Int J Pharm Bio Sci 2015 Oct; 6(4): (B) 744 - 754

Research Article Microbiology

International Journal of Pharma and Bio Sciences

ISSN
0975-6299

rDNA ITS1 SEQUENCE ANALYSIS BASED GENOTOXICITY ASSESSMENT OF DICOFOL BY USING *CULEX QUINQUEFASCIATUS* (DIPTERA: CULICIDAE)

LOVLEEN

Mosquito Cytogenetics Unit, Panjab University, Chandigarh-160014, India Department of Zoology, School of Biotechnology and Biosciences, Lovely Professional University, Phagwara-144411, India

ABSTRACT

Present research work is attributed to mutagenicity assessment of dicofol by using *Culex quinquefasciatus,* exposed to LC_{20} for 24 hours. To achieve present targets, rDNA ITS1 sequence of treated as well as control individuals was amplified by using specific forward and reverse primers with sequences FR 5´-C C T T T G T A C A C A C C G C C C G T-3´ and RP 5´-G T T C A T G T G T C C T G C A G T T C A C-3´. Sequence alignment of treated and control stocks was done by the Clustal W software programme, subsequently it was observed that dicofol induced a total of 334 alterations in rDNA ITS1 sequence, which included 33 deletions, 49 additions, 120 transitions and 132 transversions. Futhermore, dicofol caused maximum deletions and additions of cytosine base.

KEY WORDS: Culex quinquefasciatus dicofol, genotoxicity, rDNA ITS1.

LOVLEEN
Mosquito Cytogenetics Unit, Panjab University, Chandigarh-160014,India Department of Zoology,School of Biotechnology and Biosciences,Lovely Professional University,Phagwara-144411,India.

INTRODUCTION

The environmental pollution caused by the tremendous use of pesticides has become a concentration drawing topic among the scientific community. Although, the extensive use of pesticides has enhanced the economic potential in terms of increased production of food and amelioration of vector borne diseases, yet they have resulted in serious health implications such as immune suppression, hormone disruption, diminished intelligence, reproductive abnormalities and cancer[1-2]. Pesticide formulations are generally made up from complex mixtures of different types of chemicals which include active and inert ingredients. Although, inert ingredients are generally added for the stability of pesticide formulation and have no pesticidal activity, but such components may be biologically active chemicals which may be very toxic component[3]. Pesticides drastically influence the integrity of the hereditary material which ultimately interfere in expression of genetic imprints directly or indirectly [4] . Keeping these implications in view, genotoxicity assessment of such chemicals has become crucial before their actual application in the fields. Nowadays, various *in vivo* and *in vitro* innovative techniques, based on different protocols are used for genotoxicity assessment studies of various mutagens, by which genotoxicity of such chemicals are measured in term of gene mutations, chromosomal alterations and DNA damage. With advancement of molecular genetics, the number of new PCR based techniques, for *in vitro* amplification of genomic DNA have been developed which are used for functional analysis of genes, diagnosis of hereditary diseases, phylogenetics of species and assessment of DNA damages[5-6] .In relevance to this present investigation deals with genotoxicity assessment of dicofol on rDNA ITS1 sequence of *Culex quinquefasciatus.* Dicofol is a persistent, lipophilic organochlorine pesticide introduced in 1957 for commercial uses. It is a narrow spectrum insecticide used for the control of mites on a number of fruit trees, vegetables, ornamental plants and other economically important field crops. Its deleterious effects on genetic imprints and reproductive potential have been studied extensively, however, limited investigations have been executed to assess its drastic impacts on DNA. Therefore, motivated by particular cognition, present research investigation is executed, which is genotoxicity evaluation of dicofol pesticide. The objective of the present research was achieved through amplification of rDNA ITS1 sequence with the application of PCR using specific forward and reverse primers. Different protocols are used extensively for genotoxicity evaluation of different mutagens by using different test organisms. Correspondingly, mosquito genome has proved quite efficient analytical material for the genotoxicity assessment of various environmental contaminants and has been widely used in various evaluation researches. Different investigators have used larval salivary polytene chromosomes of various *Anopheline* species and genome of *Culex quinquefasciatus*, as analytical tool for mutagenicity evaluation of various chemical agents like antitumour and anticancerous drugs, aromatic amines, mitostatic drugs and salts of various heavy metals like nickel, mercury and lead[7-14]. In such studies, induced damages to genetic material have been measured in term of increased percentage frequency of chromosomal aberrations. The deleterious effects of these compounds were observed in the form of various types of aberrations such as inversions, translocations, breaks, deletions and a synapses of somatically paired homologues of polytene chromosomes. From the present studies and those carried out by other researcher, it is evident that changes in DNA caused by genotoxic chemicals can be monitored using different molecular markers at biochemical as well as molecular levels[15]. Recent developments in molecular biology by the applications of DNA fingerprinting and gene amplification with polymerase chain reaction (PCR) techniques have offered new possibilities for detecting even single base DNA damage [16-17] . In a study, DNA damage induced

