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EXPLOITATION OF AGRICULTURAL AND FOOD INDUSTRY BY-PRODUCTS AND WASTE MATERIAL THROUGH THE APPLICATION OF MODERN PROCESSING TECHNIQUES

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First section

IDENTIFICATION, ASSESSMENT AND PROCESSING OF AGRICULTURAL AND FOOD INDUSTRY BY-PRODUCTS AND WASTE MATERIAL

STUDY OF APPLICABILITY OF NATURAL PATHOGENES FOR CONTROL OF MULBERRY PYRALID *GLYPHODES PYLOALIS* WLK

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ABSTRACT:
Mulberry pyralid *Glypodes pyloalis* Wlk. is a serious pest of Uzbekistan mulberry plantations. Use of chemical pesticides has many lacks - their toxicity and mutagenity for the human, animals, including beneficial insects, and environmental contaminations. Effect of entomopathogenic bacteria, fungi, viruses and nematodes on mulberry pyralid is investigated in the work. These agents are harmless to the human, animals and plants but effectively kill pests. These pathogenes can defeat also a mulberry silkworm cultivated in sericulture of Uzbekistan only during spring of each year. Taking into account ecological preferences of bioagents for pest control, very important for densely populated agricultural complexes of the Republics, an opportunity of their use after cocoons harvesting which comes in Uzbekistan at the end of May was investigated. As a result of such research only those preparations will be chosen which under effect of biotic and abiotic factors of environment of arid zone will completely lose the virulent ability before beginning of a new silkworm breading season.
In the work following preparations have been investigated by screening: bacterial *Bacillus thuringiensis* - Lepidocid, Bitoxibacillin, Sonit K., fungi (rases *Aspergillus flavus, Beauveria bassiana, Beauveria tenella, Penicillium sp., Scopulariopsis brevicaulis* and *Fusarium sp.*), isolated from dead mulberry pyralid larvae, baculoviral preparations – AcMNPV, SeMNPV, AcAaIT, *Limantria dispar* virus and *Agrotis segetum*, *Pieris brassicae*, *Pyrausta stricticalis*, *Neodiprion sertifer* granuloviruses and also Entonem-F, a preparation of entomopatogenic nematode.
All specified preparations differently kill mulberry pyralid but only virus preparations AcMNPV, SeMNPV, AcAaIT not affected to a mulberry silkworm. Last finding is important for early pest control. The investigated stability of bacterial and nematode preparations against biotic and abiotic factors was the least for Entonem-F (1-2 days), approximately 1 month for Lepidocid and 1.5 months for Bitoxibacillin. Work on fungi resistance to environmental factors is continued. Bacterial and fungous preparation can be used only after finishing of silkworm breading season.
The undertaken approach to the control of the serious pest of mulberry plantations modern biocontrol methods will allow increase economic efficiency and ecological safety.
Keywords: mulberry pyralid, pathogens, pest control, biocontrol.

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INTRODUCTION

A comparative effect of some baculoviral, bacterial, and nematodal insecticide preparations on mulberry pyralid *Glyphodes pyloalis* Walker, a serious pest of mulberry plantations are studied in the work. Possibility of their practical use in biocontrol of the pest are discussed.

Uzbekistan is one of the world cocoon and silk producer (it was a main cocoon and raw silk producer and exporter in the former USSR). Mulberry pyralid *Glyphodes pyloalis* Walker (*Lepidoptera*: pyralidae, the synonyms: leaf-roller *Margaronia pyloalis* Walker or *Diaphania pulverulentalis* Hamson) is of long standing and significant pest of sericulture in Japan, on Formosa, in Burma, India, Malaysia and China. In Uzbekistan for the first time its appearance was registered in the middle of 90-th years of past centuries in Dzharkurgan district of Surhandarya province has quickly increased area of its habitation to other regions, particularly was spread in Kashkadarya, Syrdarya and Tashkent provinces and completely in Fergana valley [1].

Moreover the problem is important because mulberry tree is used for a long time for soil protection, land surveying and decorative arrangement, as renewed resource of valuable timber and preparation of traditional medicine, as well as a plant that gives tasty and healing fruits.

Use of chemical insecticides for control of mulberry pyralid is ineffective and does not give the expected results. The irreparable consequences of use of chemicals includes not only the reduction of former potential mulberry pyralid entomophages but also enlarges the population of mulberry and cotton plantings pests. Taking into account secondary and tertiary influences of used chemicals, application of these plant protection means must be careful and rational, especially in Central Asian region, where main population is densely concentrated in agro-industrial oasis zones [1].

In our previous works (2001-2005) the material and experimental base for laboratory rearing of insects used as host for isolation of entomapatogenic microorganisms and screening of different preparations was created. Besides this the following potential natural enemies of mulberry pyralid were revealed and studied: - entomophages *Bracon hebetor* Say, larvae of lacewing *Chrysopa carnea* Steph and ladybugs *Adonia variegate* Goez, ants *Formica rufa* and different type spiders *Aranei*, as well as some unidentified yet hymenopterous from *Elasmidae* family and dipterous, supposedly *Leucopis bona* Rohd. Bracon and lacewing are commercially cultivated types in Uzbekistan. Bracon was studied as a host for mulberry silkworm, mulberry pyralid and other agricultural pests and as producer of neurotoxic venom having the same effect as caracurt venom [2].

Baculoviral preparations, the most specific pathogenic organisms of insect diseases, possible to consider as the most perspective for mulberry pyralid control. For a long time baculoviruses as biopesticides already are used for protection of forests and agricultural plantings. They do not effect to human, animal, plants and environment. In nature they flexibly regulate over grown up population of insects. They are not widely used because of their slow action and close specificity to hosts. Due to modern biotechnological approaches high effective entomopatogenic microorganisms (transgenic baculoviruses and bacterias) have been developed. These bioinsecticides present the real alternative to chemical agents in pest control [3]. Genetically modified (GM) baculoviral constructions contain transgenes (genes, encoding hormones, enzymes or enzyme inhibitors, insectotoxins, etc.), which significantly improve insecticide ability of wild baculovirus. In some countries these GM bioinsecticides are tested in wide-ranging field tests and have shown high efficiency. In preliminary study effect of wild and recombinant baculoviruses (AcMNPV and AcAaIT preparations from California University, Devis, USA) which have not high specificity to host was preliminary studied on some pests of Uzbekistan [4]. It was shown that these preparations can be used against plant growing pests, including cotton growing and sericulture. Effect of wild AcMNPV and SeMNPV (commercial preparation) and recombinant AcAaIT baculoviruses on mulberry pyralid and silkworm at feeding by natural and artificial diets was studied by us later in comparative aspect [5,6]. Investigated baculoviruses did not effect on development and productivity of industrial and perspective breeds of silkworm. As to other entomopatogenic preparations - bacterial on base of *B.turingiensis*. (basically commercial preparations), fungous isolated from mulberry pyralid and other insects and propagated in artificial media, as well as nematode (commercial) preparations, so all of them defeats silkworm too in different degree. Their use is possible within regions remote from sericulture zone. On the other hand use of bacterial and other biopreparations existed in sericultural Bulgaria. In this connection we undertook preliminary studies of the possible applicability abovementioned preparations in our arid zone with sharply-continental climate and high level of solar radiation.

MATERIALS AND METHODS

Mulberry pyralid larvae of different instars of natural distributions were collected and transferred from the infected territory of Tashkent province. Some experiments was carried out directly in the infected zone of Fergana province. Mulberry silkworm larvae of "Orzu", "Ipakchi 1", "Ipakchi 2 and other breeds were reared in laboratory conditions using mulberry tree leaves and artificial diets [7,8].

The following commercial and natural preparations were tested in study of entomopathogenic properties: bacterial (Lepidocid, Bitoxybacillin and Sonit K – Russian production, Bti – Germany), viral: alfalfa looper virus (AcMNPV, University California Devis, USA), Virin-NS - gypsy moth (*Limantria dispar)* virus, Virin-diproin – *Neodiprion sertifer* virus, Virin-OC – noctuid *(Agrotis segetum)* granulosis virus, Virin –GLM - *Pyrausta stricticalis* granulosis virus, *Pieris brassicae* virus and also entomopathogenic nematode preparation Entonem F – all are Russian production.

Study of entomopathogenic activity of the preparations was carried out as described early [6]. During infection by the feeding diet an a aliquot of preliminary prepared fresh solutions of the preparations were added to defined weight of a feed (mulberry tree leaves or artificial diet), thoroughly mixed up or soaked and put to plastic container or Petri dish with preliminary sorted by age and counted off (10-25 studied insects in each replication), by 3 replication in a variant (for 1 preparation). The incubation was carried out in thermostat at 25^{o}C, relative humidity - 75% and natural photo-period. Mortality of studied insects was fixed every day at the same time. The data were worked up statistically and by PC (MExcel).

Artificial nutritious diets for insects were prepared according to the descriptions in work [7] with some modifications (for mulberry pyralid larvae) from local materials, chemicals from "Dyets", "Sigma-Aldrich" and "Fluka" Companies and sterilized distilled water.

RESULTS AND DISCUSSIONS

Laboratory tests of biopreparations. Last years an alternative ecologically pure methods for agricultural pest control including microbiological pathogens of pest diseases (bacteria, fungi, viruses, nematodes, etc.) which are harmless for environment, animals and human being are developed and applied. Bioinsecticides in contrast to chemicals do not affect entomophages which kill pests inaccessible to effect of pathogens. Thus entomophages and entomopatogenic agents work coordinated. They do not give chance for further development of pest population increasing of ecological safety and economical effectiveness of plant protection system [9]. Preliminary tests of wild and recombinant baculoviral preparations [5,6] showed high effectiveness and selectivity of effect on mulberry pyralid, but not effecting mulberry silkworm. This fact allows to hope to use this phenomenon practically for early stage of mulberry pyralid biocontrol that is prohibited for chemical preparations due to feeding of mulberry silkworm. In the represented experiments the spectrum of tested viral preparations is enlarged. On the other hand study of entomopatogenic bacterial, fungous and nematode preparations on this pest for the purpose to find those which could be effectively used after sericulture season (end of May, beginning of June) when under biotic and abiotic factors of environment the pathogens will totally loss their virulence have been started for the first time. In this case the protection of mulberry trees will get ideal natural agents for control both mulberry pyralid and whole complex of mulberry pests.

The results of effect of viral preparations in comparison with *Autographa californica* Speyer are represented in Table 1. Among the tested preparations cabbage white butterfly virus, Virin-diproin, AcMNPV and recombinant AcAaIT [5,6] can be chosen for further investigations. In contrast to chemical insecticides the baculoviral preparations will be possible use for early pest control.

Table 1

Effect of viral preparations on mulberry pyralid (M±m; n=3)

Name of pathogene and its dose/ concentration	Mortality in registration days, %							
	2	3	5	7	9	12	15	18
Virin NS $5x10^6$/larvae	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 $5x10^6$/larvae	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
Pieris brassicae vir. Diluted 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
Vir.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 $4x10^4$/larvae	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 (contr.)	0	0	0	0	3.3±3.3	6.6±3.3	10.0±5.7	13.3±3.3

Results of effect of commercial bacterial preparations and also Acarin, preparation of avermektine nature are represented in Table 2. The preparations had different damage activity.

Entomo-pathogenic nematode preparation, Entonem F had the same effect. Suspension of the preparation 100 times diluted killed mulberry pyralid for 80-100% during 3-4 days after single oral or contact infection. This preparation effects on all postembrional stages of insect development.

Table 2

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

Name of pathogene and its concentration	Mortality in days after infection,%						
	1	3	5	7	9	12	15
Lepidocide 0.025%	3.3±3.3	6.6±3.3	13.3±3.3	13.3±3.3	16.6±3.3	33.3±8.8	43.3±6.6
Bitoxybacillin0,025%	33.3±13.3	80.0±11.5	80.0±11.5	80.0±11.5	80.0±11.5	80.0±11.5	80.0±11.5
Sonit-K 0,025%	0	60.0±10.0	63.3±8.8	63.3±8.8	66.6±8.8	66.6±8.8	70.0±5.7
Bti, granula 0,025%	0	10.0±5.7	23.3±3.3	30	30	30	30
Acarin 0,2%	0	80.0±5.7	100	100	100	100	100
H_2O (control)	0	0	0	0	3.3±3.3	6.6±3.3	10.0±5.7

Extralaboratory preliminary tests of some preparations. Such tests of some preparations were carried out within non-sericultural zones of Tashkent city and Tashkent province. For example, one of middle size mulberry trees at the Institute of Zoology garden was sprayed by bacterial preparation in such a way that treated zones will not be overlapped but remained on solar side of tree, with the same insolation. Infected leaves after spraying and drying were fed once to insects and observed for effect of the preparation. After this test of leaves for pathogenicity else some tests for residual virulence of the preparations were carried out after their exposition to environmental external conditions. These preliminary experiments showed that lepidocide is less toxic and less stable (kept its activity 1.0-1.5 months) and bitoxibacillin is more stable which also totally lost its activity after 1.5-2.0 months.

Entomopathogenic preparation of nematodes has the least stability to environmental factors (2 – 4 days). Similar experiments will be continued.

Use of entomophags [1] and pathogenic parasites in system of integrated pest management against new pest of mulberry tree seems to be perspective. Thus the strategic approach for protection of mulberry tree against dangerous pest – mulberry pyralid will enlarge exploitation possibilities of present-day insect biocontrol means.

The important element of rational and economical pest control is using of present-day technology of application of plant protection means, change outdated equipment which does not allow conduct uniform treatment of linear mulberry plantings, particularly with thick crown of a tree that often is a reason for ineffective use even modern insecticides.

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BioR – A NEW PREPARATION FROM *SPIRULINA* BIOMASS FOR REPRODUCTIVE FUNCTION REGULATION OF SIRE BULLS AND BOARS

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ABSTRACT:
The biotechnological obtaining of the new bio remedies from vegetal, non toxic and ecologically pure sources for the correction of the reproductive function of domestic animals is very important for increasing of the efficiency of the technologies of artificial insemination. The new remedy for the correction of spermatogenesis index of sire bulls and boars has been elaborated. The protocol of *BioR* preparation summarizes the biotechnological and pharmacological multi complex cycle of product obtaining. Is described the original procedures of the *Spirulina* cultivation and industrial procedure of extraction from biomass, separation, fractionation and purification of the bioactive substances and standardization of the obtained preparation for industrial application.
The *BioR* administration induces the increasing of the ejaculate volume (up to 25%), provides an increasing of the concentration and spermatic cell density (up to 20%) and intensifies the sperm mobility (up to 30%) at the sire bulls and boars. Also, *BioR* contributes to an increasing of the stability and viability index of the seminal cells after freezing-thawing in comparison with control.
Keywords: *Spirulina*, biotechnologies, remedy, spermatogenesis regulation, sire bulls, boars.

INTRODUCTION

The program of assuration of the increased requerements of the animal nutritional products obtaining includs the valorification of the genetic performant basis and application of some efficacy technologies of artificial fertilisation with the freezing-thawing or refrigered seminal material. In this context the using of elite reproducers and acceleration of the genetic progress in animals effectiv plays an important role.

The technology of seminal material crioconservation, used in present in zootechny, has a necessity to be improved because up to 40-50% of the seminal cells don't restablish to its biological integrity after thawing, the technological process are influenced by an intensification of the lipids peroxidation and intensive accumulation of the peroxidation products in seminal plasma, which cause the structural-functional dereglations of gamets at diverse levels of organisation.

The using of some natural biopreparations, obtained from spirulina, can serve as an alternatively way for: the spermatogenesis regulation, increasing of the resistance of seminal material in the crioconservation conditions, as well as for the increasing of the efficacity of the artificial fertilisation technology. The prospective of the valorification of these preparations in this field is confirmed by the results of investigations of its bioactive substances with valuable composition and diverse sanogenic properties (in specilal, antioxidant) [ ].

The aim of the investigations is the elaboration of a new remedy for reproductive function correction of sire bulls and boars, using the cyanobacteria *Spirulina platensis* biomass as a source of bioactive substances .

MATERIALS AND METHODS

The strain of the cyanobacteria *Spirulina platensis* CNM-CB-02 from the National Collection of Non-pathogenic Microorganisms of Republic of Moldova of Institute of Microbiology and Biotechnology of Moldova Academy of Sciences, has been used as a source for BioR preparation obtaining. *Spirulina* has been cultivated in the regime of accumulation on SP-1 nutritional medium [10]. Cultivation during 144 hours, respecting the cultivation parameters as following: temperature—30ºC, light intensity—3000 lx during first 72 hours and respectively - 32ºC, 4000 lx in the next 72 hours of the cultivation process.

BioR preparation's physical-chemical properties and composition have been determined using the methods described in Romanian Pharmacopoeia, ed. X, 1998 [4].

The pharmaceutical industrial liquid form – *BioR 0,5% injectable solution,* administrated during 10 days in the doses of 0,1ml and 0,2ml/100kg of body weight, has been used in the experimental investigations. The efficiency of the preparation on the sperm production index at its administration to the sire bulls and boars in the experimental period has been estimated in the relation with the registered index in the pre experimental period. The evaluation of the included in study parameters, ,has been carried out by the laboratory examination of the ejaculate during 10 days of administration and in the following 50 days after stopping of it administration (the period of the spermatogenesis cycle at the bulls) and 40 days (the period of the spermatogenesis cycle at the boars).

The seminal material has been collected by the method of artificial vagina. The bulls have been explored in the regime of 2 days per week, double ejaculates. The seminal material from the reproductive boars has been collected in the regime of one ejaculate at an interval of 4 days. The ejaculate volume (ml) has been determined by the measuring cylinder. The sperms mobility in the ejaculate has been determined visually by microscope IMV with platinum at T +37^{0}C, the magnitude X300, in a drop of 2, 9% Na citrate solution, estimated by the sperms number with rectilinear movement forward from total number of present in the visually space and evaluated in balls in conformity with qualimetric scale of 10 balls. 1 ball = 10% of sperms with rectilinear movement forward. The sperms concentration (billions sperms/ml of the sperm) has been determined by the electronic photometry IMV.

OBTAINED REZULTS

The BioR preparation is a preparation obtained by the elaboration of some biotechnological innovative models including cyanobacteria *Spirulina platensis* cultivation, methods and procedures of extraction, fractionation and purification of bioactive principles. The stages of technology of this preparation obtaining represent an complex technological and pharmaceutical industrial cycle including: the cultivation of the cyanobacteria *Spirulina platensis*, procedures of bioactive substances extraction (from biomass), separation and purification of BioR (active substances) as well as its industrial pharmaceutical forms obtaining and standardization (Fig.1) .

BioR preparation contains such bioactive substances as: amino acids and oligopeptides, intermediary products of carbohydrates and lipids metabolism, macro- and essential microelements. They poses cytoprotector action by cell and lysosome membranes stabilization: through glutathione exchange normalization and maintenance of optimum balance between lipid peroxidation and antioxidant systems (reduction of quantity of free oxygen radicals, dienic conjugates, malonic aldehide), and also the antioxidant potential increasing, including enzymatic and non enzymatic componentes (increasing of superoxid dismutase, catalase, glutathione enzymes level, normalization of reduced gluthatione level, tocopherol level, SH-groups level, etc.). Preparation's aminoacides, oligopeptides and the microelements Mn, Fe, Zn, Cu, Se, Cr etc., stimulate tissues regeneration processes and also stimulate the cellular and humoral immunity.

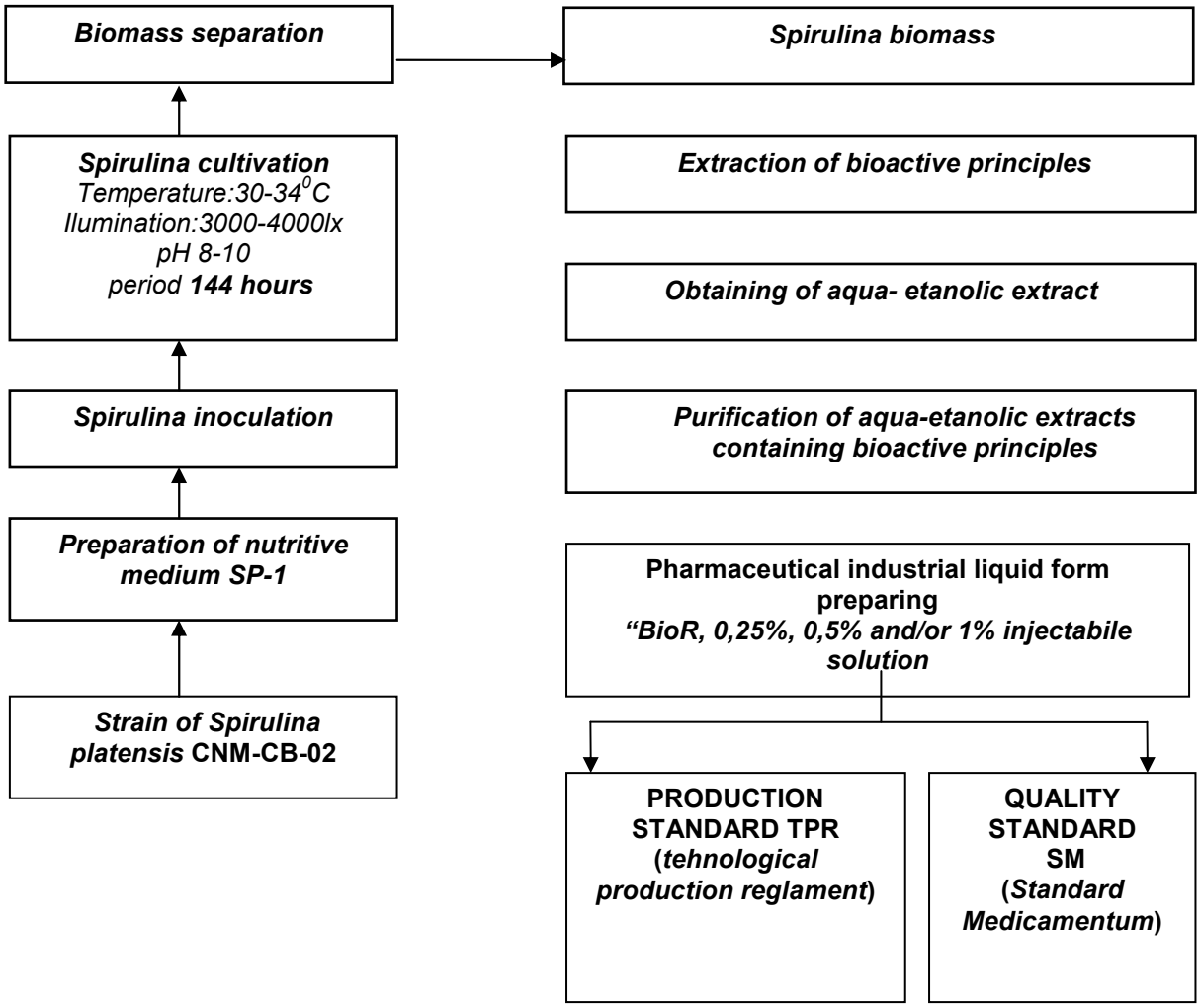

Fig.1. Integrated scheme of BioR preparation production.

The action of BioR on quantitative and qualitative indexes of spermatogenesis has been investigated for elaboration of the methods of the correction of reproductive functions of sire bulls and boars reproducers.

The results of BioR administration to the sire bulls in the doses of 0,1-0,2 ml/100kg of body weight demonstrate the positive effects of the preparation on the essential characteristics of the ejaculate: increases the seminal material concentration (with up to 28-31%) and mobility (up to 10%) (Fig.2). The increasing of criologic index of seminal material, such as longevity up to 133%, mobility up to 122% and absolute index of surviving up to 134% after decongelation of the seminal cells has been detected (Fig. 3).

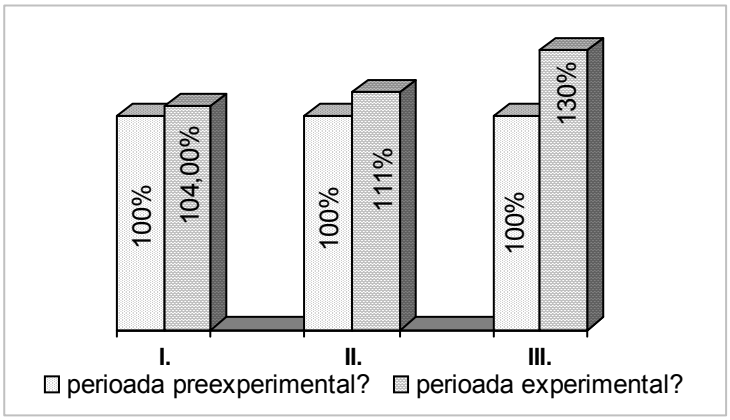

Fig. 2. Usual spermogram indexes of sire bulls in the case of administration BioR preparation (I.- ejaculate volume II.- seminal cell conc..; III.- spermatic cell mobility).

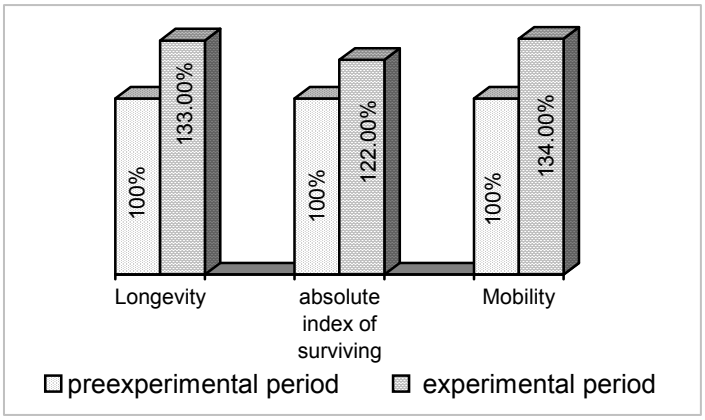

Fig. 3. Criobilogic indexes of sire bulls in the case of administration BioR preparation .

The administration of the BioR preparation, 0,5% injectabile solution to boars in the doses of 0,1-0,2ml/100 kg body weight contributes to the progresion of spermatic mobility (up to 11%), increasing of ejaculate volume (up to 25,14%) and assures the enhansing of concentration (up to 20%) as well as density of the spermatic cells.

