Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2016, 8(7):298-305

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Monitoring of organochlorine pesticide residues in Asian pear, Kiwi, Ridge gourd, Cauliflower and Bitter gourd by gas chromatography

Devendra Kumar* and Raj Kumar

Department of Chemistry, Institute of Basic Sciences, Dr. B.R. Ambedkar University, Khandari Campus, Agra-282002

ABSTRACT

Although pesticides are important not only for agricultural purpose but also in the management of vector borne diseases. But their widespread use has resulted long-standing effects on human health. The determination of the organochlorine pesticides (OCPs) in foods such as fruits and vegetables is our interest because of their toxic nature, persistence and tendency to bioaccumulate. In spite of the ban of DDT and HCH in India, these pesticides are still in use, both in domestic and agricultural purposes. In this research work we have tried to monitor residual concentration level of 19 organochlorine pesticides (OCPs) including isomers of benzene hexachloride (α-BHC, β-BHC, γ-BHC, δ-BHC), heptachlor, heptachlor epoxide, aldrin, γ-chlordane, endosulfan I + α-chlordane, dieldrin+p,p'-DDE, endrin, endrin aldehyde, endrin ketone, endosulfan II, endosulfan sulfate, p,p'-DDT, p,p'-DDD, methoxychlor and decachlorobiphenyl (DCBP) in two fruits (Asian pear and Kiwi) and three vegetables (Ridge gourd, Cauliflower and Bitter gourd) by using Gas Chromatography with Electron Capture Detector(ECD). Fruits (Asian pear and Kiwi) and vegetables (Cauliflower, Ridge gourd and Bitter gourd) were contaminated either with two or more organochlorine pesticides. However, the concentrations of detected pesticides were far below the maximum residue limit (MRL) values but continuous consumption of such fruits and vegetables can cause different diseases in human being.

Key words: Organochlorine pesticides (OCPs), Gas-chromatography (GC-ECD), Fruits, Vegetables

---

INTRODUCTION

India is home of approximately 16% of the total world's population [1]. The increase in population growth has led to drastic increase in demand for food supply which has also led to immeasurable rise in the use of pesticides and fertilizers [2]. In India 56.7% people are engage in agriculture and therefore high amount of pesticides is used in agriculture [3-5]. The consumption pattern of pesticides in India is different from that of the rest of the world. Approximately 2 million tons of pesticides are consumed worldwide each year, of which India accounts for 1% [1]. Among the different types of pesticides, organochlorine pesticides (OCPs) have been in wide use due to cheaper in cost and broad-spectrum toxicity [6]. Although India has low rate of consumption of OCPs as compared to other developed nations, but their continuous and indiscriminate use has resulted severe health hazards [1] like neurologic deficits [7,8], cancer [9]—especially non-Hodgkin's lymphoma and leukemia [11] and limb defects [10] and reproductive impairment [11]. Thus, in continuation of our previous work [12-14] an effort has been made to monitor the residual concentration level of selected organochlorine pesticides in Asian pear, Kiwi, Ridge gourd, Cauliflower and Bitter gourd.

EXPERIMENTAL SECTION

All glasswares were thoroughly washed with deionized water and then rinsed with acetone and dried in oven (150 ^{o}C) for overnight before use. Solvents like acetone, acetonitrile, dichloromethane, n-hexane and ethyl acetate were distilled before use. Adsorbent neutral alumina, florisil and charcoal were activated before use. Anhydrous sodium sulfate was purified with acetone and heated for 4 h at 600 ^{o}C in a muffle furnace to remove possible phthalate impurities. Purified extracts of fruits and vegetables were analyzed by GLC equipped with capillary column using ^{63}Ni electron capture detector (ECD). A stock solution of standard pesticides used for GC study was procured from Dr. Ehrenstorfer Gmbh Chemicals, Germany.

1. Estimation of pesticides in fruits

1.1 Sample collection and preparation: The sample consists of 1 kg of each fruits, i.e Asian pear and Kiwi, was collected from local market. Each sample was refrigerated at 5^{o}C and analyzed within two days of collection. In order to measure the right concentration of pesticides reaching within the human body, the household processing like- washing and peeling off covering etc. were carried out. Each fruit was washed for a few minutes under tap water and dried with the help of filter paper. After drying each fruit was peeled carefully, cut into small pieces and blended with the high speed warring blender to make a fine paste.

