THE ROLE OF BASIC RESEARCHES IN SOLVING OF TOPICAL PROBLEMS OF SERICULTURE AND SILK PROCESSING. BIOTECHNOLOGICAL ASPECTS

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Purposes of the study:

To develop and apply biotechnological approaches and methods in sericulture and silk technology.

Directions of the study:

I. Development and use of artificial diets (AD) for mulberry silkworm (MS) from local raw materials.

II. Study of applicability of mulberry pyralid (MP) biocontrol methods in system IPM.

III. Development and use of eco-friendly, sources – and energy saving technologies of cocoons processing and silk wastes. New direction of silk materials use.

I. Research tasks on development of artificial diets:

- Chemical study of mulberry tree leaves and potential components of artificial diets for mulberry silkworm.
- Physiological and biochemical aspects of digestion and respiration in mulberry silkworm.
- Microbiological researches in preparing, preservation and use of artificial diets for mulberry silkworm.
- Biotechnology of preparing AD and mass rearing of mulberry silkworm for sericulture and scientific researches.
- Study of fields of possible use of artificial diets for mulberry silkworm.

Table 1

Content of crude protein, sugars, lipids, ash and humidity in some materials and components of artificial diet, (%)

№	Sample	Crude protein	General shugars	General lipids	Ash	Humidity
1.	Mulberry leaves «Tadjik seedless » (05.05.86)	25.36	8.47	1.50	8.48	9.32
2.	- " - - " -Mixed varieties (May 1989)	29.71	9.00	3.20	11.23	8.32
3.	Chlorella	43.87	*	*	7.35	8.11
4.	MS larvae (V instar, flour)	43.50	0.76	19.70	3.98	7.00
5.	MS pupae (flour)	68.44	-	10.70	5.20	7.25
6.	MS pupae (defatted flour)	82.45	-	Сл.	6.75	8.51
7.	Fish flour	83.32	-	15.80	3.42	6.76
8.	Soybean oil meal	48.75	11.90	-	6.68	7.33
9.	Dry skim milk	30.60	34.80	*	7.48	7.19
10.	AD “Yamanasi” (Japan, young instars)	25.63	10.43	3.20	9.27	8.75
11.	AD “KIodo Shirio» (Japan)	20.12	9.68	2.90	8.73	9.23
12.	AD UzNIISH (Uzbekistan)	25.31	13.46	*	10.12	10.48
13.	Excrements (III instar)	11.25	4.88	1.50	9.28	7.42
14.	- " - (V instar)	13.67	2.46	-	19.72	7.42

Table 2

Amino acid compositions of mulberry leaves, some components and artificial diets (* - essential and ** - needed amino acids for MS artificial diets)

Amino acids, r/100 g protein	Defatted soybean meal	Cotton seed isolate	Spring mulberry leaves	Mulberry leaves by Cherno et al.	AD of UzNIISH		"Sirku-meita" AD	"Kiodo Siryo" AD
					for young instars	for elder instars		
ASP**	15.3	1.2	15.5	15.92	12.7	11.3'	12.8	12.7
THR*	4.0	3.5	5.3	4.6	3.9	3.9	4.0	3.9
SER	4.9	6.6	4.5	4.98	4.5	5.2	4.7	4.8
GLU*	16.7	15.9	13.7	16.11	17.2	17.8	16.8	16.2
PRO**	6.1	Сл.	4.7	4.90	8.5	10.1	8.9	8.8
GLY	4.2	6.0	5.0	4.41	4.5	4.0	4.5	5.1
ALA	4.5	2.8	6.1	6.31	4.8	4.0	4.6	5.3
CYS	1.5	0.5	1.0	Сл.	1.3	0.9	1.1	1.1
VAL*	4.4	6.6	5.5	6.02	4.3	4.2	4.3	4.7
MET*	0.8	1.4	0.9	Сл.	0.8	0.9	0.8	0.7
ILE*	4.1	5.7	4.7	4.0	4.1	4.2	4.1	4.4
LEY*	7.9	11.2	8.3	9.20	7.9	8.2	7.8	8.0
TYR	3.5	3.2	3.5	2.85	3.0	2.9	3.0	3.0
PHE*	5.2	9.7	4.9	4.22	4.9	5.1	4.9	5.1
HIS*	3.4	7.0	2.8	1.7	3.1	3.1	3.0	2.8
LYS*	5.0	4.4	6.3	6.1	6.6	5.8	6.7	5.7
AMM	0.4	-	0.4	Сл.	0.4	0.4	0.5	0.5
ARG*	7.7	14.1	6.8	5.48	7.0	7.4	7.2	7.0

Table 3

Analysis of fatty acids of mulberry leaves, in lipid preparations of larvae, pupae and in components of artificial diets (—, trace concentration)

Fatty acid	Mulberry leaves powder			Lipids		Oil			Artificial diet			
	spring feed mix	fall feed mix	fall Tadjik seedless	pupae	larvae V instar	cotton	soy bean	mul-berry seeds	Kiodo Siryo	Sirkumeita		Uz NIISH
										On net	powder	
12:0	—	—	0.1	—	—	—	—	—	—	0.2	—	—
14:0	0.2	0.6	0.2	0.2	—	—	0.8	—	0.9	0.5	0.5	0.4
14:1	1.2	1.9	0.4	—	—	—	—	—	0.5	0.5	1.0	—
15:0	0.6	0.5	0.5	—	—	—	—	—	0.3	—	0.2	—
16:0	28.2	24.8	18.5	9.9	28.6	11.4	25.4	9.3	28.1	24.3	27.1	15.9
16:1	0.2	0.5	0.2	0.3	—	—	—		1.9	1.8	0.8	0.5
16:2	1.0	—	1.4	—	—	—	—	—	0.6	0.6	1.2	—
17:0	—	—	0.1	—	—	—	—	—	—	—	2.2	—
!7:1	—	—	—	—	—	—	—	—	—	—	7.7	—
18:0	2.2	2.9	2.4	31.5								
18:1	2.2	2.2	2.0	19.8	41.1	22.8	18.1	5.8	11.8	5.4	4.3	16.1
18:2	18.9	17.2	18.6	9.3	3.6	54.5	52.2	81.3	20.8	19.3	17.2	40.4
18:3	41.9	49.4	53.6	29.0	25.8	8.2	0.5	—	27.3	44.9	32.1	19.0
20:0	1.2	—	0.8	—	—	—	0.2	—	0.6	—	0.4	0.7
20:2	2.2	—	1.2	—	—	—	0.4	—	3.1	—	1.6	3.1
Σ S	32.4	28.8	22.6	41.6	29.5	14.5	28.8	12.9	34.0	27.5	41.8	20.9
Σ U	67.6	71.2	77.4	58.4	71.5	85.5	71.2	87.1	66.0	72.5	58.2	79.1

Table 4

Proteolytic activity (PA, E/g) of some enzyme preparation in hydrolyses of different substrates (38°C, pH 10, M/± m, n = 3)

Enzyme preparations	Casein	Albumin	Sericin	Soya been concen trate	Soya been isolate	Cotton seed protein
1. Protosubtilin Г3х	137/11.3	16.7/1.3	58.7/4.9	36.7/3.0	38.4/3.2	13.5/1.1
2. Alkaline protease *Bacillus subtilis*	85.9/7.6	22.5/2.0	38.0/3.1	61.3/5.0	59.4/5.1	17.6/1.5
3. Proteolytic complex *Aspergillus oryzae*	572/43	147/12	142/11	409./35	411/36	85.8/7.71
4. Alkaline protease *Torula thermofila*	98.5/8.2	36.3/3.1	**	78.1/6.6	78.4/6.9	**
5. MS Intestine juice*	5.2/0.41	3.8/0.32	**	11.6/0.97	12.8/1.0	**
6. MS Intestine *	1.1/0.09	0.6/0.04	**	3.2/0.25	4.9/0.35	**

* PA of intestine juice as E / mg. and PA of intestine as E / 1 intestine .

** Not determined.

Fig.1

Study of kinetics of hydrolysis of cotton (a) and soybean (b) isolates and proteins of mulberry leaves acetone powder (c) under effect of proteolytic enzymes of intestine (1), intestinal juice (2) and their mixture (3)

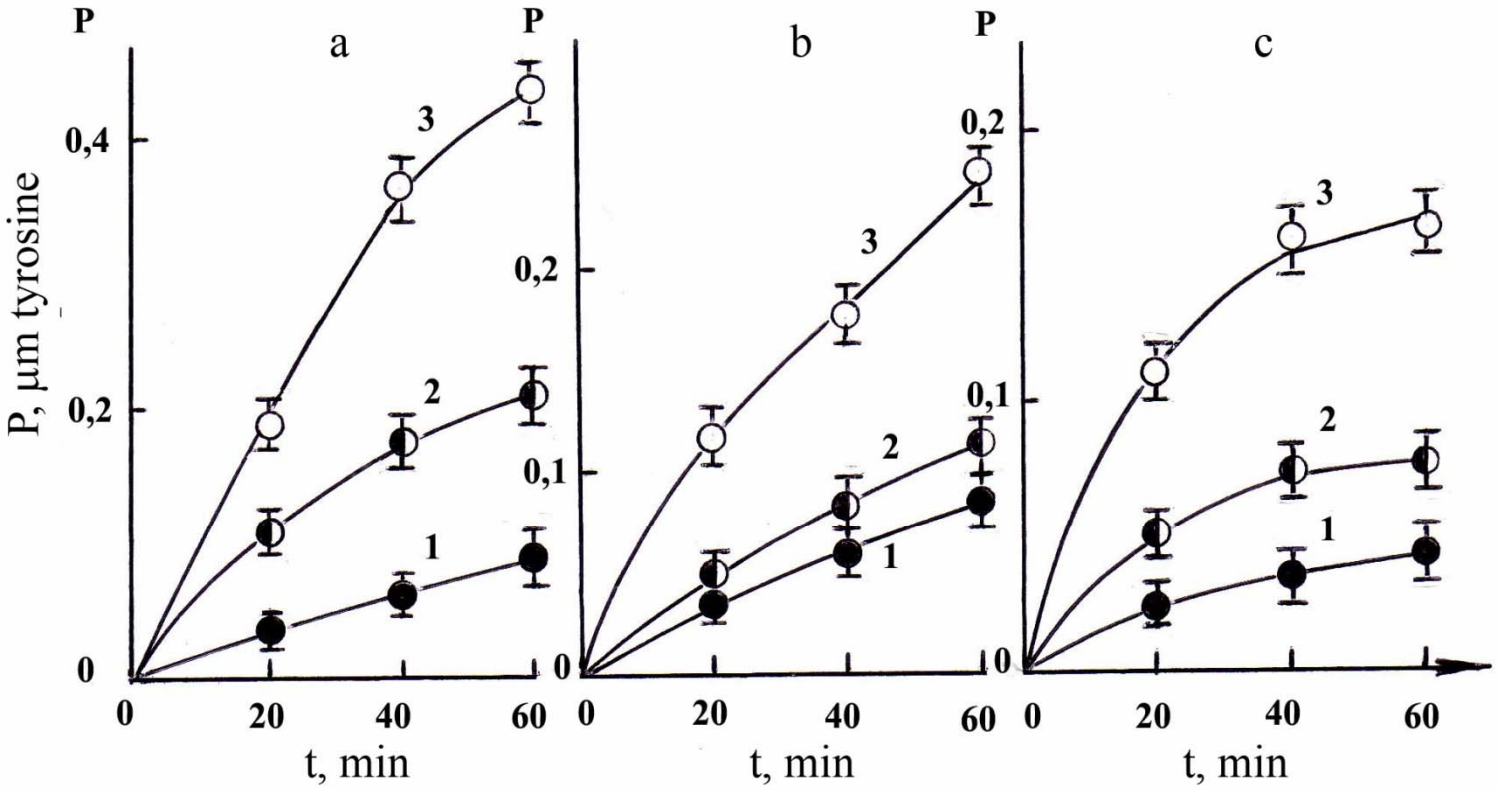
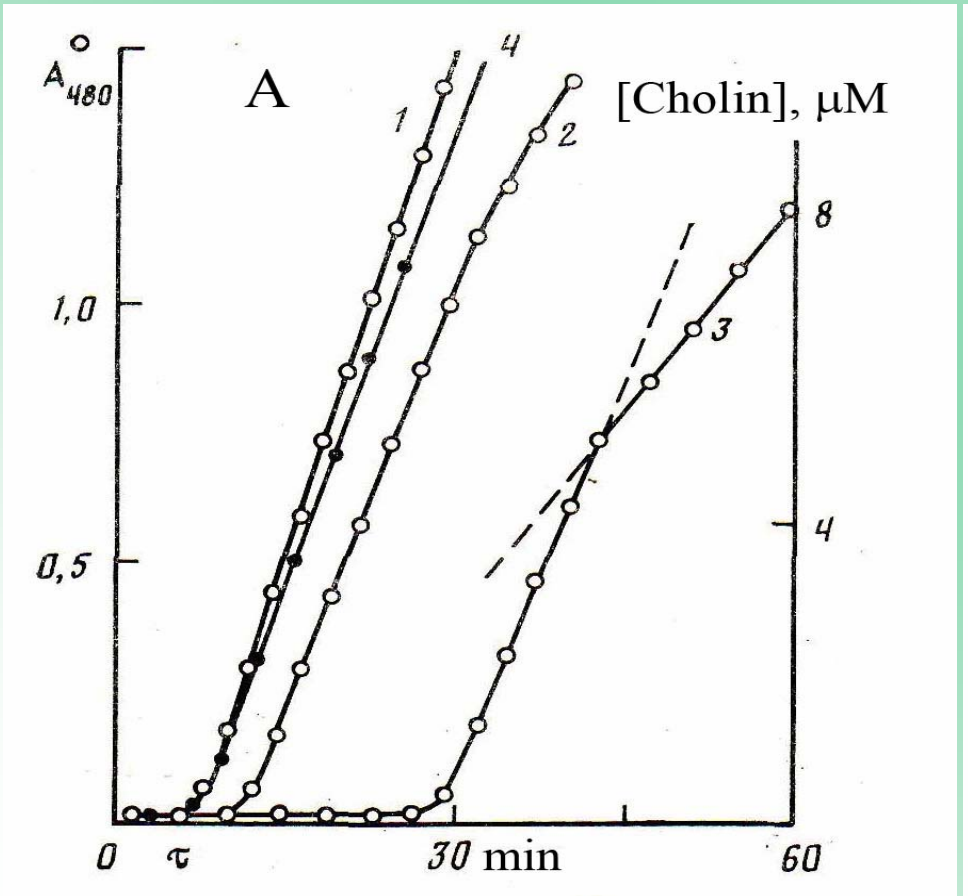


Fig. 2

Time courses of lysolecithine hydrolysis by phospholipase D (PLD) in absence (A) and presence of SDS (B)

A. - 1.2.3 – Preparations of cabbage leave, radish and cotton seeds, correspondingly; 4- choline release. B. - 1- without SDS. 2.3.4.5 –in present of 0.2 мМ SDS; 3 – 0.5 мМ; 4 – 0.7mM; 5 – 1.0 mM, correspondingly; [S]= 2 mM. [Ca^{2+}]=20mM, *Str. chromofuscus* PLD– 2.25 mkg.



