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EFFECT OF METHYL VIOLET TO MULBERRY SILKWORM SOME BREEDS EGGS

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ДЕЙСТВИЕ МЕТИЛОВОГО ФИОЛЕТОВОГО НА ЯЙЦА НЕКОТОРЫХ ПОРОД ТУТОВОГО ШЕЛКОПРЯДА

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Abstract. To liquidate the larvae, the methyl violet ($C_{24}H_{28}N_3Cl$), used against fungal diseases in fish farming, was used. The goal is to establish physiological responses in different phases of development after treating local and introduced mulberry silkworm breeds of with aqueous solutions of methyl violet. Exogenous exposure to eggs led to the formation of a negative reaction. It has been established that treatment of silkworm eggs with water and a 0.001% solution of methyl violet (from October 20 to November 15, 2018) reduces the weight of the silk layer of cocoons with statistical accuracy by 33.0% (−78.5 mg) and 22.5% (−49 .0 mg) respectively ($p<0.001$). A negative effect also occurs in other physiological indicators: there is a difference in the weight of caterpillars, cocoons and pupae, and a slight deviation occurs in the weight of caterpillars before cocooning in the aquatic version. The results of this research can be used in developing issues such as identifying more stable and promising mulberry silkworm breeds in the conditions of Azerbaijan, as a prerequisite for predicting the successful introduction of mulberry silkworm.

Аннотация. Для ликвидации личинок использовали метиловый фиолетовый ($C_{24}H_{28}N_3Cl$), применяемый против грибковых заболеваний в рыбоводстве. Цель — после обработки грен местных и интродуцированных пород тутового шелкопряда водными растворами метилового фиолетового установить физиологические ответные реакции в разных фазах развития. Экзогенное воздействие на яйца привело к формированию отрицательной реакции. Установлено, что обработка грен водой и 0,001% раствором метилового фиолетового (с 20.10 по 15.11.2018 г.) снижает массу шелкового слоя коконов со статистической точностью на 33,0% (−78,5 мг) и 22,5% (−49,0 мг) соответственно ($p<0,001$). Отрицательное влияние имеет место и по другим физиологическим показателям: наблюдается разница в весе гусениц, коконов и куколок, а также небольшое отклонение происходит в весе гусениц перед коконированием в водном варианте. Результаты настоящих исследований могут быть использованы при разработке таких вопросов, как выявление более устойчивых и перспективных пород тутового шелкопряда в условиях Азербайджана, как предпосылка для прогнозирования успешной интродукции тутового шелкопряда.

Keywords: silkworm eggs, silk layer, methyl violet, mulberry silkworm breeds.

Ключевые слова: грены, шелковый слой, метиловый фиолетовый, породы тутового шелкопряда.

Productivity of mulberry silkworm depends on biotechnological indices of variety and hybrids. Breeders have obtained mulberry silkworm breeds and hybrids with high productivity by

Т. 10. №5. 2024
https://doi.org/10.33619/2414-2948/102

introducing dedicated technologies. However, despite the high biotechnological indicators obtained from them a number of diseases in the processing, feeding stage, silkworm eggs preparation, particularly diapause and wintering periods and their vulnerability to influences of unfavorable conditions still remain as limiting factors.

As you know, it is possible to obtain superior physiological outcomes at the subsequent development phase by ensuring normal development as a normal development phase of any breeds [1-4].

It is a well-known fact that protection of mulberry silkworm from silkworm eggs pathogens is done through various methods [5-7] and may not be so effective, as long as physiological reactions and biochemical reactiveness of various varieties are different. Given this, our current studies have put to test a new method preventing growth of saprogenic fungi in order to enhance living capacity of silkworm larvae. So, methyl violet ($C_{24}H_{28}N_3Cl$) used against such fungi during the incubation of sturgeon spawns was used to liquidate insect larvae.

