IJABR

International Journal of Advanced Biotechnology and Research (IJABR)
ISSN 0976-2612, Online ISSN 2278–599X,
Vol-10, Issue-1, 2019, pp41-46
http://www.bipublication.com

Research Article

The Study of the Process of Discovery of the Spores of Pathogen of Silkworm Nosematosis using Established technical Means

[1]Burlakov V.S., [2]Shvetsov N.N., [3]Zuev N.P., [4]Zuev S.N., [5]Zadnepryansky I.P., [6]Naumkin V.N. and [7]Podchalimov M.I.

[1]Doctor of Agricultural Sciences (Advanced Doctor), Professor of the Department of Electrical Equipment and Electrotechnology in Agriculture, honored inventor of the USSR, laureate of the state prize of Turkmenistan in the field of science and technology, Belgorod State Agrarian University named after V.Ya. Gorin, 308503, setl. Maysky, Belgorod district, Belgorod region, Vavilov St. 1,
[2]Doctor of Agricultural Sciences (Advanced Doctor), Professor, Professor of the Department of General and Private Zootechnics, Belgorod State Agrarian University named after V.Ya. Gorin, Belgorod district, Belgorod region, setl. Maysky, Vavilov St. 24, Faculty of Technology, Russia, 308503,
[3]Doctor of Veterinary Sciences (Advanced Doctor), Professor of the Department of Non-Negative Pathology, the Belarusian Agrarian University named after V.Ya. Gopin, st. Vavilova, d. 1., p. Maysky, Belgorodsky District, Belgorodsky Oblast, Poccia, 308503,
[4]Candidate of Biological Sciences, Senior Lecturer, Department of Informal Pathology, Belgorod State Agrarian University named after V.Ya. Gorin, setl. Maysky, Belgorod district, Belgorod region, Vavilov St. 1, Russia, 308503,
[5]Doctor of Agricultural Sciences (Advanced Doctor), Professor of the Department of General and Private Zootechnics, Belgorod State Agrarian University named after V.Ya. Gorin, Russia, 308503, Belgorod Region, Belgorod District, setl. Maysky, Vavilova St., 1,
[6]Doctor of Agricultural Sciences (Advanced Doctor), Professor, Professor of the plant and vegetable breeding, Belgorod State Agrarian University named after V.Ya. Gorin, Belgorod district, Belgorod region, setl. Maysky, Vavilov St., 24, Agronomy faculty, Russia, 308503,
[7]Doctor of Agricultural Sciences (Advanced Doctor), Professor, Professor of the Department of Vocational Education, Kursk Institute of Educational Development, Kursk, Sadovaya St., 31, Russia, 305004

[Received: 27/11/2018; Accepted: 30/12/2018; Published: 02/01/2019]

ABSTRACT:

In recent years, the loss of the sericulture from silkworm diseases, nosematosis (pebrine) on feeding is up to 20 %, which causes great economic losses. Therefore, to detect possible contamination, after the natural death of butterflies and their drying, they are subject to mandatory microanalysis in the production of breeding and industrial graine. With the existing manual method of preparation of drugs and imperfect technical means on this technological process, it is impossible to detect sufficiently high-quality spores of infectious diseases of silkworm on nosematosis. In order to improve the efficiency, the results of studies of the process of preparation of drugs for industrial microanalysis are presented. These studies contributed to the creation of fundamentally new, effective technical means for this process. At detection of spores a laying is burnt.

Keywords: quality of grinding, the grinding module, fractions, nosematosis (pebrine), virus, edroz, microanalysis, microsporidia.

INTRODUCTION:

In the sericulture the most dangerous and prevalent disease is nosematosis (pebrine), the pathogen of which is transmitted to offspring transovarially and germinative [1]. Sick butterflies contain more spores of nosematosis, so after their natural death and drying they are subject to mandatory microanalysis. At detection of spores infected feeding is destroyed and the location and inventory is disinfected. However, with the existing mainly manual labor in the process, it is very difficult to observe and control the fulfillment of these conditions. Machines for grinding butterflies such as MRB and Pradella systems do not meet the quality requirements of the preparations. The rest of the operations are manual, which create the risk of ingress of charged particles and re-contamination of another batch of the test material [2]. Taking into account that the method of grinding butterflies does not meet the requirements of the agro process, it is required to find the most effective method of grinding and determine its technical parameters.