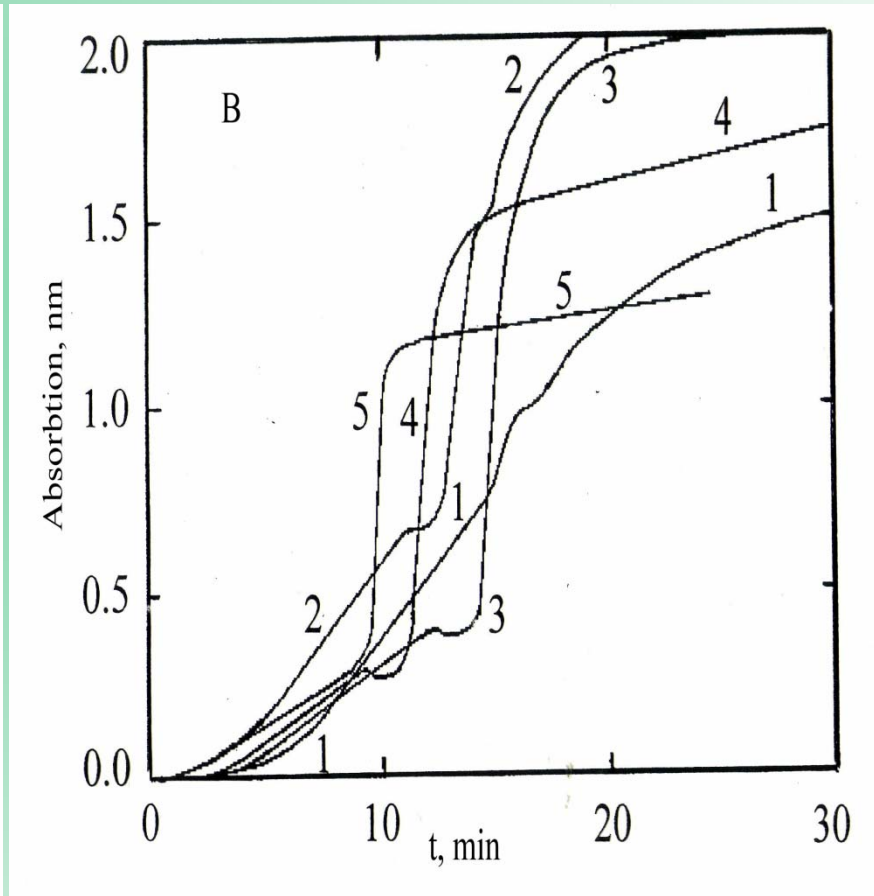


Fig.3

Structure of micelles and formation of clusters in reaction of LPC hydrolysis by phospholipase D (scheme 1)

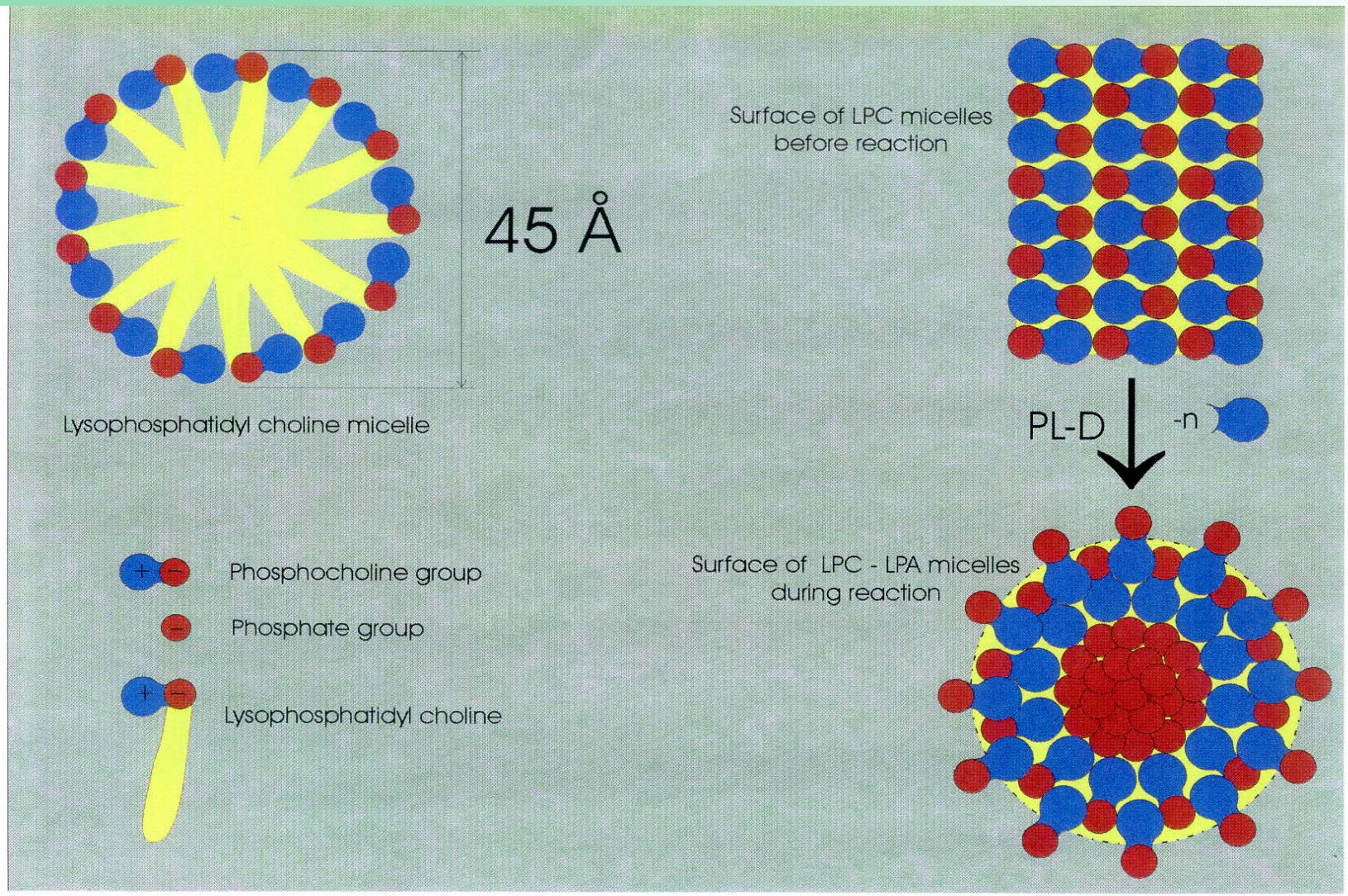


Fig.4

Interfacial activation and vector properties of phospholipase D at hydrolysis of lysolecithin (scheme 2)

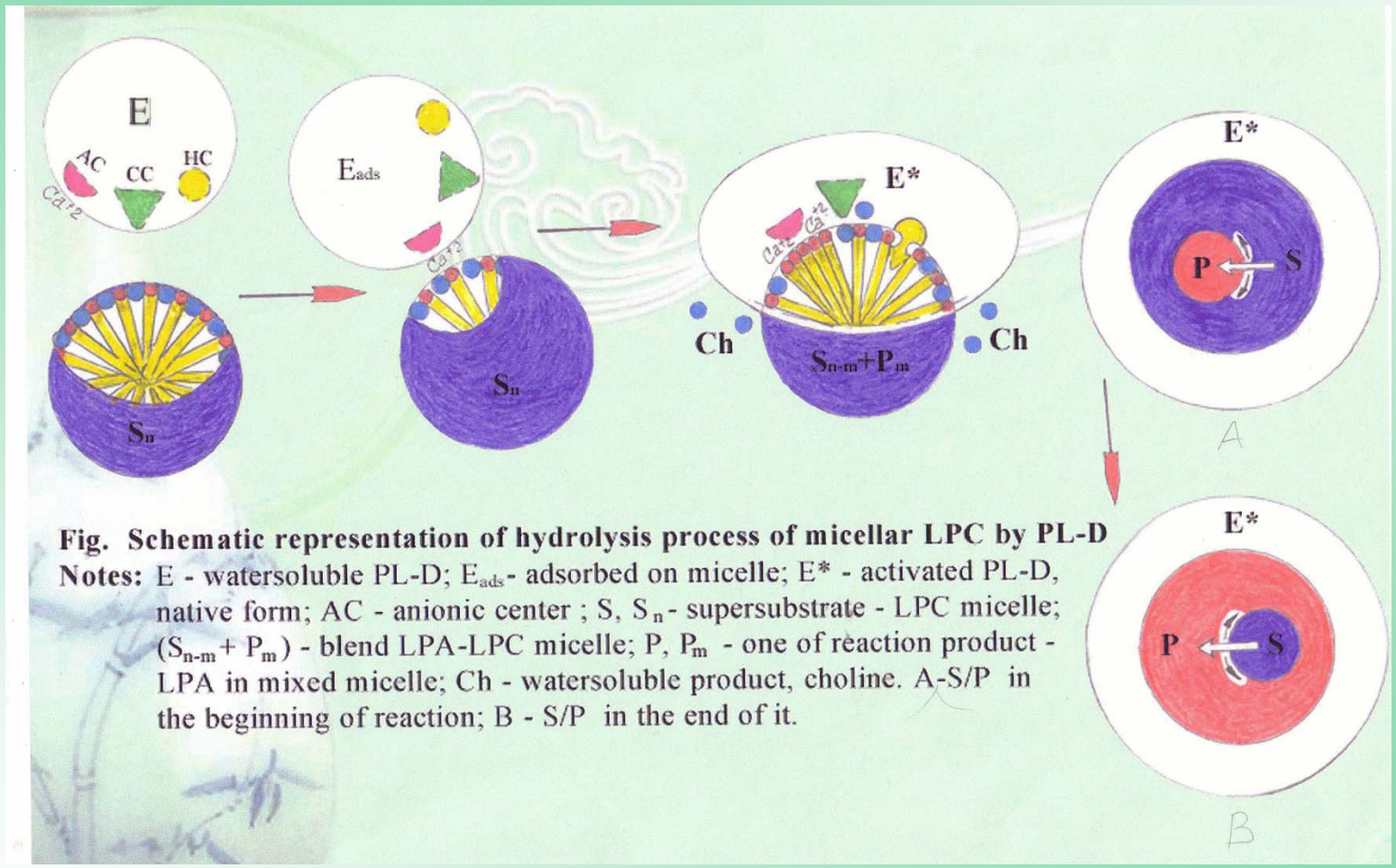


Fig. Schematic representation of hydrolysis process of micellar LPC by PL-D

Notes: E - watersoluble PL-D; E_{ads} - adsorbed on micelle; E* - activated PL-D, native form; AC - anionic center ; S, S_n - supersubstrate - LPC micelle; $(S_{n-m} + P_m)$ - blend LPA-LPC micelle; P, P_m - one of reaction product - LPA in mixed micelle; Ch - watersoluble product, choline. A-S/P in the beginning of reaction; B - S/P in the end of it.

Fig.5

Interphacial activation and formation of clusters of products in reaction of hydrolysis of phospholipids by phospholipases (scheme 3)

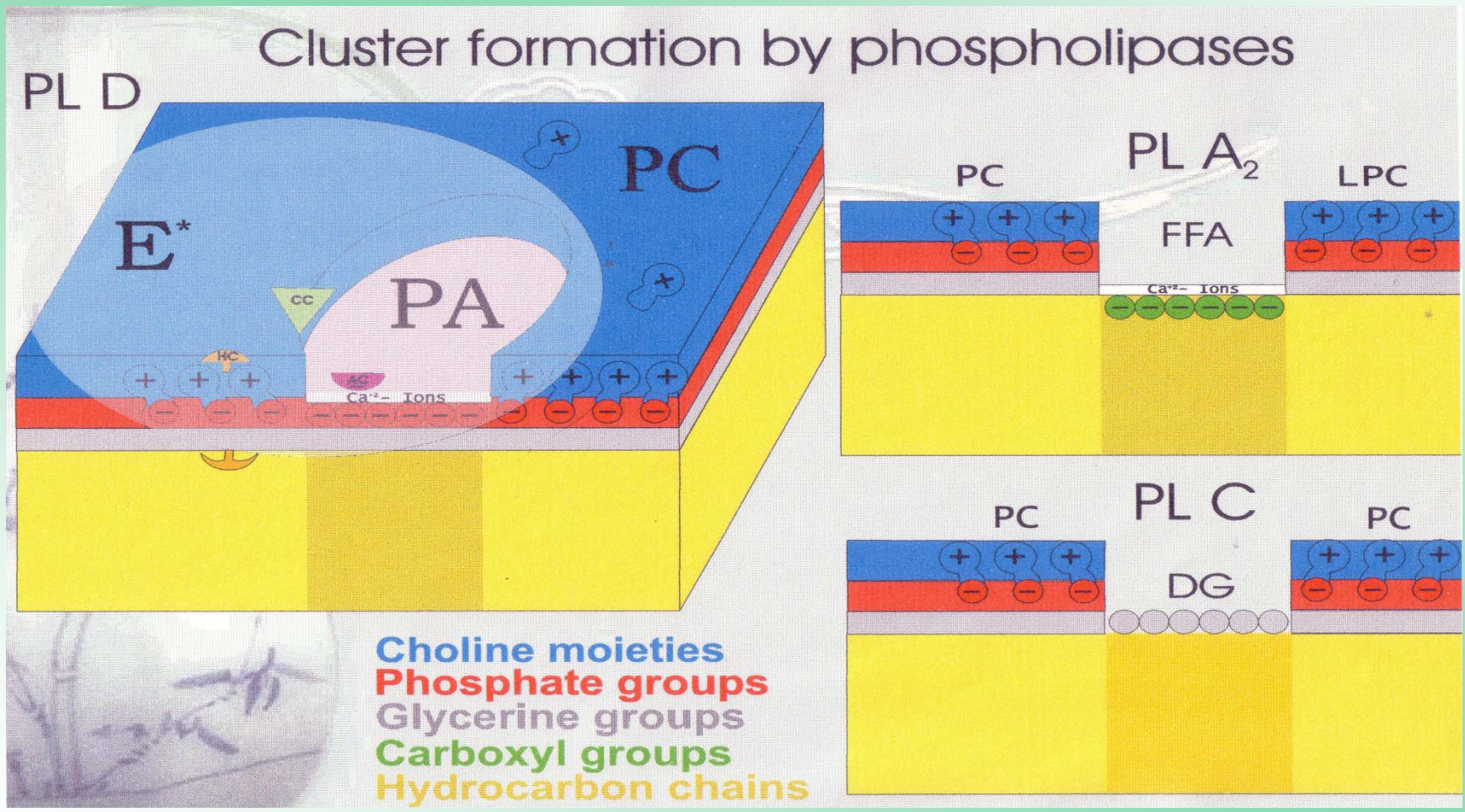
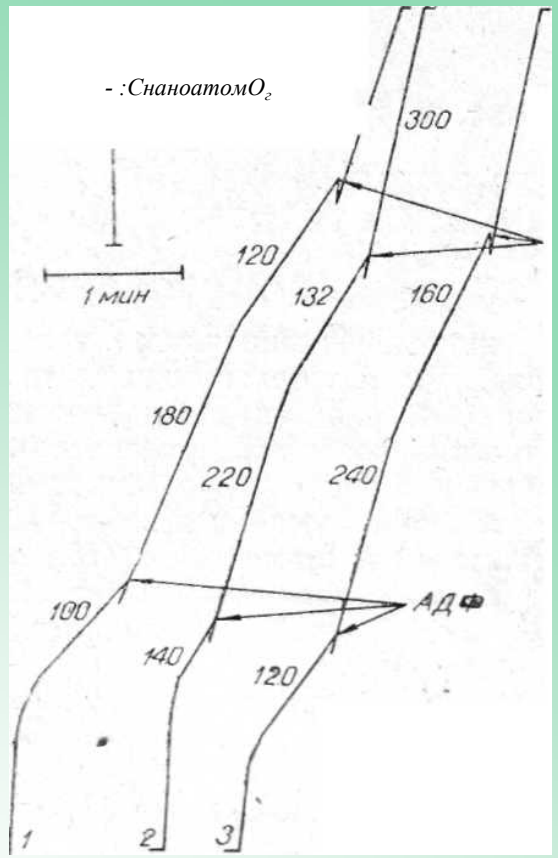


Fig.6

Respiration and oxidative phosphorylation of mitochondria mulberry silkworm digestive tract



Incubation medium: 1-sucrose 0.23 M.
tris HCl - 5 mM. pH 7.4; KH_2PO_4- 5 mM and
ЭГТА - 2 mM; 2 and 3 – same composition of
the medium, but in presence of 50 mM KCl;
but in presence of 3 – in presence of 5 mM $MgCl_2$.

