Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(7):533-539

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Mutagenicity analysis of ethionusing*Culexquinquefasciatus* (Diptera: Culicidae)

Lovleen[ab]

a).Mosquito Cytogenetics Unit, Department of Zoology, Panjab University, Chandigarh, India
b). Department of Zoology, School of Biotechnology and Biosciences, Lovely Professional University, Phagwara,

---

ABSTRACT

Pesticides are used widely in the agriculture industry to control various types of pests but at the same time, their extensive applications have caused both environmental and public health concerns. Usually the genetic toxicities of pesticides are determined by several factors, such as their bioaccumulation or persistence in the environment, their metabolism in humans and their reactivity with cellular bimolecules such as DNA, RNA and proteins. Hence, genotoxicity evaluation of such chemical has become basic requisite before their enormous application on agricultural products. In relevance to this, the present topic of research deals with genotoxicity assessment of ethion at molecular level by exposing genome of Culex quinquefasciatus to LC_{20} of concerned chemical for 24 hours. The rDNA ITS1 sequence of treated and control samples were amplified by using forward and reverse primers having nucleotide 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 RF 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. Amplified products were sequenced and subsequentlyaligned with help of Clustal W software programme. A total of 321 different types of alterations were detected in rDNA ITS1 sequences obtained from ethion treated stocks which included 41 insertions, 48 deletions, 111 transitions and 121 transversions. According to these values it was evident that maximum numbers of errors were in the form of transversions while insertions were minimum. Furthermore, it was observed that in ethion treated individuals a decrease in GC content and an increase in AT content were observed as compared to controls.

INTRODUCTION

Numerous agrochemicals like fungicides, insecticides and herbicides are being sprayed in the agricultural fields to prevent crop damages due to various types of pests. Among insecticides, organophosphates are in extensively use by farmers due to, effectiveness and broad-spectrum activity against pests but simultaneously, these chemical possess detrimental consequences in the form of potentially toxicity to other nontarget species, and readily accumulation in human body tissues. Therefore, proper handling and appropriate dose implications are necessary measured which should be undertaken to reduce the potential risk of these pesticides. Pesticides constitute a heterogeneous category of chemicals specifically designed to generally act selectively against certain organisms without adversely affecting others. However, the improper implementation of such synthetic chemicals may engender drastic consequences, therefore, most pesticides are generally remain a toxic risk to living organisms including human beings. Consequently, their genotoxic evaluation has become crucial aspect that should be conducted out on intensive levels so that specific recommended exposure limit of these formulations can be concluded in such a way that predicted concentration for application, should be lethal to target pests but should not have any destructive consequence neither to other species of animals nor to environment.

Pesticides have been considered potential chemical mutagensas a large number of reports are available which rightly defend that such compounds possess potential to inducing mutations, chromosomal alterations or DNA damage in living organisms.Suchreports in scientific literature have essentially focused the genetic toxicity of such formulations in occupationally exposed populations which including pesticide manufacturing workers, applicators, floriculturists and farm workers. These studies mainly include the biological monitoring of these agrochemicals through comet assay, chromosomal aberrations study (CA), sister-chromatid exchanges (SCE) and micronuclei formation (MN) and DNA fingerprinting etc. Pesticides are widely used chemicalswhich have ill consequencessuch as carcinogenesis[1], neurotoxicity [2] and reproductive [3] and developmental effects [4].Therefore, present research work is attributed to genotoxicity assessment of an organophasphate pesticides ethion using *Culex quinquefasciatus* genome exposed at LC_{20} level for 24 hours. The extraction and purified samples of DNAof treated and control stocks were amplified by using specific forward and reverse primers. The forward primer was a oligomer of 20 bp long nucleotide of sequence PF 5'-C C T T T G T A C A C A C C G C C C G T-3´ while reverse was 22 bp long having sequence RF 5 ´-G T T C A T G T G T C C T G C A G T T C A C-3´The amplified products were aligned by Clustal W software programme. Various types of aberrations such as additions, deletions, transitions and transversions were observed in treated individual as compared to control individuals. Further effects on AT:GC contents were also studied in pesticides exposed individuals than that of control.Ethion, an organophosphate insecticide, was selected for present research execution, and it is extensively used to kill mites, leafhoppers, maggots and foliar feeding larvae in a wide variety of food, fiber and ornamental crops. It is a nonsystemic and broad spectrum pesticide which was registered in 1965 for commercial purposes. Ethion is highly to moderately toxic compound, persistentcontinuation, in a particular environment depends upon climatic conditions; half life period of ethion is 1.3 to 8 weeks, whereas, EPA, on the basis of toxicity of the concerned compound, designated it, as class II formulation. Mode of action of ethion is to inhibit acetylcholinesterase enzyme (AChE) which is required for breaking acetylcholine neurotransmitter molecules into choline and acetate group. Blockage of AChE, ultimately results in accumulation of acetylcholine neurotransmitter at the synaptic junction which eventually caused continuous stimulation of nerve impulses, consequently, resulting hyperexcitation, voluntary muscles become twitched, ultimately the insects are killed.