This article can be downloaded from www.ijpbs.net

in barley seedlings treated with Cd, was in the form of variations in the PCR generated DNA band intensity, loss of normal bands and appearance of new bands as compared to those produced from normal seedlings[18]. Likewise, in a another investigation, it was reported that the genotoxicity of heavy metals in *Phaseolus vulgaris* was expressed in the form of several missing bands and appearance of new bands in the electrophoretic profiles from the treated material as compared to nontreated controls[19]. While using *Vicia faba* cells to know the effects of some synthetic dyes, It was noticed a number of qualitative modifications in the integrity of DNA in the form of variations in band intensity, loss of normal bands and appearance of new bands from the treated material[20]. During present study, after sequencing of PCR amplified products of treated and control individuals, the aligned was done through Clustal W software programme. Various types of nucleotide alterations such as additions, deletions, transitions and transversions were observed in the rDNA ITS1 sequence of treated individuals in comparison to controls.

MATERIALS AND METHODS

Test organism; For the execution of the targeted objectives, common house mosquito, *Culex quinquefasciatus,* taxonomically considered as a member of the *Culex pipiens* species complex, is taken as a test model to evaluate mutagenic instinct of dicofol, at sublethal exposure extent. *Culex quinquefasciatus*, a medium-sized mosquito characterized by conspicuously dark proboscis, thorax, wings as well as tarsi, thirteen segmented scaless flagellum, likewis, abdomen exhibit distinctive marking of pale, narrow, rounded bands. To colonize test organism, gravid females of *Culex quinquefasciatus* were captured with the help of appropriate mouth aspirator, from cattle sheds of village Nada sahib, approximately 20 km southeast of Chandigarh, subsequently captured mosquitoes, were repositioned to insectory under controlled conditions of temperature and humidity. Species identification was done by transferring each gravid female imago, separately to different test tubes and was examined with 5X magnification hand lens, following appropriate identification key[21]. Captured blood fed female were allowed to oviposit egg rafts in water filled petridishes, already placed in the breeding cages at 25 ± 1ºC, 70 ± 10% humidity and 12h/12h photoperiods. The rearing of larvae, hatched from egg rafts, was accomplished by sprinkling finely powdered protein rich diet, made up from dog biscuits and yeast extract mixed in 6:4 ratio[22-23] on surface of rearing medium which was changed daily to avoid scum formation, subsequently, colonization of *Culex quinquefsciatus* was accomplished in insectory. *Pesticide information:* Dicofol used in the present experiment is an organochlorine pesticide with IUPAC name 2, 2, 2 –trichloro-1, 1, bis (4-chlorophenyl) ethanol, CAS No. 115-32-2, molecular formula $C_{14}H_9Cl_5O$ and molecular weight 370.5. Chemical structure of concerned pesticide is elucidated in Fig 1. To achieve present targets, a packet of 100 ml available under the brand name Hilfol 18.5 EC (Hindustan Insecticide Limited, India) was used as such because main targets of present research is to evaluate genotoxicity of chemical formulation which is going to field. *Standardization of dose and mode of exposure*; Before executing present research, standaridization of concentration was done and stock solution (1%) of aforemention mentioned pesticide was formulated. For LC_{20} calculation, the second instar larvae were given exposure of dicofol for 24 hours in serial dilution of 1% stock solution. Same procedure was repeated for three times where, average of mortality observed in each experiment was taken as criterion for dose standardization. On the basic of observed mortality, the exact value of this dose was calculated by applying probit analysis (Fig 2) which was 4.07 µl/ml for *Culex quinquefaciatus*[24]*.* For execution of present explorations larvae of *Culex quinquefasciatus* were given exposure to statistically analyzed LC_{20} aliqout, for continuity of 24 hours, subsequently, exposed larvae were transferred to distilled water, reared by feeding on a protein rich diet. *DNA*