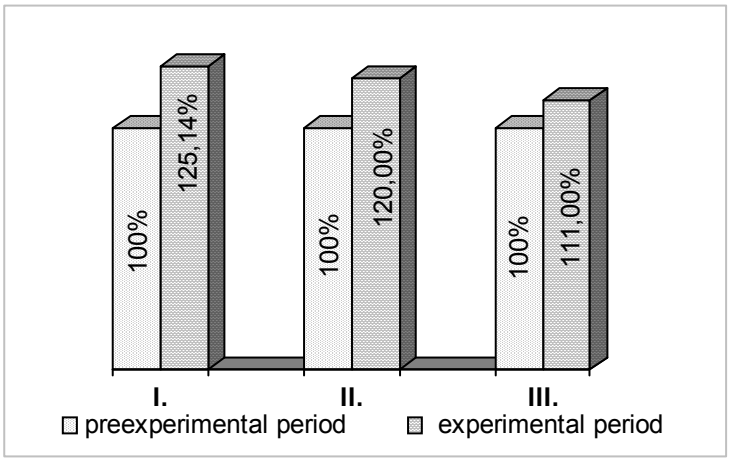

Fig. 4. Usual spermogram indexes of boars in the case of administration BioR preparation (I.ejaculate volume II.- seminal cell conc..; III.- spermatic cell mobility).

Thus, BioR it's an preparation obtained by inovative tehnology with the exploration and production of an accessible vegetal source of bioactive substances and can be included in the therapeutic profilactic scheme of the zootechny branch in the aim to increase the quantitative and qualitative indexes of sire bulls and boars spermatogenesis.

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NEW REMEDIES FOR BEES

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ABSTRACT:
The new generation sanogenic products for apiculture - *Apispir* and *BioR*^{Sp} from *Spirulina* biomass exploration biotechnologies were developed.
Apispir is the feed additive which contains proteins, including essential and immunoactive amino acids, as well as polysaccharides complex, macro- and microelements.
BioR^{SP} is the remedy with cytoprotector action on cell and liposome membranes stabilization: through glutathione level normalization and maintenance of optimum balance between lipid per oxidation and antioxidant systems, through the increasing of antioxidant potential, including enzymatic and non enzymatic components, normalization of reduced glutathione, tocopherol and SH-groups level, etc. The remedies have the regenerative, immunomodulating, anti-inflammatory and anti- viral activities.
The new apiculture products are the advantages:

- Sanogen, natural and ecologically unpolluted products;
- Stimulate growth, reproduction and pollination effect of bees;
- Increase the immune status of bees and the resistance to the incidents of CCD (Colony Collapse Disorder).

Keywords*: Spirulina*, biotechnologies, feed additive, remedy, bees.

INTRODUCTION

The apiculture is an important branch of national economy in the aspect of crops pollination (more than 90) and agriculture products obtaining.

The recent years the apiculture suffers because of the big lost of production, caused by the infectious diseases that contribute to the diminishing of fertility and productivity, increasing of the rate mortality of bees. There are the most widely spread infectious diseases: european and american foulbrood, septicemia, mycosis and virosis which conduct to the bees death [7,11].

At the beginning of 2006 the more actual problem remaining without solutions continues to be CCD - Colony Collapse Disorder, an anomaly that attains the accidental proportions, the decreasing of bees family number from 2,4 mln to 900 thousands was registered in USA, disappearing of 10 mil. in Taiwan, – with 25% in Germania, and in any cases the lost was to 80% [1,3-6].

Diverse feed additives are used in bees nutrition for the maintaining of their families and for the productivity increasing [7,10]. The products are expensive, contain the synthetic amino acids and can be rapidly exposed to the oxidation of proteic and lipidic components that cause bees diarrhea after its consummation and affect the honey quality.

The antibiotics, antifungicides and antiviral drugs are used in the eradication of families' bees' infections in the exceptional cases. There are some disadvantages of these remedies: synthetic nature, the possibility of accumulation in the honey and the danger to penetrate into the human body, the less effects and the high cost [7].

Thus the main principles of application of technological procedures in the apiculture include the elaboration of the new methods for ensuring and promotion of vitality and bees families health, prevention of appearance and increasing of bees families resistance against infection.

The aim of the investigation was the elaboration of some new sanogenic products for apiculture by biotechnological exploration of cyanobacteria *Spirulina platensis* biomass.

MATHERIAL AND METHODS

The strain of cyanobacteria *Spirulina platensis* CNM-CB-02, from National Collection of Non-Pathogenic Microorganisms of Republic of Moldova of Institute of Microbiology and Biotechnology of Moldova Academy of Sciences, is used as source for obtaining of *BioR*^{Sp} preparation and feed additive *Apispir*. *Spirulina* was cultivated in the regime of accumulation, on SP-1 nutritional medium [8]. Cultivation during 144 hours, respecting the cultivation parameters as following: temperature—30ºC, light intensity—3000 lx during first 72 hours and respectively - 35ºC, 4000 lx in the next 72 hours of the cultivation process.

The physico-chemical properties and composition of *BioR*^{Sp} preparation and feed additive Apispir were determined by the methods, described in [2,8].

In the experimental investigations *BioR*^{Sp} preparation and feed additive *Apispir* were used conform the scheme:

0,5 ml *BioR*^{Sp} preparation (10mg dry substance/ml) have been added to 500ml syrup of sucrose (1:1) and used as supplement for autumn bees families nutrition.

1-2ml *Apispir* (10mg dry substance/ml) feed additive have been added to 1000ml syrup of sucrose (1:1) and used as supplement for spring bees families nutrition.

OBTAINED RESULTS

The methodology and strategy of our investigations allow to valorificate the spirulina biomass for obtaining of two sanogenic products for apiculture – feed additive *Apispir* and *BioR*^{Sp} *preparation* (Fig.1).

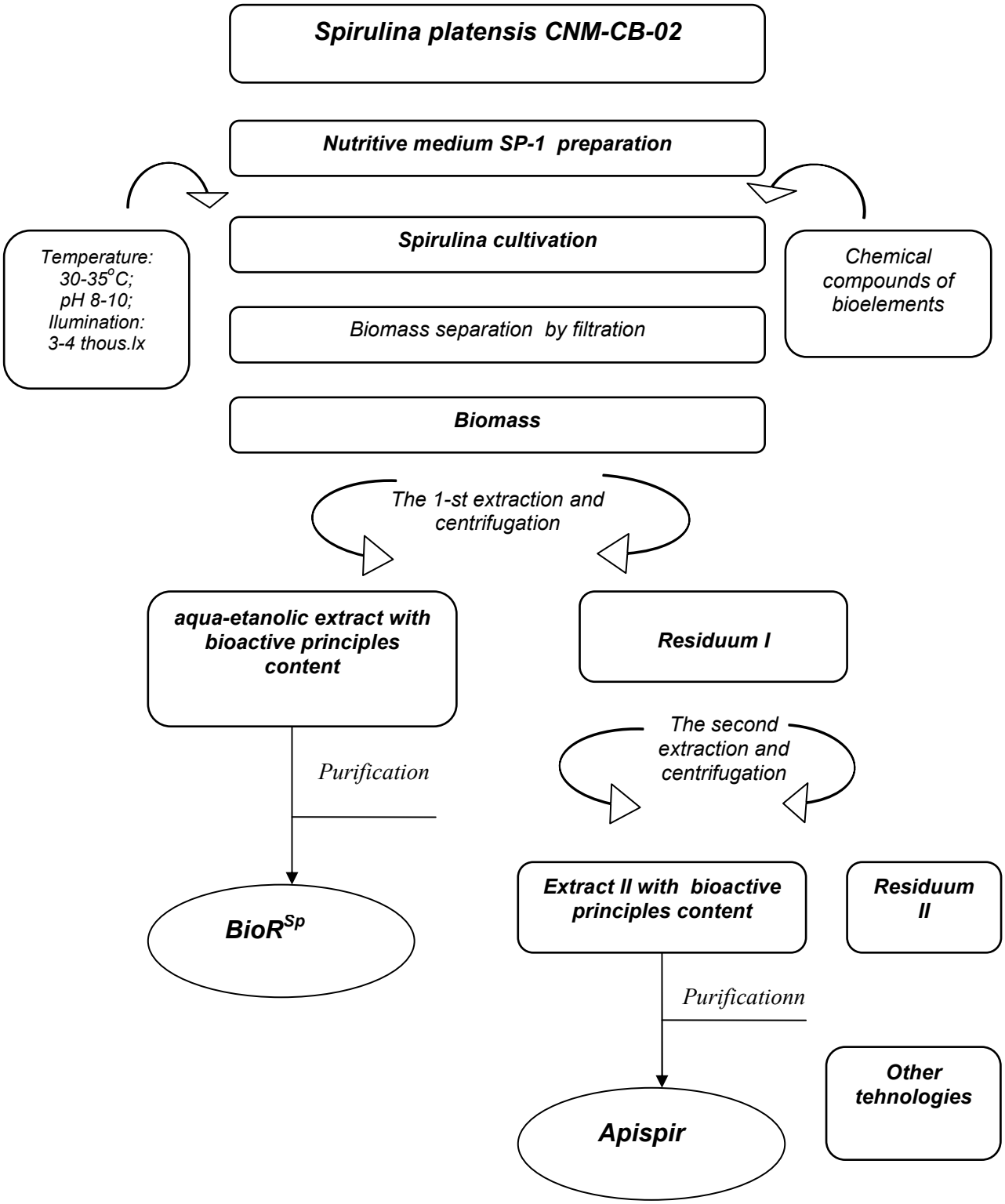

Fig. 1. Integrate tehnological scheme of *BioR*^{Sp} preparation and feed additive *Apispir* obtaining.

BioR^{Sp} preparation contains the low molecular mass substances such as: free amino acids and oligopeptides (up to 10kDa), carotenoids, pheophytin, intermediary compounds of carbohydrates and lipids metabolism, macro- and antioxidant microelements. The preparation demonstrates the antioxidant properties and the capacity to stabilize the cellular and lissome membranes by the normalization of glutathione metabolism. The presence of amino acids and

oligopeptides, as well as such microelements as Mn, Fe, Zn, Cu, Se, Cr stimulates the process of regeneration of tissues and has a positive effect on cellular and humoral immunity.

APISPIR contains the proteins, carbohydrates (including sulfated polysaccharides), microelements. The action mechanism of this product can be attributed to the properties of the including substances. The proteic composition offers the total essential amino acids content with a high efficiency (up to 87%) and utilization (92%). There are the following amino acids with immunostimulative and antioxidant action): aspartic and glutamic acids, alanine, cysteine, glycine and serine, threonine, thriptophan and valine.

The utilization of *BioR*^{Sp} preparation during autumn nutrition for honey reserves supplementing for bees wintering, provides an activation of physiological and biochemical processes in the organisms of working bees (Tab.1).

Table 1. Dinamics of some biochemical indices on bees after *BioR*^{Sp} administration in autumn (in %).

Variant	Biochemical indices					
	ALT	**AST**	**LDH**	**Al. Ph**	**A. Ph**	**T.Pr.**
Before supplem.	100,0	100,0	100,0	100,0	100,0	100,0
Control (pure syrup)	97,8	100,0	102,1	71,6	23,2	77,5
BioR^{Sp}	126,1	200,0	174,0	93,1	57,1	102,6

***ALT** – alaninaminotransferase (mmol/h.l); **AST** – aspartataminotranspherase (mmol/h.l); **LDH** – lactatdehydrogenase (mmol/s.l); **Al. Ph.** – alkaline phosphatase (nmol/s.l); **A. Ph.** – acide phosphatase (nmol/s.l); **T.Pr**. – total proteins.*

The active components of preparation increased the physiologic capacities of working bees. The bees' families were in good conditions after wintering, without some signs of diarrhea of bees or nutrition oxidation and fermentation.

The utilization of *Apispir* as feed additive for spring nutrition has stimulated the cowering spawn of queen, increasing of growing of the new generation and the quantity of collected honey. The remedy was consumed by the bees, increasing up to 18-29% the number of young generation, the honey quantity, collected after acacia pollination, up to 22-53% (Fig.2).

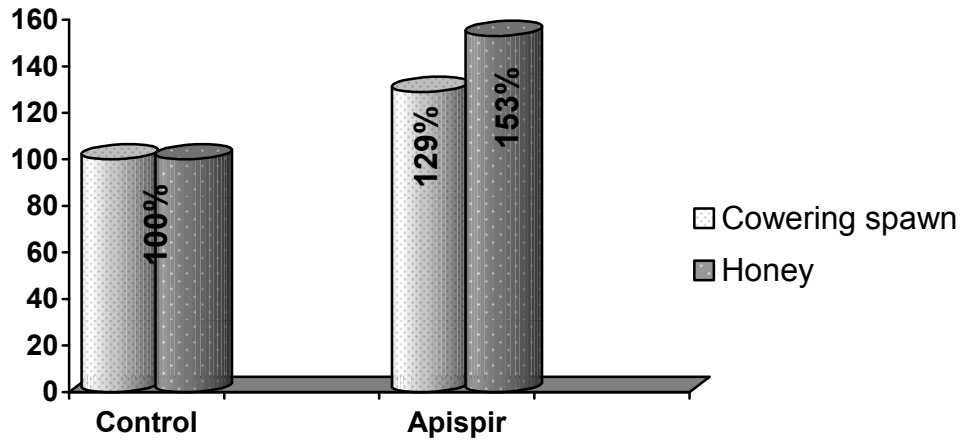

Fig.3. The cowering spawn and honey quantity obtained after *APISPIR* in spring administration.

In conclusion, *APISPIR* and *BioR*^{Sp} represent the new sanogenic, ecological products, without adverse effects with high efficacy used in less quantity by their production technology and bioactive components. The remedies can be used in the apiculture for stimulation of bees families growing, increasing of apiculture products quantity, enhancing of bees immune status, eradication of CCD (Colony Collapse Disorder).

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INFLUENCE CAVITATION ON THE WOOD SAWDUST FLUIDIZED IN WATER

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ABSTRACT:
In the given work influence pre-cavitational and cavitational modes of current on the large in water large and fine wood sawdust has been investigated. Also the comparative analysis of the basic components included in wood is carried out. The received data testified to change of percentage of cellulose and lignine in wood sawdust.
Besides research pH distilled water and solutions in pre-cavitational and cavitational modes of current which spent in comparison with their usual heating is submitted. Synthesizing peroxide of hydrogen in a pre-cavitational mode of current with small concentration resulted in saturation of water by electrons, and with artificial increase of concentration of H_2O_2 the charging condition became close to neutral. It has been received, that than above concentration of an oxidizer, water is especially structured. On the basis of it assumptions have been made, that depending on a ratio synthesized peroxide of hydrogen in pre-cavitational process and artificial addition there was a various mechanism of formation of ion-radicals.
Keywords: cavitation, water, cellulose, lignine.

INTRODUCTION

As is known high gradients of velocity in water result in chemical reorganization of molecules of water, for example, composition of hydrogen peroxide. It is necessary to add, that at high gradients of velocity can arise cavitational the phenomena which result in the even greater molecular reorganization [1-6].

MATERIALS AND METHODS

In this work influence of the cavitation on large-sized and small-sized wood sawdust in water has been investigated. In a experiment made measurement of temperature of water with sawdust, and then there was a selection of sawdust and their chemical analysis was made. In result the following data submitted in tables 1 and 2 have been received.

Table 1. Structure of the basic components of wood of a pine before and after cavitational processings in the neutral medium (small-sized sawdust)

Sample	Cellulose	Lignine
Initial wood	47,2	33,7
Wood with the cavitation (during experiment)	49,0	29,9
Wood with the cavitation (after experiment)	50,9	29,0

Table 2. Structure of the basic components of wood of a pine before and after cavitational treatment in the neutral medium (large-sized sawdust)

Sample	Cellulose	Lignine
Initial wood	53,0	30,8
Wood with the cavitation (through 15 minutes)	48,2	36,8
Wood with the cavitation (through 30 minutes)	48,8	37,2
Wood with the cavitation (through 45 minutes)	48,7	37,6

The experiments on research pre-cavitational and cavitational modes of current of a liquid were spent with the help of two centrifugal pumps with electric motor "S" (2 kw) and with more powerful electric motor "L" (> 10 kw). As researched liquids used water with the microflora, distilled water, and various solutions: 1% and 0,01% solutions hydrogen peroxide and 0,1 %-ный a solution of a sulfuric acid.

As a cavitator, creating a high gradient of velocity of a liquid, various nozzles were used. In nozzles dense chaotic packed beds from balls in diameter of 0,01 m and 0,02 m were used. So, in a nozzle executed as a cylindrical metal tube of long 0,2 m and internal diameter of 0,05 m, porcelain balls in diameter of 0,01 m were fallen asleep. And in a nozzle long 0,67 m and internal diameter 0,045 m, were fallen asleep 30 % of porcelain balls in diameter of 0,01 m and 70 % glass - by diameter of 0,02 m. Average value of velocity of stream V_o in the empty channel of a hydrodynamical contour with a nozzle and motors "S" and "L" made 1 m/s and 4 m/s. Corresponding Reynolds's number for a nozzle from packed beds the porcelain balls, determined on radius of a ball r = 0,005 m:

$$\mathrm{Re} = 2r \cdot V_o / \nu \cong 10^4, \quad (1)$$

Where $\nu = 10^{-6}$ – viscosity of water, V_o = 1 m/s. At such numbers Re a mode of current is turbulent and in the empty channel, and in nozzles with balls, therefore velocity of a liquid is practically identical on section of packed beds. The gradient of velocity inside packed beds from balls can be estimated from formula [7]:

$$dV/dr \cong V_o /(\varepsilon r_e), \quad (2)$$

Where ε = 0,4 - porosity of chaotic packing, $r_e = (2/3)\varepsilon r/(1-\varepsilon)$ = 0,44 r - equivalent radius of pore spaces.

Substituting in (2) numerical values of average velocity for different motors and diameter of the ball equal of 0,01 m, we shall receive for motor "S": $dV/dr \cong 10^3$. For motor "L" gradient of velocity will be approximately in 4 times more. We shall find number of the cavitation χ for our case, considering, that cavitation has arisen on last rows of packed beds from balls:

$$\chi = 2(p - p_1)/(\rho v^2), \quad (3)$$

where $p = p_o/19$ - the current pressure in packed beds, having approximately 20 rows of balls with radius by 0,5 cm, p_o - pressure of the pump before an input in packed beds (for motor "S" equal $2{,}6 \cdot 10^5$ Pa, for motor "L" – $7 \cdot 10^5$ Pa. Difference of pressure after packed beds it is neglected as this superfluous pressure did not exceed 20 cm of a water column), p_1 – is a pressure of sated vapours which following work [5] can be put close to the minimal pressure at which arises cavitation. The cavitation at work of centrifugal pumps arose approximately from temperature 50 °C, that it was possible to estimate from a level of noise influences. Pressure of sated vapours waters at temperature 50 °C is equal $0{,}12 \cdot 10^5$ Pa. Then corresponding numbers of the cavitation χ_{S50} and χ_{L50} for the pump with motor "S" and "L" of a formula (3) will be equal

$$\chi_{S50} = 3{,}4 \text{ and } \chi_{L50} = 3{,}1 \quad (4)$$

During experiment on research of current of a liquid in the closed hydrodynamical contour in pre-cavitational and cavitational modes every five minutes made measurements of temperature and consumed electric energy, and through each five degrees selected tests of water and solutions for definition pH and density.

RESULTS AND DISCUSSION

Measurement of density of water and solutions has shown, that with increase in temperature the density of liquids on the average monotonously decreased. However for 1% solution hydrogen peroxide at heating by the pump in an interval of temperatures 55-70 °C and for 0,1% solution of a sulfuric acid in a close interval of temperatures 50-65 °C there was a local increase in density of a liquid with rise in temperature. Such behavior is probably associated with cluster changes of structure of water owing to the various sizes of complexes with hydrogen peroxide and anions of a sulfuric acid (sulfate and hydrosulfate-ions) in this range of

temperatures. Otherwise, at heating by the pump in this range of temperatures there is a reorganization of structure of molecular chains to more dense to the packed segments. We shall notice, that this reorganization occurs just in that range of temperatures where it is considered to be cavitational influence by the most effective.

Research pH distilled water and solutions in pre-cavitational and cavitational modes of current spent in comparison with their usual heating («idle» experiment). At usual heating distilled water with rise in temperature pH insignificantly increased (with 5,5 up to 6,3), that probably corresponds to result of autoprotolyse at this temperature. Theoretical estimations predict the opposite tendency of change pH from temperature, but it is suit only special conditions of preparation of distillate and its further heating [8]. In conditions of heating of a distilled water with the help of the pump as has shown experiment, in the beginning there was a sharp jump pH of a medium in alkalescent area, and then small rectilinear growth of a hydrogen parameter to increase in temperature. And change pH increased almost in 2 times and it occured for shorter time interval, than at «idle» experiment of heating. Similar dependence pH at heating by the pump has been received in work [6], without an explanation of similar change of acidity. In figure 1 schedules of change pH of 0,01 % solution hydrogen peroxide, 1 % solution hydrogen peroxide and 0,1 % solution of a sulfuric acid are submitted depending on their usual heating («idle» experiment) and heating with the help of the pump.

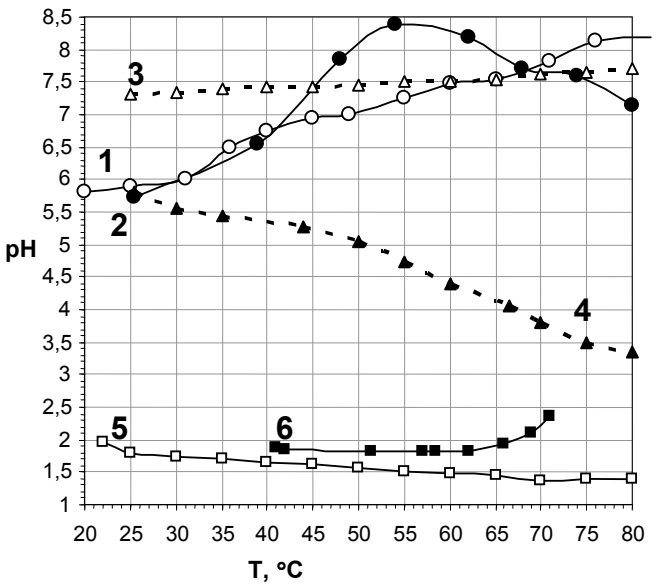

Fig. 1. pH of 0,01% and 1% hydrogen peroxide solution, and 0,1% sulphuric acid solution at the heating and its heating with the help of the cavitator.

1 - 0,01% solution H_2O_2 ("idle" experiment)

2 - 0,01% solution H_2O_2 at cavitational heating with a nozzle

3 - 1% solution H_2O_2 ("idle" experiment)

4 - 1% solution H_2O_2 at cavitational heating with a nozzle

5 - 0.1% solution H_2SO_4 ("idle" experiment)

6 - 0.1% solution H_2SO_4 at cavitational heating with a nozzle

From the received data it is visible, that in process of the cavitation the important factor is not only temperature, but also concentration of hydrogen peroxide. At usual heating pH of 0,01% and 1% solutions of hydrogen peroxide is displaced in the alkalescent medium (pH 7,5-8,2). At heating liquids by the pump with motor "L" law of a hydrogen parameter sharply changes: at high concentration of hydrogen peroxide pH sharply decreases (up to pH = 3,5) (line 4 at fig. 1), and at low concentration - pH increases and has a maximum in the alkaline medium at 55 °C (line 2 at fig. 1). These changes, most likely, are corresponded to the beginning intensive cavitational processes and the mechanism of composition and decomposition of hydrogen peroxide in pre-cavitational and cavitational modes.

The contents in water of 0,1% solution of a sulfuric acid also changed character of change of pH. At usual heating («idle» experiment) there was a downturn pH of medium up to 1,4 (line 5 at fig. 1). And at heating with the help of the pump pH practically did not vary (pH 1,8) up to temperature 60 °C, and then grew up to pH 2,4 (line 6 at fig. 1). After 60 °C active reorganization of molecular structure of water began and there was a partial neutralization of

ions of hydrogen at the expense of generation of hydroxide-ions owing to the beginning of the cavitation.

The cavitation is good way of mixing of immiscible liquids. The solution of permanganate of kalium and oils rape, mixed with the help of the cavitation, stood during one year and was not stratified as against a solution mixed in the usual way. In the same way it was possible to receive not stratified emulsion of turpentine in water. Influence of the cavitation on emulsions, most likely, results in composition of steady complexes from organic and inorganic liquids. It is possible to be convinced if to estimate by a photocolorimeter received emulsions. From comparison of results of these measurements it is possible to conclude, that the mix after cavitation remains more optically dense, that, probably, is corresponded to its greater stability. And though in due course the optical density decreases, it occurs more slowly, than in a usual mix. The found out law is confirmed also with measurement pH emulsion of turpentine in water up to the cavitation and after 15 minutes of the cavitation.

CONCLUSIONS

1. The cavitational influence on wood sawdust has led to raised contents of a lignine, that takes an opportunity of creation of wood particle board without formaldegyde pitches.

2. The cavitational influence on various liquids have allowed to receive homogeneous emulsion of oil and water, and also to achieve partial mixture of organic and inorganic substances.

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THE ROMANIAN RESEARCHES REGARDING DEVELOPMENT OF SERICULTURE'S BIOPRODUCTS

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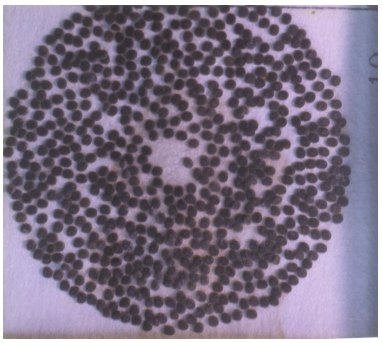
ABSTRACT
In Romania, there are nowadays, research scientifical acitivities concerning development in a profitable way of sericulture waste and by products. As a result of interdisciplinary collaboration between C.S. SERICAROM and different national research instituts (Institut of Food Chemistry, National Institut for Textile and Leather, Research National Institut for Chemistry and Farmacology), there were obtained new commercial products for the local and international market (embrional extract from silkworm eggs, cosmetic cream with pupae oil). During 1990 – 2008, there were obtained three patents, under the licence of C.S. SERICAROM and C.S. SERICAROM Research Department.
Keywords: sericulture, by products, patent

The main final sericulture product is the natural silk fiber. The silk cocoon production is the most important source for sericulture farmers and companies' income. The sericulture involves a large scale of interdependent technologies from which results different by products and waste. These ones may be turned into new commercial products with a high useful value.



