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Evaluation of mutagenic potential of acetamiprid by dominant lethal test on *Culex quinquefasciatus*

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Abstract: Acetamiprid, a member of the neonicotinoid insecticide family, is a new class of insecticide which has recently entered the market place. It is very selective and provides outstanding control of sucking pests such as aphids and whiteflies of major crops. In the present investigation, dominant lethal test (DLT) is adopted for the evaluation of the genotoxic effects of acetamiprid using *Culex quinquefasciatus* as an experimental model. In this experiment, the males hatched from larvae treated with LD_{40} were cross mated with normal females and the results were based on the number of hatched and unhatched eggs laid by these females. The statistical analysis of the results for LD_{40} treated groups gave the values of 37.526±3.886 as against 5.23±0.77 from the nontreated groups which indicated significant dominant lethality of $p<0.01$. These results indicated that exposure of pesticides even at small dose level proved deleterious to the genome of mosquito and its subsequent generation.

Keywords: Dominant lethality, Acetamiprid, *Culex quinquefasciatus*

INTRODUCTION

The neonicotinoids are the newest major group of insecticides, which includes acetamiprid, imidacloprid, clothianidin, dinotefuran, nitenpyram, thiacloprid, and thiamethoxam (Tomizawa and Casida, 2005). Neonicotinoids have proved to be ideal alternatives to organophosphates and carbamates (Elbert *et al.*, 1998) with much lower rate of application as compared to traditionally used insecticides (Schmuck, 2001). In the present study, acetamiprid is selected which is used against sucking insects, such as aphids and whiteflies on leafy vegetables, cole crops, citrus, cotton, ornamentals, and fruiting vegetables. Mammals and humans are exposed to its residues during agricultural practice or when it enters the food chain. Once entered in the body it attacks on the central nervous system of insect by binding of acetylcholine, the major excitatory neurotransmitter in insects to the nAChRs, that further cause excitation and paralysis, followed by the death of the insect. Acetamiprid has relatively low acute and chronic toxicity in mammals with no evidence of carcinogenicity, neurotoxicity, mutagenicity and/or endoctrine disruption (USEPA, 2002). Data related to its toxicity is very scarce. But some of the recent studies conducted by Kocaman and Topaktas (2007, 2009) on human peripheral lymphocytes in culture showed various types of chromosomal aberrations and sister chromatid exchanges. In the last few years several toxicity testing procedures have been designed, modified and improved in such a way that they are acceptable to toxicologists (Lu, 1991; Anderson and Conning, 1993). In recent years, a number of *in vivo* and *in vitro* protocols have been successfully used to evaluate the genotoxic potential of suspect environmental mutagens (Amer and Ali, 1968; Gaulden and Liang, 1982; Jain and Sarbhoy, 1988). Among them, dominant lethal test (DLT) is one such *in vivo* procedure which is used for evaluating the mutagenic potential of pesticides on the progenies of the treated parents. It is based on the frequency of viable and nonviable embryos produced from crosses between treated males with untreated females in which dominant lethal effect is manifested in the form of embryonic deaths. Therefore, this test also helps to determine the sensitivity of the germ cells to the chemical mutagens (Manna and Sarkar, 1998).

In the present investigations, a mosquito *Culex quinquefasciatus* was considered an ideal test system as it has a high reproductive potential and only six as the diploid number of chromosomes, whereby abnormalities present in the germ cells can be easily detected along with visible phenotypic changes in the adults. These mosquitoes lay eggs in groups (egg rafts) in which it is convenient to observe all the eggs laid by an individual. In order to meet the present objectives the dominant lethality of acetamiprid was evaluated by at LD_{40} dose level. Although, this dose is considered sublethal yet it prove high enough to cause detectable effect.

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MATERIALS AND METHODS

Acetamiprid ((E)-N- [(6-chliro-3-pyridyl)]-N-cyano-N-methyl acetamidine) is commonly sold in the form of white solid powder (Aventis Crop Sciences, U.S.A) under CAS no. 135410-20-7 and molecular formula $C_{10}H_{11}ClN_4$ (Fig. 1) and molecular weight of 222.68. For the present study, LD_{40} was calculated by probit analysis and were found to be $8.9x10^{-4}$ µl/ml (Finney, 1971, Fig. 2).

