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# ABSTRACT

of the dissertation for the degree of Doctor of Philosophy

# **BIOLOGY OF THE GIANT PEACOCK MOTH (SATURNIA PYRI DENIS ET SCHIFFERMÜLLER), DEVELOPMENT OF A MASS REARING METHOD FOR USE IN SERICULTURE**

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#### **INTRODUCTION**

**Relevance and degree of completion of the topic.** Special attention is paid to improving sericulture and cocoon production in our country. The restoration and development of traditional farms as a branch of the non-oil sector is included in the State Programs<sup>1</sup> of our Republic ("State Program for Socio-Economic Development of the Regions of the Republic of Azerbaijan in 2014-2018", "On the Development of Cocooning and Sericulture in the Republic of Azerbaijan for 2018-2025 State Program", "Strategic Roadmap for the Production and Processing of Agricultural Products in the Republic of Azerbaijan") has been reflected.

The action plan for the implementation of the "State Program for the Development of cocooning and Sericulture in the Republic of Azerbaijan for the years 2018-2025", approved by the Decree of the President of the Republic of Azerbaijan No. 3406 dated November 27, 2017, to fulfill the tasks set for 2019 - In 2021, at the Sheki Regional Scientific Center of the Azerbaijan National Academy of Sciences, research was carried out in the direction of breeding wild silkworm species and obtaining valuable "wild" silk, which is an advanced field in modern sericulture. For this purpose, Saturnia pyri (Denis et Schiff., 1775) wild silkworm mass reproduction has been carried out.

Obtaining environmentally clean materials with universal properties remains one of the main problems of our time. For this reason, sericulture scientists in the modern world are developing practical methods to use natural and regenerated silk fibroins obtained from wild silkworm cocoon silk fibers as biomaterials and nonwoven biomimetic materials, respectively. For this purpose, in 1986, a society called "The Japanese Society for Wild Silkworms" (JSWS) was established and seminars on wild silkworm species were started<sup>2</sup>. The purpose of creating that society is to obtain silk threads and silk proteins from the cocoons of various types of insects that

<sup>&</sup>lt;sup>1</sup> Azərbaycan Respublikasında baramaçılığın və ipəkçiliyin inkişafına dair 2018-2025-ci illər üçün Dövlət Proqramı [Mətn]: Azərbaycan Respublikası Prezidentinin 2017-ci il 27 noyabr tarixli Sərəncamı ilə təsdiq edilmişdir//Azərbaycan. – 2017, 28 noyabr. – s.2.

<sup>&</sup>lt;sup>2</sup> What is the Japanese Wild Sericulture Society?: [Electronic resource] /ed. by Dr. Hiromu Akai; The Japanese Wild Sericulture Society Chairman's Greetings. – Tsukuba: JSWS, – 2012. URL: https://jsws-yasan.com/about-jsws/

exist in the world and produce cocoons and to study the technologies of using them in fundamental and applied fields.

Thus, the real reason for new innovative business plans in the field of sericulture, especially wild silkworm breeding, is that, unlike the mulberry silkworm, the products obtained from wild silkworm species are of specific importance for the fields of modern medicine and high technologies and it's possible to source intelligent materials with a wide range of profiles from them. A significant part of wild silkworms, which produce natural silk, which is of high value for trade and agriculture, such as modern medicine, belongs to the family Saturniidae Boisduval, 1837.

Studies have shown that wild silk, which is found in the mountainous region of northwestern Azerbaijan, can be used for this purpose and can be used for industrial purposes.

**The object and subject of the study.** The object of the study is the giant peacock wild silkworm (*Saturnia pyri* Den. & Schif., 1775), belonging to the Lepidoptera order and Saturniidae family, and its subject is its biology and mass rearing.

The purpose and tasks of the research. The main goal of the research work is to study the biology of S.pyri, a wild silkworm species common in the northwestern region of Azerbaijan, to develop the technology of rearing it in laboratory conditions and to investigate the possibility of obtaining its silk, to protect the sensitive species with economic potential and to ensure the restoration of its natural resources, under different nutritional conditions. During feeding with the cherry tree (Prunus avium L., 1755) leaves offered as a fodder plant, researching several physiological and ecological characteristics, searching for effective methods to increase their vitality and productivity, and studying the important properties of silk for sericulture to study the cocoon of S.pyri silkworm. The study also explored the organic and mineral composition to evaluate the nutritional value of silk-free pupae. At the same time, the presented scientific work, and the experiments conducted with the S.pyri silkworm were carried out in 2019-2023 under identical conditions with the mulberry silkworm (Bombyx mori L, 1758).

To achieve this goal, the following tasks were set:

- Development of the method of collection of *S. pyri* moths from nature in the northwestern region of Azerbaijan and mass breeding in laboratory conditions;

- The research of the biology of *S.pyri* silkworm, a study of possibilities of regulating its number in its natural habitat;

- The comparative study of the dependence of the bioecological and physiological characteristics of *S.pyri* and *B.mori* silkworms on feeding conditions in the same laboratory condition;

- Comparative study of physical and chemical properties of cocoon shell and pupae of *S.pyri* and *B.mori* cocoons.

**Research methods.** For capture in its habitat, the *S.pyri* moth has been using the light trap method<sup>3</sup>. During the morphometric analysis of all developmental stages, biometric parameters were calculated using the MegaStat statistical analysis program for Microsoft Excel. To determine the quantitative and qualitative indicators of the research objects, the food activity of caterpillars was carried out by aktography, and oxygen consumption of pupa was carried out using a simple respirometer using the Warburg method. The obtained cocoons were processed in a water-based softening solution containing 10g/l of carbonate and bicarbonate, specially prepared for the calculation of the proteins in the cocoons. The nitrogen in the pulp was obtained by the Kjeldahl method, and the oil was extracted in a Soxhlet apparatus. The mineral composition of the pupa was analyzed by the X-ray fluorescence method.

### Main points presented to the defense of the dissertation:

1. Studying the biology and morphology of the moth *S.pyri* in the northwestern region of Azerbaijan in laboratory conditions is a promising direction of great theoretical and practical importance for obtaining a quality laboratory population;

2. It is possible to protect the population by reintroduction S.pyri caterpillars of a certain age to suitable habitats in their natural habitat;

3. Based on the effect of feeding conditions on the bioecological

<sup>&</sup>lt;sup>3</sup> Денисова, С.И. Взаимоотношения китайского дубового шелкопряда с кормовыми растениями в Беларуси / С.И.Денисова. – Витебск: ВГУ имени П.М.Машерова, – 2016. – 158 с.

characteristics and important industrial qualities of *S.pyri* silkworm, the most relevant method for mass rearing of silkworms is feeding on the bouquet;

4. It is possible to study the main properties of *S.pyri* cocoon for sericulture and for this purpose to obtain an effective softening solution for unwinding silk fibers;

5. By studying the biochemical and mineral composition of wild *S.pyri* silkworm pupa, the possibility of using it as a new and efficient product for the food and feed industry can be evaluated.