The usual by products and waste, resulted out of sericulture activy are:
- silkworm's eggs processed into proteic extract used in the pharmaceutical industry with hepatoprotean action, and also in the food industry;

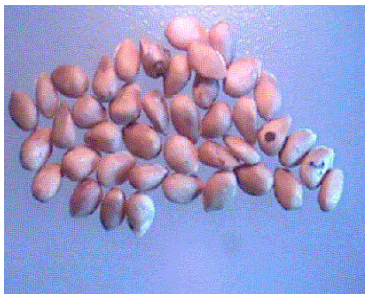
- silkworm larvae from different evolution stages, used as total proteic extract in the pharmaceutical industry (antidiabetic action) or in the food industry;

- silkworm pupae, with high fat content - over 30%, are used as chr ysalis oil to obtain cosmetic products, soap and as proteic powder for valuable fish fooder;

- silkworms rearing waste, compound of excreta, mulberry leaves waste and larvae exuvia, are used as chlorophyl paste in the pharmaceutical industry and cosmetics in order to obtain carotenoides, phytol or alcoholic fractions;

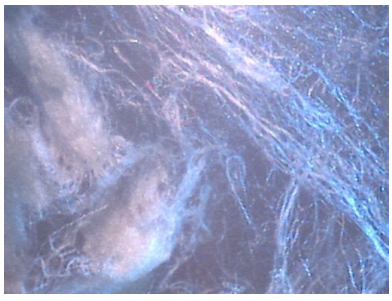
- mulberry leaves are used fresh or dryed, as antidiabetic tea and as a pouder proteic pulvis are used to obtain diverse food products (sweet products, bread, refresment juices, natural tinctorial additive;

- mulberry fruits are used fresh, dryed or frozen in the food industry to obtain different sirups, tonic wine, ameretto or vermouth wine, vinegard and different sweet products (mamelade, chocolate, frosting, jelly and fondant). Mulberry fruit juice it is also used as natural tinctorial additive for food and pharmaceutical industries;



- mulberry root biomass is a valuable raw material for the pharmaceutical industry, due its high flavone and fenol content;

- by products and waste resulted in the silk cocoons proceeding may be used in textile, leather, pharmaceutical, cosmetics and food industies as proteic, precipitant jellies, atomizated or liophylized



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(embrional extract from silkworm eggs, cosmetic cream with pupae oil). During 1990 – 2008, there were obtained three patents, under the licence of C.S. SERICAROM and C.S. SERICAROM Research Department:

- patent nr. A61K35/56/106842/1991 titled Biomorus (Stimulating compozition with antidiabetic function)
- patent nr. B1-111905/1997 , named Humanofort B. It is a natural biostimulant, having a catablolizing and rebalancing effect of endocrine functions on consumer.
- patent nr. 120747/2007, named “Mulberry dryed fruits powder - obtaining procedure and its utilisation”. It’s a natural raw material for sweet products industry

In Romania, there were also obtained and implemented other paternts to develop by products and sericulture waste, such as “Crisalys oil and obtaining procedure”, licenced under C.S. BIOTECHNOS S.A. Bucharest, patent nr. 110202/A61K35/64/1997.

Insect Farm Company produces and sells medicaments, based on proteic extract from live pupae – Bombyx mori.

In the present, there are examinate for approval, patent applications concerning alcoholic extract from mulberry root biomass and fooder receipt for pisciculture based on crisalys and mulberry leaves.

Out of secondary ingredients obtained from sericulture waste, shall be developed new commercial products, with positive effects regarding sericulture’ rentabilisation.

This field shall be an important development opportunity for research and technological improvement, with a valuable economical unlimited impact.

NEW NUTRACEUTICALS FROM SPIRULINA

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ABSTRACT:
It is known the significance of the bioelements (iron, zinc, chromium, selenium, iodine etc.) for human body. The fluctuations of these elements provoke such diseases as anemia, diabetes, osteoporosis, prostate cancer, affections of thyroid gland, etc.
One of the solutions to this problem – is to make a diet of human optimally containing bioactive ingredients as nutraceutical natural complexes satisfying needs of the man for essential substances, such as zinc, chromium, selenium, iron, and some other vitamins, proteins, polysaccharides, amino acids, polyunsaturated fatty acids etc.
Our field of interest for *Spirulina* is connected on it exploration and elaboration of the new technologies of obtaining of alternative natural nutraceuticals, containing bioelements and other valuable substances with high therapeutic effects.
The levels of accumulated elements in biomass at the *Spirulina* cultivation have been studied and the optimal cultivation conditions have been established. Distribution of some metals in diverse fractions of biomass components have been demonstrated too.
The new nutraceuticals for human: *„SpiruZinc"* (as imunomodulating effect), *„SpiruFier"* (as anti anemic effect)*, „SpiruSelen"* (as anti cancer effect) and *„SpiruCrom"* (as anti diabetic effect) obtaining from *Spirulina* biomass, were developed.
Keywords*: Spirulina*, biotechnologies, nutraceuticals.

INTRODUCTION

There are many cumulative causes that contribute to the increasing of incidence of some diseases and the decreasing of immune function of human body, as well as the evident metabolic deregulations. There are some of them: the intensity of modern mode of life, stress, malnutrition, less quality of food, incorrect nutrition, interaction between the toxic exogenous and endogen factors and agents. Microelements have an important role in these processes. Their quantities over or below the optimal level unleash some diseases and influence their pathogenesis [1-3; 13-15].

More than 20 years *Spirulina* is explored as food supplement. World Health Organization and the 3-rd international Congress of Science and Food Technology defined spirulina as an essential non-conventional source of up to 50 bioactive substances, that assures the normal unfolding of vital processes in the human and animal body. The spirulina contains such bioactive substances as: phycobiliproteins, sulfated polysaccharides, carotenoids (in special β–caroten), vitamins (α-tocoferol, ascorbic acid), immunoactive amino acids, gama-linolenic acid, enzymes, including superoxid dismutase, peroxidase, catalase and some microelements with imunomodulator, antiradicalic and antioxidative activity [4;6;8-10].

The products on the basis of spirulina biomass, obtained by innovative biotechnologies of spirulina cultivation, are characterized by the prognosticated and valuable content of bioactive compounds and essential bioconverted microelements with sanogenic effects such as: iron, zinc, selenium and chromium. That way, the possibility of the polyvalent therapeutic effect of spirulina bioadditives can be explored in the diverse clinical situations.

Aim of investigations: Elaboration of some technologies of the new nutraceuticals obtaining in the basis of spirulina biomass with the prognosticated content of bioactive compounds and bioelements

MATERIALS AND METHODS

The object of study: the strain of cyanobacteria *Spirulina platensis* CNM CB-02. The culture of *Spirulina* has been inoculated in Zarrouk modified nutritive medium [7]. The *Spirulina* cultivation has been effectuated in Erlenmeyer retorts (which 0,25L of suspension), during 144 hours, respecting the cultivation parameters as follows: temperature - 30ºC, light intensity - 3000 lx during first 72 hours and respectively - 32ºC, 4000 lx in the next 72 hours of the cultivation process.

One of some coordination compounds of Fe(III), Zn(II) and Cr(III) have been used as supplement to nutritive medium in 2 portions on the first and third day of cultivation: [Fe_3O-Gly] and [$Zn(CH_2Cl)COO_2 \cdot 4H_2O$ in the concentrations of 30-50mg/l (½+½); $Zn(HSeO_3)_2 \bullet H_2O$ and $[K_2Cr_2(SO_4)_4] \bullet 12H_2O$ in the concentration 30mg/l (½+½).

The physico-chemical properties, bioactive substances and bioelements contents in the elaborated products have been determined by the procedures described before [5;12].

OBTAINED RESULTS

The technologies of production of some nutraceuticals represent the final result of the ample biotechnological investigations of cyanobacteria *Spirulina platensis* exploration, including:

- ✓ Study of the conditions and parameters of process of accumulation and distribution of the bioelements: zinc, iron, selenium and chromium in the bioactive components of spirulina biomass, obtained at the cultivation in presence of coordinative compounds or salts of this bioelements;
- ✓ Elaboration of the procedures of biomass production with prognosticated content of bioactive substances and bioelements at spirulina cultivation in the conditions of control.
- ✓ Elaboration of the integrate technological schemes of nutraceuticals industrial production (spirulina cultivation in presence of zinc, iron, selenium and chromium compounds), biomass with prognosticated content of bioactive principles and bioelements production, obtaining and biomass powder formation, quality standardization , etc.) (Fig.1).

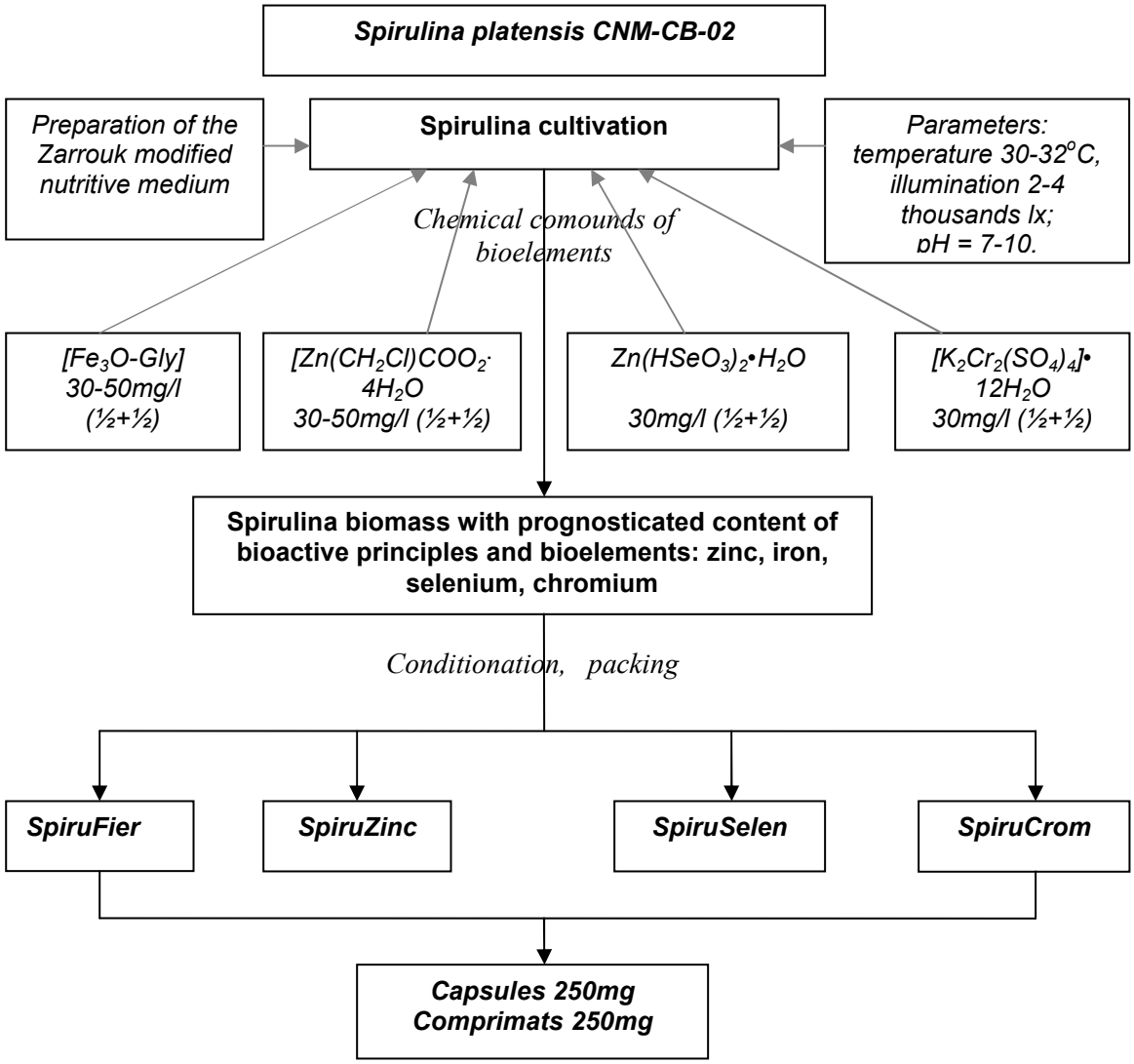

Fig.1. Integrate technological scheme of the nutraceuticals production at the spirulina cultivation in the conditions of control.

Such, application of biotechnological innovative methods for spirulina cultivation in conditions of control the new nutraceuticals have been elaborated: *SpiruZinc* (on basis of spirulina biomass with prognosticated content of bioactive principles and bioconverted zinc); *SpiruFier* (from spirulina biomass with prognosticated content of bioactive principles and bioconverted iron); *SpiruSelen* (on basis of spirulina biomass with prognosticated content of bioactive principles and bioconverted selenium) and *SpiruCrom* (from spirulina biomass with prognosticated content of bioactive principles and bioconverted chromium).

SpiruFier, capsules or comprimats 500mg contain PROTEINS (58,9-68,0%), including oligopeptides up to 10kDa (29,4-41%) ; FREE AMINO ACIDS (4,8-5,1%); CARBOHYDRATS (10,9-12,2%) LIPIDS (>5,0%) ; PHYCOBILIPROTEINS (12-14,2%) - 6,5-8,7% of C- phycocyanin and 3,5-5% of alophycocyanin; IRON (1,0-1,15%). The product can be used for the immune system fortification and completion of iron deficiency in the case of irondeficient anemie and other diseases (which clinical situation is associated with irondeficient anemie).

SpiruZinc, capsules or comprimats 500mg contain *PROTEINS* (61,0-68,0%) : Valuable compounds with nutraceutical effect, the content of immunoactive and antioxidant amino acids (up to

80% from total amino acids) is more relevant: aspartic and glutamic acids, alanine şi cysteine, glycine and serine, threonine, triptophan and valine; CARBOHIDRATS (10,9-12,2%), the substances with immunostimulative, antiviral and anti-inflammatory effect; LIPIDS (5,46-7,05%), in special : GAMA-LINOLENIC ACID (1,1 -1,4%) an essential acid, precursor of eicosotrienic acid ($C_{20}\Delta^{8,11,14}$), which takes part in the formation of the prostaglandin PGE_1 –the product used in immunology last years. The arahidonic acid ($C_{20}\Delta^{5,8,11,14}$) is important as precursor in the synthesis of prostaglandins PGG_2, PGE_2 şi $PGF_{2\alpha}$; PHYICOBILIPROTEINS (9-10,9%), are the compounds with antioxidant and immunostimulative action, in special C- phycocyanin (4,9-5,9% from biomass); ZINC (0,3-0,4%) - an essential bioelement with antioxidant and immunostimulative properties. SpiruZinc can be used for immune function correction in the case of some desease of diverse etiology (viral hepatites, lung tuberculosis, cancer, etc.).

SpiruSelen, capsules or comprimats 500mg contain: PROTEINS (>67%); FREE AMINOACIDS (>5,8%); CARBOHIDRATS (>17,3%); LIPIDS (>4,7%); PHYICOBILIPROTEINS (>14,8%), including >9% C- phycocyanin and >5,8% alophycocyanin; B-CAROTEN (0,26%); SELENIUM (0,027%). The products has the hepatoprotective and immnumostimulative proprieties, and can be used in the treatment of pathological state caused by the free radicals accumulation and chronic viral hepatites type B and C, mixt-hepatites (C and B, B and D, C and herpes virus), hepatic liver cirrhosis, some forms of cancer and other infections, as well as body detoxification from heavy metals, improving of the male and female fertility.

SpiruCrom, capsules or comprimats 500mg contain PROTEINS (>68%); FREE AMINOACIDS (>3,8%) ; CARBOHIDRATS (>20%) ; LIPIDS (>5%) ; PHYICOBILIPROTEINS (>14,1%), including >8,3% C- phycocyanin and >5,8% alophycocyanin; CHROMIUM (0,3%). The product can be used in the treatment of carbohydrates and lipids metabolism deregulation, in the case of the diabetes mellitus (non insulinodependent) associated with microvascular, renal and ocular diseases.

In conclusion, the products *SpiruZinc, SpiruFier, SpiruSelen* and *SpiruCrom* by their bioactive components assure the high biological and therapeutically effects and present a vast prospective of application as nutraceuticals in the complex therapy of the diverse etiology diseases and of the metabolism disorders caused by the biolements deficiency.

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MULBERRY STALKS: AN ALTERNATE RAW MATERIAL TO PARTICLE BROAD INDUSTRY

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BENEFITS OF INTERMITTENT CROP DRYING TECHNOLOGIES

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ABSTRACT:
In case of crop, the intermittent drying technology can basically be used successfully in two cases. One case is when the materials to be dried have different components of alternate drying properties. Another case is when the internal energy of the material bed is used to continue the drying during possible intermissions.
As an example of the first case, alfalfa is a plant used for animal feeding consists of two different components as leaf and stem. During artificial drying of alfalfa the water concentration of leaves falls faster than that of stems as because of their different drying characteristics. However, during the break periods (intermissions) of drying the water concentration of the components equalizes, i.e. drying of stems proceed to dry while leaves are being rewetted which finally yields a homogeneous product. It is the reason that the intermittent drying is beneficial to avoid large differences between the water concentrations of stems and leaves and so the quality degradation.
In order to follow entirely the moisture change in the components and finally to determine the moisture and temperature distribution along the height of an alfalfa in a fix-bed layout, separate models for the drying of the leaf and stem components of alfalfa are to be used including their particular sorption isotherms. These models should be combined with air mass and enthalpy balance equations for the fixed bed.
Using solar energy is a promising solution in attaining the technical, economical and environmental demands raised in the course of drying processes. It implies studying the fitting of solar radiation availability and the intermissions required during alfalfa drying.
Concerning to the case, when the internal energy of the material bed could serve an extraordinary input to the drying process, it implies to reduce the total amount of the energy to be used. That solution can be applied successfully for corn drying. Drying measurements were performed in order to make a comparison for the effective drying time in both continuous and intermittent processes. It was clearly identified that the intermittent solution is more beneficial.
Beside the energy saving possibilities the intermittent drying has another advantage, i.e. the quality of final products. During of the measurements the corn surface temperature was measured with the help of an infrared temperature device. It was experienced, that it is possible to control the corn surface with the help of air flow rate and drying air temperature for receiving a better quality of final product. It is very important for example for seed corn, since at high temperatures the chemical and physical properties of the material may change.
Avoiding the problem of overheating in continuous drying, a lower drying air temperature should be applied. It is not the case at intermittent drying as because due to the intermissions the average grain temperature will be less by 10-15 °C compared to the continuous case under the same input conditions.

Keywords: crop drying, alfalfa, energy, lower drying.

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EXTRACTION OF COTTON LINTERS BY SAW AND ROLLER TYPE DELINTER: IT'S EFFECT ON QUALITY OF PAPER

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ABSTRACT:

In India, cottonseed production during year 2007-2008 is estimated as 10.3 million tones. The seeds when scientifically processed yield linter as important by-product apart from hulls, oil and meal. At present, linters are extracted by saw type delinter. Attempt was made to extract linters by double roller (DR) gin which is extensively used for ginning cotton in India. Objectives of present study is to (1) measure the power requirement for extracting linters, recovery of linters by saw type delinter and double roller (DR) gin and (2) assess the properties of linters, and paper prepared from linters. The cleaned cottonseed (DR ginned) was used for the delinting trials on the saw type delinter and roller gin. Microcontroller and PC based experimental setup was specially designed to measure the power requirement in both the machines. Experiments were conducted and analyzed as completely randomized design replicated two times. It was observed that the average linter recovery, processing capacity and power requirement(for extracting 100 kg linters) on saw type delinter & DR gin were found to be 4.86% and 0.85%, 307 and 301 Kg cottonseed/h, 67.5 and 111 Kwh respectively.

Keywords: extract linters, delinting.

CROP INSURANCE– A SOCIAL PANACEA FOR SERICULTURISTS

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ABSTRACT:

The changing economic environment has triggered a renewed interest in crop and agricultural insurance programs. The applicability of insurance in given situation is based on consideration of whether it is a cost-effective means of addressing a given risk. Agricultural insurance is most often quoted tools and in practice, is almost an adjunct in managing the risks related with farming. Crop insurance is a branch of this mechanism geared to covering losses from adverse weather and similar natural perils beyond the control of growers. Sericulture has become popular among rural households who practice this culture to earn their livelihood. The eco-friendly activities of the industry satisfy the equity concerns to a great extent. Silkworm crop insurance has been popular through different five year plans covering enumerable sericulture farmers in its fray.
Silkworm rearing is prone to losses due to diseases, climatic fluctuations and set-back of other natural perils. The silkworm crop insurance covers any loss occurring during any stage of silkworm rearing, i.e., from egg to cocoon. Insurance coverage in Sericulture has become a cross legged mechanism in sustaining the lives of Seri-culturists all over, leading to development of the industry. It is a fact; the insurance does not directly increase a grower's income. It helps managing risks to this income.

Keywords: Crop Insurance, Sericulture, Social Panacea

INFLUENCE OF SOME BIOMASS PLANTS ON NERVOUS CENTRAL SYSTEM

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ABSTRACT:

Some herbs and spices have been utilized since antiquity for their culinary qualities, and in addition, have also been used for their preservative and medicinal properties. Within the past years there has been increasing interest in the discovery of either novel plant secondary metabolites with distinct biological activity or re-evaluation of already known substances with new biological activities. The characterization of such compounds has high priority within medicine and pharmacology in the search for new and more efficient compounds for the treatment of various diseases, in particular in treatment of nervous central system disorders. In the same time, the isolation of bioactive compounds from whole plants is laborious and often an obstacle for pharmacological studies.
Screens using high-performance liquid chromatography (HPLC) coupled to efficient testing of bioactive compounds (activity profiling) has already led to the identification of promising natural compounds that could be chemically modified to increase their activity. The aim of this study was to characterize the therapeutic properties of some biomass plants on the nervous central system.
A review of the literature revealed that, other than for their volatile oils, the chemical composition of these specific species has rarely been reported. That is why, the phenolic content, chemical composition, and in vitro antioxidant (iron reduction and chelation, 1,1-diphenyl-2-picrylhydrazyl radical scavenging, ascorbate-iron-(III)-catalyzed hydroxyl radical-mediated phospholipid degradation inhibition and nonsite- and site-specific hydroxyl radical- mediated 2-deoxy-*D*-ribose degradation inhibition) properties of water–soluble and alcohol – soluble extracts were examined.

Keywords: herbs, spices, bioactive compounds, volatile oils

THE ANTIOXIDANT POTENTIAL OF A FLAVONOID RICH FRACTION OF *APIUM GRAVEOLENS L.* (CELERY) LEAVES

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ABSTRACT:

Plant species of the family *Apiaceae* are known to accumulate flavonoids mainly in the form of flavones and flavonols. (1) The aim of this study was the obtainment of a flavonoid rich extract of celery leaves and the determination of its antioxidant potential. *A. graveolens L* leaves were extracted with methanol by maceration method and the methanol extract was further precipitated with ethyl ether and filtered. The filtrate was partitioned with ethyl acetate giving a flavonoid rich fraction. Thin-layer chromatography analyses confirmed the presence of flavonoid glycosides, as well as unidentified compounds. Total phenolic of the celery extract was determinated by Folin-Ciocalteu method as the gallic acid equivalent and were to be 0,050%. The flavonoid content expressed as apigenin was determined by aluminium chloride colorimetric method and were to be 18,62%. The possible antioxidant activity of the extract was investigated using hydrogen peroxide-induced luminol chemiluminescence assay. At the 10^{-2}g/mL concentration of the extract, the scavenging effect were found to be 88,7% and decreased to 29% at 10^{-4}g/mL concentration. Quercetin was used as positive control. The results concluded that the celery fraction enriched in flavonoids has antioxidant potential and needs to be further tested.

Keywords: *Apium graveolens L*, flavonoids, antioxidant

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STUDIES CONCERNING THE ASSOCIATION OF ACTIVE PRINCIPLES FROM THREE HERBAL SPECIES FOR THE OBTAINMENT OF SOME PRODUCTS WITH ACTION ON CNS

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ABSTRACT:

In the previous studies we presented the possibility of obtainment of active fractions from indigen herbal species containing triterpenes, phenolic acids, flavones that can act alone or in synergy on the central nervous system. The aim of this study consists in assessing the sedative activity of some products obtained by processing of a mix of three herbal species or by association of the obtained fractions from each species. The herbal material is represented by the flowers of *Lavandula angustifolia Mill*. and *Achillea millefolium L*, leaves from *Melissa officinalis L*, aerial parts from *Origanum vulgare L* and from a mixture of leaves and flowers of *Crataegus monogyna Jacq*. and *Crataegus oxyacantha L* or combinations of threse of these species.
The same method was used for extraction of the active principles, followed by concentration of the extractive solutions, precipitation of the products resulted by combining the active fractions coming from each species. The obtained bioactive products were analytical characterized and tested in vivo for the pharmacological activity regarding the action on CNS. It was concluded that, according to the chemical composition, the present products have sedative action on CNS.

Keywords: CNS, therapeutical product, sedative action

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RESEARCHES REGARDING MICROBIAL ANTAGONISM IN BIOLOGICAL CONTROL USED FOR *LYCOPERSICON ESCULENTUM* L. AND *SOLANUM MELONGENA* L.

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ABSTRACT:

The use of microbial antagonism constitutes one of the primary modalities for biological control involved in plant protection in accord with the principles of sustainable agriculture. The concept of sustainable agriculture requires the maintenance of the attack generated by phytopathogen agents under the economic harmful level. Nevertheless, this objective is difficult to achieve as phytopathogen agents benefit from a genuine capability to continuously adapt under the applied control methods. Consequently, there is a clear need to identify and permanently update the methods for controlling the phytopathogen agents causing qualitative and quantitative losses.

