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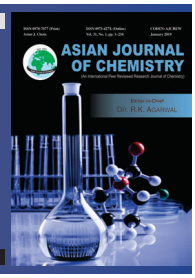

Monitoring of Organochlorine Pesticides Residues in Five Fruits using Gas Liquid Chromatography Equipped with Electron Capture Detector (GLC-ECD)

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India is the largest producer of fruits as they are important part of their economy. But they are badly affected by insect-pest attack during harvesting. Farmers use a large amount of pesticides to protect them but due to their longer persistence they found in fruits in small quantities. This paper described an analytical methodology for the monitoring of 20 organochlorine pesticides in 5 fruits *viz*. pineapple (*Ananas comosus*), apple (*Malus pumila*), plum (*Prunus domestica*), papaya (*Carica papaya*) and mango (*Mangifera indica*) by using gas liquid chromatography equipped with electron capture detector (GLC-ECD). During the monitoring work it has been found that each fruit was contaminated with pesticides. Pineapple was found contaminated with β-BHC, δ-BHC, heptachlor epoxide, dieldrin, endrin and methoxychlor; apple was found contaminated γ-BHC, δ-BHC, heptachlor epoxide, dieldrin, endrin, endosulfan II and methoxychlor; plum and papaya were found contaminated with γ-BHC, β-BHC, δ-BHC, heptachlor epoxide, 4,4′-DDE, endrin, endosulfan sulfate and methoxychlor; mango was found contaminated with β-BHC, δ-BHC, 4,4′-DDE, endrin, endosulfan sulfate and methoxychlor. However, the detected concentration of pesticides was below the MRL values but their long term use can cause serious health problems.

Keywords: Organochlorine, Pesticides, Fruits.

INTRODUCTION

Fruits are important components of the human diet since they provide essential nutrients, like vitamins and minerals that can help to keep us healthy [1]. In India estimated that per capita fruits availability is around 200.6 g per day which is below the recommended quantity 230 g per capita per day [2]. To protect the fruits from the insect-pest attack and to increase the yield, generally, farmers used large amount of chemical pesticides. The worldwide consumption of pesticides is about 2 million tons per year out of which 45 % is used by Europe alone, 25 % is consumed in the USA and 25% in the rest of the world. India′s share is just 3.75 %. The usage of pesticides [3] in India is only 0.5 kg/ha while in case of Korea and Japan it is 6.6 and 12.0 kg/ha, respectively. Globally, the pesticides [4] cover only 25 % of the cultivated land area. Amongst the pesticides (total), HCH (only γ-HCH is allowed), DDT are most commonly used pesticides which account for 70 % of the consumption of pesticides. These pesticides remain the choice of small farmers because they are cost-effective, easily available and display a wide spectrum of bioactivity [4]. The excessive use of pesticides is of major concern [5]. Pesticides can be acutely toxic and their contamination is a worldwide public health concern and main international trade problem [6]. They can cause harmful or lethal effects after one single episode of ingestion, inhalation or skin contact. The symptoms are evident shortly after exposure or can arise within 48 h [7]. Pesticides can cause respiratory tract irritation [8], sore throat and/or cough allergic [9] sensitization, eye and skin irritation [10], nausea [8], vomiting [11], diarrhea [10], headache [12], loss of consciousness [13], extreme weakness [14], death [15]. Several studies [16-18] on organochlorine pesticides in different matrices including food commodities have been reported, which indicated that exposure of organochlorine pesticides (OCPs) has been associated with human health risk of gestational diabetes [19], insulin resistance [20], diarrhea [21], skin conditions [22], reproductive problem [23], depression [24] and cancer [25]. Keeping in view of the above adverse effects on human health it has been decided to monitor the pesticide residue concentration in different fruits. In continuation of our previous work

[26,27] in this study, we have monitored 20 organochlorine pesticides in pineapple (*Ananas comosus*), apple (*Malus pumila*), plum (*Prunus domestica*), papaya (*Carica papaya*) and mango (*Mangifera indica*).

EXPERIMENTAL

All glassware was thoroughly washed by deionized water and then rinsed with acetone and dried in oven (150 ºC) for overnight before use. Solvents like acetone, acetonitrile, ethyl acetate, dichloromethane and hexane were distilled before use. Adsorbents neutral silica gel and charcoal were activated before use. The extracted and purified samples of fruits were analyzed by gas liquid chromatography equipped with capillary columns using 63Ni electron capture detector (GLC-ECD). Minor equipment such as rotary evaporator, mechanical shaker and waring blender, *etc*. were also used during extraction. A 2.0 µL solution of standard was injected to record the chromatogram of pesticides.

