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EFFECT OF ETHYL METHANESULFONATE ON VIABILITY AND COMMERCIAL CHARACTERS OF SILKWORM *BOMBYX MORI* L., AND THEIR INHERITANCE

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ABSTRACT

Two races of mulberry silkworm namely Pure Mysore and NB_4D_2, at the age of fifth instar first day were treated with different doses of ethyl methanesulfonate (EMS) by oral injection. The treated larvae along with their respective controls were allowed to continue development. Silkworms of both the races exhibited gradual reduction in the viability as the dose of EMS increased. The F_1- F_4 progenies were obtained by selfing the moths emerged from the EMS treated silkworms and control sets separately. The experimental results clearly indicated that the silkworm batches treated with lower doses like 2.5 and 5 mM of both the races exhibited improvement in commercial characters when compared to their respective controls as well as 10 mM sets. In addition, the altered characters exhibited regular inheritance from generation to generation.

Keywords: Ethyl Methanesulfonate, Viability, Commercial Characters, Mutation, Inheritance

INTRODUCTION

Studies on the mutagenesis of silkworm, *Bombyx mori* L., have been in progress for the last few decades to synthesize new gene combinations with improved commercial qualities. In recent years, genetic manipulation has emerged as one of the major tools to improve the commercial qualities. Majority of the work has been

carried out to induce mutations in silkworm through physical mutagens rather than chemical mutagens. Of the physical mutagens, radiations have been extensively exploited to bring a change in the genotype of silkworm **[1]**. Tanaka, 1934, **[2]** was the first one to induce mutations in silkworm after the popular work of the artificial transmutation of the gene by Muller, 1927, **[3]**. Since then, many efforts have been made by various scientists including Tazima, 1964, **[4]** and numerous interesting results have been accumulated. Some mutants were entirely new and contributed to the construction of chromosome maps. Also, in recent years Datta *et al*., 1981, **[5]**, Sitarama Iyengar *et al*., 1981, **[6]**, and Subramanya and Sreerama Reddy, 1982, **[7]** have succeeded in the induction of beneficial mutations in the silkworm, *Bombyx mori* L. using radiations. Haung and Onimaru, 1986, **[8]** have succeeded in inducing the translocation of Z chromosome segment to the W chromosome in silkworm by the irradiation of pupae with X-rays. Lee *et al*., 1989, **[9]** induced sex limited cocoon color character by translocation of yellow blood gene Y (II- 25.6) onto W chromosome by gamma irradiation. Lakshmikumari, 1995, **[10]** reported that the gamma rays induced mutations including chromosomal aberration, besides alteration in the physiological and biochemical constituents and irradiation at lower doses improved the commercial character of silkworm, *Bombyx mori*. Bhoopathy and Muthukrishan, 1985, **[11]** studied the effect of a chemical mutagen, diethylsulfate on growth, economic character and mutagenicity in mulberry silkworm. However, studies on chemical mutagenesis in general and role of chemical mutagens on commercial characters in particular are rather scarce. Therefore, the present investigation was carried out in this line.

MATERIALS AND METHODS

Two mulberry silkworm races namely Pure Mysore (multivoltine), NB_4D_2 (bivoltine) at fifth instar first day, weighing about 0.577 gm and 1.050 gm respectively and, a well known monofunctional ethylating agent, ethyl methanesulfonate (EMS) were used for the study.

The silkworm rearing of both parents, as well as from F_1 to F_4 progeny was conducted in the laboratory following the method described by Krishnaswami, 1978, **[12]**. In order to select required doses of the chemical various concentrations of EMS like 2.5, 5, 10, 20, 40, 80, 120, 160, 320 and 640 mM were employed. Three different concentrations of EMS like 2.5, 5 and 10 mM were selected after preliminary studies at chromosome level, viability of larvae from

treatment to moth emergence, hatching percentage and viability of F_1 progeny.

Forty µl of final concentration of EMS freshly prepared in 0.75 % NaCl solution was administered separately to each worm in to the gut by 'oral injection'**[13, 14, 15]**. The control worms received the same amount of NaCl solution only. For each concentration, 60 worms in triplicate were taken. After treatment, the larvae were allowed to continue larval development, spinning and pupation. To evaluate the viability of larvae from the level of treatment with EMS up to the stage of moth emergence three criteria were considered *viz.*, number of cocoons harvested, number of cocoons with live pupa and number of moths emerged.

