INTERNATIONAL JOURNAL OF ADVANCED BIOLOGICAL RESEARCH

www.scienceandnature.org

PESTICIDES EFFECT ON GENETIC COMPONENTS: A GENOTOXIC STUDY ON CULEX *QUINQUEFASCIATUS* BY APPLYING DOMINANT LETHAL TEST

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ABSTRACT
The use of pesticides to control weeds, insects, and other pests has resulted in a range of benefits, including increased food production and reduction of insect-borne disease, but has posed challenge to maintain human health and safe environment. Exposure to the pesticide may cause genotoxic effects on the target as well as nontarget organisms including man. Therefore, the genotoxic evaluation of such pesticides has become a priority area of research. In the present investigation, the genotoxic effect of imidacloprid and acetamiprid was studied, which belong to a new class of neonicotenoids pesticides. For this purpose, dominant lethal test (DLT) was adopted to evaluate the genotoxicity of both the insecticides on the reproductive potential of using *Culex quinquefasciatus* as an ideal test system. Dominant lethal test (DLT) is used to evaluate the mutagenic effect of pesticides on the progenies of treated parents. In this experiment, the males hatched from larvae treated with LD_{20} 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 imidacloprid gave the values of 31.56 ± 3.28 and that of acetamiprid gave the value 23.76 ± 1.84. The results obtained from both the insecticides indicated significant dominant lethality of $p<0.01$. These results indicates the risk of mutation by of imidacloprid and acetamiprid even at lower doses.

KEYWORDS: Imidacloprid, Acetamirpid, Dominant lethal Test, *Culex quinquefasciatus*.

INTRODUCTION

During the past six decades industrialisation and agricultural development had been the chief sources of pollution. Rapid industrialisation and green revolution have introduced a large variety of chemicals into our environment. According to some estimates, as many as 500 – 1000 new chemicals are being added annually (Sharma, 1997). Several of these chemicals get biomagnified in organisms or get biologically transformed into more toxic compounds which are divided into inorganic contaminants and heterogeneous groups of organic compounds. Many of them interact with the living cells at the genomic level and induce qualitative and quantitative alterations in them which ultimately interfere with the integrity of genome (Belfiore and Anderson, 2001; Theodorakis, 2001; Staton *et al*., 2001). Out of all the categories of chemical formulations, insecticides are the largest group consisting of organophosophates, carbamates, pyrethroids, neonicotenoids and organochlorines depending upon their chemical composition, properties and the category of the organic compounds from which they are synthesized. Neonicotenoids are synthetic analogues of the natural insecticide nicotine which is an active component of tobacco. Nicotine causes higher level of toxicity in mammals and limited insecticidal spectrum which ultimately lead to the development of a newer group called neonicotinoids. Neonicotenoids are broad spectrum insecticides for the effective control of aphids, whiteflies and other insects, especially homopteran pest species and chewing insects belonging to order Coleoptera and Lepidoptera (Elbert *et al.,* 1990; Takahashi *et al.,* 1992). They are also used for the control of cat and dog fleas (Mullins, 1993; Wang *et al.,* 1995; Nauen *et al.,* 1998). Neonicotinoids have proved to be ideal alternatives to organophosphates and carbamates (Elbert *et al.,* 1998) with much lower amount needed of application as compared to traditionally used insecticides (Schmuck, 2001). In the present research work, an attempt was made to evaluate the genotoxic potential of imidacloprid and acetamiprid by using the genetic material of Culex quinquefasciatus. The larvae of these species were exposed to LD_{20} of imidacloprid and acetamiprid.

Imidacloprid which is a neonicotinoid, was the first to be registered for use as a pesticide in U. S. A. in 1994. It is a novel insecticide derived from a nitromethylene insecticidal chemical called nithiazine which is closely related to tobacco toxin nicotine. It is used against soil, seed, timber and animal pests as well as for foliar treatment of crops such as those including cereals, cotton, grains, legumes, potatoes, pome fruits, rice, vegetables and grasses. Imidacloprid acts as agonists at the insect nicotinic acetylcholine receptor (nAChR). The another insecticide selected for the present investigations was acetamiprid which is more commonly used neonicotinoid sold under the brand name of 'Sharp', a product of Aventis Crop Science (now Bayer) U.S.A. In addition to 'Sharp' it is also sold as Assail, Intruder, Mosiplan, Rescate and