**The scientific novelty of the research.** As a result of detailed research, the dissertation work is the first complex research work dedicated to the rearing of the giant peacock silkworm in closed conditions (laboratory, rearing house) for sericulture.

To patent this innovation as an innovative method in the field of sericulture industry, an application was submitted to the Patent and Trademark Expertise Center under the Intellectual Property Agency of the Republic of Azerbaijan, our claim document entitled "Application of the giant peacock moth (*Saturnia pyri*) in the sericulture" dated 24.02.2020 was entered into the center on 16.02.2022 with the number 2020 0031 and was registered in the state register on 16.02.2022, and the patent number I 2022 0005 was submitted to the invention under the name "Wild silkworm breeding method".

For the first time:

- The biology of the *S.pyri* moth, which inhabits the northwestern region of our country, was studied from the egg stage to the mature imago stage, and morphometric parameters and development dynamics were evaluated;

- It was proposed to develop and implement a set of measures aimed at protecting and increasing the number of wild silkworms *S.pyri* in its natural habitat;

- The physical and chemical parameters of the cocoon of the *S.pyri* silkworm were studied, and the composition and effective duration of the softening solution for opening the cocoon thread were determined;

- The chemical and biochemical composition of the pupa of the *S.pyri* silkworm was studied, and its nutritional value as a feed

additive was evaluated.

**Theoretical and practical significance of the research**. The research has shown that the wild silkworm *S.pyri* can be mass-produced and harvested from its cocoons on an industrial scale. It has been found that the value of cocoon silk and fibroin is not inferior to the cocoons of known silk grown for analogy and can obtain its high-quality silk.

The silk of the S.pyri silkworm can be mainly used in the textile industry. The fabric woven from it will have a natural brown color and shine, high strength, and resistance to high temperatures. Civilian and military clothing, coverings, etc., are made of fabric that can be successfully used in preparation. The latest medical achievements prove that silk fibroin produced by wild silkworms, which is the basis of high-tech biomaterials used for the preparation of matrices, scaffolds, and other biomimetic materials and devices in the field of cell-tissue engineering and regenerative medicine, has superior properties than the fibroin of *B.mori* silk. For this reason, the silk of the S.pyri silkworm can make great contributions to our country in such innovative fields in the future. As a result of the analysis of the S.pyri pupa released from the cocoon, nutrients such as protein and fats were found in its content, which can be used as feed additives in various fields. Generally, products corresponding to each stage of S. pyri silkworm rearing and silk processing - eggs, feces, exuvium (eliminated old shell), etc., can be used as waste-free technologies. The research issue is a promising and innovative project for modern sericulture and cocoon production in Azerbaijan. Shortly, the creation of one of the new divisions of the silk industry in the country in this direction will help revive the silk industry in Azerbaijan and increase the production of silk materials, create thousands of new jobs, and attract new capital to the country. It is known that there is a great demand for these products among domestic and foreign consumers, mainly in the field of biomedicine and nanotechnologies.

**Approbation and application of the research.** The main provisions of the dissertation work were heard and discussed in the form of a report at the Institute of Zoology of the Ministry of Science and Education of the Republic of Azerbaijan and the Sheki Regional Scientific Center of ANAS, at the annual reporting meetings of the

Academic Council, at the scientific seminar of the Institute and the Center, as well as at International scientific and practical conferences.

- 9th BACSA International Conference "Sericulture preservation and revival – problems and prospects-2019", "SERİVİVAL" 2019, (April 7th–12th 2019, Batumi, Georgia);

- The 25th International Congress on Sericulture and Silk Industry "Silk beyond the Textile" (November 19th – 22nd 2019, Tskuba, İbaraki, Japan);

- ANAS Institute of Zoology "Fundamental and applied scientific research in Zoology: Current issues, achievements, and innovations" scientific-practical online conference, (October 6th, 2021, Baku, Azerbaijan);

- Veterinary Research Institute of the Ministry of Agriculture of the Republic of Azerbaijan "Veterinary science in the 21st century innovations towards the future" International Scientific and Practical Conference (November 25th-26th 2021, Baku, Azerbaijan);

- NGO "Belarusian Entomological Society", National Academy of Sciences of Belarus, SRPA "Scientific and Practical Center of the National Academy of Sciences of Belarus for Bioresources", etc. IV International Scientific and Practical Conference "Results and Prospects for the Development of Entomology in Eastern Europe" (December 1st–3rd 2021, Minsk, Belarus);

- Livestock Science and Research Institute of the Ministry of Agriculture of the Republic of Azerbaijan "Modern Problems and Innovations of Livestock Concepts" International Scientific and Practical Conference (December 22nd-24th 2021, Goygol, Azerbaijan);

- 10th BACSA International Conference "Regeneration of sericultural industries in 21st century" "REGESERI" 2023 (April 24th– 27th 2023, Soufli, Greece);

- ANAS and MSERA Institute of Molecular Biology and Biotechnologies International Conference on "Heydar Aliyev and the nature of Azerbaijan" (June 19th-20ty 2023, Baku, Azerbaijan).

19 scientific works, including 8 articles, 10 theses, and 1 patent, were published in local and foreign publications related to the dissertation work.

**The organization where the dissertation was performed**. The work was performed at the "Applied Zoology Center" and "Derrid Invertebrates" laboratory of the Institute of Zoology of the Ministry of Science and Education of the Republic of Azerbaijan.

The structure and size of the dissertation work. The total volume of the dissertation consists of 169 pages and 203035 characters, including an introduction (15616 characters), 5 chapters (172035 characters), a conclusion (9689 characters), results (3701 characters), practical proposals (1994 characters), bibliography and appendices. 174 literature materials were used in the dissertation. The text part of the dissertation contains 26 pictures, 5 graphs, and 19 tables.

## **CHAPTER I. LITERATURE REVIEW**

The application of the species of the family Saturniidae of the order Lepidoptera in the world sericulture and the biological characteristics of the six most common species are reported. For the first time in the world and in Azerbaijan, *S.pyri* was presented for a similar purpose, exploring foreign and local sources of scientific literature about the wild silkworm, and collecting enough information about the research work.

### CHAPTER II. MATERIAL AND METHOD OF THE RESEARCH

The primary material of *S.pyri* wild silkworm moths was collected using a light trap in a specially protected natural area around Bash-Shabalid village in the Sheki district, which is located on the southern slope of the Greater Caucasus in the north-western region of the country on May 28th, 2019 (Figure 1). In the years that followed, *S.pyri* eggs were laid by laboratory moths.

In 2022, to prevent inbreeding depression among the laboratory population, females from the laboratory population were crossed with males from the wild.