extraction and PCR amplification; For PCR based rDNA ITS1 sequence studies during present experiment, the LC_{20} exposed larvae were reared upto adult stages. The freshly emerged unfed adults of both treated and control stocks were used for DNA extraction by phenol-chloroform extraction method by following suitable protocol[25] while integrity of extracted samples was checked by following standaridized procedure[26] . The extracted and purified DNA (Fig 3) was amplified by using two specific forward and reverse primers having sequence FP 5 ´-C C T T T G T A C A C A C C G C C C G T-3´ and RF5 ´-G T T C A T G T G T C C T G C A G T T C A C-3´. For PCR amplification, 25 ml reaction mixture was prepared by mixing 10X PCR buffer, dNTP’s mixture Primer (10 mM each), Taq polymerase, $MgCl_2$, distilled water and DNA template[27] A negative control having all components except template DNA was also placed to avoid any experimental error. The reaction mixture was amplified by programme of 36 cycles consisting initial denaturation of 10 minutes at 94°C followed by 35 cycles programmed as denaturaion for 1 minute at 94°C, annealing for 1 minute at 59°C and final extension at 72°C for one minute. Finally, extensions each for 10 minutes were given at 72°C. The amplified PCR products were analyzed through electrophoresis in 2% agarose gel and visualized under UV transilluminator (Fig 4). A gene rular was also loaded in first well along with amplified products of treated and control individuals to compare base length of amplified PCR products.

RESULTS AND DISCUSSION

Figure 4 represents the amplified PCR products of dicofol treated and control samples. After amplification of about 40 different samples of treated and control stocks, a single sharp and prominent band of 850 bases was obtained for each sample during electrophoresis. The amplified PCR products from treated and control stocks were sequenced and then aligned by using the Clustal W software programme (Fig. 5). After analyzing the sequence data of treated and control stocks it was found that dicofol induced a total of 334 mutations in this sequence, which included 33 deletions, 49 additions, 120 transitions and 132 transversions. It was observed that dicofol caused maximum deletions and additions of cytosine base. It was further observed that dicofol caused continuous deletions of 11 bases from base number 614 to 624 that is deletion of TCCCACCCCCC while continuous additions of 15 bases was noticed between base 323 and 324 in the form of CCCAGTGGAGCCCAG. In case of transitions and transversions it was noticed that the pesticide effected more of guanine and cytosine bases as it had more tendency to replace guanine with adenine and cytosine with guanine. It was noticed that in dicofol treated individuals there was a decrease in GC content and increase in AT content as compared to the condition in the sequence from the controls. It was also observed that dicofol caused more additions than deletions in the treated stocks (Table 1). The genotoxic potential of concerned pesticide has been well supported by numerous studies carried out in the field of genotoxicity. In an *in vitro* study, dicofol was found to cause a statistically significant increase in sister chromatid exchange frequency in cultured human lymphoid cells[28] at concentration 10^{-6} M for 48h . Dicofol was observed to possess carcinogenic properties as it has significantly increased the cases of prostate cancer in farmers engaged in handling this pesticide[29] In another investigation, dicofol was noticed to induce chromosomal breaks which ultimately resulted in increased frequency of micronuclei formation in pesticide exposed samples of *Tilapia rendali* [30]. Like any other tumour promoting agent, it also caused protein kinase-C induction in murine embryo fibroblast cells [31]. Additionally, there are numerous studies attributed to reproduction inhibiting properties of dicofol in various organisms such as it was observed that, at concentration 500mg/kg/d, dicofol decreased the production of normal spermatocyte, spermatogonia and spermatozoa in rats due to its antiandrogenic and