1.2 Extraction and Clean up: 50 g of fine paste of Asian pear was taken out and subjected to extraction with 100 ml [15] acetone for three times. The extract was filtered with the Buchner funnel. The filtrate was concentrated in vacuum up to 5 ml and then transferred to 500 ml separating funnel. 50 ml saline water (2 %, w/v) was added to it and shaked for 50 min. The extract was then exchanged into dichloromethane layer by liquid-liquid partitioning (3×50 ml). The organic layer was separated out from the separating funnel and passed through a layer of sodium sulfate (5 g) and evaporated to dryness (2- 5 ml) in rotatory evaporator. Clean up was performed by column chromatography. Glass column (30×1.5×1.5 cm) was filled with anhydrous sodium sulfate (2 g) + neutral alumina (5 g) + florisil (1 g). The column was prewashed with n-hexane. The concentrated extract was dissolved in 10 ml hexane–acetone (9:1v/v) and subjected to column cleanup. The column was eluted with 25 ml dichloromethane-acetone (8:2 v/v). The extracts was again evaporated to dryness (2-5 ml) in rotatory evaporator and finally dissolved in 10 ml hexane-acetone (9:1 v/v) for the chromatographic analysis. Similar method was adopted for Kiwi.

2. (2) Estimation of pesticides in vegetables

2.1. Sample collection and preparation: 1 kg of each vegetable, i.e. Ridge gourd, Bitter gourd and Cauliflower, was collected from local market, refrigerated at 5ºC and analyzed within 2-3 days of collection. Sample was washed for few minutes under tap water and dried with filter paper. After drying each vegetable was chopped into small pieces. A subsample of 50 g was separate out and homogenized with 100 ml acetonitrile [16] in a high speed blender.

2.2. Extraction and Clean up: The blended sample of Ridge gourd was transferred into a separating funnel and shaked gently for 1 h with 100 ml mixture of n-hexane and dichloromethane (3:2 v/v). The separating funnel was allowed to be held at vertical position for about 30 min to obtain two distinct layers of n-hexane and dichloromethane. n-hexane layer was separated out. This process was repeated three times. The collected extract in the layer of n- hexane was dried up to dryness (2-5 ml) in a rotatory evaporator. The dry residue was dissolved in 10 ml n-hexane and subjected for clean up by column packed with florisil and activated charcoal (5:1 w/w). The extract was eluted with 50 ml mixture of ethyl acetate:n-hexane (3:7 v/v). Eluted extract was dried up to dryness and redissolved in 5 ml n-hexane and then injected to GC-ECD for analysis. Similar method was adopted for Bitter gourd and Cauliflower.

RESULTS AND DISCUSSION

First by running the stock solution of standards (Fig.1), we have determined retention time and peak areas for standards corresponding to 5 ppm concentration. In the chromatogram of standards, the peaks of different isomers of benzene hexachloride (BHC) were found at Rt values 5.210, 5.834, 6.003 and 6.676 which correspond to α-BHC, β-BHC, γ-BHC and δ-BHC respectively. The peaks of heptachlor and heptachlor epoxide were noticed at Rt value 8.285 and 11.637 respectively. The peak at Rt value 9.731 was found for aldrin. The peak of γ-chlordane was found at Rt value 12.934. The peak at Rt value 13.825 was found for endosulfan I +α-chlordane. Rt value of the peak of dieldrin+ p, p'-DDE was found at 15.241. The peaks at Rt values 16.704, 18.787 and 24.278 correspond to endrin, endrin aldehyde and endrin ketone respectively.

The peaks of endosulfan II and endosulfan sulfate were found at Rt values 17.385 and 20.584. The peaks of p,p'-DDD and p,p'-DDT were found at Rt values 18.173 and 21.026. The peak at Rt value 26.181 was found for methoxychlor. Rt value of the peak of decachlorobiphenyl (DCBP) was found at 42.525.

During the monitoring work chromatogram of Asian pear (Fig. 2) exhibited a number of peaks from those four peaks at the Rt values 5.83, 6.59, 8.28 and 9.85 resemble with the Rt values of β- BHC, δ-BHC, heptachlor and aldrin respectively which indicated the presence of β- BHC, δ-BHC, heptachlor and aldrin pesticides in the sample of Asian pear.

