First Balkan workshop "Possibilities for Using Silkworm and Mulberry for Non-Textile Purposes"

PROCEEDINGS

23 – 26 September 2008, Plovdiv, Bulgaria

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Organizing committee:

President:
Assoc. Prof. Dimitar Grekov, PhD – Rector, Agricultural University, Plovdiv, Bulgaria

Members:

1. Assoc. Prof. Panomir Tzenov, PhD – BACSA President and Director of SES – Vratza, Bulgaria
2. Dr Maria Ichim – Director, Bioengineering, Biotechnology and Environment Protection Institute – BIOING S. A, Bucharest, Romania
3. Dr Evripidis Kipriotis – Director, Agricultural Research Station, Komotini, Greece
4. Mr. Ayhan Karagozoglu – General Manager, Sericultural cooperative Kozabirlik, Bursa, Turkey

Venue and Dates:

Agricultural University, Plovdiv, Bulgaria

Programme:

DAY 1 (Tuesday 23 September 2008)
Arrival to Plovdiv

20.00 – Welcoming dinner

DAY 2 (Wednesday 24 September 2008)
Place: Agricultural University, Plovdiv

9.00 – 10.00	Registration
10.00 - 10.15	Opening – Assoc. Prof. Dimitar Grekov, PhD – Rector, Agricultural University, Plovdiv, Bulgaria
10.15 - 11.00	Plenary lecture: "Global trends in mulberry and silkworm use for non – textile purposes" by M. Ichim, D. Tanase, P. Tzenov and D. Grekov
11.00 - 11.30	Session for scientific articles
11.30 – 13.00	Round table discussion on the mulberry and silkworm products use for non – textile purposes.
13.00 – 15.00	Visit the Agricultural university vine - cellar and wine tasting
15.00 – 15.30	Round table discussion on the formulation of bi/multilateral joint sericultural project proposals with participation of Balkan countries.

15.30 – 16.30 20.00 Dinner

DAY 3 (Thursday, 25 September 2008)

Cultural program for the foreign guests

DAY 4 (Friday, 26 September 2008)

Departure

List of participants

№	Name	Post	Institution
1	Assoc. Prof. Dimitar Grekov, PhD	Rector	Agricultural University, Plovdiv, Bulgaria
2	Assoc. Prof. Panomir Tzenov, PhD	President & Director	Black, Caspian Seas and Central Asia Silk Association; Sericulture and Agriculture Experiment Station, Vratza, Bulgaria
3	Ms. Maria Ichim, PhD	Director	Bioengineering, Biotechnology and Environment Protection Institute – BIOING S. A, Bucharest, Romania
4	Mr. Liviu Ichim	Scientist	Bioengineering, Biotechnology and Environment Protection Institute – BIOING S. A, Bucharest, Romania
5	Mr. Liviu Luka	Scientist	Bioengineering, Biotechnology and Environment Protection Institute – BIOING S. A, Bucharest, Romania
6	Ms. Doina Tanase, PhD	Senior researcher	Commercial society "Sericarom", Bucharest, Romania
7	Mr. Ayhan Karagozoglu	General manager	Sericultural company "Kozabirlik", Bursa, Turkey
8	Mr Durmus Yılmaz	Member of the ruling board	Sericultural company "Kozabirlik", Bursa, Turkey
9	Mr Hasan Ucar	Financial oditor	Sericultural company "Kozabirlik", Bursa, Turkey
10	Mr Yilmaz Ekinci	Technologist	Sericultural company "Kozabirlik", Bursa, Turkey
11	Mr. Evripidis Kipriotis, PhD	Director	Agricultural Research Station of Komotini, Greece
12	Mr. George	Technician	Agricultural Research Station of Komotini, Greece
13	Mr. Konstantinos Bladenopoulos, PhD	Researcher	Cereal Institute, Thessaloniki, Greece
14	Mr. Simeon Beshkov	President	Bulgarian Sericulture Association, Sofia

15	Assoc. Prof. Zdravko Petkov, PhD	Researcher	Sericulture and Agriculture Experiment Station, Vratza, Bulgaria
16	Ms. Jolanda Vasileva, PhD	Researcher	Sericulture and Agriculture Experiment Station, Vratza, Bulgaria
17	Ms. Diana Pantaleeva	Researcher	Sericulture and Agriculture Experiment Station, Vratza, Bulgaria
18	Mr. Javor Ralchev	Main accountant	Sericulture and Agriculture Experiment Station, Vratza, Bulgaria
18	Assoc. Prof. Teodora Staikova, PhD	Researcher	Plovdiv University, Bulgaria
19	Prof. Dr Ivanka Lecheva	Vice – rector	Agricultural University, Plovdiv, Bulgaria
20	Assoc. Prof. Vasko Koprivlenski, PhD	Vice – rector	Agricultural University, Plovdiv, Bulgaria
21	Assoc. Prof. Hristina Jancheva, PhD	Vice – rector	Agricultural University, Plovdiv, Bulgaria
22	Ms. Krasimira Ivanova	PhD student	Agricultural University, Plovdiv, Bulgaria

PLENARY PAPER

GLOBAL TRENDS IN MULBERRY AND SILKWORM USE FOR NON – TEXTILE PURPOSES

By

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Paper contributed to the
First Balkan workshop on
"Possibilities for Using Silkworm and Mulberry for Non-Textile Purposes"
23 – 26 September 2008, Plovdiv, Bulgaria

INTRODUCTION

The paper is a review of the main scientific research concerning global trends in mulberry and silkworm use for non – textile purposes in order to preserve and develop the sericulture resources.

Sericulture, an art of the rearing silkworms, is practiced at economic scale in more than 25 countries all over the globe. Sericulture is an agricultural industry, yielding beside the main product (silkworm cocoons) a series of sub-products and secondary products having exceptional economic value, if done properly.

The main 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 wastes. These ones may however be turned into new commercial products with a high useful value.

A major concern of the silk industry is the need to make the most efficient use of natural fibers, but also to utilize the waste products. Silk waste itself arises from damaged cocoons or from cocoons, which are difficult to unreel, together with waste fiber from the processes preparatory to spinning.

In many of the sericultural countries after the silk reeling, the pupae 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, not enough purposeful work has been carried out on the utilization of secondary wastes of silk industry, which will definitely enhance the profit of silk industry.

From the available scientific studies from China, India and Japan, an attempt has been made to collect the information on existing status of secondary waste products utilization and processing. Hence, it is necessary to describe an approach for proper utilization of secondary waste products of silk industry *i.e.*, sericin, pupae, moths, silkworm rearing wastes and silk fiber waste from all varieties of silk.

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 diversification of exploitation methods for mulberry plantations and silkworm production. The R&D activities firstly targeted the available fields cultivated with different mulberry varieties and their agroproductive potential regarding the total biomass production and the development of technologies and methods of processing the silkworm, silk wastes and mulberry productions.

Mulberry (Morus spp.) leaves have long been the traditional feed for the silkworm (*Bombyx mori*). There is evidence that sericulture started about 5 000 years ago and hence the domestication of mulberry. Mulberry has been selected and improved for leaf quality and yield over the centuries. Through silk production projects, mulberry has been taken to countries all over the world, and it is now spread from the temperate areas of northwest and central Asia, Europe and North America through the tropics of Asia, Africa and Latin America to the southern hemisphere (southern Africa and South America). There are mulberry varieties for many environments, from sea level to altitudes of 4 000m (FAO, 1990), and from the humid tropics to semi-arid lands, such as in the Near East with 250 mm of

annual rainfall and the southwestern United States (Tipton, 1994). Mulberry is also produced under irrigation. Although the majority of silk production projects have had limited duration because of silk processing constraints and limited market opportunities, mulberry trees have remained in most places where they have been introduced.

Mulberry is an indispensable crop for silkworm production. In Japan, sericulture had been such an important industry that mulberry was not used for any other purposes. However, with the recent decline of sericulture industry, mulberry has been re-evaluated for other purposes, such as medicinal, fruit and animal production.

Currently, importance of natural products is being revitalized to alleviate various health discrepancies. The link between health and diet is well documented and the consumers' trend reflects conscience towards their dietary habits. Probing these links has led to the emergence of functional, nutraceuticals and pharma foods, now taking hold over global nutrition market. Phytochemicals in diet could provide protection against several threats like free radical formation, degenerative disorders and lifestyle related diseases but still role of array of active ingredients should be unveiled. The review is intended to focus on rich phytochemistry of *Morus alba* L., its antioxidant potential, inhibition of LDL oxidation, neurodegenerative disorders and mode of action in boosting skin tone. It has a unique nutritional profile containing proteins, phenolics, flavonoids and anthocyanins that enhances its significance as promising nature's functional tonic.

Considering the economic conditions after EU accession, meaning stimulating subsidiaries granted, the sericulture activity in the European countries will be re-launched due to the fact that it is one of the industrial/agricultural activities that supplement 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-over (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 sericulture 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.

In Bulgaria the research team of Sericulture Experiment Station, Vratza has made a research on the identification and evaluation of the silkworm rearing waste products. It was 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. 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 nitrogen percentage in the compost, prepared by silkworm rearing wastes is 1.68 %.

There have been put the beginning of using the mulberry for phytoremediation of heavy metal polluted soils. It was detected that regardless the high contents of lead and zinc estimated in the food, excrements and silkworm body as a whole the contents of heavy metals in the cocoon shells and silk were negligible. Therefore it is concluded that the mulberry – silkworm producing system could be used as a biological method of cleaning and utilization of heavy metal polluted soils.

A project for the possibilities for using mulberry fruits has also been initiated recently at the SES - Vratza. Other direction of mulberry using in Bulgaria recently became for production of high quality wood for furniture industry.

In Romania, there are nowadays, scientifically research activities concerning development of a profitable way of sericulture waste and by products utilizing. As a result of interdisciplinary collaboration between different companies and national research institutes, there were obtained new commercial products for the local and international market (embryonic extract from silkworm eggs, cosmetic cream with pupae oil).

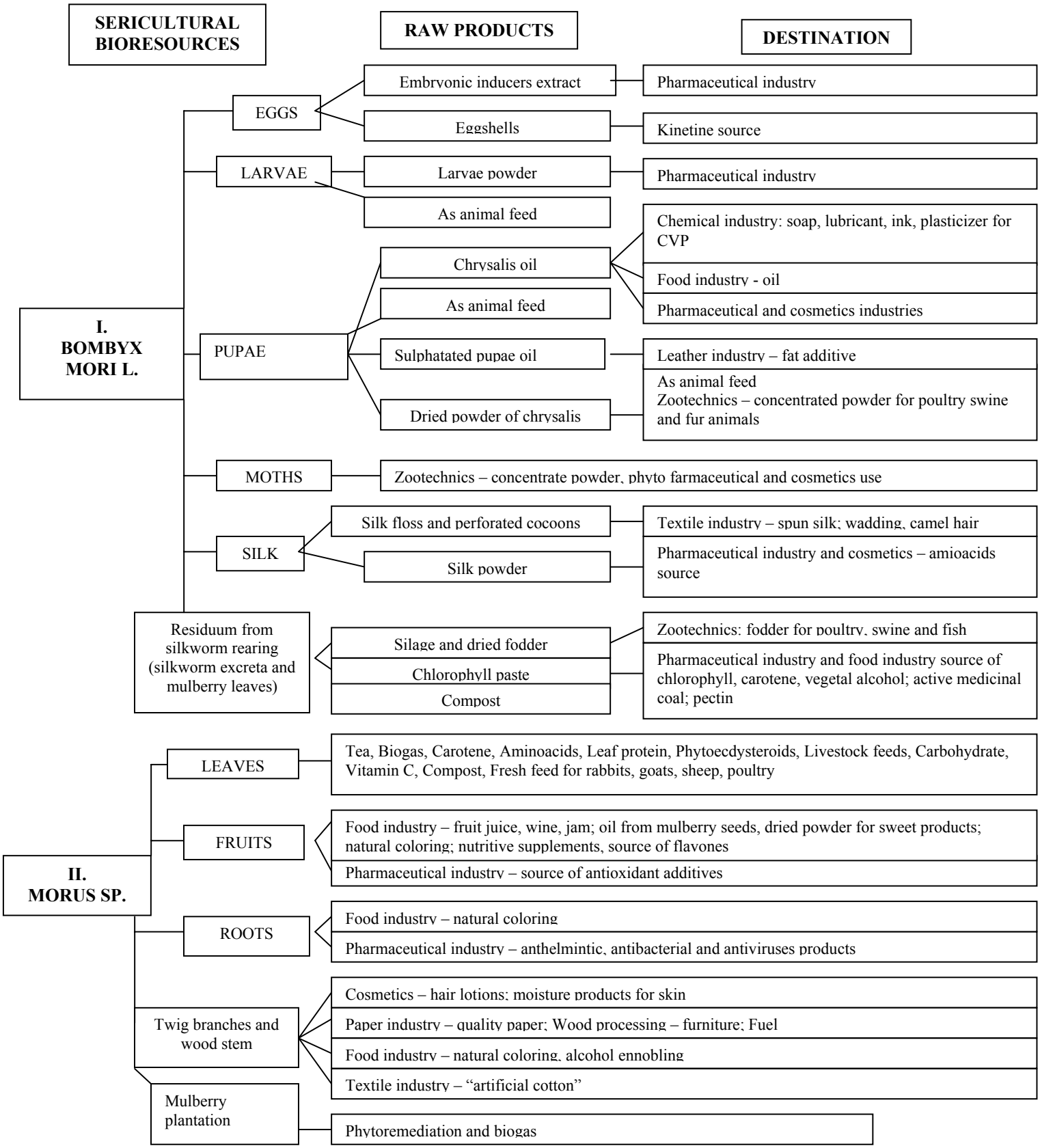
During 1990 – 2008, C. S. SERICAROM obtained three patents: nr. A61K35/56/106842/1991 titled Biomorus (Stimulating composition with ant diabetic function); nr. B1-111905/1997, titled Humanofort B. It is a natural biostimulant, having a catabolizing and rebalancing effect of endocrine functions on consumer; nr. 120747/2007, titled “Mulberry dried fruits powder - obtaining procedure and its utilization”. It is a natural raw material for sweet products industry.