Of the cocoons harvested, only uniform and healthy cocoons were selected and processed for the preparation of disease free layings **[16, 17, 18]**. The layings of NB_4D_2 race were treated with hydrochloric acid to get immediate hatching **[19, 16]**. The economic parameters selected for present study from F_1 to F_4 generations included fecundity, hatching percentage, yield of cocoons by number and weight per 10,000 larvae brushed, cocoon weight, shell weight, shell ratio, filament length, denier and effective rate of rearing (ERR). In each replication, 500 larvae were kept after third moult. Evaluations of these characters were carried out for F_1, F_2, F_3 and F_4 generations of both Pure Mysore and NB_4D_2 races.

The experimental data obtained were subjected to one way ANOVA **[20]** and Scheffe's post hoc test **[21]**.

RESULTS AND DISCUSSION

The present results clearly indicated that the concentration of EMS at 640 mM caused cent per cent lethality within few minutes, 320 and 160 mM concentrations leads complete lethality within few hours. The dosages of 120 and 80 mM also found to be disastrous and proved to be highly toxic causing severe damage to the meiotic chromosomes **[1]** besides high larval mortality. The fecundity in 20,40 and 80 mM EMS treated batches was 331, 60 and 37 eggs in Pure Mysore and 445, 301 and 224 in NB_4D_2 races respectively though the ovarioles were filled with eggs. In addition to high larval mortality, the hatching per cent was 10, 6.06 and 0.0 % in Pure Mysore and 4.01, 1.04 and 0.0 % in NB_4D_2 races respectively. Apart from this, total chromosomal aberrations at 20 and 40 mM concentrations in Pure Mysore race at pachytene, metaphase I and metaphase II were 43.08, 72.31 and 77.43, and 50.28, 84.58 and 86.18 per cent respectively. On the other hand, in NB_4D_2 race the total aberrations at pachytene, metaphase I and

metaphase II were 38.64, 66.48 and 68.31, and 46.30, 73.03 and 76.93 percent respectively **[14]**. Besides, at higher concentration the size of the egg was reduced to a significant extent in both the races, and very rarely, shape was affected in NB_4D_2 race. The concentration at 10 mM showed a sub-lethal effect **[1]**.

In Pure Mysore and NB_4D_2 races, the viability per cent was high in control worms and gradual reduction was observed as the concentration of EMS increased. Though the biomass is more and larval duration is less, the viability per cent was less in case of NB_4D_2 race when compared to Pure Mysore race **(Table 1 & 2)**. This indicates that the NB_4D_2 race is more sensitive than Pure Mysore race at physiological level.

The F_1 progeny of 2.5 and 5 mM EMS treated sets of Pure Mysore race laid more number of eggs when compared control as well as 10 mM sets. Similar pattern of fecundity was observed even from F_2 to F_4 generation. In case of NB_4D_2 race, the F_1 progeny of control sets exhibited highest fecundity and gradual reduction was observed as the concentration of EMS increased. However, from F_2 to F_4 generation the fecundity was highest in 2.5 and 5 mM sets when compared to control as well as 10 mM sets. The hatchability in F_1 progeny was highest in control sets of both Pure Mysore and NB_4D_2 races, and gradual reduction was observed as the concentration of EMS increased. However, hatchability was gradually increased from F_2 to F_4 generation and 2.5 and 5 mM sets exhibited highest hatching at F_3 and F_4 generation. The larval duration was same in all experimental sets of both the races from F_1 to F_4 generation. However, in case of NB_4D_2 race, the 2.5 mM set exhibited 12 hours reduced larval duration at F_2 generation and it was transmitted to F_3 and F_4 generations. In case of cocoon yield per ten thousand larvae brushed by number as well as by weight, again 2.5 and 5 mM EMS treated sets of both the races exhibited superiority over remaining experimental sets in all the four generations. Almost similar pattern was observed even in cocoon weight, shell weight and shell ratio except 5 mM set of NB_4D_2 race, wherein slight reduction was noticed in cocoon weight at F_3 generation. In addition, 10 mM EMS treated worms of Pure Mysore race exhibited more cocoon weight than control set at F_2 generation. Again in case of filament length, the 2.5 and 5 mM EMS treated sets of both the races exhibited superiority over remaining sets except 5 mM set, wherein significant reduction over control was noticed. However, the denier was more in these sets. The denier was also more in all EMS treated

sets from F_1 to F_4 generations. The effective rate of rearing was also more in 2.5 and 5 mM sets when compared to control as well as 10 mM sets.