Extraction of pesticides in fruits

Collection and sample preparation: The samples consisted of 250 g of each fruits, *i.e*. pineapple, papaya, apple, plum and mango were collected from local market. Each sample was refrigerated at 5 ºC and analyzed within few days of collection. In order to assess the right concentration of pesticides reaching within human body, household processing like washing, peeling off covering, *etc*. were carried out. Each fruit was washed for few minutes under tap water and dried with filter paper. After drying, each fruit was chopped into small pieces and a representative sample (250 g) was macerated with 25 g anhydrous sodium sulfate in waring blender to make a fine paste.

Extraction for pineapple (*Ananas comosus*): A macerated sample of pineapple (50 g) was extracted with 100 mL dichloromethane by using mechanical shaker for 2 h. The extract was passed from the layer of sodium sulfate by using funnel. The filtrate was concentrated under vacuum up to 30-40 mL and then transferred to a 500 mL separating funnel. Then aqueous solution of NaCl (10 %, 50 mL) was added and shaken gently for 2 h. The extract was exchanged into ethyl acetate (3 × 50 mL) by liquid-liquid partitioning. The organic layer was separated out and again passed through a layer of sodium sulfate (5 g). The filtrate was evaporated up to dryness (2-5 mL) in rotary evaporator and dissolved in 10 mL hexane.

Extraction for apple (*Malus pumila*), plum (*Prunus domestica*) and papaya (*Carica papaya*): A fine paste of each fruit (50 g) was taken and subjected to extraction with 100 mL acetone (3 × 100 mL). The extract was filter with the help of Buchner funnel. The filtrate was concentrated under vacuum upto 5 mL and then transferred to a 500 mL separating funnel. 50 mL saline water (2 %, w/v) was added to it and shake for 50 min. The extract was exchanged in to 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). The extract was evaporated to dryness (2-5 mL) by using rotary evaporator. The concentrated extract was redissolved in 10 mL of *n*-hexane.

Extraction for mango (*Mangifera indica*): A fine paste (50 g) was subjected for extraction with 50 mL acetonitrile-ethyl acetate-hexane (8:1:1 v/v) in a waring blender. The extract was collected by filtration with Buchner funnel. The fruit residue was again subjected for extraction with two times. The collected extract was evaporated under vacuum upto about 5 mL and transferred to the separating funnel (500 mL). 50 ml saline water (2 % w/v) was added and shaken for 30 min. The extract was exchanged into dichloromethane layer by liquid-liquid partioning (3 × 50 mL). The extract was passed through a layer of sodium sulfate (5 g) and evaporated to dryness in rotary evaporator. The concentrated extract was dissolved in 10 mL of hexane-acetone (9:1).

Purification: The collected extracts were subjected for clean up by column packed with silica gel:activated charcoal (5:1 w/w)/silica gel. Each extract was eluted with 50 mL of *n*-hexane then subjected to GC-ECD for analysis of pesticides.

RESULTS AND DISCUSSION

First by running chromatogram of standard of pesticides chosen for monitoring work, a retention time of the peaks for pesticides and their peak areas corresponding to 2.0 µg/µL concentration was determined (Table-1).

TABLE-1
RETENTION TIME AND PEAK AREA OF ORGANOCHLORINE PESTICIDES OF STANDARD

Peak	Pesticides	Ret. time	Area	Area (%)
1	α-BHC	8.481	747866	0.3123
2	γ-BHC	9.775	178423	0.0745
3	β-BHC	12.041	17788716	7.4293
4	δ-BHC	13.533	23197482	9.6882
5	Heptachlor	15.042	13099995	5.4711
6	Aldrin	16.753	15246076	6.3674
7	Heptachlor epoxide	18.379	15052379	6.2865
8	γ-Chlordane	20.223	13459524	5.6212
9	α-Chlordane	21.342	14431111	6.0270
10	Endosulfan I	21.925	25859986	10.8001
11	4,4'-DDE	23.068	6975083	2.9131
12	Dieldrin	23.154	18779776	7.8432
13	Endrin	24.095	13244260	5.5313
14	4,4'-DDD	24.667	12228021	5.1069
15	Endosulfan II	25.065	4210779	1.7586
16	Endrin aldehyde	25.353	8823023	3.6848
17	4,4'-DDT	26.463	10972013	4.5823
18	Endosulfan sulfate	26.718	8989607	3.7544
19	Methoxychlor	28.545	12990256	5.4252
20	Endrin ketone	29.325	2963671	1.2377

