International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Impact Factor (2012): 3.358

Sensitive Spectrophotometric Determination of Chlorpyrifos in Different Environmental and Biological Samples

Vindhiya Patel[1], Manish Kumar Rai[2a*], Prashant Mundeja[3], Joyce Rai[4]

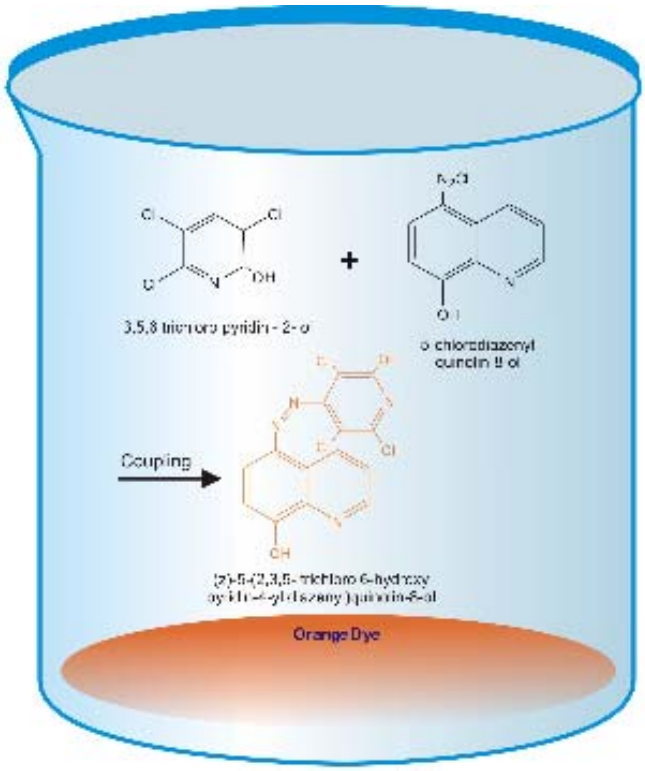
[1, 3]Research Scholar, School of Studies in Chemistry, Pt. Ravishankar Shukla University Raipur, (Chhattisgarh), 4912010, India

[2]Associate Professor, School of Studies in Chemistry, Pt. Ravishankar Shukla University Raipur, (Chhattisgarh), 4912010, India

[4]Chhattisgarh Council of Science and Technology, MIG 25, Indrāvati Colony, Raipur (Chhattisgarh), 492007, India

Abstract: ***A highly sensitive spectrophotometric method was developed for the determination of organophosphorous insecticide, chlorpyrifos, which is widely used. This method is carried out on the basis of alkaline hydrolysis of chlorpyrifos to 1, 2, 4-trichloropyridine (TCP), followed by coupling with diazotized 2-amino-8-hydroxy quinoline in alkaline medium. The absorption maxima of the orange dye formed was measured at 420 nm. Beer's law was obeyed over the concentration range of 1 µg to 8 µg (0.01-0.08 mgL^{-1}) in a final solution of 10 mL. The molar absorptivity, Sandell's sensitivity and Correlation coefficient was found to be 9.04 x 10^{9} mol^{-1} cm^{-1}, 0.010 µg cm^{-2} and 0.897 respectively. The Standard deviation and Relative standard deviation are 0.00811 and 2.45%.The method was simple, cheap, sensitive and selective. The method was satisfactorily applied to the determination of chlorpyrifos in various environmental and biological samples.***

Keywords: Spectrophotometry, chlorpyrifos, diazotization, environmental and biological samples



This method is based on alkaline hydrolysis of chlorpyrifos a popular pesticide which is coupled with diazotized 2-amino - 8- hydroxyl quinoline to get orange dye, which is studied spectrophotometrically at 420nm.

