

## EFFECT OF EXTRACT *TRIBULUS TERRESTRIS* L. AND TECHNOLOGICAL FEATURES OF THE *BOMBYX MORI* L. FED WITH ARTIFICIAL DIET

Tsvetelina NIKOLOVA

Faculty of Agronomy, University of Forestry, Sofia, Bulgaria

\*Corresponding author: c.alipieva@abv.bg

### Abstract

Cultivation of mulberry silkworm is economically efficient branches of agriculture. It originated in ancient times. The strong dependence on nutritional requirements and especially the seasonality and range of distribution of mulberries are limiting factors for the cultivation of mulberry silk butterfly larvae. Due to its peculiarities, *Morus alba* develops well and yields high leaf yields at certain seasons of the year in our country. The resulting leaf mass is of high nutritional value. The artificial food for *Bombyx mori* L. enables cultivation during any season of the year, regardless of the external climatic conditions. Some plant extracts are nutritional stimulants and improve nutritional intake, growth and even disease resistance. The purpose of this study is to test a hybrid 11xBB1xH2xHB2 created in the Scientific Center on Sericulture, Vratsa, Bulgaria on susceptibility to artificial food with added extract of *Tribulus Terrestris* L. as a stimulant. Tracking the most important biological, reproductive and technological features of silk larvae and butterflies. Artificial food was widely accepted by *Bombyx mori* L. Higher values were observed in the experimental groups fed with artificial food and added extract of *Tribulus terrestris* L. We observed the larval growth rate and vitality.

**Key words:** *Tribulus terrestris* L., *Bombyx mori* L., artificial diet, mulberry silkworm.

### INTRODUCTION

Silk butterfly is one of the most beneficial insects. Nutrition of great importance in sericulture. A very important factor is the quality of the mulberry leaf, on which the normal development and growth of the larvae depends. The species *Bombyx mori* L. is an insect that receives all the nutrients it needs to develop properly from the leaves of the mulberry (Nasreen, 1999).

Nutrition is the only factor that almost individually enhances the quality and quantity of silk butterfly cocoon production and productivity (Laskar and Datta, 2000)

In their studies, Gobena and Bhaskar (2015) found that larvae fed with mulberry leaves and added plant extracts had better growth and development than the control groups.

Mulberry leaves treated with plant extracts have different effects on growth, development and reproduction.

Aqueous extracts from *Lantana camara*, *Parthenium hysterophorus* and *Tridax procumbens* (Hipparagi et al., 2001), *Tribulus terrestris* (Murugesh and Mahalingam, 2005), *P. hysterophorus* (Rajashekaragouda et al.,

1997), *P. hysterophorus* and *Tridax procumbens* (Mahesha et al., 1999b), *Psoralea coryleifolia* and *Phyllanthus niruri* (Shubha, 2005), *Withania somnifera* (Bhaskar et al., 2004) have beneficial effects on *Bombyx mori* L.

Sangamithirai (2014) found that larvae fed with mulberry leaves treated with spirulina extract gave better results. All signs related to the production of cocoons and their quality are significantly influenced.

*Bombyx mori* L. can also be fed with artificial food. The introduction of technology for the use of artificial mixtures in the practice of breeding, testing and creation of high-performance hybrids suitable for cultivation with artificial food, extends the area and the possibilities for growing and experimenting with laboratory conditions of *Bombyx mori* L.

The use of artificial food has some advantages, such as reducing the care and costs of large mulberry plantations, expanding the range and growing possibilities of *Bombyx mori* L. regardless of the season, increasing the economic efficiency, reducing the cost of the final product - silk.

The creation and use of semi-synthetic nutrient blends makes it possible to obtain high results in

development, viability and productivity. In many ways, artificial food may be more favorable than natural food. (Ovesenska, 2000) Feeding larvae during the first age with artificial food is widespread in Japan and South Korea as it provides healthy and viable third-generation larvae and saves labor costs.

In India, artificial food is used to feed larvae during the first ages during seasons when the quality of mulberry leaf is poor.