Pristine. It belongs to first generation neonicotinoids which have contact and systemic activity via foliar applications (Horowitz *et al.,* 1998). It was registered in 2002 for pest control of leafy vegetables, cole crops, fruiting vegetables, pome fruits, cotton, citrus, stone fruits and ornamentals plants. Acetamiprid and imidacloprid possess the same physico- chemical peoperties, but acetamiprid happens to be more hydrophilic (Buchholz and Nauen, 2001). 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 (Evans 1977; Gaulden and Liang 1982; Kurth and Bustin 1985; Jain and Sarbhoy 1988; Crumpton *et al.* 2000). 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. Primarily, this effect is linked with the chromosomal damage (structural and numerical abnormalities) but gene mutations and other toxic effects cannot be excluded. 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 imidacloprid and acetamiprid was evaluated by applying LD_{20} dose of pesticide. Although, this dose is considered sublethal yet it prove high enough to cause detectable effect.

MATERIALS AND METHODS

Imidacloprid (1-(6-chloro-3 pyridylmethyle)-N-nitroimidazolidin-2 ylinedeneamine 1 -[(6-chloro-3-pyridinyle) methyle] -N-nitro-2-imidazolidimine) is sold in the colourless liquid (Bayer Environmental Science, Australis) under CAS no. is 138261-41-3 and molecular formula C_9 H_{10} Cl N_5 O_2 (Fig. 1) and molecular weight 255.7. 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. 2) and molecular weight of 222.68. For the present study, LD_{20} for imidacloprid and acetamiprid of *Culex quinquefaciatus* was calculated by probit analysis and were found to be 1.1 x 10^{-3} µl/ml and 2.63x10^{-3} µl/ml respectively (Finney, 1971, Figs. 3, 4).

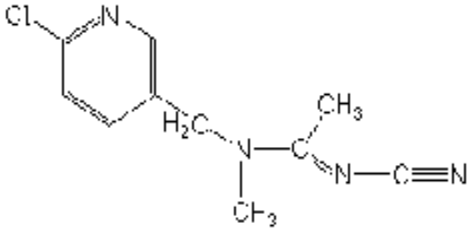

Fig. 1: Chemical structure of imidacloprid

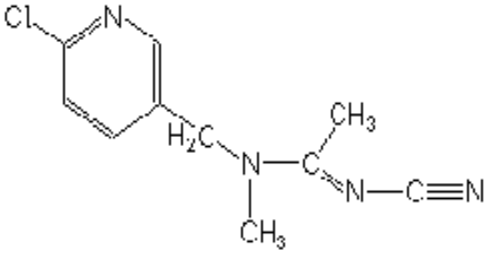

Fig. 2: Chemical structure of acetamiprid

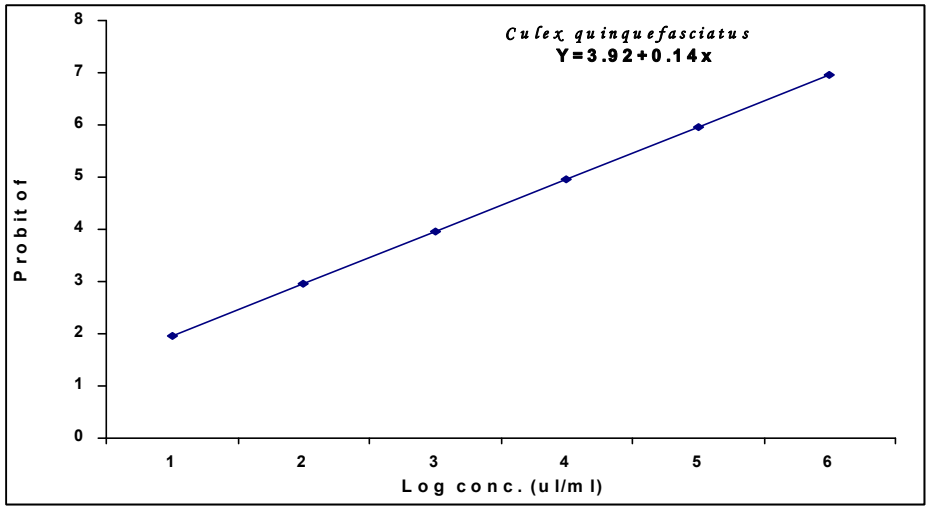

Fig 3: Relationship between the probit of kill and LD_{20} doses of Acetamaprid showing the regression line represented by the equation Y= a+ bx