1. Introduction

The massive use of pesticide had attracted attention of world toward their toxicity, persistence, and potential adverse effects on the ecosystem even if present at very low concentration levels.[1] Chlorpyrifos (O, O-diethyl O-3, 5, 6-trichloropyridin-2-yl phosphorothioate) is one of the widely used organophosphate insecticides. Recent studies show that this organophosphate chemical is consistently present in air, rain and surface water.[2] Most of the chlorpyrifos products are used in the kitchen, bathroom, and agricultural field still though it is banned for residential use.[3] Chorpyrifos is a insecticide also used for soil treatment as pre-planting and planting course and seed treatment as a foliar spray and dormant spray. Chorpyrifos has been registered in India in 1968 under insecticides Act of India for regular use in the country.[4] Investigations shows that chlorpyrifos affects the human nervous system inhibits the cholinesterase enzyme , chemist studied their use as nerve gases.[5] It persists in soil for 60–120 days and degrades there primarily through microbial action.[6] The common degradation pathway for chlorpyrifos involved the formation of TCP has half life from 65 to 360 days.[7] It is estimated that 20-50% of crops are saved from infestation through the use of pesticides and more than 4% of fruits and vegetables imported exceed concentration levels considered safe for human consumption.[8] The persistent use of chlorpyrifos has led to widespread contamination of water and soils, resulting in serious damage to non-target species .[9-10] Chloropyrifos was spiked into sterilized soil and aged in microcosms for up to 120days.[6] However, the amount of pesticides used must be closely regulated to ensure public safety, since the most common type, the organophosphates consequently pose a threat to human health.[11] Chlorpyrifos is directly toxic to the nervous system. In addition, it is transformed inside animals to chlopyrifos-Oxon, which is potent cholinesterase inhibitor.[12] In pregnant laboratory animals, chlorpyrifos exposure caused fetal death.[13]A study reported an association between umbilical cord plasma chlorpyrifos levels and fetal birth weight decrease among minority women living in new York city during pregnancy also their result indicated that prental chlorpyrifos

exposure have impaired fetal growth.[14] They inhibit an enzyme, acteylcholinesterase (AChE), that breaks down acetylcholine, chemical involves in transmitting nerve impulses across the junctions between nerves. Without functioning AChE, acetylcholine accumulates; producing rapid twitching of involuntary muscles convulsions, paralysis, and ultimately death.[3] Symptoms of acute exposure to organophosphate or cholinesterase-inhibiting compounds may include the following: numbness, tingling sensations, incoordination, headache, dizziness, tremor, nausea, abdominal cramps, sweating, blurred vision, difficulty in breathing or respiratory depression and slow heartbeat. Very high doses may result in unconsciousness, incontinence, and convulsions or fatality. There have been reports on the effects of chlorpyrifos on the reproductive and endocrine system.[15] Many advance technology are introduced in estimation of the residue of pesticides in a variety of samples, environmental matrixes by Thin Layer Chromatography, High performance thin layer chromatography, Gas chromatography, High performance liquid chromatography etc.[16-17] But this sophisticated equipment need much care and maintenance which is not possible in every laboratory and is too costly to be in reaches of common man. So some alternative methods using easily available reagents and equipment such as spectrophotometer are on process. And in this queue one more finding is discussed hereby so that everyone can protect oneself from such harmful chemical spread around in the environment.

2. Experimental

2.1 Apparatus

A systronics UV-Vis spectrophotometric model 104 with matched silica cells was used for all spectral measurements. A Systronics pH meter model 335 was used for pH measurements. A Remi C-854/4 clinical centrifuge force of 1850 rpm with fixed swing out rotors was used for centrifugation.

