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Ismatullaeva DA
Candidate of Agricultural Sciences, Uzbekistan Research Institute of Sericulture, Uzbekistan

Ziyaeva Ya M
Senior Researcher, Uzbekistan Research Institute of Sericulture, Uzbekistan

ST Valiev
Candidate of Agricultural Sciences, Uzbekistan Research Institute of Sericulture, Uzbekistan

Correspondence
Ismatullaeva DA
Candidate of Agricultural Sciences, Uzbekistan Research Institute of Sericulture, Uzbekistan

Another way of silkworm nosematosis control

Ismatullaeva DA, Ziyaeva Ya M and ST Valiev

Abstract
In this article the results of research, aimed at finding the period of the most marked reaction in the development of silkworm eggs were given, in order to increase the effectiveness of nosematosis control by applying modern antiseptic drugs of domestic production.

Keywords: Silkworm, larvae-hatchers, nosematosis, spore, grena (eggs)

Introduction
As it is known, there are a lot of works in literature, devoted to usage of various (medicinal, veterinary, etc.) preparations for bees nosematosis control (Ostrovsky, 1959, Stetsenko, 1968, Gavrilov, 1968, 1982; Smirnovaperegud, 1971; Madatov, Mershchiev, 1973, 1975, 1977, Orbic, 1985). These authors tested a whole arsenal of chemical reagents to control bees nosematosis and determined that sulfanilamide preparations, their combinations with antibiotics, fumagillin, urotropine, etc., are effective to control them.

In contrast to works devoted to the use of preparations for the control of bees nesematosis, it is not enough works of application of chemotherapy against nosematosis (pebrine) of silkworm (Chil-Hakobyan *et al*., 1962, 1966; Rusu, Popa, 1965; Wafa, Kotby, 1971; Mladen, 1973; Khakhanov, Verbitskaya, Atabekova, 1979; Chandra, Kundu, 1982; Orbic, Tomas, 1985; Kashkarova *et al*. 1982, 1983, 1988, 1989, etc.).

Despite the enormous material damage to the silkworm, caused by pebrine, the research on this very necessary issue at the end of the 20th century was scanty and sketchy.

One of the most significant works in this direction was the work of the researchers of the Institute of Microbiology of Armenia (Chil-Akopyan, Bobikyan *et al*., 1963; Chil-Hakobyan, Puchinyan, 1966). The abovementioned authors tested more than 28 antibiotics on infected silkworm eggs. The drugs were tested on the infected eggs during the wintering period - in December, and showed that none of the drugs has an anti-pebrine effect. The reason for the negative conclusion in the studies of Armenian microbiologists is the lack of knowledge of silkworm biological development and its obligate parasite, the causative agent of pebrine. During the beginning of silkworm eggs wintering, the embryo and, together with it, pebrine causative agent are at rest. And the rest stage for pebrine causative agent represents a spore form, which is hardly vulnerable to all kinds of influences. Studies have been proven with the help of microbiological methods that the preparations penetrated into silkworm eggs and, in particular, in its embryo and subsequently found in larvae. Therefore, there was no reason to think that the preparations did not penetrate into silkworm eggs.

Consequently, the reasons for the unsuccessful trial lie in the insufficient significance of the host-parasitic relationship of silkworm with pebrine causative agent.

Evidence of this served studies of AI Khakhanov, GA Verbitskaya, KS Atabekova (1979), performed on silkworm eggs, rejected by the State Control during the end of diapause, high efficiency of the tested drugs has been proved. Silkworm eggs processing in the period of embrio spring development allowed to reduce the infection for several times. Method for treating of infected eggs with sulfopyridazine + aminoquinol or chlorotetracycline was recommended to production by authors.

Works on pharmaceuticals usage in eggs phase of silkworm were continued by Kashkarova LF, Khakhanov AI, Andoskina LT. and others (1983, 1986, 1987, 1989) [3].

It was established by aforesaid authors that among pharmaceuticals the most effective, improving the quality of eggs are the preparations of the nitrofuran series and sulfonamides, as well as their combinations.

