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Evaluation of toxic potential of acephate and chlorpyrifos by dominant lethal test on *Culex quinquefasciatus*

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Abstract

The present paper deals with the toxicity evaluation of pesticides acephate and chlorpyrifos by applying dominant lethal test (DLT) on mosquito *Culex quinquefasciatus* taken as an experimental model. For this, the adult male mosquitoes emerging from LC_{20} 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 acephate treated groups was 9.49±1.50 as against 3.92±0.41 in the control groups and chlorpyrifos treated groups gave the value 9.94±1.92 as against 4.26±0.35 in the control groups. The results indicated that these pesticides induced significant ($p<0.05$) dominant lethality.

Key words

Acephate, Chlorpyrifos, Dominant lethality, *Cx. quinquefasciatus*

Introduction

Organophosphate insecticides are among the most widely used synthetic chemicals for the control of agricultural and domestic insect pests. The use of these pesticides has resulted in worldwide increase in food production, the control of disease carrying vectors and insect pests. Acephate and chlorpyrifos are two widely used organophosphate pesticides both in agricultural and non-agricultural arenas. The toxicity of acephate and chlorpyrifos like other organophosphate pesticides is attributed specifically to the inhibition of the enzyme acetylcholinesterase (Ecobichon, 1996). Therefore, their presence in the environment could be hazardous to nontarget organisms including humans. Acephate have been shown to induce chromosomal aberrations and micronuclei in bone marrow and peripheral blood erythrocytes of chick (Jena and Bhunya, 1994). Chaudhry *et al*. (2006) found abnormal increase in the incidence of intercalary heterchromatic linkages in the polytene chromosomes of treated larvae of mosquito *Anopheles subpictus*. Ozkan *et al*. (2009) also reported significant increase in the chromosomal aberrations, DNA damage and decreased mitotic index in cultured human peripheral blood lymphocytes. Mehta *et al*. (2008) and Yin *et al*. (2009) evaluated the genotoxic potential of chlorpyrifos and found that it caused a dose-dependent increase in DNA damage in the liver and brain cells of rats, and in erythrocytes and liver cells of Chinese toad, *Bufo bufo gargarizans* tadpoles. Coupled with DNA damage, Salazar-Arredondo *et al*. (2008) observed a number of sperm abnormalities in healthy human males *in vitro* while Farag *et al*. (2003), Tian *et al*. (2005) and Joshi *et al*. (2007) noticed decrease in embryo cell number leading to fetal death in rats and formation of micronuclei due to chromosomal breaks in treated rats and mice. In *Drosophila melanogaster*, Nazir *et al*. (2001) and Gupta *et*

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al. (2007) noticed a significant increase in the incidence of embryonic mortality, delayed emergence of adults from pupae and a severe reduction in survivorship of chlorpyrifos effected flies. The dominant lethal test (DLT) is *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; Chaudhry and Lovleen, 2010; 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. The present study evaluated the toxicity of acephate and chlorpyrifos 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). In order to select a dose for the assessment of toxicity of a chemical, it is desirable that it should neither be too strong that it kills all the test organisms nor too weak to give misleading data about its safe use. Therefore, in the present experiments, Lethal concentration 20 (LC_{20}) of both the pesticides was found to be an ideal concentration. This concentration was standardized by treating fixed number of second instar larvae for 24 hrs with different serial dilutions of 1% stock solution prepared in double distilled water. At the same time, parallel controls were also maintained in distilled water. Three replicates of 20 larvae were taken for each test and the percentage mortality was calculated from the number of larvae which survived out of the treated stock. The LC_{20} values in *Cx. quinquefasciatus* for 75% SP acephate and 95% EC chlorpyrifos as calculated by Probit analysis was 5 and 3.46 mg ml^{-1} respectively. To perform dominant lethal test early fourth instar larvae were treated with 5 mg ml^{-1} acephate (LC_{20} dose) by rearing them in pesticides containing distilled water for 24 hrs 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 3.46 ml ml^{-1} chlorpyrifos (LC_{20} dose). 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±SE. 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

On an average, each egg raft contained 197 eggs in the control groups out of which 189 hatched while 8 remained unhatched whereby the mean percentage frequency of unhatched eggs was 3.92. However, for acephate treated stocks the average number of eggs laid was 194 out of which 175 hatched while 19 remained unhatched whereby the mean percentage frequency of unhatched eggs was 9.49. Here the mean percentage frequency of unhatched eggs in the normal stocks was as low as 3.92 as compared to treated stocks in which the frequency of unhatched eggs had increased to 9.49. Accordingly, the percentage frequency of dominant lethality induced due to acephate was found to be 9.49 in the treated groups as against 3.92 in the control groups (Tables 1, 2). In another set of experiments for chlorpyrifos, each egg raft on an average contained 208 eggs in the control groups out of which 199 hatched while 9 remained unhatched whereby the mean percentage frequency of unhatched eggs was 4.26. However for chlorpyrifos treated stocks, the average number of eggs laid was 194 out of which 174 hatched while 20 remained unhatched. With this, the mean percentage frequency of unhatched eggs was 9.94. Here the mean percentage frequency of unhatched eggs was as low as 4.26 in the normal stocks as compared to treated stocks in which the frequency of unhatched eggs had increased to 9.94. Accordingly, the

