

Use and application of herbal preparations against pebrin disease in *Bombyx mori* L. populations.

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ABSTRACT

The serious threat posed by pebrin disease of mulberry silkworm to sericulture, the limitations of existing control methods, and the relevance of searching for herbal alternatives are substantiated. The aim of the study is to study in the condition *in vitro* and *in vivo* effects of selected plant extracts from the rich vegetation of Azerbaijan against pebrin spores and to develop safe and effective herbal preparations for use in sericulture farms.

The aim of this study was to evaluate the prevention and treatment of pebrin disease by a number of plant extracts used in traditional medicine (on worms emerging from naturally diseased grenae of the *Namazli-2* genus). The diseased grenae had been divided into 3 parts. The first part - worms that were treated with grenae based on the existing methodology and emerged from the affected grenae were given feed soaked in plain water twice a day until the end of feeding. The second part - worms that emerged from the affected grenae were given feed soaked with herbal preparations (wormwood, mint, garlic extract, citric acid) twice a day starting from the first feeding. The third part - worms that emerged from the diseased grenae that were not affected by the preparations were given feed soaked with the above herbal preparations twice a day starting from the first feeding.

As a control for the experiment, in 3 repetitions, 150 worms in each repetition, worms that emerged from naturally diseased grenae and also from healthy grenae were fed with leaves soaked in plain water twice a day.

While the total mortality from Pebrin disease was 14.23% in the control, this indicator was 4.00-7.00% in the experimental variants. The survival rate of worms was also higher in the experimental variants (94.00-96.00%) compared to the control (85.78). If the cocoon yield from one box was 72.0 kg in the diseased control variant, the cocoon yield increased to 80-85 kg in the trial of herbal preparations.

INTRODUCTION

Silk has been a natural fiber highly valued by humans since ancient times and has been widely used in the production of various textile products (El-Sayed NAA 1999). In modern times, the unique properties of silk (hygiene, elasticity, strength, etc.) have expanded its application areas, which has increased the demand for raw silk production worldwide. One of the main ways to increase silk production is to develop effective control measures against diseases of mulberry silkworms (*Bombyx mori* L.). One of the factors causing significant material damage to sericulture in Azerbaijan is various infectious diseases occurring

in mulberry silkworm broods: flasheria, septicemia, muscardina, jaundice and pebrin (nosematosis). The occurrence of these diseases in mulberry silkworm broods causes a loss of cocoon yield of up to 10-100%.

Among these diseases, pebrin (nosematosis) disease occupies a special place, the causative agent of which is *Nosema bombycis*, which causes great economic damage to sericulture farms (Atabekova, K.S (1981).

Recent studies show that pebrin disease still remains a serious problem in many silk-producing regions. Although a number of methods of combating nosematosis have been developed in the current period, they have not found wide application in carrying

out completely radical measures against the disease due to their shortcomings. Therefore, the development of a treatment method for mulberry silkworms against pebrin disease is an issue of important scientific, practical and economic importance.

Azerbaijan has a very diverse wealth of plants used in the treatment of various diseases, which have been studied by pharmacists and pharmacologists of the republic over the past 35-50 years, and a large number of plants have been included in scientific medicine.

It should be noted that currently in a number of countries, ancient folk medicine has been enriched with modern knowledge and has begun to be applied again. In Sweden, France, Germany, China and a number of other countries, medicinal plants are widely used in pharmacy. In world practice, they are trying to replace chemical drugs in medicine with natural remedies made from plants. All this shows that even in technologically advanced countries, the use of natural medicines is gradually gaining more and more supporters.

In many countries, a number of herbal remedies are now widely used in veterinary medicine for infectious diseases such as eymeriosis, coccidiosis in animals, and nosematosis in bees.

The aim of this study is to identify plants with potential effects against pebrin disease from the rich vegetation of Azerbaijan and to assess their impact on *Bombyx mori* L. populations.

