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# DOMINANT LETHAL TEST BASED GENOTOXICITY EVALUATION OF PROPOXUR AND METHYL PARATHION IN *CULEX QUINQUEFASCIATUS*

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## Abstract

Propoxur and methyl parathion are nonsystemic insecticide used for the control of various chewing and sucking insects. Exposure to these pesticides may cause genotoxic effects in nontarget organisms including man. In the present set of investigations, mutagenicity of pesticides propoxur and methyl parathion was studied by applying dominant lethal test (DLT) on mosquito *Culex quinquefasciatus* taken as an experimental model. For this, larvae were exposed to $LC_{20}$ dose of pesticide for 24 hours after which they were transferred to chemical free water for further growth. The adult male mosquitoes emerging from treated larval stock were allowed to crossmate with normal virgin females under controlled conditions of mosquito rearing laboratory along with the parallel controls, separately for each pesticide. The eggs obtained from such females were allowed to hatch after which they were examined under suitable magnification. The number of unhatched eggs was taken as the measure for calculating the dominant lethality caused by the pesticides and the data was analyzed statistically by applying Student's t-test. The statistical analysis of the results for propoxur treated groups was 8.68±0.58 as against 3.20±0.69 in the control groups and methyl parathion treated groups gave the value 10.20±3.40 as against 4.23±2.76 in the control groups. The results indicated that these pesticides induced significant ($p<0.05$) dominant lethality.

## INTRODUCTION

In recent years considerable attention has been focused on the adverse effects of environmental toxicants on the reproductive process of animals. Pesticides are some of the compound frequently released into the environment due to their wide spread use in agriculture. Therefore, their presence in the environment could be hazardous to nontarget organisms including humans. Thus the screening of pesticides for their mutagenic activity by using appropriate tests and experimental animal models is important research activity. In reference to this, induced chromosomes aberrations, comet assay, sister chromatid exchange and related genetic tests have been successfully carried out to evaluate the genotoxic potential of suspected environmental mutagens (Atienzar and Jha, 2006; Naravaneni *et al*., 2006, Chaudhry *et al*., 2007; Chaudhry and Bhinder, 2009). The dominant lethal test (DLT) is one such *in vivo* method used for assessing the harmful effects of physical and chemical mutagens on the progenies of the treated parents on the basis of the frequency of viable and nonviable embryos produced from the effected parents (Manna and Sarkar, 1998; Chaudhry *et al*., 2009; Bhinder and Chaudhry, 2011). Mosquito *Culex quinquefasciatus* Say a test organism can be easily reared in the laboratory conditions, has short life cycle and lays eggs in groups (egg rafts) in which it is convenient to examine all the eggs laid by a mosquito. Propoxur have been shown to decrease the level of enzyme acetyl cholinesterase and increased the acetylcholine concentration in wistar rats (Weisbroth *et al*., 1983; Kobayashi *et al*., 1994). It also increased DNA damage in human lymphocytes in culture (Undeger and Basaran, 2005). Hreljac *et al*. (2008) evaluated the genotoxic potential of methyl parathion and found that it induced DNA damage and reduction in cell proliferation in human hepatoma HepG2 cells. In various tissues of rats it significantly decreased acetylcholinesterase activity (Ismail and Ismail, 2009). The present study evaluated the genotoxicity of propoxur and methyl parathion by dominant lethal test (DLT) in mosquito *Culex quinquefasciatus*.