The developed methods of pebrine control do not allow complete disinfection and require improvement.

Proceeding from the assumptions in the literature that the vegetative forms of the parasite -planonts and merons predominate in silkworm eggs during the first 5 days from the moment of oviposition (according to the data of AI Khakhanov, 1956), it was set the task- to find the most favorable period in the development of eggs for the use of modern antiseptic agents.

Material and Methods of Research

Ipakchi 2 larvae were bred according to the generally accepted technology for white-cocoon breeds. On the first day of the 5th age, larvae were artificially infected with nosematosis by means of feeding with mulberry leaves treated with a suspension of spores of pathogen Nosema Bombycis N. with a titer of 100 spores / mm3. Infection was carried out once. After infection, larvae were fed till cocoons mounting. After carrying out papillonage and obtaining the necessary amount of silkworm eggs, the latter were thoroughly mixed and divided into 8 parts. Each part of eggs was treated with drugs at a certain period of its development, starting from the I-day and ending with 8-day age. New drugs "Himix", "Seppedez" were tested. Silkworm eggs treatment was carried out in aqueous solutions of the preparations at a temperature of + 25 °C for two hours. In the control variant, eggs were kept for 2 hours in ordinary water. After the treatment, the eggs were dried and stored according to conventional technology until the spring of the following year. In the spring of 2017, before the incubation, four samples of eggs on 100-pieces were counted from each variant. The counted samples were incubated, the percentages of fresh cocoons hatching were determined, etc. Hatching larvae were microscopically analyzed for the determination of residual infection, where each larva was microscoped. Microanalysis was carried out in a conventional light microscope using a phase-contrast device. In each drug, at least 50 fields of vision were viewed.

Results and Its Discussion

Data on the percentage of start of insect rearing start, infected by nosematosis and treated with antiseptic drugs were given in table 1.

Table 1: The indices of insect rearing start, infected by nosematosis and treated with preparations

№ пп	Name of preparation	Concentration (%)	Eggs hatchability (aver. from 3 replications) (%)	Hatchability increase in comparison with control (%)
1	**2**	**3**	**4**	**5**
1-day-old eggs				
1	Septadesis	0,15	79,6	1,3
	Septadesis	0,2	79,0	0,7
	Chemics	0,15	78,6	0,3
	Chemics	0,05	80,0	1,7
2-days-old eggs				
2	Septadesis	0,15	80,6	2,3
	Septadesis	0,2	80,3	2,0
	Chemics	0,15	81,0	2,7
	Chemics	0,2	80,0	1,7
3-days-old eggs				
3	Septadesis	0,15	82,0	3,7
	Septadesis	0,2	81,3	3,0
	Chemics	0,15	79,6	1,3
	Chemics	0,2	83,3	5,0
4-days-old eggs				
4	Septadesis	0,15	82,0	3,7
	Septadesis	0,2	83,3	5,0
	Chemics	0,15	83,0	4,7
	Chemics	0,2	82,3	4,0
5-days-old eggs				
5	Septadesis	0,15	83,3	5,0
	Septadesis	0,2	82,7	4,4
	Chemics	0,15	83,7	5,4
	Chemics	0,2	81,7	3,4
6-days-old eggs				
	Septadesis	0,15	87,3	9,0
	Septadesis	0,2	89,3	11,0
	Chemics	0,15	91,0	12,7
	Chemics	0,2	89,7	11,4
7-days-old eggs				
7	Septadesis	0,15	92,3	14,0
	Septadesis	0,2	91,0	12,7
	Chemics	0,15	90,7	12,4
	Chemics	0,05	88,0	9,7
8-days-old eggs				
8	Septadesis	0,15	91,7	13,4
	Septadesis	0,2	89,0	10,7
	Chemics	0,15	91,3	13,0
	Chemics	0,2	89,3	11,0
9	Control (with infection)	-	78,3	

As seen from table data, in the experimental variants, the start of insects rearing ranged from 78.6 to 92.3%, and in the control variant it was 78.3%. In processing 1-day-old eggs with drugs, an increase in the percentage of start of insect rearing is observed in comparison with the control by 0.3-1.7%. Processing of 2 and 3-day-old eggs, makes it possible to increase the start of insect rearing for 1.3-5.0% in comparison with the control. By processing 4 and 5-day-old eggs, an increase in start of insect rearing is observed by 3.4-5.4% in comparison with the control. The best result was obtained when 6, 7 and 8-day eggs were treated by preparations, where the start of insect rearing percentage was 9.0-13.4%.