In the chromatogram of standard (Fig. 1), the peaks of different isomers of benzene hexachloride (BHC) were found at R_t values 8.481, 9.775, 12.041 and 13.533 which correspond to α-BHC, γ-BHC, β-BHC and δ-BHC, respectively. The peaks of heptachlor and heptachlor epoxide were found at R_t value 15.042 and 18.379, respectively. The peak at R_t value 16.753 was found for aldrin. The peak of γ-chlordane and α-chlordane were found at R_t value 20.223 and 21.342. The peaks at R_t value 21.925, 25.065 and 26.718 were found for endosulfan I, endosulfan II and endosulfan sulfate, respectively. The R_t value of the peak of dieldrin was found at 23.154. The peaks

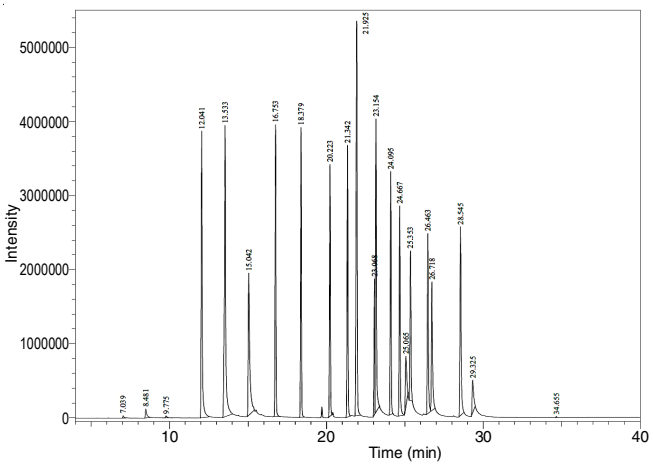

Fig. 1. Gas chromatogram of standards

at R_t values 24.095, 25.353 and 29.325 correspond to endrin, endrin aldehyde and endrin ketone, respectively. The peaks of 4,4′-DDE, 4,4′-DDD and 4,4′-DDT were found at R_t values 23.068, 24.667 and 26.463. The peak at R_t value 28.545 was found for methoxychlor.

During the monitoring work chromatogram of pineapple (Fig. 2) exhibited number of peaks from those six peaks at the R_t value 12.024, 13.522, 18.372, 23.139, 24.083 and 28.545 resemble with the R_t values of β-BHC, δ-BHC, heptachlor epoxide, dieldrin, endrin and methoxychlor respectively which indicated the presence of β-BHC, δ-BHC, heptachlor epoxide, dieldrin, endrin and methoxychlor in pineapple.

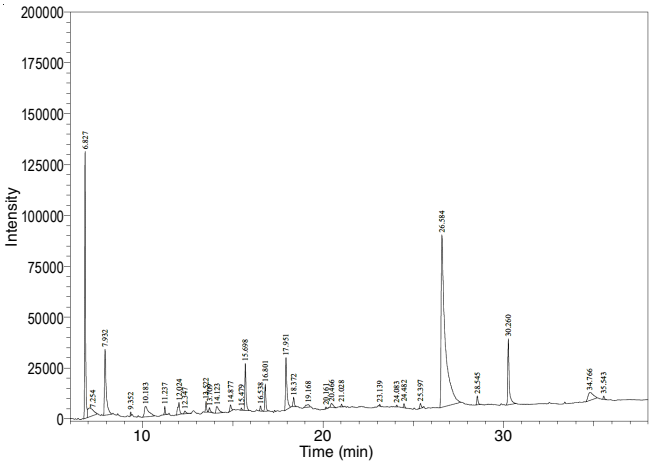

Fig. 2. Gas chromatogram of pineapple

The chromatogram of apple (Fig. 3) exhibited number of peaks from those seven peaks at R_t value 9.771, 13.523, 18.374, 23.135, 24.081, 25.046 and 28.546 were very near to the R_t value of γ-BHC, δ-BHC, heptachlor epoxide, dieldrin, endrin, endosulfan II and methoxychlor respectively which indicated the presence of γ-BHC, δ-BHC, heptachlor epoxide, dieldrin, endrin, endosulfan II and methoxychlor pesticides in apple.

