Antifertility Activity of Apron 35SD in Silkworm *Bombyx mori*

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ABSTRACT

The antifertility effects of Apron 35SD (Methyl D, L-N-(2-6-dimethyl-phenyl)-N-(2'-methoxyacetyl-alaninate), a new chemical fungicide produced by Ciba-Geigy, in silkworm *Bombyx mori* has been investigated. 50 μl of 50, 100, 200 and 400ppm of the chemical in 0.75% NaCl were injected into five day old male and female pupae at the wingbud region. The moths emerged out of these were mated to untreated moths of opposite sex, and female moths of both the crosses were allowed to lay eggs. The results showed that when males were treated, the chemical induced significant amount of dominant lethals. The crosses involving the treated females produced a large percentage of unfertilized eggs. The percentage of dominant lethals and unfertilized eggs were increased proportionately with the increase in the concentration of the chemical administered. Thus Apron 35SD is both mutagenic and antifertility agent in silkworms.

Key words: Silkworm *Bombyx mori*, Apron 35SD, dominant lethals, antifertility.

Introduction

Human beings are often exposed to a wide spectrum of both synthetic and naturally occurring chemicals. A number of them are mutagenic and therefore have hazardous effects on the human population. They enter into biological system by various ways. Apron 35SD (Methyl D,L-N-(2-6-dimethyl-phenyl)-N-(2'-methoxyacetyl-alaninate) of Ciba-Geigy Ltd. Basle, Switzerland is widely used fungicide to protect maize, sorghum, millet and sunflower against the systemic diseases caused by downy mildew. According to product information of Ciba-Geigy Ltd., Apron 35SD is less toxic to mammals and virtually non-toxic to fishes and birds. However, the preliminary studies of Pai *et al*(1) in silkworm *Bombyx mori* on the effect of Apron 35SD indicate that it can produce certain genetic effects. With these background information, studies have been conducted to analyse the effects of Apron 35SD in *B. mori*.

Materials And Methods

Indigenous bivoltine races of Kalimpong-A available at Sericulture Research Project, Department of Studies in Zoology, University of Mysore, Manasagangotri, Mysore-570006, formed the material for the present experiments. 50 μl of the concentration of 50, 100, 200 and 400 ppm of Apron 35SD in 0.75% NaCl were injected into the five day old male and female pupae separately at their wingbud region as described by Tazima(2). Male and female pupae injected with 50 μl of 0.75% NaCl crossed with untreated moths of the opposite sex formed the control. Five replicates of five each pupae were taken for experiments and the pooled data is represented in Tables 1 and 2. The treated pupae were allowed to develop further. After the emergence of the moths, treated males were crossed with untreated female moths and treated female moths were crossed with untreated male moths. The egg layings were prepared from the above crosses were permitted to hatch only after subjecting the eggs to hot acid treatment to break the diapause artificially(2). The number of embryonic deaths in the cross involving treated males and untreated females and unfertilized eggs in the cross between treated female and untreated males were calculated. In silkworm *B.mori*, the unfertilized eggs will have yellow colour while fertilized but unhatched eggs will be of dark grey colour, which can be distinguished with ease. The occurence of unhatched eggs was accounted for dominant lethals and the unfertilized eggs for unfertility due to the chemical administration. All the experiments were carried out at a standard laboratory conditions and the maintenance of the experimental specimens was followed as per the Krishnaswami(3) procedure. The results obtained thereby were subjected for statistical analyses by following the ANOVA test.

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Results

Table 1 reveals the data on the Apron 35SD induced dominant lethals in silkworm *B.mori*. Table shows that a significant amount of dominant lethals were induced when male moths of chemical treated batches were crossed to untreated female moths. It can also be seen from the same table that the induction of dominant lethals induced is directly proportional to the concentration of the chemical tested.

TABLE-1

Percentage of Dominant lethals induced by Apron 35SD in silkworm *B. mori*

Concentration (ppm)	Eggs counted	Eggs unhatched	Percentage of dominant lethals
Control (0)	1579	48	3.04
50	1062	36	3.39*
100	1369	125	9.13*
200	1446	168	11.62*
400	1352	173	12.80*

'*' = Statistically significant at 0.005 level by ANOVA.

Table 2 incorporates the results on the effect of Apron 35SD on fertility in silkworm *B.mori*, wherein 5 day old male and female pupae were injected with the chemical. Table reveals that there is no significant reduction in fertility when the cross involves treated males and untreated females. However, significant reduction in fertility was noticed when the cross involves treated females and untreated males. The percentage of increase in unfertility was directly proportional to the concentration of the chemical used.

TABLE-2

Effect of Apron 35SD on fertility in silkworm *Bombyx mori*.

Concentration (ppm)	Males treated			Females treated		
	Eggs Counted	Un-fertilized	Unfertility percentage	Eggs Counted	Un-fertilized	Unfertility percentage
Control (0)	1926	13	0.67	1926	13	0.67
50	1062	08	0.75	1513	73	4.82*
100	1369	12	0.88	963	76	7.89*
200	1444	13	0.90	1414	163	11.53*
400	1352	15	1.11	1199	232	19.35

'*' = Statistically significant at 0.005 level by ANOVA.

Discussion

Dominant lethals test is one of the widely used protocols (1, 2, 4, 5) to analyse the mutagenic effects of a chemical in animal test system. Tazima(2) considers the silkworm as one of the best non-mammalian test system for mutagenic studies. Further, there are several reports on mutagenecity of many chemicals on silkworm *B. mori*(2). The present studies reveal that Apron 35SD is mutagenic in silkworms at the concentrations tested. As is known from earlier workers(4), that injection at the pupal stages have more direct effects on the spermatids than injecting the chemical in any other stage of development, because of the spermatogenic activity is observed in the early pupal stages and perhaps the same reason may be attributed to the increased percentage of dominant lethals proportionate with the increased concentration of the chemical employed.

Fertility is one of the fitness parameters used in evaluation in toxicity studies. Fertility serves as a good indicator of various somatic effects caused by a chemical in the test system(5). Present studies reveal that fertility reduced from 99.33% to 80.61%, a statistically significant variation, when cross is between treated female and untreated males. However, reciprocal cross, where treated males and untreated females are involved, did not show a significant reduction in fertility.

Conclusion

Apron 35SD is mutagenic and an antifertility agent in silkworm *B. mori* at the concentrations tested.

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