Table 5

Metabolic reactions of MS intestine's mitochondria with different oxidative substrates (M±m. n=5)

Oxidative substrates	Oxidation speed, nano-g-atom O_2/min x mg				ADP/O	OC
	V_2	V_3	V_4	V_{DNF}		
Succinate	70±5	113±10	65±5	166±15	1.8±0.08	1.7±0.09
α - Ketoglutarate	44±3	90±6	53±4	95 ± 8	1.3±0.09	1.7±0.10
Glutamate	40±4	70±5	38±3	68±7	1.5±0.10	1.8±0.09
Malate	38±3	80±6	46±4	123±10	1.4±0.11	1.8±0.09
Citrate	36±3	52 ±4	34±3	58±5	1.3±0.12	1.6±0.08
α -Glycerophosphate	28±2	36±3	33±3	33±3	—	—
β -Glycerophosphate	34±3	36±3	36 ±3	36±4	—	—
β – Hydroxibutirate	38±4	32±4	30±4	25±3	—	—
NAD. H	38±4	35±3	35±3	35±3	—	—
NAD.H + Cytochrom c	87±7	87±7	87±8	92 ±13	—	—

Table 6

Velocity of respiration (mkg-atom O_2/ min x g) of mulberry silkworm in different embryonic and postembrionic stages of development (M±m. n=3)

Eggs	II instar	III instar	IV instar	V instar	Cocoons	Pupae
Fresh-laid 1st day (yellow) 0.720 ± 0.046 2nd day (pink) 0.312 ± 0.021 3rd day (buff) 0.483 ± 0.026 Before larva hatching $8.07 \cdot 10^{-2} \pm 0.55 \cdot 10^{-2}$	II instar begining $4.78 \cdot 10^{-2} \pm 0.31 \cdot 10^{-2}$	III instar begining $2.57 \cdot 10^{-2} \pm 0.18 \cdot 10^{-2}$	IV instar begining $2.35 \cdot 10^{-2} \pm 0.13 \cdot 10^{-2}$	V instar begining $1.32 \cdot 10^{-2} \pm 0.63 \cdot 10^{-2}$ Before cocoon spinning $3.77 \cdot 10^{-3} \pm 2.20 \cdot 10^{-4}$	4th day of spinning $1.76 \cdot 10^{-3} \pm 0.98 \cdot 10^{-4}$	Before exit of butterfly ♀ $-1.65 \cdot 10^{-2} \pm 0.89 \cdot 10^{-3}$ ♂ $-1.20 \cdot 10^{-2} \pm 0.53 \cdot 10^{-3}$

Table.7

Composition of artificial diet for young instar larvae of mulberry silkworm by receipt MS-001

N	Components of the diet	Component amount, g
1	Mulberry leaf (powder)	250
2	Soybean defatted flour	360
3	Starch	75
4	Sugar	80
5	Cellulose powder	150
6	Agar-agar	75
7	Salt mixture	30
8	Citric acid	40
9	Ascorbic acid	10
10	Sorbic acid	2.6
11	B group vitamins	2.0
12	Soybean oil	15
13	β-sitosterol	2.0
14	Antiseptics, antibiotics	2.5
	Total:	1.1 kg

Fig.7

Scheme of technological process of obtaining of ready artificial diet (AD)

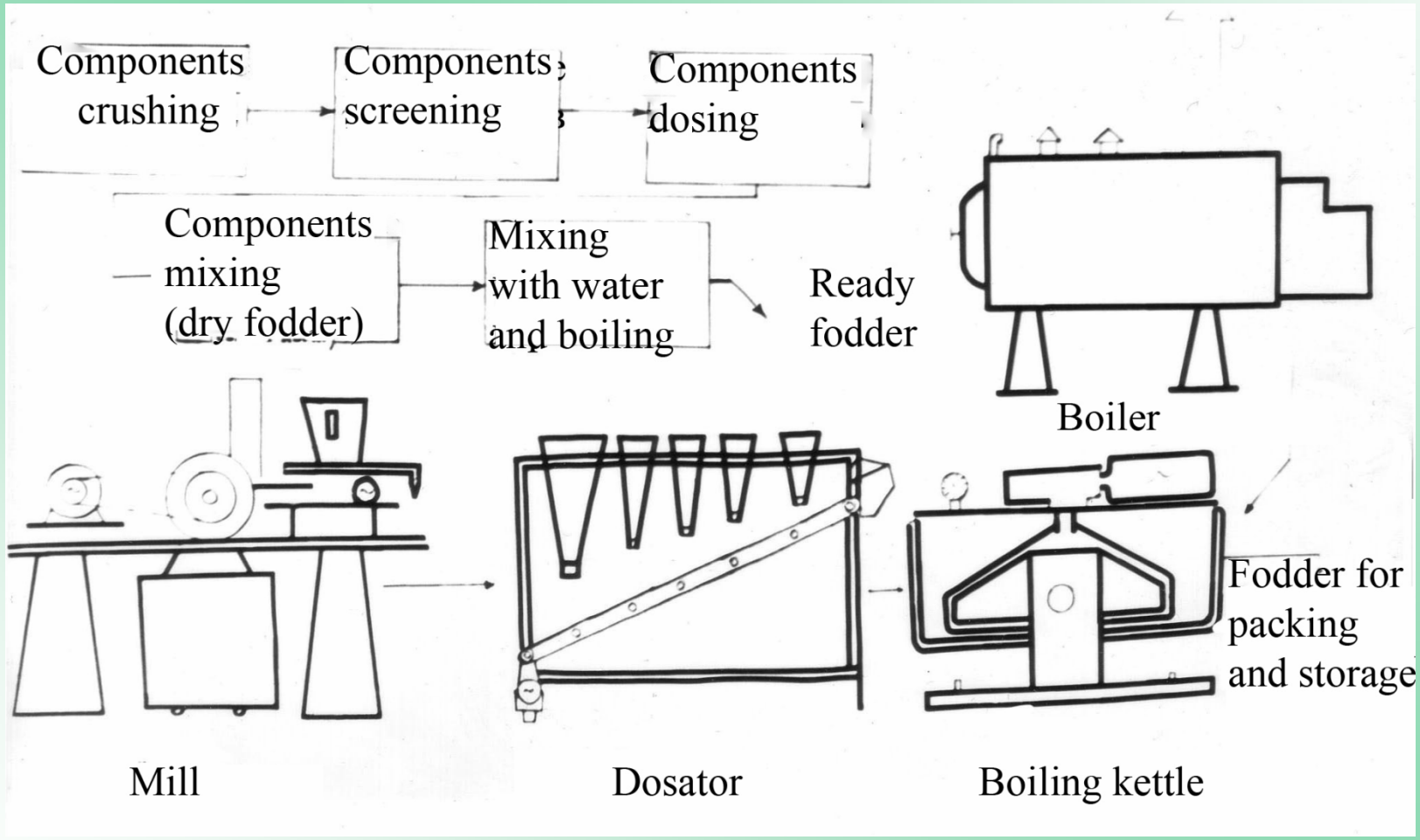


Fig. 8

Granulated and plate forms of artificial diet for mulberry silkworm larvae of young and elder instars

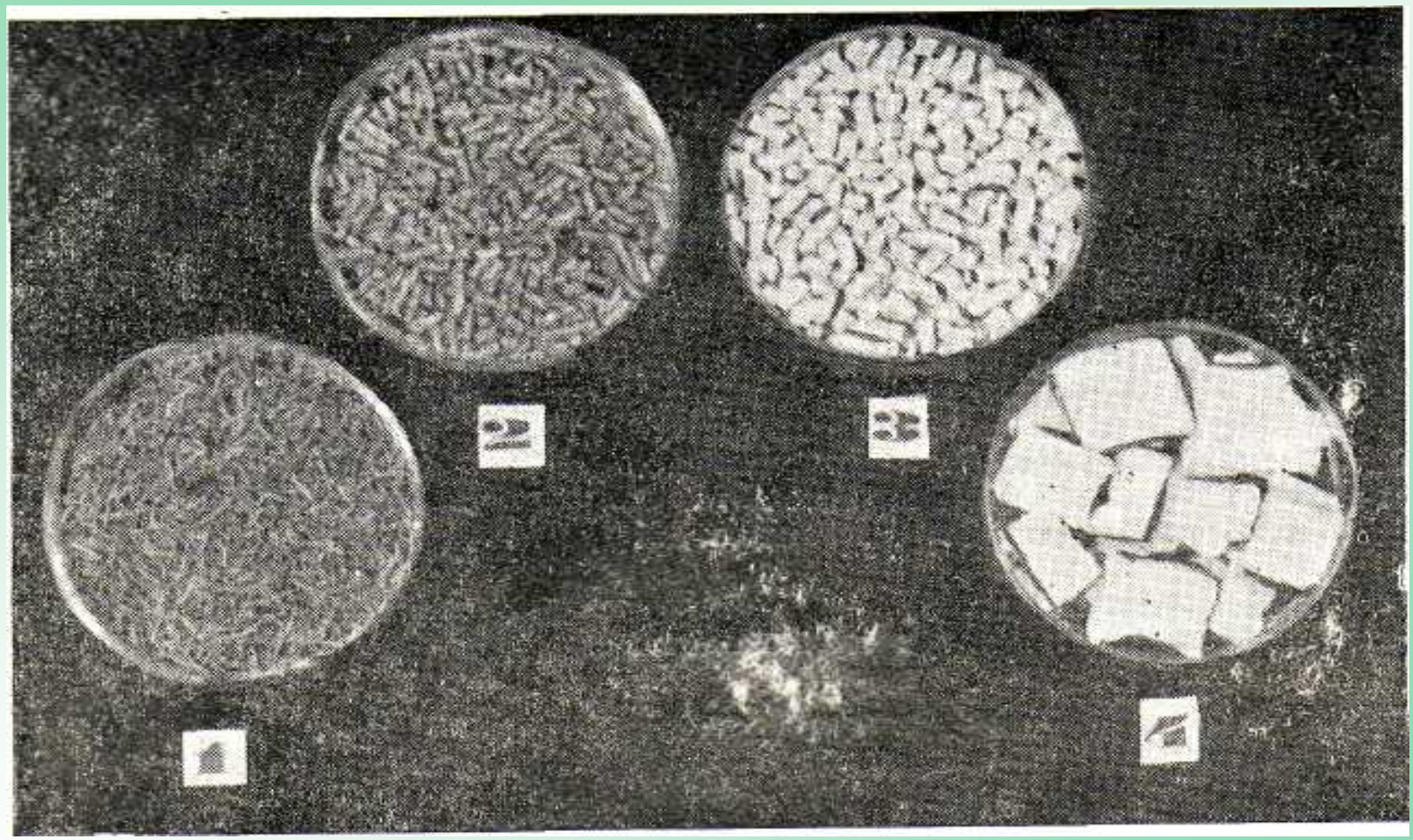


Table 8

Test of cellulose containing components of artificial diet at mixed rearing of silkworm (n=3)

Cellulose containing material	% of whi-ten lar-vae	Duration of larva period, days			Larvae vitality and amount of waved cocoons, %			Average weight of larvae by instars, cocoons and cocoon shell, g						Co-coon : shell ratio, %
		1-3	4-5	To-tal	4	5	Co co-ons	2	3	4	5	Co-co-on	Coco on/ shell	
Cellulose powder (CP) CP-HNO_3	98	13.6	14	27.6	96	96	86	0.019	0.143	0.63	1.69	2.12	0.468	22.1
Cellulose dust	100	14	13	27	100	90	80	0.010	0.066	0.48	1.36	2.28	0.483	21.2
Saccharified cellulose	100	14	13	27	100	90	60	0.014	0.066	0.37	1.48	2.28	0.478	21.0
CP from cotton stem	100	14.6	13	28.6	100	90	60	0.012	0.091	0.46	1.60	1.96	0.419	21.4
Powder of corn shaft (core)	96	14.6	14	29.6	96	90	86	0.013	0.087	0.38	1.40	2.02	0.463	22.9
Powder of mulberry stems	100	14	15	29	100	10 0	96	0.012	0.074	0.42	1.60	2.16	0.454	21.0
Blues	92	16	15	31	96	86	60	0.014	0.070	0.29	1.30	2.32	0.480	20.7
Control -leaf of mulberry	96	16	14	30	96	96	86	0.009	0.067	0.30	1.27	2.03	0.453	22.3

Table 9

Dose-depended effect of macrocyclic compounds on growth, development and productivity of mulberry silkworm (n=3)

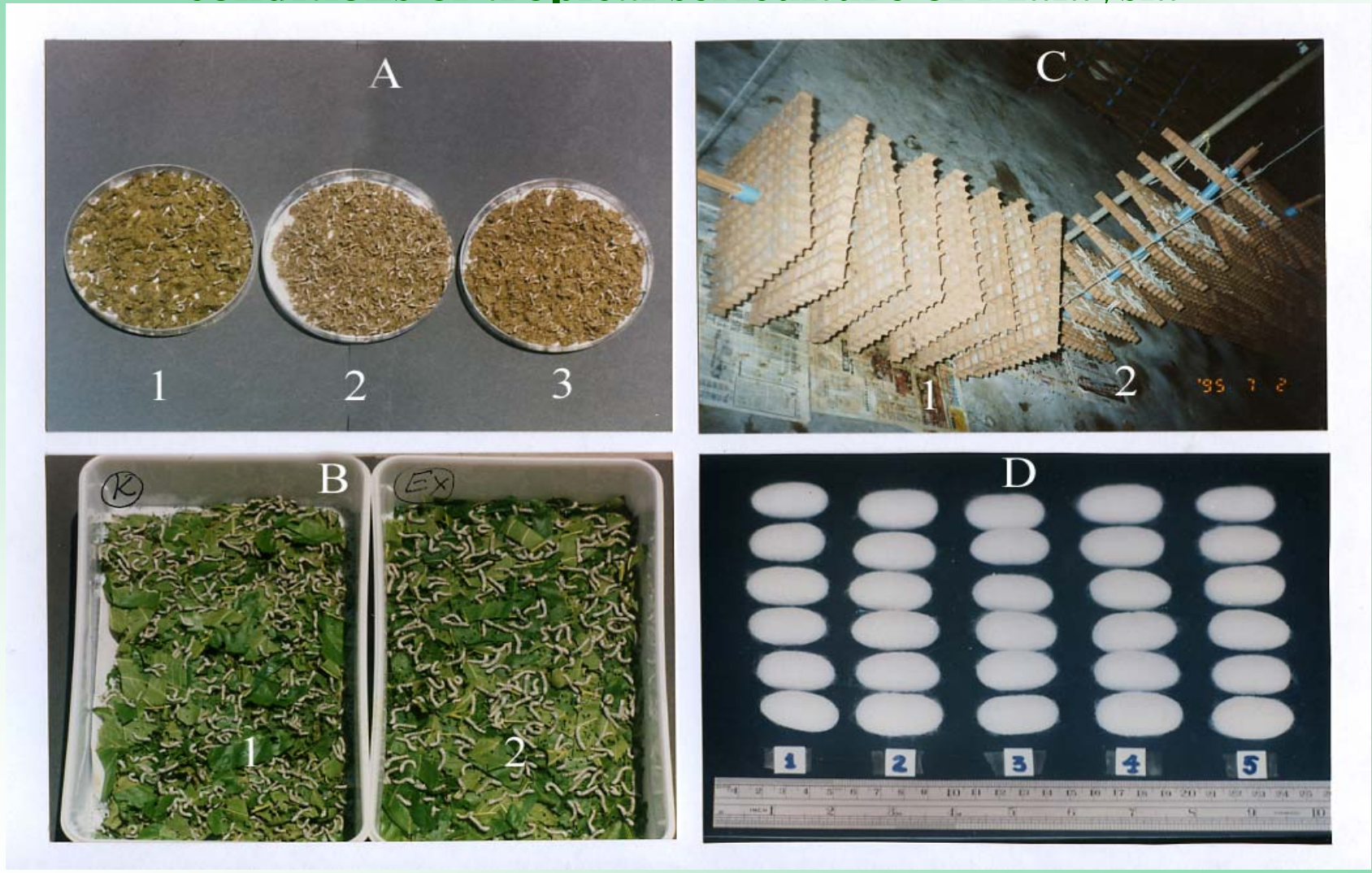
	Macroheterocyclic polyethers	%, larvae whitening	Instar duration, days					Larvae vitality by instars, %					Average weight of larvae in the beginning of each instar, g				Cocoon average weight
			1	2	3	4	Σ	2		3	4	5	2	3	4	5	
1	ВП-83-0.5 mg	70	4	3	4	6	17	100		100	95	90	0.029	0.12	0.37	1.41	2.24
2	ДБ18Кр6-1mg	80	4	3	4	6	17	100		100	85	85	0.025	0.15	0.34	1.21	1.86
3	ДА-ДБ18Кр6 - 2.5mg	80	4	3	4	5	16	100		100	100	95	0.026	0.13	0.42	1.32	1.81
4	ДВ-ДБ18К6-1mg	80	4	3	4	6	17	100		100	90	90	0.025	0.11	0.38	1.03	1.65
5	БК-224-0.5mg	80	4	3	4	6	17	100		100	100	90	0.024	0.11	0.32	1.26	1.91
6	БК-224-2.5mg	80	4	3	4	6	17	100		100	100	90	0.026	0.11	0.36	1.17	1.97
7	Control - artificial diet	70	4	3	4	7	18	100		90	90	80	0.021	0.10	0.24	1.02	1.68

Fig. 9

Feeding of mulberry silkworm (III instar) by artificial diet on the japanese rearing facility

Fig. 10

Effect of «EVA» feeding additive on development of mulberry silkworm on artificial diet and mulberry leaves in conditions of tropical sericulture of Malaysia



Recommendations on spheres of possible non-productive use artificial diet for mulberry silkworm

Mulberry silkworm as test-organism:

- - In research institutes on bio-tests of new chemical compounds and biopreparations, on genetics and selection, on physiology and biochemistry, zoology, epidemiology, mutagenesis and cancerogenese, on pharmacology, immunology, toxicology, medicine, feeding, in ecological, space and other extreme studies and also in zones of technogenic disasters.
- - In research institutes and sericulture stations for all-year studies on genetics, selection, struggle with mulberry silkworm diseases, on revelation of biopreparations and biostimulators for mulberry silkworm, for eggs grading, etc.