Five scientifically research and development projects were financed by the Romanian Ministry of Education and Research, in the frame of National Research Programs, having as purpose the experimental exploratory testing concerning the obtaining of bioproducts from sericulture waste.

Thanks to the Ministries of Research and Technology in Romania and Bulgaria, which approved the contract bilateral "Application of modern methods for processing the products and sweepings resulted from sericulture in order to obtain new ecological agriculture food" for a period of two years, between partners Institute Bioengineering, Biotechnology and Environmental Protection – S.C. BIOING S.A. - Bucharest, Romania and Sericulture Experiment Station-Vratza - Bulgaria, will develop scientific research activity in obtaining of bioproducts from sericultural wastes.

MATERIALS AND METHODS

The paper was developed by following visual plot for utilization of non-textile products and wastes resulted from sericulture.

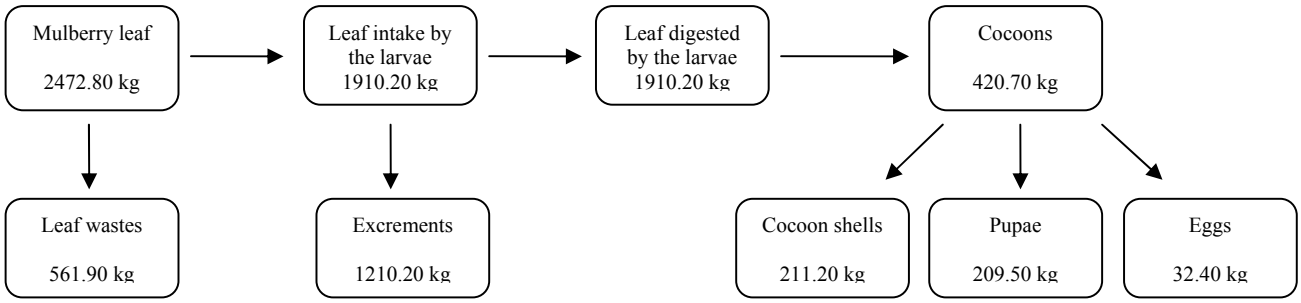


According to this visual plot, experimental researches from over 150 scientific papers were studied, analyzed and presented in this paper.

RESULTS AND DISCUSSIONS

As a first step in this study, it was made the identification and evaluation of the silkworm rearing waste products.

The data reveal that the dry matter balance from one ha mulberry plantation was as follows:



From the figures 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 fifth 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 (**P. Tzenov, Z. Petkov, Y. Vasileva, D.A. Pantaleeva- Bulgaria, M. Ichim-Romania,** 2008).

The multi-purpose nature of the mulberry makes it suitable for incorporating in the agro-forestry systems. However, since mulberry is not a nitrogen fixing plant and since the leaves will be harvested for livestock feeding, recycling of the nitrogen and other nutrients removed from the soil will be necessary.

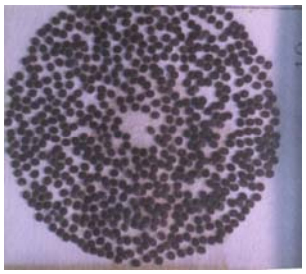
The global trends in mulberry and silkworm use for non-textile purposes relive the following aspects concerning the sericulture bioresources:

I. BOMBYX MORI L.

I.1. Bombyx mori L.:

Silkworm eggs – containing 56% albumin, 19.2% fats, 7.7% sugars. They are used:

- as they are, by eating, serving as male sexual stimulator (in popular tradition)
- as extract, rich in proteins, embryo inductors, glycoproteins, B1 and B2 vitamins with energizing and hepatic protector action, hypolipidic and hypoglycemic effect. This extract is sold in Romania as the Humanofort B product.



Silkworm's eggs processed into proteic extract used in the pharmaceutical industry with hepatoprotean action, and also in the food industry. In Bulgaria some people believe that the silkworm eggs, if eaten by heavy alcohol drinkers then they give up drinking completely

because start feeling alcohol disgust. However this fact has not been proved scientifically.

I.2. Bombyx mori L.

Larvae – used for feeding young animals, reptiles, as proteic flour having the role of dietary supplement; also as etheric extract having a high bombycisterol (a cholesterol isomer) content; the Florence lily, used as surgical thread.

Silkworm larvae from different evolution stages are used as total proteic extract in the pharmaceutical industry (anti diabetic action) or in the food industry as supplementary nutraceutical.

Traditionally, silkworm has been utilized as a diabetic medicine in the oriental countries such as China, Korea, and Japan, and recently many researches have proved the blood glucose-lowering effect of the silkworm. However, a diverse research is urgent to maximize the medicinally effect of the silkworm. A recent research result (Ryu et al., 2005) showed that the maximum blood-glucose lowering effect of the silkworm can be obtained when the silkworm was prepared at the 3rd day of fifth instar, manufactured by freezing dry method, and taken in the form of powder rather than others. Although the powdery silkworm has been proved in its pharmacological mechanism, no study has yet illustrated the major components in the silkworm and their content, which exerts blood glucose-lowering effect, until recent past. However, now the substances are turned out to be the four blood glucose-lowering substances as well as the major component, DNJ (1-deoxynojirimycin), which are nitrogen compounds.

It was also found that the silkworm contains the highest content per gram of DNJ among per gram of silkworm, mulberry leaves, and mulberry fruit, syncarp, suggesting that high concentration of DNJ is accumulated in the silkworm body.

A clinical study on this aspect revealed that overall there was no effect of the powdery silkworm on the normal candidates due to the natural glucose metabolism. In the experiment intended for diabetic patients, the most optimizing result was obtained in the dose of 900mg per day, during meal rather than after meal.

The powdery silkworm has been designated as one of the agricultural products strictly controlled by Korean government for the better quality.

Recently (Sung, 2002), lots of new techniques to produce a new sericulture products such as Genus *Cordyceps* of entomopathgenoic fungi, silkworm powder for lowering blood sugar and Nuegra, a natural alternative to Viagra by using silkworm, have been developed in Korea to overcome the declining industry and the mulberry plantation has now revived to the increasing cultivation.

I.3. Bombyx mori L.

Pupae – they result form the cocoon reeling process and contain: 50-60% proteins, 25 – 35% fats, 8 – 10% sugars, E, B1, B2 vitamins, nicotinic acid, pantothenic acid, calcium, phosphorous, copper, iron and selenium. From the pupae, the following products are obtained:

- Pupae oil, used in the pharmaceutical industry (anti-inflammatory, anti-tumefying effect, lymphatic circulation stimulant, used in the treatment of: sinusitis, otitis, bronchitis, asthma, tuberculosis, urinary infections and in post – surgery situations. In Japan, a product based on chrysalis oil is called Seratiopeptidase, while in China a product is called Gan Mo Le. From the same chrysalis oil, superior sodium and potassium soaps are obtained; varnishes and dyes used in the textile and tannery industry, which replace the red Turkish oil are also obtained (the Lei Mi Hang product, used in China); lubricants, printing inks, plasticizer for PVC are amongst its other uses. In Romania, the chrysalis oil is produced by SC. Biotehnos SA Bucharest. The residue formed during the chrysalis oil's extraction is used as natural organic fertilizer and as food for poultry, pigs, fish and furbearer animals.

The silkworm pupae due to their high fat content (over 30%), are used as chrysalis oil to obtain cosmetic products (cream, soap, lotion, emulsion) and as proteic powder for valuable animal's fodder.

In some Asian countries like Korea, China, Japan, Thailand etc. the silkworm pupae are used as delicious human food.

I.4. Bombyx mori L.

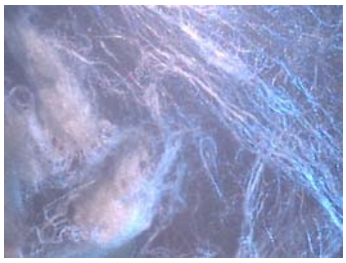
Moths or silkworm butterflies, the Bombyx mori species, result during the technological flux of obtaining silkworm eggs; they are used to prepare pharmaceutical products for trauma and convalescence recovery, or to strengthen the masculine functions (by fermentation or extraction of silkworm oils). The butterfly oil contains: 75% fatty oils, from which more than 50% are unsaturated fatty acids, having a boiling point of 201 ^{0}C, iodine index of 132 and volumetric weight of 0.928. The silkworm moth oil can be used to obtain textile dyes and superior soaps. The extraction residue can be used in obtaining of monosodium glutamate or as fodder. The butterflies can also yield Cellular Cytochrome C for pharmaceutical use, uric

acid or hormones and sex messengers of the PTTH (hormone of central nervous system) and DH type (sexual hormone).

I.5. Bombyx mori L.

Sub-products from silk manufacture – represented by silk residues, non-usable cocoons or lint. From these, there can be obtained:

- wool and thread of camelhair type (spun silk)
- silk powder for cosmetic products or for pharmacological amino-acids (glycine, alanine, serine, thyrosine, aspartic acid, glutamic acid).
- silk waste water from the reeling mill, containing soluble silk (sericine).



By products and waste resulted in the eggs production and silk cocoons proceeding may be used in textile, leather, pharmaceutical, cosmetics and food industies as proteic, precipitant jellies, atomizated or lyophilized powder.

From these silk residues, the following raw materials can be obtained:

- proteic gels;
- proteic precipitates with inorganic salts;
- microfibers;
- atomized proteic powders;
- lyophilizated proteic powders;
- proteic agents used for textiles and leathers' finishing;
- hydrolyzed silk protein (as polypeptide is used for cosmetic ingredients; they excellent perform as moisturizer, smoother and protector for both skin and hair, applied technology of hydrolyzed protein up to now) (**Ikeda Norizaku, JPN**).

I.6. Bombyx mori L.

The remaining materials from silkworm rearing are of the following types:

•excreta, containing: 7.35% water, 13.88% crude protein, 1.44% raw fats, 15.41% raw cellulose, 47.15% substances without nitrogen; it can be used as organic fertilizer, as chlorophyll source (by alcoholic extraction) or as drug for heart diseases in the traditional Chinese medicine.

•residuum of silkworm rearing composed of vegetable mulberry remnants and excreta, that can be used as organic fertilizer (as compost or fodder for animals during winter – silage this method is largely used in Japan for feeding sheep, goats and cattle).

II. MORUS sp.

The main use of mulberry globally is as feed for the silkworm but, depending on the location, it is also appreciated for its fruit (consumed fresh, in juice, alcoholic drink or as preserves), as a delicious vegetable (young leaves and stems), for its medicinal properties in infusions (mulberry leaf tea), for landscaping and as animal feed etc.