Over the entire study period, an average of ~2700 pcs. caterpillars of giant peacock moth (*S.pyri*) and ~3000 pcs. of mulberry silkworm (*B.mori*). For the purity of the experiment, *S.pyri* caterpillars were given only cherry leaves (*Prunus avium* L., 1755) during feeding, while *B.mori* caterpillars were given black and white mulberry leaves (*Morus nigra* L. and *Morus alba* L.).

The mulberry silkworm *B.mori* eggs were obtained because of the mating of the ShZEM-4 mulberry silkworm breed that emerged from the cocoons at the end of the spring-summer feeding season at the Sheki Regional Scientific Center of ANAS.



Figure 1. The research area

To fulfill the tasks, all studies were carried out according to the scheme shown in Scheme 1.

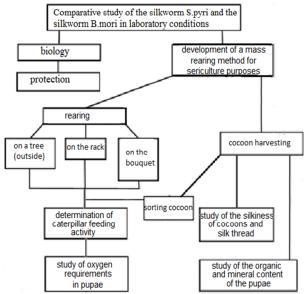
Biological indicators of insects were determined using methods accepted in biology.

Morphometric measurements were accomplished using an MSB-9 stereomicroscope equipped with a digital caliper (accuracy 0.01 mm) and an eyepiece micrometer. Mass estimation was carried out on a Mettler Toledo analytical balance (accuracy 0.002 g). Measurements were performed using the MegaStat statistical analysis program for Microsoft Excel using criteria with a confidence level of P=95% with a 0.96 significance coefficient, with n≥10 replicates.

The experiments were performed at a temperature of  $+25\pm2^{\circ}$ C, a relative humidity of  $70\pm10\%$ , and a photoperiod of 12 hours.

The percentage of egg survival, the viability of caterpillars, the

growth dynamics of caterpillars, and the sex ratio of individuals were calculated using the appropriate formulas, and the results are presented in tables.



Scheme 1. General study plan

The survival rate of eggs is determined by the following formula:

$$D = \frac{Q_1 - Q_2}{Q_1} \ge 100\%$$
 (2.1)

- where Q1 is the number of eggs laid in incubation, Q2 is the number of destroyed eggs, and D is the percentage of egg survival.

The formula used to calculate the viability of caterpillars is:

$$Y = \frac{T_1}{T_2} \times 100\%$$
 (2.2)

- where T1 is the number of caterpillars after molting, T2 is the number of caterpillars at the end of the previous instar, and Y is the percentage of survival of caterpillars.

The relative growth of caterpillars was calculated using the Schmalhausen formula (1939):

$$M = \frac{M_2 - M_1}{0.5t(M_1 + M_2)} \times 100\%$$
(2.3)

- where M1 is the weight at the early instar, M2 is the weight at the late larval instar, t is the number of instar days, and M is the growing percentage.

The sex ratio was calculated by the following formula<sup>4</sup>:

$$C = \frac{F_{Q}}{(F_{Q} + F_{\sigma})} \times 100\%$$
 (2.4)

- where  $F^{\bigcirc}_{+}$  is the number of females,  $F^{\bigcirc}_{-}$  is the number of males, C is the percentage of sex ratio.

To figure out the feeding activity of the silkworm, a device called an aktograph was used, which measures the feeding and excretion activity of caterpillars over a certain period in closed and outdoor conditions. To study the amount of oxygen consumed by living pupas, a simple respirometer device was used according to the Warburg method<sup>5</sup>.

The researched technological indicators of silkworm cocoons (cocoon weight, percentage of silkiness, amount of silk fibroin) were determined by methods adopted in sericulture. *S.pyri* silkworm cocoons were sorted and the effect of nutrition on cocoon quality was studied.

To determine silkiness, the total mass of 200 random cocoons of different sizes was weighed, after cutting and releasing the pupa, the mass of the cocoon shell was determined and the percentage of silkiness was calculated using the following formula<sup>6</sup>:

$$I = \frac{B_p}{B} \ge 100\%$$
 (2.5)

- where Bp is the mass of the cocoon shell, B is the mass of the complete cocoon, and I is the percentage of silkiness.

<sup>&</sup>lt;sup>4</sup> Денисова, С.И. Влияние смены кормового растения на рост и выживаемость гусениц шелкопрядов (*Antheraea pernyi* G.-M., *Lymantria dispar* L., *Endromis versicolora* L.) // Веснік ВДУ, Серия 73, Биология, – 2013. №1. – с. 58–62.

<sup>&</sup>lt;sup>5</sup> Климиашвили-Нуцубидзе, К.З. Влияние условий выкормки на эколого – физиологические и хозяйственно-полезные признаки у дубового шелкопряда: / диссертация на соискание ученой степени кандидата биологических наук. // – Тбилиси, 1952. – 142 с.

<sup>&</sup>lt;sup>6</sup> Gowrisankar, R., Dasari, S., Anas, M. Silk Reeling Techniques: Exploring Traditional and Advanced Methods // Journal of Experimental Agriculture International, - 2023. vol.45, №9, - p. 91-99

To determine the amount of fibroin in the cocoon fiber of *S.pyri* 122.75, the cocoon shells were freed from the pupa and other visible mechanical particles, and dried at a temperature of  $(80\pm2)^{\circ}$ C to constant weight. The resulting dry cocoon shell was softened with an aqueous solution obtained by mixing equal volumes of sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>) and sodium bicarbonate (NaHCO<sub>3</sub>) salts separately at a concentration of 10 g/l. Fibroin was calculated by comparing it with the mass of the co-coon membrane using the following formula:

$$F = \frac{L}{B_{p}} \times 100\%$$
 (2.6)

where Bp is the mass of the cocoon shell, L is the mass of unfolded silk fibers, and F is the percentage of fibroin in the cocoon silk.

The amount of moisture in the pupae was determined by the difference in wet and dry mass.<sup>7</sup> The ash-to-fat ratio was calculated using the method of Maurice R. Marshall.<sup>8</sup> Analysis by David B. Min and Wayne S. Ellefson was used to determine the fat content of the pupa<sup>9</sup>. Protein was determined using the Kjeldahl method<sup>10</sup>. The nitrogen was determined because the analysis reflects the crude protein content of the food. Determination of fiber was carried out according to the method of Kurschner and Hanek<sup>11</sup>. Virgin oil was extracted using cold pressing and extraction methods. Extraction was carried out with hexane solvent in a Soxhlet apparatus: experiments were carried out in 1-3 replicates. During the experiments, it was found that a complete release of oils occurs at a temperature of 65°C. To calculate the extraction yield, the amount of oil produced was calculated using the following formula:

<sup>&</sup>lt;sup>7</sup> Новиков, Д.А., Новочадов, В.В. Статистические методы в медико-биологическом эксперименте (типовые случаи) / Д.А.Новиков, В.В.Новочадов, – Волгоград: Вол.ГМУ. – 2005. – 84 с.