MATERIALS AND METHODS

The quality of the prepared preparations in the experiments was evaluated by the degree of grinding of the samples, measurements of the particle size of the crushed material using a microscope. The pounded samples of butterflies, without addition of water, were divided into fractions, by means of lattice classifiers, the mass of each fraction and the total mass of a sample was defined. Abdomenm, cephalothorax and wings of each butterfly are separated, which are crushed in portions by adding water. After the liquid is drained, leaving a precipitate (1.5-2.0 cm), which is thoroughly mixed with a stick and a drop is put on the slide. Each drug is viewed in at least 10 fields of view of the microscope.

The main indicators characterizing the process are the quality of preparations, sanitary and hygienic requirements and productivity. The pounded samples of butterflies, without addition of water, were divided into fractions by means of lattice classifiers.

The mass of each size fraction and the total mass of the sample were determined. The module grind (K_N) were found of the offered dependencies:

$K_N = M_f / M_s$, (1)

where M_f - mass fraction, M_s- sample mass.

The ground samples were divided into five fractions with particle sizes: up to 0.2 mm, 0.2-0.5 mm, 0.5-1.0 mm, 1.0-1.5 mm and more than 1.5 mm.

The quality of washing was assessed visually by viewing the washed mortars and pistils with a microscope with a total magnification of × 600 and phase-contrast device KF-4 in ten fields of view of the liquid droplet from the mortar after washing. Washing was evaluated by a rigid division of the results into two groups: “quality” - the absence of particles of butterflies in the preparation and “poor” - the presence of particles of butterflies.

Experiments were carried out in the conditions of graine production on rocks and on hybrids of silkworm zoned in Turkmenistan, B-1, B-2, T-3, T-4, T-13, T-16, and their direct and reverse hybrids and Ukrainian hybrids, B-1 ul. x B-2 ul., B-2 ul x B-1 ul, B-1 ul, B-2 ul, UN, UF, UN x UF, UF x UN, etc.

Physical and mechanical properties of dry butterflies were studied in order to obtain the necessary data for the design of the device.

The coefficients of static friction were determined experimentally with the well-known formula:

$f = tg\alpha$ (2)

The friction force was determined by the formula:

$T=fN$ (3)

The normal pressure as a component of the sample mass was calculated by the following equation:

$N=M_s \times \cos \alpha$, (4)

where f – coefficient of friction,

α – the angle of the plane when the sample is shifted,

T – friction force,

N – normal pressure,

M_s – sample mass.

For the reliability of the experiments, the coefficients of static friction were determined by fixing two angles α min and α max – the beginning of sliding of the first and last butterflies from the inclined plane, i.e.

$f_n = tg\ \alpha\ min \ldots tg\ \alpha\ max$ (5)

To determine the coefficient of friction samples of butterflies crushed to a module $K_N = 0.8$ were used.

The coefficient of sliding friction was determined by the formula:

$fs= tg\ \varphi - 2S / gt^2 \cos \varphi$, (6)

where φ – the angle of the plane to the horizon,

S – the way taken by the sample,

t – the time of the way passage.

The length of the way was chosen based on the dimensions of the machine equal to 0.3 m.

The efficiency of shock loads was studied on spring copra. The device provides impact velocity from 3 m/s to 15 m/s.

The work of the impact was calculated by the formula:

$A = mv^2 / 2 \times n$, (7)

where m – sample mass,

v – impact velocity,

n – number of impacts.

The most responsible assembly unit of the device for preparation of drugs is the mixer. Performance, quality and purity of preparations depend on design parameters of the mixer and modes of its operation.

The volume of the cylinder of the mixer is chosen based on capabilities of processing the maximum number of butterflies (100 pieces at a time) on one product. Conventionally, the cylinder of the mixer is divided into three areas – the lower impeller blades, the reflecting edges and the area of the top impeller blades. Based on this, the cylinder volume will be:

$V_c = 3V_{100} + V_i + 2V_e$, (8)

where V_{100} – the volume occupied by the 100 butterfly in the cylinder,

V_i – the volume of the impeller,

V_e – the volume of reflective edges.

The estimated height of the cylinder:

$h = Vc / \pi R^2$. (9)

For research two mixers with cylinders with a height of 100 mm and 80 mm, a volume of 785 cm^3 and 628 cm^3, with corresponding impellers and reflecting edges were produced.

Different amounts of material (butterflies) were loaded into the mixer – 2, 5, 10, 25, 50 and 100 (PCs.), which corresponds to the number of butterflies on the drug taken in the graine production.

The rotation speed of the impeller spindle in the range (200 min^{-1}...5000 min^{-1}) were changed with a set of replacement pulleys on the motor shaft. The grinding time of the sample was changed from 5 s to 20 s, with an interval of 5 s. The experiments were performed in a 30-fold repetition.