Subject to the foregoing, the purpose of this study was directed towards the accomplishment of researches concerning the protection of tomato plants (*Lycopersicon esculentum* L.) and aubergines (*Solanum melongena* L.) using the microbial antagonism with the aim to biologically control the phytopathogen agents identified as pertaining to some bacteria strains of *Xanthomonas campestris pv.vesicatoria*, of *Pseudomonas syringae pv. tomato* and of *Erwinia carotovora*, by further applying treatments containing basic biopreparates of *Bacillulus* sp. obtained at INCDCF- Bucharest.

Keywords: microbial antagonism, phytopathogen agents, biologically control

MAGNETIC NANOPARTICLES OBTAINED BY BIOTECHNOLOGY

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ABSTRACT:

The new millennium has a peak in the scientific and technological area devoted to the nanomaterials and nanotehnologies. Field is fabulous both through the prism of perspectives of scientific investigation and for the technological applications.

Nanomaterials have pushed the manipulation limit of matter at atomic and molecular level. They have different physical properties, chemical, electronic, magneto-electric from those of classical materials used in science and technology, just because of their extreme size.

Study of the obtaining methods and the manifested property is essential for the development of technical and technological in future. The application field of nanomaterials is large.

An important area of research is the synthesis of nanoparticles with different chemical composition, size and morphology. The form of nanoparticles is a priority area of methods of synthesis. It is necessary to develop methods of synthesis of nanoparticles without the use of toxic chemical processes.

As a result, researchers in the field of synthesis and "assembling" nanoparticles have sought to draw on the biological systems. This is not surprising, because many bodies, so unicellular and pluricellular may create inorganic materials, intra or extra cellular.

Through transmission electronic microscopy (TEM) we could see that the processes of synthesis of nanoidal and colloidal metallic particles by biosynthesis using microorganisms in the medium culture with high concentration of iron ions is different from those with silver ions.

From the dimensional viewpoint the range of the particle size are between 5 and 50 nm.

Keywords: nanoparticles, colloidal metallic particles, biosynthesis.

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Second section

IDENTIFICATION, ASSESSMENT AND PROCESSING OF SERICULTURAL BY-PRODUCTS AND WASTE MATERIAL

IDENTIFICATION AND POSSIBLE UTILIZATION OF SOME SILKWORM REARING WASTE PRODUCTS

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ABSTRACT
Sericulture implies significant quantities of secondary and waste products which may be processed in order to generate additional incomes that will even double or triple the incomes obtained from the main activity. As a first step in this direction however should be the identification and evaluation of the silkworm rearing waste products which is the aim of the present paper. We estimated that the silk production performs only 8.54 % from the dry matter mulberry leaf yield obtained by one hectare plantation. On the other hand the different wastes such as leaf remnants, excrements and the pupae give 2061.60 kg of dry matter or 83 %. That means the most part of mulberry leaves is transformed as waste products. The abundant feeding amount of the silkworm larvae leads to increase of the share of waste products. The results obtained manifested that from 125.70 kg nitrogen contained in the mulberry leaves from one hectare, 37.40 kg (29.75%) are transformed into silk. The nitrogen which went into the waste products (leaf wastes, excrements and pupae) is 79.90 kg or 63.56 %. In the silkworm rearing wastes the highest percent of crude protein and crude fats is contained in the leaves litters and the silkworm excrements – 20.51 %, 12.20% and 2.73% and 2.75 % respectively. The crude protein and fats content is comparatively higher in the mature shootlets leading the total wastes material (rearing bed and branches) to have 9.54 % crude protein and 1.77 % crude fats. The highest nitrogen content was detected in the compost prepared by silkworm rearing bed from 3rd and 4th instars, followed by those from whole larval rearing period bed and the 5th instar larval rearing bed. The nitrogen percentage in the compost, prepared by a mix of mulberry branches and rearing bed which actually represents the mean which is produced as wastes from the silkworm rearing is 1.68 %. Regardless the high contents of lead and zinc detected in the food, excrements and silkworm body as a whole the contents of heavy metals in the cocoon shells and silk are negligible. It is concluded that when the mulberry leaves are polluted by heavy metals the silkworm rearing wastes should not be utilized because the main traces of the heavy metals remained there.
Keywords: silkworm rearing, wastes, dry matter, compost, processing

1. INTRODUCTION

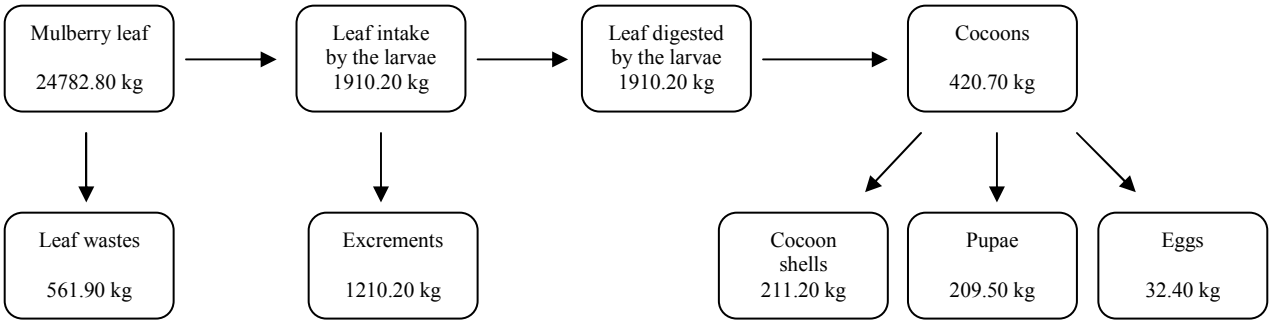
The sericulture is one of ancient and economically effective sub-branches of agriculture both in Bulgaria and Romania. The historical manuscripts manifest that the sericulture was practiced as early as during 7th-11th century A.D., but it was especially strongly developed in the end of 19th and first half of 20th century, when in some years the fresh cocoon production reached up to around 4.000 t. Considering the economic conditions after EU accession, meaning stimulating subsidiaries granted, the sericultural activity will be re-launched due to the fact that it is one of the industrial/agricultural activities that supplements the incomes of the people from rural areas.

Sericulture implies significant quantities of secondary and waste products such as: perforated silk cocoons, *Bombyx mori* chrysalides, bedding left-overs (larval dejections and mulberry plant waste), superseded cocoons, surplus mulberry leaf, springs, root and wood biomass, mulberry fruits, mulberry root biomass etc. In order to ensure a profitable sericultural activity, it is necessary to process these secondary and waste products in order to obtain biologically active substances with important uses in: pharmaceutical, cosmetic, paper and cellulose, and organic agricultural food industries. By applying some modern methods for processing the secondary and waste products from sericulture additional incomes that will even double or triple the incomes obtained from the main activity will be created.

As a first step in this direction however should be the identification and evaluation of the silkworm rearing waste products which is the aim of the present paper.

2. DRY MATTER BALANCE IN THE SILKWORM REARING

Our studies revealed that the dry matter balance from one ha mulberry plantation was as follows:



From the figures given above it may be concluded that from 2472.80 kg mulberry leaves as dry matter obtained from one hectare plantation only 620.70 kg are digested by the silkworm larvae and further transformed in 211.20 kg of silk/cocoon shells. Therefore the silk production is only 8.54 % from the dry matter mulberry leaf yield. On the other hand the different wastes such as leaf remnants, excrements and the pupae give 2061.60 kg or 83 % of all dry matter that means the most part of mulberry leaves is transformed as waste products.

Our investigations have clearly brought out that when the silkworm larvae are fed by abundant amount of mulberry leaves during the 5th instar the dry matter leaf ingestion and digestion per one hectare of plantation decrease, and consequently it lead to decline of the dry matter production as cocoons and eggs and increase of the share of waste products.

3. NITROGEN BALANCE IN THE SILKWORM REARING

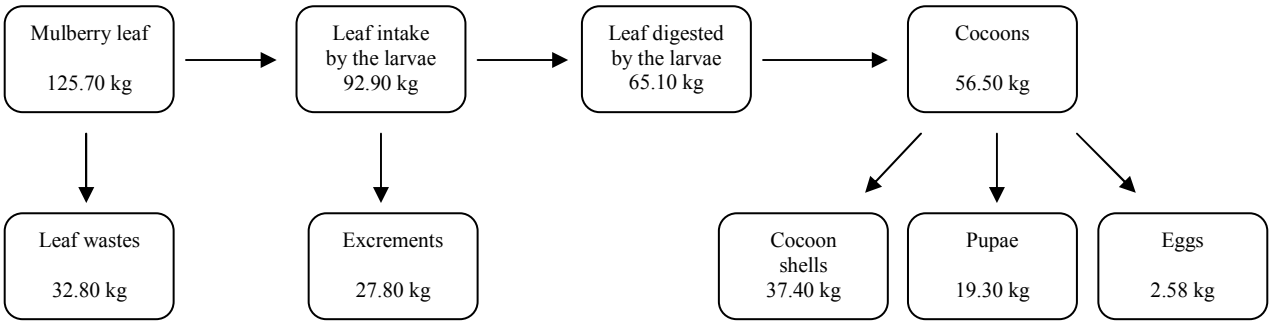
In Table 1 are given data about the nitrogen percentage in the dry matter of mulberry leaves, different products and wastes from the silkworm rearing.

The significantly higher nitrogen content in the food digested compared with the food ingested we consider as normal due to the nearly two times higher nitrogen digestibility as the dry matter as a whole digestibility.

Table 1
Percentage content of nitrogen in the dry matter of mulberry leaves, different products and wastes from the silkworm rearing.

Samples	Season of silkworm rearing	
	Early spring	Late spring
Mulberry leaves	4.45	3.90
Mulberry leaves ingested	4.33	4.23
Mulberry leaves digested	8.37	9.96
Matured silkworm larva	11.25	10.50
Pupa	9.50	9.22
Cocoon shell	17.10	17.10
Moth	10.25	10.00
Eggs	10.50	10.00

We also studied the nitrogen obtained per one ha of mulberry plantation balance. It was detected the following nitrogen content in the mulberry leaves, silkworm products and wastes from one ha mulberry plantation:



The results obtained manifested that from 125.70 kg nitrogen contained in the mulberry leaves from one hectare, 37.40 kg (29.75%) are transformed into silk. The nitrogen which went into the waste products (leaf wastes, excrements and pupae) is 79.90 kg or 63.56 %.

4. IDENTIFICATION AND ANALYSES OF THE SILKWORM REARING WASTE PRODUCTS

The chemical composition of the mulberry leaves, shootlets and the silkworm rearing wastes is presented in Table 2.

Table 2
Chemical composition of the mulberry leaves, branches and litter of silkworm rearing in %

Samples	Crude protein	Crude fat	Crude fiber	Crude ash	NES
Mulberry leaves	23.95	3.64	12.60	11.80	48.63
Mature branches	7.77	1.50	43.09	4.87	42.77
Worm excrements	12.20	2.75	15.58	12.51	56.97
Leaves litters	20.51	2.73	16.23	11.32	49.10
Shoot feeding litter (leaves and excrements)	15.81	2.74	15.98	11.99	53.55
Branch feeding litter (branches, leaf litter and excrements)	9.54	1.77	37.19	6.41	45.10

It is evident from the data that in the wastes the highest percent of crude protein and crude fats is contained in the leaves litters and the silkworm excrements – 20.51 %, 12.20% and 2.73% and 2.75 % respectively. The rearing bed composed with leaves litters and excrements in a proportion of 43.48 : 56.52 contains average amounts of crude protein and fats. The crude protein and fats content is comparatively higher in the mature shootlets leading the total wastes material (rearing bed and branches) to have 9.54 % crude protein and 1.77 % crude fats. According to Wakasawa et. al. (1984) and Harada et al. (1993) the nitrogen content in the excrements of hens is 6.18 %, in chicken broilers 4.00%, in pigs 3.61 % and in the caws 2.19 %. The data about the mineral composition of the raw and composted silkworm rearing wastes are presented in Table 3.

Table 3
Mineral composition of the raw and composted silkworm rearing wastes in %

Variant	Moisture	N	Ether extract	Mineral residue	P_2O_5	CaO	MgO
Mineral composition of the raw wastes							
Excrements	10.75*	2.45	3.71	10.97	1.41	2.73	0.75
Leaves litters	12.82*	4.53	4.51	9.17	1.55	1.84	0.58
Mineral composition of the compost							
Litters from the silkworm rearing in 3rd instar	57.08	4.14	9.92	28.87	2.16	9.87	0.96
Litters from the silkworm rearing in 4th instar	69.02	4.35	10.08	30.48	1.99	10.85	1.12
Litters from the silkworm rearing in 5th instar	76.41	2.86	5.65	24.49	1.69	8.48	1.66
Litters from the silkworm rearing during whole period	65.24	3.02	5.37	30.13	1.98	8.76	1.89
Cutted mulberry branches	55.07	1.07	1.13	7.06	0.33	2.46	0.47
Mix of mulberry branches	50.88	1.68	0.65	12.42	0.46	4.17	0.17
Litter from the silkworm rearing in 4th and 5th instars + wood shavings	72.74	1.40	3.69	16.26	0.81	6.85	0.50

* Water content of the excrements and leave litters varied considerably depend on ecological conditions and technology of the silkworm rearing

Our results manifest that the nitrogen content in the silkworm excrements is 1.95 – 2.45 % and this content is near to those in the caws while the average nitrogen content in the silkworm rearing wastes is 1.53 %.

According to Kawada (1981) the qualitative compost should have more than 70 % organic matter, over 1.2 % nitrogen, over 0.5 % phosphorous and more than 60 % moisture. The data presented in Table 3 show that all our experimental groups of composting meet the standard requirements for nitrogen, organic matter and phosphorous content.

The highest nitrogen content was detected in the compost prepared by silkworm rearing bed from 3rd and 4th instars, followed by those from whole larval rearing period bed and the 5th instar larval rearing bed.

The nitrogen percentage in the compost, prepared by a mix of mulberry branches and rearing bed which actually represents the mean which is produced as wastes from the silkworm rearing is 1.68 %. This value is near to the detected by Harada et. al. (1993) in compost prepared by caw rearing wastes, which is 1.35 – 2.30 %. The same authors found 1.94 % nitrogen content in the compost from the hen growing wastes, 4 % in chicken broilers and 2.22 – 2.92 % from pig growing wastes.

5. EFFECT OF THE MULBERRY LEAVES POLLUTION WITH HEAVY METALS ON THE BASIC AND WASTE PRODUCTS FROM THE SILKWORM REARING

The results obtained from our experiments are presented in Tables 4 and 5. Silkworms from experimental groups were fed with leaves dipped in lead or zink water solutions for 15 min.

Table 4
Content of lead in the food, excreta and silkworm *Bombyx mori L.* organism

Samples	Control № 1 group	Control № 2 group	Experimental group
	mg/kg dried matter		
Mulberry leaves	6.62	-	145.70***
Leaf remnants	3.91	3.13*	252.97***
Excreta	4.55	3.38*	316.90***
Matured larvae	2.28	1.94	19.20***
Cocoon shells	4.19	5.32	6.72*
Pupae	3.74	5.72**	10.09***
Cocoon filaments and by products	3.23	0.46***	2.58
Water after cocoon reeling (ppm)	0.007	0.000	0.020*
Moths	2.09	7.43***	7.89***
Eggs	2.21	2.43	6.39***

*P<5%, **P<1%, ***P<0.1%

Table 5
Content of zink in the food, excreta and silkworm *Bombyx mori L.* organism

Samples	Control № 1 group	Control № 2 group	Experimental group
	mg/kg dried matter		
Mulberry leaves	34.45	-	100.10***
Leaf remnants	41.00	30.15**	109.25***
Excreta	20.78	20.50	67.55***
Matured larvae	48.03	51.90	106.88***
Cocoon shells	5.53	0.00***	0.00***
Pupae	126.13	109.50*	328.50***
Cocoon filaments and by products	36.00	28.20**	37.93
Water after cocoon reeling (ppm)	1.18	1.20	1.33
Moths	107.05	50.45***	175.13**
Eggs	207.20	226.00	340.75***

*P<5%, **P<1%, ***P<0.1%

High contents of lead (316.90 mg/kg DM) were detected in the excrements of the larvae fed on polluted mulberry leaves. It was of interest to note that the content of lead in the excrements of experimental group was markedly higher than that in the mulberry leaves supplied. A similar relationship between the contents of Zn, Cu, Fe, Mn, and Cd in different feeds for pigs and their faeces had been reported by Harada et. al. (1993).

There were comparatively high contents of lead in the experimental matured larvae, pupae, moths and eggs, but these contents were less than those, observed in mulberry leaves, leaf remnants and excreta. The content of lead in the cocoon filament and by products was low and comparable to the control. As indicated in table 5 the contents of zinc in the samples of mulberry leaves, leaf remnants and excreta, taken from the experimental group were 2.91, 2.66 and 3.25 times respectively higher than those in the control.

The differences between zinc content in the faeces and matured larvae were considerably lower than those, observed in lead, accordingly zinc had not probably been excreted in a such great extent through the excrements as lead.

It was observed also a comparatively lower content of zinc in the cocoon shell and silk products and higher content in the pupae, moths and eggs in the experimental group. It was important to note that there were almost no any differences in term of contents of lead and zinc in the cocoon shells between experimental and control groups.

Our results were similar with those reported by Masui and Matsubara (1984), that the content of cobalt had increased markedly in all larval tissues, except for the silk glands, when the silkworm larvae were fed on artificial diet, contained excess amounts (50 ppm) of cobalt.

It had been demonstrated that a large part of lead and zinc intake from the food was excreted through the excrements, but a too high quantities of them were accumulated in the larval body, and thereafter passed into the pupae, moths and eggs as well.

Considering the above it is clear that regardless the high contents of lead and zinc detected in the food, excrements and silkworm body as a whole the contents of heavy metals in the cocoon shells and silk are negligible.

Therefore when the mulberry leaves are polluted by heavy metals the silkworm rearing wastes should not be utilized because the main traces of the heavy metals remained there.

6. CONCLUSIONS

1. The silk production performs only 8.54 % from the dry matter mulberry leaf yield obtained by one hectare plantation. On the other hand the different wastes such as leaf remnants, excrements and the pupae give 2061.60 kg of dry matter or 83 %. That means the most part of mulberry leaves is transformed as waste products. The abundant feeding amount of the silkworm larvae leads to increase of the share of waste products.

2. The results obtained manifested that from 125.70 kg nitrogen contained in the mulberry leaves from one hectare 37.40 kg (29.75%) are transformed into silk. The nitrogen which went into the waste products (leaf wastes, excrements and pupae) is 79.90 kg or 63.56 %.

3. In the silkworm rearing wastes the highest percent of crude protein and crude fats is contained in the leaves litters and the silkworm excrements – 20.51 %, 12.20% and 2.73% and 2.75 %, respectively. The crude protein and fats content is comparatively higher in the mature shootlets leading the total wastes material (rearing bed and branches) to have 9.54 % crude protein and 1.77 % crude fats.

4. The highest nitrogen content was detected in the compost prepared by silkworm rearing bed from 3^{rd} and 4^{th} instars, followed by those from whole larval rearing period bed and the 5^{th} instar larval rearing bed. The nitrogen percentage in the compost, prepared by a mix of mulberry branches and rearing bed which actually represents the mean which is produced as wastes from the silkworm rearing is 1.68 %.

5. Regardless the high contents of lead and zinc detected in the food, excrements and silkworm body as a whole the contents of heavy metals in the cocoon shells and silk are negligible. It is concluded that when the mulberry leaves are polluted by heavy metals the silkworm rearing wastes should not be utilized because the main traces of the heavy metals remained there.

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SILKWORM (*BOMBYX MORI*) PUPAE MEAL, A WASTE OF REELING FACTORIES. AMINO ACID CONTENT AND ITS DIGESTIBILITY IN FOWL

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ABSTRACT:
The essential amino acids content and their digestibility was determined for the silkworm cocoon reeling factory wastes, specifically for silkworm pupae, in reference to their use in fowl nutrition. For the determination of amino acid digestibility, digestibility trials with geese had been carried out. The chemical analysis on three samples of different origin indicated a satisfactory content in amino acids essential for animal nutrition. In an estimation of grams of amino acid per 100 grams of protein contained, lysine gave values of 6.39-7.27, methionine 2.34-2.50, arginine 5.34-5.84, histidine 2.54-2.91, leucine 5.99-6.92 and isoleucine 3.19-3.51. The determination of amino acid digestibility by trials with geese showed corresponding coefficients of digestibility of 94.10% for lysine, 94.57% for methionine, 93.61% for arginine, 89.14% for histidine, 91.75% for leucine and 95.48% for isoleucine. Both the content and the digestibility of the rest of the amino acids showed very satisfactory values for animal origin feedstuff.
Keywords: Silkworm pupae meal, cocoon reeling factory wastes, amino acids, gees feeding.

INTRODUCTION

The killed pupa represents approximately about 43-50% of the total dry cocoon weight, at the stage they are driven to reeling factories (Tzenov and Zaharieva, 1997; Kipriotis et al., 1999). The chemical composition and the possible uses of this industrial by-product were studied by many researchers in the past (Chervencova, 1975; Datta and Chaterji, 1997; Kipriotis et al. 2000). This by-product exists in large quantities in countries where industrial reeling is experienced and as research has shown it contains organic matter with remarkable protein content, fats and moderate fibre content. This composition, in connection to the long term preservation under natural conditions after drying, creates good possibilities for use in animal feeding.

In the majority of the relative research works, it has been described a quite good behaviour of this product to such uses, without though a deeper investigation in connection to its chemical composition and its specific nutritional characteristics. Some deeper investigation is given by Gomathi et al., (1994) where the ***in vitro*** digestibility is estimated around 60-75,5 %. Further on Kipriotis et al. (2000) investigated, apart of the chemical analysis, the ***in vivo*** digestibilities of the organic matter, the nitrogenous compounds and the fatty and fibrous compounds, the metabolisable energy contents and the coefficient of the total energy use of this by-product.

In the present work a further investigation was carried out with the determination of the essential amino acid content and their digestibilities in fowl.

MATERIALS AND METHODS

For the determination of the chemical composition and the essential amino acid content of pupae meal had been used dried samples from different Bulgarian reeling factories and their mean values were estimated. The amino acid content was estimated through a Shimadzu AAA-881 amino acid analyser, after hydrolysis of the samples by acid, expressed both as dry matter %, and as grams per 16 grams of Nitrogen (thus 100 grams of protein). For the digestibility determination was used a homogenised mixture of all the different origin samples. With this average sample digestibility trials had been carried out on 12 adult gees, (Anser domesticus), two years old, with an average live weight of 6 kg. The gees formed two groups of 6 birds each,

in an eight day trial, under feeding and fasting treatments. All the birds during the trial period were kept individually in 1m X 1m slatted floor cages, with special feces collection device, and they entered the treatment after a 48 hour starving period.

To the birds under the feeding treatment were given daily 90 grams of the mixed and homogenised pupae meal sample, in three equal doses of 30 grams, directly to the crop through catheter, in eight (8) hour intervals. (07.00 pm. 15.00 am and 23.00 am).

All the excreta of each bird, feces and urine together in the case of fowl, were collected after each meal. The total 24 hour collected amount was mixed and homogenized and a 50 gram sample of it was deep freezed (-18 C^o) for further laboratory analysis. Laboratory analysis was carried out after defreezing and vacuum drying the samples in a Buchi Rotavapor apparatus, with the classical processes of Nitrogen, fat and energy determination, the synthesis given in table 1.

For the calculation of the digestibility coefficient of each amino acid was used the Sibbald equation, (Sibbald and Morse, 1983; Sibbald, 1987), and as it was modified for the case of birds by Penkov, (Penkov et al., 1996), according to which:

$$\text{Amino acid digestibility coefficient} = 100 \times \frac{\mathbf{AA - (FAA - FFAA)}}{\mathbf{AA}}$$

Where **AA** = Grams of amino acid consumed per bird

FAA = Fecal Amino Acid (grams of amino acid investigated in the feces of each fed bird)

FFAA = Fecal Amino Acid for Fasting birds (grams of amino acid investigated in the feces of each starved bird)

The equation was performed separately for each bird and finally was used the mean of each group. The price of AA for the case of starving birds is equal to zero. The values of the chemical analysis and the nutritional parameters were analysed statistically by analysis of variance (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

As it is being observed in the results of the chemical analysis of the material used for the feeding of the birds (table 1), there appear some differences in their protein content, most possibly due to the different origin of the initial materials and the different processing methods in the reeling factories. The highest protein content, 58,28%, comes from the Harmanli, Bulgaria reeling factory, followed by 54,76% and 56,02% respectively for the Plovdiv and Pazardjik, Bulgaria, samples. In general the analysis proves that the investigated samples represent a material reach in protein, which could be an interesting supplement in animal feeding.

The amino acid determination further proved this evidence, giving very interesting contents of amino acids for all the samples (Table 2). For Lysine the taken values were 3,58-3,99%, for Histidine 1,47-1,66%, and for Arginine 2,99-3,20%. Asparagine gave respectively values between 5,44% and 6,12%, and the differences between them were statistically significant ($P \leq 0.05$). For Threonine the values were 2,43-2,55%, and for Serine 2,83% and 3,39% respectively, and again the differences between them were statistically significant ($P \leq 0.05$). For the case of Gloutamine the given values were 5,71% - 6,04%, with no statistical differences. Proline, Glycine and Alanine gave values with statistically significant differences between them ($P \leq 0.05$) ranging between 2,55%-3,11% for Proline, . 2,83%-3,93% for Glycine and 3,11%-4,09% for Alanine respectively. From the rest of the determined amino acids only Tyrosine gave values with statistically significant differences them were ($P \leq 0.05$), within the range of . 3,21%-3,75%. For the rest of them the obtained values were 0,25%-0,32% for Cystine, 2,40%-2,68% for Valine, 1,31%-1,39% for Methionine, 1,83%-1,92% for Isoleucine, 3,43%-3,79% for Leucine and 2,57%-2,71% for Phenylalanine.