## MATERIALS AND METHODS

Gravid females of *Culex quinquefasciatus* were collected from the cattle sheds in the village complex of Nadasahib, 20 kms South-east of Chandigarh. They were allowed to lay eggs in water filled petridishes placed in the breeding cages after which these eggs were allowed to hatch and a colony of larvae and adults was raised under suitable conditions of temperature and humidity in the mosquito rearing laboratory (Singh *et al*., 1975; Clements, 1996). Propoxur is a type of nonsystemic insecticide which is among the most widely used pesticides for home and outdoor use in gardens and lawns. Its molecular formula is $C_{11}H_{15}NO_3$, molecular weight 416.3 and CAS number is 114-26-1. Similarly, a methyl parathion is also a nonsystemic insecticide used for the control of chewing and sucking insects. It has a molecular formula of $C_8H_{10}NO_5PS$, molecular weight 263.2 and CAS number is 298-00-0. To perform dominant lethal test early fourth in star larvae were treated with $LC_{20}$ dose of propoxur by rearing them in pesticides containing distilled water for 24 hours after which the treated larvae were transferred to pesticide free distilled water for further development. Similarly, parallel controls of larvae were also reared in distilled water up to adult stages. Then the fixed number of treated adult males and virgin nontreated females were kept in breeding cages and were allowed to crossmate. After which the males were discarded while the females were fed on the blood of mice by holding the mice in a restrainer cage kept in the breading cages containing the experimental stocks. After three to four days these females laid eggs in water filled petridishes placed in the cages. In a separate set of experiments similar procedure was followed for treatment of the stocks with $LC_{20}$ dose of methyl parathion. After two to three days of maturation, the eggs laid by each female were carefully examined under suitable magnification. The eggs with open opercula were considered as hatched while those with closed opercula were taken as unhatched. The number of unhatched eggs was taken as the measure for calculating the dominant lethality caused by the pesticides. The percentage frequency of induced dominant lethality was calculated by dividing number of unhatched eggs in one egg raft with total number of eggs in the egg raft and quotient being multiplied with 100. The mean percentage, standard deviation and standard error were calculated for each group and the results were expressed as mean±SEM (standard error of the mean). The significance of dominant lethality as per the differences between control and test groups were determined by the Student's t-test and values of $P<0.05$ were taken to imply statistical significance.

**RESULTS AND DISCUSSION**

In the present set of experiments, five egg rafts of each control and treated individuals were studied. All the eggs were examined and hatched and unhatched eggs were counted for evaluating the percentage frequency of lethal mutations which produced nonviable eggs (Figs. 1 and 2). Mean percentage frequency of unhatched eggs was as low as 3.20 in the normal stocks as compared to propoxur treated stocks in which the frequency of unhatched eggs had increased to 8.68. However in another experiment the mean percentage frequency of unhatched eggs in the control stocks was 4.23. As compared to which it was increased to 10.20 in the methyl parathion treated stocks. Accordingly, the percentage frequency of dominant lethality induced due to propoxur was found to be 8.68±0.58 in treated as against 3.20±0.69 in the control groups while methyl parathion produced dominant lethality up to 10.20±3.40 in the treated as against 4.23±2.76 in the control groups (Table 1). From these values it was revealed that significant ($p<0.05$) dominant lethality was induced by both the pesticides. In the studies carried out so far, there had been a general consensus that the genetic basis of dominant lethality is mainly the induction of structural and numerical chromosomal anomalies which tend to induce nonviable zygotes, early embryonic deaths, sterility and semi sterility in the offspring's of effected parents (Chaubey *et al*., 1999). In similar studies on the effects of MMS, glycidamide, deltamethrin and decarbazine chromosomal abnormalities of different types were found to be responsible for the production of dominant lethal (Generoso *et al*., 1996; Shukla and Taneja, 2000; Adler *et al*., 2002). In some of the earlier studies, it was reported that propoxur increased the frequency of sister chromatid exchanges and micronuclei in human lymphocytes (Gonzalez *et al*., 1990). Oral administration of different dose concentrations of propoxur also induced micronuclei formation and chromosomal aberrations in bone marrow cells of Swiss albino mice and rat (Agrawal, 1999; Siroki *et al*., 2001). With higher doses up to 20 mg/kg propoxur produced brain cholinesterase inhibition in adult rats (Padilla *et al*., 2007). Lin *et al*. (2006) found chromosomal aberrations and sister chromatid exchanges in Chinese hamster ovarian cells. When similar studies were carried out to by Rupa *et al*. (1990, 1991) to evaluate the genotoxic potential of methyl parathion, a significant increase was seen in the increased frequency of sister chromatid exchanges and other chromosomal aberrations in human peripheral lymphocytes of smokers. It also produced increased number of abnormal sperms in swiss albino mice (Mathew *et al*., 1992). This pesticide was also responsible for significant increase in chromosomal aberrations in rat bone marrow cells, fish gill tissue and human peripheral lymphocytes (Kumar *et al*., 1993; Vijayaraghavan and Nagarajan, 1994; Das and John, 1999). Cakir and Sarikaya (2005) while evaluating the genotoxicity of this particular pesticide by employing wing somatic mutation and recombination test in *Drosophila melanogaster* found it to be quite genotoxic for the nuclear genetic content of this fly. In mammalian models like the Wister rats Narayana *et al*. (2005) observed a significant decrease in sperm count, increase in the abnormal sperms and decrease in the ascorbic acid levels in the testis of methyl parathion treated individuals. Salazar-Arredondo *et al*. (2008) and Pina-Guzman *et al*. (2009) have reported DNA damage in human spermatozoa from healthy volunteers exposed to it and decreased fertility in mice respectively.

