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Biological Characteristics of some Mutant Mulberry Silkworm (*Bombyx mori* L.) forms

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Abstract: Based on the results obtained and the analysis made we could draw the following conclusions: the factors temperature and laser beams used reduced the biological traits of mulberry silkworm; the reduction of silkworm viability in result of temperature and laser beam treatments was race specific; gamma irradiation had a slight effect on the silkworm biological traits and no race differences were established; the use of ethyl methane sulphonate led to an increase in the main biological traits

Key words: biological characteristic, mutant *Bomyx mori* L., laser treatmens, gamma irradiation.

Introduction

Sericulture is an animal husbandry branch in agriculture of great economic importance for Bulgaria. As a source of a valuable raw material for the textile industry, it provides significant earnings for a great number of farmers.

In the last years, the silkworm production in our country decreased significantly – from 1 925 t in 1976 to 145 t in 1985, ranking Bulgaria in the 11-12 place in the world and first to second in Europe.

Beside the classical methods of breeding (selection and hybridization), the evolution of modern animal breeding programs, including mulberry silkworm breeding, provided sufficient information about the use of induced mutagenesis for increasing the genetic diversity and progress of source populations in the development of highly productive silkworm races and lines with valuable economic traits. The use of physical and chemical mutagens in silkworm breeding is of a special interest, as silkworms are very suitable for such studies having a number of advantages over the other farm animals.

Mutagenic factors are divided into physical and chemical ones. The group of physical mutagens comprises different types of ionizing- and not ionizing radiation, high temperatures, mechanical treatments, etc. The chemical mutagens are a great number of chemicals. With the progress of breeding, a third group of mutagens was formed – biological mutagens (viruses, bacteria and other infectious agents and the toxins released by them). According to a number of authors (Manchev and Iolov, 1981; Sugai and Hanaoka, 1972; Sugai and Takahashi, 1981; Azizov, 1988; Fugo and Arisawa, 1992) the high temperature (32^0C) during the prepupal stage, especially when combined with insufficient air exchange, led to male sterility due to spermogenesis disturbances.

The effect of doses below 1 Gy (Ovessenska and Panayotov, 1986) was less studied. Moreover, here as objects of treatment were usually used silkworm eggs and more rarely the other ontogenetic stages of Bombyx mori L. Irradiation of 3rd instar larvae on days 2-3 and 5th instar ones on days 2, 4 and 7, as well as of nymphs and silkworms, was reported by Naumov and Mihailova (1977, 1981). Irradiation of silkworms at the start of the 5th instar with 1, 2, 3, 4, and 5 Kr/h was also made by Kurata (1983).

Ovessenska (1984) established that preincubation irradiation of eggs with a helium-neon laser increased their hatchability by 1.84%, silkworm viability by 2.69%, raw cocoon yield by 1.84%, and silk ratio percentage by 8.14%.

The objective of the present work was to study the biological traits in some new mutant forms of mulberry silkworm, *Bombyx mori* L.

Material and Methods

The experiments were carried out in the experimental base of sericulture at the Department of Animal Husbandry, the Agricultural University in Plovdiv, during the period 2001-2003. The main biological traits of two mulberry silkworm races – Plovdiv 14 and Plovdiv 15, created at the Agricultural University, were tested. The methods of work involved treatments with low and high temperatures, gamma-irradiation, irradiation of larvae with He-Ne laser beams, and treatment with ethyl methane sulphonate (EMS). Egg incubation and silkworm rearing were done using a standard methodology adopted by the Department of Animal Husbandry.

Results and Discussion

The treatments with the aforementioned factors (except EMS), used to increase mulberry silkworm viability, were made at the larval stage. Therefore, egg hatchability in all variants and the control did not differ significantly in the first year of the study. Its value for Plovdiv 14 was 95.31 %, and for Plovdiv 15 - 95.65 %.

As for silkworm viability, the most important trait for cocoon yield formation, it was higher in Plovdiv 15, as compared to Plovdiv 14, showing values of 97.17 % and 96.35 %, respectively (Table 1).

The viability of race Plovdiv 14 was highest when silkworms were treated with ethyl methane sulphonate – 98.51%, on the average for the period tested. It varied in the separate years from 98.09% to 98.95%, the highest value being

recorded in the first year of the study. In this variant, the viability increase was 2.16% as compared to the control.

The viability in the variant irradiated by gamma rays showed a value of 94.99%. Here, the highest viability was observed during the year of the treatment – 97.75%. In the next year, a viability collapse (90.00%) occurred, while in the third year a significant increase – 97.17%, was registered. The averaged value for the period of the study was by 1.36% lower than that for Plovdiv 14 (control).

The treatment with low and high temperatures had a negative effect on silkworm viability, the effect of high temperature being stronger than that of the low one. Immediately after temperature treatment, viability decreased significantly – 29.64 %. In the next years it increased but still remained lower than that of the control.