The gravid females of *Culex quinquefasciatus* Say were collected from village inhabitation of a rivulet, 20 kms East of Chandigarh. They were allowed to lay eggs in water filled petridishes placed in the breeding cages. The egg rafts obtained in this way were allowed to hatch and the larvae were reared on a protein rich diet consisting of a mixture of finely powdered dog biscuits and yeast powder in the ratio of 6: 4 respectively. A colony was raised under suitable conditions of temperature and humidity in mosquito rearing laboratory (Krishnan, 1964; Singh *et al.*, 1975). Fixed number of freshly hatched healthy fourth instar larvae were treated with LD_{40} dose of the pesticide by rearing them in acetamiprid containing rearing medium for 24 hours after which they were transferred to pesticide free water and allowed to grow upto adult stages. Similarly, parallel controls of larvae were also reared upto adult stages and the freshly hatched adults of both the sexes were fed on 10% sucrose/ glucose solution. The treated males were crossmated with nontreated females after which the females were provided with a blood meals by trapping a mice in a restrainer cage before keeping the same in the breeding cage (Muro and Goyer, 1969). After 4-5 days, females laid eggs which were allowed to hatch and after one week all the eggs were examined under suitable magnification of a dissecting microscope. The eggs with open opercula were considered as hatched while those with closed opercula (Figs. 3, 4) were taken as unhatched. The frequency of unhatched egg was taken as the criterion to evaluate the effects on the viability of embryos. Based on these figures the percentage frequency of induced lethality was calculated by applying the following formula.

Percentage frequency of unhatched eggs =

$$\frac{\text{No. of unhatched eggs in an egg raft X 100}}{\text{Total no. of eggs in an egg raft}}$$

Statistical analysis: The whole experiment was repeated five times and the statistical analysis was carried out by applying Student t- test using significance level of 0.01.

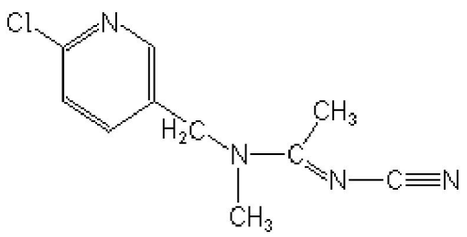

Fig. 1. *Chemical structure of acetamiprid.*

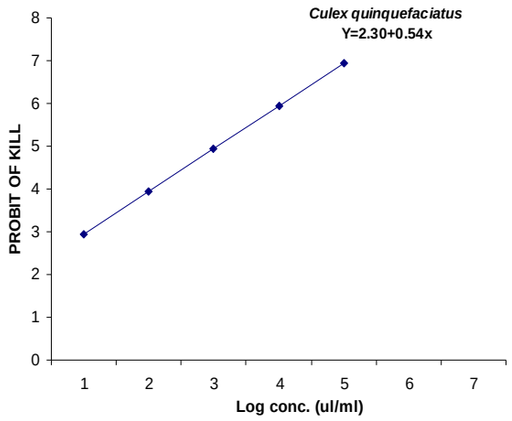

Fig. 2. *Relationship between the probit of kill and LD_{40} of acetamiprid showing the regression line represented by the equation Y= a+ bx.*

RESULTS AND DISCUSSION

Assessment of dominant lethal mutations through crossing experiments is a widely accepted parameter for determining the genotoxicity of environmental mutagens (Suter, 1975; Manna and Sarkar, 1998). In the present set of experiments, the percentage frequency of lethal mutations which produced nonviable eggs is presented in Tables 1 and 2. Table 1 represents the percentage frequency of unhatched eggs of nontreated individuals of C*ulex quinquefasciatus*, whereas Table 2 represents the percentage frequency of unhatched eggs of C*ulex quinquefasciatus* treated with LD_{40} of pesticide.