The distribution of the amino acid values shows the same fluctuations in the case of the value expression in connection to the total nitrogen content of the samples, and more specifically the expression per 100 grams of protein or respectively 16 grams of Nitrogen (16gr N X 6,25 = 100gr protein) (table 2).

In this case the slight differences between the two ways of expression most possibly derive from the different contents of the samples in non proteinic nitrogenous compounds which influence the total Nitrogen contents and are transformed by calculation to total protein.

In general though the amino acid contents of the samples, either expressed as % of the total weight, or in accordance to the nitrogen contents, follow the same dispersion, as it comes out from figures 1 and 2.

The digestibilities of all the determined amino acids of the mean pupae meal sample, in connection to the total protein digestibility, which were calculated through the digestibility trials, appeared in all cases very high, as it is shown in table 3 and figure 3.

Digestibility for total protein reached 85,03 % and this price was used as control for the evaluation of the determined amino acid digestibilities. In all cases the amino acid digestibility was higher than 81 %, which is considered as very high compared to the total protein digestibility. The only exception came from glycine, who's digestibility just reached 77,38 %. The higher digestibility values came from isoleucine (95,48 %), methionine (94,57 %), and lysine (94,10 %). For the rest of the amino acids the digestibility values ranged between 81,23 % (alanine), and 93,61 % (arginine), all the mean values ranging around 91 %.

To evaluate though more accurately this by-product by means of animal nutrition, it would be necessary to compare these results to other similar, but unfortunately such results could not be found in the literature.

CONCLUSIONS

The high contents of the studied in this work material in a basic nutritional substance, like protein, in connection to the high contents of the essential in animal nutrition amino acids, which competes the most of the well known of animal origin feeding stuffs, and the observed high digestibilities of all, put this material in a very interesting position for animal feed composition.

Its use in animal feeding, as it was shown by the feeding trials, appears promising and it has to be examined in the next steps its biological value. The any observed differences on its composition show that there must exist influences deriving from the origin of the material and the way of its processing during reeling. These observations evidently underline the necessity of a deeper investigation which will involve, apart of the nutritional studies, also the details of the industrial processing and treatment of the raw materials. Such a study will complete the knowledge around this product and will further contribute to its better and safer use in animal feeding.

Table 1

Chemical composition of silkworm pupae meal

Chemical composition of samples	Origin of samples			
	Harmanli Reeling unit (Bulgaria)	Plovdiv Reeling unit (Bulgaria)	Pazardjik Reeling unit (Bulgaria)	X±Sx
Dry matter %	93.84	94.29	94.06	94.06±0.13
Ash %	3.06	2.88	3.89	3.28±0.31

Crude Protein% (N * 6.25)	58.28	54.76	56.02	56.02±0.73
Ether extract%	30.27	28.47	28.14	28.96±0.66
Crude fiber %	8.26	9.13	10.11	9.17±0.53
Non prot.Nitrog. Compounds %	0.13	4.76	1.84	2.224±1.35

Table 2. *Determination of the amino acid content of three silkworm pupae meal samples, coming from Bulgarian cocoon reeling factories*

Amino acid	Origin of samples						x±Sx	
	Harmanli		Plovdiv		Pazardjik			
	In D.M. %	g/16 g N	In D.M. %	g/16 g N	In D.M. %	g/16 g N	In D.M. %	In 16 g .N
Lysine	3,99	6,97	3,98	7,27[a]	3,58	6,39[b]	3,85±0,135	6,87±0,258
Histidine	1,66	2,90	1,47	2,54	1,63	2,91	1,59 ± 0,059	2,79 ± 0,122
Arginine	3,19	5,57	3,20	5,84	2,99	5,34	3,13 ± 0,068	5,58 ± 0,140
Asparagine	5,66	9,88[a]	5,44[a]	9,93	6,12[b]	10,92[b]	5,74 ± 0,200	10,24 ± 0,339
Threonine	2,43	4,24	2,55	4,66	2,55	4,55	2,51 ± 0,040	4,48 ± 0,126
Serine	2,83[a]	4,94[a]	3,17	5,79[b]	3,39[b]	6,05[b]	3,13 ± 0,163	5,59 ± 0,335
Gloutamine	5,71	9,97[a]	6,04	11,03[b]	5,92	10,57[b]	5,89 ± 0,096	10,52 ± 0,307
Proline	2,55[a]	4,45[a]	3,11[b]	5,68[b]	2,60[a]	4,04[a]	2,75 ± 0,179	4,72 ± 0,493
Glycine	2,83[a]	4,94[a]	3,59[b]	6,56[b]	3,93[b]	7,02[b]	3,45 ± 0,325	6,17 ± 0,631
Alanine	3,11[a]	5,43[a]	3,81[b]	6,86[b]	4,09[b]	7,30[b]	3,67 ± 0,291	6,53 ± 0,564
Cystine	0,32	0,56	0,30	0,55	0,25	0,45	0,29 ± 0,021	0,52 ± 0,035
Valine	2,40	4,19	2,68	4,89	2,55	4,55	2,54 ± 0,081	4,54 ± 0,202
Methionine	1,39	2,43	1,37	2,50	1,31	2,34	1,36 ± 0,024	2,42 ± 0,046
Isoleucine	1,83	3,19	1,92	3,51	1,84	3,23	1,86 ± 0,028	3,31 ± 0,101
Leucine	3,43	5,99[a]	3,79	6,92[b]	3,63	6,48[b]	3,62 ± 0,104	6,46 ± 0,269

Tyrosine	3,21 [a]	5,60 [a]	3,75 [b]	6,85 [b]	3,61 [b]	6,44 [b]	3,52 ± 0,162	6,30 ± 0,368
Phenylalanine	2,65	4,63	2,57	4,69	2,71	4,84	2,64 ± 0,041	4,72 ± 0,062
Crude Protein	58,28		54,76		56,02		56,02 ± 0,727	

a,b : Means in the same row and for the same expression, with different superscripts differ significantly (P<0.05)

Table 3. Digestibility of silkworm pupae meal amino acids in trials with gees

Amino acid	*Digestibility %*		
	X	**Sx**	**S%**
Lysine	**94,10**	**0,49**	**0,99**
Histidine	**89,14**	**0,97**	**2,45**
Arginine	**93,61**	**0,61**	**1,45**
Asparagine	**92,25**	**0,83**	**2,01**
Threonine	**91,69**	**0,51**	**1,24**
Serine	**86,28**	**1,23**	**3,18**
Gloutamine	**91,17**	**0,76**	**1,87**
Proline	**91,42**	**0,74**	**1,81**
Glycine	**77,38**	**2,06**	**5,96**
Alanine	**81,23**	**1,72**	**4,72**
Cystine	**88,90**	**2,33**	**2,62**
Valine	**88,38**	**1,03**	**2,60**
Methionine	**94,57**	**0,19**	**0,20**
Isoleucine	**95,48**	**1,80**	**4,21**
Leucine	**91,75**	**0,72**	**1,74**
Tyrosine	**89,15**	**0,98**	**2,46**
Phenylalanine	**92,17**	**1,10**	**2,66**
Crude protein digestibility	**85,03**	**1,32**	**3,47**

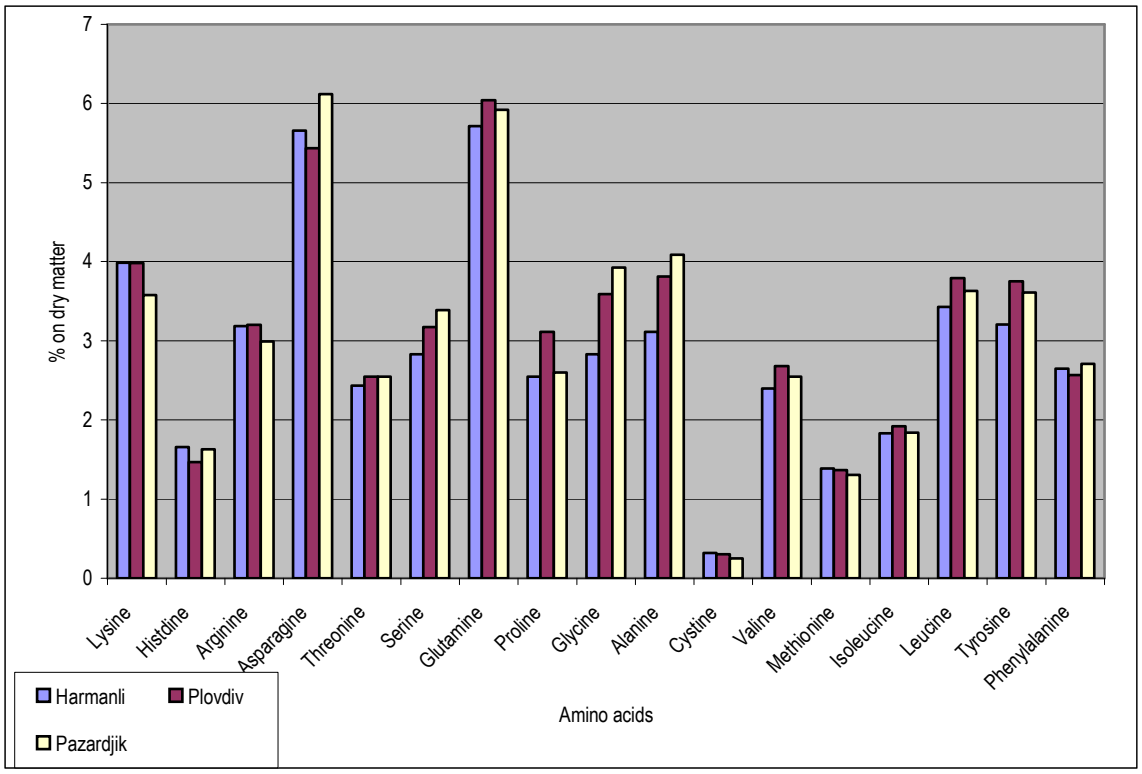

Figure 1. Amino acid content of silkworm pupae meal (% on dry matter)

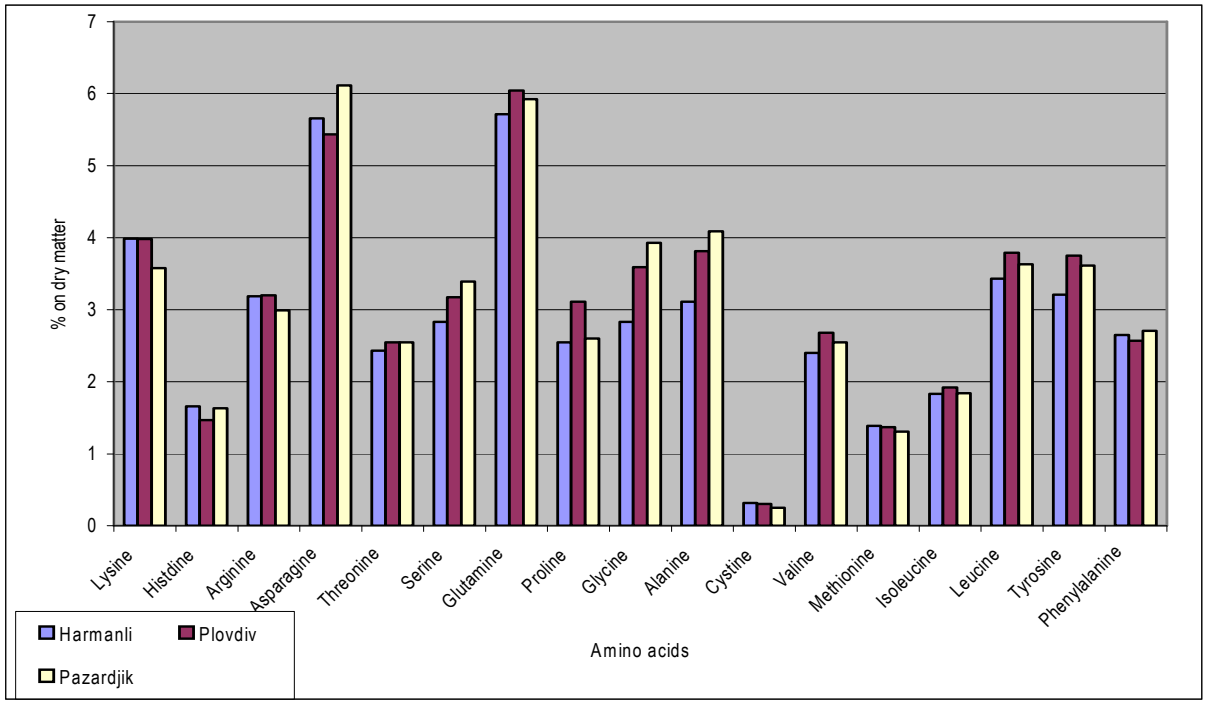

Figure 2. Amino acid content of silkwoem pupal meal (grams/16 grams of nitrogen)

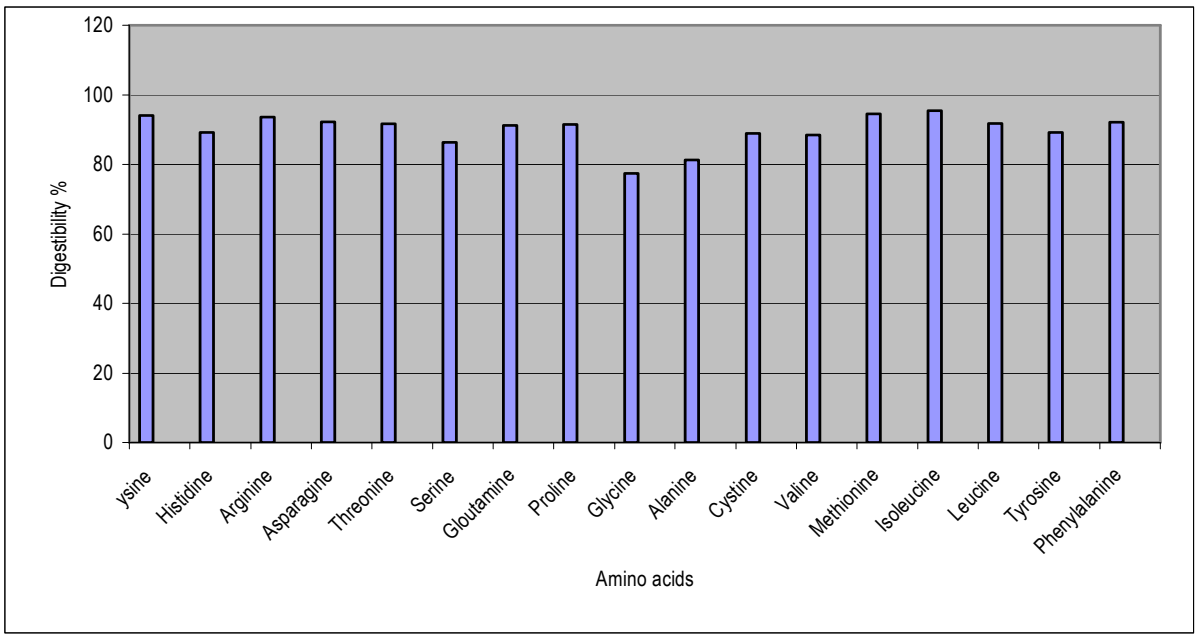

Figure 3. Digestibility % of amino acids in silkworm pupae meal

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THE SECONDARY INGREDIENTS FROM MORUS PLANTS' LEAVES AND SEEDS

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ABSTRACT:
The paper work presents the results of some secondary ingredients evaluation for marigold inflorescence and leaves (Calendula officinalis) as control and for mulberry seeds and leaves (Morus sp).
It was determinate the inflorescences and leaves carotenoids content (β-carotene and xantophile) and the seeds vegetal glycerides content, by both modern and classical methods (HPLC).
The plants were cultivated using a modern warming germinal bed system. The paper work recommends Morus plants as raw materials source for carotenoids extraction.
Keywords: secondary ingredients, marigold, mulberry, carotenoid, modern germinal bed

INTRODUCTION

The experimental researches were made in the frame of National Research – Developing Plan, financed by the Ministry of Education and Research from Romania.

One of the experimental objectives was to make sericulture a profitable activity, by developing a program that is able to use the Morus sp cultivated fields for an intercalary cultivated system with other medicinal plants.

Out of cultivated medicinal plants in Romania, it was selected for this experiment Calendula officinalis, known as marigold. This plant is widely used in phyto pharmacological industry from our country, being easier and secure to get profit out of it. The experimental work intends to develop Morus plants as raw materials source for carotenoids extraction. Regularly, the Morus plants are used for silkworms rearing.

Another project objective was to experiment a modern germinative worming bed system for an optimum administration of soil temperature and assurance of a maximum viability of Calendula officinalis and Morus sp plantlets, multiplied through a germinal method of this system. These plants are used to set up new pure or intercalary crops.

MATERIAL AND METHODS

The biological material was represented by Calendula officinalis seeds out of a mixture of different species and mulberry hybrids seeds, harvested from the female species Ichinose and Ukraine 107 (2006 harvest) and 16 different mulberry species for leaves. The germinative worming bed installation, APTMA type, covered a total surface of 10 m2, corresponding to 10 x 1m2 experimental plots.

The mulberry plants used to set up an intercalary crop, are rooted cuttings type and the experimental plots have drop irrigation equipment. (Photo 1)

Photo 1: Mulberry rooted cutting in the experimental plot with drop irrigation system
(photo authors: Constantinescu Marilena and Tanase Doina, 2007)

The biological material was represented by Calendula officinalis L. seeds out of a mixture of different species and mulberry hybrids seeds, harvested from the female species Ichinose and Ukraine 107 (harvest of 2006 year) and 16 different mulberry species for leaves.

The evaluation researches for Morus sp plants' secondary ingredients were made by carotenoids leaves content determination (β-carotene and xantophile) and the seeds vegetal glycerids content. There were compared the obtained values with the results of University of Agricultural Sciences and Veterinary Medicine Cluj Napoca (Adela Pintea 2004) research for the same evaluation of marigold plants inflorescence ingredients and leaves.

The total lipids extraction method it was represented by the classic one (Folch, 1957) for inflorescences and leaves (methanol: chloroform extraction, 2:1 report). To obtain oil out of seeds it was used Soxhlet extraction method (petroleum ether extraction). The fat acids were identified by chromatography gas method, in the following conditions: gas-chromatography HP 5890 II/5972 GC-MSD, 70 eM, Wiley specter library, HP5MS capillary column with a 30 mm length, interior diameter – 0.25 mm, shadowgraph thickness – 0.32 um, Helium bearer gas – 1 ml/minute.

For carotenoids pigments separation, it was used the HPLC method with a Kontron 322 pumps system, a chromatograph column for the Zorbax ODS – 5(C18) inverted stage, with 250 mm length and 4.6 mm diameter. The detection was made using an array detector Waters 990.

RESULTS AND DISCUSSIONS

There were obtained the following experimental results regarding the marigold mature inflorescences total carotenoids content as control, is seen in Table 1

Chemical structure	Carotenoides	Tocoferols	Sterols
Content ml/g	0.544	1.2	1.36

The table data are experimental data obtained by University of Agriculture Sciences and Veterinary Medicine, Cluj Napoca (Pintea Adela, 2004). Comparatively, there were used the data regarding the carotenoids content from the scientifically literature; in 1960 Movcean, C.D. mention these quantities of carotenoids content for 100 g cannular flowers reported to the absolute dry mass: β-carotene – 0.224 g, licopine – 0.336 g, violaxanthine – 0.150 g, rubixanthine – 0.070 g.

The carotenoids content (carotene and xantophile) of mulberry leaves are presented in Table 2.

Morus species	**Carotenoids mg / g D.S.**	**Morus species**	**Carotenoids mg / g D.S.**
China 1	0.57,71	Kayrio Nezumigaeshi	71,94
China 2	0.6128	**Ukraine 9**	**0.9617**
Thie-ba	0.6783	Ukraine 107	0.6874
China 32	0.7351	Eforie	0.83,38
China 199	0.8626	**Lugoj**	**0.9418**
Ichinose	0.6816	Calafat	0.8745
Kokuso 21	0.6324	Galicea - fem.	0.7656
Ken-Mochi	0.8200	Galicea - masc.	0.8171

There are distinguished the carotenoids content values between 0.5771 – 0.9617 mg/g D.S. (dry substance). These values are comparatively close to the ones of UASVM Cluj Napoca and other documentation sources.

From mulberry seeds there were extracted the lipids as vegetal glycerids (Photo 2), having the following fat acids content (% D.S.):

- palmitic acid (C16:0) – SFA 9,45 %
- stearic acid (C18:1) - SFA 4,30 %
- oleic acid (C18:1) - MUFA 6,88 %
- linoleic acid - PUFA
  similar with linolic acid (C18:2) 79,36 %
- linolenic acid (C18:3) samples

Photo 2: Total oil extraction from mulberry seeds
(Photo author: Tanase Doina, 2005)

The chemical analyse distinguish the prevalent linolic acid content similar as name with linoleic acid – 79.36%, essentially polyunsaturated greasy acid, which can not be produce by the human body. These types of essential greasy acids (the mentioned one and alfalinoleic acid) have a role in cellular membrane forming, funtions and integrity maintenance, participating to the cholesterol metabolim maintenance in the human body, being precursor of ecosanoids who includ prostaglandines, tromboxans and leucotriens which work as local hormons for sickness, accension healing, blood coagulation and other functions.

The data from scientifical literature (Konovalova, O.A. et al, 1990), distinguish the presence in marigold plants of oleanoic acid, in free and glycozide forms. Also, the scientifical literature mention that the Calendula mature seeds content between 50 – 60 % calendic acid. Once the calendic acid content from marigold is increasing, it is mention a dramatic decreasing of linoleic and oleic acids content, while the stearic acid doen't present a content significant variation. (Cromack et al, 1997).

Comparatively, the data regarding the greasy acid chemical content of Morus plants seeds with the mature seeds of marigold plants, distinguish the essential greasy oils diversity from the both vegetal biomass, anallysed in quantitatives proportions which can justify the therapeutic qualities, chemical compounds diversity of these species with phytopharmaceutical destination.

CONCLUSIONS

1. The carotenoids and vegetal glycerids content of Morus plants leaves and seeds presents comparatively values of mature seeds and marigold leaves (Calendula officinalis L.).
2. The Morus plants leaves and seeds may become the raw material source to obtain carotenoids and vegetale oils with a high level of essential greasy oils content.
3. The Morus plants may be used in intercrops with marigolds plants, the main production haveing as destination using them in the phytopharmaceutical industry.

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SILKWORM PUPAE: A VALUABLE BYE-PRODUCT OF SERICULTURE

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ABSTRACT:

With increasing cost of production in sericulture, there is a need to augment alternative income sources by adding value to bye-products to keep the sector viable. Though attempts have been made to utilize bye-products like silkworm excreta, rearing bed waste, molted skin, host plant shoots, sericin, tasar cocoon peduncle, silkworm pupae, silkworm moths etc., same needs to be exploited on commercial scale. Of these bye-products, utilization of silkworm pupae needs immediate attention in view of its production in huge quantities. Value addition through this important bye-product of sericulture in human nutrition, poultry, piggery, fishery, industry etc., is discussed. Outcome of studies conducted in India towards development of package for pupae collection and storage for pupae oil extraction/ purification, utilization of silkworm pupae as protein supplement in poultry etc., is discussed.
Further, in view of spread of eri culture out of North-Eastern States, need and potential for utilization of eri silkworm pupae is elaborated. Studies on nutritional status and surface lipid content of pupae/ pre-pupae, drying of pupae and extraction/ refining of pupae oil besides its physico-chemical properties are briefly discussed to support the potential for value addition in this sector. Keeping in view the pilot studies conducted so far, areas to be focused in future besides the constraints are discussed.

Keywords: Bye-products, value addition, nutritional status, pupae oil

DELAYED RIPENING MULBERRY AS SECONDARY SERICULTURE PRODUCT FOR ENTREPRENEURSHIP

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ABSTRACT:

Besides income by producing silk cocoons, it has bye- products value also, such as mulberry fruit, wood, Silkworm litter, silk waste and Pupae atc. Traditionally mulberry fruit has been used as a medicinal agent. Mulberry fruits apart from minerals, vitamins and Sugars, in particular it is known to contain cyanin. Studies with anthocyanins in laboratory animals suggest antioxidant activities as a dominant feature, but also cardiovascular protection, immune enhancement, antiviral activity and stress reduction as potential health benefits. Fresh mulberries are so fragile and perishable. That's why most mulberry growers are unable to make shipping to distant places for large scale entrepreneurship. On going studies suggest that the delayed ripening fruits has been genetically modified (G.M) to control the ripening process. And has longer shelf life.

In view of this, techniques were optimized for agrobacterium mediated transformation. Apical meristamatic tip was dissected from axenic *Morus alba* shoot cultures. These apical meristems were bombarded (1100 ǿ) with naked tungstan particles in a biolistic gun at a distance of 10 cms under pressure (-25 in Hg) to maximize biochemical wounding. This procedure result in increasing the Agrobacterium mediated transformation. These apical meristems were infected and co-cultivated in the presence of acetosyringone to induce the agrobacterium virulence genes with 16 hrs culture of agrobacterium tumefaciens strains LBA- 4404 harbouring ACC synthase gene, antisense with NPT-II as selective agent for two days at 27^0C in the dark. Following co-cultivation the apical meristems ware then transferred to selection medium containing Kanamycin and carbencillin. After three weeks, regenerated shoots were transferred to rooting medium containing 100 mg/l Kanamycin and 500 mg/l carbencillin. Plantlets forming roots on this selective medium were transferred to pots for hardening the plants.

Keywords: Delayed ripening, Mulberry, Agrobacterium, Entrepreneurship Fruit, ACC synthase.