Mulberry silkworm as biologically active substances (BAS) producer:

- - In genetically engineered developments where mulberry silkworm are used as producer of genetically modified biopolymers (fibroin and sericine), high effective medical peptide preparations, hormones, enzymes and other products (interferon, growth hormone, bioinsecticides, etc.).
- - In engineering enzymology and biotechnology (isolation of enzymes, their inhibitors, and other bioregulators from mulberry silkworm eggs, larvae and pupae and obtaining of nano-matrix forms on the base of silk fibroin hydrogels.
- - Obtaining of animal protein, carbohydrate, lipid and other BAS.

Mulberry silkworm as training aid: For study of biology, physiology, biochemistry and zootechnics of this insect and for development of practical skill on sericulture in primary and secondary schools, in colleges and universities.

II. Tasks on development of mulberry pyralid biocontrol methods

- Evaluation of infection rate of Uzbekistan regions;
- Role of entomophages in mulberry pyralid biocontrol and search of their often occurred species.
- Role of entopathogenic agents in mulberry pyralid control and definition of their effective species.
- Joint use of pathogenes and low impact chemistry preparations in control of mulberry pyralid.
- Effect of negative temperatures on mulberry pyralid during cold winters.

Fig. 11
Mulberry pyralid *Glyphodes pyloalis* Walker and stages of its development

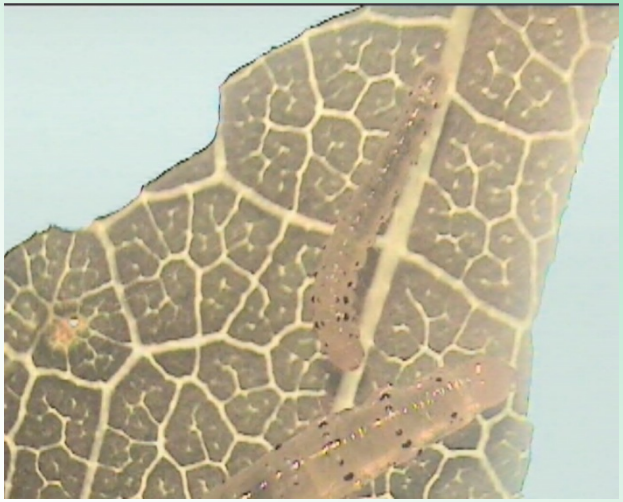
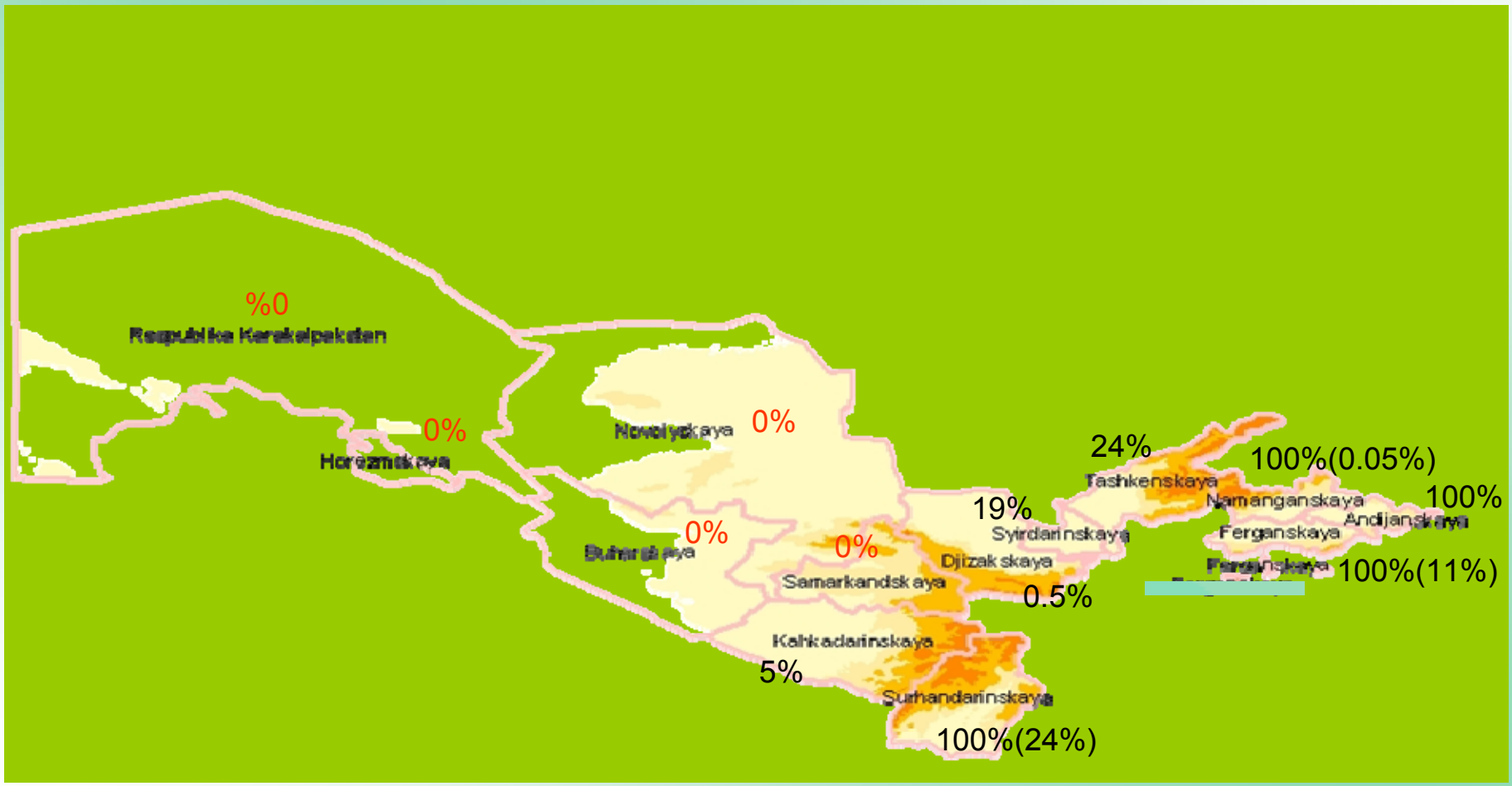


Fig.12

Mulberry plantations infected by pest in Uzbekistan (Buka district, Tashkent province, 2006)

Fig. 13

Mulberry pyralid infection in Uzbekistan provinces, % in 2006(2008)



Entomophages which are often occur on mulberry trees infected by mulberry pyralid

- Bracon *Bracon hebetor* Say
- Golden-eyed leswing *Chrysopa carnea* Steph.
- Lady beetles *Adonia variegata* Goez
- Spiders *Aranei,* different species
- Ants *Formica rufa*
- Hornets *Vespa orientalis* and waspes *Pompilidae*
- New parasites – hymenopterous from elasmid family (*Elasmidae*) and dipterous – fly *Leucopis bona* Rohd.

Table 10

Paralyzing effect of *Bracon hebetor Say* (A) venom gland extract and its fractions (B), obtained by gel-filtration (Sefadex G-100,1) on larvae(2), pupae (3) and imago (4) of mulberry silkworm.

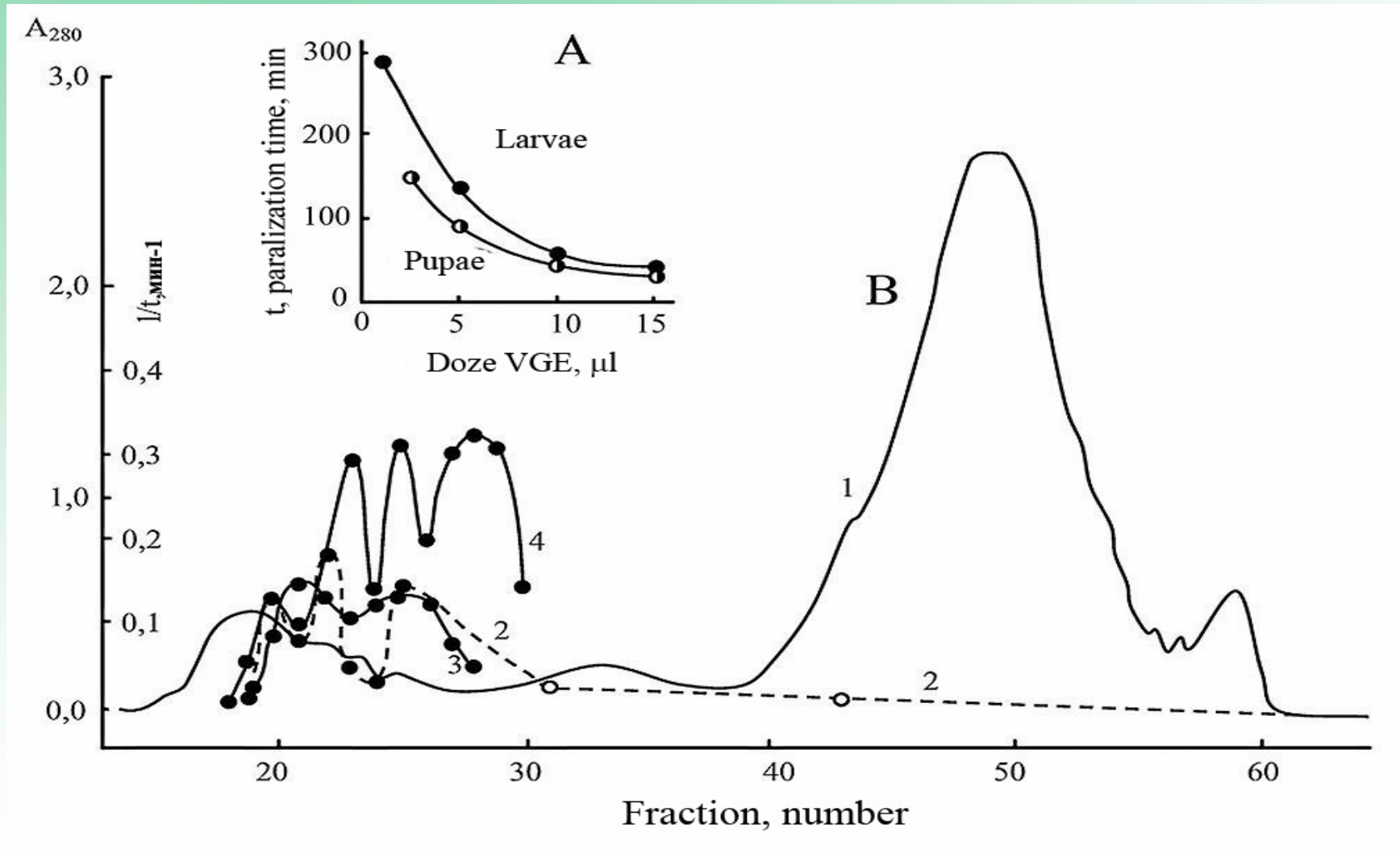


Fig. 14

New parasite of mulberry pyralid, hymenopterous from *Elasmidae* family

Fig. 15

New parasite of mulberry pyralid - fly *Leucopis bona* Rohd.:

1 – larva; 2-pupae; 3 – adult pest

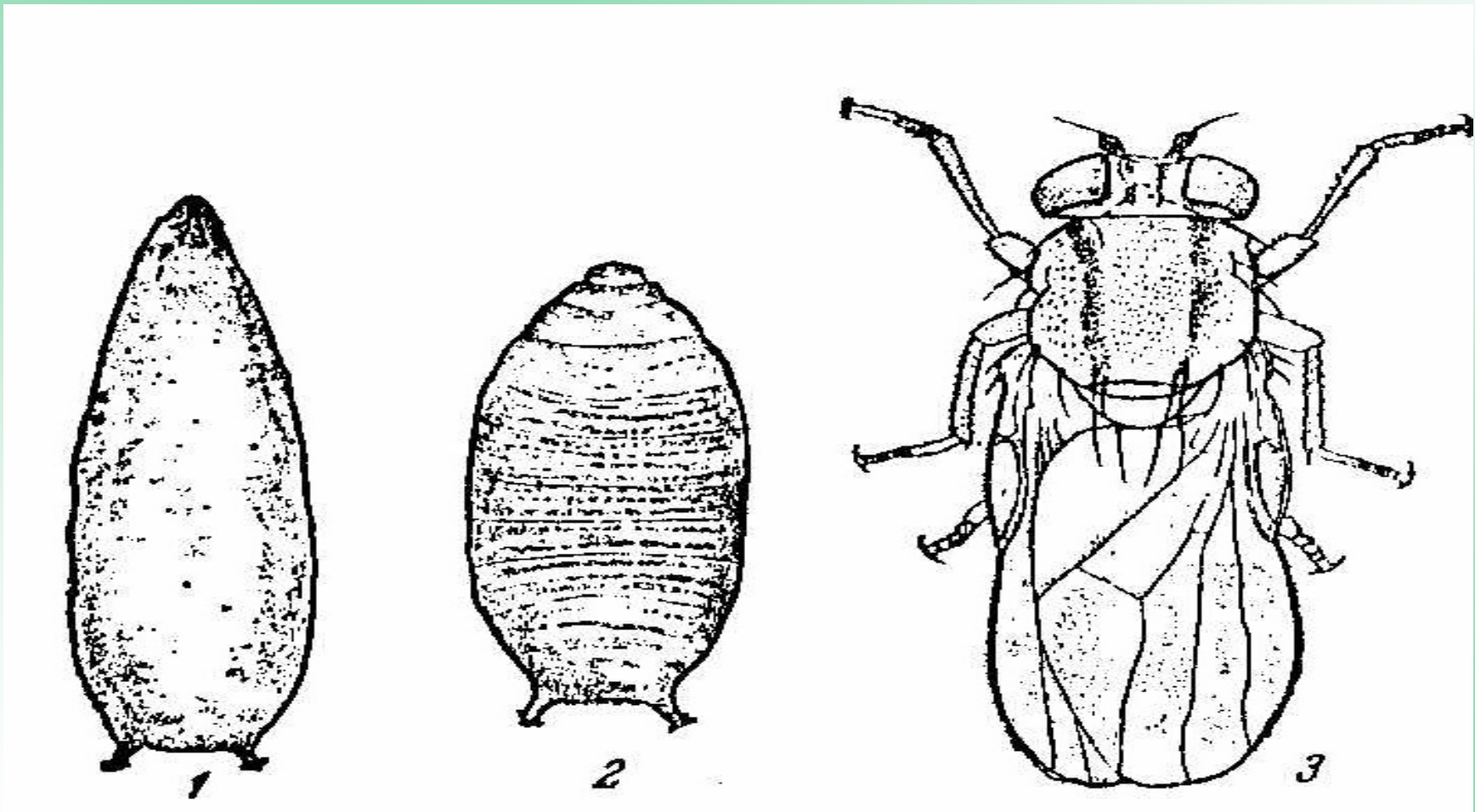


Fig. 16

Pathogenes to control mulberry pyralid.
Structure and entomopathogenic activity of natural baculoviruses

