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Genotoxicity evaluation of carbaryl using *Culex quinquefasciatus* (Diptera: Culicidae).

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ABSTRACT

The present investigation is attributed to genotoxicity assessment of a carbamate pesticide carbaryl, at nucleotide level by comparing rDNA ITS1 sequence of treated groups with control stocks. To achieve objective, second instar larvae of Culex quinquefasciatus were exposure to LC20 of carbaryl for duration of 24 hours. The detrimental consequences of concern pesticide, were analyzed after in vitro amplification of rDNA ITS1 by polymerase chain reaction technique using specific forward and reverse primers having sequence of FP 5′-C C T T T G T A C A C A C C G C C C G T-3′ and 5′-G T T C A T G T G T C C T G C A G T T C A C-3′respectively. Subsequently to sequence alignment with clustalW software, various alterations in form of additions, deletions, transitions and transversions were observed in pesticide treated samples. During present research about 390 alterations were detected in pesticide exposed groups, comprised 18 additions, 58 deletions, 142 transitions and 172 transversions of bases. Furthermore, carbaryl possess tendency to induced maximum deletions of adenine base and maximum addition of thymine nucleotide. In case of transitions and transversions, carbaryl had a maximum potential of affecting the guanine base.

Keywords: Carbaryl, Culex quinquefasciatus, rDNA ITS1 sequence.

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INTRODUCTION

For overwhelming production of agriculture commodities, the use of pesticides, chemical fertilizers and other synthetic chemicals has increased exponentially. Among all synthetic agro- chemicals, pesticides occupy a major position which constitutes a heterogeneous category of chemicals particularly designed to control various types of pests. The use of such synthetic chemicals has greatly enhanced the production of agricultural commodities by acting against pests in agricultural fields [1] but simultaneously their drastic consequences on genetic material of living organisms cannot be ignored. Although, pesticides are generally designed specifically against certain target organisms but such chemicals are generally toxic to other nontarget organisms including human beings. Such agrochemical formulations sometime induce irreversible damage to hereditary imprints, therefore can prove lethal to survival of living organism. These chemicals are generally reactive chemicals which can associate with various nucleophilic sites of cellular biomolecules such as DNA, RNA or proteins and damage these cellular components resulting in interference of their normal functioning [2]. The induced DNA damage can potentially lead to detrimental genetic and reproductive abnormalities or can cause numerous chronic diseases [3-4].

Genotoxic consequences of such xenobiotic agents can be assessed by a great variety of short term studies based on different protocols using different test systems such as microbes, plants and animals. Nowadays, commonly used techniques are comet assay, sister chromosomal exchanges analysis, micronucleus assay, chromosomal aberrations study. Recently, innovative techniques and advancement in molecular biology have provided different methodologies to detect DNA damage even upto nucleotide levelsCitterio, S., Aina, R., Labra, M., Ghiani, A., Fumagalli, P., Sgorbati, S. and Santagostino, A., 2002. Soil genotoxicity: a new strategy based on biomolecular tools and plants bioindicators. Environ. Sci. Tech. 36, pp. 2748–2753. Full Text via CrossRef | View Record in Scopus | Cited By in Scopus (26) by following Polymerase Chain Reaction (PCR) technique [5-8]Citterio, S., Aina, R., Labra, M., Ghiani, A., Fumagalli, P., Sgorbati, S. and Santagostino, A., 2002. Soil genotoxicity: a new strategy based on biomolecular tools and plants bioindicators. Environ. Sci. Tech. 36, pp. 2748–2753. Full Text via CrossRef | View Record in Scopus | Cited By in Scopus (26). The present topic of research deals study of the detrimental consequences of carbaryl pesticide on the genomic component of Culex quinquefasciatus through the amplification of ribosomal DNA Internal Transcribed Spacer 1 (rDNA ITS1) sequence by polymerase chain reaction (PCR) technique. The genotoxic potential of carbaryl was assessed at LC20 level with exposure duration of 24 hours and mutagenic tendency of concerned pesticide was manifested in the form of various alterations such as additions, deletions, transitions and transversions in rDNA ITS1 sequence of exposed individuals. Further impact of concerned chemical on GC:AT contents of treated stocks were also analyzed.