The low temperature treatment showed the same tendency – a very low viability in the first experimental year and a gradual increase during the subsequent years, its value in the year 2003 being 93.48%.

The lowest viability was established when larvae were treated with laser beams. Its values ranged from 56.18 % to 60.48 % for the years 2001 and 2003, respectively. As averaged for the period of the study, the viability obtained in result of laser treatment was 58.25%, being 38.26 % lower than that of the control.

The results obtained for the silkworm race Plovdiv 15 repeated those for Plovdiv 14.

The highest stimulating effect was obtained with ethyl methane sulphonate. Here, the average viability for the period of the study was 98.39%, or by 1.22% higher than that in the control (97.17%).

Gamma-irradiation of larvae led to an insignificant decrease of viability. Its value was 95.14% versus 97.17% in the control.

Like in Plovdiv 14, the high-temperature treatment of this race showed a stronger negative effect than that of the low temperature. In the variants of both temperature treatments, very low viability was registered, its values increasing in the subsequent years. Nevertheless, the data estimated for the year 2002 were lower than those for the same year in the control.

The lowest viability for the period studied was established in the variant irradiated by laser beams - 57.98 %. This value was by 39.19 % lower than that in Plovdiv 15 (control).

Table 1. Silkworm viability - %

RACE	PLOVDIV 14				PLOVDIV 15			
Treatment with:	2001	2002	2003	$\bar{x}\pm S\bar{x}$	2001	2002	2003	$\bar{x}\pm S\bar{x}$
Low temperature	32.06	76.19	93.48	67.24±22.40	42.58	80.09	93.15	71.94±15.15
High temperature	29.64	70.09	85.46	61.73±16.64	24.96	65.03	87.27	59.09±18.23
Gamma rays	97.75	90.00	97.17	94.99±3.06	94.48	96.60	94.34	95.14±0.90
Laser beams	56.18	58.09	60.48	58.25±1.24	53.79	59.13	61.02	57.98±2.16
EMS	98.95	98.49	98.09	98.51±0.25	97.99	98.69	98.48	98.39±0.21
Control	96.20	96.74	96.11	96.35±0.20	96.50	97.20	97.80	97.17±0.38

Data for larval period duration are given in Table 2.

During the first year of the experiment, the high temperature treatment shortened the larval stage in Plovdiv 14 by 34 hours and in Plovdiv 15 by 76 hours.

Gamma irradiation of Plovdiv 14 led also to reduction in the larval stage duration – by 672 hours versus 682 h for the control.

The other factors used (low temperature, laser beams and ethyl methane sulphonate) lengthened the larval stage as compared to the control. The least larval stage lengthening was obtained when using ethyl methane sulphonate – 700 hours. Here, the larval period increased by 8 hours.

Table 2. Larval stage duration - h

RACE	PLOVDIV 14				PLOVDIV 15			
Treatment with:	2001	2002	2003	$\bar{x}\pm S\bar{x}$	2001	2002	2003	$\bar{x}\pm S\bar{x}$
Low temperature	840	808	744	797±28.22	864	840	792	832±21.17
High temperature	648	672	672	664±8.00	600	696	715	670±35.59
Gamma rays	672	768	708	716±34.29	708	768	756	744±22.45
Laser beams	794	818	800	804±7.21	816	804	776	798±11.85
EMS	700	704	696	700±2.31	700	678	696	691±6.77
Control	682	680	696	686±5.03	676	680	700	685±7.42

Under the effect of laser beams the larval stage lasted 794 hours, its longest duration being registered when a low temperature treatment was used – 840 hours for the race Plovdiv 14.

During the second year of the study, the low temperature treatment induced larval stage shortening, while all other factors lengthened the larval period duration as compared to the respective values in the preceding year. In the year 2003, the shortest larval stage was registered in the low-temperature variant – 672 hours versus 696 hours for the control. The longest larval stage was established in the race Plovdiv 14, irradiated by laser beams – 800 hours. When compared with the preceding year, all variants showed a significant reduction in their larval stage duration.

For the race Plovdiv 15 it could be noted that its larval stage in the first year of the experiment was by 76 hours shorter when a high-temperature treatment was used, as compared to the control (676 h). The longest larval stages were registered in the variants treated with a low temperature and laser beams – 864 and 816 hours, respectively.

In the next year, the larval stage in the EMS variant lasted 678 hours, or 2 hours less than that in the control. In all other variants, the larval stage was lengthened from 16 to 160 hours in comparison with the control.

In the year 2003, the larval stage duration for Plovdiv 15 varied from 696 hours in the EMS variant to 792 hours in the variant using a low-temperature treatment. Compared to the preceding year, shorter larval stages were registered in result of treatments with low temperatures, gamma rays and laser beams.

Table 3 shows data for the raw cocoon weight which is an important technological trait.