EXTRACTION, QUANTIFICATION AND UTILIZATION OF CERTAIN SECONDARY WASTE FROM TROPICAL TASAR SILKWORM *ANTHERAEA MYLITTA* DRURY

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ABSTRACT:
Industries produce products and wastes or bye products. This is true for silk industry and tasar silk industry also. Bye- products / secondary wastes of an industry are often brought to purposeful utilization to augment the profits. Indian silk industry is based on insect species *Antheraea mylitta* Drury and dozens of primary and secondary host plants. There are possibilities of a number of wastes, if we consider the whole process of tasar industry ranging from raising and maintenance of food plants, utilization of leaves for rearing, sorting of cocoons, preservation of seed cocoons, preparation of seeds (disease free laying), reeling spinning and weaving. The present communication is restricted to a few wastes or seemingly waste products from pupal stage which itself is a waste once subjected to reeling process. The secondary wastes referred in this communication are cocoonage (proteolytic enzymes elaborated by silkworm moths for its eclosion and becoming a waste if the seed cocoons are cut to facilitate emergence), pupa powder and pupa oil. Details of methods of extraction, quantification and possible utilization have been dealt in the present communication.
Keywords: Secondary waste, Extraction, Utilization, *Antheraea mylitta.*

INTRODUCTION

Secondary wastes or bye products of an industry are often brought to purposeful utilization to augment the profits. This vital aspect so far seems to have been neglected in tasar industry. Cocoonage (proteolytic enzymes elaborated by silkworm moths) acts on gummy substance of the cocoons and hydrolyses it into amino acids. Then cocoon fibres become loose and can be reeled and pupae from the cocoons will be obtained alive which can be further utilized for moth emergence, coupling and egg laying. The present process of reeling kills the pupae which are thrown away without any utilization though they are rich in proteins, carbohydrates, oils and minerals. Literature reveals that some studies have been done on the utilization of pupae of mulberry silkworm *Bombyx mori* L. (Basavanna et al., 1967 & Nagaraj and *Basavanna*, 1969). However no purposeful work has been carried out on the utilization of secondary wastes which will definitely enhance the profit of tasar industry. Hence, a study has been undertaken for the extraction, quantification and utilization of certain secondary wastes i.e. cocoonage, pupa powder and pupa oil of tropical tasar silkworm *Antheraea mylitta* Drury.

MATERIALS AND METHODS

The liquid (cocoonage) from 3,000 emerging moths of *Antheraea mylitta* was collected by means of a method similar to that of Kafatos and Williams (1964) with slight modifications in the sense that instead of capillary tubes, glass tubes bent in 'V' shape at an angle of 45° were used. The liquid was removed from the tubes within 10 to 15 minutes. The quantity of the liquid discharged by each moth which varied from 0.2 ml to 0.6 ml was stored at -10° C for study.

The pupae oil was extracted by Soxlet apparatus using petroleum ether (boiling point: 40-60° C). The extraction of the oil is completed in two hours. The oil was collected in a glass container and analysed for physical–chemical properties. To find out the composition of the pupae oil, the oil was hydrolysed with alcoholic sodium hydroxide (3.8 N). The sodium salts of the saponified oil were passed through a column of cation exchange resin (Amberlite IR-120) and the free acids are eluted from the column by repeated washing with alcohol. The mixture of free acids are then chromatographed on a column of silicon gel eluting with different proportions of benzene, ether and methanol mixtures and the fractionation was monitored by the help of thin layer chromatography (TLC) using solvent system, chloroform : methanol (96: 4, v/v). By repeated column chromatography followed by TLC, the compounds were separated. Nitrogen

content of the pupae powder was determined by Kjeldahl's method and crude protein was calculated by multiplying nitrogen percentage by 6.25. Carbohydrates and total minerals were estimated by the method of AOAC (1955).

RESULTS AND DISCUSSION

The physical and chemical properties of cocoonage as well as its effect on the softening of the tasar cocoons were tested. It is colourless and transparent. It changes into yellowish brown colour when exposed to air for 3-4 hours. It is basic in nature.

Effect on the softening of the tasar cocoons:

Normally, the tasar cocoons are prepared for reeling after soaking in 0.1% sodium carbonate solution for 12 hours followed by cooking at 15 lb/ sq. inch pressure for 3 hours. Cooking in high temperature affects adversely the tensile strength of the silk fibre. As such if this cocoonage is utilized for reeling, it may enhance the quality of the thread.

A batch of 10 cocoons separately was soaked in 1%, 5% and 10% aqueous solutions of this liquid (cocoonage) for 18 hours. It was found that the cocoons treated with 10% solution were completely reelable while those treated with 5% solution were partially reelable. The cocoons treated with 1% solution were not reelable. Thus cocoonage which becomes a waste if the seed cocoons are cut to facilitate emergence may be utilised in cold reeling of tasar cocoons. An investigation is also required to find out a substitute or to prepare an analogue of the cocoonage.

The chemical composition of the pupae powder has been presented in Table 1. It is evident from the table that the oil free pupae powder contains 80% protein, 10% carbohydrate and 10% minerals contents. Hence, it may be utilised in preparation of nutritious biscuits for human beings. Further, it can also be utilised as poultry feed and in pisci culture after taking a systematic study.

The physico- chemical properties and chemical composition of pupae oil have been depicted in Table 2 & 3 respectively. Judging from the physico- chemical characteristics, the pupae oil of *A. mylitta* appears to be suitable for manufacture of soap. The saponification value of the oil (169.2) is comparable to the I.S.I. specification for Mahua oil (187.2).

To explore the possibilities of utilizing pupae oil in soap making, the samples of washing soaps were prepared on laboratory scale using 100% oil and following the cold process. For preparation of soap, a known weight (100g) of the pupae oil was taken in a beaker. To this, 100 ml. of 25% aqueous sodium hydroxide was added slowly at room temperature with vigorous shaking until it was completely saponified. After saponification of oil, sodium silicate (5g) was added as filler with good stirring. The whole mass was then kept for setting in wooden moulds. The time required for satisfactory setting of the soap mixture was about 4 days. The soap formed was fairly hard and could be cut into desired shapes. The soap prepared has good cleaning properties and produced profuse lather with tap water.

In view of acute shortage of suitable oils for soap manufacture, the pupae oil has a distinct industrial future. The samples of the soap were prepared using simple facilities available in the laboratory and it is expected that better results can be obtained by employing suitable equipments and modern know-how of the soap making process.

Thus, it is inferred that extraction, quantification and utilization of the secondary waste will enhance the profit of tasar industry. Cocoonage and pupae powder may be utilised in cold reeling of tasar cocoons, preparation of nutritious feed for human beings, chicken and fish respectively. Pupae oil has a distinct industrial future. It may be utilised successfully in soap industry.

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Table1. Chemical composition of *Antheraea mylitta* pupae powder (Percentage on oven dry basis)

Sl. No.	Constituents (%)	Dried pupae powder	Pupae powder after extraction of oil
1.	Total protein	63-65	80
2.	Carbohydrate	7-8	10
3.	Total mineral	7-8	10
4.	Oil	20-25	-

Table2.Physico-chemical properties of pupae oil

Sl.No.	Physico-chemical properties	Solvent extracted oil from pupae of *A. mylitta*
1	Colour	Light yellow
2	Odour	Cod-liver oil like
3	Yield percent on pupae powder	20-25 %
4	Acid value	1.1
5	Saponification value	169.2
6	Unsaponifiable matter percent by weight	4.8 %
7	Ester value	168.0

Table3. Chemical composition of pupae oil

Sl. No.	Components	Pupae oil of *A.mylitta*
1	Saturated straight chain acids of C_{12},C_{14},C_{16}andC_{18} carbon atoms	34%
2	Unsaturated straight chain acids of C_{16}andC_{18} carbon atoms	30%
3	Hydroxy unsaturated straight chain acids of C_{16} and C_{18} carbon atoms	32%

UTILIZATION OF SECONDARY WASTE PRODUCTS OF SILK INDUSTRY

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ABSTRACT:
Proper utilization of secondary waste products of silk industry can generate extra income in addition to the silk the main out put in sericulture industry. Major wastes of silk industry are sericin, pupae, pupae skin, moths, silk worm excreta and silk fibre waste. It has been essential that ways and means are shown to the Indian sericulture to boost their income and by-products utilization could fill the bill. Realization of waste during mulberry and non-mulberry silk reeling is quite high (about 35-40 % of the raw silk reeled) and availability of mulberry dry pupae in India is approximately equal to raw silk production of 16525 tons. Pupae can be properly used to extract oil to the extent of 20 % (3305 tons). The large quantity of waste that accumulates in silk reeling process in India could be utilized to produce value added by-products with adoption of improved technology/process and the cost of silk production can be reduced by utilizing the wastes of the silk industry. Sericin powder can be prepared or extracted from silk industry waste water which can be a valuable ingredient for food items.The pupa skin which is available in abundantly in the reeling and grainage industries as a waste can be utilized as a high potential commercial raw material for various industries, including pharmaceuticals. Moths emerged from the double cocoons may be used for the preparation of commercial DFLs of multi x bivoltine as well as bivoltine hybrids. Silkworm pupae provide a source of human food and poultry feed. Their nutritional values are astonishingly high, containing large quantities of proteins (80%), fats, carbohydrates and vitamins. It has been atomized for use in cosmetic powders and it is also being used in production of soaps, shampoos, rinses and setting lotions as silk proteins have excellent properties such as giving the hair elasticity, lusture and firm lasting shape. The silk worm excreta provide a source of manure for agricultural products. Silk fibre waste obtained during processing of cocoons and reeling operations can be suitably used for conversion it into spun yarn (Ghicha, Katia, Jhuri, Balkal and core spun yarn) by spinning process. It has been evaluated that secondary waste products of silk industry can be exploited industrially in order to earn more profit in Indian sericulture industry.
Keywords: Silk, Reeling, Sericin, Pupae, Pupae skin, Moth, Silk worm excreta, Silk fibre waste.

INTRODUCTION

India is a second largest producer of silk in the world, next to the China and has a 13.4 % share in the global raw silk production. A major concern of the silk industry is the need to make the most efficient use of natural fibres, particularly in terms of utilizing waste products of silk industry. Silk waste itself arises from damaged cocoons or from cocoons which are difficult to unreel, together with waste fibre from the processes preparatory to spinning.

The present process of reeling kills the pupae which are thrown away without any utilization though they are rich in proteins (80%), fats, carbohydrates and vitamins. Literature reveals that some studies have been done on the utilization of by- products of silk industry (Sonwalkar, 1998 & Raju, 1996). However no purposeful work has been carried out on the utilization of secondary wastes of silk industry which will definitely enhance the profit of silk industry. In the present study an attempt has been made to collect the information on existing status of secondary waste products of Indian silk industry and on the basis of processing of cocoons. Hence, this paper describes an approach for proper utilization of secondary waste products of silk industry *i.e.*, sericin, pupae, pupae skin, moths, silk worm excreta and silk fibre waste from all four varieties of silk.

MATERIAL AND METHODS

Raw Materials

All four varieties (mulberry, tasar, eri & muga) of reelable and defective cocoons were collected from Central Tasar Research and Training Institute, Ranchi and different silk wastes with other by-products were obtained after processing (stifling or drying, cooking of cocoons and reeling) of cocoons.

Methodology

The study was conducted on sample basis and post cocoon parameters. All four varieties of reelable cocoons were dried separately and cocoon weight, shell weight, pupa weight were analyzed. The data was tabulated and analyzed by calculating percentage. The information on existing status of secondary waste products was collected from the selected respondents *i.e.*, office bearers of the post cocoon technology section/division, silk producers and was broadly categorized under two heads, namely, technical information, information about secondary waste products and their marketability. Technical information included type of machines and techniques for drying, cooking, reeling/spinning of cocoons and their availability. Information about secondary waste products of silk industry included waste generated in silk sector and their status. Marketing included types of by-products, methods of marketing and income. The datas were collected on existing status of secondary waste products of Indian silk industry.

RESULTS AND DISCUSSION

Information regarding type of machines and techniques for drying, cooking, reeling/spinning of cocoons and their availability were studied. It was found that for stifling of green cocoons mostly hot air drying machine consist of a chamber for cocoons, fitted with heating, air ventilation and air circulation facility are in use. For cooking of mulberry cocoons pan and circular pressurized cooking methods are in use whereas for cooking of tasar, eri, muga cocoons pressure cooker or traditionally methods are used. Silk reeling in India is carried out on Charkha, Cottage Basins (bivoltine raw silk recovery 66.3- 69.5 % and waste (%) on silk weight 14.1 - 16.7) and multiend reeling machine [bivoltine raw silk recovery 76.6 – 80.0 % and waste (%) on silk weight 7.2- 9.9] for mulberry reeled yarn and wooden Charkha, pedal/motorized reeling-cum-twisting four spindles machine [raw silk recovery 55.8 – 65.0 % and waste (%) on silk weight 35 - 40] for tasar reeled yarn. Spinning is carried out on Pedal/motorized Spun Wheel, Ambar Charkha, and other traditionally methods for various spun yarn. Natural fancy doupion silk can be made from double cocoons. Eri cocoons are open mouthed with a discontinuous filament, which make them suitable only for spinning. Approximately 90 percent of eri cocoons are hand spun in Assam, India. North eastern region is India's biggest consumer of eri (endi) pupae and live pre- pupae are sold in plastic bags, by weight (kg.) in local markets or by tin container at rural bus stops. The tribal communities rear eri and their source of income from ericulture is categorized into three heads i.e.(i) from eri spinning 48 %, (ii) from eri pupae 37% and (iii) from eri waste 15 %. Marketing information have found the eri pupae market with hundred of kilograms of silkworm pre-pupae fetching as much as around Rs. 70 per kg in urban areas, though average price in rural areas is Rs. 35 per kg.

Table 1: Chemical composition of silk filament	
Fibrion	72-81 %
Sericin	19-28 %
Fat and wax	0.5-1.0 %
Colouring matter + ash	1.0- 1.4 %

The chemical composition of silk filament is given in the Table 1.Cocoon filament is a typical protein fibre consisting of two filaments (brins) of fibroin cemented with sericin, besides other matters such as wax, fatty acids, inorganic matters and colours. The percentage of sericin and fibroin content present in cocoon filament is also influenced by the silkworm races.

Wastes generated in silk sector

The main object of silk industry is to produce silk cocoons by rearing the silkworm and produce raw silk. During the conversion or the processing of cocoons into raw silk filament, generation of waste at every stage is inevitable. The secondary wastes products of silk industry are sericin, pupae, pupae skin, moths, silk worm excreta and silk fibre waste. Wastes generated during various stages of silk production are:-

a) Floss generated during the deflossing of cocoon.
b) From defected cocoon (double, stained, malformed, flimsy etc.) which are sorted out before reeling.
c) Cut/Pierced cocoons which are produced during reproduction.
d) Peduncle waste from tasar cocoon.
e) Waste generated during cooking, reeling and re-reeling.

The large quantity of waste that accumulates in silk reeling process in India could be utilized better to produce value-added by-products. Realization of waste during mulberry silk reeling is around 35 % of raw silk and availability of mulberry dry pupae in India is approximately equal to raw silk production of 16525 tonnes. Pupae can be properly used to extract oil to the extent of 20 % (3305 tonnes) [1]. During rearing, pierced cocoons and double cocoons are created. Silk reeling generates brushing waste, end-missing cocoons and pupae. Other rejected cocoons and waste are re-processed into flow silk and spun silk yarns.

Table 2: Existing status of silk production and generated silk waste in India

Year	Production of Raw silk in Tonnes			Generation of silk waste in Tonnes		
	Mulberry	Wild/Vanya	Total	Mulberry	Wild/Vanya	Total
1995-96	12884	1025	13909	4022	208	4230
1996-97	12954	1172	14126	4000	247	4247
1997-98	14048	1188	15236	4215	235	4450
1998-99	14260	1284	15544	4250	253	4503
1999-00	13944	1270	15214	4153	248	4401
2000-01	14432	1425	15857	4237	305	4542
2001-02	15842	1509	17351	4655	319	4974
2002-03	14617	1702	16319	4514	336	4850
2003-04	13970	1772	15742	3764	373	4137
2004-05	14620	1880	16500	3587	365	3952
2005-06	15445	1861	17306	3705	297	4002
2006-07	16525	1950	18475	N.A.	N.A.	N.A.

(Source: Man made Textile in India, January 2007, N.A: Not available)

It is clear from Table 2 that the total raw silk production and silk wastes of mulberry is higher than that of non-mulberry silk, but during 2001-02 to 2005-06 (downward) decreasing of generated mulberry silk waste due to development of efficient cocoon cooking and reeling machines.

Utilization of secondary wastes products

Sericin: Sericin was extracted with 75% (v/v) ethanol to obtain crude powder. A novel effective technology for preparation and characterization of sericin powder extracted from silk

industry wastewater or degumming wastewater which is important for potential application of sericin to food manufacture [2].

Pupae: Silkworm pupae are a by-product of reeling industry and it is estimated that in case of mulberry silkworm, annually 1.5 lakhs tonnes of pupae are produced which is generally waste material. In some parts of India the silkworm pupae are regarded as delicious food for human due to their nutritional values [3].In dry eri pupae, the main constituents of eri pupae are found to be 62 % crude protein, 44 % soluble protein, 25 % total lipid and 5.2 % ash content. Eri culture is performed traditionally by the tribal communities of Assam and they rear eri for obtaining silk for clothing and to consume pupae as food item. The different items of cusine that can be prepared out of eri pre-pupae and pupae include fry, Pakori/chop, cake etc. The oil portion of eri pupae can be extracted from dry pupae powder by solvent extraction method. Eri pupae oil can be utilized in food industry as well as the source material for oleo-chemical industries [4].Waste silkworm pupae (SWP) generate vast resources of nutrients for livestock and poultry. SWP is one of the unconventional top class proteins (65-75%) and lipid feed which is a waste product of silk industry and is obtainable four times in a year. The effects of Silk Worm Pupae on growth and egg production performance are good. The efficiency by the birds receiving SWP was better as compared to the control. The study demonstrated that cheaper waste silkworm pupae could be an excellent substitute of costly protein concentrate in formulating diets for layers leading to increase profitability. The growth performance, egg production performance and profitability almost linearly increase up to 6% dietary levels [5]. In sericulture industry the most generated waste is pupae which can be utilized as inoculums in the fermenting media to produce protease enzyme through submerged fermentation [6].

Pupae skin: In Karanaka State of India, it is estimated that fresh pupa available from cocoons i.e. @ 80 % of weight of cocoon and pupae skin available from dry pupae i.e. @ 2% of weight of the dry pupa which is available in abundantly in the reeling and grainage industries as a waste can be utilized as a high potential commercial raw material for various industries, including pharmaceuticals [7].

Moths: Double cocoon formation is known to be caused by high density of worms on mountage which is genetical and is more common in uni and bivoltine strains. Indian bivoltines spin about 2-6 % of double cocoons. Double cocoons are processed to make doupion silk. Usually, double cocoons have one male and one female pupa. The moths emerged from the double cocoons may be used for the preparation of commercial DFLs of multi x bivoltine as well as bivoltine hybrids [8].

Silk worm excreta: The silk worm excreta provide a source of manure for agricultural products.

Silk waste fibres: The silk wastes form the bivoltine can be reutilized by extracting the fibroin powder using either formic acid or calcium chloride which may find many uses in diversified field like pharmaceuticals, food industry, cosmetics and bio-medical field [9, 10]. The natural silk wastes solution was prepared, using a high-frequency electromagnetic field which have been used to improve the properties of the locally produced Polyacrylonitrile fibres[11]. Due to the economic aspect, silk waste is not discarded and is used in producing yarns such as Spun silk, Noil silk, Ghicha, Jhuri, Balkal/Peduncle, Katia and core spun yarn etc. Using silk wastes and other raw materials like filaments and employing the non-woven production techniques like self/chemical/mechnical bonding diverse products could be produced.

CONCLUSIONS

In addition to the chief products of the silk industry, there are hosts of secondary waste materials (sericin, pupae, pupae skin, moths, silk worm excreta and silk fibre waste) eliminated during silk manufacturing, which have commercial value. Thus, using these wastes in an effective way is like fringe benefits to the silk producer. Silk waste fibres can be utilized to manufacture core spun yarns or silk blend yarns /fabrics such as silk/wool, silk/cotton, silk/jute,

silk/viscose etc. and it can be reduced the use of pure silks in the fabric without affecting the lusture and maintain demand of natural silks. Silk waste fibres can be used to develop non-woven product by chemical/mechanical bonding. Silk lovers get new products in a competitive way. It will generate market demand for diversified products with natural designs. The sericin, pupae and its content can be sold for fertilizer, feed stuff for animals, food industry, pharmaceuticals, cosmetics, bio-medical field and other agricultural purposes. This is of increasing importance in developing countries such as India, where the proper utilization of secondary waste products of silk industry can generate extra income in addition to the silk the main out put in sericulture industry and minimizing the cost of silk production. A systematic approach for its marketing would generate sustainable self employment for the villagers.

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WASTE PRODUCT OF ERI PUPAE - AN ALLURING DISH TOO

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ABSTRACT:
Most of the natural plant and animal products are serving as human food and around the many parts of the World the silkworm too exploited as potential diet. Ericulture, an subsidiary occupation of rural hilly tribal populace and the waste by-products of the culture is of immense potential in generating the sources of human food, chocolates, animal food, fertilizers, medicines, cosmetics etc., The advantage of taking out the live pupae without damaging the quality of silk, made the tribal communities use the pupae as rich food source. The richness of the pupae in protein (60%), fats (25%), Aminoacids (8%) Vitamins and minerals, made their use as food as the daily requirement of balanced diet. In terms of food value it is scoring on par with meat and fish. The eri pupae smoked in ash and hot coal, boiled in salt water followed by semi drying, looks like blackened peanuts or cashew nuts and can be used as ready to eat dishes. North eastern region of India, the live pupae are sold in local market at Rs. 25-60 per kg over different seasons approximately earning Rs. 20,000 per hectare per year remains hidden due to its localization. The percentage of earning from the eri culture other than its fabric amounts to 50 % indicates the potential of by-products, especially as cusines like fry, Pakori, Chop, Cake etc. The prospect of utility of eri pupae as an alluring dish in addition to its use in chocolate industry, animal feed, fertilizer, medicinal and cosmetic industries definitely encourages the producers of eri silk. This could ensure additional income for the eri rearers and in the upliftment of the socio- economic condition of the poor tribal people. Due to high richness of protein, fats, vitamins, minerals and completely free from cholesterol the eri pupae is the best diet to north eastern region of India, particularly Nagaland state. This potentiality can be exploited commercially by making use of systematic processing of the products.
Keywords: Eri silk worm, Eri culture, Pupae, by-product, cusine, alluring dish

INTRODUCTION

India's utilization of by products has not kept pace with the strides the country achieved in sericultural fronts over the years. There has been remarkable growth of these by product extracting activity since 1970s, because of realization on cost benefit ratio. Many by products presently discarded as wastes, which can be put to better use for amelioration of human beings and generation of value-based products thereby catapulting the industry to a more profitable and economically viable position. The total utilization of silkworm host plants, wastes of rearing, grainages, cocoons, pupal matter through different marketable products and such an integrated exploitation of the total resources will certainly make the Sericulture more sustainable (Herbert H.Ross et al, 1982, Shiva Prakash, 1988, Hisao Aruga, 1994, Katti et al, 1996, Satyanarayana Raju, 1996, Majumder, 1997, Mani, 1997, Sang Mi Han et al, 2002, Satyendra Kr Choudhury, 2003, Chinnaswamy et al, 2004). The production cost of silk can be reduced either by standardization of processing methods or utilizing the wastes of the industry and/ or applying the combination of both. The by product utilization deserves special mention and can be highly useful to Indian sericulture industry, which helps in socio economic up-liftment of the rearers, who are the mostly the rural poor people. Phyto technology for effective utilization of crop products and post harvest technology for conversion of wastes / by products to high value utilities (Majumdar, 1997) will cut down cost of production, avoid pollution, recycles resources is of today's high crave, because of ever growing population and their demanding wants.

PRESENT SITUATION

The rearing of eri silkworm is a heritage and almost a prerogative for the tribes in north eastern India. Ericulture is an ideal activity for development as subsidiary occupation in providing supplementary income to a large number of rural hilly and tribal populations (Alok Sahay et al, 1997, Chaoba Singh and Suryanarayana, 2003, Suryanarayana and Chaoba Singh, 2005). Majority of the host plants of eri silkworms are available in the region and the agro climatic conditions have restricted ericulture to limited pockets only. However, the recent studies

on biodiversity in wild silk moths of India have indicated a massive potential for ericulture in north eastern India. Presently, 1.20 lakh families, mostly belongs to economically poorer sections are involving in the practice and 96% of total erisilk production of the country is from this region. The ericulture is a village craft and the rearing, spinning and weaving activities are handed over from one generation to the other as the erisilk fabric, unique in appearance and aesthetic in appeal and also the national dress of the Assamese in North East India. The silkworm pupae are rich in biochemical compositions and can provide a source for staple food. Their high nutritional values of silkworm pupae with good quantities of proteins, fats, carbohydrates, minerals and vitamins, the chrysalis of ericulture is more important as delicacy than its silk (Chaoba Singh and Suryanarayana, 2003). Unlike other silkworms, eri pupae can be isolated from cocoons without damaging the silk as the cocoons were opened at one end. Further, this processes will facilitates smooth spinning of yarn in addition to the maintenance of cleanliness. Eri pupae is great delicacy and dietary staple for Rabhas, Bodos, Abor, Miri, Kachari, Garos, Khasi, Naga, Adis, Mizos, Syntengs tribals of Tibeto Burman and Indo mongoloid origin of North East India (Chaoba Singh and Suryanarayana, 2003). Tribals consuming the eri pupae for their taste, were unknowingly consuming rich protein food material, which was proved later by scientists (Alok Sahay et al, 1997). In Meghalaya, Karbi Anglong areas of Assam, pupae are dried and smoked in ash and hot coal and in other areas the boiled and semi dried pupae look like blackened pea nuts/ cashew nuts, palatable as peanut shells. The delicious food [endi] items like fry, pakori/chop and cake etc., can be prepared from pupae and in powder form this will be used in soups and sauce as protein source. Silkworm pupae were eaten by Chinese as food (Roychoudhury and Joshi, 1995) and in Japan cakes are prepared and sold as silkworm pupal cakes due to their high nutritive value (Majumder, 1997). In Hong-Kong, China, Korea and Japan the healthy silkworm larvae are sterilized, vacuum dried and sold as commercial food (Ramakanth and Anantha Raman, 1997). In Africa, the mature larvae of Saturniids used as a garnish in raw, dried and powdered form for human consumption and the dried product of pupae, the peaggie and also the roasted pupae are consumed as food in Western United States. Hence the scope of systematic commercialization of the dietary value of the pupae will boost Sericulture in general and ericulture in particular by generating additional income to the rearers.