<sup>&</sup>lt;sup>8</sup> Marshall, M.R. Ash Analysis. In: Food Analysis / M.R. Marshall. – Boston, MA: Springer, – 2010. – p. 105-115.

<sup>&</sup>lt;sup>9</sup> Min, D.M., Ellefson, W.C. Fat Analysis. In: Food Analysis / D.M. Min, W.C. Ellefson. – Boston, MA: Springer, – 2010. – p. 117–132.

<sup>&</sup>lt;sup>10</sup> Mariotti, F. Converting nitrogen into protein - beyond 6.25 and Jones' factors. Critical reviews / Food Science and Nutrition, - 2008. vol. 48, № 2, - p. 177–84.

<sup>&</sup>lt;sup>11</sup> Dampanaboina, L., Yuan, N., Mendu, V. Estimation of Crystalline Cellulose Content of Plant Biomass using the Updegraff Method / J Vis Exp. – 2021. May; vol. 15, № 171.

$$H_{\rm m} = \frac{K_{\rm y}}{N} \ge 100\% \tag{2.7}$$

- where Ky is the mass of oil, N is the mass of the sample, and  $H_m$  is the percentage of production yield.

The amount of carotene (provitamin A) in the fat of the pupa was determined using the spectrophotometric method by calibration with a standard solution ( $K_2Cr_2O_7$ ); Vitamin E was determined by thin layer chromatography on Silufol paper plates (Czech Republic) impregnated with a stable layer of silica gel<sup>12</sup>. The composition of mineral elements was analyzed by X-ray fluorescence using an Omega 4000 instrument (Innov-X, head office: Massachusetts, USA, General Director: Don Sackett) at the National Center for Nuclear Research JSC (Baku, Azerbaijan)<sup>13</sup>.

### CHAPTER III. BIOLOGY OF THE SATURNIA PYRI 3.1. Biomorphological characteristics of Saturnia pyri species.

According to the results of scientific research and experiments, the biology of the silkworm *S.pyri* in Azerbaijan has not been sufficiently studied. Taking into account the need for a more detailed study of the morphological characteristics of the species, from 2018 theoretical, and starting from 2019, practical research on the mass breeding of *S.pyri* silkworm in laboratory conditions was started.

According to the latest literature data, the family Saturniidae includes 2,349 species in 169 genera and nine subfamilies. Systematics of the giant peacock moth:

Type: Arthropoda (von Siebold, 1848) Ortega-Hernández, 2016 Class: Insecta Linnaeus, 1758

Order: Lepidoptera (Linnaeus, 1758) Capinera, John L., 2008 Family: Saturniidae Boisduval, 1837

Genus: Saturnia Franz Paula von Schrank, 1802

<sup>&</sup>lt;sup>12</sup> ГОСТ 30417–2018. Масла растительные. Методы определения массовых долей витаминов А и Е. Дата введения 2020-01-01.

<sup>&</sup>lt;sup>13</sup> Hutton, L.A. Electrochemical X-ray fluorescence spectroscopy for trace heavy metal analysis: Enhancing X-ray fluorescence detection capabilities by four orders of magnitude / L.A. Hutton, G.D. O'Neil, T.L. Read [et al.] // Analytical Chemistry, – 2014. 86 (9), – p. 4566-4572.

Scientific name: Saturnia pyri Denis et Schiffermüller, 1775

Information on the phenology of the *S.pyri* silkworm is given in Table 1. It is known that phenology depends on environmental factors such as temperature, humidity, and climatic conditions. The percentages of survival rates for all metamorphosis stages of the *S.pyri* silkworm are given in Table 2. Caterpillars with a life cycle consisting of five larval instars (L1-L5) were calculated by studying the development days for each age and their mass at the beginning and end of the age, and the percentage of age-appropriate growth was determined. The obtained results are presented in Table 2.

Table 1

Phenology of the wild silkworm *Saturnia pyri* (Den. et Schiff, 1775) (Sheki region, 2019–2023 average)

March	March			May			June			July			August			September	<b>1</b>	Diapause	
III	Ι	Π	III	Ι	Π	III	Ι	II	III	Ι	Π	III	Ι	Π	Ш	Ι	Π	III	
0	0																		
	+	+	+	+	+	+													
		•	٠	•	•	•	•												
						_	_		_	_	_	_	_						
												0	0	0	0	0	0	0	0

#### Table 2

# The percentage of survival rates of the *Saturnia pyri* (Den. et Schiff, 1775) wild silkworm in all stages of development

Development stages	Quantity	Period of development, (days)	Quantity	Survival rates, (%)
eggs	259	$9.20 \pm 0.44$	255	98.57±0.25
L-L5	251	48.66±0.33	233	92.91±0.30
late L5	231	$4.85 \pm 0.28$	229	99.13±0.21
pupa	224	320.00±0.23	223	99.55±0.31
$\bigcirc$ imago	122	6.57±0.37	-	-
👌 imago	96	9.83±0.31	-	-
result	-	392.54±0/23	-	86.10±0.26

# Table 3

(Den. et Schiff, 1775) during the larval stage									
	Larval	Mass of cat							
Larval instars	development period, (days)	early L instar	late L instar	Growth, (%)					
L1	6.3±0.17	$0.005 \pm 0.001$	$0.01 \pm 0.01$	11,11±0,01					
L2	8.1±0.44	$0.05 \pm 0.01$	$0.10{\pm}0.04$	8.33±0.04					
L3	9.3±0.16	$1.20{\pm}0.08$	3.07±0.22	9.41±0.23					
L4	10.6±0.11	$4.08 \pm 0.02$	7.12±0.22	5.26±0.21					
L5	13.4±0.57	$10.29 \pm 1.01$	15.50±0.54	3.015±0.012					

## Developmental dynamics of the wild silkworm *Saturnia pyri* (Den. et Schiff, 1775) during the larval stage

The length of bodies of females and males at the imago stage is  $245.50\pm1.79/241.34\pm2.23$  mm, forewing length  $285.34\pm1.21/272.25\pm2.03$  mm, angular in this case, the wing size is calculated to be approximately  $173.67\pm1.45/2$  152.1 $\pm$ 31.79mm (Figure 2).

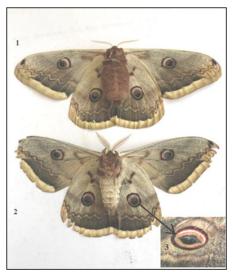
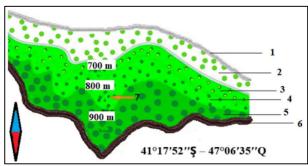


Figure 2. Saturnia pyri (Den. et Schiff, 1775) moths (ventral side): 1 -  $\bigcirc$ ; 2-  $\Diamond$ ; 3- eye (oculi macula)

### 3.2. Conservation of Saturnia pyri moth.

In 2018-2023, as a result of investigations conducted within the framework of scientific research work, it was determined that there is

a danger of decreasing the quantity of local *S.pyri* population in the specially protected area where the primary materials were collected (Scheme 2).