The results for the race Plovdiv 14 in the year 2001 showed that all factors used reduced the cocoon weight from 0.264 g in the gamma-irradiation variant to 1.228 g in the high-temperature treatment. An insignificant increase in cocoon weight (1.826 g versus 1.820 g for the control) was observed only in the egg treatment with ethyl methane sulphonate.

Table 3. Raw cocoon weight - g

RACE / Treatment with:	PLOVDIV 14				PLOVDIV 15			
	2001	2002	2003	$\bar{x}_{\pm S}\bar{x}$	2001	2002	2003	$\bar{x}_{\pm S}\bar{x}$
Low temperature	0.689	1.590	1.764	1.348±0.33	0.729	1.467	1.826	1.341±0.32
High temperature	0.592	0.985	1.322	0.966±0.21	0.426	1.005	1.860	1.097±0.42
Gamma rays	1.556	1.710	1.859	1.708±0.11	1.580	1.728	1.510	1.606±0.08
Laser beams	1.268	1.306	1.459	1.344±0.06	1.495	1.502	1.483	1.493±0.01
EMS	1.826	1.869	1.873	1.856±0.02	1.951	2.069	1.993	2.004±0.03
Control	1.820	1.860	1.840	1.840±0.01	1.900	1.950	1.860	1.903±0.03

The values registered in the next experimental year were higher than those in the preceding one, but here again a cocoon weight increase was established only in the EMS variant – 1.869 g versus 1.860 g for the control.

In the third experimental year, the cocoon weight increased in all variants tested, but its highest values were obtained when gamma rays and ethyl methane sulphonate were used – 1.859 g and 1.873 g, respectively, as compared to the control in that year. The lowest cocoon weight was found in the Plovdiv 14 variant with a high temperature treatment – 1.322 g.

All tendencies established for Plovdiv 14 were valid for Plovdiv 15, too. Here also a significant cocoon weight reduction was observed in the first experimental year, and only in the EMS variant a higher cocoon weight was registered – 1.951 g versus 1.900 for the control.

In the next years of the study, the values of cocoon weight increased and in the year 2003 the EMS variant exceeded significantly the control (by 0.133 g). In the high-temperature variant the cocoon weight was equal to that of the control (1.860 g). The cocoon yield per 1 box of eggs was formed as a product of egg hatchability, silkworm viability and cocoon weight.

Table 4 shows that only in the EMS variant the cocoon yield in all three experimental years exceeded that of the control, the highest yield being observed in the year 2002 – 36.296 kg for the race Plovdiv 14 and 40.262 kg for the race Plovdiv 15. In all other variants the cocoon yields per 1 box of eggs were significantly lower than those of the controls.

Table 4. Cocoon yield per 1 box of eggs - kg

RACE	PLOVDIV 14				PLOVDIV 15			
Treatment with:	2001	2002	2003	$\bar{x}_{\pm S}\bar{x}$	2001	2002	2003	$\bar{x}_{\pm S}\bar{x}$
Low temperature	4.180	17.573	29.662	17.138±7.36	5.979	16.024	30.974	17.659±7.26
High temperature	3.414	7.199	21.642	10.752±5.55	2.065	6.349	24.963	1.126±7.02
Gamma rays	27.242	30.320	32.150	29.904±1.75	28.030	26.300	29.467	27.932±1.12
Laser beams	13.841	14.741	17.145	15.242±0.99	15.624	17.256	17.583	16.821±0.61
EMS	35.627	36.296	36.226	36.050±0.21	37.697	40.262	38.677	38.879±0.75
Control	33.136	34.609	33.650	33.798±0.43	35.093	36.445	34.606	35.381±0.55

Comparing the cocoon yields between the separate years showed that they increased reaching their highest value in the last year of the study.

Conclusions

Based on the results obtained and the analysis made we could draw the following conclusions:

- The factors temperature and laser beams used reduced the biological traits of mulberry silkworm;
- The reduction of silkworm viability in result of temperature and laser beam treatments was race specific;
- Gamma irradiation had a slight effect on the silkworm biological traits and no race differences were established;
- The use of ethyl methane sulphonate led to an increase in the main biological traits.

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BIOLOŠKE KARAKTERISTIKE MUTANTA DUDOVE SVILENE BUBE (*Bombyx mori* L.)

-originalni naučni rad-

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Rezime

U radu je prikazan efekat temperature, laserskih i gama zraka i etil-metan-sulfonata na biološke karakteristike dudove svilene bube.

Rezultati ispitivanja ukazuju na sledeće: temperatura i laserski zraci su smanjili biološka svojstva dudove svilene bube; smanjenje životnih funkcija kod svilene bube kao posledica efekta temperature i laserskih zraka, je specifična karakteristika vrste; gama zračenje je imalo mali uticaj na biološke osobine svilene bube; primena etil metan sulfonata dovela je do jačanja glavnih bioloških svojstava.