2.2 Reagents

All the reagents used were of Anala. R grade or of the best available quality. Double distilled water was used throughout the procedure. Chlorpyrifos (Excel crop care limited , Mumbai(Maharashtra)): 1 mg mL^{-1} was prepared in double distilled water. Working solutions were prepared by dilution of the stock solution with distilled water. Sodium Hydroxide: 8 mole L^{-1} aqueous solution was used. Sodium nitrite: 2% m / v solution was prepared. 2-amino 8-hydroxy quinoline: 1% in 10 v / v hydrochloric acid was prepared. Diazotized 2-amino 8-hydroxy quinoline: to 10 mL 2-amino -8-hydroxy quinoline, 1 mL of 2% sodium nitrite was added and the solution was kept in a brown bottle. This remained stable for 4 hr when kept at 0-5 ^{o}C.

2.3 Preparation of Analytical Curve

An aliquot of test solution containing 1 to 8 µg in 10 mL of chlorpyrifos was taken in a 25 mL graduated tube and 10 mL of 8 mole L^{-1} sodium hydroxide was added to it. The solution was then heated for 3 minutes for complete hydrolysis. Then, 1 mL of diazotized 2-amino- 8-hydroxy quinoline was added and shaken thoroughly and kept at 0-5 ^{o}C for 10 minutes for full color development and orange color was obtained (Scheme 1). The solution was then diluted to the 10 mL with water and absorbance was measured at 420 nm against a reagent blank.

2.4 Determination of Chlorpyrifos in polluted water

Water samples from rivers receiving run off from various agricultural fields, where chlorpyrifos were sprayed are collected. Then these samples are filtered through Whatman No. 40 filter paper. Now the water is evaporated to dryness and the residue was dissolved in 10ml of double distilled water. Aliquot of water samples were taken in 25 mL graduated tube, followed by the addition of 10 mL of 8 mole L^{-1}sodium hydroxide and analyzed as described above.

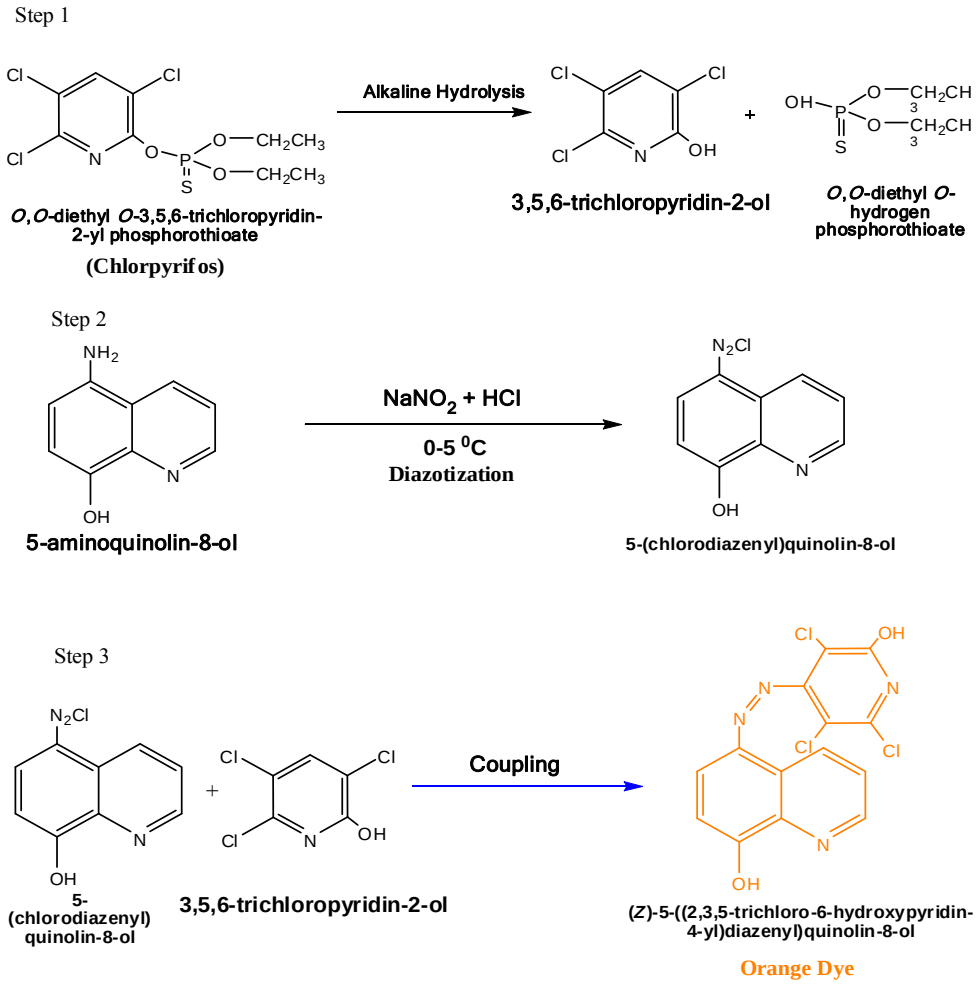
2.5 Determination of Chlorpyrifos in Different Fruit , Vegetables and soil.

