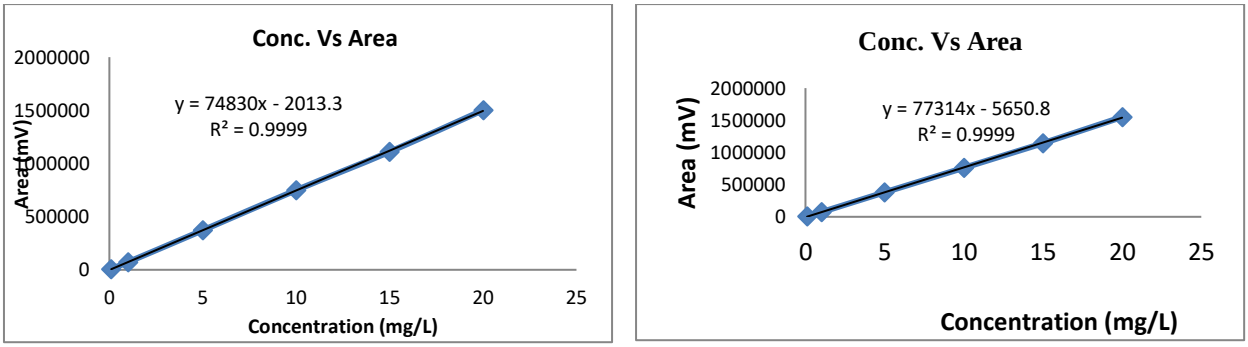

Fig. 2: Linearity Curve for Acetamiprid and Bifenthrin

4. PRECISION

4.1 Preparation of Standard Solution

The Linearity standard solution; standard 5mg/L was prepared and used for the precision determination.

4.2 Preparation of Sample Solution

An amount of 18.20, 18.21, 18.20, 18.19 and 18.18 mg Test substance was weighed accurately into clean and dry 10 mL volumetric flasks, dissolved the contents with mobile phase and made up to the mark with the mobile phase. These solutions were equivalent to 1820, 1821, 1820, 1819 and 1818 mg/L respectively. An aliquot of 0.1 mL sample solutions was taken into 10 mL volumetric flasks, diluted with mobile phase and made up to the mark with the mobile phase These solutions were equivalent to 18.20, 18.21, 18.20, 18.19 and 18.18 mg/L respectively. These prepared solutions were injected into HPLC. The results are presented in Table 2-3.

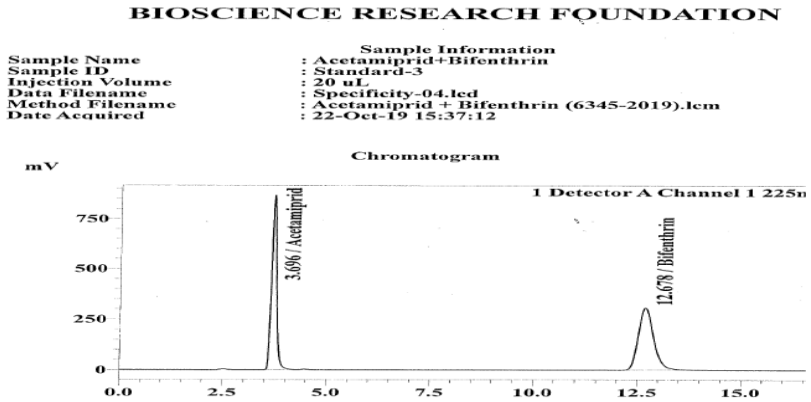

Fig. 3: Typical Chromatogram for Acetamiprid & Bifenthrin (separation in the specificity run)

Table 2: Precision (Acetamiprid)

Code	Sam. Conc.(mg/L)	Std. Conc. (mg/L)	Samp. Area (mV)	Std. Area	Avg. Std. Area (mV)	Purity of Calibration sol.	A.I. Content (%)	Avg. Content (%)
P-Std-R1	-	5	-	371278	370980	100	-	-
P1R1	18.20		314883	-			23.32	23.32
P1R2			314818				23.31	
P2R1	18.21		314995				23.31	23.31
P2R2			314899				23.31	
P3R1	18.20		314885				23.32	23.32
P3R2			314815				23.31	
P4R1	18.19		314847				23.33	23.33
P4R2			314898				23.33	
P5R1	18.18		314910				23.35	23.35
P5R2			314895				23.34	
P-Std-R2	-		-	370681			-	-
Mean								**23.32**
SD								**0.0143**
% RSD								**0.0613**

Table 3: Precision (Bifenthrin)