In Bulgaria, artificial food can be used to grow larvae in the first and second age, especially in the autumn season, when the quality of the mulberry leaf is lower and then switches to feeding with mulberry leaves until the cocoon is wrapped.

It takes 110 kg of dried artificial food to grow a box until the cocoons are wrapped. Due to the relatively high cost of artificial food, it is not economically justifiable to feed cocoons to produce cocoons throughout the larval period (Tsenov, 2012).

Saviane (2014) works on both larvae feeding patterns and monitors some of the most important indicators and adaptive ability of larvae to artificial food when degrading the properties of the mulberry leaf.

Zhou (2008) proves that artificial nutrition alters the amounts of proteins associated with the immune system, digestion and nutrient uptake, energy metabolism and silk synthesis in poor nutrition and nutrition in silkworms.

Feeding on artificial food results in fewer cocoons, a lower quality of silk thread, a lower survival rate of young larvae and insufficient resistance to specific pathogens in silkworms fed on artificial food.

Murugesu (2007) uses an extract of *Tridax procumbens*, *Tribulus terrestris* and *Parthenium hysterophorus* and reports significantly greater larval weight and lower mortality than artificial food prepared with distilled water.

*Tribulus terrestris* L. is widespread in Western Europe, Asia and China, with significant differences in the ratio of active plant substances in different geographical areas. In our country, a number of scientists have worked with *Tribulus terrestris* L. to test its effect on various experimental animals (pigs, rabbits, chickens). Biologically active substances in the plant, even in minimal quantities, have a significant effect (positive or negative) on the organism of the

animals tested. (Valchev, 2008; Dimitrov 1987; Surjiska, 2005)

## MATERIALS AND METHODS

The study was conducted at the training and experimental facilities of the Faculty of Agronomy at the University of Forestry, Sofia, in March 2019. The eggs were laid for incubation on March 20, 2019.

The silk larvae feeding experiment used artificial food containing dried mulberry leaf flour provided by Center on Sericulture, Vratsa, Bulgaria. Artificial food is prepared according to the method recommended by the manufacturer. Five days after turning, the cocoon was collected and weighed with the help of an electronic balance and a caliper.

***Tribulus terrestris* L. extract.** B 1 liters of water will begin to sprout / 10 g of *Tribulus Terrestris* L. herbs in test group 1 and 5 g in test group 2 / After 1 h, distilled water is added to the boil, and then it is left to boil for 24 h. It is roasted through filter paper and stored at low temperature.

### **Method of preparation of artificial food**

Distilled water or herb extract is added to the dry substance. 250 g dry substance + 700 ml, 800 ml *Tribulus terrestris* L. extract is homogenized with a mixer and placed in a 2 cm thick box. The resulting mixture was subjected to a heat treatment and cooled. The finished food is stored in a sealed container at 2-5°C until fed. The food can be stored for up to 40 days without losing its nutritional quality. *Tribulus terrestris* L. extract of different concentration was added to the artificial larval food of the experimental group. Control larvae are fed with artificial food without additives, in which the nutrient mixture is prepared only with distilled water. The incubation and cultivation of the larvae was carried out according to the generally accepted methods in our country (Petkov, 1982; Petkov, 1995), which aims to accelerate the development of the embryo in the egg. After reaching stage IV, the eggs were re-stored at 2-5°C. The hatching started 11 days after their incubation bet. The experiment was performed with one control and two experimental groups of 50 booms. Growing and feeding of the larvae was carried out in boxes in a specialized room up to the age of five, including at established temperature and humidity according to Petkov (1980) (Table 1), which is a universal breeding regime.

Table 1. Mode for silkworm rearing with artificial food

Ages	Temperature, °C	Humidity%
I	27	85
II	26-27	85
III	25-26	80-85
IV	24-25	70-75
V	20-24	65-70

Immediately before each meal, the artificial food is cut into strips of size appropriate to the age of the larvae. The gambling of the food is done after every sleep and after eating. Some of the most important biological, reproductive and technological features of silkworms and butterflies were monitored; Measuring the mass of a silk cocoon, Measuring the mass of a mature beetle.