NUTRITIVE VALUES

Pupae contain crude protein (55-60 %), total lipids (26%), free amino acids (5-8%) and 100 gms of dried eri silkworm pupae can provide 75% daily protein requirement of human individual (Chaoba Singh and Suryanarayana, 2003). The vitamins like pyridoxal, riboflavin, thiamine, ascorbic acid, folic acid and minerals like calcium, iron and phosphorus make the pupae more nutritive (Roychoudhury and Joshi, 1995, Chaoba Singh and Suryanarayana, 2003). In terms of protein, fat, vitamins and calories the eri pupae are equal to meat, but for certain degree of indigestibility. However, the bio chemical analysis reveals that the pupae are of highly nutritive value and found better than the protein of soya bean, fish or beef. The exoskeleton of pupae contains large amounts of crunchy chitin, which can supplement cereal diet of rural people. During the months of March and May every year the rearers will harvest pupae from cocoons, boil in the evenings and subsequently put them for drying and the methods will vary at different places. The use of pupae in chocolates, in chilli sauce has vast potential for commercializing the concept. The domestic silkworm pupae have rich nutritive values with water, protein, fat, glycogen, chitin and ashes (**Table-1**) and rich proportion of essential amino acids, vitamins, minerals and lipid fractions (**Table-2**).

OTHER USES

The pupae oil has got wide uses in oelo chemical, soap and food processing industries. The eri pupae are being used as poultry or fish feed in other states. When used as poultry feed hens improved their egg laying capacity with impact on the colour of the egg yolk (Hisao Aruga, 1994). Pupal fat is good raw material in soap industry, glycerin and cosmetics and fat free pupae used as feed of carps and fish for better yields. Pupal protein is used as raw material for preparing amino acids and flavoured products with high nutritive value (Hisao Aruga, 1994) and fertilizer can be generated from the pupal excreta (Shiva Prakash, 1988). The n-triacontanol, a plant growth promoter is found in good quantities and being extracted from silkworm and the artificial fibres and membranes are also prepared from proteins of pupae (Majumder, 1997). Chitin, a component of pupal skin used in different applications like additive to increase the loaf volume in wheat flour bread, in post operational treatments such as conchotomy, deviatomy, polypectomy because of its easy usebility, less hemophase, greater pain relief and fastens healing of wounds (Katti et al, 1996). Chitin found as potent antimicrobial against Staphylococccus aureus, Klebsiella pneumoniae, Asppergillus niger etc., anti fungal against Trichophyton equinum, its buffering activity against acids, as food additive to control carcinogenicity of food stuffs (Katti et al, 1996). Chitin was also used as Immunoadjuvant (antiviral agent), bacteriostatic agent, fungistatic agent, anti-sordes agent in preventing carcinogenic bacteria from teeth and bio compatible membrane to check bleeding in major surgeries (Katti et al, 1996). Silkworm proteins in the form of Serratio peptidase is used in pharmaceuticals for anti-inflammatory, anti-tumefacient action i.e.inflammation of acute sinusitis, tonsiloctomy, oral surgery, during filling, cleaning and taking out teeth. Serratio peptidase was used as enzyme tablets along with antibiotics in treatments for bronchitis, pulmonary tuberculosis, urinary tract infection and to control post operative inflammations in orthopedics (Teotia, 1988). Certain proteins of silkworm and pupae used as specialty diets for cardiac and diabetic patients because they are easily digestible and reduces cholesterol and blood sugar by providing additional energy and Shinki Fibroin, the derivative of silkworms is used in protein and amino acid extraction in Japan (Ramakanth and Anantha Raman, 1997).

SCOPE FOR EXPANTION

In North East India, there is no control, restriction, rules and regulations on the harvesting and sale of pupae and that was informal, but in recent years the trade is commercially increasing. At rural bus stops, the pupae were sold in kgs kept in baskets, which can be eaten dry as crisp or hydrated again and served as stew or fried. The marketing surveys reveal that hundreds of kgs of pupae were sold at Rs.25/- to 60/- and edible pupae alone will fetch Rs.19440=00 per ha/yr (Chaoba Singh and Suryanarayana, 2003). Source of income from ericulture will be from spinning (48%/, from eripupae (37%) and from eri waste (15%) (Alok Sahay et al, 1997, Chaoba Singh and Suryanarayana, 2003, Suryanarayana and Chaoba Singh, 2005). The eri pupae contain 16% oil and can be extracted from dry pupae powder by solvent extraction method using petroleum ether and this oil with higher amount of unsaturated fatty acids is of great use in oleo chemical industries and the better safonification value over rice-brawn oil is advantageous for use in the soap industries (Shiva Prakash, 1988). Important characteristics of oil like refractive index-1.47 at 30 0C, acid value-67.37, safonification value-150.88, iodine value-174.91, cholesterol %- 0.36 made its great prospect of utility in food industry (Satyendra Kr Choudhury, 2003). One of the main objectives of ericulture of the region was production of eripupae for human diet being rich source of proteins, vitamins and hormones but the diversification and commercial approach was lacking (Chaoba Singh and Suryanarayana, 2003, Suryanarayana and Chaoba Singh, 2005). Further, the potential of silk pupae oil extraction and related activities is not fully attained and still there is wide scope for

exploitation. By increasing the production of eri silk and making the availability of eri pupae in large quantities, the concept of their dietary utilization can be commercialized. The prospect of utility of eripupae and its oil in other profitable areas will definitely encourage the producers of eri silk to enhance its production, because of the importance of chrysalis as delicacy and silk considered as a by product. The pupal skin which is available abundantly in the reeling and grainage sectors as a waste can be utilized as commercial raw material for various industries, including pharmaceuticals (Katti et al, 1996, Sang Mi Han et al, 2002).

CONCLUSION

By-product utilization hopefully can play a crucial role in the coming years to make the sericulture an economically viable proposition enabling it to withstand competition from other cash crops. The useful utilization of byproducts through indigenously available processing techniques brings additional income from Sericulture by cutting down the cost of the silk in addition to the socio economic up-liftment of the rearers. Need for introduction of integrated processing complexes with redesigning of present practices deserves special mention to make the practice more attractive and people to participate enthusiastically due to additional income. The operation of nutritive values of pupae through methodical dispensation certainly boosts up the ericulture as versatile and sustainable among the commercial crops. The effective by-product utilization from Indian sericulture industry in general and from ericulture in particular can play a crucial role in making it viable and to withstand the competitive competition.

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Table-1 : Biochemical constituents of pupae in percentage of domestic silkworm (Hisao Aruga, 1994 & Roychoudhury and Joshi, 1995)

Pupal form	Water	Fat	Protein	Glycogen	Chitin	Ash
Dried	7.18	29.57	49.98	4.65	3.73	2.19
Sqashed	6.32	15.20	60.77	5.78	4.63	2.73
Fat free	5.49	00.47	72.82	6.92	5.55	3.27

Table-2 : Particulars of essential amino acids (mg/g), vitamins, minerals and lipid fractions in deoiled pupae of domestic silkworm (Roychoudhury and Joshi, 1995 & Alok Sahay et al, 1997)

Essential amino acids		Vitamins	Minerals	Lipid fractions
Amino acid	Qty. (mg/g)			
Glycine	14	Pyridoxal	Calcium	Glycerol trioleate
Aspartic acid	21	Riboflavin	Iron	Neutral lipid
Glutamic acid	05	Thiamine	Phosphorus	Phospholipids
Serine	05	Ascorbic acid	-	Cholesterol
Threonine	07	Folic acid	-	Lionolenic acid
Alanine	94	Nicotinic acid	-	-
Lysine	05	-	-	-
Arginine	19	-	-	-
Valine & Metionine	199	-	-	-
Leucine & Isoleucine	500	-	-	-

EFFICACY OF PROLIFIC COMPOST PRODUCED BY RECYCLING THE SERI-FARM RESIDUE

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ABSTRACT:

Advent of modern technologies to exploit the full potentials of high yielding varieties of mulberry (Morus. sp), though has helped in improving the productivity has also adversely affected the soil health and resulted in eco-imbalance.
In order to reach higher productivity, conserve the natural resources and revive soil health, replacement of harmful chemical fertilizers with safe organic inputs to the maximum extent is the need of the day.
Victory 1 (V1) mulberry variety require 350 kg of Nitrogen every year to exhibit its full potentiality in qualitative and quantitative parameters. If this has to be met in toto, 30 MT compost has to be applied twice a year. However, non availability of such a huge quantity of compost with required Nitrogen levels (2%) might divert back the cultivators to indiscriminate use of chemical fertilizers.
Silkworm rearing conducted by utilizing one hectare of mulberry also generates 25 MT seri-farm residues, which could be effectively recycled in the farm itself for converting it as prolific compost with as much high Nitrogen content as 1.26 to 2.46 % in different composting methods. Besides, the soil micro-organism population is retained to the optimum levels on application of compost produced by recycling the seri-farm residues.
The paper discusses the methodologies adopted to effective utilization of seri-farm residue for compost making and its efficacy on productivity.

Keywords: Seri-farm residue, Compost, Nitrogen level

ECONOMICS OF RECYCLING SERI-FARM RESIDUE FOR COMPOST MAKING

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ABSTRACT:

The major attraction of sericulture is its farm based activity which highly suits small and marginal farm holdings with low capital investment. However, consequent to the evolution of high yielding mulberry varieties, the cultivators are forced to use voluminous chemical fertilizers to gain maximum productivity, which besides deteriorating soil health also culminate the environment. To avoid this, farmers resort to organic farming. But, the limiting factor is availability of such a huge quantity of compost (30 MT/ ha/ yr) at lower cost.
Combating the above problem by effectively recycling the seri-farm residue generated in the very mulberry farm itself can induce economic feasibility with better cost benefit ratio. While 25 MT of seri-farm residue is generated by operating 1 hacter of mulberry, its convergence cost ranges from Rs 80/- to 100/- per MT and compost worth Rs 1.5 lakhs can be produced with assured cost benefit ratio of 1:3.5, 1:2.4 and 1:2.3 in pit composting, windrow composting and vermin composting respectively.
The paper discusses the expenditure involved in three different methods of composting and the financial benefits accrued while converting the seri-farm residue into good quality compost.

Keywords: Seri-farm residue, Economics, Cost benefit ratio

EXCRETION OF LITTER BY TROPICAL TASAR SILKWORM, ANTHERAEA MYLITTA DRURY DURING RAINY CROP

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ABSTRACT:

In India, cottonseed production during year 2007-2008 is estimated as 10.3 million tonnes. The seeds when scientifically processed yield linter as important by-product apart from hulls, oil and meal. At present, linters are extracted by saw type delinter. Attempt was made to extract linters by double roller (DR) gin which is extensively used for ginning cotton in India. Objectives of present study is to (1) measure the power requirement for extracting linters, recovery of linters by saw type delinter and double roller (DR) gin and (2) assess the properties of linters, and paper prepared from linters. The cleaned cottonseed (DR ginned) was used for the delinting trials on the saw type delinter and roller gin. Microcontroller and PC based experimental setup was specially designed to measure the power requirement in both the machines. Experiments were conducted and analyzed as completely randomized design replicated two times. It was observed that the average linter recovery, processing capacity and power requirement(for extracting 100 kg linters) on saw type delinter & DR gin were found to be 4.86% and 0.85%, 307 and 301 Kg cottonseed/h, 67.5 and 111 Kwh respectively.
During cleaning of linter, it was observed that in case of DR gin, the linter % is 15.8% higher than the saw type delinter (76.83%) and found to be 89.03%. The cleaned linter samples were kiered with 3% NaOH in an autoclave for 3 hours and washed thoroughly then converted into pulp. Pulp samples were bleached using hypochlorite 4% for 2 hours at room temperature. Air dried bleached and unbleached linter samples were beaten in a valley beater upto 300 ± 25 CSF and sheets of 60±2 GSM were prepared. For bleached paper, studies revealed that Burst Factor, Tear Factor, Breaking Length, Double Folds, and Brightness in DR gin are far better than saw type delinter and found to be 16.67 and 10 (kg/cm^2), 210.8 and 197 ($mN.m^2/g$), 1925 and 1094(meter), 11and 2, 78.5 and 74.6 (%) respectively. For unbleached paper, studies revealed that Burst Factor, Tear Factor, Breaking Length, Double Folds, and Brightness in DR gin are also far better than saw type delinter and found to be 18.3 and 13.3 (kg/cm^2), 278.8 and 190.4 ($mN.m^2/g$), 2513 and 1263 (meter), 20 and 5, 60.8 and 48.6 (%) respectively. Looking at better product properties with less productivity and higher energy requirement, the design modification in DR gin is needed in future.

Keywords: Antheraea mylitta, Terminalia, Shorea, Eco-race, Instar, Excretion, Waste.

SILKWORM PUPAE– A POTENTIAL MULTI-PRODUCT FEED

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ABSTRACT:

Many insects are excellent and nourishing food. Mankind eats many curious things including oysters, shrimps, whelks, cockles and even snails uncooked. Use of edible insects has been reported in India from centuries. There are at least 52 species reported belonging to 45 genera, 26 families and 10 orders. Nutrient analysis of atleast 20 species in India encourages insect/insect pupae consumption especially in view of the fact that many people cannot afford fish or other animal meat. Amongst these, pupae of silkworms of ***Bombyx mori*** and ***Bombyx*** species (Bombycidae family) and ***Antheraea mylitta***, ***Antheraea paphia***, ***Antheraea roylei***, ***Antheraea assamensis***, ***Samia cynthia*** and ***Samia ricini*** (Saturnidae family) which produce silk, are treated as high-protein food in India and their rearing residue as pond fish-feed. Besides, other silkworm species also have great multiple-product potential in the region. Tribes residing in the fringe and core regions of the forest are invariably the silkworm eaters in the form of CHIND KIRA or GURMARI KIRA. They suffice their nutrient supplement through these insect feed. Silkworms are naturally the healthiest insects one can get to feed themselves. One cannot just beat the low fact content and the nutritional value of a silkworm. They and their pupae are a high source of Calcium, Protein, Iron, Magnesium, Sodium and Vitamins B1, B2 and B3. The guaranteed analysis say the silkworm consists of Crude Protein (Min) 45%, Crude Fat (Min) 20%, Crude Fiber (Max) 5%, Moisture (Max) 12% and Ash (Max) 5%. Silkworm larvae are building a strong demand as they are simple to care that require dry environment, excellent for pregnant females due to their high calcium content and also produce life saving proteins.
The subject rests in darkness precisely because the people who practice the habit of consuming larvae or pupae as food are not those of whom the civilization reaches. It is also feared that the spread of civilization may lead to the total abolition of these interesting practices before we know about them.

Keywords: Silkworm, Pupae, multi-product feed.

BY PRODUCT UTILIZATION IN ERICULTURE INDUSTRY

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ABSTRACT:
Among the commercially exploited wild sericigenous insects for silk production *viz.,* tasar, oak tasar, eri and muga, eri silkworm is domesticated. Ericulture is believed to have originated in India by 1600 B.C. Eri silkworm *Samie cynthia ricini* has been reported from the Palearctic and Indo-Australian bio-geographic region. Ericulture has been introduced in Korea, Indonesia, Thialand, Vietnam, Laos, Cuba, Uruguay, Egypt, France, Italy and many other countries. In India, eri moths are predominantly found in the sub-Himalayan belt of North-east India from the lowest level of plains up to an altitude of 5000' ASL with temperature ranging from 13ºC to 38ºC and also in the dense semi-deciduous forests of central and eastern India. In India, it is mostly confined to Assam, Meghalaya, Nagaland and Manipur states and also practiced in a small quantity in states like, Andhra Pradesh, Bihar, Orissa, West Bengal, Uttar Pradesh, Madhya Pradesh, Uttaranchal, Jharkhand etc.
The production of Eri raw silk in India has gradually increased from 95 MT in 1950-51 to 1485 MT during 2007-08 registering a significant increase. The domesticated indoor rearing of eri silkworm under controlled conditions, hardy nature of silkworm, thermal property and incredible durability of the fabric, nutritive value of eri pupae, and large production of vegetable biomass for fire wood, roofing material etc, have attributed towards the successful growth of the eri sector. Amongst all the wild silks, eri silk production is gradually increasing over the years for the past five decades and accounts for nearly 78% of total non-mulberry silk production and 7% of total raw silk production in India. The eri cocoons unlike other cocoons are open ended, hence, the traditional eri rearers spin the cocoons on traditional device "takli" after degumming and the spun yarn thus produced is woven into fabric on their own loin looms. Its matt finish combined with a natural sheen makes its appearance unique. The special thermal properties of eri silk supplement the requirement of warm clothing to some extent. Of late, introduction of new machine for spinning of eri cocoons has given rise to finer yarns paving the way to a multiplicity of designs and products including blends. The new technology of slivering eri cocoons for mill processed spun yarn has also opened up several new possibilities in creative weaving. The eri pupae have a high calorific value of 460 kcals/100g on dry basis and 133 kcals/100 g on fresh weight basis, which is higher to Cows milk (69 kcals/100 ml), eggs (163 kcals/10 g), Chicken (120 kcals/100 g), White sugar (385 kcals / 100 g) and raw Carrot (42 kcals/100 g). Further, the eri pupae are rich in protein (53.3%), fats (25.6 %) and carbohydrates (4.4%). Pupal protein contains all essential amino acids including high concentration of leucine, which is important in human nutrition. The pupa also has sufficient quantities of calcium, iron and other minerals required for growth and development, all together making it comparable to meat and fish. Consumption of pupae can substantially supplement the protein starved rural populace, beside usage in poultry and fish feed in addition to income generation through silk production. The traditional pupal recipes basically involve frying, deep frying, baking and boiling with spices. The pupae in these cases are used fresh and the food prepared is highly perishable. Value addition can be enhanced by suitable preservation methods and by conversion of silkworm pupae into convenient processed products for wider market acceptability in different regions. Another important byproduct is the eri pupal oil, which has a refractive index (at 30^{o}C) of 1.47, comparable to other common vegetable oils. Rancidification in pupil oil is slower because of its low saponification value (150.88). These properties of eri pupal oil have vast application in food, medicine and oleochemical industries.
With the perspective for value addition and export of the silk products and multiple uses of eri silkworm pupae for human diet, medicine, industry etc., eri culture has tremendous potential in the non-traditional niches not only in India but also many other countries across the world where food plants like castor & tapioca are traditionally cultivated for agricultural purpose for production of castor beans and tubers respectively, where partial use (25-30%) of foliage can make this a regular source of supplementary income to the farmers.
Though Ericulture industry is mainly promoted for the production of Eri Spun Silk for textiles purpose, however during the course many waste like eri pupae, stems, rearing waste, silk waste etc. are also generated which can be harnessed for a variety of purposes. The paper discusses the assessment & processing of secondary waste products in Ericulture.

Keywords: Eri Silkworm, *Samie cynthia ricini,* Castor, Tapioca, Eri Silk, silkworm pupae, waste products.

Among the commercially exploited wild sericigenous insects for silk production *viz.,* tasar, oak tasar, eri and muga, eri silkworm is domesticated. Ericulture is believed to have originated in India by 1600 B.C. Eri silkworm *Samie cynthia ricini* has been reported from the Palearctic and Indo-Australian bio-geographic region. Ericulture has been introduced in Korea, Indonesia, Thialand, Vietnam, Laos, Cuba, Uruguay, Egypt, France, Italy and many other countries. In India, eri moths are predominantly found in the sub-Himalayan belt of North-east India from the lowest level of plains up to an altitude of 5000' ASL with temperature ranging from 13ºC to 38ºC and also in the dense semi-deciduous forests of central and eastern India. In India, it is mostly confined to Assam, Meghalaya, Nagaland and Manipur states and also practiced in a small quantity in states like, Andhra Pradesh, Bihar, Orissa, West Bengal, Uttar Pradesh, Madhya Pradesh, Uttaranchal, Jharkhand etc.

The production of Eri raw silk in India has gradually increased from 95 MT in 1950-51 to 1485 MT during 2007-08 registering a significant increase. The domesticated indoor rearing of eri silkworm under controlled conditions, hardy nature of silkworm, thermal property and incredible durability of the fabric, nutritive value of eri pupae, and large production of vegetable biomass for fire wood, roofing material etc, have attributed towards the successful growth of the eri sector. Amongst all the wild silks, eri silk production is gradually increasing over the years for the past five decades and accounts for nearly 78% of total non-mulberry silk production and 7% of total raw silk production in India.

The eri cocoons unlike other cocoons are open ended, hence, the traditional eri rearers spin the cocoons on traditional device “takli” after degumming and the spun yarn thus produced is woven into fabric on their own loin looms. Its matt finish combined with a natural sheen makes its appearance unique. The special thermal properties of eri silk supplement the requirement of warm clothing to some extent. Of late, introduction of new machine for spinning of eri cocoons has given rise to finer yarns paving the way to a multiplicity of designs and products including blends. The new technology of slivering eri cocoons for mill processed spun yarn has also opened up several new possibilities in creative weaving.

The eri pupae have a high calorific value of 460 kcals/100g on dry basis and 133 kcals/100 g on fresh weight basis, which is higher to Cows milk (69 kcals/100 ml), eggs (163 kcals/10 g), Chicken (120 kcals/100 g), White sugar (385 kcals / 100 g) and raw Carrot (42 kcals/100 g). Further, the eri pupae are rich in protein (53.3%), fats (25.6 %) and carbohydrates (4.4%). Pupal protein contains all essential amino acids including high concentration of leucine, which is important in human nutrition. The pupa also has sufficient quantities of calcium, iron and other minerals required for growth and development, all together making it comparable to meat and fish. Consumption of pupae can substantially supplement the protein starved rural populace, beside usage in poultry and fish feed in addition to income generation through silk production. The traditional pupal recipes basically involve frying, deep frying, baking and boiling with spices. The pupae in these cases are used fresh and the food prepared is highly perishable. Value addition can be enhanced by suitable preservation methods and by conversion of silkworm pupae into convenient processed products for wider market acceptability in different regions. Another important byproduct is the eri pupal oil, which has a refractive index (at 30^{o}C) of 1.47, comparable to other common vegetable oils. Rancidification in pupil oil is slower because of its low saponification value (150.88). These properties of eri pupal oil have vast application in food, medicine and oleochemical industries.

With the perspective for value addition and export of the silk products and multiple uses of eri silkworm pupae for human diet, medicine, industry etc., eri culture has tremendous potential in the non-traditional niches not only in India but also many other countries across the world where food plants like castor & tapioca are traditionally cultivated for agricultural purpose for production of castor beans and tubers respectively, where partial use (25-30%) of foliage can make this a regular source of supplementary income to the farmers.

Though Ericulture industry is mainly promoted for the production of Eri Spun Silk for textiles purpose, however during the course many waste like eri pupae, stems, rearing waste, silk waste etc. are also generated which can be harnessed for a variety of purposes. The paper discusses the assessment & processing of secondary waste products in Ericulture.

ULTRASTRUCTURAL CHANGES DURING INFECTION OF CYTOPLASMIC POLYHEDROSIS VIRUS IN MIDGUT CELLS OF TASAR SILKWORM, *ANTHERAEA MYLITTA* (D)

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ABSTRACT:

The virus, Cytoplasmic Polyhedrosis Virus (CPV) produces polyhedral inclusion bodies (PIBs) in the cytoplasm of midgut columnar epithelium of silkworm larvae, *Antheraea mylitta*. Upon ingestion, these PIBs are solubilized in midgut lumen due to alkaline pH and release viral genome that binds to the receptor on the epithelium. Electron microscopic study revealed sub cellular alteration in the midgut columnar cells, showing swollen mitochondria, heavy vacuolization and disorganized, shortened and confluent microvilli. However, the virus particle first appeared in virogenic stroma with many mature virions were occluded in to crystalline protein forming polyhedra in to the midgut cells cytoplasm.

Keywords: Cytoplasmic Polyhedrosis Virus, polyhedral inclusion bodies, *Antheraea mylitta*(D). Midgut, columnar epithelial cell, microvilli

THE SWOT METHOD FOR ENERGETIC POTENTIAL OF MORUS BIOMASS PLANTS

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ABSTRACT:
The paper work presents the research results regarding the economical assessment of energetic potential from mulberry biomass, belonging to different Morus specie, of C.S. Sericarom – Research Department's patrimony.
The moriculture represents the mulberry plants cultivation, with multiple destinations – sericulture, phyto remediation, phyto pharmaceutical, agro alimentary production, etc. By the SWOT method, it was estimated the economical potential of Morus sp. plants' biomass, for an intensive cultivation system, as regenerated energy resource. This biomass can be the energetic source, by using biomass conversion technologies in order to obtain bio fuel (burning and gasification).
The mulberry intensive plantations' agro productive potential is between 20.000 – 100.000 kg biomass/ha (vegetative and wooden biomass); the mulberry plants regenerate annually in a constant manner the vegetative biomass, starting with the 3rd year after planting.
Keywords: mulberry varieties, biomass, energy, biogas.

INTRODUCTION

The moriculture represents an obtaining phytotechnology of some products with higher commercial value, being part of natural silk production system. Since the silkworms rearing activity is decreasing at international and national level, it was necessary a diverstification of exploatation methods for mulberry plantations. The R&D activities firstly targeted the available fields cultivated with different mulberry varieties and their agroproductive potential regarding the total biomass production.

There were made researches regarding mulberry plants utilization for phytoregenerable activity (environment decontamination, polluted with traffic Pb), phytopharmaceutical (therapeutic products obtained from mulberry roots, fruits and leaves), agroalimentary production (half-finished sugar product from mulberry fruit and proteic concentrate out of mulberry leaves). Lately, it was evaluate the economical potential of Morus plants as regenerable energy source, for an intensive cultivation system.

