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Growing mulberry silkworm with artificial diet with added extract *Tribulus terrestris* L.

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Abstract

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Growing silk butterflies is ancient and cost-effective sub-sectors of agriculture. A limiting factor in the growth of the larvae of the mulberry silk butterfly is the strong dependence on the nutritional needs and especially the seasonality and prevalence of the mulberry tree. Because of its biological and morphological features, *Morus alba* develops well and with high yields of high nutritional value but only at certain seasons of the year. Artificial feeding makes it possible to grow in any season of the year, regardless of outside climatic conditions. Some plant extracts are food stimulants and improve nutritional intake, growth, and even resistance to disease. The purpose of this study is to test breeds and hybrids created by them on susceptibility to artificial food supplemented with *Tribulus terrestris* L. as a stimulant, and to follow the most important biological, reproductive and technological features of silkworms and butterflies. *Bombyx mori* L. perceived artificial food with great willingness. High values were observed in the experimental groups, as well as an intensity of growth and vitality. As a result of the stimulating action of the extract, the number of eggs increased, unfertilized eggs were not observed as well as non-viable embryos in them.

Keywords: artificial diet; mulberry silkworm; *Bombyx mori* L.; *Tribulus terrestris* L.

Introduction

The development of the silkworm was related to the cultivation of the mulberry tree. A very important factor is the quality of the mulberry leaf, this affects the normal development and growth of the larvae. Availability of mulberry leaf of high nutritional quality, under temperate climatic conditions, is restricted to the spring-summer season, a situation that represents a limiting factor in selecting *Bombyx mori* L.

Silkworm is a monophagous insect that has special significance in sericulture industry. It totally depends on the mulberry leaves at their larval stage. Mulberry leaves are a traditional food for silkworm larvae due to presence of protein (Bhattacharyya, 2016). They use fresh, tender and easy to eat mulberry leaves.

The quality of the mulberry leaves deteriorates over time and they become stiff. Artificial food replaces fresh mulberry leaves completely.

Silkworms (*Bombyx mori* L.) reared on artificial diets have great potential applications in sericulture. Dong (2017) provide important insights into the regulation of silkworm metabolism and silk protein synthesis when silkworms adapt to an artificial diet.

„Artificial diet“ was developed for the first time in Japan and spread throughout the world. Mulberry flour is the main ingredient in the composition of the artificial food. It includes many other ingredients such as soybean meal, corn starch, citric acid, ascorbic acid, agar - agar, trace elements and vitamins, sitosterol, antibiotic and preservatives. Through it can be grown larvae year round - even in winter. Preparation and disinfection of the premises and inventory is necessary, as the risk of the occurrence of diseases is much higher than the fresh mulberry leaf feding. It is used mainly in research in

betting on various experiments with larvae of the mulberry silkworm. The use of artificial food also has several advantages, such as reducing the care and cost of large mulberry trees, expanding the area and the opportunities for growing *Bombyx mori* regardless of the season, increasing economic efficiency, lowering the cost of the final product - silk.

The creation and use of semi-synthetic food blends allows for high results in development, viability and productivity. In many ways, artificial food may be more beneficial than natural food (Ovesenska, 2000).

Feeding larvae in small ages with artificial food is very popular in Japan and South Korea as it provides a healthy and vibrant worm elderly and saves labor costs. In India, artificial food is used to feed larvae in small ages in seasons with bed quality of mulberry leaf.

Japan and China could make enviable headway in this field. It did not take long to realize that all existing silkworm breeds and hybrids might not thrive on artificial diet, instead they should be made adapted to the diet through breeding process. The strategy saw a number of silkworm strains and their hybrids exclusively evolved for rearing on artificial diet at least during the young development stages. The economic traits of silkworm breeds/hybrids reared on artificial diet during the young stages were at par with that of leaf reared silkworms. The origin of the concept, its materialization, development of related technologies and its refinement over the decades are reviewed.

According to Bhattacharyya a complete rearing of the silkworm on artificial diets was first achieved in 1960. At that time, on artificial diet based rearing, the larval growth was poor, mortality was high, development was delayed, and cocoons were small. At present there is little difficulty in rearing the silkworm on artificial diets and obtaining cocoons with good quality in a laboratory condition.

Manimuthu (2010) evaluate the influence of the dietary supplementation of the herbal tonic "Alloe" containing principally the extract of *Aloe vera* during its fifth instars of silkworm. The cocoon characteristics of the *Bombyx mori* L. commercial cross breed race L x CSR2 were evaluated. One of the alternative ways of improvement of larval growth and cocoon production is enrichment of mulberry leaves with supplementary nutrients.

Thangapandiyan (2019) investigates the impact of spirolene on the signs of *Bombyx mori* L. All the treated groups other than control groups determined a significant increase of the larvae weight, cocoon weight, filament weight and filament length parameters indicating the positive effects of the added nutrients in the mulberry leaves during the larval development.