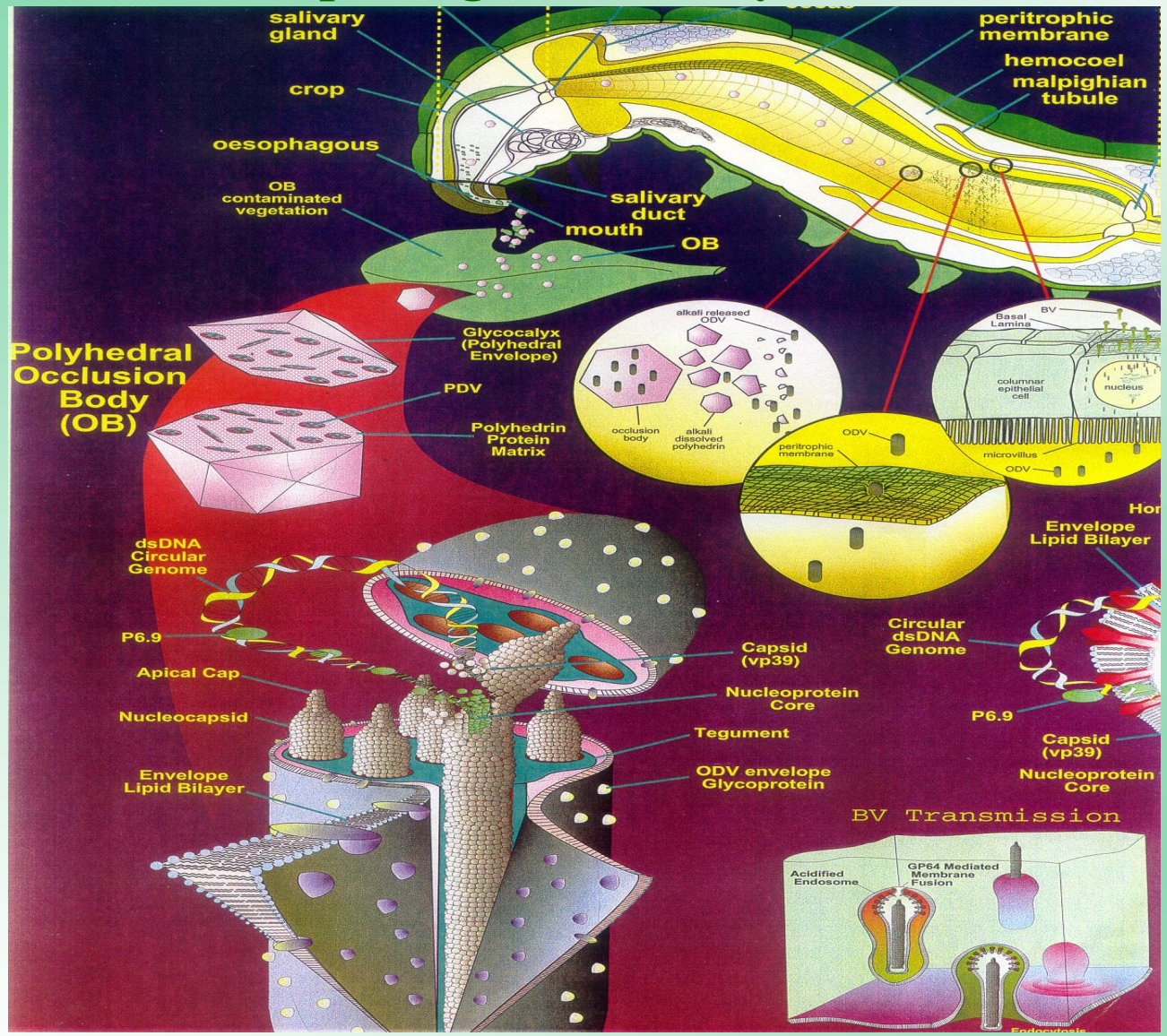


Fig. 17

Recombinant (A. AcAaIT) and wild (B. AcMNPV) baculoviruses of Californian loopers *Autographa californica* Speyr

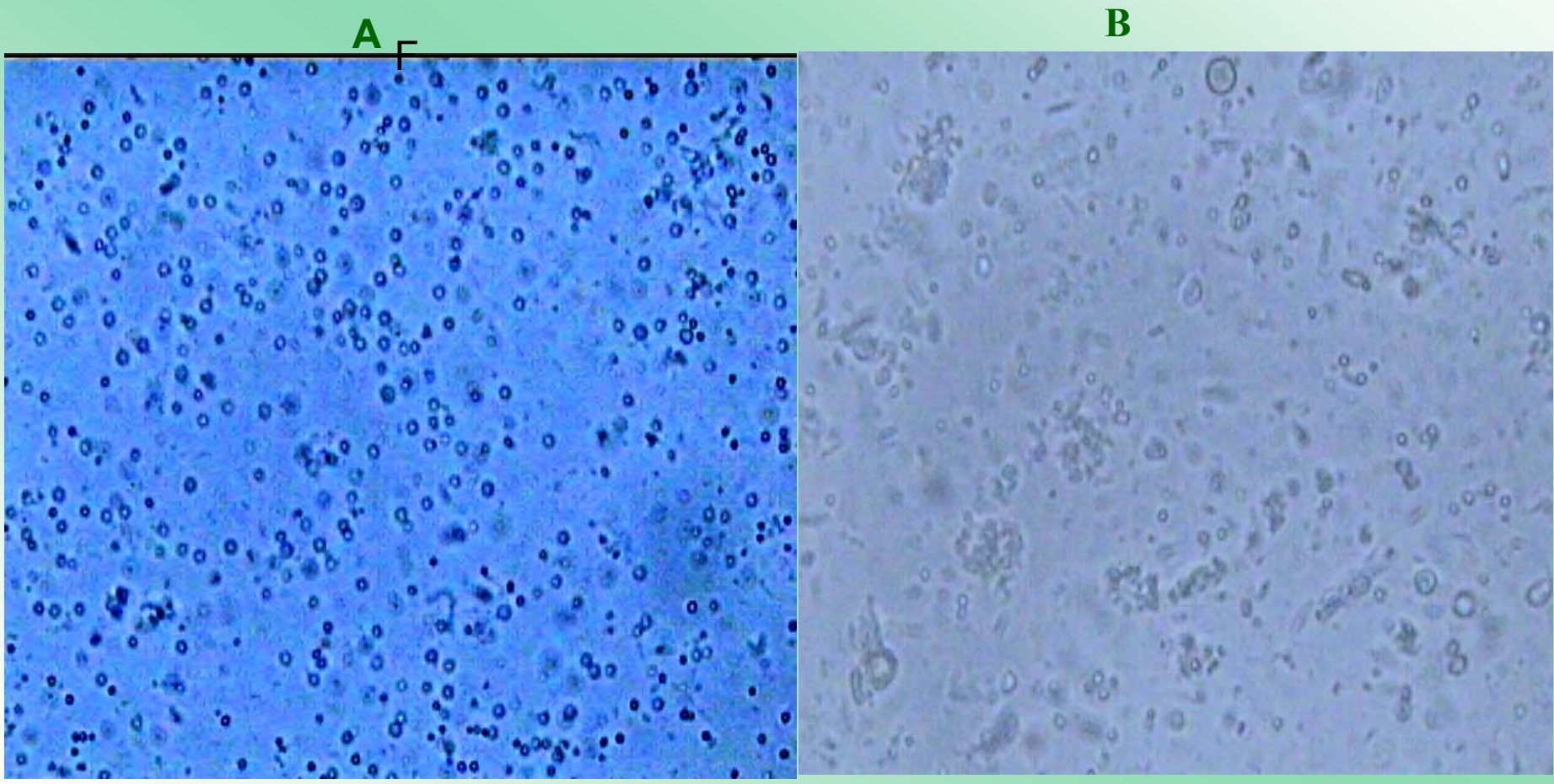


Fig.17

Effect of wild (AcMNPV) and recombinant (AcAaIT) baculoviruse based insecticides on different instars mulberry pyralid larvae

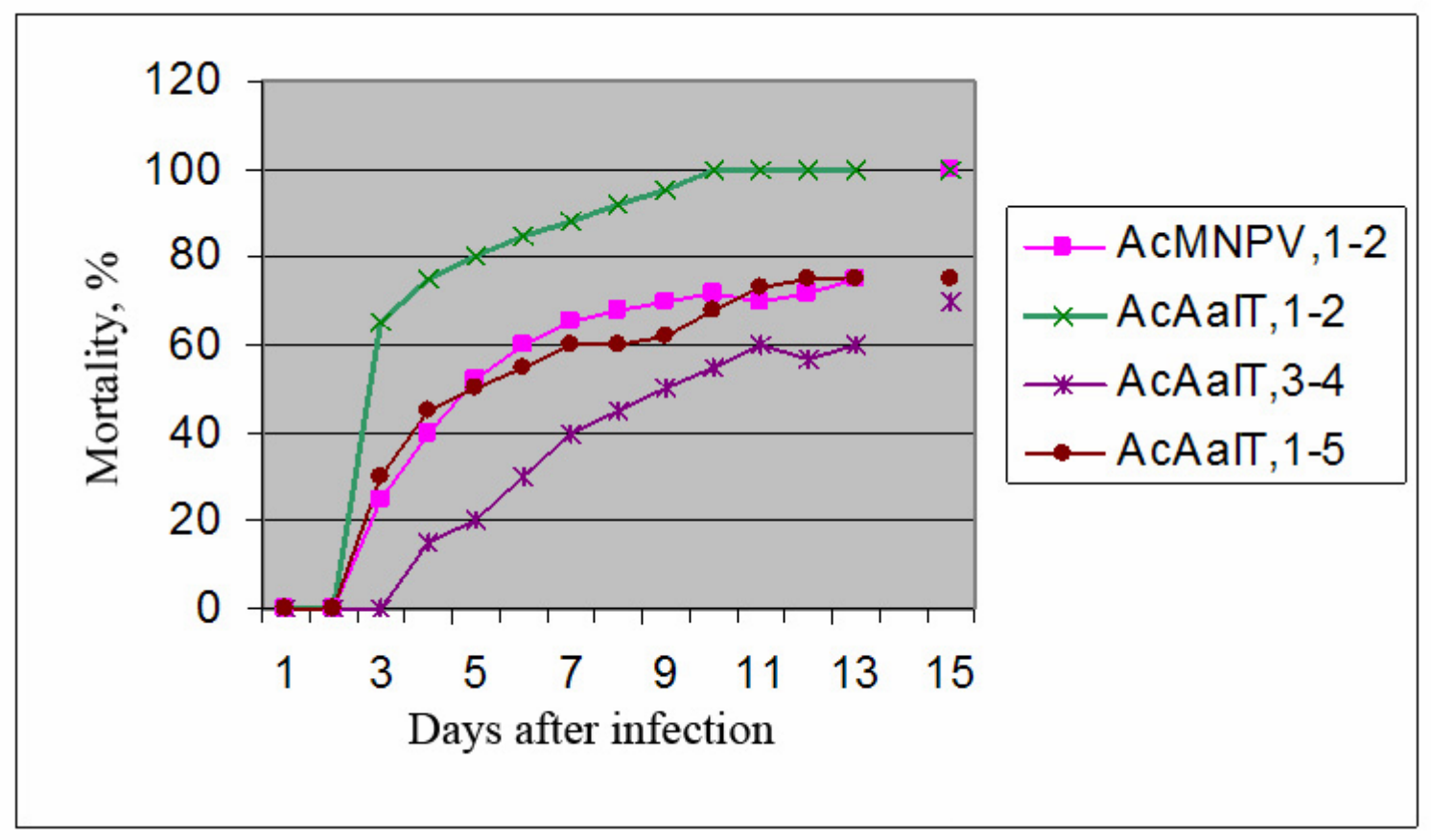


Table 11

Effect of Russian viral preparations on mulberry pyralid (M±m; n=3; *- P<0.05)

Viral preparations	Mortality in indicated days after infection, %							
	2	3	5	7	9	12	15	18
Virin NSh $5x10^6$/larva	0	20.0±10.0	23.3±8.8	26.6±12.0	40.0±10.0	43.3±8.8	46.6±8.8	50.0±10.0
Virin Diproin $5x10^6$/larva	0	13.3±13.3	26.6±12.0	36.6±12	46.6±12	50.0±11.5	50.0±11.5	50.0±11.5
Virus *Pieris brassicae* 1/5	10.0±5.7	20.0±0.0	23.3±3.3	40.0±0.0	46.6±3.3*	53.3±6.6	56.6±8.8	60.0±11.5
Virus *Agrotis segetum* 1/5	10.0±5.7	23.3±8.8	26.6±6.6	30.0±5.7	36.6±8.8*	36.6±8.8	36.6±8.8	36.6±8.8
AcMNPV $4x10^4$/larva	0	20.0±0.0	26.6±6.6	46.6±8.8	60.0±5.7	73.3±3.3*	80.0±5.7*	83.3±8.8
H_2O (Control)	0	0	0	0	3.3±3.3	6.6±3.3	10.0±5.7	13.3±3

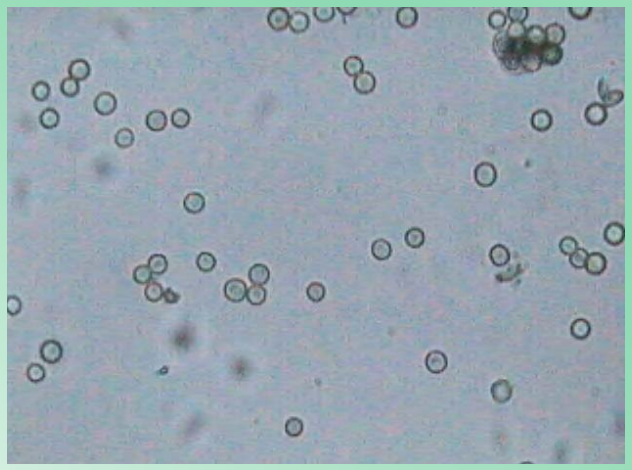
Table 12

Effect of bacterial preparations *B.thuringiesis* on mulberry pyralid (M±m; n=3)

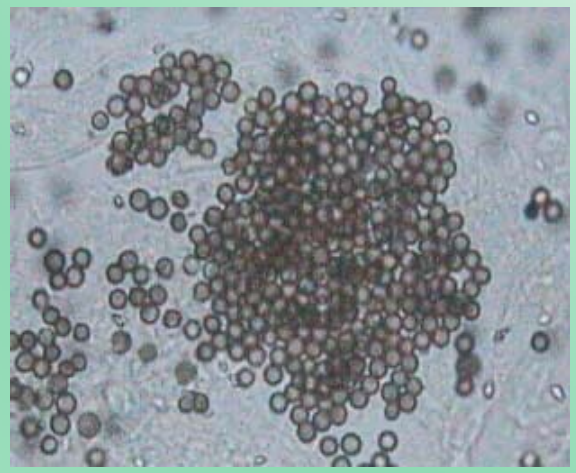
Preparation and its concentration	Mortality in indicated days after infection, %							
	1	3	5	7	9	12	15	18
Lepidocide 0.025%	3.3±0.3	6.6±0.3	13.3 ±3.3	13.3 ±3.3	16.6 ±3.3	33.3 ±4.8	43.3 ±6.6	43.3 ±6.6
Bitoxy-bacillin 0.025%	33.3 ±3.3	80.0 ±11.5	80.0 ±11.5	80.0 ±11.5	80.0 ±11.5	80.0 ±11.5	80.0 ±11.5	80.0 ±11.5
Sonit-K 0.025%	0	60.0 ±7.0	63.3 ±8.8	63.3 ±8.8	66.6 ±8.8	66.6 ±8.8	70.0 ±5.7	70.0 ±5.7
Control, H_2O	0	0	0	0	3.3 ±3.3	6.6 ±3.3	10.0 ±5.7	13.3 ±3.3

Fig. 18

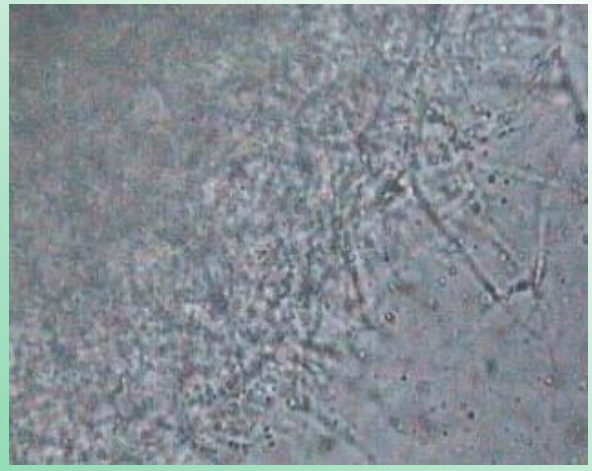
Fungal cultures isolated and purified for tests on mulberry pyralid and mulberry silkworm



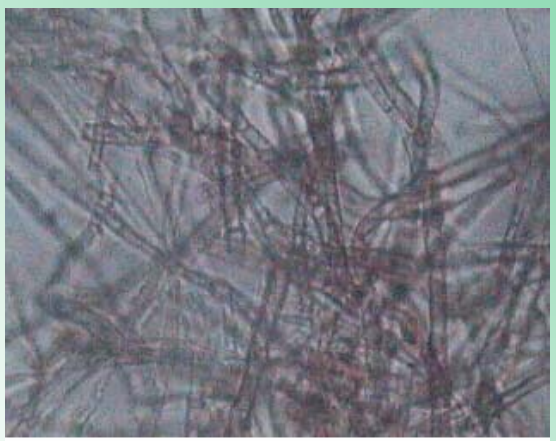
A. Flavus



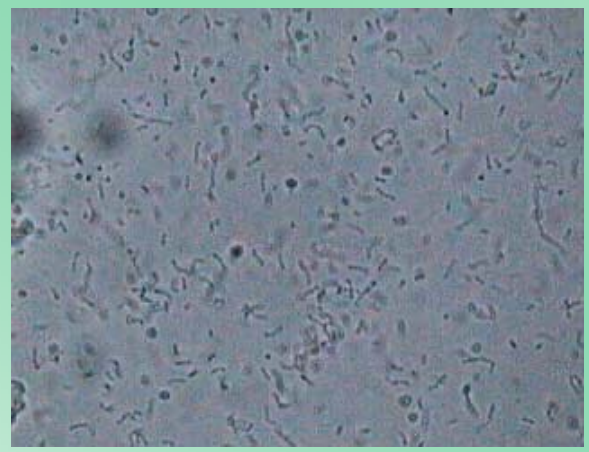
Penicillium sp.



S. brevicaulis



Fusarium sp.