II.1. MORUS sp. Leaves

There are many countries where mulberry is utilized traditionally as a feed in mixed forage diets for ruminants, such as in certain areas of India, China, Afghanistan, Bulgaria, Georgia, Azerbaijan etc. In Italy

there have been several studies on the use of mulberry for dairy cows and other domestic animals (Vezzani, 1938; Maymone, Tiberio and Triulzi, 1959; Bonciarelli and Santilocchi, 1980; FAO, 1993) and in France a research project was undertaken to introduce mulberry in livestock production (Armand, 1995). However, it was only in the 1980s that specific interest in the intensive cultivation and use of mulberry as animal feed started in Latin America. It is surprising that a plant that has been improved for leaf quality and yield to feed the silkworm, which has high nutritional feed requirements, has received such limited attention from livestock producers, technicians and researchers.
Like several significant breakthroughs in science and technology, the discovery of the value of mulberry as a high-quality feed in Latin America happened serendipitously. A Costa Rican farmer of Chinese origin, whose silk project had failed, fed mulberry leaves to his goats and was impressed by the palatability of the leaves and by the performance of his animals. He communicated his observations to scientists at the Tropical Agriculture Research and Training Center (CATIE), who were receptive to the farmer's news and forward thinking enough to include mulberry in their tree fodder evaluations and later in agronomic and animal performance trials (J. Benavides, personal communication). In Africa, the International Centre for Research in Agroforestry (ICRAF) in Kenya and the Livestock Production Research Institute in the United Republic of Tanzania have also conducted successful agronomic and animal trials by themselves, apparently without being aware of the interest elsewhere.

Even though there have not been conducted any systematic researches on the use of mulberry leaves for the domestic animals feeding in Bulgaria it is very common in the rural areas that the farmers use the leaves and shoots of mulberry trees for feeding sheep, goats, caws, rabbits etc.

The *Morus* species is known especially for the utilization of leaves in sericulture, as well as for the utilization of different organs as prime source for the obtaining of some active pharmacological products. In conformity with plant organs, the chemical composition of vegetal material is different.
Mulberry root bark is used in traditional medicine, especially in Asian regions. Modern medicine has confirmed the therapeutic potential of the products obtained from *Morus sp.* root bark, products which have a specific pharmaceutical activity of some fractions obtained from mulberry root bark containing components soluble in water and n-butyric alcohol and about vegetal product “sohakuhi”, have been demonstrated that the two fractions have cathartic, analgesic, diuretic, antitussive, antiedemic, sedative, anticonvulsant, hypotensive activities.

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 (semi-finished sugar product from mulberry fruit and proteic concentrate out of mulberry leaves). Lately, it was evaluated the economical potential of Morus plants as regenerable energy source, for an intensive cultivation system.
Studies have been recently conducted on the characterization of mulberry leaves for biogas and comparison of these values with other tree foliages (Devarajan, 1999). Degradation of biogas was studied by the *in vitro* gas production technique (Menke *et al*., 1979) showed that the potential biogas production in young leaves was 60.6 ml/200 mg while the rate of degradation was 0.0703. The corresponding values for the mature leaves were 35.4 ml and 0.0624 respectively, indicating the fall in fermentability with maturity. The potential gas production for the young leaves was highest among the forages studied and the rate of biogas production lower compared with only *Moringa oleifera* (Table 1), suggesting high nutritive value of the young leaves. The fermentability of the mature leaves was also high and

comparable with *Leucaena* leaves. The high rate of biogas production for mulberry indicates high intake potential of this forage.
Chemistry of mulberry leaves enumerate that it contains some antimicrobial agents like kuwanon G and leachianone etc. Mulberry extracts or components especially flavonoids i.e. quercetin, rutin and isoquercitrin scavenge free radicals showing potential against oxidative stress. Presence of prenylated flavonoids further strengthened its antioxidant claims. Additionally, these antioxidants provide cardiovascular protection as these inhibit LDL oxidation and thus atherosclerosis. Likewise, some other components such as 1-deoxynojirimycin (DNJ) and Moran 20K have been reported to be effective against hyperglycemia and lipid peroxidation in diabetics. Mulberry leaves as protein source in food formulations and neuroprotective functions can be used against neurodegenerative disorders such as Alzheimer and Parkinsonism. Furthermore, it also demands special consideration to improve skin tone. Chemopreventive potential has been highlighted in some studies but still researchers should pay attention to validate the findings to enhance meticulousness.

Mulberry leaves can also be used in poultry rations. Incorporation of shade-dried mulberry leaves in layers' mash to the extent of 6 percent showed an increase in egg production with desirable yolk colour without any adverse effect on body weight and egg quality (Narayana and Setty, 1977). Mulberry leaves, owing to their high carotene content, can form a valuable source of vitamin A for the health of poultry birds and increased egg production.
The effect of supplementing mulberry leaves ad libitum to concentrate diets of Angora rabbits on wool production has been studied by Singh, Goel and Negi (1984). The average intake of mulberry leaves was 10.4 g/day/kg W 0.75 while the total DM intake was 29.5 g/day/kg W 0.75. The digestibility coefficients for DM, CP, CF and NFE were 69, 66, 72 and 78 percent respectively. The nutritive value of mulberry leaves (percent in DM) calculated by difference was digestible CP 9.8 and total digestible nutrients 64. The results indicated that mulberry leaves could be advantageously incorporated in the diets of Angora rabbits for wool production. Mulberry leaves may be supplemented up to a level of 40 percent of the DM with impunity.
The use of high protein mulberry tree fodder should be encouraged to provide supplementation of crop residues and natural pastures, thereby increasing productivity and the overall use of available on-farm biomass. Mulberry tree forage is well accepted by ruminants, pigs, poultry and rabbits. There is a need for systematic research on the optimization of the use of this tree forage and for developing strategies for its optimum supplementation under different feeding situations. The promotion of mulberry should be viewed in context of a holistic farming systems approach with the aim to increasing farmers' incomes, generating employment and conserving the environment. This is also an attractive option to achieve an integration of silk production and livestock rearing. Acceptance of these strategies could reduce the need for land clearing and pasture establishment in the fragile areas of the world that are prone to erosion following clearing.
Mulberry has the potential to play a valuable role in world agriculture. It is an extremely versatile plant that can fulfill a number of roles in smallholder agricultural production. Its value is multifaceted and the potential for increasing and diversifying its use is enormous. However, its value and benefits as a high-quality supplement to low-quality roughages in ruminant feeding systems have not been widely known nor fully exploited. There is a wide genetic diversity in this species which has wide-ranging soil and climatic adaptation - a large number of provenances are available that grow under different soil and climatic conditions. Systematic studies are warranted to evaluate these provenances in order to know the superior genotypes, collect and maintain germplasm, and conduct agronomy and management studies. Such studies include: environmental adaptation; establishment and propagation; defoliation management of trees; planting density, cutting intervals and cutting heights in intensive forage

production systems; seed production in different agroforestry systems (e.g. agrosilviculture, agrosilvi-horticulture, silvipasture, energy plantation, boundary plantation, alley cropping and perennial cropping). This will improve biomass production with high nutrients for livestock feeding and extend the ecological range of the plant. The future role and value of mulberry will depend on the outcome of these programs.

The use of crop residues as basal diets for fattening cattle and lambs has been promoted in China during the last decade with much success (FAO, 1995a; FAO, 1998b). Farmers generally supplement with high levels of concentrates, including cereal grains and oilseed meals. It is important to find alternative supplements (FAO, 1995b). With growing lambs, alternative ways of using mulberry foliage would be welcomed by farmers when income from sericulture is low. Mulberry leaves are relished by sheep and goats and have a high nutritive value with a protein content of about 20 percent of DM (FAO, 1998). Roothaert (1999) observed that dairy heifers had higher voluntary intake, and thus higher potential of milk production, when consuming mulberry fodder rather than the cassava tree (*Manihot glaziovii*) and Leucaena (L*eucaena diversifolia*). Mulberry leaves could be considered as an appropriate supplement for sheep fed by a basal diet of ammoniated straw, replacing partially or totally the oilseed meals, which could then be used in monogastric diets. However, there is little information on this subject.

Mulberry leaves have a high nutritional content, which is higher in spring than in the autumn. When used as a supplement for an ammoniated rice straw diet, the mulberry berry may fully substitute for rapeseed meal, but attention should be paid to the negative associative effect between rapeseed meal and mulberry leaves when supplemented together. The benefits resulting from supplementation with mulberry leaves included an increased intake of basal diet, less consumption of concentrate and an increased income. However, the growth rate of lambs on the ammoniated straw diets in the present study was not very high, regardless of the supplement. One of the reasons may be that straw intakes were not high. Further study is needed to investigate the response to the increasing percentage of mulberry leaves in diets for lambs.

Mulberry, traditionally used as feed for silkworm, has been the subject of research at the Faculty of Agrarian and Veterinary Sciences, São Paulo State University (FCAV-UNESP), with the objective of feeding goats. Its favorable characteristics for a high intake by goats include high protein content (similar to alfalfa), and good green biomass production (5-8 tones/ha/cut) throughout the year, including the dry season.

Mulberry has been shown to have a considerable potential for feeding goats, both from the biological and economic points of view, since it is well accepted by these animals, has high contents of CP and TDN and a good green biomass production per unit area with a deep root system, which allows good production throughout the year including the dry period (Takahashi, 1998).

Oxidation of low-density lipoprotein (LDL) has been implicated in atherogenesis. Antioxidants that prevent LDL from oxidation may reduce atherosclerosis. We investigated LDL antioxidant activity and extracted compounds of mulberry (*Morus alba* L.) leaves. The LDL antioxidant activity of 60% ethanol extracted of mulberry leaves, which inhibits human LDL oxidation induced by copper ion, was determined on the basis of oxidation lag time and calculated as epigallocatechin 3-gallate equivalents (58.3 μmol of EGCG equivalent/g of dry weight). Three flavonol glycosides [quercetin 3-(6-malonylglucoside), rutin (quercetin 3-rutinoside) and isoquercitrin (quercetin 3-glucoside)] were identified as the major LDL antioxidant compounds by LC-MS and NMR. The amounts of

these flavonol glycosides in mulberry leaves and mulberry-leaf tea were determined by HPLC. Our results showed that quercetin 3-(6-malonylglucoside) and rutin were the predominant flavonol glycosides in the mulberry leaves.
Mulberry leaves are used fresh or dried, as antidiabetic tea and as a powder proteic pulvis are used to obtain diverse food products (sweet products, bread, refreshment juices, and natural tinctorial additive.

II.2. MORUS sp. Fruits

The chemical composition of white (*Morus alba* L.), red (*Morus rubra* L.) and black (*Morus nigra* L.) mulberry fruits which grown in the East Anatolia Region of Turkey was investigated. The highest total phenolic and flavonoid contents were observed in black mulberry (1422 mg gallic acid equivalents/100 g fresh matter and 276 mg quercetin equivalents/100 g fresh matter). *M. alba* had the highest total fat content (1.10%), followed by *M. nigra* (0.95%) and *M. rubra* (0.85%), respectively. The major fatty acids in mulberry fruits were linoleic acid (54.2%), palmitic acid (19.8%) and oleic acid (8.41%), respectively. The total soluble solids content of mulberry species varied between 15.9% (*M. rubra* L.) and 20.4% (*M. alba* L.), acidity between 0.25% (*M. alba* L.) and 1.40% (*M. nigra* L.), pH between 3.52 (*M. nigra* L.) and 5.60 (*M. alba* L.), ascorbic acid 19.4 mg/100 g (*M. rubra* L.) and 22.4 mg/100 g (*M. alba* L.), respectively. Mineral compositions of the mulberry species were 0.83% N, 235 mg/100 g P, 1141 mg/100 g K, 139 mg/100 g Ca, 109 mg/100 g Mg, 60 mg/100 g Na, 4.3 mg/100 g Fe, 0.4 mg/100 g Cu, 4.0 mg/100 g Mn and 3.1 mg/100 g Zn, respectively. (**Sezai Ercisli and Emine Orhana**)

Mouro is the spirit beverage that comes from the distillation of fermented fruits of the mulberry tree (*Morus nigra* L.). *Mouro* is also the Greek common name of this fruit. Usually, it is used for the production of syrups, jams and jellies. In Greece, it is also used for the production of the traditional aromatic *mouro* distillate. To gain a better perspective of this, it was collected helpful information regarding the production process and it also was analyzed. The alcoholic title, even though it meets – except for one sample – the official minimum limit of 37.5% vol. it varies substantially from 35.48 to 45.59% vol. Methanol, acetaldehyde, ethyl acetate and ethyl lactate, which at high concentration can affect negatively the quality of the distillates, vary from 107 to 198, from 21 to 79.4, from 6.2 to 1031.7 and from traces to 51.21 g/hl AA, respectively. Mineral concentrations, mainly that of lead (Pb), presented significant fluctuations; the measured levels, however, do not pose a threat for consumer safety. On the other hand, the desirable concentrations of the higher alcohols (>140 g/hl AA), the favorable esters, such as ethyl octanoate (1.3 g/hl AA) and ethyl decanoate (1.4 g/hl AA) and, mainly, the relatively high concentrations of 2-phenylethanol (traces to 12.73 g/hl AA) seems to distinguish the *mouro* distillate. (**E. H. Soufleros Ageliki S. Mygdalia and P. Natskoulis**)