Goal — after processing silkworm eggs belonging to local and introduced mulberry silkworm breeds with methyl violet liquefied solutions, physiological response reaction of other development phases was established.

Study material and methodology

Study of physiological response reaction of silkworm larvae to keeping conditions was performed on local Vatan and introduced Sverico-sari, Oragase breeds. The studies were carried out at the Sericulture center newly founded under the faculty of Soil Science and Agrochemistry of the Azerbaijan State Agricultural University in 2018-2023.

The study material was stored and fed under normal lighting conditions with temperature and relative humidity regimes prescribed in relevant tables. 100% diapause (dark color, dented) larvae were used in 5 pairs (1♀+1♂) in each series. After the diapause processing was performed using methyl violet ($C_{24}H_{28}N_3Cl$) liquefied solutions (0,01% and 0,001%) and experiments were performed (1 experiment and 2 examination — placed on dry-paper and processed with distilled water) in 3 series.

At each series, the development rate, skin changing times, caterpillar weight, cocoon weight, cocoon and silk layer weight, moth flight time, productivity per female moth, diapause start time in larvae were noted. Caterpillar recuperation capacity was determined by way of calculating them by beginning and end of the experiment and was expressed in % ratio relative to the initial number. Notes were also taken of the death ratio of cocoon spinning individuals [9, 10].

Statistical analysis of the outcomes [11] was conducted and mean arithmetic index (x), mean arithmetic error (sx), variance accuracy index relative to trial (tf) were determined.

Discussion and analysis of the study results

Outcomes of the study aiming to assess success of introducing new silkworm breeds and find out more prospective breeds adapted to new setting prove that the physiological responses to altered keeping conditions of silkworm eggs may be different (Table 1, 2).

The obtained experimental results accurately show that, processing silkworm eggs with methyl violet liquefied solutions 0,01% and 0,00% has led to response reactions in most of them.

Thus, the exogenous influence has a positive effect on the percentage of survival, the dynamics of the weight of caterpillars and cocoons, as well as the productivity of moth, and most importantly, the processes of the formation of the state of physiological rest — diapause.

Т. 10. №5. 2024
https://doi.org/10.33619/2414-2948/102

Table 1

PHYSIOLOGICAL RESPONSE OF THE EGGS OF SILKWORM LOCAL VATAN BREED TO DIFFERENT KEEPING CONDITIONS
(larvae from 03.07.2018; * — mean daily temperature and relative humidity)

Versions	*Caterpillar emerging date*	*Caterpillar age and skin changing date*	*Caterpillar weight, mg (x±sx)*	*Cocoon spinning date and caterpillar weight, mg*	*Cocooned coconut weight, mg (x±sx)*	*Cocoon weight, mg (x±sx)*	*Weight of the silk later, mg (x±sx)*	*Weight difference between trial and experimental versions*			*Butterfly flight time, larvae number(1♀)*	*Diapause formation Date and %*	*Death in %*
								mq	*%*	*tf*			
Trial: 50 unit, No sterile	20.04. 2019	IIy - 31.04. (18⁰C,68%)*	-	**1)** 30.05. 3070,0±10,7	1932,0±47,9	1700,0±32,8	220,5±11,9	-	-	-	16.06. 25⁰C80%;	22.06. 6,67%	53,3
		IIIy -08.05. (18⁰C,77%)*	122,3±7,7	(21,5%) **2)**08.06.	2148,0±55,9	1852,0±41,2	235,7±10,5				18.06.		
		IVy-15.05.	392,5±11,7	2937,5±17,9							299,8±42,5		
		(19⁰C,90%)* Vy -21.05.	936,7±33,7	(45,4%) **3)**10.06.	1509,5±28,8	1230,0±31,8	260,0±14,9						
		(25⁰C,70%)*		1745,4±28,0(33,1%)									
experiment: H2O 100 unit., Effect time: 20.10 - 15.11.2018.	29.04. 2019	IIy - 06.05. (18⁰C,70%)* IIIy -14.05.	- 136,2±11,4	30.05. 2740,9±22,1	1760,5±22,7	1340,7±33,0	340,5±11,4	**1)**120 **2)**105	55,4	7,2 p<0,05	14.06. 25⁰C78%;	24.06. 76,5%	0,0
		(20⁰C,75%)* IVy-20.05. (19⁰C,80%)*	388,2±16,2					**3)**81	44,5	15,4 p<0,001	17.06. 256,0±19,8		
		Vy -25.05.	850,9±17,8						30,9	4,3			
		(25⁰C,70%)*								p<0,001			
Experiment: Violet-K 0,001% 100 unit., date:20.10-15.11.2018.	29.04. 2019	IIy - 07.05. (15⁰C,80%)* IIIy -15.05.	- 210,5±14,5	02.06. 2650,9±32,3	1995,3±34,0	1420,5±11,0	460,5±12,5	**1)** 240	109	14,2 p<0,001	16.06. 25⁰C80%;	24.06. 80,7%	12,5
		(20⁰C,70%)* IVy-21.05. (20⁰C,75%)*	395,7±10,5 915,7±22,3					**2)** 225	95,4	13,8 p<0,001	20.06. 1470,5± 7,9		
		Vy -26.05.						**3)** 201	77,1	10,3			
		(25⁰C,78%)*								p<0,001			