Schem 2. Landscape division of the Bash Shabalid biogeocenosis: 1- border of the natural protection area; 2- hazelnut garden; 3- forest-meadow landscape; 4- meadow-steppe landscape; 5- mountain-forest landscape; 6- mountain range; 7- monitored area

Therefore, it was decided to draw up a conservation plan .:

- 1. reducing the death rate;
- 2. conducting monitoring and investigations;
- 3. increasing the number and population;
- 4. improvement of environmental education of the population;
- 5. Conservation of critical habitats for S.pyri.

# CHAPTER IV. DEPENDENCE OF ECOLOGICAL AND PHYSIOLOGICAL CHARACTERISTICS OF SATURNIA PYRI SILKWORM ON NUTRITIONAL CONDITIONS

# 4.1. Effect of feeding methods on silkworm development dynamics

For this purpose, approximately 600 newly hatched larvae of each silkworm species were selected and divided into three equal experimental batches consisting of 200 *S.pyri* and 200 *B.mori* caterpillars. While feeding the caterpillars of the *S.pyri* silkworm, wetting of the leaves with clean water was carried out, and it was determined that wet food is more favorable for S.pyri silkworms than dry food.

Feeding was initiated on cherry and mulberry trees, respectively, under laboratory conditions of controlled temperature, relative humidity, and a 12-hour photoperiod on shelves, bouquets, and under variable environmental conditions outdoors. The results are presented in Table 4.

The 168 cocoons were obtained from 200 *S.pyri* silkworms fed by the bouquet method, which was the highest indicator among the three applied methods.

For each batch of *S.pyri* and *B.mori* silkworms fed on shelves, bouquets, and trees, the mass of caterpillars was measured and averaged to study the development dynamics. The obtained results showed that the rearing and yielding of silkworm *S.pyri* in natural conditions, and silkworm *B.mori* in closed conditions, on the general principle, are more successful.

Table 4

### The dependence of the development periods of the caterpillars of the wild silkworm *Saturnia pyri* (Den. et Schiff, 1775) and the caterpillars of the mulberry silkworm *Bombyx mori* (Linnaeus, 1758) on feeding methods

		•	,		0				
Developmental		Species of silkworms (600 of each type)							
	1	Developme	ent of <i>S.pyri</i> c	aterpillars,	Development of B.mori caterpillars,				
periods of the silkworm			(in days)	_	(in days)				
		shelf	bouquet	tree	shelf	bouquet	tree		
L1 activity		4.30±0.33	$4.20\pm0.10$	-	$3.00{\pm}0.57$	4.0±0.12	-		
LI	molting	$2.20 \pm 0.33$	$2.30\pm0.13$	-	$2.30 \pm 0.66$	$2.80{\pm}0.18$	-		
L2	activity	7.30±0.23	$6.50 \pm 0.09$	-	3.60±0.33	$3.30{\pm}0.18$	-		
L2	molting	$38.00 \pm 0.24$	$2.80{\pm}0.44$	-	$2.30{\pm}0.16$	$2.10{\pm}0.16$	-		
L3	activity	$7.80{\pm}0.18$	$6.10{\pm}0.12$	7.00±0.12	6.00±0.13	$6.10{\pm}0.44$	7.10±0.43		
LS	molting	3.10±0.24	$3.60 \pm 0.37$	3.40±0.32	$2.20{\pm}0.33$	$2.50 \pm 0.33$	3.60±0.13		
L4	activity	8.40±0.33	$7.40{\pm}0.34$	7.00±0.21	7.10±0.33	$8.80 \pm 0.35$	8.90±0.43		
L/4	molting	3.60±0.36	$3.10{\pm}0.53$	4.50±0.20	$3.80{\pm}0.18$	3.10±0.16	3.60±0.33		
L5	activity	$14.70{\pm}0.12$	13.50±0.29	$12.50 \pm 0.29$	5.60±0.19	$6.50 \pm 0.14$	7.70±0.39		
LJ	molting	-	-	-	-	-	-		
late L5		5.50±0.29	$4.10\pm0.11$	4.30±0.17	4.70±0.21	$5.80 \pm 0.11$	5.5±0.43		
common caterpillar stage		51.80±0.84	46.12±0.71	49.90±0.81	33.80±0.61	37.40±0.61	42.80±0.11		
	U	numbe	er of <i>S.pyri</i> co	coons	number of <i>B.mori</i> cocoons				
С	cocoon	114	168	147	189	55	14		
				•					

**Note:** Experiments were conducted at +25±1°C temperature, 70±10% relative humidity, and 12-hour photoperiod.

### Table 5

The mean mass of caterpillar and pupa at the late L4-L5 instar of wild silkworm *Saturnia pyri* (Den. et Schiff, 1775) and mulberry silkworm *Bombyx mori* (Linnaeus, 1758)

Species of	The eve of	Average mass index, (g)				
silkworm	measurement	shelf	bouquet	tree		
	late L4	3.114±0.270	4.501 ±0.037	5.012 ±0.043		
S.pyri	late L5 (the eve of spinning the cocoon)	12.001±0.032	$18.046 \pm 0.027$	19.001 ±0.206		
	Pupae	$4.502 \pm 0.145$	5.390±0.010	5.820±0.013		
	late L4	$1.262 \pm 0.011$	0.962±0.015	0.952±0.115		
B.mori	late L5 (the eve of spinning the cocoon)	4.503±0.015	3.502±0.215	3.706±0.019		
	Pupae	1.741±0.216	$0.822 \pm 0.101$	$0.840 \pm 0.015$		

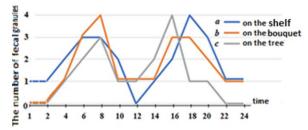
**Note:** Experiments were conducted at +25±1°C temperature, 70±10% relative humidity, and 12-hour photoperiod.

In comparison, the results of all three feeding methods for batches of *S.pyri* silkworms showed that *S.pyri* silkworms fed on trees and bouquets and at the end of L5 (before cocoon spinning) had an average mass in the range of  $18.524\pm0.5g$ . This is about 1.5 times more than the average mass of each individual in the batch fed on the shelves.

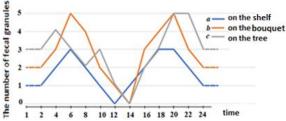
The study of the influence of rearing conditions on the feeding activity of caterpillars was carried out by counting the excrement granules secreted by silkworms as a result of leaf-feeding processes at certain hours of the day. For that purpose, the aktograph was used, and as can be seen from the obtained results, the general principle remained relevant in this experiment as well: *S.pyri* larvae fed on a bouquet and tree took more food more actively than the batch fed by the shelf method (Graph 1, 2).

By studying the oxygen consumption of silkworm pupae separately, it was possible to determine the feasibility and prospect of feeding using different methods.