Code	Sam. Conc.(mg/L)	Std. Conc. (mg/L)	Samp. Area (mV)	Std. Area	Avg. Std. Area (mV)	Purity of Calibration sol.	A.I. Content (%)	Avg. Content (%)
P-Std-R1	-	5	-	379847	380774	100	-	-
P1R1	18.20		381447	-			27.52	27.52
P1R2			381533				27.53	
P2R1	18.21		381626				27.52	27.53
P2R2			381816				27.53	
P3R1	18.20		381393				27.52	27.51
P3R2			381300				27.51	
P4R1	18.19		381776				27.56	27.56
P4R2			381861				27.57	
P5R1	18.18		381496				27.55	27.56
P5R2			381711				27.57	
P-Std-R2	-		-	381701			-	-
Mean								**27.54**
SD								**0.0232**
% RSD								**0.0844**

The formula for Active content Calculation:

$$A.I.Content\ (\%) = \frac{\text{Sample Area x Std. Conc. (mg/L)}}{\text{Average Std. Area x Sample Conc. (mg/L)}} \text{x Purity (P) \%}$$

5. ACCURACY (% RECOVERY)

The recovery processes and the recovery determination was validated with three fortification levels of processes.

5.1 Preparation of Standard Solution

The standard solution prepared for linearity (10 mg/L of Acetamiprid and Bifenthrin mixture) was used as a standard in per cent recovery determination.

5.2 The preparations were analyzed under HPLC (fortification level 0.1 & 1 mg/L). The results are presented in Table 4-5.

Formula:

$$Fortified\ Area\ =\ Detected\ Area\ -\ Blank\ Sample\ Average\ Area$$

$$Recovered\ Concentration\ (mg/L) = \frac{\text{Standard Concentration (mg/L)}}{\text{Standard Average Area}} \times \text{Fortified Area}$$

$$Recovery\ \ (\%) = \frac{\text{Recovered Concentration (mg/L)}}{\text{Fortified Concentration (mg/L)}}$$

The above preparations were analyzed under HPLC and checked for recovery (%). The results are presented in following table 4 and 5

Table 4: Recovery (Acetamiprid; level 1& 2)

Fortification Level	Std. Conc. (mg/L)	Std. / Sample area (mV)	Mean Std. Area (mV)	Detected Conc. (mg/L)	Fortified Conc. (mg/L)	Recovery (%)	Avg. Recovery (%)	SD	% RSD
Std-R1	5	370856	371158	-	0.1	-	99.23	0.20	0.20
F1R1		7365		0.0992		99.22			
F1R2		7390		0.0996		99.55			
F1R3		7364		0.0992		99.20			
F1R4		7363		0.0992		99.19			
F1R5		7349		0.0990		99.00			
F2R1		74831		1.0081	1	100.81	100.81	0.19	0.19
F2R2		74830		1.0081		100.81			
F2R3		75057		1.0111		101.11			
F2R4		74735		1.0068		100.68			
F2R5		74696		1.0063		100.63			
Std-R2		371460		-		-			
Mean							**100.02**	**0.19**	**0.19**

Table 5: Recovery (Bifenthrin; level 1& 2)

Fortification Level	Std. Conc. (mg/L)	Std. / Sample area (mV)	Mean Std. Area (mV)	Detected Conc. (mg/L)	Fortified Conc. (mg/L)	Recovery (%)	Avg. Recovery (%)	SD	% RSD
Std-R1	5	382933	383210	-	0.1	-	99.05	0.24	0.24
F1R1		7599		0.0991		99.15			
F1R2		7586		0.0990		98.98			
F1R3		7563		0.0987		98.68			
F1R4		7610		0.0993		99.29			
F1R5		7600		0.0992		99.16			
F2R1		75254		0.9819	1	98.19	98.72	0.33	0.34
F2R2		75783		0.9888		98.88			
F2R3		75711		0.9879		98.79			
F2R4		75941		0.9909		99.09			
F2R5		75618		0.9866		98.66			
Std-R2		383486		-		-			
Mean							**98.89**	**0.29**	**0.29**

6. LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTIFICATION (LOQ)

From the Linearity Standard Solution concentration of 5 mg/L was used in these LOD & LOQ determinations. From this solution, 1 mg/L solution was prepared and further diluted to get the 0.01 & 0.1 mg/L concentration solutions were prepared. The dilution details were given in Table No. 6 and the determined area, calculations are in table 7 and 8.