Pebrin (nosematosis) is one of the most widespread and harmful infectious diseases of mulberry silkworms. The causative agent of the disease is transmitted from generation to generation (through grenae) through spores. Worms emerging from infected grenae infect healthy worms during feeding, causing mass mortality and significant economic losses.

Various physical, chemical and biological methods have been used to combat Pebrin disease. In order to obtain healthy cocoons, Louis Pasteur proposed the cellular method in 1868. This method involves examining each female moth individually under a microscope and destroying the eggs of that moth if pebrin spores are detected. Although this method is effective, its long time, high labor intensity and the requirement of special equipment limit its widespread application.

Studies have shown that when the grenae and pupae of mulberry silkworms are exposed to sublethal temperatures for a certain period of time, the tolerance of the next generation to high temperatures increases, the survival rate increases, and the productivity and quality of the cocoons improve (Khakhanov, A.I., Kashkarova, L.F., Andoskina, L.T., Parishev, B.A. 1983). It is thought that the effect of sublethal temperatures strengthens the immune system of the worms and increases their tolerance to stress.

In recent times, one of the main directions in the fight against pebrin disease has been the exposure of grenae and pupae to sublethal temperatures. As a result of the conducted studies, it was determined that by exposing sick pupae of mulberry silkworms to pebrin for 16 hours at a temperature of 33-34°C, the technological basis for the production of grenae free of pebrin spores has been created (Kuzmina, E.V. 1988). This method allows to obtain healthy offspring by limiting the development of the pathogen.

High temperatures have been used in sericulture not only against pebrin, but also against other infectious diseases. According to information, exposure of grenae to 34°C for 24 hours can help prevent viral flasheria and cytoplasmic jaundice. It has also been observed that the ability to prevent cytoplasmic jaundice increases in worms fed at 35°C in an artificial nutrient medium (Marchishina, E. 1997). Although thermal treatment methods offer promising approaches for disinfection of grenae, their potential effects on grenae quality require careful monitoring.

According to the literature, various chemical disinfection methods and a number of ways to combat infectious diseases of mulberry silkworms have been developed, affecting the grenae.

Important research has also been conducted by Azerbaijani scientists in this direction. In particular, Khakimova and Asanov, after storing the grenae taken out of the refrigerator 20 days before spring incubation for 3 days at a temperature of 15-16°C and a relative humidity of 75-80%, treated it with a solution of FAA preparations, the main composition of which is K, Na and NH₄, for 2.5 hours at a temperature of 24-25°C. As a result, the

percentage of butterflies infected with pebrin decreased by 3-12 times compared to the control group.

In the studies conducted by Kashkarova L.F., Khakhanov A.I. et al., pebrin-infected (38.1%) grenae were treated with solutions of various drugs (chloridin, quinine, piperazine, urotropine, streptoside, emetine, nuflein). The grenae removed from the refrigerator was stored at a temperature of 15-16°C for 3-4 days before being treated with the solution. The least infection was observed in newly hatched caterpillars in the variants of 1.0% chloride (81.1%), 0.5% piperazine (91.3%) and 0.5% emetine (83.2%), which is significantly lower than in the control group (water).

Khakhanov and colleagues tested Fumagillin (0.8; 0.08; 0.008%) and Monomycin (600, 400, 200 thousand units per 1 liter of distilled water) preparations in various concentrations against pebrin disease of mulberry silkworm. Based on the results obtained, the authors recommended using both preparations only in industrial feeding. This limitation is probably due to the special conditions required for the application of the preparations or the impracticality of use in private farms.

As a result of the studies conducted, the therapeutic and toxic effects of synthetic preparations called *Nozematol-1* and *Nozematol-2* against pebrin disease of mulberry silkworms were studied. It was found that when 20-40 grams of *Nozematol-2* were added to 1 kg of leaf meal, the level of pebrin infection of butterflies decreased by 5-10 times compared to the control group. Although these results indicate the effectiveness of synthetic preparations, concerns about their potential side effects and continued use make the search for plant-based alternatives urgent (Garayev, I.I., Ahmadov, E.A. 2005).