Anthocyanins in the fruits of mulberry (*Morus alba* L.) were extracted and separated by high-speed counter-current chromatography (HSCCC) using a biphasic solvent system. The five compounds were identified. The antioxidant activity of crude mulberry anthocyanins (CMA), C3G, C3Ga, C7G, C3RG and C3RGa was investigated by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging method. The results showed that CMA, C3G, C3Ga, C3G and C7G have higher scavenging ability on DPPH. At the concentration of 0.10 mg/mL, the DPPH radical scavenging rates of C3G, C3Ga and C7G were about 88% of vitamin C, while C3RG and C3RGa were about 60% of it. CMA had the same DPPH radical scavenging rate as vitamin C or the five anthocyanin monomers when the concentration reached 0.40 mg/mL, which shows that CMA is an excellent antioxidant agent. (**Q. Du J. Zheng and Y. Xu**)

Five major mulberry varieties [M-1 – M-5] cultivated in Korea were assessed for their polyphenolic composition using spectrophotometric methodology, and tested for antioxidant potential by some different assays. The total polyphenol (TP) was found from 2235 to

2570 μg/g gallic acid equivalents, total anthocyanin (TA) content to vary from 1229 to 2057 μg/g, coloured anthocyanins (CA) from 126 to 190 μg/g, and total flavanol (TF) from 16.4 to 65.4 μg/g catechin equivalents except Mocksang (M-5). The ethanolic extract from mulberry fruit shows a rapid and concentration-dependent increase of antioxidant activity. Especially, the antioxidant activities of M-2 and M-4 are higher than those of the others in a hemoglobin-induced linoleic acid system. (**Song-Hwan Bae and Hyung-Joo Suh**)

Berry extract contains high amounts of anthocyanins and is commonly used in diet or in some therapeutic applications. In this study, it was observed that cyanidin 3-rutinoside and cyanidin 3-glucoside (extracted from *Morus alba* L.) exerted a dose-dependent inhibitory effect on the migration and invasion, of highly metastatic A549 human lung carcinoma cells in absence of cytotoxicity. The results showed that cyanidin 3-glucoside and cyanidin 3-rutinoside treatments could decrease the expressions of matrix matalloprotinase-2 (MMP-2) and urokinase-plasminogen activator (u-PA) in a dose-dependent manner and enhance the expression of tissue inhibitor of matrix matalloprotinase-2 (TIMP-2) and plasminogen activator inhibitor (PAI). Further analysis with semi-quantitative RT-PCR showed that these alterations were all on the transcriptional level. Further, a treatment of cyanidin 3-rutinoside and cyanidin 3-glucoside also resulted in an inhibition on the activation of c-Jun and NF-κB. Together, these result suggested that anthocyanins could decrease the in vitro invasiveness of cancer cells and therefore, may be of great value in developing a potential cancer therapy. (**Pei-Ni Chen, Shu-Chen Chu, Hui-Ling Chiou, Wu-Hsien Kuo, Chui-Liang Chiang and Yih-Shou Hsieh**).

The neuroprotective effects of cyanidin-3-*O*-β-d-glucopyranoside (C3G) from the mulberry fruits on neuronal cell damage. A 1% HCl–MeOH mulberry fruit extract was shown to have a cytoprotective effect on PC12 cells that had been exposed to hydrogen peroxide. The extract inhibited the cerebral ischemic damage caused by oxygen glucose deprivation (OGD) in PC12 cells. The neuroprotective effect of the mulberry fruit extract was further demonstrated in vivo using a mouse-brain-injury model with a transient middle cerebral artery occlusion (MCAO). C3G was isolated as a neuroprotective constituent from the mulberry fruit extract. Compared with the control group, C3G had neuroprotective effects on the PC12 cells exposed to hydrogen peroxide in vitro and on cerebral ischemic damage in vivo. (**Tong Ho Kang, Jin Young Hur, Hyun Bok Kim, Jong Hoon Ryu and Sun Yeou Kim**)

From mulberry seeds there were extracted the lipids as vegetal glycerids 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) traces. (Tanase Doina, Constantinescu Marilena, Pau Elena, Ungureanu C.)

Mulberry fruits are used fresh, dried or frozen in the food industry to obtain different syrups, tonic wine, amaretto or vermouth wine, vinegar and different sweet products (marmalade, chocolate, frosting, jelly and fondant), oil from mulberry seeds. Mulberry fruit juice it is also used as natural alcoholic extract additive for food and pharmaceutical industries.

From the mulberry fruits after alcoholic fermentation and further distillation it is made a perfect hard alcoholic drink.

II.3. MORUS sp. Roots

The studies from the last years present the identification and isolation from *Morus sp.* root bark of many chemic compounds of different classes with pharmaceutical activity, which can be used in therapeutics. For example, a polysaccharide isolated from *Morus alba* root bark has an immunomodulating activity. Also, there have been isolated, physical-chemical described and pharmacological tested many flavonoides, compounds with classic flavonic structure, such as isoquercetin, quercetin and morin, or with much more complex structure, such as: phenyl flavonoides, morusin from *Morus nigra*, with analgesic properties, kuwanon G and H, from *Morus alba* with hypotensive activity, kuwanon G has also a strong antimicrobial activity for caryogenic bacteria: *Streptococcus sorbinus, S. sanguis* or *Porphyromonas gingivalis*.

Another polyphenols which has been isolated from *Morus Alba* root bark with pharmadynamics activity are: mulberroside C with weak antiviral activity for the virus Herpes simplex type 1; oxyresveratrol with inhibitory effect for enzymes such as cyclooxygenose-2 or mulberroside A with ant oxidative activity. From variety Ichinose root bark have been isolated two polyphenols from the water soluble fractions: ethyl β-resorcylate with antimicrobial activity for all type of fungus and plant's pathogen bacteria and 5,7-dihydroxychromone with selective antimicrobial activity.

From root bark was isolated chemical compounds with nitrogen such as polyhydroxilated alkaloids: polyhydroxilated piperidine, polyhydroxyl-non-tropane and polyhydroxy-pyrrolidine alkaloids: 1-deoxynoiirimicine which can prevent the diabet and obesity; and glycoproteins such as moron 20K which contains 20% serine and cysteine, the same as insulin.

The hypoglycemic activity of the flavonoids rich fraction of 70% alcohol extract of *Morus alba* root bark (MRBF-3) was evaluated after its oral administration to streptozotocin-induced diabetic rats. Diabetes was induced by injection of 60 mg kg^{-1} i.p. The administration of MRBF-3 to streptozotocin (STZ)-diabetic rats for 10 days in a dose of 200 and 400 mg kg^{-1} day^{-1} was not significant. However, administration of MRBF-3 for 10 days (600 mg kg^{-1} day^{-1}) significantly reduced the amount of the glucose from control level (379 ± 9 mg/dl) to a lower level (155 ± 8 mg/dl) and significantly increased the insulin level from control (10.8 ± 0.3 μU/ml) to a high level (15.6 ± 0.3 μU/ml). The measurement of produced lipid peroxides (expressed as the amount of thiobarbituric acid (TBA) reactive substance, nmol TBARS/ml serum) indicated antiperoxidative activity of MRBF-3. The oral administration of MRBF-3 to STZ-diabetic rats significantly decreased the lipid peroxides from 6.3 ± 0.8 to 5.1 ± 0.7 nmol TBARS/ml serum. The phytochemical investigation of MRBF-3 resulted in the isolation of four hydrophobic flavonoids with one or two isoprenoid groups ($\log P = 5–9$): morusin, cyclomorusin, neocyclomorusin, and kuwanon E, a 2-arylbenzofuran, moracin M, and two triterpenes, betulinic acid and methyl ursolate. The data obtained from this study revealed that MRBF-3 may protect pancreatic β cells from degeneration and diminish lipid peroxidation. However, this is the first biological screening of *Morus alba* root bark; further future merit studies including clinical study will be necessary in order to confirm the results obtained from this study. (**Abdel Nasser B. Singab Hesham A. El-Beshbishy, Makiko Yonekawa, Taro Nomura and Toshio Fukai**)

The antioxidant activity and liver protective effect of *Morus bombycis* Koidzumi were investigated. Aqueous extracts of *M. bombycis* Koidzumi had higher superoxide radical scavenging activity than other types of extracts. The aqueous extract at a dose of 100 mg/kg showed significant hepatoprotective activity when compared with that of a standard agent. The biochemical results were confirmed by histological observations indicating that *M. Bombycis* Koidzumi extract together with CCl_4 treatment decreased ballooning degeneration.

The water extract recovered the CCl_4-induced liver injury and showed antioxidant effects in assays of $FeCl_2$–ascorbic acid-induced lipid peroxidation in rats. Based on these results, we suggest that the hepatoprotective effect of the *M. bombycis* Koidzumi extract is related to its antioxidative activity. (**Ying-Shan Jin, Jae-Hoon Sa, Tae-Heum Shim, Hae-Ik Rhee and Myeong-Hyeon Wang**)

The fraction obtained from *Morus* bark have presented antimicrobial activity in the presence of Gram + bacteria (*Staphylococcus aureus* and *Streptococcus salivarius*) and lack of activity for Gram – bacteria (*Pseudomonas aeruginosa*) and some fractions have weak antifungal activity for *Candida albicans*.

Examining the antimicrobial activity of the fractions, no matter how they were obtained and the concentration in flavones expressed in ruthozide, it was observed the fact that there is a correlation between the antimicrobial activity for Gram + bacteria and the concentration in flavones. So as: F I (water extraction) which contains 0% flavones (ruthozide) presents a weak antimicrobial activity for *Streptococcus salivarius*; F II (30% alcohol extraction) and F VIII (chloroform extraction, the compression of extractive solution till at residue, extraction of some active principles from residue with ethylic alcohol 30% till at 80 ml volume of extract, at the reflux for 30 minutes) which contain 0.055% and 0.049% flavones presents moderate antimicrobial activity for *Staphylococcus aureus* and *Streptococcus salivarius*; F III (70% ethylic alcohol extraction), IV (30% ethylic alcohol, the compression of extractive solution under small pressure till 105 ml, the addition of 45ml alcohol 96%, standing-by 48 hours, centrifugation and supplemented of the volume till 150ml extract with 30% ethylic alcohol), V (70% ethylic alcohol, the compression of extractive solution under small pressure till 105ml, the addition of 45 ml ethylic alcohol 96%, standing-by 48 hours, centrifugation and supplemented of the volume till 150 ml extract with 24% ethylic alcohol), VI (acetone 70%, the compression of extractive solution under small pressure till at the volume of 105 ml, the addition of ethylic alcohol 95%, standing-by 48 hours, centrifugation and the addition of the volume at 150 ml extract with ethylic alcohol 30%) and VII with concentration of 0.085%, 0.090%, 0.150%, 0.0140% and 0.059% of total flavones present surely antimicrobial activity for *Staphylococcus aureus* and *Streptococcus salivarius*. (**Maria Ichim; Svetlana Colceru-Mihul; Doina Tanase**).

A prenylated flavonoid, moralbanone, along with seven known compounds kuwanon S, mulberroside C, cyclomorusin, eudraflavone B hydroperoxide, oxydihydromorusin, leachianone G and α-acetyl-amyrin were isolated from the root bark of *Morus alba* L. Leachianone G showed potent antiviral activity (IC_{50}=1.6 μg/ml), whereas mulberroside C showed weak activity (IC_{50}=75.4 μg/ml) against herpes simplex type 1 virus (HSV-1). Their structures were elucidated by spectroscopic methods. (**Jiang Du, Zhen-Dan He, Ren-Wang Jiang, Wen-Cai Ye, Hong-Xi Xu and Paul Pui-Hay But**)

The 70% alcohol extract of *Morus alba* L. root bark was fractionated over cellulose CC eluted with water, 50% methanol and finally with 100% methanol to yield 3 fractions (MRBF-1, MRBF-2 and MRBF-3), respectively. In continuation of chromatographic purification of 70% alcohol extract fractions of the Egyptian *M. alba* L. root bark, 4 compounds namely: mulberroside A, 5,7,2′-trihydroxyflavanone-4′-*O*-β-d-glucoside and albanols A and B were isolated from MRBF-2 for the first time from the Morus plant. Experimentally induced atherosclerosis was produced by feeding rats a diet enriched in coconut oil (25% by weight) and cholesterol (2% by weight) for 21 days. Then, hypercholesterolemic rats were orally administered (MRBF-1, MRBF-2 and MRBF-3 fractions) in a dose of 500 mg kg^{-1} day^{-1} for 15 successive days, in order to evaluate their expected hypocholesterolemic activity. Lipid profile parameters such as plasma total cholesterol, LDL-C, VLDL-C, LDL : HDL ratio and triglycerides, as well as plasma and liver lipid peroxides and glutathione-*S*-transferase enzyme levels, serum paraoxonase enzyme level, LDL oxidation, LDL aggregation and LDL

retention, were measured. Plasma and liver glutathione-*S*-transferase enzyme levels were unaffected in all studied groups. The results revealed that the administration of (MRBF-2 and/or MRBF-3) fractions resulted in alleviation of atherosclerotic state. Administration of MRBF-3 significantly retained plasma and liver peroxides towards their normal levels, and also, produced significant increase in resistance towards major atherogenic modifications; namely LDL oxidation, LDL aggregation and LDL retention by 44%, 30%, and 33%, respectively. Thus, it can be concluded that the consumption of MRBF-2 and (MRBF-3, in some extent) fractions of *M. alba* L. root bark 70% alcohol extract may act as a potent hypocholesterolemic nutrient and powerful antioxidant via the inhibition of LDL atherogenic modifications and lipid peroxides formation in hypercholesterolemic rats. (**Hesham A. El-Beshbishy, Abdel Nasser B. Singab, Jari Sinkkonen and Kalevi Pihlaja**)

Mulberry root biomass is a valuable raw material for the pharmaceutical industry, due to its high flavones and phenol content.