Table 6: Dilutions (LOD & LOQ) for LOD – Acetamiprid and Bifenthrin

Stock Concentration (mg/L)	Dilution Volume (ml)	Final Volume (ml)	Final Concentration (mg/L)
1.0	1	10	0.1
0.1	1	10	0.01

Formula:

LOD = Average + (3 x Standard Deviation)
LOQ = Average + (10 x Standard Deviation)

Table 7: Limit of Detection (LOD) And Limit Of Quantification (LOQ) Of Acetamiprid

Sample ID	Std. Conc. (mg/L)	Std./ Sample Area	Average Std. Area	A. I. Content (mg/L)
L&L-Std-R1	5	371645	372504.5	-
LOD-R1		750		0.0101
LOD-R2		764		0.0103
LOD-R3		749		0.0101
L&L-Std-R2		373364		-
			MEAN	0.0101
			SD	0.0001
			LOD	0.0105

Sample ID	Std. Conc. (mg/L)	Std./ Sample Area	Average Std. Area	A. I. Content (mg/L)
L&L-Std-R1	5	371645	372504.5	-
LOQ-R1		7344		0.0986
LOQ-R2		7326		0.0983
LOQ-R3		7313		0.0982
L&L-Std-R2		373364		-
			MEAN	0.0984
			SD	0.0002
			LOQ	0.1004

Table 8: Limit of Detection (LOD) And Limit of Quantification (LOQ) Of Bifenthrin Example Calculation: (LOD& LOQ)

Sample ID	Std. Conc. (mg/L)	Std./ Sample Area	Average Std. Area	A. I. Content (mg/L)
L&L-Std-R1	5	383631	383748	-
LOD-R1		760		0.0099
LOD-R2		756		0.0099
LOD-R3		760		0.0099
L&L-Std-R2		383865		-
			MEAN	0.0099
			SD	0.0000
			LOD	0.0100

Sample ID	Std. Conc. (mg/L)	Std./ Sample Area	Average Std. Area	A. I. Content (mg/L)
L&L-Std-R1	5	383631	383748	-
LOD-R1		7502		0.0977
LOD-R2		7519		0.0980
LOD-R3		7659		0.0998
L&L-Std-R2		383865		-
			MEAN	0.0985
			SD	0.0011
			LOQ	0.1097

LOQ (Bifenthrin) R1

$$\text{A.I Content } \left(\frac{\text{mg}}{\text{L}}\right) = \frac{\text{Std. Conc. (mg/L)} \times \text{Sample Area}}{\text{Average Std. Area}}$$

$$= \frac{5 \times 7502}{383748} = 0.0977 \text{ mg/L}$$

$$LOQ = \text{Mean Value} + (10 \times \text{SD})$$

$$0.0985 + (10 \times 0.0011) = 0.1097 \frac{\text{mg}}{\text{L}}$$

7. ACTIVE CONTENT ANALYSIS OF ACETAMIPRID AND BIFENTHRIN

7.1 Preparation of Standard solution

Standard concentration 5 mg/L was prepared and used as a standard in concentration analysis.

7.2 Preparation of Sample Solutions

The formulation sample (30 mg/L) was prepared and dissolved by sonication and diluted appropriately and injected into HPLC.

$$Bifenthrin/Acetamiprid\ (mg/L) = \frac{Concentration\ of\ standard\ (mg/L)\ x\ Area\ of\ sample\ solution\ x\ Dilution\ Factor}{\text{Area of standard solution}}$$

8. Conclusion

8.1 Specificity

The blank, standard and the sample peaks were not co-eluted each other of acetamiprid and bifenthrin; hence the specificity was achieved as per the guideline SANCO 3030/99 Rev.4 requirement.

8.2 Linearity

The Linearity correlation co-efficient is achieved NLT 0.99 as per SANCO 3030/99 Rev.4 guideline.

8.3 System Precision

The system precision is achieved as the % RDS for 5 replicates observed as0.06 & 0.08 % RSD for acetamiprid and bifenthrin; which is very well comply with the guideline requirement. (SANCO 3030/99 Rev.4 was NMT 15% RSD).

8.4 Horwitz equation

The % RSD is within limit according to the modified Horwitz equation (Acceptable Limit <1.67% RSD for 23.32% active analyte as per SANCO/3030/99 Rev.5)

The % RSD is within limit according to the modified Horwitz equation (Acceptable Limit <1.63% RSD for 27.54% active analyte as per SANCO/3030/99 Rev.5

8.5 System Recovery

The system recovery 98.72% to 100.81% were achieved for, hence the minimum requirement of the (SANCO 3030/99 Rev.4).

8.6 Details of the Laboratory work were carried out

Bioscience Research Foundation, Sengadu village & Post, Via Manavalanagar, Kandamangalam – 602002, Kanchipuram District, Tamilnadu, India Ph: +91 44 27658293/94/95/96, Email: brfchennai@gmail.com