## RESULTS AND DISCUSSIONS

Figures 1 and 2 show higher values of signs under the action of the added extract to the larval feed. The results obtained by us confirm those of Murugesh, (2007), that *Tribulus terrestris* extract significantly increases larval mass. The controls show slightly lower values.

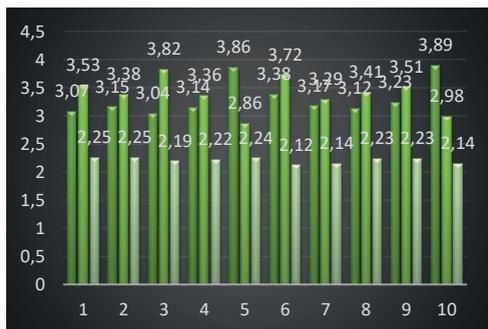


Figure 1. Weight of larva V age (g)

Fresh cocoon weight, mg depends on the mass of the pupa by cocoon shell weight, mg and ekzuvialnata skin of the larva in a V age.

Female pupae are heavier and heavier cocoon shell weight. The mass of the cocoon is determined by the mass of the pupa.

The cocoons in the experimental groups have a higher mass and especially in the experimental group 2, where the effect of the added extract on the food has the greatest influence.



Figure 2. Mass of silk cocoon

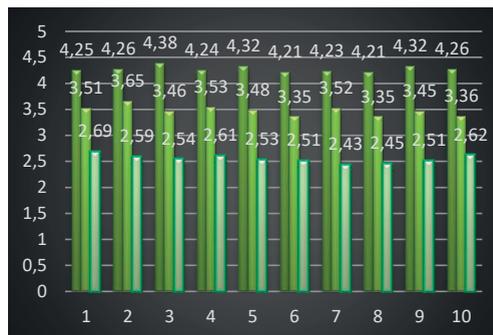


Figure 3 Average length of cocoon

The size of the cocoons depends on the breed or hybrid and the growing conditions. Cocoons with female pupa beans are larger than those with male.

Higher trait values were observed in both experimental groups. In test group 1 the values were from 4.21 to 4.38 and lower in test group 2 from 3.35 to 3.65. These higher values are most likely due to *Tribulus terrestris* L. added to the diet. The lowest values reported were in the control group from 2.43 to 2.69 mm.

Table 2. Shell ratio (%)

	Silk ratio± SD (%)
Control	22.50 ± 2.02
Experimental Group 1	24.03 ± 1.52
Experimental Group 2	23.97 ± 1.83

Shell ratio is the content of silk, which is determined by the percentage by weight of the silk sheath to the mass of the cocoon. And in these signs, there is a difference in values.

In the control group, shell ratio lower than 22.50% than in the experimental groups. The highest values were reported in test group 1 of

24.03% with higher concentration in the extract and values of 23.97% in group 2.

Shell ratio of cocoons depends on the hybrid used, the sex of the pupa in the cocoon and the growing conditions during the larval period. In cocoons with male pupa, the values of the trait are higher than the cocoons.

## CONCLUSIONS

Based on the results obtained from the cultivation of *Bombyx mori* L. with artificial foods and the added extract of *Tribulus terrestris* L., the following conclusions are reached:

Artificial food was perceived by the larvae with great pleasure because of the high percentage of active substances of the herb and well absorbed in the form of an extract. High values were observed in the intensity of growth and vitality of the larvae. They pack cocoons that do not differ from the breed's characteristic features. Due to the stimulating effect of the extract added to the food.

All treated groups, other than the control group, identified a significant increase in larval and cocoon mass, indicating the positive effects of the herbal extract added to the mulberry leaves. for larvae fed with artificial food and plant extract added.

The results obtained confirm that the active substances in the plant, even in minimal quantities have a positive effect on the organism of the investigated larvae.