Т. 10. №5. 2024
https://doi.org/10.33619/2414-2948/102

A comparative analysis of the results proves that the expression form, i.e. the level, of the response is different in the local Vatan and introduced Sverico-sari and Oragase breeds — which is clearly evident in the dates of hatching and molting of the caterpillars.

It is interesting that the effect of exogenous influence is recorded from the small age of the caterpillars to the fifth age, in the absence of sharp hygrothermal changes in the feeding conditions in the introduced breeds, but this response effect, which was found later between the test and experimental options, weakens, as a result, the caterpillars change their shell to the fifth age. When we reconcile this determined effect with the results obtained for the local Vatan breed, it is possible to see that there is no serious deviation, only in the experimental variants, the caterpillars hatch 9 days later than the eggs. Undoubtedly, this causes deviations in the history of molting, and a difference of 5-7 days is determined in this physiological process compared to the control. However, regardless of the hygrothermic parameters of the feeding conditions, the shell change at the age of V takes place with a difference of 3-4 days compared to the control variant, although it is the same in the caterpillar phase.

Considering that all the experiments were carried out on eggs laid at the same time, then it is possible to explain that the determined physiological response to the impact in young caterpillars is both endogenous and exogenous in nature. That is, the process of molting at these ages is carried out under the influence of internal and external factors, and the simultaneous molting of those caterpillars at the age of V shows that the endogenous mechanism of regulation prevails: the hormonal regulation of the process depends on the date of hatching of the caterpillars, regardless of the temperature and relative humidity of the storage and feeding conditions [12-15].

It is known that the assessment of the physiological state and biochemical reactivity of the body is carried out mainly according to weight indicators. The results prove that the caterpillars of the domestic breed treated with 0.001% aqueous solution of methyl violet differ from the caterpillars of the introduced breed by insignificant fluctuations in their mass. can be explained by intensive nutrition and biochemical reactivity of the body.

In the introduced breeds of mulberry silkworm, the response of eggs after methyl violet treatment (i.e. exogenous effect) according to the weight indicators is different, and this is more clearly expressed in the Sverico-sari breed. Thus, keeping eggs in H_2O for 26 days led to an intensive increase in the weight of caterpillars: compared to the control option (eggs placed on paper in a dry environment), the difference was 16.4% in the third age, 143.3% in the fourth age, and 27.3% in the fifth age accounted for 3% (Table 2). However, the difference in the weight dynamics determined in the caterpillar phase in the other introduced Oragase breed compared to the control was +43.7% (III age), +65.0% (IV age) and −36.2% (V age).