Although chemical disinfectants are effective in neutralizing pebrin spores, there are concerns about their negative effects on the environment and silk fiber. Therefore, the search for more natural and environmentally safe control methods continues.

In recent years, the increasing interest in the antimicrobial and antiparasitic properties of herbal preparations has raised the issue of their potential application against pebrin disease (Golyshenkov, P.P. 1984). Some researchers have suggested that certain herbal extracts may have a positive effect on the survival rate of pebrin-infected worms. However, the mechanisms of action of these preparations and their efficacy against hereditarily transmitted pebrin infection have not yet been sufficiently investigated.

The aim of this study was to evaluate the role of a number of herbal extracts used in traditional medicine in the prevention and treatment of pebrin disease (on worms from naturally infected grenae of the *Namazli-2* strain). The results of this study may contribute to the development of natural and sustainable control strategies for the sericulture industry.

It should be noted that in a number of countries, ancient folk medicine has been integrated with modern scientific knowledge and has begun to be re-applied. In Sweden, Germany, China and other countries, medicinal plants are widely used in pharmacy. Modern advances in the field of phytochemistry and pharmacology allow us to understand the biologically active substances of plants used in traditional medicine and their mechanisms of action.

Literature data provide numerous examples of the widespread use of herbal preparations in the medical and veterinary fields. In particular, a number of natural remedies are used in beekeeping against nosematosis. It has been established that adding 10 mg of citric acid to bee feed once a week reduces the tolerance of spores in the intestine. For the prevention of nosematosis, beekeepers widely use garlic (in the form of alcohol extract, fresh juice, aqueous solution). Biologically active substances such as allicin in garlic are thought to have a potential effect against parasitic infections.

There is also experience in using plants such as red hot pepper (containing capsaicin), wormwood and chamomile against nosematosis in beekeeping. The antimicrobial and antiparasitic properties of various biologically active substances contained in these plants can ensure their effectiveness. Usually, aqueous or alcoholic extracts of herbal preparations are mixed with sugar syrup and given to bees in the evening (Garayev, I.I., Musayeva, M.R. 2005).

These experiments used against nosematosis in beekeeping, the treatment of mulberry silkworm with herbal preparations against

pebrin disease may provide promising scientific directions. The study of plants with similar biologically active substances and the study of their effect on mulberry silkworm may be promising. Studies conducted by Hajiyeve and Eminov have shown the therapeutic potential of a number of plants (cumin, mint, three-leaf clover, chamomile, etc.) against helminthiasis in animals. The authors observed that giving 25 grams of a mixture of these plants in the form of juice and decoction to each animal for 10 days has a lethal effect on helminths. This study once again confirms that biologically active substances contained in various plant species can be effective against parasitic infections.

Herbal preparations successfully used against helminthiasis in animals may also be potentially effective against pebrin disease due to the substances they contain that have a broad spectrum of antiparasitic effects. The above facts indicate the importance of using herbal preparations against pebrin disease in mulberry silkworms and the need to conduct scientific research in this direction.

Material and methods

The main objective of this study is to develop effective methods for the treatment and prevention of pebrin disease of mulberry silkworms using herbal preparations on a natural (hereditary) basis.

To achieve this goal, the following tasks have been identified:

1. Evaluation of the in vivo condition effects of wormwood (*Artemisia absinthium* L.), mint (*Mentha piperita* L.), garlic (*Allium sativum* L.) extracts and various concentrations of citric (*Citrus limon* (L.) Osbeck) acid against pebrin disease of mulberry silkworm (*Bombyx mori* L.).
2. Determination of the optimal application duration of selected herbal preparations.
3. Determination of the therapeutic efficacy of the preparations depending on the application duration and concentration.
4. Identification of herbal preparations with the highest therapeutic efficacy.
5. Evaluation of therapeutic efficacy by applying selected preparations to mulberry silkworms emerging from naturally pebrin-infected grenae.