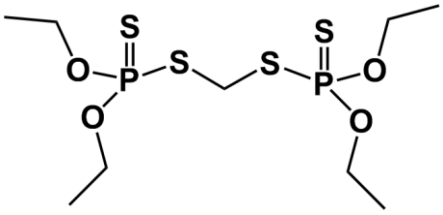
Nowadays, different biomarkers are implemented to assess the drastic consequences of different environmental mutagens on genetic imprints and reproduction potential of various experimental models. Correspondingly, mosquito genome has proved quite efficient analytical material for genotoxicity assessment of various environmental contaminants and in such studies, induced damages to genetic material have been measured in term of increased percentage frequency of chromosomal aberrations [5-8].

Numerous *in vitro* and *in vivo* investigation are available in literature review, which provide insight into a variety of chronic genotoxic consequences and other deleterious effects of various pesticides on different test models [9-12]but only limited studies has been carried out on evaluation of clastogenic and cytotoxic effects of ethion on genetic material, therefore motivate by this cognition present research has been executed.The present genotoxicity assessment studies has been designed to investigate the induced mutagenicity of ethion, in comparison to natural population.

EXPERIMENTAL SECTION

Pesticide tested— Ethion, belongs to organothiophosphate family of organophosphate insecticide which is commercially used to control various types of pests on different crops. Concerned pesticide possess following characteristics

IUPAC name O,O,O',O' – Tetraethyl S,S' methylenebis(phosphorodithioate, CAS number 563-12-2,
Molecular formula $C_9H_{22}O_4P_2S_4$
Molecular weight 384.5



Chemical structure of ethion is elucidated in Fig.1 [13]. For execution of present exploration, a packet of 100 ml available under trade name ethion (M/S Krishi Rasayan Exports Ltd, India) was procured from market, and was used as such because the basic motive of present investigation was an evaluation of clastogenic properties of this commercial formulation, which is really going to field.

Experimental test organism—Culex quinquefasciatus was selected for present investigation and to achieve the target, the blood fed female of this species 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 identified by examined with help of 5X magnification hand lens, by following suitable identification key[14]. Thereafter, those gravid femaleslaid eggs in water filled petridishes already placed in mosquito breeding cages at 25 ± 1ºC, 70 ± 10% humidity and 12h/12h photoperiods [15-16]. Egg rafts obtained from them were allowed to hatch in BOD incubator set at optimal conditions of temperature and humidity. The larvae hatched from them were fed on protein rich diet made from dog biscuits and yeast extract mixed in ratio 6:4, quantity of feed was adjusted according to density and stages of larvae .
Standardization of dose and mode of exposure— For $LC_{20,}$ second instarlarvae were 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 monitored after 24 hours and only larvae whose bodies were completely devoid of the exuviae were recorded as alive. The exact LC_{20} value was calculated by applying probit analysis [17] which was 14.45 µl/ml for ethion during present research (Fig. 2). The mortality in the control group was taken to be the natural response rate.

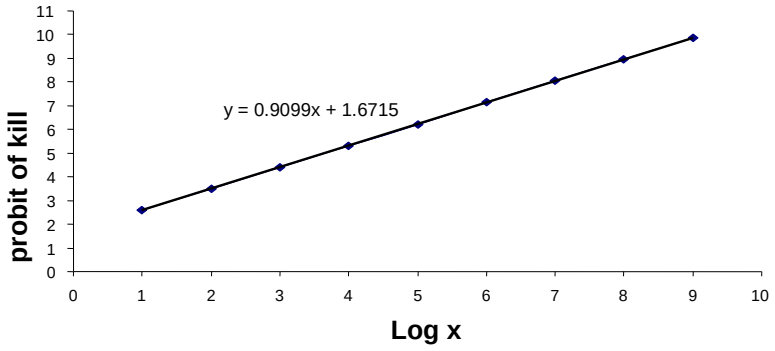


Fig.2. Relationship between the probitof kill and dose of ethion for *Culex quinquefasciatus*