B. tenella

Table 13

Effect of isolated fungi on mulberry pyralid (M±m; n=3)

№	Fungi preparation	Mortality (%) in indicated days after infection						
		2	3	5	7	9	11	14
1	*Aspergillus flavus*	0	10	23.3±3.3	33.3±3.3	53.3±3.3	73.3±3.3	100
2	*A. oryzae*	0	13.3±3.3	36.6±3.3	46.6±3.3	56.6±3.3	76.6±3.3	100
3	*Beauveria tenella*	0	16.6±3.3	26.6±3.3	36.6±3.3	86.6±3.3	100	-
4	*B. bassiana*	0	0	23.3±3.3	43.3±3.3	86.6±3.3	100	-
5	*Scopulariopsis brevicaulis*	0	0	23.3±3.3	63.3±3.3	86.6±3.3	100	-
6	*Fusarium sp.*	0	16.6±3.3	36.6±3.3	60±	100	-	-
7	*Beauveria tenella* strain 85	0	26.6±3.3	70±	90±	100	-	-
8	*B.bassiana* strain 134	0	16.6±3.3	56.6±3.3	80±	100	-	-
9	Control (H_2O)	0	0	0	0	0	0	10

Table 14

Effect of Entonem-F preparation on V instar mulberry pyralid larvae (M±m; n=3)

№	Preparation, dilution	Days after infection						
		1	3	5	7	9	11	14
1.	EPN-1/10	10	5.3±0.9	2.0±1.0	1.0±0.5	1.0±0.5	0.6±0.3	0.6±0.3
2.	EPN-1/100	10	8.3±0.8	6.6±1.2	6.0±1.0	5.0±1.0	3.3±1.2	3.3±1.2
3.	EPN-1/1000	10	9.6±0.3	9.3±0.6	8.3±0.6	6.6±0.3	6.3±0.6	6.0±1.0

Practical recommendation in biocontrol of mulberry pyralid *Glyphodes pyloalis* Wlk.

- Use of effective mulberry agrotechnics
- Decrease of use of chemical insecticides
- Use of entomophages produced by biofactories
- Involvement by attraction of other active entomophages of mulberry pyralid: ladybugs, spiders, ants, hornets, wasps and others.
- Use of wild and recombinant viral preparations of *Autographa californica* in early control of mulberry pyralid.
- Use of some bacterial, fungal, nematodal agents and low impact chemicals after cocoons production season.
- This IPM will be more effective after cold winters.

* To prevent transfer of infected and died mulberry pyralid larvae to rearing rooms with mulberry leaves.

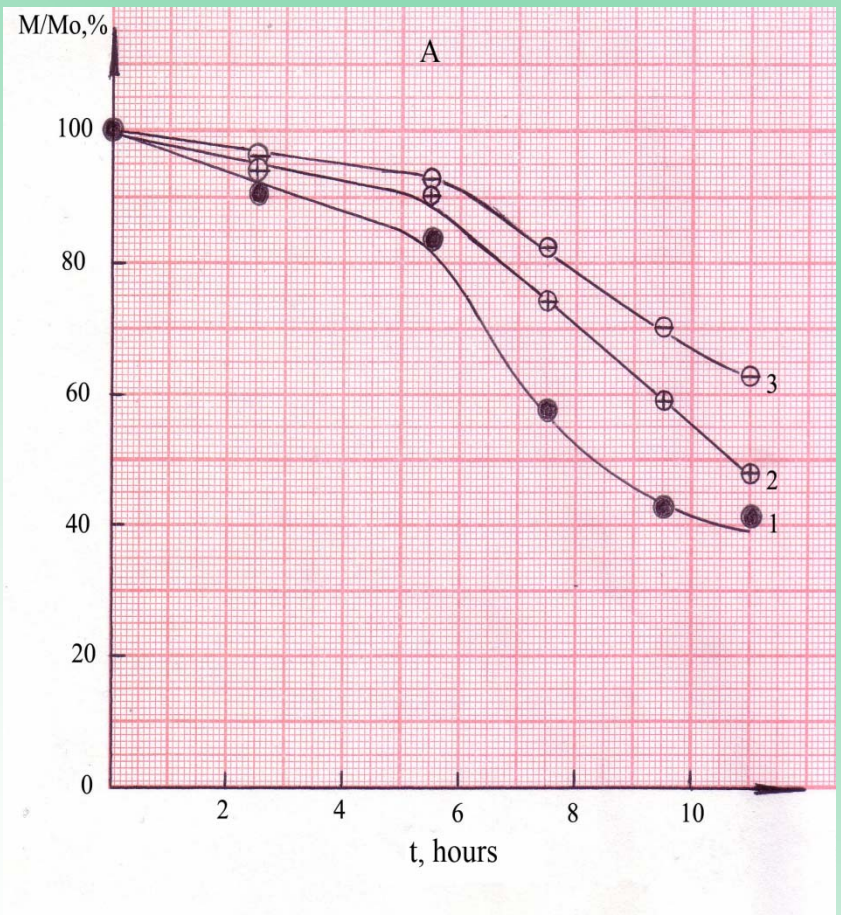
III. Tasks of research on rational use of silk materials

- To apply bioprotective methods of sufocating, drying and sterilization of mulberry silkworm pupae and cocoons.
- To develop obtaining of silk sorbents, immobilized on and into them proteins, enzymes, medicines, etc.
- To develop chemical and biochemical methods of silk protein hydrolysis and fields of their application.
- To determine and develop new directions of silk application in pharmacy and bionanotechnology.

Fig.19

Bioprotective infrared drying of cocoons at one-three layered loading (A) and consumer forms of artificial diet (B) for silkworm

Б



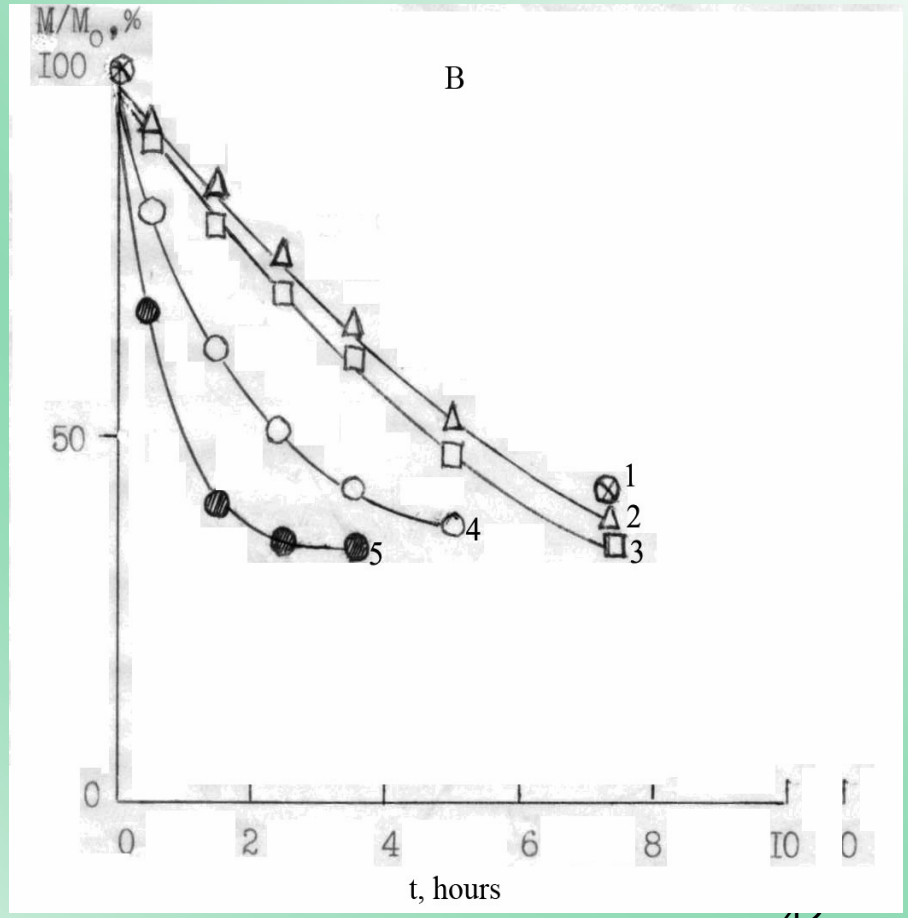


Table 15

Phospholipase A_2 activity in preparations of pupae suffocated and dried by different methods

Method of drying	pH			
	9.0	10.0	11.0	10.0 without $CaCl_2$
1. Sublimation	17.5	32.5	17.8	35.0
2. Heat drying	5.3	11.2	4.8	13.1
3. Superhigh frequency (SHF) drying	9.7	18.8	7.5	20.0
4. IR-drying by the proposed method	15.0	28.2	13.4	31.6

Table 16

Alteration of average weight of larvae grown on artificial diet with components dried by different methods

Drying methods, AD components	Average larvae mass by instars, g			
	II	III	IV	V
I. **Sublimation**				
1. ML powder	0.0205	0.089	0.280	0.720
2. Defatted pupae powder	0.0190	0.090	0.282	0.780
3. Sericine powder	0.0202	0.037	0.273	0.750
4. Control	0.0195	0.083	0.265	0.730
II. **SHF drying**				
1. ML powder	0.0163	0.067	0.205	0.658
2. Defatted pupae powder	0.0158	0.059	0.196	0.530
III **IR-drying**				
1. ML powder	0.0192	0.085	0.273	0.700
2. Defatted pupae powder	0.0195	0.087	0.276	0.720
3. Sericine powder	0.0197	0.090	0.270	0.700
4. Control	0.0190	0.085	0.260	0.680

Table 17

List of products and semiproducts obtained from wastes and by-products of sericulture and silk technology

Larvae Pupae	Fibrous wastes	Chitinous wastes	Waste waters	Excrements	Bedding
Food proteins Chitin. Enzymes Inhibitors of Protease Antimicrobial substances Neutral and Polar lipids Vitamins Minerals Secondary metabolites Other BAS	Fibroin hydrolyzates Peptides Amino acids Fibroin hydrogel Biosorbents Functional Proteins for bio-nanotechnology Immobilized medicines, proteins and enzymes Fibroin composites	Chitin Chitozan Fibroin Hydrolyzates Biosorbents Biostimulators Functional biopolymers Mixed composites	Sericin Lipids Pigments Minerals Sericin hydrolyzates Peptides Amino acids	Uric acid Chlorophyll Phytol. Fodders Enzymes Melanins Organic fertilizers Compost	Uric acid Chlorophyll Phytol. Fodders Enzymes Vitamins Organic fertilizers Compost

Fig. 20

Gel-chromatographic analysis of silk fibroin acid hydrolyzates

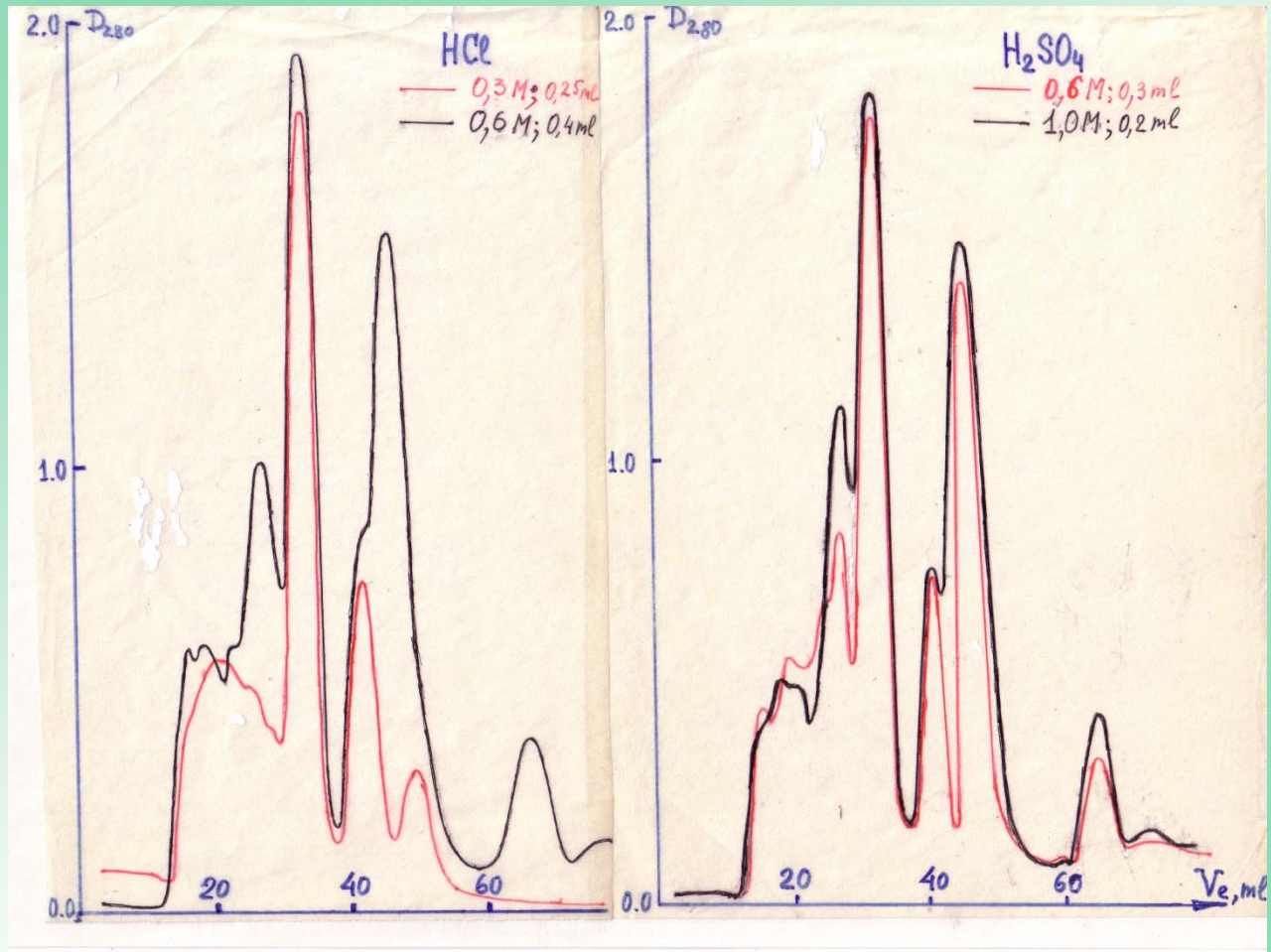


Fig. 21

Analysis of fibroin hydrolyzates obtained by enzymatic decomposition (Sephadex G-10).