Research Materials. In the experiment, healthy and diseased cocoons of the Namazli-2 mulberry silkworm strain created at the Azerbaijan Scientific Research Institute of Sericulture were used.

Preparation of Herbal Preparations

- **Wormwood extract:** Dried wormwood herb (aerial parts) was boiled in distilled water (1:10 ratio) for 30 minutes, then cooled and filtered. It was prepared at a 1% concentration and fed to mulberry silkworms.
- **Mint extract:** Dried mint leaves were boiled in distilled water (1:10 ratio) for 20 minutes, then cooled and filtered. It was prepared at a 2% concentration and fed to mulberry silkworms.
- **Garlic extract:** Fresh garlic cloves were crushed, soaked in distilled water (1:5 ratio) for 24 hours and filtered. It was prepared at a concentration of 1% and fed to mulberry silkworms.
- **Citric acid solution:** A 5% citric acid solution was prepared in distilled water and fed to mulberry silkworms.

For the test experiments, sick grenae were taken to work with each preparation. The grenae were treated with the solutions of the preparations (mint - 25%, wormwood - working solution, lemon

- 4%, garlic - working solution) in sterile bags for 2 hours at 15-18 °C, dried, and incubated 30 days before incubation.

As a control for the experiment, in 3 repetitions, 150 worms in each repetition, worms that emerged from natural grenae and also from healthy grenae were fed with leaves soaked in plain water twice a day.

After the start of the experiment, constant observation was carried out on the experiment until the worms turned into cocoons (until they emerged). The worms that remained under development and died by day were examined individually under a microscope according to the repetition of the variants, and the therapeutic effectiveness of the preparations was determined.

During the work, 10 female and 10 male cocoon samples were taken from each variant to study the biological indicators of feeding after cocoon collection, and 25 female and 25 male cocoons were taken to study the technological indicators of cocoons.

Discussion of the work: In this study, natural diseased and healthy grenae of the mulberry silkworm strain *Namazli-2*, created by breeders of the Azerbaijan Scientific Research Institute of Sericulture, were used. Some morphological and biological characteristics of the *Namazli-2* strain are as follows:

- grenae revival - 86.90 %,
- worm viability - 93.30 %,
- feeding period - 28.7 days,
- live cocoon mass - 1.73 g,
- silkiness of live cocoon - 23.31 %,
- cocoon yield from one box of grenae - 28.1 kg.

The grenae of the *Namazli-2* strain are ash-colored, sticky, the worms are spotted, and the cocoons are white and oval.

Experiments were conducted as follows to study the therapeutic effects of the tested herbal preparations on naturally diseased grenae and worms emerging from these grenae.

Considering that the low percentage of grenae resurrection is considered as an indicator of its infection with pebrine disease spores, the current study investigated the therapeutic effect of preparations applied against pebrine disease in mulberry silkworms of the species *Bombyx mori* (L.). For the test experiments, an appropriate amount of naturally diseased grenae for each variant (preparation) was taken and placed in pre-prepared sterile bags, removed from hibernation 30 days before spring incubation, treated with the above-mentioned concentrated solutions of the tested preparations at a temperature of 15-18° for 2 hours, then dried and incubated.

After the diseased grenae were treated in a solution of the appropriate herbal preparations, samples were taken from each variant in 3 replicates, 100 grenae in each replicate, to study the regenerative capacity of the grenae. At the same time, in the experiment, 3 samples of 100 grenae in each replicate were taken from the natural healthy grenae, which were treated with ordinary water (distilled) and to study the regenerative capacity (table 1).