DNA extraction :For PCR based rDNA-ITS-1 sequence studies, freshly emerged adults of both the treated and nontreated stocks were transferred individually to eppendorf tubes and stored at -20˚C before DNA extractions. The freshly hatched adult specimens of both treated and control stocks were used for DNA extraction using phenol-chloroform extraction method [18]. For PCR reaction mixture was prepared by mixing10X PCR buffer, dNTP's mixture forward and reverse Primer (100 mM each), Taq polymerase, $MgCl_2$, double distilled water and the extracted DNA was amplified by using two specific forward and reverse primers 5 ´-C C T T T G T A C A C A C C G C C C G T-3´ and reverse primr sequence 5 ´-G T T C A T G T G T C C T G C A G T T C A C-3´ [19]. A negative control was also kept which contain all reagents except extracted DNA. The bands were visualized by extraphorased in 2% agarose (Fig. 3,4). A standard gene rular was also mounted along with them.The amplified DNA samples of both treated and control stocks were sequenced and the data obtained was aligned by Clustal W software programme [Fig.5]. By comparing the sequences of ITS1 from treated and control samples, the alterations present in the form of additions, deletions, transitions, transversions and AT: GC ratios were calculated and recorded for their comparative frequency of occurrence.

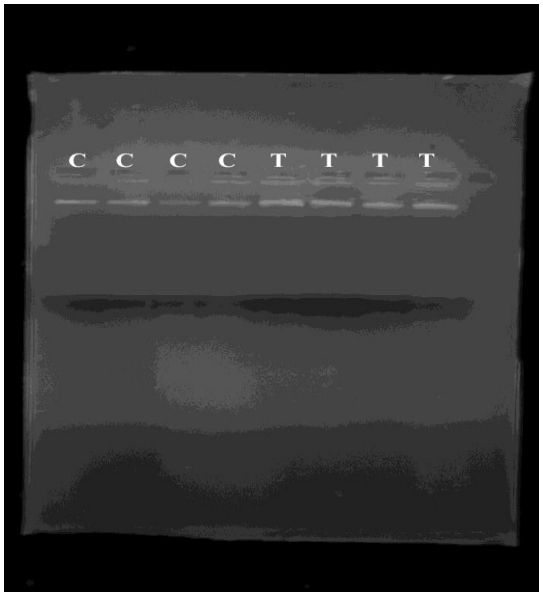


Figure 3.DNA bands from control (C) and Ethion treated (T) individuals of *Culexquinquefasciatus*

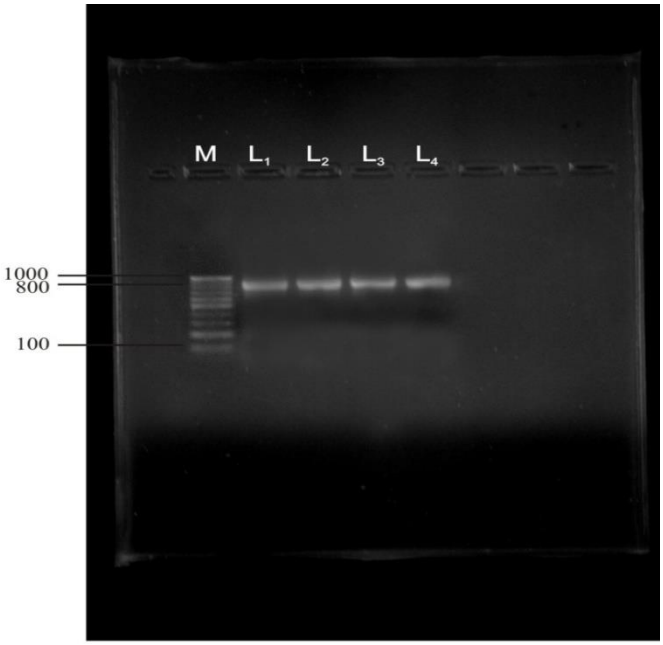


Figure 4.PCR amplified DNA bands from control (C) and ethion treated samples (T) of*Culexquinquefasciatus.* Lane M: Standard gene ruler, Lane L_1: DNA band from control stock, LaneL_2: DNA band from control stock, Lane L_3: DNA band from treated stock, Lane L_4: DNA band from treated stock