Another clear indicator of the dynamics of the development of living organisms depending on the influence of different environments is the use of oxygen (biological oxygen consumption), the intensity of the process with the decomposition of components, and the release of carbon dioxide.



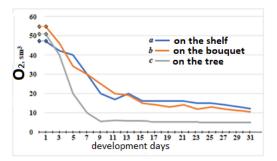
Graph 1. Feeding activity of wild silkworm *Saturnia pyri* (Den. et Schiff, 1775) at different hours of the day: a- shelf rearing; b- bouquet rearing; c- outdoors (on the tree) rearing



Graph 2. Feeding activity of the mulberry silkworm *Bombyx mori* (Linnaeus, 1758) at different hours of the day: a- shelf rearing; b- bouquet rearing; c- outdoors (on the tree) rearing

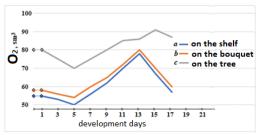
As a result of the measurements made on the live pupae of *S.pyri* and *B.mori* silkworms fed under three different conditions (Graph 3 and Graph 4), it was determined that the oxygen volume decreased from the first day. This reduction occurs due to the process of histolysis, which is expressed by the slowing down of the metabolism from the end of the last instar of the silkworm to the beginning of the pupation period.

After histolysis, histogenesis begins, during which the pupa's oxygen demand increases. Correspondingly, the dependence of the amount of oxygen consumed by *S.pyri* and *B.mori* silkworm pupae fed under three different conditions on the development days is given.



Graph 3. The dependence of the amount of oxygen consumed by the pupae of the wild silkworm *Saturnia pyri* (Den. et Schiff, 1775) fed in three different conditions during 1 month: a- on the shelf; b-on the bouquet; c- on the tree

It should be noted that the regularity of increased oxygen consumption in pupae during the histogenesis process was reflected for both types of silkworms, regardless of the applied rearing method (shelf, bouquet, and tree), and the normal developmental stages of pupae were not disturbed.



Graph 4. The dependence of the amount of oxygen consumed by the pupae of the wild silkworm *Bombyx mori* (Linnaeus, 1758) fed in three different conditions during 1 month: a-on the shelf; b-on the bouquet; c- on the tree

Thus, among the *S.pyri* pupae from silkworms fed by all three methods and then entering winter diapause, the pupae of caterpillars fed on the wood used the least oxygen. On shelves, pupae of foraged *S.pyri* silkworms and pupae fed with leaf bouquets consumed approximately the same amount of oxygen. All of this shows that *S.pyri* is a wild silkworm, for which it is not suitable to live on

shelves, even in bouquets placed in containers full of water, or to feed on trees in the field.

# CHAPTER V. A STUDY OF THE COCOON OF THE SATURNIA PYRI SILKWORM

### 5.1. Characteristics of Saturnia pyri cocoon

The cocoons of *S.pyri* used in the study, spun by caterpillars fed on cherry leaves (*Prunus avium*), were brown, irregularly shaped (pear-shaped), and consisted of two poles. After releasing the membrane of the *S.pyri* cocoon, which has a simple structure, by performing a special softening process, it was possible to separate it into several (three visible) layers. There is an eclosion hole of about 3-5 mm at the sharp pole of the cocoon. and along this hole, parallel fibers protrude in a brush form (Figure 3).

The distribution of oxalate crystals, tannin-phenol compounds, and serine in the outer and inner layers of *S.pyri* and *B.mori* cocoons was reported. In comparison, the *B.mori* cocoons don't contain mineral crystals, while the *S.pyri* cocoon contains few minerals often in the outer layer. As can be seen from Graph 5, the amount of mineral salts and tannin-phenolic compounds in the inner and outer layers of the *S.pyri* cocoon is less than that of the Saturniidae family representatives and is classified as openable. The low mineral content increases the economic importance of the *S.pyri* cocoons.

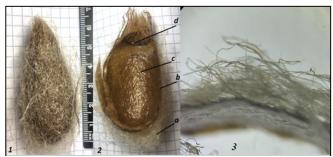
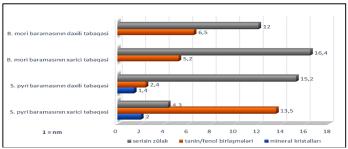


Figure 3. Description of the cocoon of wild silkworm *Saturnia pyri* (Den. et Schiff, 1775): 1-intact cocoon; 2- cocoon membrane (or shell): a- outer layer, b- middle layer, c- inner layer), d- cocoon hole (eclosion hole), 3- fragment of cocoon membrane under a light microscope appearance



Graph 5. Distribution of sericin, tannin-phenolic compounds, and mineral crystals between the outer and inner layers of *S.pyri* and *B.mori* cocoons

# 5.2. The softening of *Saturnia pyri* cocoons and determination of fibroin

One of the main indicators of cocoon quality is silkiness; to obtain an average indicator, the silkiness percentage of the cocoon shell was calculated by species on 200 alive, intact samples of *S.pyri* and *B.mori* cocoons of different sizes taken during the experiment. The results are presented in Table 6.

Table 6

Characteristics of live cocoons of wild silkworm Saturnia pyri (Den. et Schiff, 1775) and mulberry silkworm Bombyx mori (Linnaeus, 1758)

Types of	Cocoon	Cocoon shell	Silkiness, %	
silkworms	mass, g	mass, g	511K111055, 70	
S.pyri	7,15±0,09	0,804±0,03	11,43±0,10	
B.mori	2,19±0,21	0.41±0,03	21,07±0,08	

To calculate the percentage of fibroin in *S.pyri* silk fibers, the cocoons freed from the pupae were softened using sodium carbonate and sodium bicarbonate dissolved in 10 g/l of water.

The result of the investigation was that the most effective method for better degumming of the *S.pyri* cocoon and preservation of its natural color and shine is to first boil it in the prepared solution for 20 minutes and then steam it for 45 minutes. The ratio of the total amount of unfolded silk was 1: 4: 1.5 for the three layers (Figure 4).

The degummed silk fibers obtained after softening were dried to the final mass in the laboratory. The mass fraction of fibroin for both silkworm cocoons was calculated using Formula 2.6 by comparing the final mass value obtained with the mass of the cocoon shells measured before degumming. As a result, approximately  $77.15\pm0.35\%$  fibroin was determined for *S.pyri* and  $79.42\pm0.16\%$  for the *B.mori* batch taken as a control. As can be seen, the percentage of fibroin in the silk fibers of the *S.pyri* and *B.mori* silkworms is almost the same.



Figure 4. Raw silk from the cocoon of wild silkworm *Saturnia pyri* (Den. et Schiff, 1775): a- outer layer silk; b- middle layer silk; c- inner layer silk

### 5.3. Biochemical and mineral composition of Saturnia pyri pupa

During the research, the chemical and biochemical components of the pupae freed from the cocoons of *S.pyri* were studied. As can be seen from Table 7, the composition of *S.pyri* pupa is rich in nutrients.