Various samples of vegetables, fruits and soil each 5g were collected from agricultural field, where chlorpyrifos had been sprayed as an insecticide. The sample were macerated with 20 mL portions of ethanol:double distilled water(1:1)filtered through a whatman filter paper No. 40 and the filtrate was centrifuged at 1850 rpm for 10 minutes. In case of vegetables and fruits, the filtrate was quantitavely transferred in to 50 mL calibrated flask and made up to the mark with distilled water. (a) 5 milliliter aliquot were taken in a beaker, added 5 mL of 8 Mole L^{-1} sodium hydroxide and heated for 3 minutes at 30-45 ^{o}C Under optimum condition for complete hydrolysis. Then 0.5 mL diazotized 2-amino-8-hydroxy quinoline was added. Shaken thoroughly and kept at 0-5 ^{o}C for 10 minutes for full color development.

2.6 Determination of chlorpyrifos in Biological Samples.

For the determination of chlorpyrifos in various biological samples i.e. urine and blood, this method was applied. Synthetic samples were prepared by adding known amounts of chlorpyrifos to these samples and then deproteination with trichloroacetic acid was done and analyzed after applying the described process. Three replicate analysis were done and given in Table-2.

Reactionmechanism



Scheme 1: Colour reaction. The colour reaction involves the following steps: Coupling of diazotized 2-amino-8-hydroxy quinoline with 1, 2, 4-trichloropyrdine (TCP) to form orange dye showing a λ_{max} at 420 nm.

3. Results and Discussion

3.1 Spectral Characteristics

The orange color dye formed in the proposed reaction shows maximum absorption at 420 nm. All spectral analysis was carried with double distilled water as the reagent blank showed negligible absorption at this wavelength. The color system obeys beer's law in the range of 1µg to 8 µg in a final solution of 10mL at 420 nm. Fig 2 shows absorbance and concentration of chlorpyrifos ranging from 1 to 8 µg per 10 mL. The molar absorptivity, Sandell's sensitivity and correlation coefficient, were found to be 9.04 $\times 10^9$ mol^{-1} cm^{-1}, 0.010 µ g cm^{-2} and 0.979 respectively.

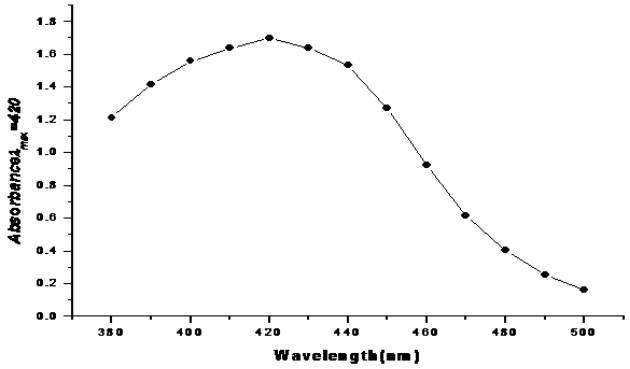


Figure 1: Absorbance curve of chlorpyrifos drawn between wavelength and Absorbance. λ_{max} obtained is 420 nm of concentration 10 µg.