MATERIALS AND METHODS

The biologic material it was represented by 16 mulberry varieties, for somatometric determinations in order to estimate the maximum biological and technological production (leaves and wood biomass) for the traditional system (8000 plants/ha) and intensive system (20000 plants/ha), specific for the Bucharest Baneasa area conditions (photo no.1). These mulberry varieties are part of the C.S. SERICAROM Research Department's patrimony, with a total number of 60 mulberry varieties.

The vegetal biomass is represented by mulberry leaves, mulberry herbaceous sprouts and mulberry wooden material. For the economical potential evaluation of Morus plants' biomass, it was used the SWOT method, to distinguish the characteristics of this type of bio resource as regenerated energy source.

The SWOT method is a diagnostic- analyze and represents a preliminary investigation that reveal it's strong and weak points (strength-weaknesses, opportunity – threats). This method content an internal analyse – regarding the productiv potential of resulted biomass from moriculture, as regenerable energy source within the units who realise its trasformation into

biogas or by direct burning and external analyse, of profit maker opportunities for biomass and/or biogas, for internal consuption or for market.

The biogas is an anaerobe fermentation product of biomass organic waste. These wastes may have different origins (animal waste, agriculture products and wastes, vegetal waists, house organic waists, residual waters, etc.). The biomass treated by anaerobe fermentation produce biogas with 60-70% marsh gas who can be used directly as fuel and also as commercial compost such as high quality fertilizer (about 5% from the initial volume).

At UE level, the biomass is recognized as major regenerated energy source, with a quota of 63% out of the total regenerated energy consumption. The technologies evolution based on biomass will be an important bench-mark of the strategies regarding the energy markets globalization. It is estimate that it can be produced about 0.5 m^3 biogas/kg of dry material. In order to evaluate the biogas obtaining potential, there were considerate as reference values, the following organic materials:

Nr. crt	Organic material	Quantity kg	Biogas / average m^3
1	Dry leaves	50	22
2	Green vegetal wastes	50	35
3	Corn cob	50	40

A sustainable technical solution, in order to obtain biogas out of household/farm stead organic sweeping, it was elaborate by the C.S. ICPT Tehnomag Cluj Napoca, Romania. In this installation, the fermentation lasts 20 days, at 30^0C, being a discontinuous installation and the raw material deposit capacity is 170 kg composite.

RESULTS AND DISCUSSIONS

Determination of agro productive capacity (mulberry leaves and total vegetal biomass production) distinguishes the following parameters values for 2 types of cultivation systems concerning 8000 plants/ha and respectively 20000 plants/ha (Table 1):

Table nr. 1

The vegetal biomass productivity of mulberry varieties

Variety	Mulberry leaves maximum biological production traditional system Kg/ha	Mulberry leaves maximum technological production traditional system Kg/ha	Mulberry leaves maximum biological production intensive system Kg/ha	Mulberry leaves maximum technological production intensive system kg/ha
Hu San 1	18160	13620	45400	34050
Hu San 2	24560	18420	61400	46050
Thie-Ba	20480	15360	55200	38400
Hu San 32	17760	13320	58500	33200
Hu San 199	18800	14100	47000	35250
Ichinose	21200	15900	53000	39750
Kokuso 21	**30720**	**23040**	**57600**	**91400**
Ken Mochi	20400	12500	51000	31250
Kayrio Nezumigaeshi	9520	7140	21300	19040
Ukraine 9	16400	12300	41000	30750
Ukraine 107	15840	11880	39600	29700
Eforie	11360	8520	28400	21300
Lugoj	19440	14580	48600	36450

Calafat	20240	15180	50600	37950
Galicea female	21200	15900	53000	39750
Galicea male	16320	12240	40800	30600

The table data show that the mulberry leaves production is between 7.140 – 30.720 kg/ha for Kayario Nezumigaeshi and Kokuso 21 mulberry varieties in the traditional system. The biomass production has values between 19.040 – 91.400 kg/ha for the same varieties in the intensive system.

The SWOT analyze distinguished:

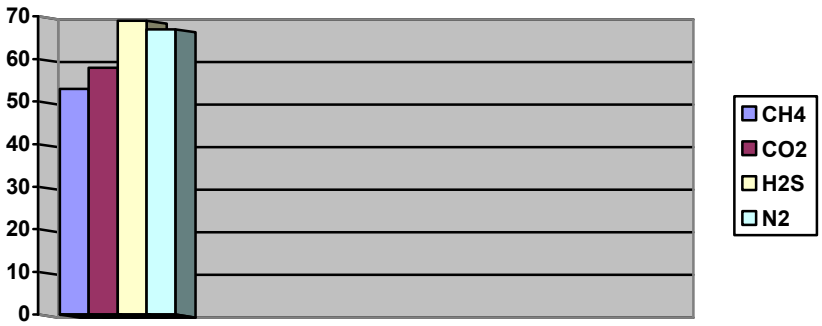
- **Strong points:** the vegetative regeneration capacity of Morus plants, after the exploitation cuttings (vegetative biomass harvesting: leaves, herbal sprouts and wood), corresponding to the spigot cutting, based on dormant bud's excitability and the very strong external radicular system;
- the rustic character of Morus plants phenotypes, these ones being able to be cultivated on different soil type (soil restriction are low) and the requests for water and nutritive elements aren't high; the chemical content of mulberry leaves indicate a high level of organic substances (protein, lipids, cellulose), according with the table 2:

Table nr. 2

Chemical content of mulberry leaves

Sample no.	Varieties	Dry substance %	Raw protein %	Lipids %	Cellulose %	Ashes %
176	Ken Mochi	-	14.38	1.16	11.12	17.21
177	Hibrid 2002	-	16.53	1.76	13.38	12.51
178	Ucraina 9	90.29	13.08	1.08	12.29	15.63
179	China 2	91.03	19.61	1.92	12.13	9.71

- the biomass vegetal price, represented by herbal sprouts and wood is 3.0 €/t, being a referential price, because this biomass is considered as waste out of silkworms rearing activity from sericultural plantations
- for mulberry plantation exploitation as regenerated energy resource as biogas, the vegetal biomass price is 27 €/t for the poplar wood used for cellulose (price available in Romania);
- existence of hashing agricultural equipment for vegetal biomass (herbal and wooden sprouts);
- the resulted fertilizers may be totally used in Morus plantations or agricultural crops;
- the biogas content, according with its fermentation period, is pointed out in graphic nr 1:



Graphic 1. Biogas evolving variations (%)

- the biogas quantity obtained from 1 m^3 vegetal biomass, is 42.5 m^3;

- the Morus plants biomass may be used in different organic mixtures in order to obtain biogas (with birds waste, animals waste, purification stations sludge, corn cob chaff, etc);
- in sericultural farms the obtained biogas may be used for rearing spaces warming or for silk cocoons drying, having in this way a close circuit of biomass from mulberry plantations;
- economical data concerning the quantitative and financial values of the utilization of mulberry biomass revealed the maximum quantum of €4000 – 10600 for pulp cellulose post industrial and about €1500 – 3000 to obtain biogas (Table 3)

Table nr 3.

Economical data concerning mulberry biomass utilization

Economical purpose of mulberry biomass utilization	Biomass production Kg/ha	Main production: kg, m3, MT/ha	Unitary production value: euro/kg/m3/MT	Total production value - euro -
Sericulture	10000	400-crude silk cocoons	8.0*	3200
Phyto remediation - cellulose pulp - biogas	20000 – 100000 20000 – 100000	200 – 530 MT 500 - 1000 m3	200.00** 0.30***	**40000 – 106000** **1500 - 3000**
Phytopharmaceutical	10000	3000	0.25****	750

*the price includes the European subvention
** price/ MT Port Europe Union
*** price for domestic consumption
**** price for free market for medicinal plants

- **Weak points:** using the Morus plants vegetal biomass as energy regenerated resource needs the existence of biogas producing installation and the co-generation ones;
- the Morus plants are perennial wood plants and need an investment of 8000 – 10000 €/ha with an exploatation period of 25-30 years;
- the investment starts to be recovered from the 3rd year, the biomass production increasing annually, with a maximum in the 15th – 20th year after plantation;
- lack of some mechanized harvesting equipments with good results; in Romania the exploatation cuttings are made with Sthil cutting machine or manually;
- the competition with other energetic plants in the fertile agricultural areas (corn, forestry wood varieties, new plants - such as Miscanthus sinensis).

CONCLUSIONS

The productive determinations and chemical analyses distinguished the mulberry plants value regarding the possibility and perspectives in using then to obtain other market products, especially biogas. The agro productive potential of mulberry intensive plantations is 20000 – 100000 kg biomass/ha, mulberry plants regenerate the vegetative biomass starting form the 3rd year after plantation. The Morus plants are a regenerated energy bio resource, obtained by direct burning or anaerobe fermentation (biogas – 42.5 m3/50 kg vegetal dry biomass).

The SWOT analysis distinguishes the positive characteristics of mulberry plants regarding the biomass regeneration and the possibility to obtain regenerated energy out of this biomass, as biogas, in rural areas.

The estimate value for profit maker biomass of Morus plants is maximum in the condition cellulose pulp destination in among of €40000 – 106000. For biogas obtaining the economical value is between €1500 – 3000 and for sericulture is about €3200.

To found a mulberry plantation for sericulture, phyto pharmaceutical and phytorepair activities needs an investment of 8000 – 10000 €/ha.

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THE SILK FIBROIN AS SOURCES FOR THE BIOMATERIALS

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ABSTRACT:
Silk from the silkworm *Bombyx mori* has been used as biomedical suture material for centuries. The unique mechanical properties of these fibres provided important clinical repair options for many applications. During the past 20 years, some biocompatibility problems have been reported for silkworm silk. More recent studies show that the regenerated silk fibroin films exhibit comparable biocompatibility *in vitro* and *in vivo* with other commonly used biomaterials such as collagen. We propose in this study the obtaining of regenerated fibroin films and their modification with different groups for some specific applications. In this respect we obtained fibroin-polyvinyl alcohol films, fibroin-poly(itaconic acid) films, fibroin-poly(2-acrylamido-2-methylpropane sulphonic acid) films, fibroin-poly(2-hydroxyethyl methacrylate-co-itaconic acid) and fibroin-poly(2-hydroxyethyl methacrylate-co-2-acrylamido-2-methylpropane sulphonic acid) films. The structure of the new biomaterials was evaluated by RAMAN spectroscopy. Contact angle measurements were performed. Cytotoxicity assays showed the good *in vitro* biocompatibility of the fibroin-based materials. The positive results may lead to the potential use of these films in bone pathology as osseous substitutes or in designing scaffolds for tissue engineering (e.g., tissue-engineered blood vessels).
Keywords: silkworm, biomedical suture material, bone pathology

1. INTRODUCTION

In the last years there has been an increasing interest in using silk fibroin in biomedical and biological applications. The reasons for using this kind of fibrous material are related to its high mechanical properties combined with flexibility, tissue biocompatibility and good oxygen permeability [1].

Natural silk fibres have excellent mechanical properties. For example, domesticated (*Bombyx mori*) silk worm fibres possess a tensile modulus on the order of 5 GPa, strengths of 400 MPa, and tensile elongations of 15% or more and are able to undergo quite large deformations in compression without kinking [1-4]. The mechanical performance of silk is even more remarkable since the fibres are produced under ambient conditions from aqueous solutions. *B. mori* silk fibres consist primarily of two components, fibroin and sericin; fibroin is the structural protein of the silk fibre, and sericin is the water-soluble glue that serves to bond fibres together. The majority of the fibroin is highly periodic with simple repeating sections broken by more complex regions containing amino acids with bulkier side chains. The basic, highly repetitive sections are composed of glycine (45%), alanine (30%), and serine (12%) in a roughly 3:2:1 ratio. These three residues contain short side chains and permit close packing of crystals through the stacking of hydrogen-bonded ß-sheets. The structure is dominated by [Gly-Ala-Gly-Ala-Gly-Ser]*n* sequences, with corresponding side groups of H, CH_3, H, CH_3, H, CH_2OH. The sericin proteins, which comprise approximately 25 wt % of the silkworm cocoon, contain glycine, serine, and aspartic acid totalling over 60%. Compositional details for the silks can be found in a number of references [4-9].

In this paperwork we propose the use of fibroin-based biomaterials as films. In this way the biocompatibility of the silk fibroin could be increased. *Bombyx mori* silk fibroin dissolves in neutral salt-alcohol systems without degradation of its molecular weight. The lithium bromide or lithium thiocyanate-ethanol system and hexafluoroisopropyl alcohol (HFIP) have been widely used to dissolve silk fibroin [10-15]. Fibroin-polyvinyl alcohol matrixes were obtained and then characterised by contact angle measurements. Next, we have functionalised the fibroin fibres with different carboxylic and sulphonic groups and then we dissolved the grafted fibres into lithium bromide solution. The films were subjected to contact angle measurements and cytotoxicity assays against macrophage cells.

2. MATERIALS AND METHODS

Materials

Silk cocoons were supplied by S.C. SERICAROM S.A Company (Bucharest, Romania). Itaconic acid (IA) and 2-acrylamido2-methylpropane sulphonic acid (AMPSA) were provided by Sigma Aldrich, St-Quentin Fallavier, France and used without any further purification. 2-hydroxyethyl methacrylate (HEMA) was purified by distillation under reduced pressure. Polyvinyl alcohol (PVA 88%) was supplied from Fluka and used as such. All other substances were of analytical or pharmaceutical grade and obtained from Sigma-Aldrich.

Functionalisation of fibroin fibre with acidic groups

Cocoons from *Bomby mori* were processed three times (40 minutes each) in 0.5% solution of Na_2CO_3 and $NaHCO_3$ solution at 95-100°C to extract sericin [12]. Then the obtained fibroin fibres were rinsed with demineralised water and dried at 37°C over night. The grafting procedure was adapted from literature [16] and mainly consisted in: the fibres are treated with ammonium cerium nitrate (Ce^{4+}) in sulphuric acid solution under inert atmosphere for 20 minutes. Then the monomer solutions of IA, AMPSA, HEMA-IA (10% molar composition of IA) and HEMA-AMPSA (10% molar composition of AMPSA) were added over the reaction medium and the temperature was raised to 45°C. After 4 hours the grafting reaction was almost complete and the fibres were rinsed with demineralised water to remove the residual cerium salt and dried over night at 37°C. The recipes are presented in table 1.

Table 1. Recipes for fibroin grafting

System	Mass ratio
Initiator / Fibroin	1/5
AI / Fibroin	2/1
AI / Fibroin	3/1
AI / Fibroin	4/1
HEMA-AI (molar ratio 90/10) / Fibroin	2/1
HEMA-AI (molar ratio 90/10) / Fibroin	3/1
HEMA-AI (molar ratio 90/10) / Fibroin	4/1
AMPSA / Fibroin	2/1
AMPSA / Fibroin	3/1
AMPSA / Fibroin	4/1
HEMA-AMPSA (molar ratio 90/10) / Fibroin	2/1
HEMA-AMPSA (molar ratio 90/10) / Fibroin	3/1
HEMA-AMPSA (molar ratio 90/10) / Fibroin	4/1

Preparation of fibroin films

1g of purified *Bombyx mori* fibroin was solubilised in 9M aqueous LiBr solution and dialysed in cellophane bags for 3 days. The clear solution was kept into the refrigerator. Polyvinyl alcohol solution was obtained by dissolving PVA in demineralised water at 50°C for 2 hours.

The fibroin and PVA solutions were mixed together at different weight ratios (100/0, 50/0 and 0/100). 10 ml of this mixture was sprayed onto polyethylene sheets to obtain thin films of fibroin-PVA films. Then they were dried in vacuum oven at room temperature over night.

The functionalised fibroin fibres were obtained as films by the same procedure described below. The fibroin-IA, fibroin-AMPSA, fibroin-HEMA-AMPSA and fibroin-HEMA-IA films were dried under vacuum at room temperature over night and subjected to further analyses.

RAMAN analysis

A Bruker spectrophotometer with ionised argon laser COHERENT Innova 90-4 (4W) device was used to perform the RAMAN analysis of the grafted fibres.

Contact angle measurements

KSV CAM 200 apparatus was used for static contact angle measurements performed on dried films. Ultrapure water droplets were used with a drop volume of 20 µl. The measurement of each contact angle was made within 10 s after each drop to ensure that the droplet did not soak into the compact. The contact angles reported were the mean of 10 determinations. Smaller contact angles correspond to increased wettability.

Cytotoxicity assays

Biomaterials biocompatibility can be verified by testing *in vitro* cytotoxicity. It was used cell line L929 of murine fibroblasts, which was cultivated in culture medium (DMEM or RPMI 1640), supplemented with calf foetal serum and antibiotics. The cells were microscopically examined for detecting cytotoxicity visible signs, cellular lysis or cellular components dimensions and conformation. Fibroin films were carbon coated (10-nm thick) with a MED 020 (Bal-Tec, Balzers, Liechtenstein) and examined with a JEOL 6301 F field emission scanning electron microscope (JEOL, Paris, France) with an accelerating 5 kV voltage.

3. RESULTS AND DISCUSSIONS

Grafting yield

Gravimetric evaluation represents the “mass gain” compared to the initial weight of the unmodified fibres, offering quantitative information on the copolymer deposited on the fibres. The grafting yield (η) was calculated using the equation (1):

$$\eta = (m_f - m_i)/m_i \times 100 \qquad \text{eq (1)}$$

where: m_i – weight of the unmodified fibre before grafting, m_f – final mass.

The values for the grafting yields were between 37 şi 40%: η_{AI}=37%, $\eta_{HEMA\text{-}AI}$=40%, η_{AMPSA}=39%, $\eta_{HEMA\text{-}AMPSA}$=40%.

RAMAN analysis

The RAMAN analysis of the modified fibroin fibres are presented in figure 1 in comparison with the virgin silk fibroin. The specific bands of the fibroin are given in table 2.

Table 2. RAMAN spectrum interpretation of the Bomby mori *silk fibroin*

Absorption band (cm^{-1})	Intensity	Assignment
645	Medium	Tirosine
830-855	Medium	Tirosine
1004	High	Phenyl-alanine and triptophan
1232-1269	Medium	III amide group
1449	Very high	CH_2 and CH_3 groups
1605	Medium	Phenyl-alanine
1668	Very high	I amide
3286	Medium	N-H

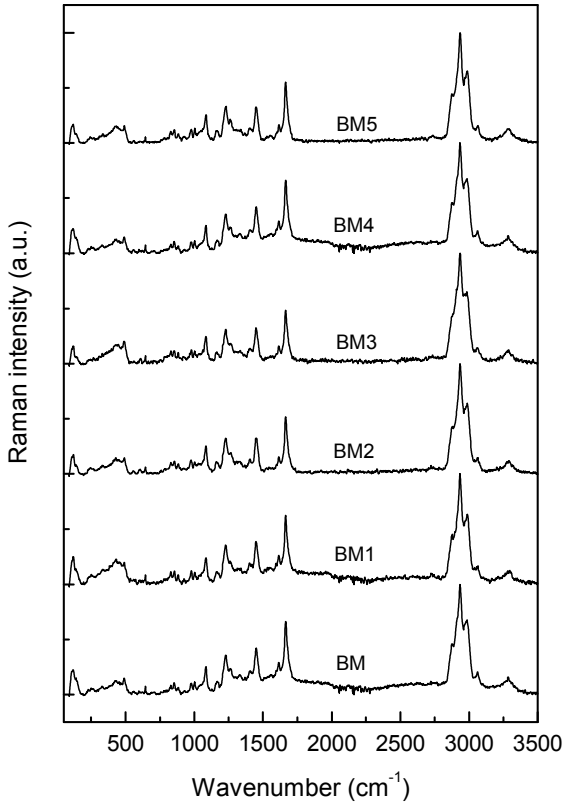

Fig.1. RAMAN spectra of the non-grafted (BM) and grafted fibroin with HEMA-AMPSA 10% and 10% molar (BM1 and BM2), HEMA-IA (BM3), IA (BM4) and AMPSA (BM5)

RAMAN spectrum of *Bomby mori* fibroin is very complex as it contains 17 natural aminoacids in which the major sequences are of Gly-Ala-Gly-Ala-Gly-Ser type. It is possible that the absorption peaks of the carboxylic and sulphonic groups to overlap the peaks of the fibroin functions. Due to this aspect it is very difficult to exactly compare the spectra of the non-grafted and grafted fibroin fibre. Nevertheless, we could observe differences in the 1000-1500 cm^{-1} region, fact that could confirm the grafting of the carboxylic and sulphonic at some extent onto the fibroin surface.

Contact angle measurements

The contact angles measured on the different silk fibroin films modified with PVA or carboxylic and sulphonic groups are listed in table 3. Smaller contact angle usually indicates that the material surface is more hydrophilic, enhancing for example the cell adhesion and proliferation.

Table 3. Contact angle values for fibroin-PVA matrix film and fibroin grafted with acidic groups

Material	Mean contact angle (°)
Fibroin film	54
PVA film	48
Fibroin-PVA matrix film	45
Fibroin-HEMA-AMPSA film	47
Fibroin-HEMA-IA film	55
Fibroin-IA film	54
Fibroin-AMPSA film	43

Data are shown as average ± standard deviation (n = 60).

Cytotoxicity using cell cultures

Numerous cells were encountered at the surface of the fibroin films after a three day period. The L929 murine fibroblast cells were mainly found in a round shape, but sometimes, they exhibited an elongated shape (figures 2 and 3). They exhibited numerous thin filopodia, and sometimes, thicker extensions were encountered, allowing communication with cells. No images of necrotic cell could be evidenced at the surface of polymeric films. The conclusion is that the fibroin-based biomaterials show no cytotoxicity against the cells. From this point of view, the films could have potential use in medicine and biology as scaffolds or bone substitutes.

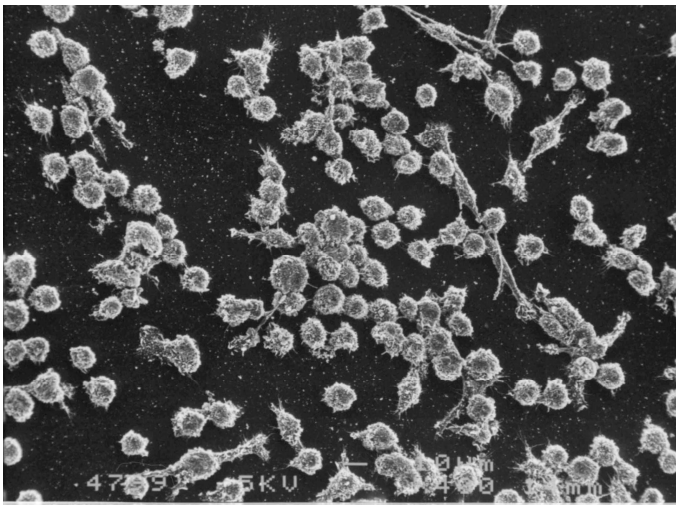

Fig.2. SEM microphotgraphs for fibroin-HEMA-AMPSA biomaterials

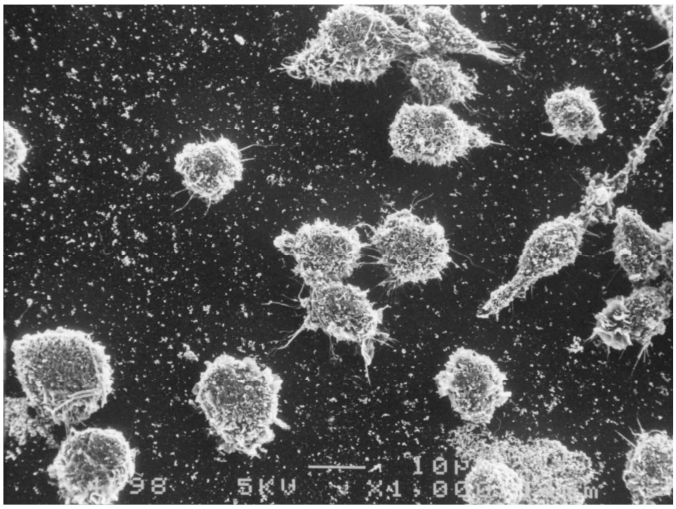

Fig.3. SEM microphotographs for fibroin-IA biomaterials

4. CONCLUSIONS

We have reported in this article the preparation of fibroin-PVA matrixes and films of fibroin grafted with acidic groups. The idea of using fibroin films proved to be very efficient in medical applications as the biocompatibility of the materials could be improved. The contact angles revealed the hydrophilic character of the fibroin films, in this way enhancing, for example, the cell adhesion and proliferation. The cytocompatibility of the fibroin films was very good. All these combined results lead to the potential use of these silk fibroin modified films in tissue engineering as blood vessel scaffolds or in osseous surgery as bone substitutes.

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SILKWORM AS BIOREACTOR FOR RECOMBINANT PROTEIN PRODUCTION

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ABSTRACT:

A bioreactor is a system in which a biological conversion is effected and carries out chemical process which involves organisms or biochemically active substances derived from such organisms. This system could produce high levels of relative products for feeding additives, vaccines and pharmaceutical. Thereby, It is perfectly the most efficient and economic platform to produce recombinant proteins. At present silkworm (*Bombyx mori* L.) is being utilized as a powerful eukaryotic model in fundamental researches and as a bioreactor for foreign protein production using baculovirus expression vector system (BEVS). Numerous reports are available addressing silkworm as an efficient biofactory for the production of heterologous proteins. This system has advantages such as needing less time than animal or plant expression systems; expressing the recombinant protein in both insect cultured cells and related insect larva on a large scale; recombinant protein is usually expressed at high levels and some of the post translational modifications are faithfully performed while this is not the case with prokaryote or yeast expression system; the recombinant virus only infects insects and their cells, while it cannot infect vertebrates or human. Thus, the recombinant protein produced is safer than that produced in mammals or their cultured cells. Therefore, BEVS is becoming the safest and most efficient expression system. Silkworm larvae are larger than larvae susceptible to AcNPV-infection thus reducing manipulation per volume hemolymph produced. Furthermore, the in vivo expression avoids the high costs of cell culture technology and suppresses the product level reached in the cell culture. At this report we point out some new approaches developed through mass production of recombinant proteins utilizing silkworm as a bioreactor and to compare them with other options available.

Keywords: silkworm, protein production, baculovirus expression vector system