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Table 1 : Values of hatched and unhatched eggs of control *Cx. quinquefasciatus*

No. of egg raft	Total no. of eggs in an egg raft	No. of hatched eggs in each egg raft	No. of unhatched eggs in each eggs	Percentage frequency of unhatched eggs	Mean percentage frequency of unhatched eggs
1	210	200	10	4.76	
2	175	170	5	2.85	
3	230	222	8	3.47	3.92±0.41
4	200	190	10	500	
5	170	164	6	3.52	

Table 2 : Values of hatched and unhatched eggs of *Cx. quinquefasciatus* exposed to acephate (5mg ml^{-1})

No. of egg raft	Total no. of eggs in an egg raft	No. of hatched eggs in each egg raft	No. of unhatched eggs in each eggs	Percentage frequency of unhatched eggs	Mean percentage frequency of unhatched eggs
1	158	148	10	6.32	
2	180	168	12	6.66	
3	225	197	28	12.44	9.49±1.50*
4	220	190	30	13.63	
5	190	174	16	8.42	

* significant at $p<0.05$

Table 3 : Values of hatched and unhatched eggs of control *Cx. quinquefasciatus*

No. of egg raft	Total no. of eggs in an egg raft	No. of hatched eggs in each egg raft	No. of unhatched eggs in each eggs	Percentage frequency of unhatched eggs	Mean percentage frequency of unhatched eggs
1	212	204	8	3.77	
2	228	216	12	5.26	
3	182	176	6	3.29	4.26±0.35
4	208	198	10	4.80	
5	214	205	9	4.20	

Table 4 : Values of hatched and unhatched eggs of *Cx. quinquefasciatus* exposed to chlorpyrifos (3.46 ml ml^{-1})

No. of egg raft	Total no. of eggs in an egg raft	No. of hatched eggs in each egg raft	No. of unhatched eggs in each eggs	Percentage frequency of unhatched eggs	Mean percentage frequency of unhatched eggs
1	240	210	30	12.5	
2	244	208	36	16.07	
3	148	139	9	6.08	9.94±1.92*
4	160	150	10	6.25	
5	180	164	16	8.8	

* significant at $p<0.05$

percentage frequency of dominant lethality induced due to chlorpyrifos was found to be 9.94 in the treated as against 4.26 in the control groups (Tables 3, 4). From these values it was revealed that significant ($p<0.05$) dominant lethality was induced by both the pesticides.

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 semisterility in the offsprings 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 lethals (Generoso *et al*., 1996; Shukla and Taneja, 2000; Adler *et al*., 2002). Pesticides significantly increases the cellular

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reactive oxygen species (ROS) production which cause chemical modifications and alterations in DNA and nucleoproteins, including modified bases and sugars and even strand breaks leading to chromosomal aberrations (Hreljac and Filipic, 2009). It is known that organophosphate pesticides are alkylating agents and alkylation of DNA bases either directly or indirectly via protein alkylation is involved in DNA disintegration. The phosphorus moiety in organophosphates is a good substrate for nucleophilic attack which might cause phosphorylation of DNA and lead to DNA damage. In some of the earlier studies, it was reported that acephate significantly affected the sperm shape abnormalities and mice implants (Farag *et al*., 2000a, b). Patnaik and Tripathy (1992) exposed larvae of *Drosophila* to different concentrations of pesticide and reported that it is genotoxic for the chromosomes of both somatic and germ cells of *Drosophila*. Gupta *et al*. (2007) reported that *Drosophila melanogaster* exhibited a dose-dependent reproductive abnormality. Delpuech and Meyet (2003) evaluated the effect of LD_{20} of chlorpyrifos on the reproduction and sex ratio of a wasp *Trichogramma brassicae* in which they found sex ratio distortion between males and females. This decrease in sex ratio was interpreted to be due to a decrease in fertilized eggs. De Silva and Samayawardhena (2005) while working on *Poecilia reticulata* observed that LC_{50} of chlorpyrifos affected the mating behavior, number of offsprings and their survival which reduced to 47% after 14 days when the individuals were treated with the 2 $\mu g\ l^{-1}$ as compared to 94% of survival in the controls. Tian and Yamauchi (2003) came across a significant increase in micronucleus production in the treated group of mice while Tian *et al.* (2005) reported that chlorpyrifos treatment given to pregnant female mice resulted in an abnormal reduction in the number of live foetuses. Salazar-Arredondo *et al*. (2008) came across DNA damage in human spermatozoa in the form of alterations in the sperm chromatin.

From the present study, it is concluded that 75% SP acephate and 95% EC chlorpyrifos are potential mutagen for the mosquito genome as they have induced significant dominant lethality. It can be suggested that these pesticides could be deleterious to the genome of other living systems.

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