RESULTS AND DISCUSSION

About 40 different samples of ethion treated and control individuals were amplified by using ITS1 sequence specific forward and reverse primers. Base length of amplified PCR products were carefully analyzed by comparing with ladder bands loaded along with them in agarose gel. After that the PCR amplified products from treated and control stocks were sequenced and then aligned by using Clustal W software programme. Various types of aberrations such as additions, deletions, transitions and transversions of bases were examined in the sequence of ethion treated stocks after comparing them with those of the controls.

Table1. Aberrations in ITS1 sequence of ethion treated individuals of *Culexquinquefasciatus*

Type of aberration	Total number of aberrations	Type of bases mutated	Total number of bases mutated	Location in the base sequence
Deletion	48 A=8 T=13 G=12 C=15	GCT A AC CGTATGAC GCG C G C TGCCCCAGT GTACCC C G T TATGATT G TT	3 1 2 8 3 1 1 1 15 1 1 1 7 1 2	1 to 3 32 171 to172 196 to 203 276 to 278 291 314 327 336 to 350 416 475 494 514 to 520 738 756 to 757
Addition	41 A=11 G=13 C=5 T=12	T C TT G CGCAC TTGGTAT G G ATTGGGT A AA GGAAT AGAGAT C	1 1 2 1 5 7 1 1 7 1 2 5 6 1	215 to 216 448 to 449 554 to 555 573 to 574 579 to 580 604 to 605 610 to 611 632 to 633 639 to 640 659 to 660 677 to 678 702 to 703 783 to 784 810 to 811
Transition	111	A→G G→A C→T T→C	32 24 30 25	204,288,292,316,367,398,425,467,485,533,538,586,613,616,626,639,648,665,682,711,727,730,733,735,736,765,773,774,785,798,801,831 5,102,189,205,214,423,524,551,577,641,662,668,706,715,740,743,749,753,762,783,787,788,819,834 16,188,191,318,522,523,541,565,588,594,596,598,610,615,635,647,653,687,688,698,700,702,703,720,728,741,797,812,823, 835 10,22,295,406,446,509,537,540,571,574,591,622,629,630,638,661,672,680,704,705,707,726,770,781,810
Transversion	121	A→T T→A T→G G→T C→G G→C A→C C→A	7 13 21 14 16 25 14 11	181,443,484,684,724,780,833 420,456,462,513,566,673,674,679,709,721,755,767,830 4,9,228,255,402,435,452,480,483,498,504,546,568,625,683,723,725,747,758,799, 840 14,268,441,491,561,623,650,652,655,666,694 ,800,820,842 15,182,263,375,419,445,482,573,583,597,603,632,692,745,748,802 11,207,384,400,426,472,531,532,544,545,593,618,620,627,667,669,691,717, 718,769,778,813,817,822,832 24,178,187,234,324,448,459,492,539,585,601,771,772,779 490,507,553,567,676,746,750,795,804,846, 849

The number of bases present in the sequence of control stocks but absent in the sequence of treated stocks were considered as deleted while those absent in the controls but present in the treated sequences were considered as additions. When a purine base was replaced with another purine base and pyrimidine base with another pyrimidine base, the substitions were considered as transitions while transversions refers to the substitution of a purine with a pyrimidine base and vice versa. In each case a prominent band of 850 base pair was observed. A total of 321 different types of alterations were detected in the sequences obtained from ethion treated stocks which included 41 insertions, 48 deletions, 111 transitions and 121 transversions. According to these values it was evident that maximum numbers of errors were in the form of transversions while insertions were minimum. A continuous stretch of deletion of 15 bases was also present from base number 336 to 350 of sequenceTGCCCCAGTGTACCC. It was

further noticed that ethion caused maximum deletions of cytosine base. This also induced continuous additions of 7 bases from number 604 to 605 having sequence TTGGTAT. When the sequence was read further, it was found that this pesticide had caused maximum additions of guanine bases. In case of ts/tvethion, affected adenine and guanine bases as in transition it induced maximum replacements of adenine with guanine while in transversion it substituted guanine with cytosine[Table 1]. It was observed that in ethion treated individuals a decrease in GC content and an increase in AT content were observed as compared to controls. It was observed that it induced more deletions than additions in the treated stocks