Carbaryl (n-naphthyl, 1-methyl carbamate) is categorized as carbamate insecticide, extensively applied for the control of pests like aphids, mites, leafhoppers, maggots and foliar feeding larvae on a wide variety of crops. It is broad spectrum insecticide, possessing anticholinesteric mode of action as it exhibit tendency to block the activity of acetylcholinesterase (AChE) reversibly. Although, numerous in vitro and in vivo investigation are available in literature review, which provide insight into a variety of acute chronic genotoxic properties and other deleterious effects of this polycyclic aromatic carbamate chemical on different test models but only limited studies has been carried out on evaluation of direct consequence of carbaryl on DNA. Therefore, motivate by particular cognition, present study has been undertaken to evaluate the impact of carbaryl pesticide on individual nitrogenous bases of rDNA ITS1 sequence of exposed organisms. Man gets exposed to it at its manufacturing stage, contact during crop dusting and routine handling. Carbaryl insecticide acts primarily by inhibiting acetylcholinesterase activity which is required for breaking acetylcholine neurotransmitter molecules into choline and acetate group. Other severe biochemical effect of carbaryl is, it reduces glutathione protein which mainly contributes to the biotransformation of xenobiotics, through reactions involving the glutathione conjugation. Carbaryl has half-life period of 28 days and due to its moderately toxic chemical properties U.S. EPA classified it as level II pesticide.

Nowadays, different biomarkers are used to analyse the potential adverse consequences of different environmental mutagens on genetic imprints and reproduction potential, using various innovative techniques on vast variety of experimental models. Correspondingly, mosquito genome has proved quite efficient analytical material for genotoxicity assessment of various chemical agents like antitumour and anticancerous drugs, aromatic amines, mitostatic drugs and salts of various heavy metals like nickel, mercury and lead etc [9-16] 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 asynapses of somatically paired homologues of polytene chromosomes.

The selected pesticide carbaryl was found potential genotoxic and detrimental reproductive inhibitors in different investigations [17-18] . During present investigation, subsequent to in vitro amplification of rDNA ITS1 sequence, alignment was done by using clustal W siftware. Thereafter various alterations of rDNA ITS1 sequence were detected which included addition, deletion, transition and transversion. Furthermore impact on GC and AT content were also reported.

MATERIALS AND METHODS

Pesticide tested

Carbaryl pesticide is synthesized from 1-naphthol and methylisocyanate (MIC).The chemical structure of carbaryl, aliphatic as well as aromatic part of the compound, has been elucidated in Fig. 1[19]. This pesticide has following characteristic.

IUPAC name: 1-naphthyl methyl carbamate,
CAS number: 63-25-2
Mmolecular formula : C12H11NO2
Molecular weight: 201.23

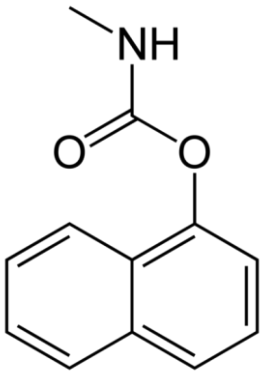

Figure 1: Chemical structure of carbaryl

Carbaryl, a carbamate insecticide, available under trade name sevin was procured from M/S S. S. Cropcare Limited, India and was used as such. The basic motive of executed investigation was an evaluation of clastogenic properties of this commercial formulation, which is really going to field.

Experimental test organism

For present targeted objectives, common house mosquito, Culex quinquefasciatus, taxonomically classified as a member of the Culex pipiens species complex, was selected as test organism to evaluate cytotoxic as well as clastogenic characteristics of carbaryl, at sublethal exposure extent. Culex quinquefasciatus, a medium-sized mosquito possessing conspicuously dark proboscis, thorax, wings as well as tarsi, and thirteen segmented scaless flagellum, likewisely, abdomen exhibit distinctive marking of pale, narrow, rounded bands toward the basal side of respective tergites. Blood fed females of Culex quinquefasciatus were collected from a village Nada sahib, about 20 km southeast of Chandigarh, with the help of mouth aspirator and were carried to laboratory in small field collection cage made of mosquito net cloth. Subsequently, those captured gravid female mosquitoes were individually transferring to different test tubes, identified by examined with help of 5X magnification hand lens, by following suitable identification keys [20-25]. After that gravid females were allowed to lay egg raft in mosquito breeding cages at 25 ± 1ºC, 70 ± 10% humidity and 12h/12h photoperiods. The eggs obtained from them were allowed to hatch in distilled water filled enamel bowl under controlled conditions of BOD incubator at optimal conditions of temperature