In the present observations, it is clearly indicated that the extent of larval viability as well as embryonic lethality in the F_1 progeny of both the races was proportionate to the concentration of EMS used. Datta *et al.*, 1978, **[22]** also found the increased frequency of dominant lethal in silkworm *Bombyx mori* as the dose of EMS increased. But, it is proved that lower doses of EMS might stimulate silkworm vitality and improves the commercial characters, which has been confirmed by the biochemical studies also **[1, 13]**. High percentage of mortality due to chemical mutagens in *Drosophila* was also reported by Browning, 1969, **[23]**, Sram, 1970, **[24]**, Slizynska, 1973, **[25]** and Auerbach, 1976, **[26]**. They are all reasoned out that high mortality was due to cytotoxic effect of the mutagen. Kerimova, 1977, **[27]** reported high mortality in mulberry silkworm due to dimethyl sulfate (DMS) and considered that it might be due to strong cytotoxic effect of the chemical. In the present investigation reduced fecundity and small size eggs in the F_1 progeny might also be due to strong cytotoxic effect of the EMS on ovarian cells as well as metabolic disturbances. In bivoltine races, two moths from the batch treated with 40 mM laid deformed eggs, which might be due to the effect of EMS on cells of ovary **[1]**.

In general, 2.5 and 5 mM sets exhibited more productivity over control in almost all characters in all the four generations, whereas higher concentration like 10 mM showed inferiority over the control as well as the batches treated with 2.5 and 5 mM EMS. These observations are also supported by the results of biochemical studies **[1, 13]**. Lakshmikumari, 1995, **[10]** showed that lower doses of gamma radiation might stimulate silkworm vitality and improve the commercial characters of silkworm. The present investigations also clearly indicated that the lower doses of EMS might bring about some changes in either the genotype of the silkworm itself or some influencing factor that may be generated due to EMS treatment; perhaps, such factor might be responsible for this alteration. As the EMS is known to be a potent mutagen **[28]**, it might have induced mutation resulting in the changed character, which is passed on from generation to generation in *Bombyx mori*. The enhanced rate of metabolism in the treated sets clearly indicated enhanced digestion, absorption, conversion and productivity. This basic knowledge can be exploited in the sericulture industry during

the evolution of new breeds, which goes a long way to improve the sericulture industry.

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Table 1: Effect of EMS on Viability of Silkworm *Bombyx mori* L. Race: Pure Mysore

Concentration of EMS (mM)	No. of cocoons harvested	No. of cocoons with live pupa	No. of moths emerged	Viability from larva to moth (%)
0.0	57	56	54	90.00
2.5	47	43	39	65.00
5	46	40	38	63.33
10	42	37	34	54.83
20	39	32	28	46.66
40	36	29	24	40.00
80	24	16	12	20.00

NOTE: For each dose 60 worms in triplicate were used; The variation between the control and experimental sets is significant at 5%level

Table 2: Effect of EMS on Viability of Silkworm *Bombyx mori* L. Race: NB4D2

Concentration of EMS (mM)	No. of cocoons harvested	No. of cocoons with live pupa	No. of moths emerged	Viability from larva to moth (%)
0.0	58	57	52	86.66
2.5	46	43	37	61.66
5	44	42	35	58.33
10	40	36	30	50.00
20	36	31	27	45.00
40	32	27	22	36.66
80	26	15	11	18.33

NOTE: For each dose 60 worms in triplicate were used; The variation between the control and experimental sets is significant at 5% level

Table 3: Effect of EMS on Commercial Characters of Silkworm and its Transmission from Generation to Generation. Race: Pure Mysore