antispermatogenic effects [32]. Dicofol pesticide was observed to influence normal steroidogenesis in alligators inhabiting dicofol contaminated lake because it has tendency to reduce the normal testosterone concentration[33]. Additionally ill consequences of dicofol on other living organisms are also well documented, aforementioned chemical has a tendency to cause an egg shell thinning ultimately resulting in failure of normal hatching in birds like *Anas platyrhychos* [34], *Strepedia risoria*[35], and *Falco spaverius* [36-37]. All this discussion supported the genotoxicity of dicofol to genetic imprints of living organisms as well as on reproductive potential of different test models. Results of present research execution indicated that dicofol induced genotoxicity on rDNA ITS1 in treated individual in comparison of control stocks. Dicofol was observed to induce considerable alterations in nucleotide sequence in the form of addition, deletion, transition and transversion of rDNA ITS1 sequence of *Culex quinquefasciatus*. Synthetic pesticides are generally associated with clastogenic and cytotoxic consequences, therefore non-chemical alternatives should be encouraged which include cultural practices such as polyculture, crop rotation, use of biological products and agents including beneficial insects which are natural enemies of target pests.

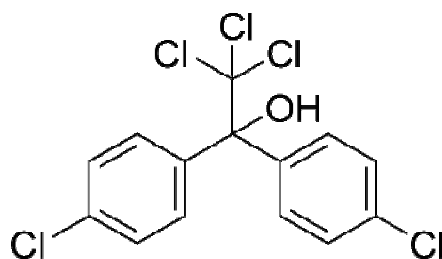

Figure 1
Chemical structure of dicofol[38]

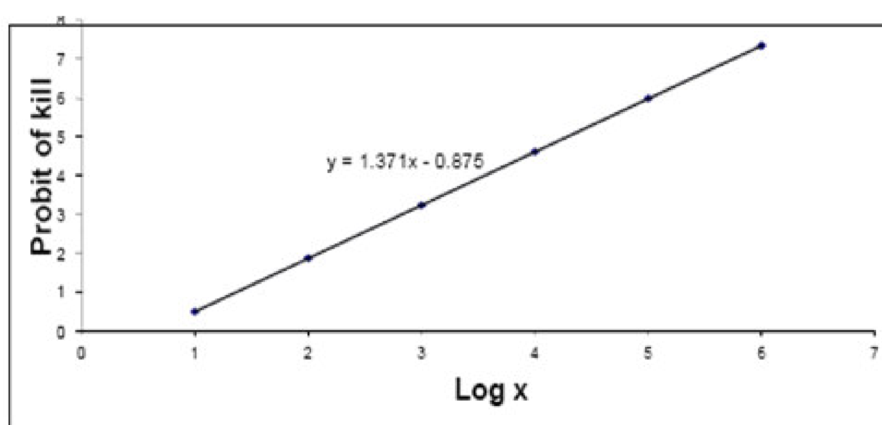

Figure 2
Relationship between the probit of kill and doses of dicofol for Culex quinquefasciatus

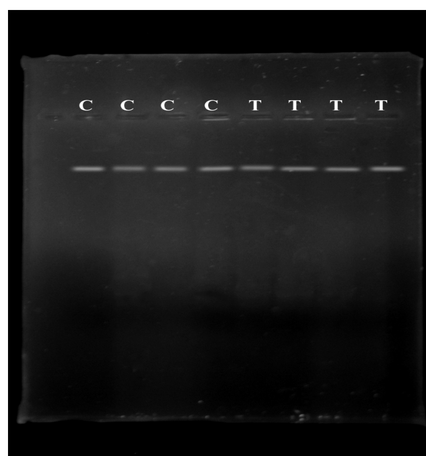

Figure 3
DNA bands from control (C) and dicofol treated (T) Individuals of Culex quinquefasciatus

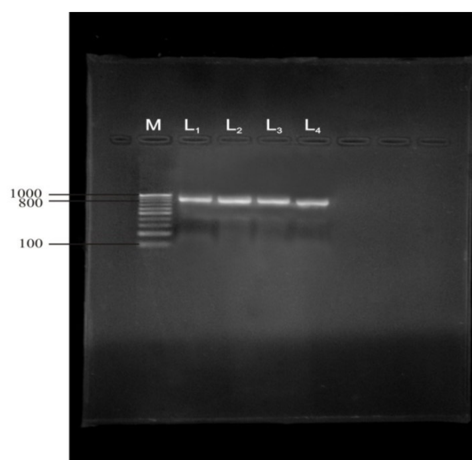

Figure 4
PCR amplified, DNA bands from control (C) and dicofol treated samples (T) of Culex quinquefasciatus. Lane M: Standard gene ruler, Lane L_1: DNA band from control stock, Lane L_2: DNA band from control stock, Lane L_3: DNA band from treated stocks, Lane L_4: DNA band from treated stocks.