The preparations used and their concentrations are as follows:

- 25% peppermint extract
- 4% citric acid solution
- wormwood - working solution
- garlic - working solution

The effect of preparation used against Pebrin disease on the regenerative capacity of grenae

Table 1

Name of the preparation used	Density of the used preparations,%	Number of repeats, units	Unresurrected grenae, in numbers	Grenae's resurrection ability
Mint	25	100	6,66	93,34
Lemon	4	100	8,66	91,34
Wormwood	Working solution	100	8,33	91,67
Garlic	Working solution	100	30	70,0
Control-healthy	-----	100	15	85,0
Control-diseased	-----	100	24	76,0

The analysis of the experiment revealed that the treatment of grenae with herbal preparations has a positive effect on its

resurrection ability. Thus, while the percentage of grenae resurrection in the control (patient) variant was 76.0%, in the

experimental variants this indicator was significantly higher (91.34 - 93.34%).

The diseased grenae was divided into three parts.

- The first part - the grenae was processed based on the existing methodology and the worms that emerged from the affected grenae were given feed soaked in water twice a day until the end of feeding.

- The second part - the worms that emerged from the affected grenae were given feed soaked with herbal preparations twice a day starting from the first feeding.

- The third part - the worms that emerged from the diseased grenae that were not affected by the preparation were given feed soaked with herbal preparations twice a day starting from the first feeding.

The experiment included control groups:

- Diseased control: Worms from naturally infected grenae (3 replicates, 150 worms per replicate) were fed mulberry leaves soaked in plain water twice a day.

- Healthy control: Worms from healthy grenae (3 replicates, 150 worms per replicate) were fed mulberry leaves soaked in plain water twice a day.

Worms emerging from diseased grenae were fed with mulberry leaves soaked in various concentrations of herbal preparations, depending on their age, twice a day. When the ready-made solution of the preparations was mixed with feed and fed to the worms, 2 ml of the corresponding preparation solution was used for every 100 worms per 10 g of freshly chopped mulberry leaves. Mulberry silkworms were fed with the preparations starting from the first feeding. Intermediate feeds were given to the worms in the usual way. Feeding the worms with the solution of the corresponding preparations was continued together until the 2nd day of the 3rd age, and from the 2nd day, in 3 repetitions according to the options, with 150 worms in each repetition, until the end of the 4th age (until the 4th sleep). In the experiment, mulberry silkworms were fed with ordinary feed (without preparations) from the 1st day of the 5th age until the end.

To study the therapeutic effect of the tested herbal preparations on worms artificially infected with pebrin spores, healthy silkworms of the *Namazli-2* mulberry silkworm breed, i.e. free from noseema spores, were fed with age-appropriate (untreated) feed according to agrozootechnical rules until the 2nd day of the 3rd age, and on the 2nd day of the 3rd age, worms were counted and 3 replicates were drawn up, with 100 worms in each replicate. Then, the worms were artificially infected after 8 hours of starvation on the same day (2nd day of the 3rd age) according to the appropriate methodology. To artificially infect mulberry silkworms with pebrin, the diseased worms were crushed in a mortar and mixed with water, filtered through 2-3 layers of gauze, centrifuged 4-5 times and cleaned of foreign impurities. The sediment was dissolved in boiled cold water to prepare a suspension containing 1000 Nosema spores per mm³. Spore counting was carried out in a Goryayev chamber. After the suspension was prepared, after 8 hours of starvation, the worms were fed by adding 2 ml of the suspension to 10 g of freshly chopped leaves, that is, they were artificially infected. 24 hours after infection, the worms were fed with the tested plant preparations. In the experiment, the preparations were given to the worms until the 5th day of the 5th age of life.

In the experiment, to determine the effect of herbal preparations on the development of pebrin disease in mulberry silkworms,

The effect of treating naturally diseased grenae with herbal preparations on the development of pebrin disease

regular observation was carried out on the worms every day until the cocooning period. Underdeveloped and dead worms were collected by age for each variant, examined individually under a microscope, and worms infected with pebrin were recorded. The therapeutic and prophylactic effectiveness of the tested preparations was determined according to the number of diseased worms (compared to the diseased control) (Table 2).