The present results of propoxur and methyl parathion induced dominant lethality in *Cx. quinquefasciatus* which is suggestive of the fact that they are effective mutagens for the genome of mosquito and could be deleterious to the genome of other living systems. It may be added that dominant lethal test is an ideal parameter for evaluating the genotoxicity of pesticides in the subsequent progenies of the effected parents. It also raises a point of caution that the exposure to these pesticides may also affect the reproductive viability in other animals including those human subjects which handle such pesticides without the desired safeguards generally recommended for their use.

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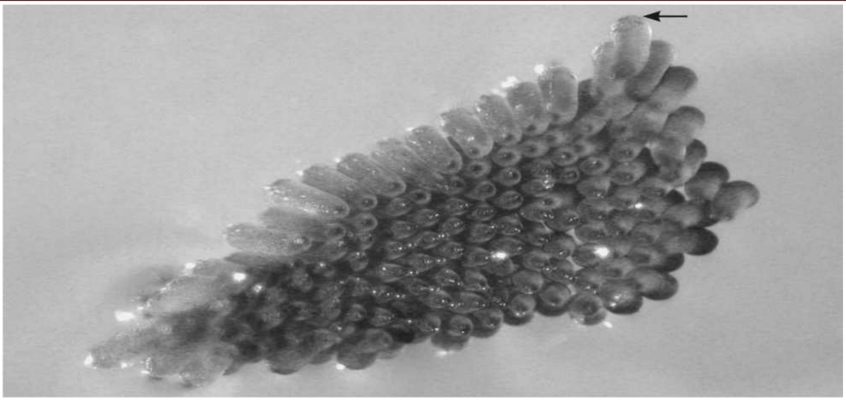

**Figure 1 Egg raft of *Culex quinquefasciatus* showing unhatched eggs with closed operculum (→)**

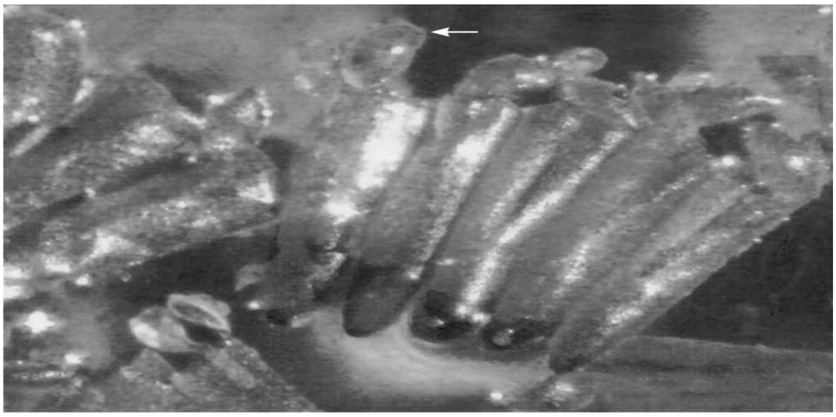

**Figure 2 Egg raft of *Culex quinquefasciatus* showing hatched eggs with open operculum (→)**

**Table 1**

**Statistical analysis of dominant lethality induced by propoxur and methyl parathion in *Culex quinquefasciatus.***

| Pesticides | Type of Stock | Number of Egg Crafts | Mean Percentage Frequency of Unhatched Eggs | Mean±SEM |
|---|---|---|---|---|
| **Propoxur** | Control | 5 | 3.20 | 3.20±0.69 |
| | Treated | 5 | 8.68 | 8.68±0.58* |
| **Methyl Parathion** | Control | 5 | 4.23 | 4.23±2.76 |
| | Treated | 5 | 10.20 | 10.20±3.40* |

SEM= standard error of the mean,

* Significant at $p<0.05$