Figure 5

Multiple sequence alignment of rDNA ITS 1 sequence of control and dicofol treated individuals (* =Aligned base pair, --- addition in control sequence, ---- deletion in treated sequence)

```
CONTROL:     --TTGTAGATTGCCTATCCTTTGTGCCCTCTGCAGTCGAACTGTTGCTAGTCCTTCCCGG 58
TREATED:     CCTTGTCGACG--TTGCATAGTGAACTCTTTGCAGGTGAACATTTGCTAGTCCTTCCCGG 58
               **** **     *      **  * ** *****  ****  *****************

CONTROL:     ATTACATTTGAATCGCTGAAGTTGACCGAACTTGATGATTTAGA-GGAAGTAAAAGTCGT 117
TREATED:     ATTACATTTGAATCGCTGAAGTTGACCGAACTTGATGATTTAGAAGGAAGTAAAAGTCGT 118
             ******************************************** ***************

CONTROL:     AACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACCGTAACACACTTCA--TACC 175
TREATED:     AACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACCGTAACACACTGCACATACC 178
             *************************************************** **  ****

CONTROL:     ATGACAGCCATAG-------TATGACAGCGTACACGTTAATGTGTTCCTGTGAGGGAAAG 228
TREATED:     ATGACAGCCACGGCATGACGTATGACAGCGTACACGT-AATGTGTTCCTGGGATGGAAAG 237
             **********  *       ***************** ************ ** ******

CONTROL:     TAGAGGAGGAAGGAAGGTCTCTCGGCCATCGTCTCTCGGC---TCTCCGCGTT-GAATGT 284
TREATED:     TATAGGAGGAAGGAAAGTCTCTCGGCTATCGTCTCTCGGCGGCTCTCCGCATTCAAATGT 297
             ** ************ ********** *************   ******* **  *****

CONTROL:     CGCA-GTTTCGC-GCACGCACAACACAACA-ACACGTGCGAC---------------TGA 326
TREATED:     CGCCCACTTCGCTGCACCCACAGCGCACCACAGACGTACTGCCCCAGTGGAGCCCAGTGA 357
             ***    ***** **** **** * ** ** * **** *  *               ***

CONTROL:     TCAGTAGCCCAGTCCGGACCCCCTCCCCGGTGATCACACACACGGTGCGCGCGCTGTGCG 386
TREATED:     TCACCAGTGCAGGCCGGACCGCCTCCCCGGTGATCACACACACGGTGCGCGTG-TGTGCG 416
             ***  **  *** ******* ****************************** * ******

CONTROL:     CTGCGCGTACGCCCTCGGCAGCCGGGAGAACAAAGCGAACGGGAGAGACCGACCGACCGG 446
TREATED:     CTGAGCACACGAGAG-ACCGACCGAACAAAACAACGGACCCCGAGGGAGGGCCCGACCGA 475
             *** **  ***       *  ***    **  **  ** *  *** **  * *******

CONTROL:     ACGGCCCCACGGTGCGCTGGCCAGTCTAGATGGCGGTACCAAACGCAGGCGT--GTGCGG 504
TREATED:     CTAGCCCCGGGGTGGGGTGGCAAGTGTATGTCGCAGTACCGAGCTCTGGTCCTGGGATGC 535
                *****  **** * **** *** **  * ** ***** * * * **     *   *

CONTROL:     CACGTTCGTCCTATGTGCGCCGTATTCTACATGCACGCAGCGC--TACC--ACGGGTTGC 560
TREATED:     TAGATCCTCCCTATGTCCACCTCTGCGTACATGCGCGCTGCGCGTTACCTCACGATTGGC 595
              *  * *  ******* * **      ******* *** ****  ****  ***  * **

CONTROL:     GCGCGGGCCGTG-CACTCGCGCCCGGGTAACCACTGGAGCGTCCCTTCACGGGGGTCCCA 619
TREATED:     GTGTGGTCCGGGGCACTCTCGAC---ACAGGGAGTAGGGC---------TGGGGG----- 638
             * * ** *** * ***** ** *     *   * * * **          *****

CONTROL:     CCCCCCCGTAAAAAAAAAGGGGGGGTGAAAATTGGGAATTCCGGCCTGTTGCTTCTCCCGC 679
TREATED:     ------CATAAAATCAGGCCCACCTGA----CGTAACTTCTAAAGCATTGTTGTTCTTTC 688
                   * *****  * *      ***     *  * ***       *** *  **   *

CONTROL:     TGGGAGTAGGTACCCGAAAACAAAATTCAAACCCCAAAGTCGGGAGAACT-TATATGCTG 738
TREATED:     TTTGAACTAGCACGCCTTCTTCTCATCCTAAACTAAAGATGGGTAGAGTAATGGAGGATG 748
             *  **    * ** *         ** * ** *  **  * ** ***    *  * * **

CONTROL:     GATGGTGCGGTGAG-ATTTTCATATGTATCAATGACACACCAGCCAGCGCTCTAAAAATA 797
TREATED:     ATCGAAGTGATCCGCGCTGCTGTATGTGTGCGCGAGAGAGAGGATGACTGTC-AGAGATG 807
                *  * * *  *   *    ***** *    ** * *   *    *  ** * * **

CONTROL:     CCGCGCTGTCGCATA-----GAGATATGT-AGCGACGTATGAAGAGGCTGCAACACTTC 850
TREATED:     ACGCGCGAGCATGTGTGCGCGAGACGTGTGAGAGAAGAGTGGCTCGCGCAATACAGCGC 867
              *****   *   *      ****  *** ** ** *  **    *      ***   *
```