9. REFERENCES

[1] Guidelines for drinking-water quality, 2nd ed. Addendum to Vol. 2. Health criteria and other supporting information. World Health Organization, Geneva, 1998.
[2] Zachary Getenga, Ulrike Dorfler, Azukalwobi, Michael Schmid and Reiner schroll; A trazine and Bifenthrin mineralization by an Arthrobacter sp. isolated from a sugarcane-cultivated soil in Kenya; Elsevier; Volume77, issue 4, October 2009, Pagess534-539.
[3] Synergistic effects caused by Acetamiprid and Bifenthrin
[4] on chlorpy rifos toxicity to early-life stages of the zebrafish
[5] Danio rerio
[6] Joanne Pérez & Inês Domingues & Marta Monteiro &
[7] Amadeu M. V. M. Soares & Susana Loureiro
[8] Joanne Perez & Ines Domingues & Marta Monteiro Amadeu M. V. M. Soares and Susana Loureiro Synergistic effects caused by Acetamiprid and Bifenthrin on chlorpy rifos toxicity to early-life stages of the zebrafish Danio rerio; Springer, Environmental Science and Pollution Research (2013) 20:4671–4680, DOI 10.1007/s11356-012-1443-6
[9] Synergistic effects caused by Acetamiprid and Bifenthrin
[10] on chlorpy rifos toxicity to early-life stages of the zebrafish
[11] T. B. Bowman; Mobility and persistence of the herbicides Acetamiprid, metolachlor and Bifenthrin in Plainfield sand determined using field lysimeters; Environmental Toxicology and Chemistry; https://doi.org/10.1002/etc.5620080605
[12] C. F. Reinhardt & K. J. Hugo; Simple regression models for the qualitative prediction of Acetamiprid and Bifenthrin leaching; South African Journal of Plant and Soil; 14:2, 58-62, DOI: 10.1080/02571862.1997.10635082
[13] Weber, J.8. & Whitacre, D.M., 1982. Mobility of herbicides in soil columns under saturated- and unsaturated-flow conditions. Weed Sci. 30, 579-584
[14] Weber, J.B. & Miller, C.T., Organic chemical movement over and through the soil. In: B.L. Sawhney & K. Brown (eds.). Reactions and movement of organic chemicals in soils. SSSA, Madison, WI, USA
[15] Synergistic effects caused by Acetamiprid and Bifenthrin
[16] on chlorpy rifos toxicity to early-life stages of the zebrafish
[17] Danio rerio
[18] Joanne Pérez & Inês Domingues & Marta Monteiro &
[19] Amadeu M. V. M. Soares & Susana Loureiro
[20] Reinhardt, C.F. and NEL, P.c., 1993. A bioassay technique for estimating Acetamiprid dissipation time in soils. Proc. 9th Symp. Pestic. Chem., Piacenza, Italy, pp. 479-488
[21] Reinhardt, C.F., Ehlers, J.G. & NEL, P.c., 1990. Persistence of Acetamiprid as affected by selected soil properties. S Air. J Plant Soil 7, 182-187
[22] Jay Chandran, K., Steinheimer, T.R., Somasundaram, L., Moorman, T.B., Kanwar, R.S. & Coates, J.R., 1994. The occurrence of Acetamiprid and degradates as contaminants of subsurface drainage and shallow groundwater. J Environ. Qual. 23, 311-319.
[23] Helling, C.S., 1970. Movement of s-triazine herbicides in soil. In: F.A. Gunther & J.D. Gunther (eds.). Residue Rev. 32, 175-210.
[24] Ehlers, 1.G., Reinhardt, C.F. and NEL, pc., 1987. Acetamiprid activity under field conditions. Appl. Plant Sci. 1, 57-60.
[25] Ehlers, J.G., Reinhardt, C.F. and NEL, P.c., 1988. Influence of selected soil properties on the activity of Acetamiprid. S. Afr. 1. Plant Soil 5, 32-36.
[26] Hougard JM, Zaim SD, Guillet P (2002) Bifenthrin: a useful pyrethroid insecticide for treatment of mosquito nets. J Med Entomol 39:526–533
[27] Laskowski DA (2002) Physical and chemical properties of pyrethroids. Rev Environ Contam Toxicol 174:49–170
[28] Smith TM, Stratton GW (1986) Effects of synthetic pyrethroid insecticides on nontarget organisms. Res Rev 97:93–119

[29] Yadav RS, Srivastava HC, Adak T, Nanda N, Thapar BR, Pant CS et al (2003) House-scale evaluation of bifenthrin indoor residual spraying for malaria vector control in India. J Med Entomol 40:58–63
[30] Brunet JL, Badiou A, Belzunces LP (2005) In vivo metabolic fate of [^{14}C]-acetamiprid in six biological compartments of the honeybee, *Apis mellifera* L. Pest Manag Sci 61:742–748
[31] Joshi DK, Gold MH (1993) Degradation of 2,4,5-trichlorophenol by the lignin-degrading basidiomycete *Phanerochaete chrysosporium*. Appl Environ Microbiol 59:1779–1785
[32] Prashanth MS, David M, Mathed SG (2005) Behavioural changes in freshwater fish, *Cirrhinus mrigala* (Hamilton) exposed to cypermethrin. J Environ Biol 26:141–144