In the chromatogram of plum (Fig. 4) eight peaks at R_t value 9.765, 12.023, 13.520, 18.361, 23.067, 24.080, 26.725 and 28.542 were very close to the R_t value of γ-BHC, β-BHC, δ-BHC, heptachlor epoxide, 4,4′-DDE, endrin, endosulfan sulfate and methoxychlor respectively which indicated the presence of above pesticides in plum.

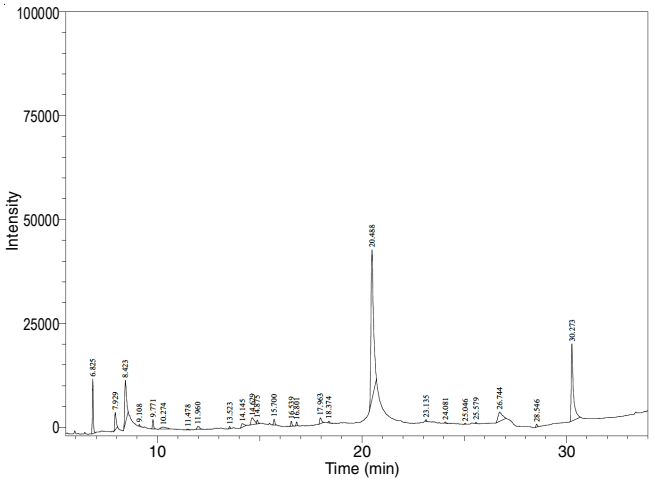

Fig. 3. Gas chromatogram of apple

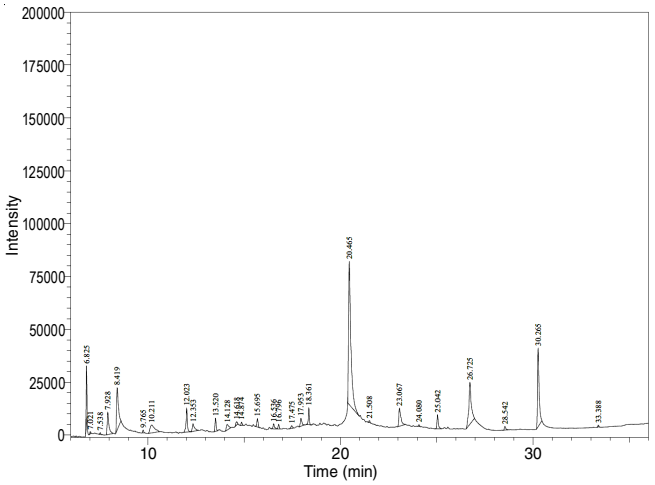

Fig. 4. Gas chromatogram of plum

The chromatogram of papaya (Fig. 5) exhibited a number of peaks from those eight peaks at the R_t value 9.765, 12.021, 13.518, 18.361, 23.060, 24.079, 26.707 and 28.544 resemble with the R_t value of γ-BHC, β-BHC, δ-BHC, heptachlor epoxide, 4,4′-DDE, endrin, endosulfan sulfate and methoxychlor respectively, which indicated the presence of γ-BHC, β-BHC, δ-BHC, heptachlor epoxide, 4,4′-DDE, endrin, endosulfan sulfate and methoxychlor pesticide in papaya.

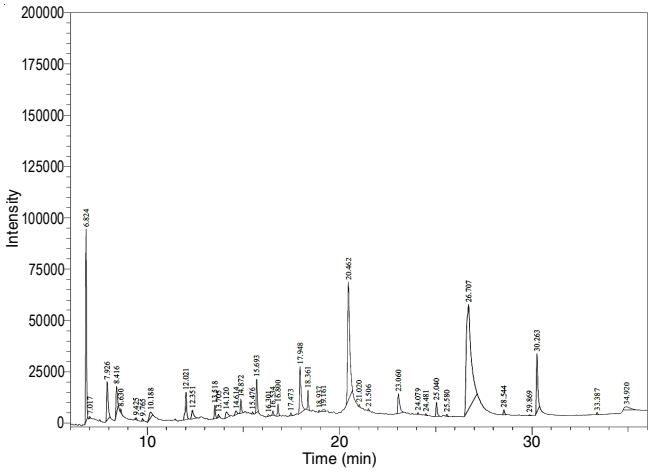

Fig. 5. Gas chromatogram of papaya

In the chromatogram of mango (Fig. 6) six peaks at R_t value 12.022, 13.518, 23.055, 24.078, 26.706 and 28.540 were very near to the R_t value of β-BHC, δ-BHC, 4,4′-DDE, endrin, endosulfan sulfate and methoxychlor respectively, which indicated the presence of above pesticides in mango. The concentrations of detected pesticides have been reported in Table-2.