Table 1
Alterations in rDNA ITS1 sequence of dicofol treated individuals of Culex quinquefasciatus

Type of aberration	Total numbers of aberration	Type of bases mutated	Total number of bases mutated	Location in base sequence
Deletion	33	GC	2	10 to 11
	A=5	G	1	178
	T=7	T	1	206
	G=5	C	1	401
	C=16	CGG	3	582 to 584
		GTCCCTTCA	9	599 to 607
		TCCCACCCCCC	11	614 to 624
		AAAT	4	646 to 649
		T	1	789
Addition	49	CC	2	Before 1
	A=8	A	1	102 to 103
	T=7	CA	2	171 to 172
	G=15	CATGACG	7	188 to 189
	C=19	GGC	3	268 to 269
		C	1	278 to 279
		C	1	288 to 289
		T	1	295 to 296
		C	1	312 to 313
		CCCAGTGGAGCCCAG	15	323 to324
		TG	2	497 to498
		GT	2	546 to 547
		TC	2	550 to 551
		G	1	571 to 572
		A	1	728 to 729
		C	1	751 to 752
		TGCGC	5	811 to 812
		G	1	820 to 821
Transition	120	A→G	32	14,187,305,307,322,405,431,454,475,486,488,538,588,596,635,716,726,730,752,758,764,768,778,782,791,793,796,809,811,817,829,832
		G→A	37	23,244,276,279,289,319,392,402,406,410,411,413,445,448,480,500,507,522,554,585,594,626,640,653,660,661,665,684,687,717,738,742,747,783,804,807,840
		C→T	27	12,17,25,28,35,255,334,378,447,495,501,504,561,563,608,659,669,672,675,676,699,712,727,745,757,781,808
		T→C	24	8,15,186,290,331,393,497,509,512,526,529,586,650,664,685,689,705,740,753,756,769,816,839,847
Transversion	132	A→T	12	492,527,542,632,686,695,696,698,701,707,739,842
		T→A	6	18,22,40,728,743,828
		T→G	18	9,19,34,169,219,339,400,499,528,557,570,671,731,733,755,803,805,848

G→T	18	41,222,231,321,474,490,511,525,555,566,577,652,677,680,681,722,785,834
C→G	26	314,335,347,398,421,434,435,455,460,462,471,506,530,589,590,592,663,692,719,766,772,774,776,786,838,846
G→C	18	300,330,403,420,426,427,477,496,503,520,637,638,639,641,642,694,749,837
A→C	17	5,288,310,412,417,424,437,446,633,655,697,700,702,750,767,833, 835
C→A	17	16,389,397,399,416,467,580,662,710,713,735,777,780,797,823,826,841

ACKNOWLEDGMENT

The author is very thankful to Chairperson, Department of Zoology, Panjab University, Chandigarh for providing the necessary facilities under the CAS programme of U.G.C. to carry out the present research work.