After determining the start of insect rearing percent, the larvae were microscopically analyzed to determine residual infection with nosematosis.

Table 2: Comparative data on nosematosis infection of fresh cocoons with a control variant

№ pp	Name of preparation	Concentration (%)	Infection rate of larvae-hatchers (%)	Infection rate in comparison with control	
				Reduction	
				Abs.%	Rel.%
1	2	3	4	5	6
1-day-old eggs					
1	Septadesis	0,15	15,9	1,1	6,5
	Septadesis	0,2	14,7	2,3	13,5
	Chemics	0,15	13,6	3,4	20,0
	Chemics	0,2	15,0	2,0	11,7
2-days-old eggs					
2	Septadesis	0,15	15,2	1,8	10,6
	Septadesis	0,2	14,5	2,5	14,7
	Chemics	0,15	14,8	2,2	12,9
	Chemics	0,2	14,1	2,9	17,0
3-days-old eggs					
3	Septadesis	0,15	14,9	2,1	12,4
	Septadesis	0,2	14,3	2,7	15,9
	Chemics	0,15	12,9	4,1	24,1
	Chemics	0,2	13,9	3,1	18,2
4-days-old eggs					
4	Septadesis	0,15	13,1	3,9	22,9
	Septadesis	0,2	15,9	1,1	6,5
	Chemics	0,15	13,2	3,8	22,4
	Chemics	0,2	14,2	2,8	16,5
5-days-old eggs					
5	Septadesis	0,15	12,9	4,1	24,1
	Septadesis	0,2	11,7	5,3	31,2
	Chemics	0,15	12,3	5,0	27,6
	Chemics	0,2	14,3	2,7	15,9
6-days-old eggs					
6	Septadesis	0,15	11,1	5,9	34,7
	Septadesis	0,2	11,6	5,4	31,7
	Chemics	0,15	10,3	6,7	39,4
	Chemics	0,2	11,5	5,5	32,3
7-days-old eggs					
7	Septadesis	0,15	8,3	8,7	51,2
	Septadesis	0,2	9,6	7,4	43,5
	Chemics	0,15	7,7	9,3	54,7
	Chemics	0,2	8,9	8,1	47,6
8-days-old eggs					
8	Septadesis	0,15	8,4	8,6	50,5
	Septadesis	0,2	9,4	7,6	44,7
	Chemics	0,15	7,3	9,7	54,1
	Chemics	0,05	10,9	5,5	33,54
9	Control (water)	-	17,0		

As shown, the results of microanalysis of infection by nosematosis of larvae-hatchers in the experimental variants were 7.3-15.9%, and in control - 17.0%. The reduction of experimental material contamination for 1.1-4.1% in comparison with the control is observed in 1, 2, 3 and 4 days-old eggs in treatment with drugs. Treatment of 5, 6, 7 and 8-days-old eggs allows reducing the incidence of nosematosis by 2.7-9.7%. The best index of reduction in infection with nosematosis is determined when 6, 7 and 8-days-old eggs are treated by preparations. Here the infection is reduced by 5.4-9.7 absolute percent or 31.7-54.7 relative percent in comparison with control variant.

Conclusions

Thus, the establishment in a fresh oviposition of a period optimal for the use of antiseptic agents, in turn, contributes to increase the effectiveness of silkworm nesematosis control. Our investigations have made it possible to establish another period in the development in eggs phase, which is favorable for the use of antiseptic drugs. This is the period when vegetative forms of parasite -planonts and meerons-predominate in the development of eggs in the first 8 days from the moment of their laying. As a result of the research, another method of silkworm nosematosis control has been developed.

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