II.4. MORUS sp. – twig branches and wood stem

Because tyrosinase catalyzes melanin synthesis, tyrosinase inhibitors are important in cosmetic skin-whitening. Oxidative stress contributes to skin aging and can adversely affect skin health, which means antioxidants active in skin cells may support skin health. We examined 25 traditional Chinese herbal medicines that might be useful for skin-whitening and skin health. Extracts (100 μg/mL) were tested for cytotoxicity on human epidermal melanocytes (HEMn); 12 exhibited low cytotoxicity. Their effects on tyrosinase and melanin inhibitory activities and free radical scavenging activities were further assessed. Phenolic contents were evaluated using Folin–Ciocalteu reagent. Four herbs, *Pharbitis nil*, *Sophora japonica*, *Spatholobus suberectus*, and *Morus alba*, exhibited potent inhibitory effects on tyrosinase (IC_{50} values 24.9,95.6,83.9, and 78.3 μg/mL, respectively). Melanin inhibition was not dose-dependent. *Sophora japonica* (IC_{50}: 14.46 μg/mL, 1,1-diphenyl-2-picrylhydrazyl (DPPH); 1.95 μg/mL, hydroxyl radical) and *Spatholobus suberectus* (IC_{50}: 10.51 μg/mL, DPPH; 4.36 μg/mL, hydroxyl radical) showed good antioxidative activities and high phenolic contents (255 and 189 mg of gallic acid/g extract, respectively). Among active anti-tyrosinase extracts, *Sophora japonica* and *Spatholobus suberectus* were especially potent in HEMn cells in terms of free radical scavenging effects and high phenolic contents, making them the strongest candidates for cosmetic application found in the current study. (**Kuo-Hsien Wang, Rong-Dih Lin, Feng-Lin Hsu, Yen-Hua Huang, Hsien-Chang Chang, Ching-Yi Huang and Mei-Hsien Lee**)

Mulberry twig branches and wood stem are used in cosmetics – for hair lotions; moisture products for skin, in the paper industry, in the wood processing – for furniture, as fuel, in the food industry – for natural coloring, alcohol ennobling and in the textile industry – for making the so called “artificial cotton”.

II.5. MORUS sp. – phyto remediation

Benzo[*a*]pyrene, a high molecular weight (HMW) polycyclic aromatic hydrocarbon (PAH) was removed from solution by *Sphingomonas yanoikuyae* JAR02 while growing on root products

as a primary carbon and energy source. Plant root extracts of osage orange (*Maclura pomifera*), hybrid willow (*Salix alba*×*matsudana*), or kou (*Cordia subcordata*), or plant root exudates of white mulberry (*Morus alba*) supported 15–20% benzo[*a*]pyrene removal over 24 h that was similar to a succinate grown culture and an unfed acetonitrile control. No differences were observed between the different root products tested. Mineralization of ^{14}C-7-benzo[*a*]pyrene by *S. yanoikuyae* JAR02 yielded 0.2 to 0.3% $^{14}CO_2$ when grown with plant root products. Collectively, these observations were consistent with field observations of enhanced phytoremediation of HMW PAH and corroborated the hypothesis that co-metabolism may be a plant/microbe interaction important to rhizoremediation. However, degradation and mineralization was much less for root product-exposed cultures than salicylate-induced cultures, and suggested the rhizosphere may not be an optimal environment for HMW PAH degradation by *Sphingomonas yanoikuyae* JAR02.

Bacterial benzo[*a*]pyrene cometabolism, a plant-microbe interaction affecting polycyclic aromatic hydrocarbon phytoremediation was demonstrated with *Sphingomonas yanoikuyae* JAR02 that utilized plant root extracts and exudates as primary substrates. (**Jeremy A. Rentz, Pedro J.J. Alvarez and Jerald L. Schnoor**)

The development of moriculture as an ecological landscape technology shall be an important approach for the sericulture re-launch.

MAJOR CONSTRAINTS AGAINST AND RECOMMENDATIONS FOR PROMOTION OF USING THE SERICULTURE PRODUCTS FOR NON –TEXTILE PURPOSES IN THE BALKAN COUNTRIES

- **Lack of tradition and experience in the scientifically based processing the waste and by – products from sericulture;**
- **Not sufficient research made on the subject;**
- **Lack of enough industrial technologies for sericulture products non-textile processing;**
- **There is an urgent need for sericulture product diversification to meet the demands on the international market;**
- **The present European system for giving subsidies to the sericultural farmers requires producing obligatory not less than 20 kg of fresh cocoons from one box of eggs. In the case that the farmers sell for example silkworm larvae instead of cocoons they can not get any EU subsidy. Therefore there should be found some other criteria to prove the amount of silkworms reared and the EU regulations for the subsidies to be changed in order to stimulate not only the cocoon production but the other products from sericulture as well.**
- **The mulberry and silkworm breeding priorities may be re-directed in order to meet the requirements of the products use for non – textile purposes. For example some new mulberry varieties with high fruit yield and quality or**

varieties with faster growth and accumulation of quality wood for the furniture industry may be created. In the silkworm new strains having bigger larvae and pupae or with higher sericin content or tolerable to artificial diet feeding or breeds with some specific qualitative traits may be evolved.

- **It is also important to develop a technology for production and supply with high quality silkworm eggs all over the year.**
- **It is necessary that the mulberry agrotechnics and harvesting systems and the silkworm rearing technologies to correspond with those new non – textile purposes product use.**
- **The silkworms use for non textile items requires in many cases to grow them all over the year, therefore the Balkan countries have to develop the silkworm artificial diet rearing system.**
- **The producers should identify long-term strategic policies, technology development, and innovations, considering the importance of the factors involved such as product standard, production technology, technology development, and product innovation.**
- **It is expected that production costs in the Balkan region will increase while more countries with lower labour costs will join the sericulture production. Therefore, it is challenged to the region producers to compete in the world markets. In order to survive in sericulture business, producers should not make cheap products with low quality to compete for the prices but to produce more sophisticated products which satisfy consumers even though they are more expensive.**
- **The strategy of Balkan region sericulture products processing industries should be to produce our own unique products which are definitely different from other countries.**
- **It is important to encourage the sericulture products processing companies to establish global relationships, such as international joint ventures and cooperative deals in the field of research and technology licensing.**
- **The producers should establish a network to make collaboration among different sectors of supply chain at the regional level. The different sectors, which include all stakeholders in sericulture products processing industry from downstream to upstream of supply chain, may be formed into a cluster according to the local which they are. The cluster system can reduce and eliminate the problem of producers by supporting each other in term of raw material, technology, equipment and marketing. So this system is more efficient in term of cost reduction, quality improvement and quick responding to consumers' needs.**
- **And final but very important is also to promote the new sericulture non – textile products in general and as natural and environmental friendly in particular.**

CONCLUSIONS

1. Proper utilization of secondary and waste products of sericultural industry can generate extra income in addition to the silk, the main output. The major wastes and by products of sericulture are sericin, pupae, moths, silkworm excreta, silk fiber waste and mulberry leaves, fruits and roots. The new commercial products have been obtaining from these raw materials with the valuable destination for pharmaceutically (anti diabetic, antiviral, hypoglycemic, hypotensive, antibacterial and antivirus products), cosmetically (skin and hair products), zootechnically (fodder for rabbits, goats, poultry, swine, sheep and fur animals), foodstuff

(oil, juice, marmalade, wine, fruit distillate, vinegar, dried fruit powder, natural coloring), ecological use (landscape, phytoremediation).

2. Considering the high potential of using the sericulture products for non – textile purposes the research institutions in the Balkan countries should pay more attention in doing research in this field in order to create new valuable items, utilizing the available mulberry and silkworm germplasm resources, mulberry plantations and silkworm rearing infrastructures.

3. The development of sericultural products use for non – textile purposes direction may be considered as an alternative way to solve partly the problem with the decline of silk production and the efficient utilization of the existing sericultural human capacity, research and production facilities.

4. It seems to be difficult and costly for new entrants to build such a large amount of know-how in relatively short periods of time. Therefore, these problems may be eliminated by regional collaboration or network establishment. Therefore, domestic, regional and international cooperation can help in sharing information on research and technologies.

5. It is necessary to establish an international working group on the sericultural products use for non – textile purposes which may include countries new comers, comparatively more developed, developing, advantage, producer, consumer and trader, to discuss, identify, and analyze major constraints and strategies for development in global aspect. This group may include experts in all areas and fields both private and government sectors.

6. As a follow up of the first Balkan workshop on "Possibilities for Using Silkworm and Mulberry for Non-Textile Purposes" a regional working group on the sericultural products use for non – textile purposes may be established, including members from all Balkan states.

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MULBERRY BIOMASS IN BULGARIA

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ABSTRACT

General productivity and balance of biomass from some local mulberry varieties have been studied with a view to fully utilization of biomass from agriculture by realization of special technological and organizational measures. It was determined that biomass yield per tree varies from 12.58 kg at local mulberry to 19.17 kg at No 106 variety in case of annually pruning of mulberry trees in intensive plantations for silkworm rearing. We consider that in Bulgaria it is possible to obtain more than 10000 t of biomass from available singly mulberry trees and several thousand da of mulberry plantations per year. Main part of this biomass - mulberry leaves (4000 t) is enough for rearing of 8000 boxes of silkworm eggs and producing of about 210 t fresh cocoons. Also it is possible to collect 130 t mulberry fruits and use them as human or animal food, or for producing several kinds of preserves. Mulberry branches and perennial wood could be used with success as alternative source of fuel. Besides they can be composted independently or together with remnants from silkworm rearing for alternative fertilization of many agricultural crops.
Key words: mulberry, biomass, by-products

INTRODUCTION

Incorporation of Bulgaria to European community impose on organization and export of ecology friendly production, which is connected with realization of technological and organizational measures for environment preservation and for fully utilization of biomass from different agricultural crops (Slavov, 1995).

Mulberry is one of the most useful plants for human and because of this it is worldwide distributed, including Bulgaria. Mulberry is characterized with very good vegetative regeneration capacity based on dormant bud's excitability and the very strong external radicular system. Mulberries are able to be cultivated on different soil type 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, and cellulose).

Main productions from mulberry - leaves are only one food for silkworm Bombyx mori L. (Kojuharov and Hristov, 1960; Petkov and Penkov, 1980). Besides this mulberry implies significant quantities of secondary and waste products such not used mulberry leaves, branches, root and wood biomass, mulberry fruits, etc. In order to ensure a profitable activity, it is necessary to process these secondary and waste products.

One of the most important goals of specialists is work out a balances of available biological resources and increased use of by-products from main agricultural crops. This will provide environment protection against pollution and decrease utilization of different chemicals and energy as well (Nedjalkov, 1995).

The aim of the present study was to determine the balance of mulberry biomass in Bulgaria and to discuss possibilities for its future use.

MATERIAL AND METHODS

The study has been carried out at Sericultural Experiment Station in Vratza. The biologic material was represented by 7 Bulgarian mulberry varieties. Maximum biological and technological production of the trees for traditional in Bulgaria system (222 plants/da, 3 x 1.5 m planting scheme and non-irrigation condition) were determined. General biomass from mulberry was separated on leaves, branches and fruits (sorosis), and on main production (useful and declined) and by-products.
Available data of nowadays mulberry fund in Bulgaria (Petkov, 2007) was used for determination of mulberry biomass balance. Energy value of mulberry branches was determined by calorimetric value of Krisha et al. (1932).