Table 7

m	mulberry silkworm <i>Bombyx mori</i> (Linnaeus, 1758)										
research	silkworm pupa										
object	moisture,	dry matter,	protein, %	fat, %	cellulose	ash,					
	%	%			fiber, %,	%					
S.pyri	49,00	51,00	52,50	27,89	10,50	2,50					
B.mori	47,00	53,00	65,70	18,06	3,50	2.88					
S.ricinii <sup>14</sup>	-	-	54.70	25,06	5,85	4,88					

Biochemical composition of the pupae of wild silkworms *Saturnia pyri* (Den. et Schiff, 1775), *Samia ricinii* (Donovan, 1798), and mulberry silkworm *Bombyx mori* (Linnaeus, 1758)

<sup>14</sup> Longvah, T., Mangthya, K., Ramulu, P. Nutrient composition and protein quality evaluation of eri silkworm (Samia ricinii) prepupae and pupae // Food Chemistry, - 2011. vol. 128, № 2, - p. 400-403. The chrysalis oil we obtained from *S.pyri* pupa was dark yellow and had a unique pleasant smell. The average melting point was 26.0 °C, and the density was 930 kg/m3. As in the case of *B.mori* pupa oil, the unsaturated fatty acids in the oil extract make this oil physiologically rich and increase its usefulness.

The amount of carotene and tocopherol was determined to find vitamins A and E in the oil of *S.pyri* pupa.

Calculations showed that the amount of  $\beta$ -carotene (Vit A) in 100g of *S.pyri* pupa oil was 572 $\mu$  $\theta$ , and the quantity of total carotenoids was 1144 $\mu$  $\theta$ .

To determine vitamin E in the oil of *S.pyri* pupa, the tocopherol content in the oil was first calculated. To determine the mass fraction of this vitamin, the content of  $\alpha$ -tocopherol was calculated according to the calibration graph by the method of thin-layer chromatography (Figure 5): it was found that 25.0mg = 25 mg% or 0.34 IU of vitamin E per 100g of fat.

The mineral content of the pupa was also checked. As can be seen from Table 8, the calculation of the mineral content of the wild silkworm *S.pyri* pupae by dry weight determined the presence of 25 chemical elements and showed that it is rich in natural macronutrients such as K, Mg, Na, Ca, Al.



Figure 5. Silica gel plate chromatography of α-tocopherol in the chrysalis oil of wild silkworm *Saturnia pyri* (Den. et Schiff, 1775)

The preliminary results of our experiments showed that the caterpillars of the *S.pyri* silkworm take minerals from the feed plants

and store them in their bodies, so how rich they are in microelements in the pupal stage directly depends on the microelements in the feed plant.

Magnesium Phosphorus Aluminum Siliceous Natrium Calcium Barium Kalium Sulfur Copper Lead Zinc chemical Iron elements Si Ρ Zn **Symbols** Κ Na Mg Ca A1 S Ba Pb Fe Cu Indicator, 376.1 311.3 701.9 101.6 156.4 611.0 34.2 7.60 3.10 0.30 3.60 4.20 3.20 (mg) Manganese Chromium Vanadium Titanium Strontium Rubidium Gallium Niobium Zirconium Yttrium Nickel chemical Tin elements Symbols v Ti Mn Ni Zr Y Nb Rb Cr Ga Sn Sr Indicator, 4,50 0,30 0,90 1,30 2,20 0,10 1,10 0,10 0,60 0,30 1,10 0.20 (mg)

# Saturnia pyri (Den. et Schiff, 1775) in a 100 g dry sample of wild silkworm pupa amount of mineral substances

Table 8

### CONCLUSION

*S.pyri* moths as the primary material of the study were obtained in 2019 from the Bash Shabalid biogeocenosis under the auspices of the Sheki Regional Scientific Center of ANAS, near the Bash Shabalid village of the Sheki region, located in the northwest of Azerbaijan by using a light trap. The generations obtained in the laboratory were used in the following research years. The biology of the obtained *S.pyri* laboratory population was studied in detail. To preserve the bioresources of the species in its natural habitat, a corresponding work plan was drawn up for protection measures for maintaining and restoring the population by releasing the caterpillars of a certain age grown in closed and constantly controlled conditions to appropriate settlements. The life cycle of monovoltine with holometamorphosis covering  $392.54\pm0.23$  days was observed in the study of biomorphological features.

The basis of the presented research work covers during the period from the feeding of cherry tree (*Prunus avium*) leaves to the beginning of cocoon spinning of the caterpillars of the *S.pyri* after incubation. During the experiments, 2600 S.pyri caterpillars were fed in two different environments:

1) indoor or laboratory conditions with controlled temperature  $(+25\pm1^{\circ}C)$  and relative humidity (70+10%) with a 12-hour photoperiod;

2) Outdoors or in natural conditions.

Under closed conditions, the caterpillars were fed with a bouquet of cherry leaves, which differed technically and physically, in two ways, on a shelf, and in water. According to the obtained results, the bouquet method was evaluated as a more convenient and effective method than the shelf method in terms of the development and productivity of caterpillars, as well as in terms of leaf consumption.

In the bouquet method, leafy branches are placed in water in the form of a bouquet in glass or earthen pots so that the leaves used as food for caterpillars remain wet during their use, and the caterpillars are fed by moving freely in these bouquets. Observations show that caterpillars prefer to be on the underside of the leaf both during the rest (especially at night) and molting. For this reason, it has been determined that the bouquet method is more effective.

When the humidity in the air decreases, water is sprayed on the bouquets. It was observed that this method caused a decrease in the feeding activity of *S.pyri* silkworms and the movements of leaving the bouquet. Spraying water on the bouquets on eves when the air humidity is below 50% increases the protection of the presence of the caterpillar in environmental conditions that change its viability up to two times. By partially increasing the weight indicators of the caterpillars, the development period between the L1-L5 age has been significantly shortened.

The arboreal method is the most natural method for the wild species *S.pyri* silkworm, consisting of the larvae feeding outdoors,

directly on the feed trees. However, due to the impossibility of proper control over the caterpillars in field conditions, it was assessed as unsuitable for mass breeding. Because they are attacked by birds, as well as night birds due to the special luminescence of their skin, in addition to the impact of heavy rain and hail.

Several experiments were conducted and results were obtained to study the effect of the mentioned breeding methods on the bioecological and physiological characteristics and productivity of *S.pyri* silkworm. It was determined that the maturation of *S.pyri* caterpillars fed in bouquets and the transition to the cocooning period took place in a shorter time compared to the shelf method.