and humidity. The larvae hatched from them were reared by feeding them on protein rich diet made from dog biscuit and yeast extract mixed in ratio of 6:4 [26-27] The feed was gently sprinkled on the surface of rearing medium whose quantity was adjusted according to stage and density of larvae. Rearing medium was changed daily to avoid scum formation for healthy growth of larvae. A colony was reared from them under controlled conditions of laboratory.

Standardization of dose and mode of exposure—LC20 was calculated on the basis of mortality of second instar larvae exposed to serial dilution concentrations of stock solution of pesticide (1%) for 24 hours. Desired concentrations of serial dilution were prepared by adding aliquots of the stock solutions in distilled water. To test each of these concentrations, three replicates of twenty larvae were kept simultaneously, with respective controls under controlled conditions of laboratory. The mortality of larvae was noticed after 24 hours and on the basis of mortality of larvae in each set the exact value of LC20 was calculated by probit analysis [28] which was 10.00 µl/ml for Culex quinquefasciatus during present study as elucidated by Fig 2. The mortality in the control group was taken to be the natural response rate. For present genotoxicity assessment study, second instar larvae of Culex quinquefasciatus were given treatment of statistically analyzed LC20 for 24 hours, subsequently, they were transferred to distilled water and were reared by feeding on protein rich diet. Present research work was carried out at LC20 level as it is sublethal dose which exhibits toxicity but does not drastically reduce the population of test organisms. The freshly emerged adults of treated and nontreated stocks transferred individually to eppendorf tubes and stored at -20 0C before DNA extractions

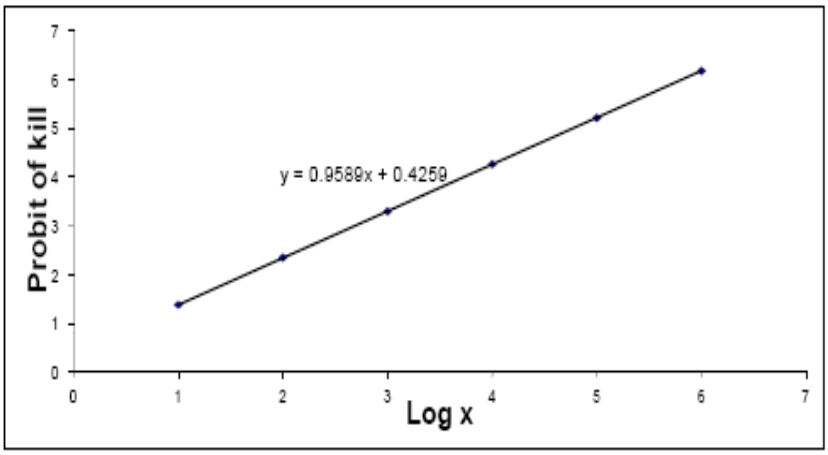

Figure 2: Relationship between the probit of kill and doses of carbaryl for Culex quinquefasciatus.

Extraction and PCR amplification of DNA - The freshly hatched unfed adult specimens of both treated and control stocks were used for DNA extraction using phenol-chloroform extraction method [29] and at a time only single individual was used for DNA extraction in each eppendorf tube. The integrity and purity of extracted samples was tested by specific protocol [30]. For PCR amplification, master mix was prepared by mixing 10X PCR buffer, dNTP mix (10mM each), MgCl2, Taq polymerase (3 units/µl), double distilled water and extracted DNA. Specific forward and reverse primers (FP, RP) were used during this amplification which were 20 base oligomers having a sequence of FP 5 ´-C C T T T G T A C A C A C C G C C C G T-3´ and 22 base long oligomers with sequence 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´respectively [31] . Subsequently, amplification of DNA was done by following specific protocol [32] according to which, each of the 25 µl of reaction mixture was loaded in a thermocycler machine, which was programmed for the initial one cycle for denaturation of DNA at 94°C for 10 min . Subsequently total 35 cycles was carried out, each consisting of each of denaturation, annealing of primer and extension of DNA at 94°C for 1min, 590C for 1 min and 72°C for 1 min respectively. This was followed by final extension of 10 minutes at 72°C. For each amplifications, a negative control consisting of all the components of reaction mixture except the genomic DNA, was also carried out so as to check the experimental errors during whole procedure. The PCR amplified products and standard DNA ladder were electrophorased in 2% agarose gel. The DNA bands (Fig.3) were observed in UV transilluminator and were photographed under polarized camera. The amplified products were sequenced and were aligned through Clustal W software programme.