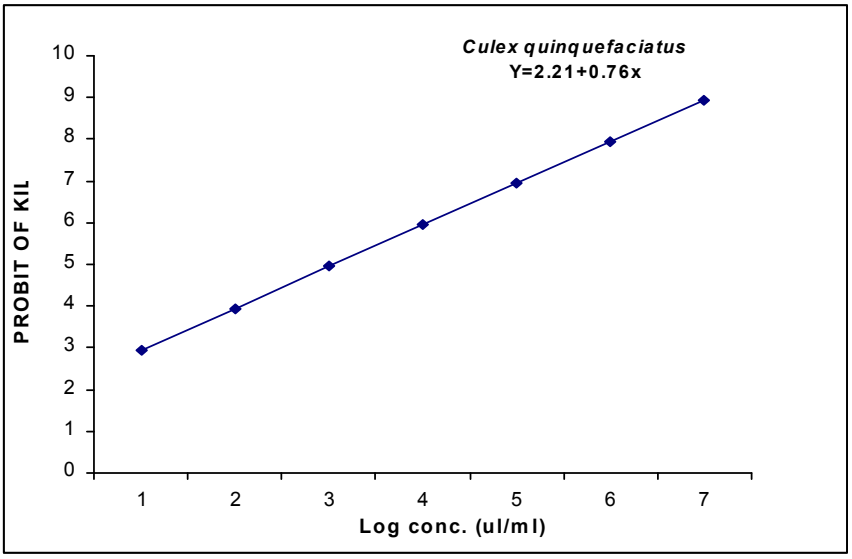

Fig 4: Relationship between the probit of kill and LD_{20} doses of Acetamaprid showing the regression line represented by the equation Y= a+ bx

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 was treated with selected dose of the pesticide by rearing them in insecticide 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 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.

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

The whole experiment was repeated five times and the statistical analysis was carried out by applying Student t-test using significance level of 0.05.

RESULTS

During the course of present research work the genotoxic effect of imidacloprid and acetamiprid was expressed in the form of eggs which did not hatch. The eggs with open opercula were considered as hatched while those with closed opercula were taken as unhatched (Figs. 5-10). This ultimately showed the damaging effect of selected pesticides on the viability of the treated gametes and their chromosomes which reduces the normal production of viable embryos

Fig. 5: Normal egg raft of *Culex quinquefasciatus* with closed opercula

Fig. 6: Egg raft with closed opercula of *Culex quinquefasciatus* treated with LD_{20} doses of imidacloprid

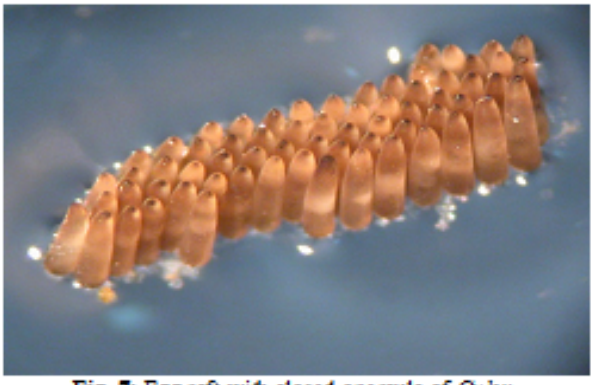

Fig. 7: Egg raft with closed opercula of *Culex quinquefasciatus* treated with LD_{20} doses of acetamiprid

Fig. 8: Normal egg raft of *Culex quinquefasciatus* with open opercula

Fig. 9: Egg raft with open and closed opercula of *Culex quinquefasciatus* treated with LD_{20} doses of imidacloprid

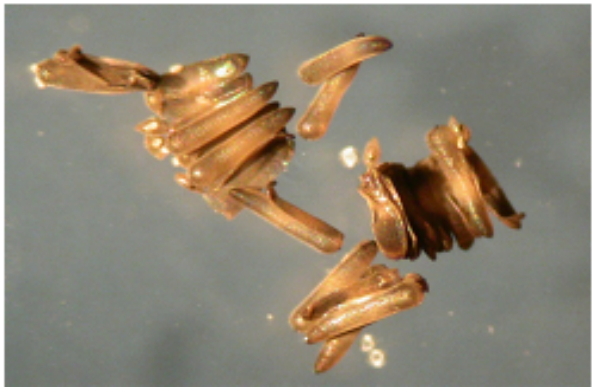

Fig. 10: Egg raft with open and closed opercula of *Culex quinquefasciatus* treated with LD_{20} doses of acetamiprid