Table 1. Percentage frequency of unhatched eggs in control stocks *of Cx. quinquefasciatus.*

No. of Egg raft	Total no. of eggs in an egg raft	No. of eggs hatched	No. of eggs unhatched	% frequency of unhatched eggs
1	112	104	8	7.14
2	118	112	6	5.08
3	103	96	7	6.8
4	95	92	3	3.16
5	100	96	4	4

Table 2. Percentage frequency of unhatched eggs of *Cx. quinquefasciatus* treated with LD_{40} of acetamiprid.

No. of egg raft	Total no. of eggs in an egg raft	No. of eggs hatched	No. of eggs unhatched	% frequency of unhatched eggs
1	100	65	35	35.00
2	92	47	45	48.49
3	115	83	32	27.83
4	107	60	47	43.93
5	97	66	31	31.96

Table 3. Statistical analysis of dominant lethality of *Cx. quinquefasciatus* treated with LD_{40} of acetamiprid.

Type of larval stock	No. of egg rafts counted	% frequency of unhatched eggs	Standard deviation	Standard error	't' value
Control	5	5.236	1.727	0.7724	7.505
Treated	5	37.526	8.689	3.886	d.f = 4

d.f. = degree of freedom, $p<0.01$

Accordingly, the average frequency of unhatched eggs was 37.44% as against 3.88% in the controls. The percentage frequency of dominant lethality was 37.52 ± 3.88 as against 5.23 ± 0.77 in the controls. The t value was 7.50 at d.f. 4 which showed considerably significant value at $p<0.01$ (Table 3, Figs. 3, 4, 5, 6). Most of the mutagens are known to have a damaging effect on the viability of the treated gametes and their chromosomes which ultimately reduces the normal production of viable embryos. This evidence was also provided by Brewen *et al.* (1975) who showed that the broken chromosomes due to the mutagenic action of chemical mutagen were eventually lost at anaphase, resulting in the death of the developing embryo. Bender *et al.* (1973, 1974) proposed a model by which chromosomal aberrations could be induced by a mutagen in the germ cells. Their studies strongly supported the view that the dominant lethality was the outcome of the loss of chromosomal material by way of deletions in the chromosomes of gametes. This was due to high frequency of damage to the male germ cell chromosomes which ultimately resulted in early embryonic deaths. Sharma *et al.* (1989) performed dominant lethal tests using *Cx. quinquefasciatus* as an experimental insect in which the extent of dominant lethality was assessed by measuring the percentage frequency of hatched and unhatched eggs in both treated