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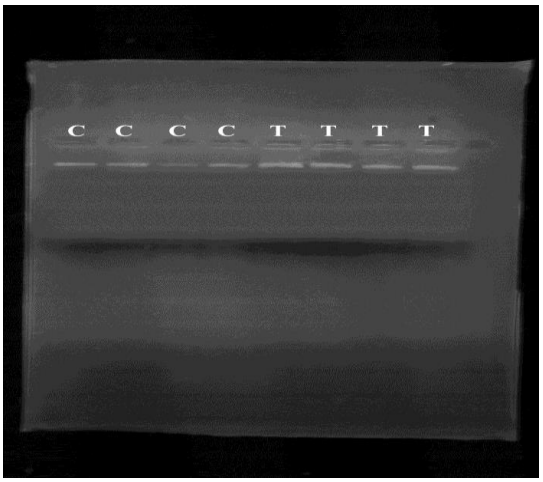

Figure 3: DNA bands from control (c) and carbaryl treated (t) individuals of culex quinquefasciatus .

RESULT

About 40 different samples of treated and control stocks were amplified by using rDNA ITS1 sequence specific forward and reverse primers for pesticide treated and control stocks. Each time, a prominent band of 850 base pair long were obtained for each sample (Fig.4). Those amplified products were sequenced and were aligned by applying Clustal W software programme (Fig.5). Various types of alterations such as additions, deletions, transitions and transversions of bases were examined in the sequence of carbaryl treated stocks after comparing them with those of the controls. The bases which were identical in treated and control groups were presented by asterisk (*) while additions or deletion were shown by dashes (--). In the carbaryl treated stocks there were about 390 alterations which were comprised of 18 additions, 58 deletions, 142 transitions and 172 transversions of bases. It was observed that carbaryl possess tendency to induce maximum deletions of adenine base. A continuous deletion of 13 bases was observed from base number 378 to 390 with sequence AAATGTACGCTGG. It was further noticed that carbaryl also induced maximum additions of thymine base. About 7 bases in between base number 185 and 186 with sequence GCCATGA were found to be added due to action of this chemical in treated stocks. As for transitions and transversions, carbaryl had a maximum tendency of affecting the guanine base. In the same the selected pesticide also induced replaced of guanine either with adenine or with cytosine. As for the GC and AT content there was no change in carbaryl treated stocks. It was also noticed that carbaryl induced more deletions than additions (Table,1).

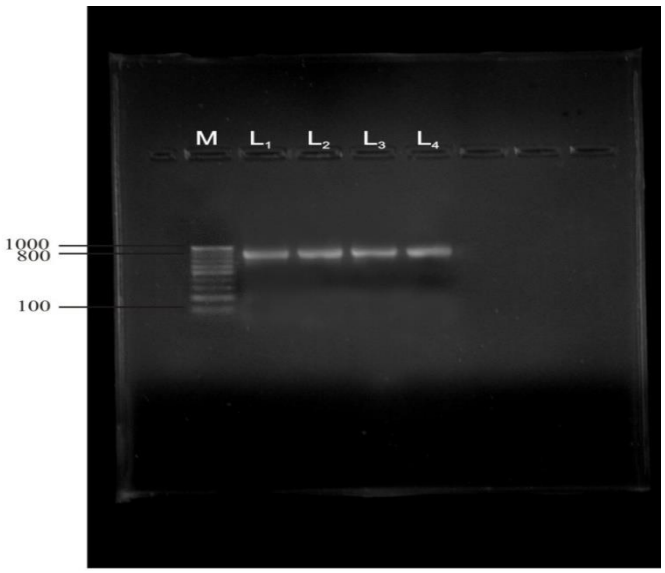

Figure 4: PCR amplified dna bands from control (c) and carbaryl treated samples (t) of culex quinquefasciatuS. Lane M: Standard gene ruler, Lane L1: DNA band from control stock, LaneL2: DNA band from control stock, Lane L3: DNA band from treated stock, Lane L4: DNA band from treated stock.