Figure 14. Multiple sequence alignment of ITS1 ofcontrol and ethion treated individuals
(= Alignedbase pair, --- addition in control sequence, ---- deletions in treatedsequence)*

```
Control: GCTTGAGGTTGGTGCCTATTGTGATCTTTGAAGGTGACATTTGCTAGTCCTTCCCGGATT 60
Treated: ---GAAGGGCCGTTGTTATTGCGCTCTTTGA-GGTGACATTTGCTAGTCCTTCCCGGATT 56
               ***   **   ***** * ******* ***************************
Control: ACATTTGAATCGCTGAAGTTGACCGAACTTGATGATTTAGAGGGAAGTAAAAGTCGTAAC 120
Treated: ACATTTGAATCGCTGAAGTTGACCGAACTTGATGATTTAGAAGGAAGTAAAAGTCGTAAC 116
         ************************************** ******************
Control: AAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACCGTAACACACTGCACATACCATG 180
Treated: AAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACCGTAACACACTGC--ATACCCTG 174
         ************************************************  ***** **
Control: ACAGCCACGCCATGACGTATGACAGCGGTACACGT-AATGTGTTCCTGTGAGGGAAAGTA 239
Treated: TGAGCCCTACTATGA--------GACCGTACACATTAATGTGTTCCTGGGAGGGCAAGTA 226
           ****   *  ***          * * ****** * **********************
Control: GAGGAGGAAGGAAAGTCTCTCGGCCATCGTCTCTCGGCGGCTCTCCGCATTCAAATGTCG 299
Treated: GAGGAGGAAGGAAAGGCTCTCGGGCATCTTCTCTCG---GCTCTCCGCGTT-GAACGTCG 282
         *************** ******* **** ******* ************ ** ****
Control: CAGTTTCGCGCACGGCACCACACAACACACACGTACTGCCCCAGTGTACCCCAGTGATCA 359
Treated_ CAGTTTCGCGCACG-CGCTACACACCA-ACACGTAC---------------CAGTGATCA 325
         ************** * * ***** ** ********               *********
Control: CCAGTCCAGTCCGGACCCCCTCCCGGGTGATCACACACACGGTGCGTGTCTGTGTGCGGC 419
Treated: CCAGTCCGGTCCGGAGCCCCTCCCCGGTGATCACACACGCCGGGCGCGTCTGTGTG-GGG 384
         ******* ******* ******** ************* * * *** ********* **
Control: TGCGCAGACGGCAGCTGCGGCGGAACTAA-GAGTAGCTAGAGATCGAGAGAGAGCGGACC 478
Treated: AGCACGCACGGCAGCGGCGGCTGTAGCACCGAGGAGCAAGCGAACGAGGGAGACCG-ACC 443
          ** * ******** ***** * * * *** *** ** ** **** **** **   ***
Control: GTCCTAACGGCCGAGTGGGTGGACTTGGCCTGCTTTATGATTGCCGGCGGTCGGAGGGTA 538
Treated: GGCGGTGCGGCATCG-GGGGGGACTGGGACCGCTA-------GTTAGCGGTCCCGGGGCG 495
         * * **** * *** ***** ** * *** *               ******   ***
Control: ATCGGGGTTGCTGGCA--GGTTCGGCCCCTCTTGTCC-TGCGTG-----ATGCGAAACAT 590
Treated: CCTGGCCGTGCTAGAATTGGTTCGTCCCTAAGTGCCGGCGCATGCGCACATGGGCGCTAT 555
            **   **** *    * ********* ** * *   * **     **** **
Control: TGGCTCCCCCAGCG-------ACTGTC-CCAGCACGGGTTGA-----TAGATTGCTTCGT 637
Treated: CGCTTTGTCCCGGGTTGGTATACTGTTGCCGGTGCCGCTCTAGGCACCGGGTTTGTCGAT 615
          *  *   ** * *       *****  ** *  * * *  *       * **  *   *
Control: TATGTCTCCTCACGCGCC---GAGGGGTGCGAGGGGGGTTTTCG--TTTAATATGCCCTG 692
Treated: TGGGTTATCTCTTGCTCTTCTAGGGAGCACGGTCACGGCAATAGAATACAGGTTGTTCTC 675
         *  **   ***  *  *      ** *  *      **   *    *  *   **  **
Control: CGGCCTCTCGC-----CTTGTGTGAGAGGAGGGCTCTATTACGATTAGAATGTGCTGGCC 747
Treated: GGTCCTTTTGTGGAATTCCACGAGGGAGAACCGTACGTGCGTGGTTGGGGT-TATTAGGA 734
          * *** * *           * * *** *     *        ** *         **
Control: TCGCCGGCTTTTCGTGCAAGTGGTAAAAGACGAATTG------CACGGTGAGTACGCATG 801
Treated: GGAACGACA--GCGTACAGGAGCCCCGGGACCCTCTAAGAGATCGCAATGAGTAAGTGGT 792
                *    *** **   *      ***    *       * *  ********
Control: ACTCTGCGTT-GCGTGCGTGGGGCGCGGTCTAGAGCTCTGTAGACACGTC 850
Treated: GGTATGCGTCCGTCTGCCTATGCTGCGGTCAGCTATTCTGGATACAAGTA 842
           * ****   *   *  *  *  ******        **   ***  *
```