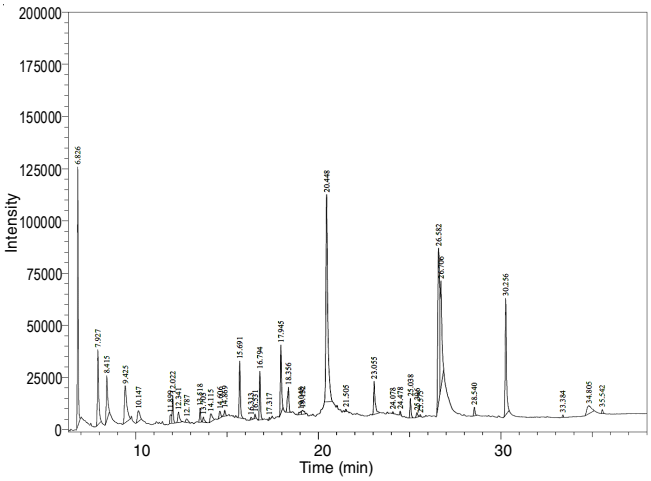

Fig. 6. Gas chromatogram of mango

TABLE-2
CONCENTRATION OF PESTICIDES IN FRUITS

Name of sample	RT value	Area of peak	Conc. of pesticides (µg)	Name of the pesticides
Pineapple	12.024	42780	0.00096	β-BHC
	13.522	21351	0.00036	δ-BHC
	18.372	22865	0.00060	Heptachlor epoxide
	23.139	2861	0.00006	Dieldrin
	24.083	3023	0.00009	Endrin
	28.545	21199	0.00065	Methoxychlor
Apple	9.771	8345	0.01870	γ-BHC
	13.523	2184	0.00003	δ-BHC
	18.374	1909	0.00005	Heptachlor epoxide
	23.135	1632	0.00003	Dieldrin
	24.081	1166	0.00003	Endrin
	25.046	998	0.00009	Endosulfan II
	28.546	3187	0.00009	Methoxychlor
Plum	9.765	5270	0.01181	γ-BHC
	12.023	54455	0.00122	β-BHC
	13.520	25470	0.00043	δ-BHC
	18.361	28926	0.00076	Heptachlor epoxide
	23.067	63036	0.00361	4,4'- DDE
	24.080	2330	0.00007	Endrin
	26.725	172138	0.00765	Endosulfan sulfate
	28.542	8742	0.00026	Methoxychlor
Papaya	9.765	4697	0.01053	γ-BHC
	12.021	67223	0.00151	β-BHC
	13.518	27315	0.00047	δ-BHC
	18.361	34347	0.00091	Heptachlor epoxide
	23.060	65544	0.00375	4,4'-DDE
	24.079	2995	0.00009	Endrin
	26.707	865959	0.03853	Endosulfan sulfate
	28.544	12652	0.00038	Methoxychlor
Mango	12.022	53251	0.00119	β-BHC
	13.518	26612	0.00045	δ-BHC
	23.055	90543	0.00519	4,4'-DDE
	24.078	3097	0.00009	Endrin
	26.706	346059	0.01539	Endosulfan sulfate
	28.540	19238	0.00059	Methoxychlor