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Control; --GCAGTCCTGCTGATCATTAAAGTCTTTG-AGGTGACATTTGCTAGTCCTTCCCGGATT 57
Treated: TTGTCGCCGTGTGCACTATTGTAGTCTTTGCAGGTGACTTTTGCTAGTCCTTCCCGGATT 60
         * * * * *  * *** ******** ******* ********************

Control: ACATTTGAATCGCTGAAGTTGACCGAACTTGATGATTTAGAAGGAGGTAAAAGTCGTAAC 117
Treated: ACATTTGAATCGCTGAAGTTGACCGAACTTGATGATTTAGA-GGAAGTAAAAGTCGTAAC 119
         **************************************** *** **************

Control: AAGGTTTCCGTAGGTGAACCT-GCGGAAGGATCATTACCGTAACACACTGCACATACCAT 176
Treated: AAGGTTTCCGTAGGTGAACCTTGCGGAAGGATCATTACCGTAACACACTGCACATACCAT 179
         ********************* ****************************************

Control: GACAGCCAT-------TGTAAGACCGCACACAAATG-TGTGTGAGCAGGAAGGAAGGACT 228
Treated: GACAGCCACGCCATGACGTATGACAGCGTACACGTAATGTGTTCCTGTGAGGGAAAG-TA 238
         ********      *** *** ** *** * *****    ** **** *

Control: CTAGGCCATAGGATGTTCTCTCTCCTCTCGTCTCGCCGCTCTTCCCCGCA--CGCACAAC 286
Treated: GAGGAGGGAAGGAAAGTCTCTCGGCCATCGTCTCTCGGCGGCTCTCCGCATTCGAATGTC 298
          *   ****  ****** * ******** **  ** ***** *** *  *

Control: TCAAC--CGCGCGAGCACCGCTCAACCAGTCACATGCGGTCCCCCTCCCCTCC-GGTTAC 343
Treated: GCAGTTTCGCGCACGCACAACACAAC-ACACACGTACTGCCCCAGTGTACCCCAGGTGAT 357
         **  ***** **** * ***** * *** * * *** *  * *** **** *

Control: CCCCACGGGGGCGTGTGGTGCCCGCCGCGGCAATAAATGTACGCTGGCACGGGAAACGTG 403
Treated: CACCAGTCCAGTCCG-GACCCCCTCCCCGGTGAT----------CACACACACCGTG 403
         * ***   *  * *  *** ** *** **       ***  * ****

Control: GAGCGGCCGGGGGGGGCGGGGAGAGGCCAGCCGAACCGAACCGCCCCGTAGAGATGCATG 463
Treated: C-GTGTCTGTGTGCGCTGCGCACACGGCAGCGGCAGCGGACCA------AGAGGAGCAAG 456
         * * * * * * * * * * * * * **** * * * * ***    **** *** * *

Control: GACGGCGGGGGTGGCTTGCGAAACGGGGTTTGGCCCGGGGGGACAACCCCGACCCTTTCC 523
Treated: GAGAACGAGAGAGACCGACCGACCGAA---CGGCCCCGGGGTGGG---CTGGCCAGT--C 508
         **  * * * * * *  * * ***   ***** ****    * * *** * *

Control: CGGCGCGCGGAGGGTCCTGGTTTATTTGCGTG-GAGTTGGTTGTGGATAACCTGCGCCGG 582
Treated: TAGATCGCGGTA---CCGAGCGCAAGCGTGTGCGGGCAGGTTCTG-----CCTGTGTCCG 560
         * *****   ** *  *  * * *** * * **** **   **** * * *

Control:  AGGACTAGAACTGTTGGGCGTCAGAGTGGTGACGAAAGAGCGCGGAGAAAAATGCGCGTG 642
Treated:  GCGCATA---CTTACATGCA-CTCAGCGCTTACCACCGGGTGCGCGTGC---TCCGGGCA 613
          * **  **   ** * * * * * * * * * * *****    * * * *