To determine the performance, the time of its full cycle was measured:

$T_c = T_1 + T_2$, (10)

where T_1 – preparation time, including the time of grinding, water supply and filtration operations,

T_2 – time of washing and drying of working bodies.

The time of additional and intermediate operations was taken into account when determining the hourly productivity, i.e.:

$T = T_c + T_3\ T_{1+2} + T_{mech}$, (11)

where T_3 – time of manual loading of the mixer with material and switching on the device.

T_{1-2} – the time of transition to the washing mode, associated with the need to remove the glass with drugs and start the device.

T_{mech} – time of working spindle speed accelerationof the impeller.

The time required to supply a dose of water corresponding to the amount of material was measured, i.e. 2, 5, 10, 50 and 100 cm^3, respectively, at the rate of 1 cm^3 per one crushed butterfly.

At detection of a pebrine, the graine of a laying with this number is burned at strict observance of sanitary and hygienic conditions.

The coefficient proposed by us - the grinding module (K_i) was from the expression:

$K_i = Mf/Ms$, where Mf – mass fraction, Ms – sample mass.

RESEARCH RESULTS

Taking into account that the purpose of continuous microanalysis is to detect mainly spores of nosematosis, the linear dimensions of which do not exceed 4 microns (in exceptional cases - up to 20 microns), the quality of grinding according to the existing technology should be recognized as unsatisfactory. For reliable search of spores in preparations, more than half of the crushed material should contain particles smaller than 0.2 mm.

At microscopying for contamination, graine plants and breeding silk stations are guided by the approved “Basic rules for the preparation of industrial graine”. Microanalysis of butterflies is carried out after 15 days after their isolation in bags. The butterfly dries completely and makes it easier to grind. The intermediate stages of the pebrine turn into spores, which are easier to detect during examination. Norm of viewing of preparations for the working day is established no more than 400...500

To the crushed mass, water is added at the rate of 2 cm^3 per 1 butterfly at compacting 2, 5 or 10 butterflies per drug or 1 cm^3 with a larger compaction. After that, the microscopist with a glass rod takes a drop of liquid from the bottom of the mortar (the density of the pebrine spores is higher than the density of water) and applies three strokes to the standard slide. Each smear (the actual drug) can be seen in 10 fields of view. The drug is considered to be infected if it detected during microscopy spores of pebrine, regardless of their number. Bags of infected cells are separated and then destroyed.

Quality grinding of silkworm butterflies with manual method (10 butterflies to the drug), using a machine of Pradell system (2 butterflies on the preparation machine used in breeding business) and our cars UPP-1 and UPP-4, expressed by the module grind on fractions is presented in table 1.

Table 1 - Quality grinding of silkworm butterflies with existing and new technologies in graine production

Fractions, mm	Grinding module K_i		
	On the Pradell system machine	Manual rubbing	Machine UPP-1 and UPP-4
Less 0,2	0,143 – 0,365	0,011 – 0,023	0,484 – 0,585
0,2 – 0,5	0,411 – 0,521	0,029 – 0,034	0,182 – 0,420
0,5 – 1,0	0,081 – 0,269	0,086 – 0,178	0,021 – 0,230
1,0 – 1,5	0,020 – 0,093	0,483 – 0,678	-
More 1,5	0,020 – 0,075	0,185 – 0,293	0,004 – 0,082

However, the table shows that when grinding butterflies on the machine, the main part of the crushed material contains particles with linear dimensions of 0.2 ... 0.5 mm, and when rubbing manually – 1.0 – 1.5 mm.

For the expression of the grinding modulus K_N is the main particle fraction with dimensions less than 0.2 mm. With the existing technology, the grinding modulus is $K_i \leq 0.023$. Even in breeding business when using the grinding machine of the Pradell system the module of grinding does not exceed 0.365 at necessary to have $K_i \geq 0.50$.

<u>Basic physical and mechanical properties of the processing material.</u> The processing material in the preparation of drugs for microscopy is air-dry silkworm butterfly. Depending on the breed, the mass of the air-dry silkworm butterfly varies considerably. Often even in the same party, received from one feeding, there are butterflies in excess of each other in weight in 2.5...3 times. Calculations performed by the formula (13) showed that the volume of the butterfly body is in the range from 0.9 cm^3 to 2.93 cm^3.

As it can be seen from table 2, the mass of the butterfly varies from 112 mg to 290 mg, and the average mass of one butterfly varies from 0.153 ± 0.009 g (Turkmen – 13) to 212.7 ± 8.7 mg (Belokokonnaya hybrid – 1 x Belokokonnaya – 2).