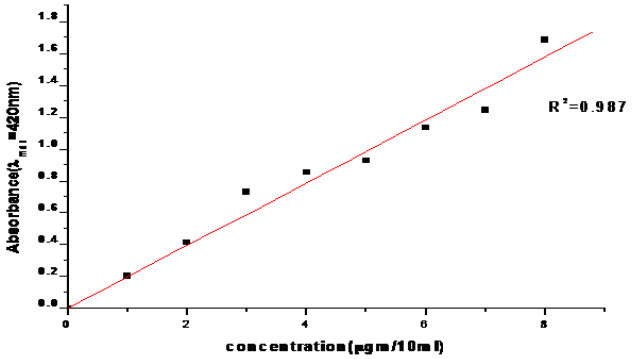


Figure 2: Calibration curve is plotted between absorbance and concentration of chlorpyrifos ranging from 1 to 8 µg per 10 mL.

3.2 Effect of pH

The effect of pH on the color reaction was studied and was found constant absorbance value obtained at a pH range of 10.5- 12 and no buffer solution was needed to stabilize the color. At pH lower and higher than this, the absorbance values decreased. It was observed that about to 10min we required for complete color development and the color remained stable for several days.

3.3 Effect of Temperature

Maximum color intensity was observed when the solution containing orange dye was observed at different temperature. The dye shows maximum absorbance at 45 ^{o}C and then gradually decreases is shown in Fig 3. The color remains at room temperature for 4days.

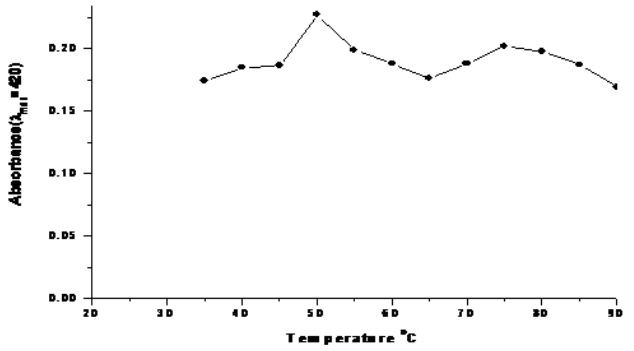


Figure 3: Effect of temperature upon the dye formed for determination of chlorpyrifos is shown with the graph plotted between absorbance and temperature in ^{o}C.

3.4 Effect of foreign Species

The effect of common foreign species and pesticides was studied to assess the validity of the method. Known amount of metal ions, and pesticides were added to the standard 2 µg of chlorpyrifos before hydrolysis and the solution was analyzed by the proposed method. The method was found to be free from interferences of most of the foreign species and pesticide (Table-1).

Table 1: Effect of Foreign Species i.e metal ion, and pesticide (Concentration of chlorpyrifos 2 µg in 10 mL)

Foreign	*Tolerance*
Cypermethrin	500
Paraquat	250
Dichlorvos	500
Glyphosphate	300
Zn^{2+}	400
Cu^{2+}	250
Pb^{2+}	500
Fe^{2+}	300

*The amount causing an error of ±2% in absorbance value.

3.5 Precision

The precision of the method was checked by determining 2 µg of chlorpyrifos in 10 mL of final solution over a period of 7days. The standard deviation and relative standard deviation of absorbance values were 0.0811 and 2.45% respectively.

3.6 Application

The proposed method was applied satisfactorily, for the determination of chlorpyrifos in various samples of polluted water, vegetables, fruits and biological fluids. The amount of chlorpyrifos found in various matrix i.e. water, rice, soil, spinach, brinjal, coriander, orange, were 1.25-1.54 µg in 5g, 3.64-3.79 µg in 5 g, 2.23-2.48 µg in 5g, 3.81-4.02 in 5g collected from field using chlorpyrifos, 4.62-4.87 µg in 5 g, 3.15-3.52 µg in 5g, 2.10-2.38 µg in 5g, 1.01-1.27 µg in 5 g purchased from market respectively.(Table-2) and (Table-3).