Arrows – standard volumes of elution of BSA, B-12 vitamin, tyrosine and glycine

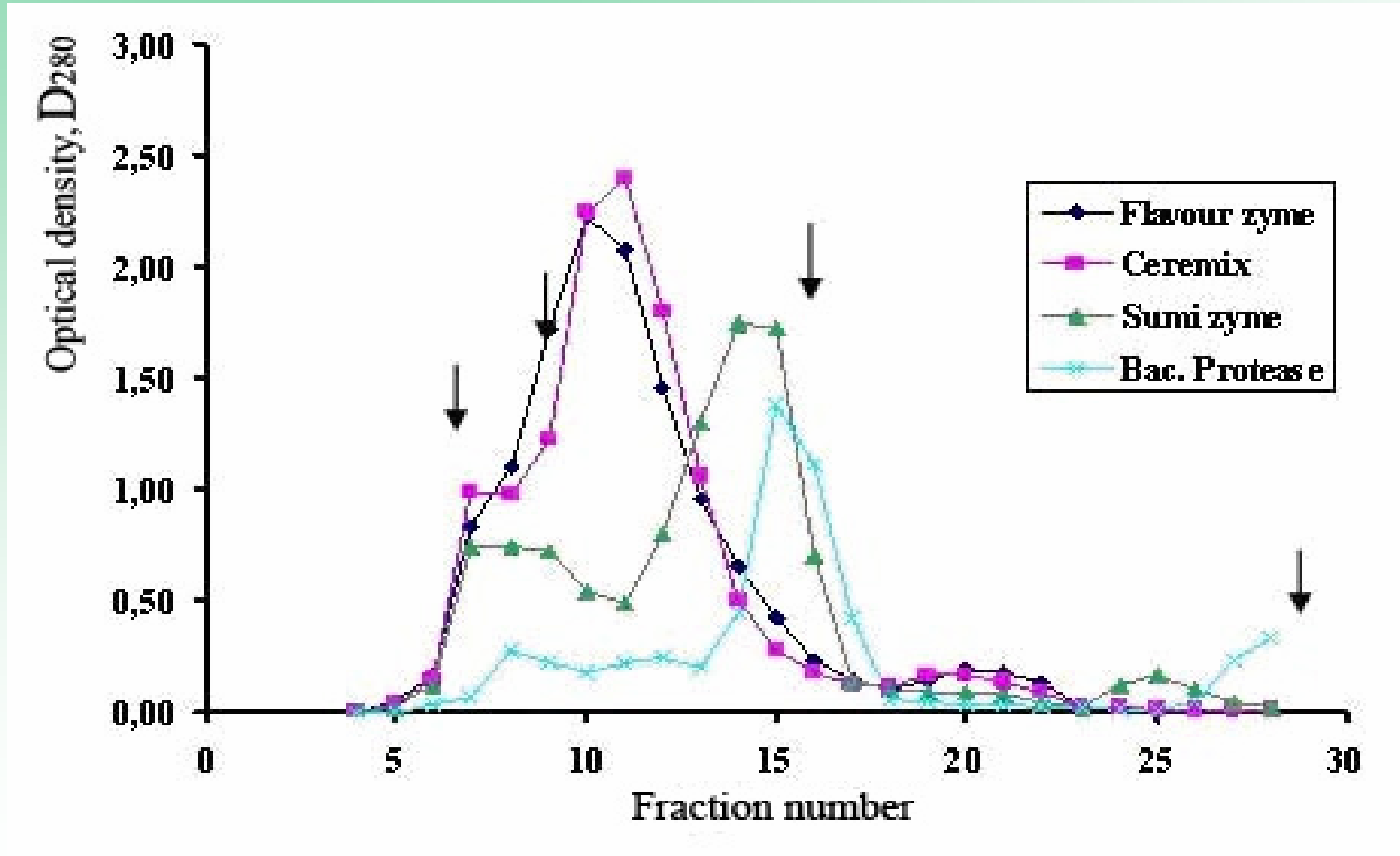


Fig. 22

Scheme of designed and working model of bioreactor "Artificial intestine"

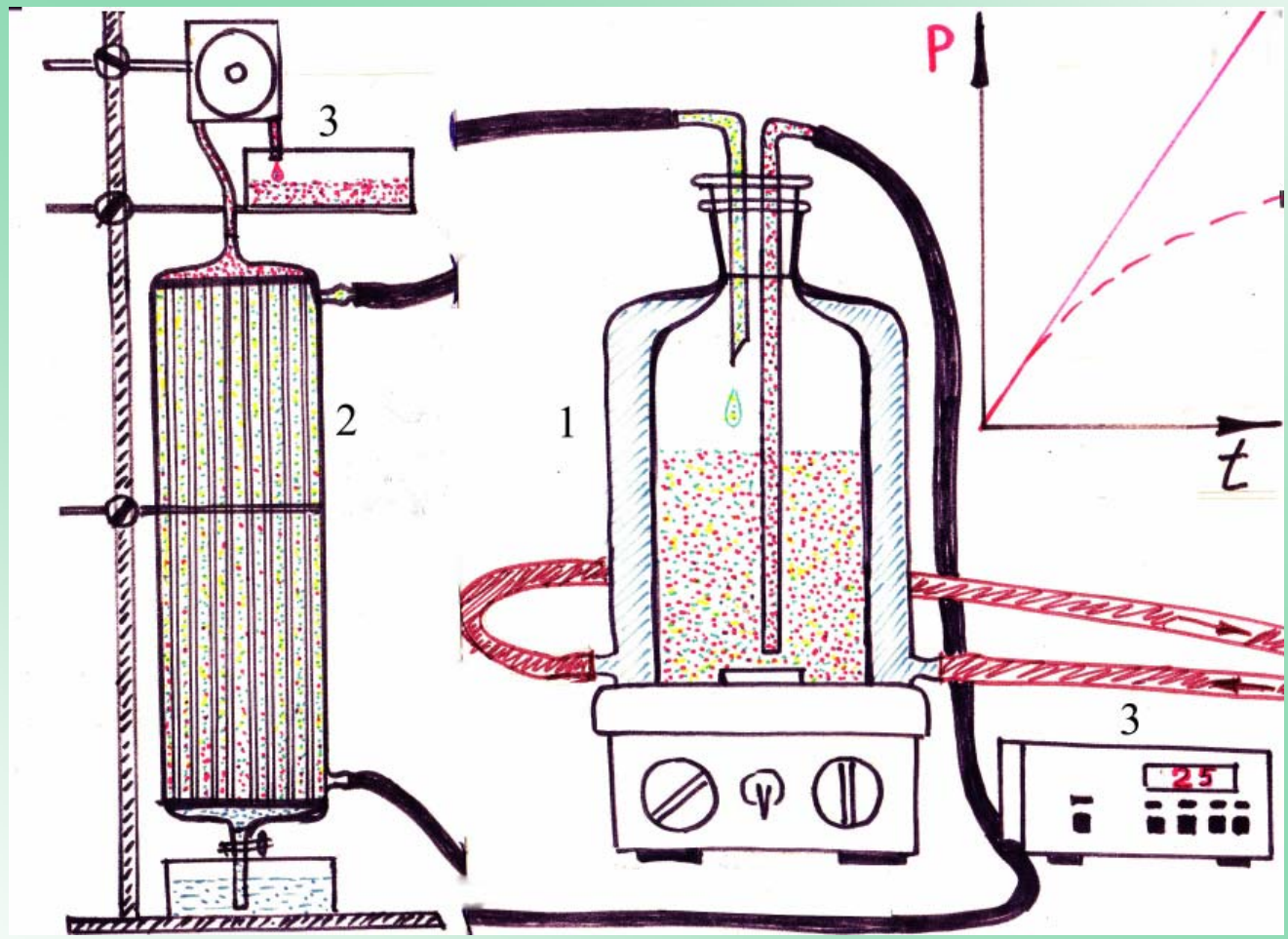


Fig. 23

Fibroin hydrolyzates (А), obtained by old (upper) and by new method (bottom). (Б) –tyrosin from chromatographic fractions; (В) – effect of UV irradiation on hydrolizates in creams; (Г) – their stability to infection by microorganisms

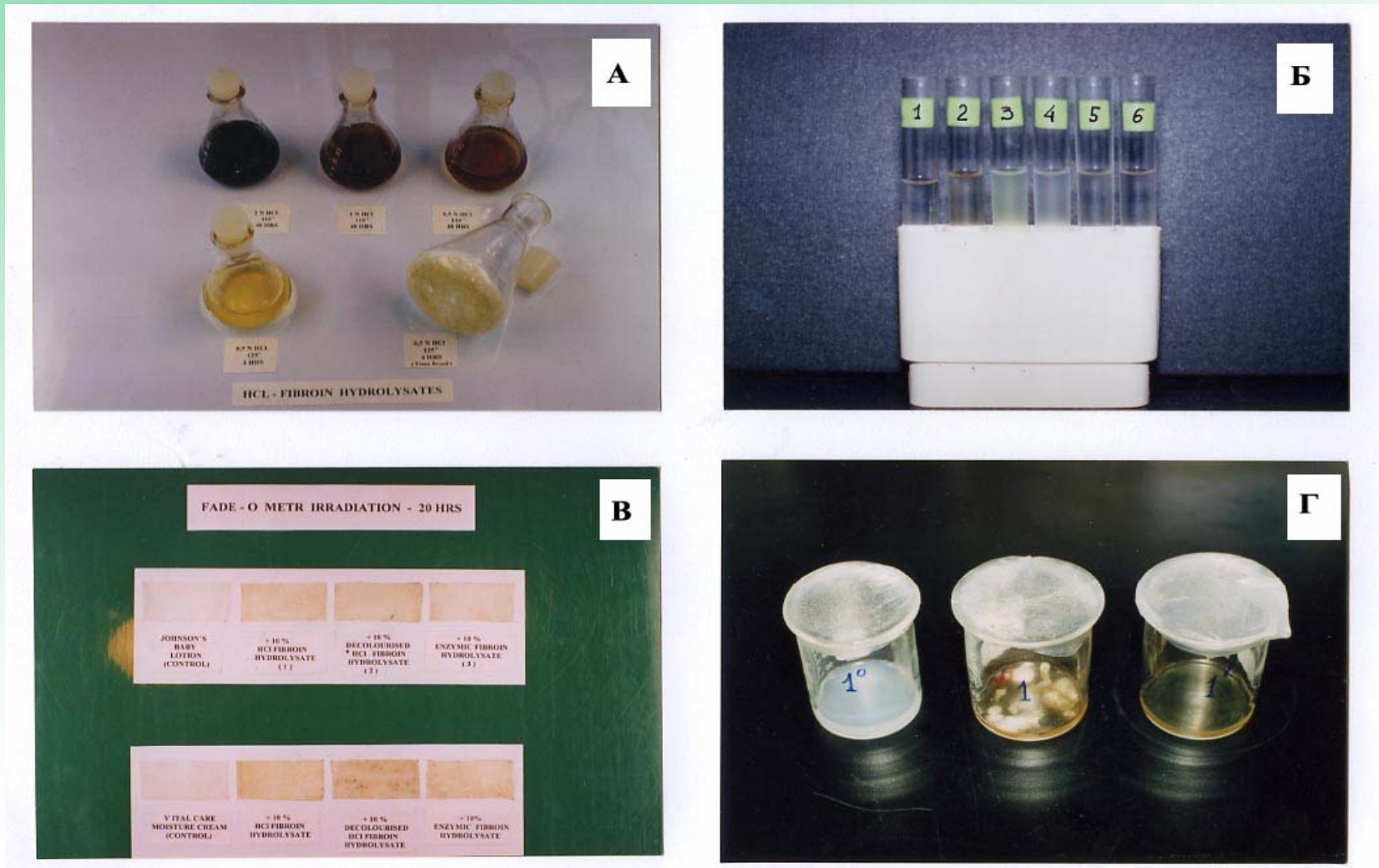


Fig. 24

SEM pictures of sorbent samples from natural silk thread: A – scale in 10 μm; B - 2 μm; C - 200 nm and regenerated fibroin; D - 200 nm.

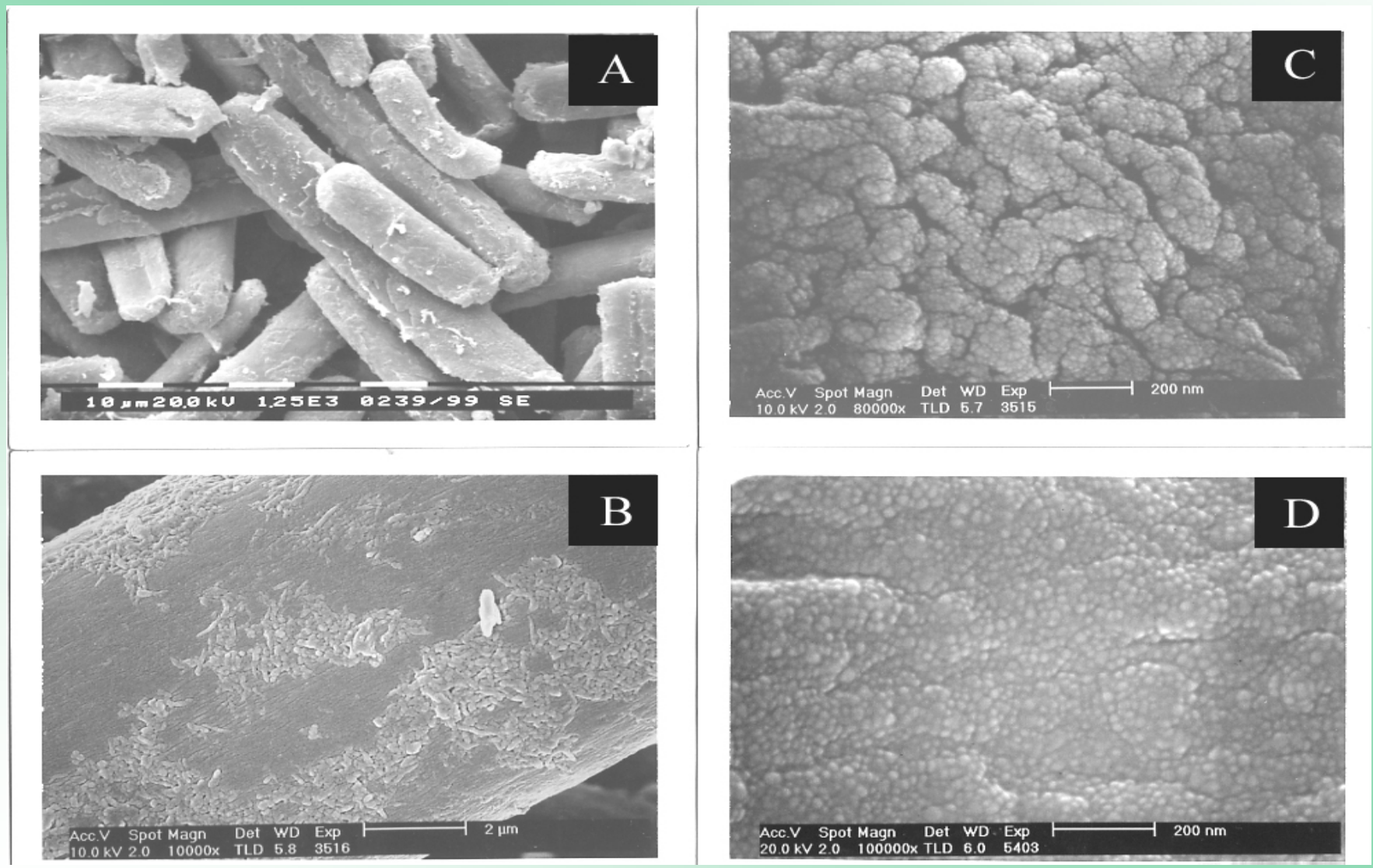


Table 18

Activity of biospecifically immobilized proteolytic enzymes on the silk at different pH

pH	Trypsin*	Chymotrypsin*	Chymopsin*	Alkaly protease *B. Subtilis* **
6.0	0	0	0.11	0.25
7.0	0	0.25	0.15	0.31
7.5	0.16	0.35	0.25	-
8.0	0.35	0.75	0.30	0.35
8.5	0.49	0.90	0.43	-
9.0	0.65	2.30	0.55	0.44
9.5	0.90	2.55	0.40	-
10.0	0.30	1.05	0.28	0.68
10.5	0.90	0.65	0.20	-
11.0	0.55	0.80	-	0.92
11.5	-	0.75	-	-
12.0	0.40	0.50	-	0.84

Fig. 25

EM pictures of nanoparticles fibroin-PAA-lipase (F-PAA-CRL, А) and F-Dextran blue (Б), F - PLD (В), F- lysozyme (Г),

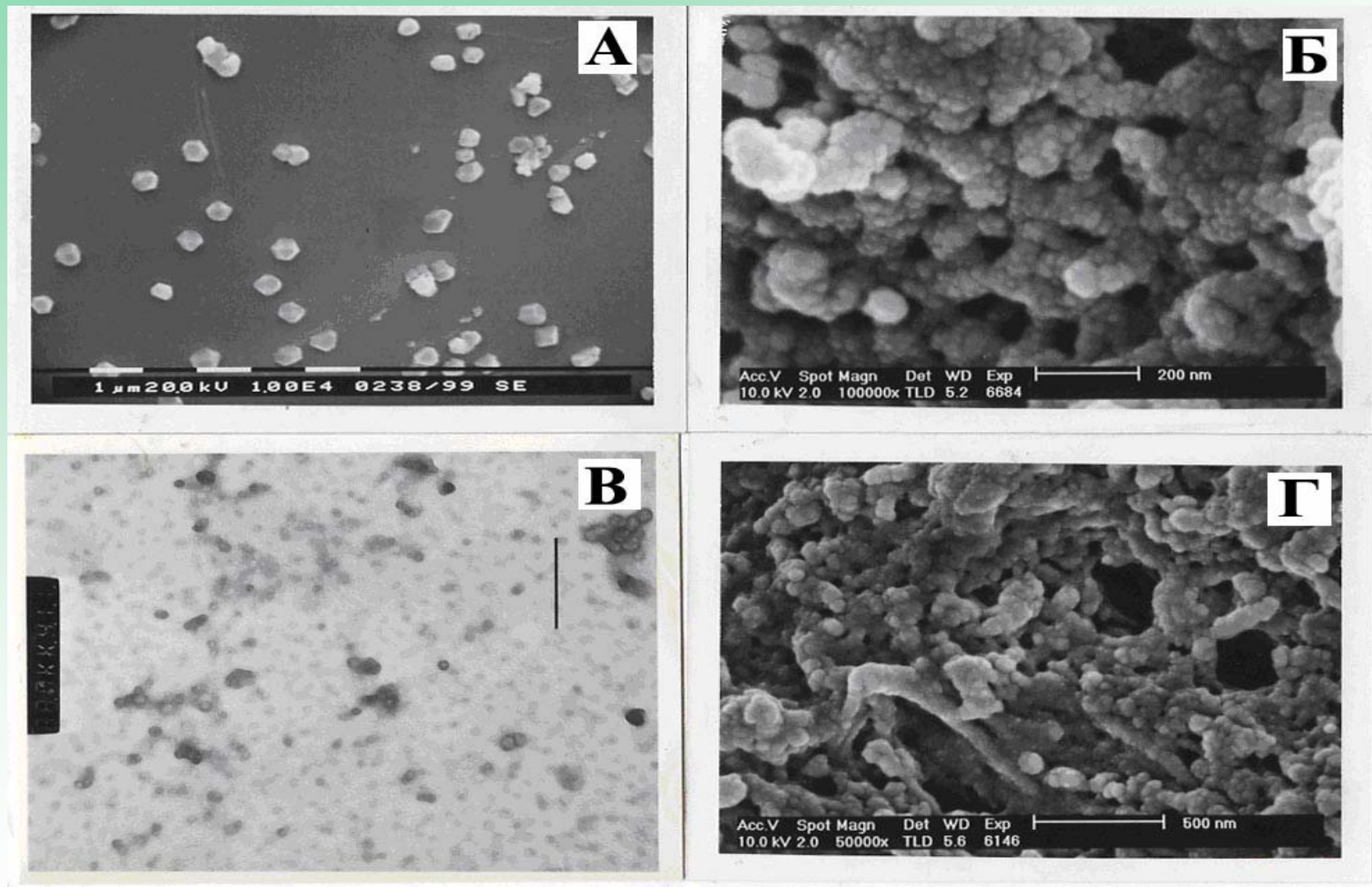


Fig. 26

SEM pictures of fibroin F- *E.coli* cells (Д), F-vitamin B_{12} (Е); F- *Saccharomyces cerevisiae* cells (Ж) and F- cytochrome c (З) composites