RESULTS AND DISCUSSION

The moriculture represents an unique phyto-technology for biomass producing with higher commercial value. Since the silkworms rearing activity is decreasing, it was necessary a diverstification of exploatation methods for mulberry plantations. There were made many researches regarding mulberry plants utilization for phyto regenerable, phyto pharmaceutical activity, agroalimentary production etc.
By products from main agricultural crops commonly can be used as forage for animals and also as manure or compost. In moriculture after silkworm rearing it is possible to obtain additionally animal forage, fuel, biogas, compost and fruits.
It was determined that biomass yield depends on mulberry variety. The biggest biomass per tree 19.17 kg was produced from No 106 variety, followed by Vratza 1 (15.29 kg), Vratza 18 (14.83 kg) and No 24 (17.43 kg). Local mulberry was characterized with comparatively low biomass production per tree - 12.58 kg, regardless of quantity and quality of leaves. Biomass obtained from 1 da mulberry plantation was varied from 1692 kg in No 59 variety to 3805 kg in No 106 variety (table 1).
According to Tzenov et al. (2008) 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 and rest dry matter must be utilized.
Most of mulberry trees are female and percentage of fruits in biomass is about 8 %. Despite their features, which made them suitable as food for human, animals and birds they do not find proper application till now (Tewari and Rao, 1990).
Mulberry fruit is a small berry, weighing 4-5 g maximum. The color depends on the cultivar. For Morus alba L., some cultivar have white fruits, other ones have black fruits, for Morus nigra L., the fruit is black, for Morus rubra L. and Morus multicaulis L. some cultivars have black fruits, some others have deep red fruits when ripen, for Morus kagayamae Koidz, the fruit is black. The fruit falls from the tree as soon as it is fully ripe. It is best, therefore, to grow the tree in short grass to cushion the fall of the fruit but to still make it possible to find and harvest. The fruit is up to 20 mm in diameter for local varieties.
The mulberry fruits has a long history of medicinal use in old medicine. Sweet and sour in flavor, mild in nature, it is related to the liver and kidney channels. Moistens and tones liver and kidneys, nourishes blood, sharpens vision, produces fluids, quenches thirst, benefits vital energy and eliminates excessive fluids. Mulberry fruit is used for liver-kidney yin deficiency,

ringing in ears, dizziness, insomnia, rheumatic pain, premature gray hair, constipation, diabetes.
Mulberry fruit can be eaten raw, cooked or used in preserves.
A delicious slightly acid flavor, it makes an excellent dessert fruit and can be eaten in quantity. The fruit is juicy and refreshing, though it must be used as soon as it is ripe (from mid-August to September) otherwise it will start to rot. The fruit can also be dried and ground into a powder. Mulberry fruit is rich in carrotene, vitamins B1, B2 and C, glucose, sucrose, tartaric acid and succinic acid.
The percentage of the leaves in total biomass was varied from 38 % in local mulberry to 55 % in Vratza 1 and 59 % in Veslets varieties. Local and hybrid mulberry phenotypes have the lowest leaf yield and their leaves are small in size and serrated. Leaf yield of old Bulgarian varieties No 3, 24, 26 and 59 is bigger and varies from 867 kg/da to 1351 kg/da. New Bulgarian varieties – Vratza 1, Vratza 18 and Veslets were characterized with the biggest leaf yield (1376 - 1722 kg/da at non irrigated condition) which is juxtaposed to the world standards. Therefore cultivation of new high productive mulberry varieties will allow more biomass production (especially leaves for silkworm rearing) and more by products for further application.
Mulberry leaves could be used for producing of tea and included in some medicines as well. Recent research has shown improvements in elephantiasis (enlargement and thickening of tissues) when treated with leaf extract injections and in tetanus (an acute infectious disease characterized by tonic spasm of voluntary muscles especially of the jaw and caused by the specific toxin of a bacterium (Clostridium tetani) which is usually introduced through a wound) following oral doses of the sap mixed with sugar. The leaves are antibacterial, astringent, diaphoretic (increase perspiration), hypoglycaemic (abnormal decrease of sugar in the blood), odontalgic (relating to or marked by toothache) and ophthalmic (relating to, or situated near the eye). They are taken internally in the treatment of colds, influenza, eye infections and nosebleeds.
One of main sources of non commercial fuel for sericulture farmers are dry mulberry branches after silkworm rearing and mulberry tree wood. In India near 64 % of generated energy is used for family purposes and part of non commercial fuel is covered by mulberry branches Chinnaswami and Haripriprasad, 1995).
Mulberry wood is one of the best. It possesses medium density hardwood with a closed, straight grain, bright yellow sapwood with a light tan heart wood. Its colour tends to turn brown with exposure to sunlight. The late growth in mulberry is full of open pores, much like ash.
It was determined that branch yield per 1 tree was varied from 4.44 kg in No 59 to 7.94 kg in No 106 variety. No 106, local mulberry, No 24, Vratza 18 and Vratza 1 varieties were characterized with biggest branches yield per da, 1308 kg, 1378 kg, 1308 kg, 1198 kg and 1170 kg, respectively. These qualities possess energy value between 1808 Kcal and 3593 Kcal according calorimetric value of Krisha et al. (1932) and could be used with success as non commerce source of fuel.
Mulberry stem could be used in medicine also. The stems are antirheumatic, diuretic, hypotensive and pectoral (something worn on the breast). A tincture of the bark is used to relieve toothache.
Nowadays in Bulgaria there are about 4000 da of mulberry plantation and 700000 single mulberry trees, which guarantee more than 12000 t of biomass annually. 8000 boxes with silkworm eggs could be reared and 210 t of fresh cocoons could be produced with available 4000 t leaves. About 31 % from the main production drop off mainly with fall of the leaves (1600 t), but has great meaning for improving of soil fertility trough increasing of organic matter in the soil.

170 t of mulberry fruits are available as by product and 130 t of them could be used as food or source for food processing industry.
Mulberry branches represent main part of mulberry by products (7000 t) and about 5800 t could be used as fuel with 26.68x109 Kcal energetic values.
A lot of mulberry branches are leaved after silkworm rearing. This quality (750 t for all country) may be composted successfully, independently or together with other remnants from silkworm rearing and used as good organic fertilizer (Tzenov et al., 2008). 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 %.
Another possibility for mulberry biomass using is for biogas producing. The biogas is an anaerobe fermentation product of biomass organic waste which can be used directly as fuel and also as commercial compost and fertilizer. These days, the biomass is recognized as one of the major regenerated energy source. According to Tanase et al. (2008) mulberry regenerate the vegetative biomass starting form the 3rd year after plantation which is a regenerated energy bio resource, obtained by direct burning or anaerobe fermentation (biogas – 42.5 m3/50 kg vegetal dry biomass). By the SWOT method, the same authors estimated the economical potential of biomass Morus sp. plants as regenerated energy resource and found that this biomass can be the energetic source, by using biomass conversion technologies in order to obtain bio fuel (burning and gasification).

CONCLUSIONS

Possibilities for producing of formidable amount of organic biomass with versatile application which mulberry hold out, allow development of sericulture as part of alternative ecological agriculture.
On the other hand by products from mulberry cultivation could be used with success more intensively, leaves for herbal and forage, fruits as human and animals food and in medicine, branches and stems as non commerce fuel.

Table 1. Biomass from 1 da mulberry plantation

Variety	Total kg	Leaves kg	Fruits kg	Branches	
				kg	Kcal
No 3	2112	1078	-	934	2608
No 24	3246	1351	587	1308	3008
No 59	1692	867	39	786	1808
No 106	3805	1680	563	1562	3593
Vratza 1	2944	1545	229	1170	2691
Vratza 18	2841	1376	267	1198	2765
Local	2775	768	629	1378	3169

Table 2. Balance of mulberry biomass in Bulgaria, t

Production	Total production			Addition production			Residues from silkworm rearing
	Total	Could be used	Remain in the field	Total	Could be used	Remain in the field	
Mulberry leaves	5600	4000	1600				
Fruits				170	130	40	
Branches				7000	5800	1200	
Residues from silkworm rearing							750
Total	5600			7170	5930		750

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SPECIAL FEATURES OF FRUITS FROM SOME LOCAL MULBERRY VARIETIES AND POSSIBILITIES FOR THEIR UTILIZATION

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ABSTRACT

Fruits from 15 local mulberry varieties of SES Vratza's gene-bank were studies with a view to fully investigation of mulberry bio-diversity in Bulgaria and of necessity for utilization of sericulture by-products.

Available female mulberry varieties could be used not only for silkworm rearing but for fruit producing as well. Mulberry fruits are suitable as human and animal food in fresh or processed state.

Mulberry varieties P 4, P 6, No 24 and Vratza 1 are especially perspective for fruit producing.

Some possibilities for utilization of mulberry fruits in food processing industry and medicine are discussed also.

Key words: mulberry, biodiversity, gene bank, fruits, food industry

INTRODUCTION

Mulberry belongs to Moraceae family. In Bulgaria are widespread trees from *Morus Alba*, *Morus Bombycis, Morus Multicaulis* and *Morus Nigra* species. Depending of cultivation mulberry is fast growing plant, tree or bush in habit. It is well known that mulberry allow yearly pruning of all branches for silkworm feeding with their leaves.

Most of mulberry trees are female, fruit-bearing and percentage of fruits in biomass is about 8 %. Despite their features, which made them suitable as food for human, animal and birds they do not find proper application and utilization till now.

Research on mulberry reproductive structure has a great meaning for choice of proper varieties and direction for their cultivation (Gatin and Ogurtzov, 1981; Mukerdjee, 1965; Petkov, 1998), especially for producing of fruit direction.

The aim of the present study was to analyzed morphological features of fruits which determined reproductive structure of some Bulgarian mulberry varieties from SES Vratza's gene bank and direction for their further utilization.

MATERIAL AND METHODS

The study was carried out at SES Vratza mulberry gene bank, which was established 40 years ago and maintains under standard cultivation (Petkov, 1998).

The biologic material was represented by 21 Bulgarian mulberry varieties. Maximum biological and technological production of the trees for traditional in Bulgaria system (333 plants/da, 3.0 x 1.0 m planting scheme and non-irrigation condition) were determined. 10 trees per variety were analyzed for main morphological character of mulberry racemes, flowers, fruits and seeds, according methods for mulberry vegetative resources investigation (Petkov, 2000). Colour and taste of mulberry fruits were determined subjective.

RESULTS AND DISCUSSION

Data from our investigation are presented at table 1 and figures 1-3.

Table 1. Morphological features of mulberry fruits

Variety	Length cm	Diameter cm	Taste	Weight of 10 fruits g	Colour
P 4	1.82	1.06	Sweet	12.87	Dark red
P 6	2.46	1.58	Very sweet	29.32	Black
P 7	1.89	1.06	Very sweet	4.10	White
P 9	1.80	1.14	Sweetish	11.30	White
P 13	1.75	1.11	Sweet	4.09	Black
P 14	2.10	1.27	Sweet	10.30	Light red
P 16	1.85	1.11	Sweetish	12.73	Dark red
P 19	1.68	1.28	Sweet	10.29	Dark red
P 20	1.87	1.15	Sweet	14.17	Black
P 22	2.07	0.97	Slightly sour	12.96	Black
P 23	1.58	1.10	Sweetish	10.97	Dark red
No 24	1.93	1.19	Very sweet	8.69	White
No 106	1.86	1.19	Sweetish	9.34	White
Vratza 1	2.20	1.20	Sweetish	14.14	Light red
Vratza 18	1.59	1.06	Sweet	10.42	Dark red

Mulberry is dioecious plant. From all Bulgarian mulberry varieties female ones are predominant, 15 varieties, followed by male varieties - 4 and P 13 and P 19 are bisexual.
Most of mulberry trees are female and percentage of fruits in biomass is about 8 %. Despite their features as food for human, animal and birds they do not find proper application till now (Tewari and Rao, 1990). Female varieties could be cultivated not only for silkworm rearing but for fruit production as well.
At predominant part of Bulgarian varieties mulberry racemes and fruits are situated on weak but more than one year branches and shoots. The length of racemes was varied from 7 mm in P 21 to 28 mm in P 10 and width from 5 mm in P 22 to 12 mm in No 106 variety. Varieties with 20-30 flowers in 1 raceme (10 varieties) and racemes with 30-40 flowers (10 varieties) are predominant.
Mulberry fruits (sorosis) are characterized with their size, shape, weight, chemical content and taste. The qualitative characters of fruits are influenced not only by variety but from ecological conditions and applied cultivation as well.
Mulberry fruit is a small berry, weighing 4-5 g maximum. The color depends on the cultivar. For Morus alba L., some cultivar have white fruits, other ones have black fruits, for Morus nigra L., the fruit is black, for Morus rubra L. and Morus multicaulis L. some cultivars have black fruits, some others have deep red fruits when ripen, for Morus kagayamae Koidz, the fruit is black. The fruit falls from the tree as soon as it is fully ripe. It is best, therefore, to grow the tree in short grass to cushion the fall of the fruit but to still make it possible to find and harvest.