Table 2 - Size and weight characteristics of silkworm butterflies

Silkworm breeds and hybrids	Length, mm limits, average	Width, mm limits, average	Weight, mg limits, average
Turkmen – 13	18,5…29,5	6,1…12,0	112…258
	23,33± 0,26	9,48 ± 0,14	161,8 ± 9,8
Turkmen – 13	18,4…29,0	7,4…12,5	113…229
	22,55± 0,24	10,39 ± 0,10	153,0 ± 9,0
Belokokonnaya – 1 x	20,0…32,2	8,0…12,7	143…290
Belokokonnaya – 2	24,42±0,24	10,76 ± 0,10	212,7 ± 8,7

The volume of the butterfly body can be calculated using a simplified geometric model using the formula:

$V=\pi R^2 (2/3R + H + 1/3h)$, (12)

where V – the volume of the body of a butterfly without wings,

R – radius of hemisphere and cylinder,

H – the height of the cylinder,

H – the height of the truncated cone.

Expressing the values h, H and R through the length L and width B in the body of the butterfly and substituting expressed in the formula (12), we obtain a formula that allows us to determine the volume of the body of the butterfly through its length and width:

$V = H * 2B/4 (L – B/2)$ (13)

The study of the effectiveness of shock loads showed that even at speeds of 14-15 m/s with a single exposure it is impossible to achieve the grinding module Ki=0.5. At medium speeds (8-9 m/s) no more than 10% of the material is destroyed.

To grind 80 ... 82% of the material to the required degree, multiple shock loads at speeds of 10...15 m/s is required.

Description of the schematic diagram of the device for preparation of drugs.

Based on the analysis of the results of the study, a schematic diagram of the device UPP-1 is made, figure 1.

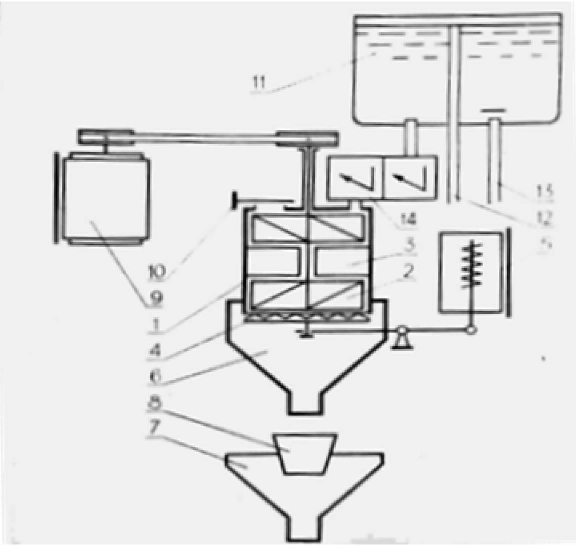

Figure 1 - A schematic diagram of the UPP-1

Machine UPP-1 performs the required operations for the preparation of drugs, increases productivity by 2-3 times. In order to improve the performance of the machine in the future, a new design of the device UPP-4 is developed.

The basis of the device UPP-1 is a mixer, which is a hollow cylinder 1, the axial line of which the working grinding body – impeller 2 is installed in the bearings. A feature of the working body is the shape of the impeller, consisting of two spaced sections of the blades with multidirectional bending.

Between the sections of the impeller on the inner surface of the cylinder reflective ribs 3 radially mounted. The bottom of the cylinder is covered by a disc filter 4. The disc filter is installed with the possibility of axial movement with the help of an electromagnet 5 in order to adjust the gap along the lower perimeter of the cylinder. A cone 6 is screwed on the mixer, under which a cup 8 is installed in the socket of the drain funnel 7 to collect the prepared material. The spindle of the impeller 2 is driven by an electric motor 9 by means of a belt drive. In the upper part of the cylinder 1 there is a loading opening, overlapped during operation by the flap 10. To supply a dose of water for the preparation of the drug and washing the working bodies above the mixer, a water tank 11 is installed, in which a constant water level with a level regulator 12 is maintained. Water in the tank is supplied from the water supply through the pipe 13. The tank connects to the mixer via a conduit through electromicroscopy 14.

Device UPP-4 has four mixer mounted like a carousel. In the mixers, various operations of the process are simultaneously carried out: in 1 – loading of the material, in 2 – grinding, mixing with water and filtration of the drug, in 3 – washing of the working bodies, in 4 – the second washing and drying. At the same time, the performance of the UPP-4 is almost 3 times increased compared to the UPP-1 machine, and the power consumption increased by 3.5 times.