As a result of the conducted research, it was found that the cocoon wrapping directly depends on the weight index of the caterpillar before the start of the process. In the experiments, the caterpillars were grouped according to the date of cocooning, as a result of which it was found that the cocooning of caterpillars obtained from control (dry) and H_2O-treated eggs occurred almost at the same time: at this time, the weight of individuals was 21.5% in the native breed, 21.5% in the introduced Sverico-sari caterpillars that start the process in the first days are 63.0%, that is, significantly heavier.

It should be noted that the exogenous effect on eggs mainly leads to an increase in the weight of caterpillars, cocoons, pupae and silk cover. The study of the effect of distilled water in the experiments was mainly related to the use of aqueous solutions of the methyl violet. However, the results of the experiments showed that water, which is the solvent of the methyl violet (check option 2), is the cause of most of the positive effect achieved. The results presented in the tables prove that the positive effect achieved on almost all physiological indicators was obtained in the H_2O variant.

Т. 10. №5. 2024
https://doi.org/10.33619/2414-2948/102

In particular, it is important to note that the increase in the weight of the silk layer was 54.4% in the H_2O variant of the domestic breed, and 80.5% in the methyl violet (0.01%) variant, with accuracy ((p <0.05-<0.001) and correspondingly was 108.8% (H_2O) — 77.1% (methyl violet 0.001%).

A similar effect was recorded in the introduced Sverico-sari breed: the weight of the silk cover was 68.2% and 79.2% (methyl violet) and 16.1% (H_2O) and 11.4% (methyl violet) occurred in the version of 0.001% solution (p<0.001%), at the same time after exposure to 0.01% aqueous solution of 2.7% methyl violet (p<0.05).

It is interesting that in the introduced Oragase breed, the changes due to this effect were different: the exogenous effect on the eggs resulted in the formation of a negative reaction. It was found that keeping wintering eggs in water and 0.001% solution of methyl violet (from 20.10 to 15.11.2018) decreased the weight of the silk layer of cocoons with statistical accuracy by 33.0% (−78.5 mg) and 22.5% (−49.0 mg) (p<0.001) causes a decrease. It is important to note that the detected negative effect also occurs in other physiological indicators, i.e., the difference in the weight of caterpillars, cocoons and pupae is observed, and a slight deviation occurs in the weight of caterpillars before cocooning in the water variant.

As a result of research, it was determined that keeping wintering eggs in distilled water and aqueous solutions of the drug for 30 days also affects the date of flight of butterflies, but at this time, Oragase breed shows differences again. So, in conditions where the hygrothermal background is almost unchanged, the exogenous influence accelerates the flight of moth from pupae.

It is an interesting fact that in the studied series, a positive response in egg-laying is recorded only in introduced breeds of mulberry silkworm: the number of eggs laid per female is 3.6-4.8 times (Sverico-sari) and 1.2 times compared to the control version (Oragase) has already been.

The obtained results clearly proved that, depending on the storage conditions of overwintering eggs, a serious reaction occurs when a state of physiological quiescence is formed. As it is known, diapause — a state of physiological rest, as in all insects, plays an important role in the life cycle and seasonal development of the mulberry silkworm. It is the diapause in the mulberry silkworm that ensures timely exit of the caterpillars from the eggs, thereby allowing them to survive unfavorable environmental conditions. However, the untimely hatching of caterpillars coincides with the lack of quality natural feed, as a result of which difficulties arise in the feeding of caterpillars in granary farms.

It was determined that keeping wintering eggs in water and 0.01-0.001% aqueous solutions of the drug for 30 days leads to an increase in the percentage of eggs in diapause. At this time, the exception is again detected in the Oragase breed, that is, against the background of the 15.0% indicator of the test option, 25.9% of diapause eggs are recorded after exposure to a 0.001% solution of the drug.