After the mulberry silkworm feeding period was completed, that is, after the worms had cocooned, on the 7th day of cocooning for the variants and replicates, the cocoons were collected from the branch, cleaned of roughness, and the dead and unborn cocoons were selected. On the 8th day of cocooning, 15 female and 15 male cocoons and their cocoon membranes were weighed for the variants and replicates, and the biological indicators of the mulberry silkworm were determined. The results of the experiment are presented in tables 1 and 2.

The table shows that the treatment of the grenae with various solutions and the feeding of the worms emerging from the grenae with feed soaked with herbal preparations had a positive effect. Thus, when the grenae was treated with 25% mint extract and the worms emerging from the grenae were given feed soaked with the same extract, the total mortality was 4.45%. When the grenae was treated with 25% mint extract only and the worms emerging from the grenae were given feed soaked with water, the total mortality was 4.00%.

The total mortality in the worms emerging from the grenae treated with 4% citric acid solution and fed with feed soaked with that solution was 4.22%. When the grenae was treated with 4% citric acid solution only and the worms were given feed soaked with water, the total mortality was 5.00%.

In comparison, the overall mortality in the diseased control group was 14.23%, while in the healthy control group this figure was 2.78%.

These results indicate that treatment of grenae with mint and citric acid solutions, and especially the addition of feeding with mint extract, significantly reduced the mortality rate from pebrin disease compared to the diseased control group.

CONCLUSION

Herbal preparations significantly increase the survival rate of silkworms. The survival rate, which was 76% in the control group, was over 91% in the groups treated with mint, citric acid and wormwood. This increases the emergence of healthy larvae.

Therapeutic feeding reduces the mortality rate of silkworms. The mortality rate, which was 14% in the control group, varied between 4-7% in the groups fed feed soaked with herbal preparations. This indicates that the preparations are effective in preventing the development of the disease.

Herbal preparations increase cocoon productivity. While 72 kg of cocoons were obtained from one box in the control group, this figure increased to 80-85 kg in the treated groups. This is a major advance for silk production.

The results of this study are very promising for the sericulture industry. In the future, the wider application of herbal preparations can significantly reduce the damage caused by pebrin disease. Further studies can further investigate the mechanisms of action of these preparations and determine optimal application methods.

Table 2.

Name of plants	Concentration of solution affected by grenae, %	Feed concentration of the solution, %	Feeding intensity, times	Number of worms in the repetition	Number of patients during the worm season, in numbers	Number of cocoons	Total mortality from Pebrin disease	
							number	%

Mint Lemon	Mint -25	Mint-2 Lemon -5	2	150	6,00	0,67	6,67	4,45
	—	Mint -2 Lemon-5	2	150	8,17	1,50	9,67	6,45
	Mint -25	Water	2	150	3,50	2,50	6,00	4,00
Mint Wormwood	Mint -25	Mint -2 Wormwood -1	2	150	5,50	1,51	7,01	4,67
	—	Mint -2 Wormwood -1	2	150	8,82	2,84	11,66	7,00
	Mint -25	Water	2	150	3,50	2,50	6,00	4,00
Mint Garlic	Mint -25	Mint -2 Garlic -1	2	150	13,49	4,34	17,83	11,89
	—	Mint -2 Garlic -1	2	150	16,17	5,49	21,66	14,44
	Mint -25	Water	2	150	3,50	2,50	6,00	4,00
Lemon Wormwood	Lemon-4	Wormwood -1 Lemon-5	2	150	5,49	0,84	6,33	4,22
	—	Wormwood -1 Lemon-5	2	150	6,67	1,67	8,34	5,56
	Lemon-4	Water	2	150	5,00	2,50	7,50	5,00
Lemon Garlic	Lemon-4	Lemon-5 Garlic -1	2	150	6,00	1,17	7,17	4,78
	-	Lemon-5 Garlic -1	2	150	7,00	3,17	10,17	6,78
	Lemon 4	Water	2	150	5,00	2,50	7,50	5,00
Control-healthy	Water	Water	2	150	2,50	1,67	4,17	2,78
Control-diseased	Water	Water	2	150	13,13	7,51	21,34	14,23

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