Table 4 - Technical characteristics of machines UPP-1 and UPP-4

Main indicators	UPP-1	UPP-4
Performance of drugs / hour	60	180
Number of microscopists per machine	1	3
The number of butterflies in one drup, PCs.	2, 25, 50, 100	2, 25, 50, 100
Power consumption, W	100	350
Single-phase power	220 в, 50гц	220 в, 50 гц
Machine weight, kg	20	38
Circumferential speed of the working body, m/s	12	15
The volume of one cylinder mixer, cm^3	628	785

REFERENCES

1. Golovko V.A., Kirichenko I.A., Kazmiruk V.V., Denisenko E.A. Diagnosis and prevention of pebrine, fight with it and various infections of the silkworm // Methodical recommendations. – 2001. Kharkov RIP “Original”, - 32 p.
2. Kirichenko I. A. The main infectious diseases of silkworm in Ukraine and measures to combat them. // - 1995.Kharkov: RIP “Original”, - 208 p.

3. Burlakov V.S., Burlakov A.S. Microanalysis at the graine plants // J. “Agriculture of Turkmenistan”. – 1973. - № 1. – P. 27.

4.Бурлаков В.С. Устройство для приготовления препаратов для микроанализа. А. с. № 376069 // Б.И. – 1973. - № 17.

5. Burlakov V.S., Burlakov A.S. Mechanization of the process of industrial microanalysis // Silk.– 1975. - № 4. – P.

6. Burlakov V.S., Burlakov A.S. Mechanization of labor-intensive processes of sericulture. // Brochure, ed. Ash. «Turkmenistan» - 1985. – 16 p.

7. Burlakov V.S., Burlakov A.S. Machine UPP-1 for preparation of drugs // Silk.– 1989. - № 4. – P.

8. Burlakov V.S. Prospects and results of work on mechanization and automation of labor-intensive processes of sericulture // Abstracts. – International Symposium “Actual problems of world sericulture”. – Kharkov. – 1992. – P. 132-133.

9. Burlakov V.S. Devices of UPP-1 and UPP-4 for industrial microanalysis on infestation of silkworm with pathogens // Materials of reports. – International scientific conference “I. I. Mechnikov’s ideas and development of modern natural science”. – Kharkov. – 1996. – P. 143-146.

10. Golovko V.A., Burlakov V.S., Kirichenko I.A. Study of the technology of preparation of drugs for microanalysis of silkworm butterflies to pebrine using the established machinery of UPP – 1 and UPP – 4 // Coll. of scientific works of the Uzbek Research Institute of Sericulture, Vol. № 4, Tashkent. – 2004. – P. 34-38.

11. Berlanesu S., Pa и E. Меры борьбы с пебриной шелковичных червей% Шелк. – 1991. - №1. – С. 28 (Prod. anim. Zootechn. Med. veter. – 1988. – T. 38, №7 – C. 40-43).

12. Vasanthara jn V.N., Munirathnamma N. Studies on silkworn diseases. III Tpizootiology of a Septictmic disease of silkworms causeb by Serratia marcescens. “I. Indian Inst Sci”, 1978, 60, №4, 33-42 – (англ.) РЖБ Энтоология, М, 1979, № 6, - С. 65.

13. Enomoto S., Moriyama H., Iwanomi S. Septictvia occurrence in coccons as ctlated to silcworm ctacing conditions. ARQ 1987. 21,1/ 117-121 (англ.) П – 26062.

14. Ohba M. Bacillus thurigiensis populations naturally occurring on mulberry leaves: a possible source of the populations associated with silkworm – rearing in sectaries. Journal – of – (1996. Vol. 80. - №1. p. 56-64).

15. Mikihiko Miura. Silkin Japan: Present and Future. http:silk in Japan Web.Web.htm – 2004. C. 1-6.

16. Gasifier system for silk industry. http://www.terrin. org/case/silk. Htm. – 1/2/2004. – C. 1-5.

17. hybrinds weave silken chase. http:www deccanherald. Com/deccanheral…-2004. – C. 2-4.

18. Jahm R. Sericulture. Department of Entomology №6 State University Last Updated: http://ww.cols.ncsu.tdu – 2003. – C. 3-6.

19. Komatsu K. Introduction to Silk (Japanese), Science House, Tokyo. – 2004. – 270 pp.

20. Sericulture. 1. General Information: 2. Proposals for 2001-2002: OKI:www.Kar.nic.in/bellary/ser. 2004. – P. 1-4.