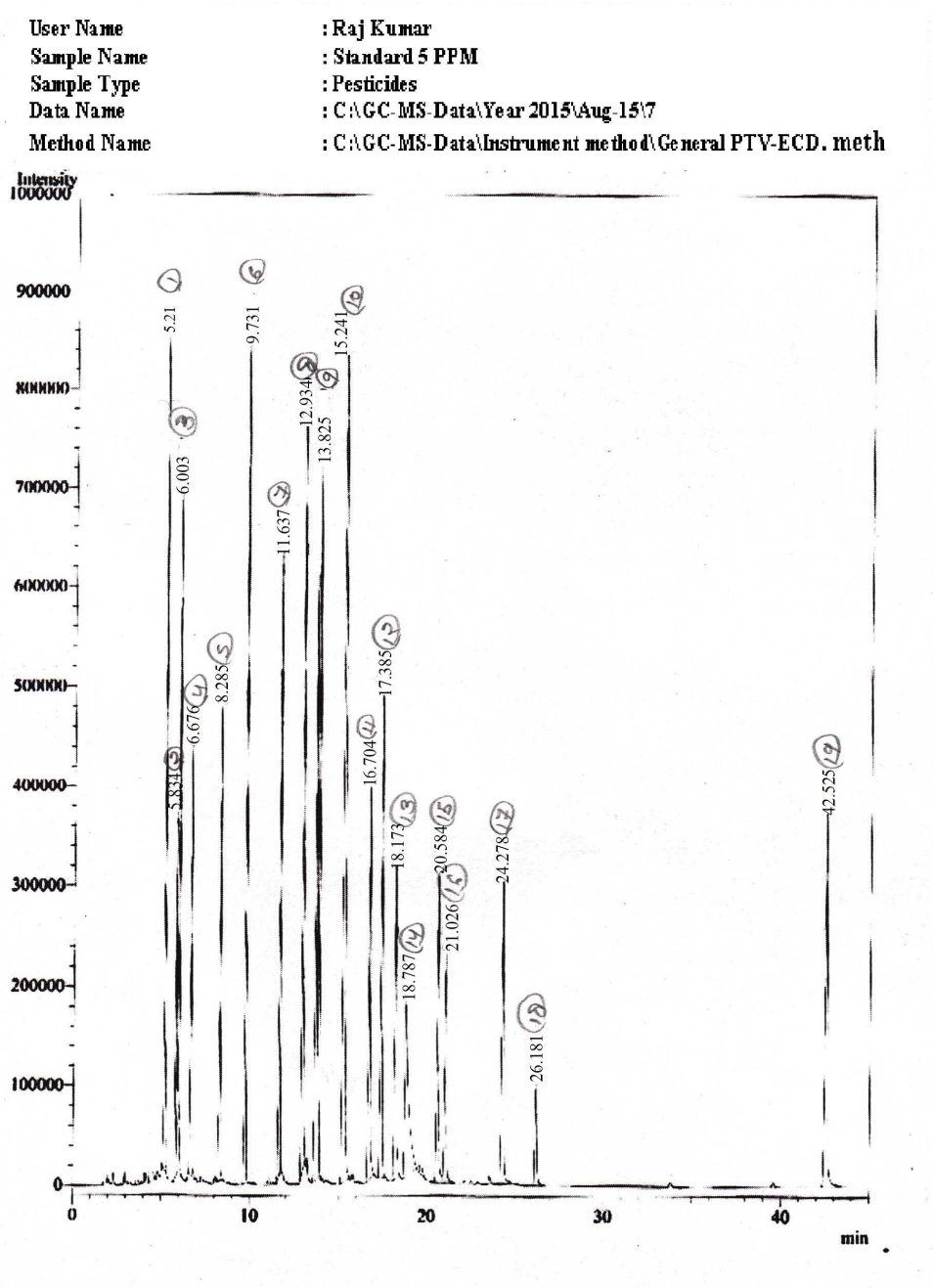

Fig.1. Gas Chromatogram of Standards of Organochlorine pesticides

C:\GCMS-data\instrumentmethod\GENERAL-PTV-ECD.meth\standard 5 ppm

Peak Table-Channel 1

Peak No	Pesticides	Retention Time	Area	Area %
1	α-BHC	5.21	3703640	5.8074
2	β-BHC	5.834	1528709	2.9921
3	γ-BHC	6.003	3264915	5.761
4	δ-BHC	6.676	2085353	3.8096
5	Heptachlor	8.285	2292675	3.9475
6	Aldrin	9.731	4346046	4.9554
7	Heptachlor Epoxide	11.637	3269756	5.5584
8	γ-Chlordane	12.934	4300941	6.8968
9	Endosulfan-I +α Chlordane	13.825	7686728	10.85
10	Dieldrin + p,p'-DDE	15.241	756334	6.6925
11	Endrin	16.704	2655909	4.7616
12	Endosulfan-II	17.385	3188425	5.1234
13	p,p'-DDD	18.173	2250920	3.9055
14	Endrin Aldehyde	18.787	2598185	4.6178
15	Endosulfan Sulfate	20.584	2186191	3.7103
16	p,p'-DDT	21.026	1608239	3.042
17	Endrin ketone	24.278	2388342	3.7033
18	Methoxychlor	26.181	751951	1.976
19	Decachlorobiphenyl(DCBP)	42.525	3456786	4.1234
			55572167	100

Table 1: Concentrations of pesticides evaluated through Gas Chromatography

S. No	Name of Samples	Rt value	Conc. (ppm)	Name of the Pesticides
1	Asian pear	5.83	0.0059	β- BHC
		6.59	0.00099	δ - BHC
		8.28	0.00067	Heptachlor