The percentage frequency of lethal mutations which produced nonviable eggs is presented in Tables 1-4. Accordingly, the percentage frequency of dominant lethality induced due to imidacloprid at LD_{20} was found to be 31.56 ± 3.28 as against 13.2 ± 1.65 in the control with 't' value 9.95 while for acetamiprid the value was 23.76±1.84 as against 5.23±0.77 from the controls, while the 't' value was 8.56 at d. f. 4. The results obtained from both the insecticides indicated significant dominant lethality. In the similar set of study, when same species of mosquito treated with different pesticides, represents, significant dominant lethality at LD_{20} dose level. These results also prove the genotoxic effects of the pesticides on subsequent generations (Chaudhry *et al.,* 2009, Bansal and Chaudhry 2011).

TABLE 1: Different sets of parallel experiments to investigate the dominant lethality in the control and imidacloprid treated stocks of *Culex quinquefaciatus*.

CONTROL

Egg rafts	Percentage frequency of unhatched eggs	Mean of percentage frequency	Standard deviation	Standard error
1	9.44			
2	12.17			
3	9.6			
4	7.5	13.2	3.70	1.65
5	10.07			

TREATED

Egg rafts	Percentage frequency of unhatched eggs	Mean of percentage frequency	Standard deviation	Standard error
1	33.75			
2	31.03	31.56	7.34	3.28
3	26.53			
4	34.13			
5	32.37			

TABLE 2: Statistical analysis of dominant lethal mutations in the treated and control stocks of *Culex quinquefasciatus*

Type of stock	Mean ± S. E.	' t' value d. f.= 8
Treated	31.56 ± 3.28	9.95
Control	13.2 ± 1.65	

S. E. = standard error
d. f. = degree of freedom
* = significant p> 0.05

TABLE 3: Different sets of parallel experiments to investigate the dominant lethality in the control and acetamiprid treated stocks of *Culex quinquefaciatus*.

CONTROL

Egg rafts	Percentage frequency of unhatched eggs	Mean of percentage frequency	Standard deviation	Standard error
1	7.14			
2	5.08			
3	6.8	5.23	1.72	0.77
4	3.16			
5	4			

TREATED

Egg rafts	Percentage frequency of unhatched eggs	Mean of percentage frequency	Standard deviation	Standard error
1	23.47			
2	29.41			
3	18.75	23.76	4.12	1.84
4	21.24			
5	25.93			

TABLE 4: Statistical analysis of dominant lethal mutations in the treated and control stocks of *Culex quinquefasciatus*

Type of stock	Mean ± S. E.	' t' value d. f.= 8
Treated	23.76 ± 1.84	
Control	5.23 ± 0.77	8.56

S. E. = standard error
d. f. = degree of freedom
* = significant p> 0.05

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). 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.

With the application of this test, damage due to these pesticides could be studied by following appropriate experimental procedures to evaluate the indirect damage caused to the germ cells leading to the effects on the developmental or embryonic stages of the insects. In fact, it is a type of dual test system which presents the indirect effect on the germ cells and direct effect on the developing embryos and/or progenies of the treated parents. The dominant lethal test is preferably carried out by treating the males of the species which are mated with virgin females. The mutations in the males are in the form of chromosomal aberrations and related genetic lesions which are carried by the spermatozoa without interfering with their viability to fertilize the egg (Bateman and Epstein, 1971). In summation it may be added that dominant lethal test is an ideal parameter for evaluating the genotoxic potential of imidacloprid and 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 acetamiprid is much higher as compared to imidacloprid which further proves the risk of acetamiprid even at lower 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 and his animals of economic importance.

ACKNOWLEDGEMENT

The authors are grateful to Chairperson, Department of Zoology and Co-ordinator of Centre of Environment and Vocational Studies, Panjab University, Chandigarh for providing necessary facilities to carry out present research work. Authors are also thankful to Photographic section of Panjab University for providing needful help to develop the photos related to the work. PGIMER, Chandigarh is also acknowleged for the support.

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