As an interesting result, it should be shown that, while a high percentage of mortality is always recorded in the test variants, regardless of the breed, the effect with the methyl violet leads to either a decrease in the percentage of mortality or its complete elimination: 23.5-100% in the local Vatan breed, Sverico-sari 65.2-36.5% in (excluding 0.01% methyl violet variant) and 47.1-39.4% in Oragase, respectively.

The comparative analysis of the obtained experimental results clearly proves that the treatment of overwintering eggs of the mulberry silkworm with pure distilled and 0.01% and 0.001% aqueous solutions of methyl violet leads to the formation of a response on many physiological indicators, which. The degree of expression of these reactions varies depending on the gender: the response is more clearly manifested during the hatching and molting of the caterpillars in the domestic breed.

Т. 10. №5. 2024
https://doi.org/10.33619/2414-2948/102

Table 2

PHYSIOLOGICAL RESPONSE OF INTRODUCED SILKWORM BREEDS TO DIFFERENT KEEPING CONDITIONS OF SILKWORM EGGS

Versions	*Caterpillar emerging date*	*Caterpillar age and skin changing date*	*Caterpillar weight, mg (x+sx)*	*Cocoon spinning date and caterpillar weight, mg*	*Cocooned coconut weight, mg (x+sx)*	*Cocoon weight, mg (x+sx)*	*Weight of the silk later, mg (x+sx)*	*Weight difference between trial and experimental versions*			*Butterfly flight time, larvae number (1♀)*	*Diapause formation Date and %*	*Death in %*
								mq	*%*	*t_f*			
Sverico-sarı Trial: Larvae dated 09.06.2018, 160 units. 33,3% -ster.	20.04. 2019	IIy - 27.04. (12⁰C,60%)* IIIy -31.04. (15⁰C,65%)* IVy-10.05. (15⁰C,77%)* Vy -18.05. (18⁰C,90%)*	- 166,5±10,6 330,8±15,1 1225,0±25,0	**1)** 26.05. 81,3%; 2250,0±31,1 **2)** 03.06. 18,7% 1417,5±42,3	981,0±0,15 946,2±0,82	802,0±1,22 756,2±5,85	138,2±0,03 96,8±8,3	-	-	-	03.06. 28⁰C55%; 06.06. 259,9±13,0 (caterpillar units 134 emerged on 04.07. and the remaining – wintering)	10.06. 20% 975 units	41,7 31.04. 5,0 02.06 (30⁰C 55%)
Experiment: H_2O Sverico-sarı: larvae units 125 dated: 09.06.2018 125-15.11. 2018.	27.04. 2019	IIy - 31.04. (15⁰C,65%)* IIIy -05.05 (18⁰C,72%)* IVy-11.05. (14⁰C,80%)* Vy -18.05. (18⁰C,90%)*	14,8±0,38 193,8±11,7 805,0±15,0 1560,0±29,0	**1)** 23.05. 1636,7±59,2 **2)** 03.09. 1794,0±22,7	1600,3±75,0 1235,0±11,3	1323,0±64,0 1048,5±43,0	232,5±12,2 173,5±14,2	94 77	68,2 79	7,7 p<0,001 4,7 p<0,001	04.06. 29⁰C75%; 05.06. 08.06 305,9±14,1 *Caterpillar yield- 0%*	10.06. 85,4%	27,2 31.04
Trial: Violet-K 0,001% -li Sverico-sarı larvate units 100: 09.06.2018.	20.04. 2019	IIy - 27.04. (12⁰C,60%)* IIIy -09.05. (15⁰C,77%)* IVy-13.05. (17⁰C,80%)* Vy -18.05. (18⁰C,90%)*	- 164,5±17,7 325,2±17,5 500,8±15,8	**1)** 22.05. 2225,0±51,7 (15,9%) **2)** 25.05. 1933,3±20,9 (24,1%) **3)** 03.06. 1175,0±0,66 (60,8%)	1150,0±35,7 1208,0±35,9 966,1±22,6	827,2±19,5 806,0±22,1 722,2±37,3	160,5±5,9 138,7±8,16 107,8±5,2	22 - 11	16,1 - 11,4	3,7 p<0,001 - 1,1 p<0,001	04.06. 29⁰C75%; 05.06. 1235,0±27,0	08.06. 36,8%	15,2