Control:  AGTGAGAGCGAGTGCGATGGATGTGATCACTACGCGCGCGACGCAGTAGATGCGAGACGC 702
Treated:  CTCCCGCACGGGTACGACTGG-GTCATC-CTTCACACGCGAA-----CGACCCACCGAAT 666
           * ** ** *** * ** *** ** * * ******    ** *

Control:  GTGGGGAGAGTGCGCGCTGCTTCGCACCACTCTGTATAATATAAGAAGAAGAAGGAGGGC 762
Treated:  AAAGAAGGGGTTTTCTTTCTTAGGACTCGCTCGCTCCTCTTTCGTAAGAGGAACGGGCAC 726
          * *** * * * *  **** *  **  **** ******* * *

Control:  GTATCGGTGGATATGTGTCGTGAGTGGCCGTGCGCGCGCGCGCCTCGCGCGTCGGTTCTT 822
Treated:  AAACAAACCT-CATTTCCCTTGGGTTGGGG-GATCACTCGGGCTTGGTGTACTGGATGGA 784
          *     *** * ******* * * * * *** ** * * *  *** *

Control:  TAGGAGAGGTTGGTCGGACAGTGTGATC  882
Treated:  AAACGGGAACTAAAGGCGCCCTCAAA--  810
          *  *  *  * * * *
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Figure 5: Multiple sequence alignment of its1 of control and carbaryl treated individuals (* =Aligned base pair, --- addition in control sequence, ---- deletion in treated sequence).

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Table 1: Aberrations in rDNA its 1 sequence of carbaryl treated individuals of culex quinquefasciatus.

Type of aberration	Total number of aberration	Type of base mutated	Total number of bases mutated	Location of base in sequence
Deletion	58	A	1	99
	A=18	A	1	226
	T=15	C	1	311
	G=12	T	1	359
	C=13	AAATGTACGCTGG	13	378 to 390
		A	1	405
		CCCCGT	6	447 to 452
		GTT	3	491 to 493
		ACC	3	509 to 511
		TC	2	521 to 522
		GGT	3	536 to 538
		GATAA	5	568 to 572
		GAA	3	590 to 592
		T	1	603
		AAA	3	632 to 634
		T	1	664
		A	1	671
		GCAGT	5	685 to 689
		T	1	764
		C	1	791
		TC	2	849 to 850
Addition	18	TT	2	2 bases before 1
	A=4	C	1	28 to 29
	T=8	T	1	138 to 139
	G=2	GCCATGA	7	185 to 186
	C=4	A	1	205 to 206
		TT	2	278 to279
		TT	2	291 to 292
		A	1	337 to 338
		C	1	555 to 556
Transition	142	A→G	35	19,197,203,215,219,231,236,284,290,318,375,442,457,484,506,508,515,557,583,621,628,630,653,663,699,709,711,731,746,752,758,785,827,829,840
		G→A	49	103,205,224,233,243,297,304,320,353,361,394,396,446,467,468,471,473,477,481,489,490,525,535,542,598,602,642,650,656,676,678,696,701,703,705,707,708,761,763,768,769,798,813,825,830,831,834,835,847
		C→T	31	2,10,15,198,214,227,273,283,291,332,343,355,374,407,411,420,513,524,552,577,579,623,702,715,719,722,729,806,810,812,815
		T→C	27	5,14,185,186,254,270,324,335,357,362,479,494,544,546,550,559,597,609,641,645,660,693,739,774,780,814,832
Transversion	172	A→T	10	20,36,190,285,534,605,674,740,743,773

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T→A	21	228,230,237,242,306,314,458,462,475,548,560,596,704,724,766,770,818,822,823,836,846
T→G	15	11,244,251,268,287,341,480,519,541,545,549,735,739,793,821
G→T	27	211,216,263,322,350,367,409,413,415,505,528,581,595,599,613,629,644,661,71,4,716,718,747,777,782,788,796,800
C→G	22	7,229,234,235,252,265,269,329,331,349,430,435,439,466,507,639,725,790,803,808,820,837
G→C	41	12,213,313,351,352,356,363,370,395,404,417,419,422,424,426,428,483,500,565,581,584,606,611,616,627,636,646,667,694,698,721,736,756,760,771,772,779,826,839,843,845
A→C	22	3,202,212,298,397,399,437,486,586,618,619,631,643,647,649,690,697,728,738,741,745,842
C→A	14	194,255,281,303,328,333,345,518,527,587,684,700,727,795