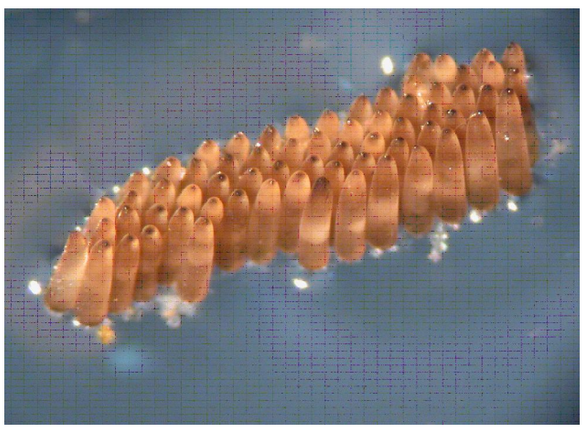

Fig. 3. *Egg raft with closed opercula of nontreated Cx. quinquefasciatus.*

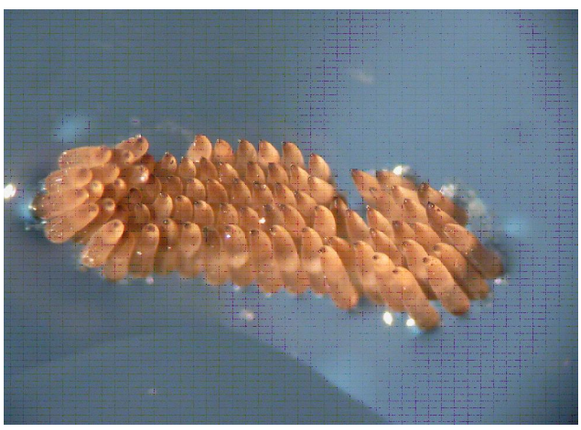

Fig. 5. *Egg raft with closed opercula of treated Cx. quinquefasciatus.*

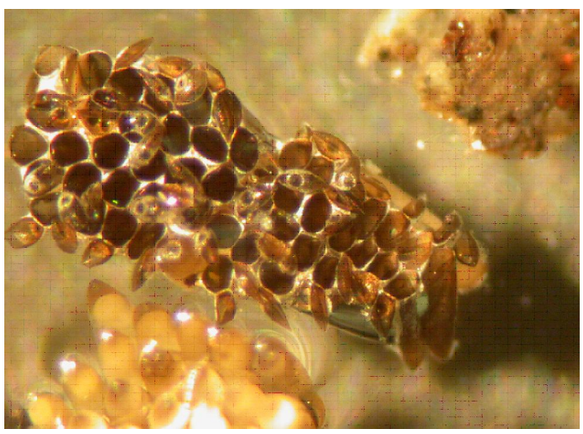

Fig. 4. *Egg raft with open opercula of nontreated Cx. quinquefasciatus.*

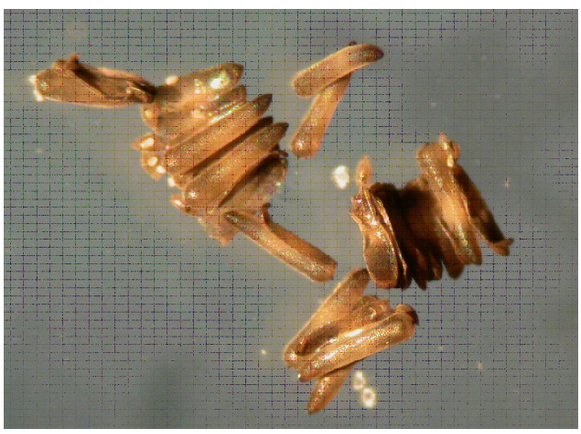

Fig. 6. *Egg raft with open and closed opercula of treated Cx. quinquefasciatus.*

and control stocks of mosquitoes. More recent, Chaudhry and her co- workers also performed similar experiments with improved protocols of cytogenetics to evaluate the genotoxicity of some pesticides such as chloropyrifos, imidacloprid, cypermethrin, carbaryl and monocrotophos F_1 generations after mating treated males with different sets of virgin females. The results showed the occurrence of significantly high level of dominant lethality (Chaudhry and Anand, 2004; Chaudhry *et al.*, 2008; Chaudhry and Lovleen, 2008; Bhinder *et al.*, 2009). Some of the recent reprints indicate that exposure to acetamiprid is related to induction of chromosomal aberrations and sister chromatid exchanges in human peripheral lymphocytes at almost all the concentrations and treatment times (Kocaman and Topaktas, 2007, 2009). To the contrary, the test carried out on lower animals like termites showed that even at low dose of 4.8 ppm of acetamiprid, more than 90% individuals died within 120 hr of exposure (Mo *et al.*, 2005). Related to such studies on the toxicity of acetamiprid, the larvae of mosquito *Culex pipiens pallens* were also exposed to pesticides, after which the results indicated that these larvae were highly sensitive to acetamiprid as their mortality rate was significantly high as compared to the normal nontreated stocks. The 1st instar larvae were the most susceptible to acetamiprid as compared to 4th instar larvae which were found to be more tolerant to the action of acetamiprid (Mo *et al.*, 2008). According to El Hassani *et al.*, (2008) and Yassine *et al.*, (2009), field studies showed that acetamiprid was also found to affect the non-target species of insects like honeybees (*Apis mellifera*). When the toxicity of acetamiprid was compared with imidacloprid and thiamethoxam among the species of termites it was found that acetamiprid was more toxic than imidacloprid but less toxic than thiamethoxam (Rust and Saran, 2008).

In summation it may be added that dominant lethal test is an ideal parameter for evaluating the genotoxic potential of acetamiprid and other pesticides at different dose concentrations which prove harmful to the genomic contents of the test organism mosquito. The present study shows that genetic damage caused by such a lower dose of LD_{40} is so high which further proves the risk of acetamiprid at further higher doses. It also raises a point of caution that, the exposure directly acting pesticide could be deleterious to the genome of other living system including man.

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