Table 2: Chlorpyrifos,in the biological samples (urine and blood)

Samples		*Amount of Chlorpyrifos added(µg)*		*Chlorpyrifos Found** (µg)*		*Recovery %*	
		X	*Y*	*X*	*Y*	*X*	*Y*
Urine	A	1	1	0.94	0.96	94	96
	B	2	2	1.68	1.83	84	91
Blood	A	1	1	0.91	0.93	91	93
	B	2	2	1.83	1.88	91.5	94

X,Y= Samples added .
*Mean of three replicate analyses.
**In µg 10 mL^{-1}
*** Amount of biological Samples=5 mL, after treatment as described in procedure section.

Table 3: Recovery of Chlorpyrifos in various environmental and agricultural samples

Samples	*Chlorpyrifos originally found*(µg) A*	*Chlorpyrifos added (µg) solution prepared in 250ml. B*	*Total Chlorpyrifos found by proposed method**** C*	*Difference (d =c-a)*	*Recovery % (d/b x 100)*
Polluted water**	1.25	1	2.17	0.92	92
	1.54	2	3.43	1.89	94
Rice***	3.64	1	4.49	0.85	85
	3.79	2	5.71	1.92	96
Soil***	2.23	1	3.18	0.95	95
	2.48	2	4.34	1.86	93
Spinach***	3.81	1	4.72	0.91	91
	4.02	2	5.96	1.94	96
Brinjal***	4.62	1	5.57	0.95	95
	4.87	2	6.51	1.64	82
Coriander***	3.15	1	4.07	0.92	92
	3.52	2	5.25	1.73	86
Apple***	2.10	1	3.01	0.91	91
	2.38	2	4.25	1.87	93
Orange***	1.01	1	1.97	0.96	96
	1.27	2	3.23	1.96	98

*Mean of three replicate analysis.** Water sample 5 mL; after treatment 5 mL aliquot was analyzed. *** Sample 5 g (Rice, Soil, Palak, Coriander taken from agriculture field and Brinjal, Apple, Orange purchased from market, 5 mL aliquot of sample was analyzed after treatment as described in procedure section.)
**** In µg per 10 mL

Table 4: Comparison with various extractive spectrophotometric methods

Method	*Reference*	*λmax(nm)*	*Beer's Law range detection limit (ppm)*	*Remark*
Anthranilic acid	(19)	450	0.5-8.18	Less sensitive
Congored	(20)	605	0.5-5.7	Toxic reagent
p-amino benzoic acid	(21)	520	0.048-0.72	Less sensitive
2-amino8-hydroxy quinoline (Proposed method)		420	0.01-0.08	Highly sensitive, selective.

4. Conclusion

The present method is sensitive, selective and cheaper spectrophotometric method for determination of chlorpyrifos in various environmental and biological samples requiring no extraction steps and thereby avoiding the toxic organic solvents.

5. Acknowledgements

Authors are thankful to the Head School of Studies in Chemistry Pt. Ravishankar Shukla University and Director General Chhattisgarh council of science and technology, for providing laboratory facilities and research grant.