DISCUSSION

The above results indicated potential detrimental consequences of carbaryl on rDNA ITS 1 sequence of exposed individuals in comparison to control stocks. The selected pesticide induced addition, delection, transition and transversion. Carbaryl exhibited maximum tendency to cause transversion of guanine nitrogenous base with cytosine. Similarly carbaryl caused prominent replace guanine nitrogenous base with adenine also. Furthermore, carbaryl also induced maximum deletion of adenine nitrogenous base. The mutagenic properties of this chemical are also well supported by other investigations carried out in field of genotoxicology. In an in vitro study, this pesticide was observed to induce unscheduled DNA synthesis in human cell culture which was due to metabolism of carbaryl to form an intermediate called diol, similar to various potential mutagens while in another in vivo study carbaryl was observed to induce frame shift mutations in Salmonella typhimurium [33-34]. The mutagenic tendency of this chemical further enhanced due to its tendency to intercalate with genetic material which ultimately resulted in faulty addition or deletion of bases during DNA replication. Carbaryl was found to activate various genes such as cytochrome P4501A1, NF-kB, HSP70, GRP78 and GADD153 in cultured human liver cells [35] .This pesticide was found to induce DNA fragmentation and numerical chromosomal aberrations of X, Y and chromosome 18 in the sperm cells of workers exposed to this pesticide, which lead to adverse effects on their reproductive system [36]. In Chinese hamster cells, carbaryl was observed to possess spindle disruption properties and noticed to elevated chromosome number, multiple chromatid exchanges and chromosomal fragments which was found to be correlated with dose and duration of exposure of this chemical [37-38] .In a study conducted on Allium cepa, it was found that carbaryl induced a number of aberrations such as c-mitosis, stickiness, vagrant chromosomes, polyploidy, delayed anaphase, end to end joining of chromosomes, chromosomal breaks, production of ring chromosomes and formation of anaphasic bridges [39]. While in cultured Chinese hamster cells, this synthetic chemical was noticed to cause depolimerization of spindles leading to uncoupling of karyokinesis and cytokinesis. This effect was concluded to be a major factor responsible for decreased

frequency of cells at anaphase and telophase stages in cultured cells. The spindle disruption properties of this chemical was due interference in the normal function of an enzyme tyrosine kinase required for normal cell division [40] .As such, despite of ubiquitous application of this pesticide, available literature unambiguously suggestive of association of quite hazardous effects to human health particularly to the workers occupationally engaged in production and handling of this pesticide. similarly in a study it was noticed that carbaryl exposed workers had significantly higher proportion of sperm head abnormalities which were related to genetic damage [41] .Decreased sperm motility and concentration were recorded in human exposed to carbaryl which concluded that these effects were due to increased lipid peroxidation which reduced the intracellular level of glutathione[42].

The data obtained from present parameter suggested that carbaryl has caused considerable damage at nucleotide level in the form of additions, deletion as well as substitution of bases as detected by application of PCR. In relevance to the present results, It is overall suggestion that use of less harmful plant based pesticides should be encouraged, beside this, application of natural predators of pests, crop rotation, multiple cropping system, use of biological products should be encouraged.