		9.85	0.00023	Aldrin
2	Kiwi	9.74	0.0041	Aldrin
		8.26	0.0085	Heptachlor
3	Ridge gourd	5.83	0.00109	β – BHC
		6.67	0.00006	δ-BHC
		8.22	0.00027	Heptachlor
		9.75	0.000095	Aldrin
4	Cauliflower	5.82	0.0003	β – BHC
		8.28	0.0001	Heptachlor
		6.59	0.00039	δ-BHC
		9.66	0.00014	Aldrin
5	Bitter gourd	5.89	0.0021	β – BHC
		9.74	0.00033	Aldrin
		8.20	0.00081	Heptachlor

CIL/ SAIF Panjab University Chandigarh

Sample Header

Data File:	R-1
Original Data Path:	C:\GCMS-data\YEAR 2015\AUG-15\7
Sample Type:	Unknown
Sample ID:	1
Sample Name:	
Acquisition Date:	08/07/15 11:10:42 AM
Run Time(min):	14.02
Injection Volume(µl):	1.00
Scans:	8410
Low Mass(m/z):	0
High Mass(m/z):	0
Instrument Method:	C:\GCMS-data\instrument method\GENRAL-PTV-ECD.meth

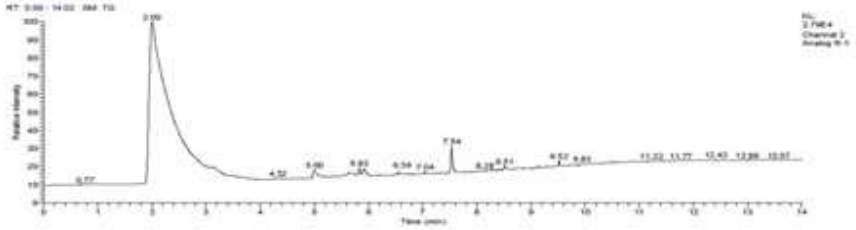

Fig. 2: Gas chromatogram of Asian pear

CIL/ SAIF Panjab University Chandigarh

Sample Header

Data File:	R-3
Original Data Path:	C:\GCMS-data\YEAR 2015\AUG-15\7
Sample Type:	Unknown
Sample ID:	1
Sample Name:	
Acquisition Date:	08/07/15 12:01:42 PM
Run Time(min):	14.02
Injection Volume(µl):	1.00
Scans:	8410
Low Mass(m/z):	0
High Mass(m/z):	0
Instrument Method:	C:\GCMS-data\instrument method\GENRAL-PTV-ECD.meth