Dose of EMS (mM)	Generation	Fecundity	Hatching (%)	Yield per 10,000 larvae brushed		Cocoon Weight (gm)	Shell weight (gm)	Shell Ratio (%)	Filament Length (mt)	Denier	ERR
				(No.)	(Kg)						
0.0	F_1	357	97.24	9365	8.790	0.940	0.118	12.55	369	1.93	93.66
	F_2	495	97.05	9266	6.702	0.789	0.115	14.57	348	1.83	92.66
	F_3	417	97.46	9333	8.820	0.950	0.119	12.52 12.67	407	1.92	93.33
	F_4	485	96.65	9300	10.881	1.176	0.149		444	1.86	93.00
2.5	F_1	394	90.72	9436	9.097	0.980	0.128	13.06	401	1.96	94.36
	F_2	498	96.07	9366	7.028	0.875	0.136	15.54	374	1.96	93.66
	F_3	419	98.44	9366	9.036	0.970	0.134	13.81	478	1.94	93.66
	F_4	508	97.76	9333	11.024	1.190	0.163	13.09	480	1.90	93.33
5.0	F_1	373	79.77	9400	8.804	0.943	0.126	13.36	386	2.07	94.00
	F_2	498	96.07	9333	6.868	0.846	0.129	15.24	370	1.94	93.33
	F_3	419	97.63	9366	9.072	0.972	0.134 0.154	13.78	477	1.93	93.66
	F_4	505	96.75	9333	10.932	1.182		13.02	456	1.87	93.33
10	F_1	351	67.11	8933	7.627	0.897	0.113	12.59	353	1.94	89.33
	F_2	460	95.42	9000	6.584	0.802	0.112	15.21	365	1.92	90.00
	F_3	410	94.27	9000	8.280	0.936	0.120	11.96	413	1.93	90.00
	F_4	481	96.06	9166	9.990	1.097	0.140	12.76	410	1.95	91.66

NOTE: For each set 500 worms in triplicate were maintained after 3rd moult; The variation between the control and experimental sets is significant at 5% level

Table 4: Effect of EMS on Commercial Characters of Silkworm and its Transmission from Generation to Generation. Race: NB_4D_2

Dose of EMS (mM)	Generation	Fecundity	Hatching (%)	Yield per 10,000 Larvae Brushed		Cocoon weight (gm)	Shell weight (gm)	Shell Ratio (%)	Filament Length (mt)	Denier	ERR
				(No.)	(kg)						
0.0	F_1	612	96.97	9400	17.400	1.880	0.378	20.11	1191	2.30	94.00
	F_2	600	96.65	9266	17.815	1.955	0.384	19.64	1368	2.09	92.66
	F_3	619	96.85	9466	18.291	1.964	0.392	19.96	1420	2.03	94.66
	F_4	588	96.64	9266	18.630	2.043	0.425	20.80	1297	2.34	92.62
2.5	F_1	591	81.81	9466	18.499	1.986	0.407	20.49	1286	2.34	94.66
	F_2	618	93.58	9300	17.843	1.958	0.390	19.91	1456	2.18	93.00
	F_3	632	97.14	9533	18.412	1.968	0.406	20.67	1236	2.32	95.33
	F_4	620	97.84	9366	19.629	2.129	0.449	21.08	1338	2.40	93.66
5.0	F_1	563	75.49	9433	18.415	1.984	0.399	20.11	1294	2.26	94.33
	F_2	609	93.04	9300	18.152	1.981	0.396	19.99	1332	2.45	93.00
	F_3	660	98.44	9500	18.147	1.942	0.409	21.06	1128	2.93	95.00
	F_4	596	98.52	9333	20.172	2.198	0.462	21.01	1322	2.67	93.33
10	F_1	546	66.39	8600	15.360	1.821	0.362	19.88	1246	2.34	86.00
	F_2	589	89.94	8833	16.403	1.891	0.360	19.37	1223	2.10	88.33
	F_3	597	93.42	9333	16.935	1.849	0.370	20.01	1229	2.14	93.00
	F_4	587	90.11	9000	17.336	1.962	0.338	19.52	1230	2.40	90.00

NOTE: For each set 500 worms in triplicate were maintained after 3rd moult; The variation between the control and experimental sets is significant at 5% level