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. C. Kaur and H.C. Kapoor, *Int. J. Food Sci. Technol.*, **36**, 703 (2001); https://doi.org/10.1046/j.1365-2621.2001.00513.x.
2. Ministry of Agriculture & Farmer Welfare, Horticulture Statistics at a Glance, Government of India (2017).
3. P.I. Devi, J. Thomas and R.K. Raju, *Agric. Econ. Res. Rev.*, **30**, 163 (2017).
4. R.M. Burkul, S.V. Ranade and B.L. Pangarkar, *Int. J. Emerg. Trend Eng. Basic Sci.*, **2**, 88 (2015).
5. I. Mahmood, S.R. Imadi, K. Shazadi, A. Gul and K.R. Hakeem, Effects of Pesticides on Environment, In: Plant, Soil and Microbes: Vol. 1 Implications in Crop Science, Springer International Publishing: Switzerland, p. 253 (2016).
6. S. Chandra, N.A. Mahindrakar, M. Kumar and P.L. Shinde, *Int. J. Adv. Res.*, **2**, 1075 (2014).
7. http://www.pan-uk.org/health-effects-of-pesticides/ (visited on 15/02/2019).
8. S.M. Bradberry, S.A. Cage, A.T. Proudfoot and J.A. Vale, *Toxicol. Rev.*, **24**, 93 (2005); https://doi.org/10.2165/00139709-200524020-00003.
9. C.L. Beseler and L. Stallones, *J. Agric. Saf. Health*, **15**, 327 (2009); https://doi.org/10.13031/2013.28887.
10. R. Sapbamrer and S. Nata, *Environ. Health Prev. Med.*, **19**, 12 (2014); https://doi.org/10.1007/s12199-013-0349-3.
11. A.A. Adewunmi and S.O. Fapohunda, *Eur. J. Biol. Res.*, **8**, 70 (2018); https://doi.org/10.5281/zenodo.1237542.
12. N. Tarannum, M. Singh and R. Hawaldar, Pesticides as an Occupational Hazard Facts and Figures, In: Handbook of Research on the Adverse Effects of Pesticide Pollution in Aquatic Ecosystems, IGI Global, p. 201 (2019).
13. M.C. Keifer and J. Firestone, *J. Agromed.*, **12**, 17 (2007); https://doi.org/10.1300/J096v12n01_03.
14. A.K. Srivastava and C. Kesavachandran, Health Effects of Pesticides, CRC Press (2019).
15. J.E. Jäger, Residues of Pesticides, In: Chemical Hazards in Foods of Animal Origin, Academic Publishers: Wageningen, p. 567 (2019).
16. D.P. Mukherjee, B. Kumar, S. Kumar, M. Mishra, R. Gaur, D. Prakash, S.K. Singh and C.S. Sharma, *Arch. Appl. Sci. Res.*, **3**, 85 (2011).
17. R.K. Kole, H. Banerjee and A. Bhattacharyya, *Bull. Environ. Contam. Toxicol.*, **67**, 554 (2001); https://doi.org/10.1007/s001280159.
18. L. Guzzella, C. Roscioli, L. Viganò, M. Saha, S.K. Sarkar and A. Bhattacharya, *Environ. Int.*, **31**, 523 (2005); https://doi.org/10.1016/j.envint.2004.10.014.
19. L. Saunders, P. Kadhel, N. Costet, F. Rouget, C. Monfort, J.P. Thome, L. Guldner, S. Cordier and L. Multigner, *Environ. Int.*, **68**, 171 (2014); https://doi.org/10.1016/j.envint.2014.03.024.
20. K.-S. Kim, Y.-M. Lee, S.G. Kim, I.-K. Lee, H.-J. Lee, J.-H. Kim, J. Kim, H.-B. Moon, D.R. Jacobs Jr. and D.-H. Lee, *Chemosphere*, **94**, 151 (2014); https://doi.org/10.1016/j.chemosphere.2013.09.066.
21. K.P. Singh, A. Malik, D. Mohan and S. Sinha, *Bull. Environ. Contam. Toxicol.*, **74**, 162 (2005); https://doi.org/10.1007/s00128-004-0563-1.
22. T. Vial, B. Nicolas and J. Descotes, *J. Toxicol. Environ. Health*, **48**, 215 (1996); https://doi.org/10.1080/009841096161294.
23. S.H. Safe, *Environ. Health Perspect.*, **103**, 346 (1995); https://doi.org/10.1289/ehp.95103346.
24. C. Beseler, L. Stallones, J.A. Hoppin, M.C. Alavanja, A. Blair, T. Keefe and F. Kamel, *J. Occup. Environ. Med.*, **48**, 1005 (2006); https://doi.org/10.1097/01.jom.0000235938.70212.dd.
25. E. Sohail, A. Waseem, W.L. Chae, J.L. Jong and H. Imitiaz, *J. Clin. Exp. Oncol.*, **26**, 98 (2004).
26. D. Kumar and A. Kashyap, *J. Chem. Pharm. Res.*, **6**, 751 (2014).
27. D. Kumar and P. Kulshrestha, *J. Chem. Pharm. Res.*, **8**, 539 (2016).