The length of mulberry fruits was varied from 1.58 cm in P 23 to 2.46 cm in P 6 and varieties with average length between 1.8 and 2.2 cm are predominant. The shape of mulberry fruits is mainly cylinder type and their diameter was varied in wide limits, from 0.97 to 1.58 cm, respectively.

The colour of mulberry fruits is an important variety distinct character for mulberry. For tested Bulgarian varieties the fruit colour was varied from white to black. P 7, P 9, No 24 and No 106 possesses white to light pink berries and the rest varieties were characterized with dark red to black coloured fruits.

Another characteristic of mulberry fruits is their taste, which varied from very sweet to light sour. P 22 variety has light sour fruits and P 6, P 7 and No 24 varieties are characterized with very sweet fruits (fig 1).

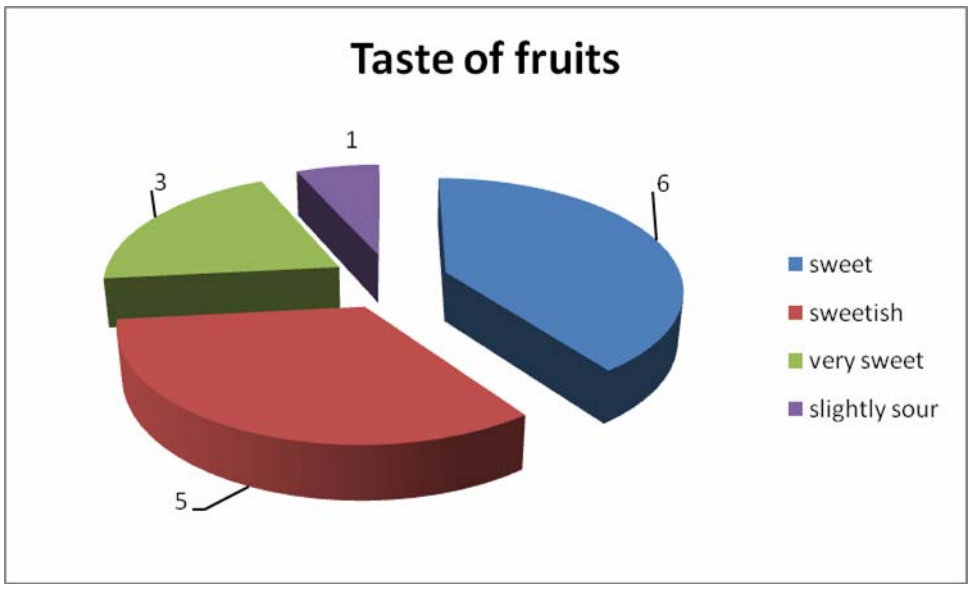

Fig. 1. Taste of fruits from Bulgarian varieties.

It was determined high variation in the average weight of mulberry fruits, which affects the fruit yield and economic value of tested cultivars. P 7 and P 13 varieties have the lightest fruits, 0.41 g and P 6 the heaviest ones – 2.93 g. A large number of varieties are characterized with average weight of fruits – from 1.0 to 1.5 g (fig 2).

The mulberry fruits has a long history of medicinal use in old medicine. Sweet and sour in flavor, mild in nature, it is related to the liver and kidney channels. Moistens and tones liver and kidneys, nourishes blood, sharpens vision, produces fluids, quenches thirst, benefits vital energy and eliminates excessive fluids. Mulberry fruit is used for liver-kidney yin deficiency, ringing in ears, dizziness, insomnia, rheumatic pain, premature gray hair, constipation, diabetes.

Mulberry fruit can be eaten raw, cooked or used in preserves. Tasty jams, marmalades, compotes, sweet and other courses, soft and strong drinks, vinegar etc could be prepared from them or with their participation. A few hundred recipes from world cuisine with mulberry fruit participation are available.

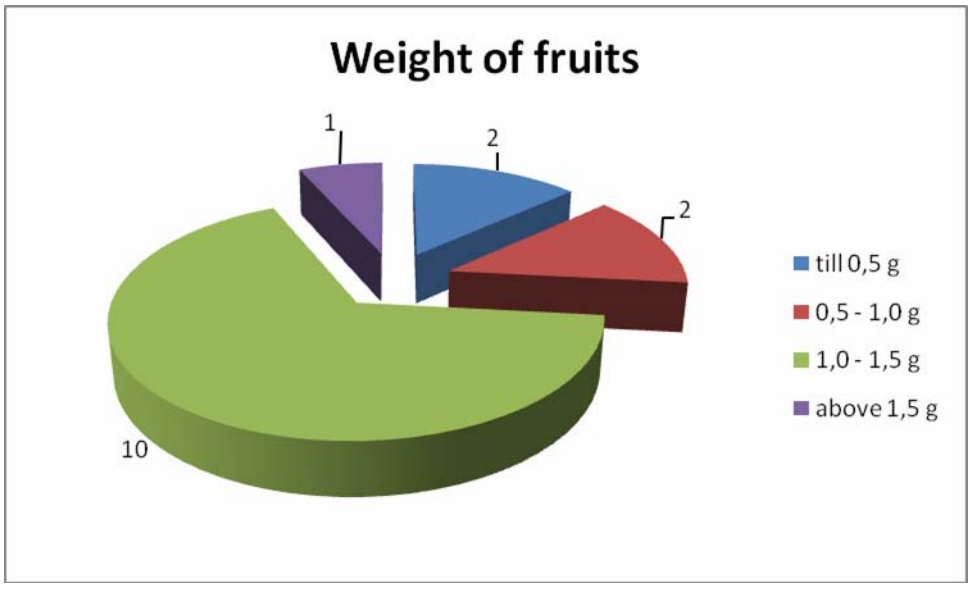

Fig. 2. Weight of mulberry fruits.

A delicious slightly acid flavor, it makes an excellent dessert fruit and can be eaten in quantity. The fruit is juicy and refreshing, though it must be used as soon as it is ripe otherwise it will start to rot. The fruit can also be dried and ground into a powder. Mulberry fruit is rich in carrotene, vitamins B1, B2 and C, glucose, sucrose, tartaric acid and succinic acid. The fruit has a tonic effect on kidney energy. It is used in the treatment of urinary incontinence, tinnitus (a sensation of noise (as a ringing or roaring) that is caused by a bodily condition), premature greying of the hair and constipation in the elderly. Its main use in herbal medicine is as a coloring and flavoring in other medicines.

Research on followed scientific-applied problems are necessary for acceleration of mulberry fruits utilization in the future:

- **Selection of new varieties for fruit production;**
- **Determination of fruit chemical and biochemical content;**
- **Observations on phonological development of mulberry flowering, pollination and fruits maturing;**
- **Development of technology for establishment new mulberry plantations for fruit production;**
- **Development of technologies for exploitation and pruning of mulberry plantations for fruit production;**
- **Development of recipes and technologies for processing of fresh mulberry fruits;**

CONCLUSIONS

From analysis of the data collected it could be concluded the presence of some significant differences in reproductive structure of Bulgarian mulberry varieties, preserved at SES Vratza mulberry gene bank.
Available female varieties could be used for fruits producing under special cultivation.
The most perspective mulberry varieties for fruit production are P 4, P 6. No 24, No 106 and Vratza 1.

Fig. 3. Mulberry fruits

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CORRELATION BETWEEN ISSR MAKERS AND COCOON QUALITY IN A MULBERRY SILKWORM, BOMBYX MORI L.

By

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ABSTRACT

The mulberry silkworm, Bombyx mori L is an important insect economically and an excellent model genetic system. Genome analysis has been initiated in the silkworm with the objectives of obtaining genetic maps using different markers systems: RFLP, RAPD, ISSR….Molecular genetic analysis of such complex traits using DNA markers would provide valuable tool for economic improvement of this insect.

To address this issue, fifteen ISSR primers were screened for their ability to reveal molecular diversity between thirty silkworm strains from two groups with deference in cocoon quality. This led to the identification of marker S12.1100 generated by one primer, which associate with cocoon quality of strains in silkworm. Carry out successful sequencing of fragment S12.1100, which similarities compared to the ones in GenBank vary from 71,608% to 89,671%. Designed primer and amplify specific marker S12 1100 and it may be to enlarge to used such marker in molecular marker-assisted breeding programs.

INTRODUCTION

Mulberry silkworm (Bombyx mori L.) is one of the important economically insects. Silk fabric has long been welcome all over the world. Silk industry play an important role by Vietnam, China, India and other developing countries. Among many factors that drive silk industry, the breeding and commercialization of high yield and quality silkworm varieties has contributed greatly to the stabilization and strengthening of this industry.

A large number of morph biochemical markers have been linked to molecular markers (Shi et al. 1995; Yasakuchi 1998; Nagaraju 2000). However, little has been done to understand the genetics of productivity traits, except in the analysis of heritability and combinatorial ability (Tazima 1964; Shibukawa et al. 1986; Rao et al. 1991; Chaterjee et al. 1993). DNA markers closely linked to a characteristic of interest could be used to select for that trait in different ways. However, within an intraspecific context, the quantitative trait loci mapping of many important agronomical characteristics require using very informative markers that allow the discrimination of a large number of bands (Tan et al. 2001). Of course, the affectivity of such marker-assisted selection depends on the precision of the phenotypic classification of the trait of interest and the degree of linkage between the marker and the trait (Nagaraju et al. 2002). Development of molecular markers is important in the silkworm for construction of linkage map, fingerprinting of strains for breeding and marker-assisted selection. In the current study we report the correlation between ISSR makers and cocoon quality in silkworm Bombyx mori L.

MATERIALS AND METHODS

Experimental material. Thirty silkworm strains divided into two groups with differences in cocoon quality used in the study were from the Seri cultural Research Center, Vietnamese Academy of Agricultural Sciences. Each strain was carried out a total of ten individual's pupae.

DNA extraction and generation of PCR profiles. Using the standard method of phenol chloroform extration, the genomic DNA was extracted from 10 pupae from more than two broods. In the extraction grinding of the tissue (0.01 g) was carried in 900µl extraction buffer (NaCl 50mM, EDTA 50mM, SDS 1%), at 56°C with 10 µl of K-proteinase (100 mg/ml). The tissue was incubated and ground periodically until there were no visible fragments; the samples were then centrifuged for 15 min at 12,500 rpm. 10 µl of potassium acetate (8M) were added to the extract before centrifugation. The aqueous phase was recovered and re-extracted with an equal volume of a chlorophorm- isoamyl alcohol mixture (24:1) for 10 min. After centrifugation at 12,500 rpm for 15 min, two volumes of absolute ethanol and 1/10 sodium acetate 3M were added to the separated aqueous phase to precipitate nucleic acids. After 15 min of centrifugation the supernatant was discarded and the pellet was air-dried. The pellet was re-suspended in TE buffer (Tris HCl 10mM, EDTA 1mM, ph: 8.0) and incubated one hour at 37°C after the addition of 10 µl ARNAse (100 µg/ml). The DNA was re-extracted with phenol-chloroform-isoamyl alcohol and ethanol precipitated as before. The DNA was re-suspended in TE buffer, quantified by optical absorbance and examined by electrophoresis in an agarose gel (0.8%) stained with ethidium bromide.

PCR was carried out with a Gene Amp® PCR System 9700, using 20 µl of reaction mixture containing 2 µl of 10x PCR buffer (20 mM MgCl2), 2 µl of 2mM dNTP, 4 µl of primer 2,5 mM, 1 µl of DNA (20ng/ µl), 0,5 µl Taq DNA polymerase (Institute of Biotechnology of Vietnam - 5U/ µl), 10,5 µl autoclaved water. The PCR schedule adopted was 1 cycle 94oC for 2 min, followed by 35 cycles of 94 oC for 3 second, 50 oC for 40 second, 72 oC for 2 min and a final extension of 10 min at 72 oC. PCR products were separated on 1,3% W/ V agarose gel in 1x Tris - boric acid - EDTA buffer. Ten microlitres of PCR products were loaded with 2 µl of tracking dye and run until the dye was 7cm - 8 cm from the wells. The profiles were stained with ethidium bromide and documented by the documentation unit of ultra violet products, U.K and photographs were later used for binary scoring. The banks were numbered sequentially from high to low molecular weight positions for each primer.