Ethiongenotoxicity has been documented by some other reports ; In a study ,it was found that ethion caused various types of chromosomal aberrations in chick bone marrow cells [20] while in another investigation observed that ethion in *Bombyxmori*, reduced RNA/DNA ratio by reducing synthesis of nucleic acid content and decrease in the protein content by reducing their synthesis [21]. The present results indicated that ethion at LC_{20} level has considerable induced damages in rDNA ITS1 sequence of exposed individuals of *Culexquinquefasciatus*. 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.

Acknowledgment
The author isvery 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] CR Michael; PH Alavanja;K Matthew; Ross RB Matthew,*CA-Cancer J. Clin***,2013**,63(2), 120–142 .
[2] Keifer MC; Firestone J. *J Agromedicine*. **2007**,12 (1),17-25.
[3] YH Chiu; MC Afeiche; AJ Gaskins; P.L. Williams; J.C. Petrozza,C; Tanrikut,R; Hauser.J. E;Chavarro. *Human reproduction*. **2015**30 (7) .
[4] VF Garry;DSchreinemachers; ME Harkins; J Griffith. *Environ Health Perspect*. **1996** 104(4), 394–399.
[5] A Chaudhry; PK Anand; Geeta; S Singh;Lovleen . *Cytologia***2006**, 71(4), 431-437.
[6] A Chaudhry;S Singh .*J ApplBiosci*.**2006**,33(1), 27-31.
[7] GP Sharma; RCSobti; A Chaudhry; KK Ahluwalia. *Cytobios***1989**,59, 131-135.
[8] GP Sharma; KG Soni;S Chaudhry . *Cytobios*, **1980**,59, 131-135.
[9] TH Roderick; NL Hawes. *Genetics*,**1974**,76, 109-117.
[10] PJ Sykes; AM Hooker; AA Morley. *Mutat Res***1999**, 427 (1), 1-9 .
[11] D Gilley; H Tanaka; BS Herbert. *Int J Bio Chem Cell Biol***2005**, 37 (5), 1000-1013.
[12] P Michailova; N Petrova; J Ilkova; S Bovero; S Brunetti; K White; G Sella. *Environ Pollut***2006**, 144(2), 647-654.
[13] https://en.wikipedia.org/wiki/Ethion#/media/File:Ethion.png
[14] R Reuben; SC Tewari; J Hiriyan; J Akiyama .*MosqSyst***1994**, 26(2), 75-96.
[15] KRP Singh; GD Brooks; MA Ansari, *J Com Dis***1974**, 6, 121-126.
[16] KRP Singh; RS Patterson; GC Labrecque; RK Razdan. *J Com Dis* **1975**,7, 31-53.
[17] DJ Finney. Probit analysis. Cambridge University Press, Cambridge,**1971**, 3, 237-245.
[18] J Sambrook; EF Fritsch; T Maniatis. Molecular cloning: A laboratory manual, 2nd ed. Cold Spring Harbor, New York **1989**.
[19] RG Sharpe; RE Harbach; RK Butlin. *Sys. Entomol* ., **2000** 25, 263-272.
[20] SP Bhunya; GB Jena. *In vivo*,**1994**, 8(6),1087-1090.
[21] SB Nath;S Raju; RPG Krishna; SRP Kumar. *J Environ Biol*, **1996**17(4), 269-272.