Trial: Violet-K 0,01% -li Sverico-sarı larvae units 80 dated: 09.06.2018	20.04. 2019	IIy - 31.04. (15⁰C,65%)* IIIy -09.05. (16⁰C,90%)* IVy-15.05. (18⁰C,77%)* Vy -20.05. (20⁰C,70%)*	14,8±0,38 77,0± 3,4 146,5±10,4 747,4±23,6	27.05. 1826,0±38,7	1500,8±63,2	947,5±0,58	142,0± 5,6	3,8	2,7	0,67 p<0,05	04.06. 29⁰C75%; 05.06. 925,0±33,5	07.06. 35,2%	56,0 (cocoon spinning) 02.06.
Oragase Trial: 03.07.2018. Larvae units 130, 2,0% - Sterile	20.04. 2019	IIy - 31.04. (15⁰C,65%)* IIIy -05.05. (18⁰C,68%)* IVy-13.05. (19⁰C,90%)* Vy -17.05. (22⁰C,85%)*	- 245,3±10,8 430,9±8,39 995,0±27,8	24.05. 1685,5±20,8	1460,0±33,5	1226,0±41,0	218,0 ±8,8	-	-	-	08.06. 29⁰C72%; 09.06. 538,9±24,3	10.06. 15,0%	17,0
Trial: H_2O Oragase 03.07.2018 Larvate units 80.effective date:20.10-15.11.2018.	20.04. 2019	IIy - 27.04. (15⁰C,60%)* IIIy -09.05. (17⁰C,80%)* IVy-15.05. (19⁰C,85%)* Vy -18.05. (22⁰C,78%)*	- 281,9±11,6 422,3±9,9 935,8±24,9	21.05. 1860,0±31,9	848,9±0,08	729,9±0,15	139,5±0,07	-79	33,0	8,9 p<0,001	02.06. 30⁰C55%; 07.06. 601,3±12,0	04.07. 6,9%	8,0
Trial: Violet-K 0,001% , Oragase 03.07.2018 Larvae units 100	20.04. 2019	IIy - 28.04. (15⁰C,65%)* IIIy -06.05. (18⁰C,68%)* IVy-15.05. (22⁰C,90%)* Vy -18.05. (22⁰C,77%)*	- 289,0±10,6 495,0±13,6 958,8±15,3	23.05. 1658,0±31,1	1351,0±21,0	1109,0±12,1	169,0±5,0	-49	22,5	-4,9 p<0,001	05.06. 29⁰C78%; 06.06. 589,5±14,0	04.07. 25,9%	6,7

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Т. 10. №5. 2024
https://doi.org/10.33619/2414-2948/102

Т. 10. №5. 2024
https://doi.org/10.33619/2414-2948/102

Against the background of insignificant fluctuation of the weight indicators of the caterpillars belonging to the local breed, a clearer response on this indicator was detected in the introduced Sverico-sari and Oragase breeds: the exogenous effect on overwintering eggs caused an increase in the weight of the silk cover with statistical accuracy against the background of an increase in the mass of caterpillars, the weight of cocoons and pupae stimulated.

As the most important result, it is necessary to pay particular attention to the reduction of the percentage of egg-laying, diapause formation, and death rate regardless of the sex of the mulberry silkworm, which is a positive response to the effect of the methyl violet.

The results of the present research can be used in the development of issues such as the identification of more sustainable and promising breeds of mulberry silkworm in the conditions of Azerbaijan, as a prerequisite for predicting the successful introduction of mulberry silkworms.

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