REFERENCES

1. Jadaramkunti UC, Kaliwal BB. Possible mechanisms for anti-implantation action of dicofol in albino rats. J Basic Clin Physiol Pharmacol.12 (3): 217-26, (2001).
2. Lind PM, Milnes MR, Lundberg R, Bermudez D, Orberg J, Guillette LJ. Abnormal bone composition in female juvenile american alligators from a pesticide- polluted lake (Lake Apopka, Florida). Environ Health Perspect. 112(3): 359-362, (2004).
3. Bolognesi C. Genotoxicity of pesticides: a review of human biomonitoring studies. Mutat Res. 543: 251-272, (2003).
4. Shugart L, Theodorakis, C. Environmental genotoxicity: probing the underlying mechanisms. Environ Health Perspect. 102:13-1,7(1994).
5. Lin CK, Tsen, HY. Use of two 16S DNA targeted oligonucleotides as PCR primers for the specific detection of *Salmonella* in food. J Appl Bact. 80: 659-666, (1996).
6. Marillia EF, Scoles, GJ. The use of RAPD markers in *Hordeum* phylogeny. Genome 39: 646–654, (1996).
7. Sharma GP, Chaudhry A, Ahluwalia KK, Sobti RC .The effects of lead acetate on the chromosomes of a mosquito.CIS.45: 19-21(1988).
8. Sharma GP, Sobti RC, Chaudhry A, Ahluwalia KK . Genotoxicity of two heavy metal compounds lead acetate and mercuric chloride in *Anopheles stephensi* liston (Diptera: Culicidae). Cytologia, 41: 733-740 (1988).
9. Sharma GP, Sobti RC, Chaudhry A, Ahluwalia KK .Chromosomal aberrations and dominant lethal in *Culex fatigans* due to mercuric chloride. Cytobios. 59: 131-135 (1989).
10. Sharma GP, Soni KG, Chaudhry S (1980). The salivary gland chromosome map of *Anopheles aconitus* Donitz (Diptera: Culicidae). Cytobios, 59: 131-135 (1980).
11. Chaudhry A, Anand PK. Evaluation of the mutagenic potential of chlorpyrifos (CPF) using polytene chromosomes of *Anopheles* mosquito. J Environ Biol, 26(1): 145-150 (2004).
12. Chaudhry A, Anand PK, Geeta, Singh S, Lovleen . Ectopic pairing of the intercalary heterochromatin in the organophosphate pesticide treated mosquito chromosome