References

[1] Fasfous, I. I. S. E.; Rehan, S. E.; Dawond, J. N.; *Jordan J. Chem*. 2012, *7*, 203.
[2] Liu, B.; Mc Connell, L. L.; Torrents, A.; *Chemosphere* 2001, *44*, 1315.
[3] Rahman, M. A.; Omar, D.; Islam, M. Z.; Morshed, M.M.; Rashid, K. N. H. B.; *Agric.Biol.J.N.AM*. 2010, *1*, 548.
[4] Kumar, P.; Singh, S. P.; Shrikant, K.; Mudhukar, D.; *Turk. J.Vet. Anim. Sci.* 2011, *35*, 219.
[5] Thompson, T. S.; Treble, R. G.; *Bull. Environ.Contam.Toxicol*. 1996, *5*, 525.
[6] Wu, X. M.; Yu, Y. L.; Li, M.; Long, Y. H.; Fang, H.; Li, S. N.; *J. Soil Sci. Plant Nutr.* 2011, *11*, 44.
[7] Venugopal, N. V. S.; Sumalatha, B.; Bonthula, S. R.; Veeribabu, G.; *Malaysian . j. Anal. Sci.*
[8] 2012, *16*, 180.
[9] Shende, C.; Inscore, F.; Sengupta, A.; Stuart, J.; Farquharson, S.; *Sens. & Instrumen. Food*
[10] *Qual.* 2010, *4*, 101.
[11] Li, X. H.; Jiang, J. D.; Gu, L. F.; Ali, S. W.; He, J.; et.al.; *Int. Biodeterior Biodegrad.* 2008, *62*, 331.
[12] Xu, G. M.; Zheng, W.; Li,Y. Y.; Wang, S. H.; Zhang, J.S.; et.al.; *Int. Biodeterior. Biodegrad*. 2008, *62*, 51.
[13] Betancourt, A. M.; Carr, R. L.; *Toxicological Sci.* 2004, *7*, 63.
[14] Chambers, J. E; Carr, R. L.; *Fund. Appl. Toxicol.* 1993, *21*, 111.
[15] Cox, C.; *J. Pesticide Reform*. 1994, *14*, 15.
[16] Zhao, Q.; Gadagbui, B.; Dourson, M.; *Regulatory Toxicol. Pharmacology* 2005, *42*, 55.
[17] Rawlings, N. C.; Cook, S. J.; Waldbillig, D.; *J. Toxicol. Environ. Health. Part A* 1998, *54*, 21.
[18] Zhang, Q.; Zhu, L.; Han, C.; Wang, J.; Xie, H.; Wang, J.; Sun, S.; *J.Food.Agric.Environ.* 2011, *9*, 659.
[19] Sherma, J.; *J. AOAC Int.* 2001, *84*, 993.
[20] 18.Janghel, E. K.; Rai, J. K.; Rai, M. K.; Gupta, V. K.; *J.Braz.Chem.Soc.* 2007, *18*, 590.
[21] Venugopal, N. V. S.; Sumalatha, B.; Bonthula, S. R.; *Orient. J. Chem.* 2012, *28*, 979 .
[22] Neil, E.; Garrett, H.; Srank, F.; Michael, D.; *Mutation Research* 1986, 168.
[23] Khan, S.; Rai, M. K.; Gupta, V. K.; Janghel, E. K.; Rai, J. K.; *J.Chem.Soc.Pak.* 2007, *29*, 37.

Author Profile

Vindhiya Patel, Research Scholar, Pursuing Ph.D., School of Studies in Chemistry, Pt. Ravishankar Shukla University, Raipur (C.G.), M.Phil. from Dr. C. V. Raman University Bilaspur, (C.G.),M.Sc. from School of Studies in Chemistry, Pt. Ravishankar Shukla University, Raipur, Chhattisgarh, India

Dr. Manish Kumar Rai, Associate Professor, School of Studies in Chemistry, Pt. Ravishankar Shukla University, Raipur (C.G.), Ph.D. from Pt. Ravishankar Shukla University, Raipur, Chhattisgarh, India

Prashant Mundeja, Research Scholar, Pursuing Ph.D., School of Studies in Chemistry, Pt. Ravishankar Shukla University, Raipur, Chhattisgarh, India

Dr. Joyce Rai, Senior Scientist Chhattisgarh Council of Science and Technology, Raipur (Chhattisgarh). Ph.D. from Pt. Ravishankar Shukla University, Raipur, Chhattisgarh, India