To clarify which of the listed different feeding methods is comfortable and less stressful for the growth of the studied bioobjects, the amount of oxygen they consume when they breathe in the pupal stage was determined. During the experiments, the amount of oxygen consumed by the pupae obtained from the caterpillars fed on the tree was taken as a normal measure. For *S.pyri* silkworms fed in the laboratory, although the indicators of the bouquet and shelf methods did not differ much, the respiration frequency of the pupa was more stable in the bouquet method.

The percentage of quality (selective) cocoons wrapped by *S.pyri* caterpillars fed in bouquets was higher than the cocoons obtained in the other two feeding conditions.

For the first time in Azerbaijan, a method of breeding wild silkworms for use in sericulture was developed and presented within the given scientific research work.

### RESULTS

1. As a result of studying the biology of the *S.pyri* moth for the first time in 2019-2023 in the northwestern region of Azerbaijan, a controlled  $\Delta t = 25\pm1^{\circ}C$ ,  $\Delta \psi = 70\pm10\%$ , 12-hour photoperiod for the development of this species in closed conditions was considered as optimal. The life cycle of *S.pyri* silkworm is 392.54\pm0.23 days, and the average life cycle for egg, caterpillar, pupa, and adult imago stages is respectively 9.20\pm0.44, 48.66±0.33, 320±0,23 and 9.83±0.31 days, the percentage of viability was 86.10±0.26%, the percentage of females was 56%, and the percentage of males was 44%. The average survival rate of *S.pyri* eggs was 98.57% with an average laying of  $300\pm35.55$  eggs per female. The caterpillars of *S.pyri* are oligophagous, the most favorable food plant is determined to be the cherry tree (*Prunus avium*, *L*) [3, 5, 13, 15].

- 2. As a result of the conducted research, a plan of measures was developed for the protection of sensitive species *S.pyri*, whose number is decreasing in nature, in the area where it is distributed, and according to the plan, 130 in 2019, 309 in 2020, and 527 in 2021 in the area where the species was initially discovered, the number of laboratory populations aged L3-L5 reintroduction of caterpillars was carried out. The monitoring results conducted in 2022-2023 according to that plan were positive [1, 9, 18].
- 3. Since *S.pyri* is a wild silkworm, it instinctively develops better under feeding conditions that are close to natural conditions, and therefore feeding caterpillars on bouquets in laboratory or rearing houses conditions is closer to natural conditions, so it is appropriate to breed them in bouquets for mass increase. It should be noted that feeding with water-sprayed leaves reduces S.pyri caterpillars' running around, doubles their survival, partially increases their body weight, and shortens the development period between L1-L5 ages. During feeding on the bouquet, S.pyri caterpillars, according to the development dynamics and duration (46.12±0.71 days), the number of obtained cocoons (168 pieces) and quality (the highquality cocoons number 68.15+0.09%), according to indicators of food activity of larva on the second day of L4 instar (28.96 feces in 24 hours) and the amount of oxygen consumed by pupa in the first month of the pupal stage, it is more favorable for the mass increase of wild silkworm indoor conditions. [5, 7, 11, 19].
- 4. As a result of the study of the cocoon of the silkworm *S.pyri*, which has a simple structure, it was determined that the cocoon membrane, which is 11.43+0.10%, consists of three layers and contains mineral salts (mainly calcium oxalate), tannin-phenol compounds (mainly in the outer layer) and the percentage of the structure protein composition of silk fibers (fibroin 77.15+0.35%), (sericin 20.5%+0.63%) was determined. All these indicators are

close to the indicators of other species used in sericulture, and in some cases superior was determined to be [2, 10,14, 16].

- 5. 5. It is suggested to boil the cocoons in an effective softening solution (a mixture of  $Na_2CO_3$  and  $NaHCO_3$  with 10 g/l of each) for 20 minutes and steam for 45 minutes to open the cocoons of saturnia. The use of this softener preserves the natural brown color and luster of S. pyri silk fibers, and it was possible to open all layers of the cocoon (including the inner layer) through the solution. The ratio of the total amount of unfolded silk was 1:4:1.5 for the three layers. Thus, the possibility of obtaining silk thread useful for various fields of the silk industry from the *S.pyri* moth has been proven [4, 10, 14, 16].
- 6. It was determined that 52.50% of crude protein, 27.89% of fat, and 10.50% of fibers are contained in the pupa of *S.pyri* silkworm. 25 mg of  $\alpha$ -tocopherol, 572 $\mu$ 0 of  $\beta$ -carotene, and 1144 $\mu$ 0 of total carotenoids, which is the biologically active form of available to-copherols, were found in 100 g of oil of *S.pyri* pupa. High amounts of K, Mg, Na, Ca, and Al macro-elements within accepted norms were detected in the pupa of *S.pyri* silkworm. The amount of trace elements such as Ti, Fe, Cu, Ba, and Zn is evenly distributed in the pupa [6, 8, 17].

### PRACTICAL RECOMMENDATION

Based on our results, we make the following suggestions:

1. The rearing house should be thoroughly disinfected before and after the silkworm feeding season under conditions of closed and controlled temperature ( $+25 \pm 1^{\circ}$ C) and relative humidity ( $70 \pm 10\%$ ). It should be ensured that farmers follow appropriate hygiene rules during the feeding period.

2. Newly hatched larvae should be fed in comfortable wide boxes, and the boxes should be covered with a damp protective cover. Starting from the end of the L2 instar, caterpillars should be transplanted into bouquets. To place bouquets of freshly cut branches, it is important to use containers filled with water with a stable bottom and a narrow neck (ceramic, if possible), and an intermediate distance should be calculated between the bouquets, allowing the silkworms not to crowd together and regulate normal air ventilation. To do this, containers with new bouquets should be placed in an empty place in contact with the previous bouquet so that the caterpillars can freely move to the new bouquet. It is necessary to wait until the caterpillars have completely moved from the old bouquets, where the leaves were eaten, to the bouquets with new branches; When collecting used bouquets, you need to make sure that there are no silkworms among the branches; it is necessary to ensure that caterpillars in a molting state remain in old bouquets until they become active; the tabletop on which the bouquets are arranged should be as clean as possible of excrement released by silkworms and old shells discarded after molting. Except for rainy and chilly days, it is necessary to spray bouquets with water from a spray bottle 1-2 times a day (until the middle of the L5 instar).

3. To unwind *S.pyri* cocoons, the first step is to make a crosssection of the cocoon and free it from the pupa. Then it is necessary to prepare a water-based softening solution with a concentration of sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>) 10 g/l and sodium bicarbonate (NaHCO<sub>3</sub>) 10 g/l and after bringing the cocoons to boiling point in this solution for 20 minutes, they should be steamed 45 minutes. Since the silk fibers in the cocoon of *S.pyri* are not continuously wound, unlike silkworm silk, the resulting pure silk fibers are preferably pre-spun on a spinning wheel for use in textiles.

### LIST OF SCIENTIFIC WORKS PUBLISHED ON THE DISSERTATION TOPIC

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