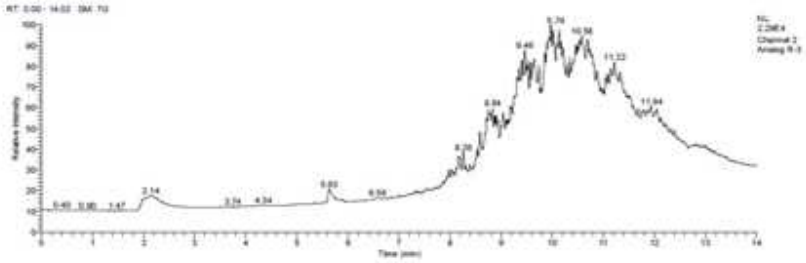

Fig. 3: Gas chromatogram of Kiwi

CIL/ SAIF Panjab University Chandigarh

Sample Header

Data File:	P-5
Original Data Path:	C:\GCMS-data\YEAR 2015\SEP-15\03
Sample Type:	Unknown
Sample ID:	1
Sample Name:	
Acquisition Date:	09/03/15 01:12:56 PM
Run Time(min):	20.52
Injection Volume(µl):	1.00
Scans:	12310
Low Mass(m/z):	0
High Mass(m/z):	0
Instrument Method:	C:\GCMS-data\instrument method\GENRAL-PTV-ECD.meth

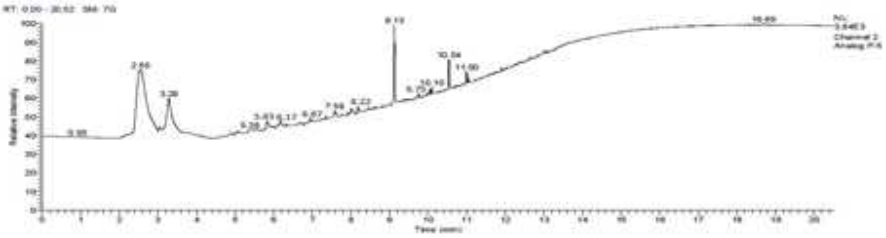

Fig. 4: Gas chromatogram of Ridge gourd

CIL/ SAIF Panjab University Chandigarh

Sample Header

Data File:	R-2
Original Data Path:	C:\GCMS-data\YEAR 2015\AUG-15\7
Sample Type:	Unknown
Sample ID:	1
Sample Name:	
Acquisition Date:	08/07/15 11:45:05 AM
Run Time(min):	14.02
Injection Volume(µl):	1.00
Scans:	8410
Low Mass(m/z):	0
High Mass(m/z):	0
Instrument Method:	C:\GCMS-data\instrument method\GENRAL-PTV-ECD.meth

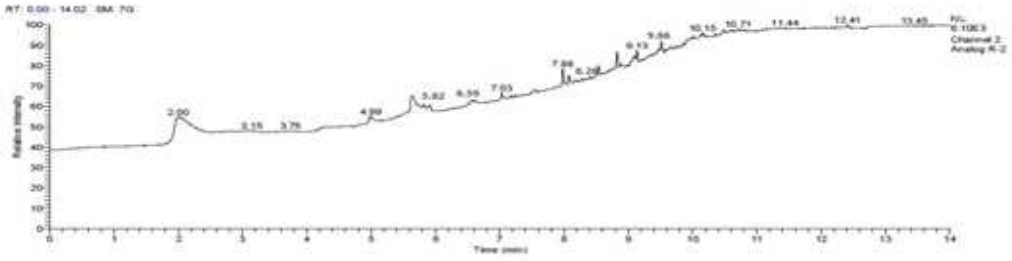

Fig. 5: Gas chromatogram of Cauliflower

CIL/ SAIF Panjab University Chandigarh

Sample Header

Data File:	R-4
Original Data Path:	C:\GCMS-data\YEAR 2015\AUG-15\7
Sample Type:	Unknown
Sample ID:	1
Sample Name:	
Acquisition Date:	08/07/15 12:19:42 PM
Run Time(min):	14.02
Injection Volume(µl):	1.00
Scans:	8410
Low Mass(m/z):	0
High Mass(m/z):	0
Instrument Method:	C:\GCMS-data\instrument method\GENRAL-PTV-ECD.meth