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REFERENCES

[1] Taylor D, Green N, Stout G. Biological Science. 3rd edition, Cambridge University Press, Australia, 1997.
[2] Crosby DG. Environment Toxicology and Chemistry. Oxford University Press, New York, 1998 .
[3] Ribas G, Surralles J, Carbonell E, Xamena N, Creus A, Marcos R. Mutagenesis 1994; 11: 221.
[4] Lander BF, Knudsen LE, Gamborg MO, Jarventaus H, Norppa H. Scand J Work Environ. Health 2000; 26(5): 436.
[5] Mullis KB, Faloona FA . Method Enzymol 1987; 155: 335.
[6] Conte LS, Miorini M, Giomo A, Bertacco G, Zironi R. J Agr Food Chem 1998; 46: 1844.
[7] Savva D. Ecotoxicol Environ Safety 1998; 41(1): 103.
[8] Citterio S, Aina R, Labra M, Ghiani A, Fumagalli P, Sgorbati S, Santagostino A. Soil genotoxicity: Environ Sci Technol 2002; 36: 2748.
[9] Sharma G P, Chaudhry A, Ahluwalia K K, Sobti R C. CIS 1988a ;45: 19.
[10] Sharma G P, Sobti R C, Chaudhry A, Ahluwalia K K. Cytologia 1988b; 41: 733.
[11] Sharma G P, Sobti R C, Chaudhry A, Ahluwalia K K . Cytobios 1989; 59: 131.
[12] Sharma G P, Soni K G, Chaudhry S . Cytobios 1980; 59: 131.
[13] Chaudhry A, Anand P K . J Environ Biol 2004; 26(1): 145.
[14] Chaudhry A, Anand P K, Geeta, Singh S, Lovleen .Cytologia 2006; 71(4): 431.
[15] Chaudhry A, Geeta. J Cytol Genet 2005; 6: 103.
[16] Chaudhry A, Singh S. J Appl Biosci 2006; 33(1): 27.
[17] Pant N, Shankar R, Srivastava SP. Human Exp. Toxicol 1996; 15(9): 736.
[18] Rani A, Sahai A, Srivastava AK, Anita R . J Anat Soc 2007; 56(4): 1.
[19] https://en.wikipedia.org/wiki/Carbaryl#/media/File:Carbaryl-2D-skeletal.png
[20] Christophers SR. The Fauna of British India including Ceylon and Burma. Vol IV. Taylor and Francis. London, UK, 1934.
[21] Barraud PJ. The Fauna of British India including Ceylon and Burma. Vol V, Taylor and Francis, London, UK, 1934.
[22] Wattal B L, Kalra N L Mosq Born Dis 1967; 9: 85.
[23] Clements AN. The biology of mosquitoes. Mc Millian and Co., New York, 1992.
[24] Barraud PJ. The Fauna of British India including Ceylon and Burma. Vol.V, Taylor and Francis, London, UK; 1934.
[25] Reuben R, Tewari SC, Hiriyan J, Akiyama J. Mosq Syst 1994; 26(2):75.
[26] Singh KRP, Brooks GD, Ansari MA . J Com Dis1974; 6: 121.
[27] Singh KR.P, Patterson RS, LaBrecque GC, Razdan RK . J Com Dis 1975; 7: 31.
[28] Finney DJ.1971. Probit analysis. Cambridge University Press, Cambridge, 1971.

[29] Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K. Short protocols in molecular biology. 4th ed, John Wiley and Sons, New York, 1999.
[30] Sambrook J, Fritsch EF, Maniatis T. Molecular cloning: A laboratory manual, 2nd ed. Cold Spring Harbor, New York; 1989.
[31] Sharpe RG, Harbach RE, Butlin RK . Sys Entomol 2000; 25: 263.
[32] Williams JGK, Kubeklik AR, Livak KJ, Rafalski JA, Tingey SV. Nucl Acids Res 1990; 18: 6531.
[33] Ahmed FE, Hart RW, Lewis N J. Mutat. Res1977: 42:161.
[34] Greim H, Bimboes D, Egert G, Goggelmann W, Kramer M. Arch Toxicol 1977; 39(1-2): 159.
[35] Delescluse C, Ledirac N, Li R, Piechocki MP, Hines RN, Gidrol X, Rahmani R. Biochem Pharmacol 2001; 61(4): 399.
[36] Xia Y, Cheng S, Bian Q, Xu L, Collin MD, Chang HC, Song L. Toxicol Sci 2004; 85(1): 615.
[37] Onfelt A, Klasterska I. Mutat Res 1983; 119: 319.
[38] Onfelt A, Klasterska I . Mutat Res 1984; 125: 269.
[39] Grover IS, Ladhar SS, Randhawa SK. Environ Pollut 1989; 58: 313.
[40] Renglin A, Harmala-Brasken AS, Eriksson JE, Onfelt A. Mutagenesis 1999; 14(3): 327.
[41] Wyrobek AJ, Watchmaker G, Gordon L, Wong K, Moore D, Whorton D. Environ Health Perspect 1981; 40: 255.
[42] Meeker J D, Singh N P, Ryan L, Duty S M, Barr D B, Harick R F, Bennett D H, Hauser R . Hum Reprod 2004;19: 2573.