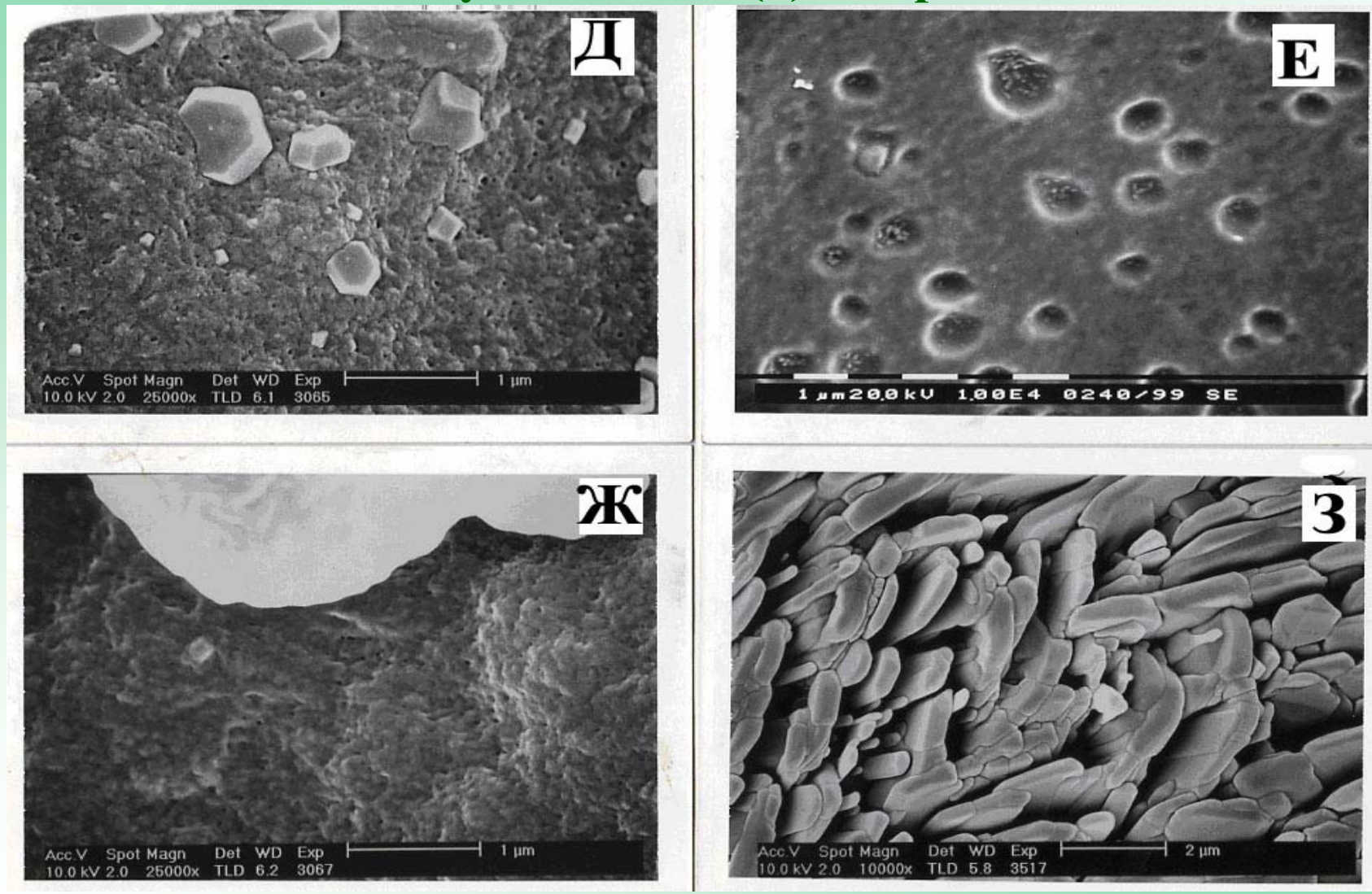


Fig. 27

Transalkylation reactions by immobilized phospholipase D in presence (A) and absence (B) of 10 mM $CaCl_2$ in isooctane-lecithin-ethanol system

A

B

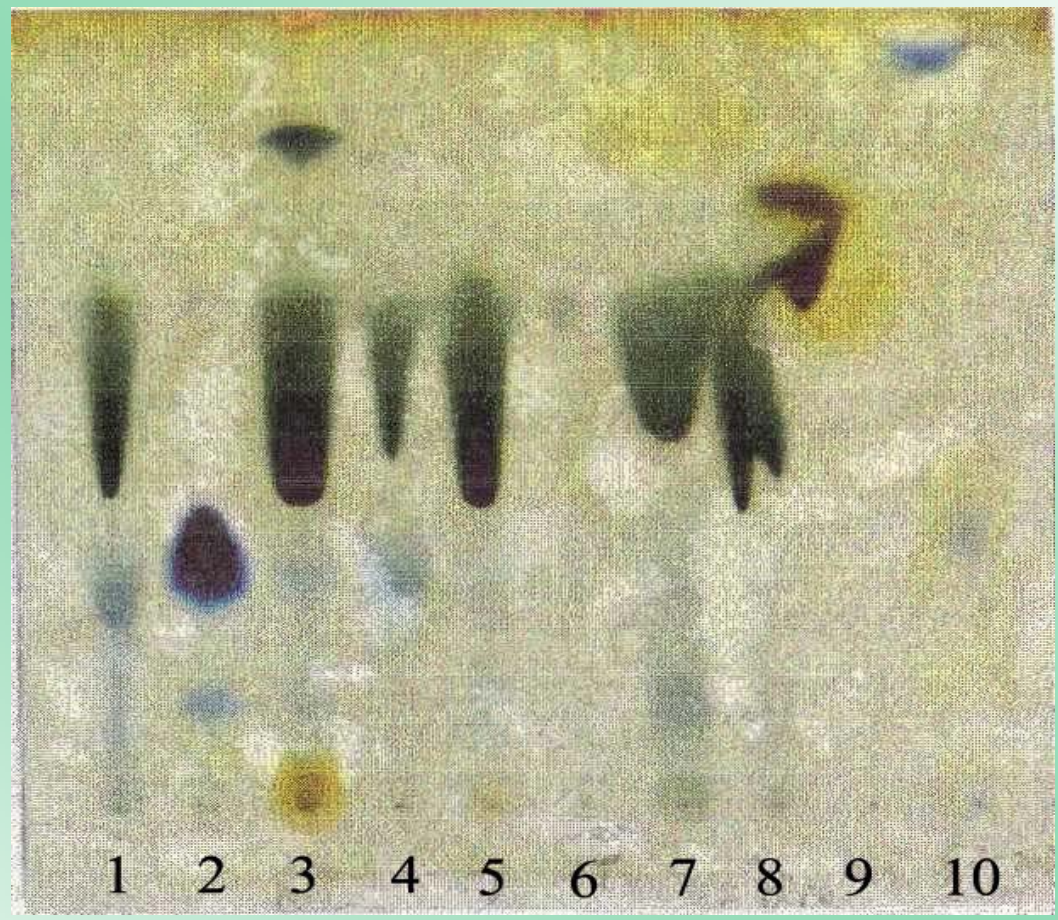


Table 19

Reaction of etherification with ketoprofen (KP) and different alcohols by immobilized lipase in isooctane environment (HPLC)

Conditions of reaction: KP – 80nM +alcohol(100µl)+isooctane(0.9ml)+15mg IL
Temperature 30°C at stirring. Time of reaction 36 hours

Samples	Retantion Time, min	Ratio of squares of picks,%
1.KP +methanol+ E	2.9; 17.7; 19.0	0.43; 49.6; 49.9
2. KP+ethanol+ E	2.9; 17.6; 18.9	~0.0; 49.8; 50.1
3. KP+propanol+ E	2.8; 17.6; 18.9	~0.0; 85.8; 14.2
4. KP +butanol+E	2.9; 17.7; 19.0	0.4; 49.7; 49.7
5. KP+octanol+E	2.9; 18.0; 19.0	10.3; 3.1; 46.6

C O N C L U S I O N S

Results of performed researches:

- Mulberry silkworm artificial diets and field of its use, methods and means of increasing sericulture productivity have been developed.
- Infection rate, reasons of fast spread of mulberry pyralid in the Republic have been determined. Biological protection methods of mulberry plantations against the pest have been developed.
- Bioprotective method of suffacation and drying of biological objects, methods of wastes utilization, methods of obtaining of biosorbents, immobilized proteins, enzymes and biologically active substances have developed.

Preliminary economic efficiency of biotechnological approaches in sericulture and silk technology

1. Artificial diet used in combined technology of silkworm rearing will lead (at other equal conditions) to centralization of cocoon production with further transfer sericulture from seasonal industry based on cottage work to all-year industry (social and economic effect).
2. Developed conception of mulberry silkworm stimulation in young instars will allow to decrease expenses more than in 50 times, to shorten cocoon production for 1-3 days and increasing mulberry silkworm vitality on 10-17%, cocoon weight – on 10-20%, shell-cocoon ration – on 2-3%, oviposition – on 10-20%, cocoon reeling – on 8-15%, thread length – on 30-100 m. This gives economy at production of 1kg raw silk in 4-6$.
3. Use of bioprotective method of suffocation, drying and sterilization of alive larvae, pupae, cocoon and biological objects allows to preserve in them in native state all complex of biologically active substances with simultaneous increasing technological parameters of cocoon unreeling and silk production quality. It is related and to mulberry silkworm artificial diet and to its components. Effect from increasing of quality.
4. Improved method of obtaining of fibroin hydrolyzates increases product output on 15 – 25%, decreases hydrolysis time from 48 to 6 hours and does not require expenses for purification. Costs of production is decreased on ~ 20%.

SUMMARY

- 1. Physico-chemical and biochemical methods of components selection for mulberry silkworm artificial diets from local raw material, methods of its preparation, storage, feeding technology, adaptation and selection criterions of mulberry silkworm breeds to it and its fields of practical application have been developed.
- 2. Biochemical, biophysical and biotechnological aspects of food digestion-absorbtion processes was studied and modeled. Asporogenic yeasts which hydrolyse cellulose to glucose were found out in intestinal contents of mulberry silkworm. The synergetic effects of interaction of water-soluble and membrane-bound proteolytic enzymes giving reasons for effective application of industrial and immobilized enzyme preparations in feeding were found out. This principle was probed and used in designed bioreactor "Artificial intestine" working in conditions of digestion-absorption simulating functions of high-efficient digestive system of mulberry silkworm.
- 3. For the first time cluster-forming and vector mechanism of activation, functioning and membrane regulation of one of lipolytic enzymes – phospholipase D was investigated. This practically expended range of mulberry silkworm development stimulators.
- 4. The conception of efficient increasing of mulberry silkworm productivity by stimulation of larvae development in junior instars when expenses for stimulation are minimal but the effect in end production is remarkable was developed.
- 5. For the first time the space experiment was performed with mulberry silkworm using artificial diet. The experiment confirmed hypothesis about possibility of silkworm breeding in any geographic region of the Earth and at any season of year. Mulberry silkworm as transgenic animal could be an efficient on-board test-organism and producer of bio-active substances.
- 6. The possibility of use of wild and recombinant baculoviruses in early control of dangerous mulberry plantings pest – mulberry pyralid, a new host of alfa-alfa looper *Autographa californica* virus was developed. At the same time these bioinsecticides don't affect silkworm larvae. Due to identity of endogenic virus of mulberry pyralid and densovirus of mulberry silkworm risk of infection of mulberry silkworm by it increases.

Continuation

- 7. Drying of artificial diet, mulberry silkworm larvae, pupae, alive cocoons and other bio-objects with preservation of native composition of biologically active substances in dried products with use of IR-irradiation on base of functional ceramics was developed. Mulberry silkworm larvae and pupae dried by this bio-protective method are a new resource of biologically active substances for medicine, food industry, fodder production and cocoons have advanced technological properties. Cuticle of larvae and pupae, pupae themselves and moths after egg laying are an additional source of chitin.
- 8. Hydrolizates of fibroin, sericin and other unusable proteins by improved chemical method up to any planned depth of hydrolysis were obtained. Properties of the final product do not require its additional purification, output of this product significantly exceeds output in traditional analogue. Improvement of the product quality was achieved at hydrolysis of protein substrates by soluble and immobilized proteases on carbon-mineral sorbents or on fibroin biosorbents. In this processes it is succeed to avoid inhibition of enzyme by reaction product in specially designed bioreactor “Artificial intestine”.
- 9. Silk biopolymers being biocompatible and biodegradable proteins have found more valuable application in the native form. Fibroin composites and biosorbents are irreplaceable material for design of biosensors, means of drug delivery, modeling of high-efficient enzyme systems similar to insects peritrophic membrane or animal intestinal mucosa, for creation analogues of transport vesicle and other parts of alive cells. Stabilized enzymes stereospecific functioning in waterless organic media are developed on the base of fibroin and could be used for stereospecific bioseparation in pharmaceutical industry.
- 10. Nanoglobular structure of silk biosorbents and composites from regenerated fibroin is determined by electronic microscopy. Observed dense-crowded nanoparticles probably represent nanocrystals grown from spontaneously formed crystallization centers in concentrated fibroin solutions in conditions of fast dehydratation at thread formation during process of cocoons waving from silkworm silk gland secrets or during sublimation of regenerated silk composites.

R E C O M M E N D A T I O N S

Artificial diets for mass rearing of mulberry silkworm in conditions of continental (Central Asia) and tropical (Malaysia) climates are produced on base of developed receipts and methods of preparation with use of products and wastes of local industry

Spheres of possible non-production use of mulberry silkworm artificial diet

Mulberry silkworm as test-organism:

- **- In research institutes on bio-tests of new chemical compounds and biopreparations, on genetics and selection, on physiology and biochemistry, zoology, epidemiology, mutagenesis and cancerogenesis, on pharmacology, immunology, toxicology, medicine, feeding, in ecological, space and other extreme studies and also in zones of technogenic disasters.**
- **- In research institutes and sericulture stations for all-year studies on genetics, selection, struggle with mulberry silkworm diseases, on revelation of biopreparations and biostimulators for mulberry silkworm, for eggs grading, etc.**
- **Mulberry silkworm as biologically active substances producer:**
- **- In genetically engineered developments where mulberry silkworm are used as producer of genetically modified biopolymers (fibroin and sericin), high effective medical peptide preparations, hormones, enzymes and other products (interferon, growth hormone, bioinsecticides, etc.).**
- **- In engineering enzymology and biotechnology (isolation of enzymes, their inhibitors, and other bioregulators from mulberry silkworm eggs, larvae and pupae and obtaining of nano-matrix forms on the base of silk fibroin hydrogels.**
- **- Obtaining of animal protein, carbohydrate, lipid and other BAS.**
- **Mulberry silkworm as training aid: For study of biology, physiology, biochemistry and zootechnics of this insect and for development of practical skill on sericulture in primary and secondary schools, in colleges and universities.**

Grazie dell'attenzione
Thanks for attention
Etiboringiz uchun rahmat
Blagodaryu za vnimanie