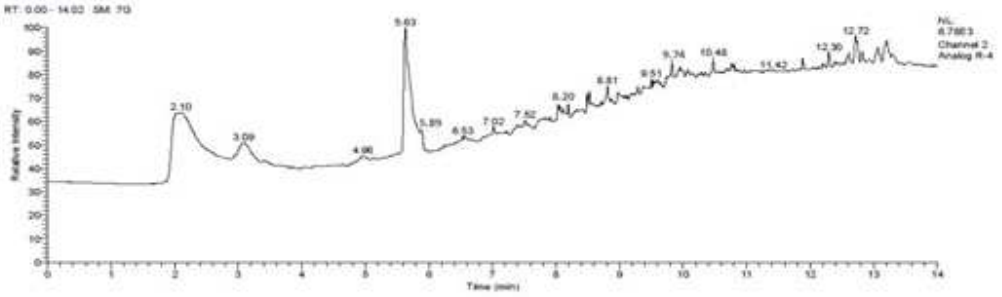

Fig. 6: Gas chromatogram of Bitter gourd

In the chromatogram of Ridge gourd (Fig. 4) four peaks resembles out of various peaks at Rt values 5.83, 6.67, 8.22 and 9.75 was very close to the Rt value of β- BHC, δ-BHC, heptachlor and aldrin respectively which indicated the presence of above pesticides in the sample of Ridge gourd.

In the Chromatogram of Cauliflower (Fig. 5) a number of peaks has been noticed out of which four peaks at Rt values 5.82, 6.59, 8.28 and 9.66 was very near to the Rt values of β- BHC, δ-BHC, heptachlor and aldrin respectively which suggested the presence of above pesticides in the sample of Cauliflower.

The chromatogram of Bitter gourd (Fig. 6) three peaks at Rt values 5.89, 8.20 and 9.74 was very near to the Rt values of β- BHC, heptachlor and aldrin respectively which indicate the presence of above pesticides in the sample of Bitter gourd.

All the detected organochlorine pesticides and their concentration levels have been reported in Table. 1.

In the present monitoring study all the fruits and vegetables samples were contaminated either two or four organochlorine pesticides. Although, the residual concentrations were far below the MRL values prescribe by Codex Alimentarius 2016 but their continuous consumption of such fruits and vegetables may cause severe diseases like cancer, neurogenetic disorder, limb defects e.t.c.

Acknowledgments
We are also thankful to Head, Department of Chemistry, I.B.S., Khandari Campus, DR. B.R.A.U., Agra for providing necessary facilities to carried out this research work. We thanks to Central Instrumentation Laboratory (CIL), Panjab University, Chandigarh for providing Gas Chromatographic studies of samples.

REFERENCES

[1] IC Yadav; NL Devi; JH Syed; Z Cheng; J Li; G Zhang; KC Jones. *Sci. Total Environ.*, **2015**, 511, 123–137.
[2] LB Abdulra'uf; GH Tan. *Food Chem.*, **2015**, 177, 267–273.
[3] PK Gupta. *Toxicology*, **2004**, 198, 83-90.
[4] Planning Commission of India, New Delhi. Government of India, Tenth five-year plan **2002- 2007**, 513–566.

[5] S Kumar; AK Sharma; SS Rawat; DK Jain; S Ghosh. *Asian J. Environ. Sci.*, **2013**, 8(1), 51- 57.
[6] A Johri; A Dhawan; RL Singh; D Pharmar. *Toxicol. Sci.*, **2008**, 101(2), 331-340.
[7] F Kamel; AS Rowland; LP Park; WK Anger; DD Baird; BC Gladen; T Moreno; L Stallone; DP Sandler. *Environ. Health Perspect*., **2003**, 111(14), 1765-1772.
[8] JA Firestone; T Smith-Weller; G Franklin; P Swanson; WT Longstreth; H Checkoway. *Arch. Neurol*., **2005**, 62(1), 91-95.
[9] JL Daniels; AF Olshan; DA Savitz. *Environ. Health Perspect*., **1997**, 105(10), 1068-1077.
[10] LS Engel; ESO'Meara; SM Schwartz. *Scand. J. Work Environ. Health*, **2000**, 26(3), 193- 198.
[11] A Agarwal; R Prajapati; OP Singh; SK Raza; LK Thakur. *Environ. Monit. Assess.*, **2015**, 187(2), 54.
[12] D Kumar; P Chauhan; Neelam. *Res. J. Agric. Sci*., **2011**, 2(3), 528-531.
[13] D Kumar; RC Sharma; P Chauhan. *Res. J. Agric. Sci.*, **2011**, 2(1), 40-43.
[14] D Kumar; A Kashyap. *J. Chem. Pharm. Res.*, **2014**, 6(6), 751-755.
[15] I Mukherjee; S Singh; PK Sharma; M Jaya; M Gopal; G Kulshrsestha. *Bull. Environ. Contam. Toxicol*., **2007**, 78, 380-383.
[16] M Bhanti; A Taneja. *Chemosphere*, **2007**, 69(1), 63-68.