(Culicidae: Diptera). Cytologia, 71(4): 431-437 (2006).
13. Chaudhry A, Geeta. Assessment of genotoxic effect of monocrotophos using mosquito genome (Culicidae: Diptera*).* J Cytol Genet, 6: 103-110 (2005).
14. Chaudhry A, Singh S. Chromotoxicity evaluation of acephate using mosquito polytene chromosomes (Culicidae; Diptera*).* J Appl Biosci, 33(1): 27-31 (2006).
15. Savva, D. Use of DNA fingerprinting to detect genotoxic effects. Ecotoxicol Environ Safety*.* 41(1): 103-106 (1998).
16. Dewolf H, Blust R, Backeljau T. The population genetic structure of *Littorina littorea* (Mollusca: Gastropoda) along a pollution gradient in the Scheldt estuary (The Netherlands) using RAPD analysis. Sci Total Environ*.* 325: 59-69 (2004).
17. Atienzar FA, Jha AN. The random amplified polymorphic DNA (RAPD) assay and related techniques applied to genotoxicity and carcinogenesis studies: A critical review. Mutat Res. 613: 76- 102 (2006).
18. Liu W, Li PJ, Qi XM, Zhou QX, Zheng L, Sun TH , Yang YS . DNA changes in barley (*Hordeum vulgare*) seedlings induced by cadmium pollution using RAPD analysis. Chemosphere. 61(2): 158-167(2005).
19. Enan MR. Assessment of genotoxic activity of para-nitrophenol in higher plant using arbitrarily primed-polymerase chain reaction (AP-PCR) Am J Biotechnol Biochem. 3 (2): 103-109 (2007).
20. Abdelmigid HM. Risk assessment of food coloring agents on DNA damage using RAPD markers. J Appl Biol Sci. 3(2): 116-123 (2009)*.*
21. Reuben , R., Tewari, S.C., Hiriyan, J. and J. Akiyama: Illustrated keys to species of *Culex (Culex)* associated with Japanese encephalitis in Southeast Asia (Diptera: Culicidae). Mosq Syst*.* 26(2), 75-96 (1994).
22. Singh, K. R. P., Brooks, G. D. and M. A. Ansari: Mass rearing of mosquitoes. J Com Dis*.* 6, 121-126 (1974).
23. Singh K R P, Patterson R S, Labrecque G C and Razdan RK: Mass rearing of *Culex pipiens fatigans* Weide *.* J Com Dis. 7, 31-53 (1975).
24. Finney DJ. Probit analysis. Cambridge University Press, Cambridge (1971).
25. Ausubel F M, Brent R, Kingston RE, Moore D D, Seidman J G, Smith J A and Struhl K: Short protocols in molecular biology. John Wiley and Sons, Inc., New York, 4th Edn. (1999).
26. Sambrook, J., Fritsch, E. F. and T. Maniatis: Molecular cloning: A laboratory manual, Cold Spring Harbor, New York, 2nd Edn. (1989).
27. Sharpe R G, Harbach R E and Butlin, RK. Molecular variation and phylogeny of members of the *Minimus* group of *Anopheles* subgenus *Cellia* (Diptera: Culicidae). Sys Entomol. 25: 263-272 (2000).
28. Sobti R C, Krishan, A. and J. Davies: Cytokinetic and cytogenetic effect of agricultural chamicals on human cells *in vitro.* Arch Toxicol*.* 52(3), 221-231(1983).
29. Settimi L, Masina A, Andrion A and O Axelson. Prostate cancer and exposure to pesticides in agricultural settings. Int J Cancer*.* 104(4), 458-461 (2003).
30. Grisolia C K. A comparison between mouse and fish micronucleus test using cyclophosphamide, mitomycin C and various pesticides. Mutat Res. 518 (2), 145-150 (2002).
31. Rotenberg SA, Weinstein IB. Two polychlorinated hydrocarbons cause phospholipids dependent protein kinase C activation *in vitro* in the absence of calcium. Mol Carcinog*.* 4(6): 477-481(1991).
32. Jadaramkunti U C and Kaliwal BB. Effect of dicofol formulation on estrous cycle and follicular dynamics in albino rats. *J.* Basic Clin Physiol Pharmacol*.* 10(4), 305-314 (1999).
33. Guillette L J, Gross T S, Masson G R, Matter J M, Percival H F and Woodward. Developmental abnormalities of the gonad and abnormal sex hormone

concentrations in juvenile alligators from contaminated and control lakes in florida. Environ Health Perspect. 102: 680-688 (1994).

34. Bennett J K, Dominguez S E and Griffis WL. Effects of dicofol on mallard egg shell quality. Arch Environ Contan Toxicol. 19(6): 907-912 (1990).
35. Schwarzbach S. The role of dicofol metabolites in the egg shell thinning response of ring neck doves. Arch Environ Contam Toxicol. 20(2), 200-205 (1999).
36. Maclellan K N M, Bird D M, Fry D M and Cowles JL. Reproduction and morphology effects of o,p’ dicofol on two generations of captive american kestrels. Arch Environ Contam Toxicol. 30(3): 364-372 (1996).
37. Wiemeyer S N, Donald R, Clark D R, Spann J W, Belisle AA and C. M. Bunck, CM: Dicofol residues in eggs and carcasses of captive American kestrels. Environ Toxicol Chem. 20(12): 2848-2851,(2001).
38. https://en.wikipedia.org/wiki/Dicofol#/media/File:Dicofol_chemical_structure.png. 3 December 2007.