## REFERENCES

Bhaskar, R.N., Sridevi, G., Devaiah M.C., Govindan, R. (2004). Evaluation of medicinal plant extracts based on cocoon and reeling parameters of silkworm, *Bombyx mori* L. (CSR2 x CSR4). *Proceedings of the National Seminar on Progress of Research on Disease and Pest Management*, 247-254.

Dimitrov, M., Georgiev, P., Vitanov, S. (1987), Application of tribestane to stutterers with sexual interference. *Veterinary Medical Sciences*, 5, 102-109.

Gobena, W., Bhaskar, R. (2015). Fortification of Mulberry Leaves with Medicinal Botanical Plant Extracts Effect on Silkworm, *Bombyx mori* L. (PM×CSR<sub>2</sub>) (Lepidoptera: Bombycidae) Larval Growth and Cocoon Traits. *Journal of Biological Sciences*, 15 (4), 199-206.

Hipparagi, G.D., Rayar S.G., Karabhantanal, S.S. (2001). Field spray of extract of botanicals on mulberry and its effect on silkworm, *Bombyx mori* L., growth and development. *Proceedings of the National Seminar on Mulberry Sericulture Research in India*, KSSRDI, Bangalore, India, 127

Laskar, N., Datta, M. (2000). Effect of alfalfa tonic and its inorganic ingredients on growth and development of silkworm *Bombyx mori* L. race Nistari. *Environ. Ecol.*, 18, 591-596.

Mahesha, H. M., Rajashekhargouda R., Rayar, S.G. (1999). Effect of aqueous extracts of few botanicals with special reference to weeds on *Bombyx mori* L. *Proceedings of the 18th Congress of the International Sericultural Commission*, 114-121.

Murugan, K., Jeyabalan, D., Senthilkumar, N., Krishna S.S., Sivaprakasam, N. (1999). Growth promoting effects of plant compounds on silkworm. *Proceeding of the National Seminar on Tropical Sericulture*, 107-108.

Muruges, K. A., Bhaskar, R. N. (2007). Efficacy of botanicals on larval growth of silkworm, *Bombyx mori* L. and its impact on silk productivity. *Bulletin of Indian Academy of Sericulture*, 11 (1), 11-15.

Ovesenska, L., Grekov, D., Panayotov, M. (2000). Tropic subtropics and temperate climate. *Academic Edition of All*, 220.

Rajashekaragouda, R., Gopalan, M., Jayaraj S., Natarajan, N. (1997). Field performance of plant extracts on mulberry silkworm, *Bombyx mori* L. *Entomon*, 22, 235-238.

Sangamithirai, A., Selvisabhanayakam, N., Susithra, P., Ganeshrabhu, V., Mathivanan, V. (2014). Studies on the quantitative parameters of silkworm *Bombyx mori* L. (Lepidoptera: Bombycidae) fed with control and spirulina treated mr2 mulberry leaves. <https://www.semanticscholar.org/paper/ORIGINAL-ARTICLE-STUDIES-ON-THE-QUANTITATIVE-OF-FED-Sangamithirai-Susithra/605268355fe4a4db1b5dd6ceaa9de8f3285970b2>

Shubha, K. (2005). Efficacy of medicinal plant extracts on stability and spread of BmNPV. M.Sc. Thesis, University of Agricultural Sciences, Bangalore, India.

Tsenov, P. (2012) Artificial food for bubbling. Silk Textile Cluster.

Valchev, G., Dimitrov, A., Grigorova, S., Zlateva, N. (2008) Testing the effect of *Tribulus terrestris* L. extract as a growth factor in rabbits. *Animal Science*, 45 (3), 96-101.

Zhong-hua, Zhou, Hui-juan, Yang, Ming, Chen (2008). Comparative Proteomic Analysis between the Domesticated Silkworm (*Bombyx mori*) Reared on Fresh Mulberry Leaves and on Artificial Diet. *American Chemical Society*, 7, 12, 5103-5111.

Zhou, Z. (2008). Comparative Proteomic Analysis between the Domesticated Silkworm (*Bombyx mori*) Reared on Fresh Mulberry Leaves and on Artificial Diet. *J. Proteome Research.*, 7 (12), 5103-5111.