Primer selection for ISSR. Fifteen primer of University of British Columbia Biotechnology Laboratory Primer kit NO 9 were selected for polymorphism and detailed were done twelve dinucleotide (AG)8, (GA)8T, (TC)8C, (TC)8A, (CT)8A, (TC)8A, (TG)8G, (AG)8 YC, (CT)8GC, (AC)8RG, (GT)8YG, (AC)8YG, two trinucleotide (ACC)6, (GYY)6 and one pentanucleotide (GGGGT)2(GGGT)2T repeat primers.

Characterization of selected PCR products. The specific amplified product of interest was excised from agarose gel and DNA was eluted with a QIA quick gel extraction kit (Fermentas). To get a sufficient quantity of DNA, the eluted DNA was used as a template for re-amplication with the same primer, re-eluted and cloned into the R/T vector in the InsT/ATM cloning kit (Fermentas). Sequencing was done using the universal sequencing primres. A DNA sequencing kit was used with an a semi-automatic DNA sequencer (ABI PRISM® 3100 Avant Genetic Analyzer, Applied Bios stems, Perkin Elmer, Avestha Gengraine Technologies PVT Ltd.). The sequence was first cleaned of any vector contamination and the presence of the ISSR primer sequence was ascertained with the help of the National Centre for Biotechnology Information (NCBI) pair-wise BLAST search (http://www.ncbi.nlm.nih.gov/blast/bl2seq. l2.html).

RESULTS AND DISCUSSION

Out 15 ISSR primers tested, twelve gave distinct polymorphic products, three primers did not yield any amplification products. The number of markers generated for genomic DNA of 30 silkworm stocks with twelve ISSR primers varied from 5 to 9 and the percentage of polymorphism ranged from 79,69 to 100,00. The degree of polymorphism appeared to be independent of the number of markers generated by a particular primer (Binh, 2008).

Association between molecular markers and productivity components

The silkworm genome was found to be an excellent subject for analysis in fingerprinting programs for the identification of markers associated with productivity characteristics. Cocoon and shell weight are two very important economic traits that researchers have tried to physically identify in the genome for more than half a century. The analysis showed the importance of the band S12.1100 associate with high cocoon quality, because this band are presence in high cocoon quality strains and absence in strains with low cocoon quality. Correlation analysis between bands and productivity characteristics showed positive and negative associations. In some cases markers for both cocoon weight and shell weight were observed. The contribution of these common markers was positive for both in some cases and positive for one and negative for the other in other cases. This result shows that a higher cocoon weight does not necessarily reflect a higher shell percentage. These characteristics seem to be controlled by different genes, confirming the evidence reported by He et al. (1998).

Cloning and sequencing the band S12.1100

With help of SCAR (Sequence Characteristic Amplified Region) technique, the process of cloning and sequencing the band S12 1100 were done. The results of S12 1100 sequence are presented in figure 1.

TCTCTCTCTC TCTCTCTAAA CAAGAATTCC ACATCATCCT GACTGCTCTG CCAAGAGCAA CCGACTATAA TGAGGTACTT 80
TTTACTCTTT AGGAAAAGAT CTTTAGGTGA AAGATCGCTC GATCCGCCTA TTGTAAAATG GTAACCGTAG AAAATAGTTA 160
TGAGAAAGTG AATGCCACTA TCCAGTTTGA GACATGAGGT AGAATTCGCC TCATTGTGTT TGGTAGCTCT GATTATATTA 240
CGTTTTTCCA TAACTTAATC TGTATCTTTG CCATAAAGAG ACTCGACAAC TTAGATTTGA CTAGAAAAAA AACAACAGAG 320
AATCGGACCT TTCCTCGTAT TTTAGCATGT TTCCACACAC ATATAGGCTA TATAAACACA ATATATTTAC TGGTCGTCTT 400
GTGAATCCGC AAGGGGAGGT ACTACCAGCC TGCCAATTTC TGTTGTGAAT TACCAATGCG TTCCAGTTTG AAGGGTGGGG 480
CAGCCACTGT AAGCCTGAGA CCTTAGAGCT CAAATTTCGA GGTGGGATTT ACGTTGTAAA TGTCTATGGG CTCCGATAAC 560
CACTTCACAT GAGGTGGGTC GTAAGCTCGT CCAACCATCT GAGCCATAAA AAACATATAT AAGTACCACA AGCTCCTTAT 640
AAATCAATCG AAATGATAGA CTGACTTGTA AAAAACAAAC TAGCATTGAA CGTCAGAGAC TGAAAATAAA CATCTAAAGA 720
TCTCAAACGT AGCTTGACGA TCGACATCAA ACTAACCGTT CTAATGTTAC CGCATTCGGT CGGTTCATGG GGGTTTTTAA 800
CTAAATTCGG TATTCGGTTT TCGTGTGACG GAAAGAAAAG CGAAGgAATC TTCATTGTTA TTACGAAGGA GACTTTTACG 880
TTCGCTACGT CCATTAGTAT TATTCATTCG TCCACGTACC TAAAGCTtGc GATTTTAATG TGAGATACGT CATCAAAATA 960
TGTTGTTGTR ATTCATTCGT CCACGTAATG CTTGCGATTT AGAGAGAGAG AGAGAGA 1017

Fig 1. The derived sequence of band S12.1100

The sequence was analyzed and compared with the similar ones in Genbank. Their sequence similarity ranges from 71.608% to 89.671% (Fig. 2)

EM_INV:AB219449; AB219449 Bombyx mori BmBR-C gene for (166400 nt)
initn: 629 init1: 629 opt: 861 Z-score: 624.0 bits: 134.5 E(): 2.7e-28
banded Smith-Waterman score: 861; 71.608% identity (71.608% similar) in
398 nt overlap (540-927:156145-156533)

```
              510       520       530       540       550       560
Sequen AAAACTGGTAGAGCTATTTACATCAATACCTGATAAGACATCTTGGGCATCTGTAGTTAT
       : : : ::::: :    ::  ::::
EM_INV TACTTGTCAGTTAGTGACGTTTATAAATTGTTACAGGACATCCTCCATGTCAATAGT--A
     156120    156130    156140    156150    156160    156170
              570       580       590       600       610       620
Sequen GGTAAACTAAATAATTTATTTAAATTTTACATAGATATTGCAGATAACTTTGTTTAAGTT
       ::: :: :: :  : ::::::::  :  : :: :  : :  : :: : ::  ::  : :
EM_INV GGTTAAGTAGAGTAATTATTTAA--TGCATATCGTCAGT-AAAATTATTTCCTTACATTA
```

```
                156180    156190     156200    156210    156220
              630      640      650      660      670      680
Sequen TGTGGTTTTTTTTCAGGTACTCTTATGTAGGGCTATAAATGAACTGGTTGTAGGATACTG
       :  ::::  ::   :: :    ::::   :   : : ::    : :::::: :  ::
EM_INV TAAGGTTGATT---AGTTTTCGTTATCGTGTTGT-TTTATCCTATAGTTGTAAGGAACAT
      156230    156240     156250    156260    156270    156280
         690      700      710       720      730      740
Sequen AGTTTCAAAGATAAAGAGGTTT-TTATGCT--TACTTTTTTTTCCTAACTAATCTGATAG
       :: :  ::  : : :  ::: :  ::::  ::  :::::::::::: :::  :::::::
EM_INV TGTGTACAAACTCAGCACTTTTGTCTTGCTTATATATTTTTTTCCTACCTATGCTGATAG
       156290    156300   156310   156320   156330    156340
          750      760      770      780      790      800
Sequen CCTTGAGAGGCTATTTCAGCGTAACCTTAACTAGTAGGTAAGCTCACGGGGCTCAAACCT
       ::::::::: : ::::::::: ::: :::::::::::: ::::::::::::::::::::
EM_INV CCTTGAGAGACGATTTCAGCGGAACTTTAACTAGTAGGTGAGCTCACGGGGCTCAAACCT
       156350    156360   156370   156380   156390    156400
          810      820      830      840      850      860
Sequen GACAACGTTGATAACACAAACCCTAGCAAGAGCCGTGCTTCGCAGAATCTACCACCGGAT
       ::  :::::: ::::::::::::::::: :::::::::::: ::::::::::::::::
EM_INV GAGGACGTTGCTAACACAAACCCTAGCAAAAGCCGTGCTTCGCTGAATCTACCACCGGAT
       156410    156420   156430   156440   156450    156460
          870             880      890      900      910
Sequen GGGAAA-------CACTGAGAAAATTCGGCGAGAAACTCAGTAGGCTGTGTCTGTGGGTT
       :::::      ::::::::: :: ::::::::::::::: ::::::::::::::::
EM_INV CGGAAACGCGACCCACTGAGAAGATCCGGCGAGAAACTCAGTGGGCTGTGTCTGTGGGTT
       156470    156480   156490   156500   156510    156520
          920      930      940      950      960      970
Sequen AATTTACTATAACTATGCGCCCGCGCAACATGCTCACCGCCCTAGCCGAGCTATGTTTTA
       ::::::::
EM_INV AATTTACTCGTCGAGCCCTTCGTCGCAAGATGACCGGGTCTTGCTTAGTCGTGCGCACAT
       156530    156540   156550   156560   156570    156580
EM_INV:AB219449; AB219449 Bombyx mori BmBR-C gene for   (166400 nt)
rev-comp initn: 872 init1: 679 opt: 853  Z-score: 617.9  bits: 133.3 E(): 5.8e-28 banded Smith-Waterman score: 853;
89.671% identity (89.671% similar) in 213 nt overlap (927-722:99333-99545)
              950      940      930      920      910      900
Seque- GGTGAGCATGTTGCGCGGGCGCATAGTTATAGTAAATTAACCCACAGACACAGCCTACTG
                          ::::::::::::  ::::::: ::: ::::
EM_INV AACCCATCGCTTGCGACGAAGGGCTCGACGAGTAAATTAACCTTCAGACACGGCCCACTG
       99310    99320    99330    99340    99350    99360
          890      880           870      860      850
Seque- AGTTTCTCGCCGAATTTTCTCAGT-------GTTTCCCATCCGGTGGTAGATTCTGCGAA
       :::::::::::: :: ::::::::      :::::: ::::::::::::::::::::::
EM_INV AGTTTCTCGCCGGATCTTCTCAGTGGGTCGCGTTTCCGATCCGGTGGTAGATTCTGCGAA
       99370    99380    99390    99400    99410    99420
          840      830      820      810      800      790
Seque- GCACGGCTCTTGCTAGGGTTTGTGTTATCAACGTTGTCAGGTTTGAGCCCCGTGAGCTTA
       :::::::::::::::::::: :::::: :::::: ::::::::::::::::::::::: :
EM_INV GCACGGCTCTTGCTAGGGTTCGTGTTAGCAACGTCGTCAGGTTTGAGCCCCGTGAGCTCA
       99430    99440    99450    99460    99470    99480
          780      770      760      750      740      730
Seque- CCTACTAGTTAAGGTTACGCTGAAATAGCCTCTCAAGGCTATCAGATTAGTTAGGAAAAA
       :::::::::::::::::::: :::::::::::::::: ::: :::: :::::::::
EM_INV CCTACTAGTTAAGGTTACGCTGGAATAGCCTCTCAAGGCTACCAGCTTAGGTAGGAAAAA
       99490    99500    99510    99520    99530    99540
          720      710      700      690      680      670
Seque- AAAGTAAGCATAAAAACCTCTTTATCTTTGAAACTCAGTATCCTACAACCAGTTCATTTA
       :::
EM_INV AAAAAGTAGTTTTTCTCAAGAGTTATTCTCAAACCTTTTAAAATATCTTAAAAACTAAAC
       99550    99560    99570    99580    99590    99600
```

Fig 2. Comparison with two sequences represented in GenBank

Amplify specific band

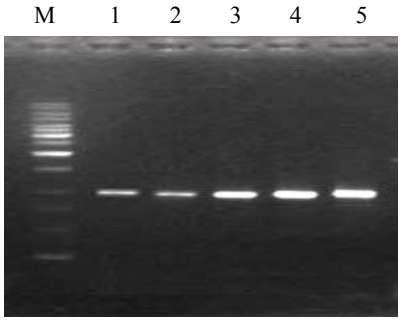

Fig 3. PCR profile generated with specific primer
M: 1kb of Fermentas
1-5: Strains with high cocoon quality

On the base of derived sequence, specific primers were design and amplify succesful (Fig 3). In conclusion, the designed primer can be used to amplify a specific marker linked cocoon quality of silkworm in order to evaluate the quality of the silkworm strains for practicing purpose.

ACKNOWLEDGEMENTS

This work was supported by the Basic Research Program (Project No 6. 106. 06) of National Scietific Council 2006-2008.

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