Hiware & Bhalerao (2008) used aqueous extracts of two medicinal plants and found interesting and pesticide results with respect to many parameters.

Fattah (2018) used alcoholic extracts (Moringa, Rosemary, Garlic, Cestrum durant „Bostachia" and four aqueous extracts (Moringa, Rosemary, Garlic and Cestrum durant on imported and local hybrid of silkworm. Aqueous extraction and using of local hybrid is more available easy and cheapest.

In Bulgaria artificial food can be used for rearing in the first and second development stages, especially during the autumn season when the quality of mulberry leaf is lower. After that they should be feed with fresh mulberry leaves to format cocoons.

For the cultivation of silkworms one box to turn the cocoon needed 110 kg dry artificial food. Due to the relatively high cost of artificial food it is not economically viable to feed silkworms throughout whole period of development (Tsenov, 2012).

Different authors tried to establish artificial diet with different composition for silkworm rearing. They used different essential food as ingredients of artificial diet.

Tribulus terrestris L. is widespread in Western Europe, Asia and China, in particular geographic areas found significant differences in the proportion of active plant substances. Used primarily in folk medicine for centuries. It has been found that the extract of this herb contains a mixture of steroid saponins which have hormone-regulating action.

In our country in South Bulgaria and Black Sea grows on sandy, stony places, on road sides as weed. The over ground of the plant contains steroidal saponins, which are which are derived metabolic substances in plants.

From the other active substances in the herb, the phenols account for about 18% of the water extract, and the vitamin C concentrations reach 900 mg per 100g. This implies certain antioxidant properties of the herb (Andrade, 2009).

Extract of *Tribulus terrestris* L. is an original non-hormonal preparation. Its active components are furostanol saponins, defined as protodioscin in the product with a content of not less than 50%.

Number of researchers have worked with *Tribulus terrestris* L. for testing of its effects on various experimental animals. Biologically active substances in the plant, even in minimal quantities, have a significant influence (positive or negative) on the body of the test animals (Valchev, 2008; Dimitrov 1987; Surdzijska, 2005, etc.).

The implementation of technologies for the use of artificial mixtures in the sericulture practice, the testing and creation of highly productive hybrids suitable for cultivation with artificial diet, expands the possibilities for growing and experimenting with Bombyx mori L. in laboratory conditions (Ito & Kobayashi, 1978).

Since the success of the rearing of silkworm larvae on artificial diet, the details of nutritional requirements of the silkworm have been clarified (Ito, 1961, 1972; Horie, 1968).

Inthis regard Avramova & Grekov (2013) tested hybrids and as a result of the established tolerance of silkworms to rearing with artificial diet, they recommended developing recipes with different content of dried mulberry leaf.

According to Guntcheva (2016), artificial nutrition has an unequivocal effect on the survival of individuals of all breeds in their age range and varies widely. Individuals are placed under and cultivation conditions, but different in their adaptive capabilities to artificial food.

Saviane (2014) working on two ways of feeding the larvae and follows some of the most important indicators and adaptive ability of larvae to artificial food in deterioration of the mulberry leaf.

Zhou (2008) proves that artificial food is changing the amounts of proteins associated with the immune system, digestion and absorption of nutrients, energy metabolism and silk synthesis in poor nutrition, and eating food in silkworms. Feeding with artificial food results in fewer cocoons, lower quality silk thread, lower survival rate of young larvae, and insufficient resistance against specific pathogens in silkworms fed on artificial food.

Murugesh (2007) uses *Tridax procumbens* extract *Tribulus terrestris* and *Parthenium hysterophorus* and has a significantly higher larval weight and less mortality than artificial food prepared with distilled water.

Brahma (2018) found that the presence of vitamins C and E added to food increased the level of protein in the silk gland.

Material and Methods

The study was conducted in the educational and experimental base of the Department of Perennials and Horticulture at the Faculty of Agronomy at the University of Forestry in Sofia in February 2019. Eggs are pledged for incubation on February 20, 2019. The tetra-hydrid еИ1хВБ1хН2хХБ2 created in the Experimental station in the town of Vratsa. In carrying out the feeding experiment on silk larvae, an artificial food meal content of dried mulberry leaf, provided by Experimental station in the town of Vratsa. Artificial food is prepared according to a manufacturer›s recommended procedure.

Extract of *Tribulus terrestris* L.

In variant 1 the herb is 5 g of a and in variant 2 - 10 g. Boiled for 1 hour and stored at low temperature.

Method of preparing artificial food

To the dry substance is added distilled water or herb extract. 250 g dry substance + 700 ml, 800 ml extract of *Tribulus terrestris* L., homogenized using a mixer and placed in a box with a layer thickness of 2 cm. The resulting mixture is heat-treated and cooled. The finished food is stored in a closed container at a temperature of 2–5°C until it is fed. Food can be stored for up to 40 days without loss of nutrition. In the preparation of the artificial food for larvae from the experimental group *Tribulus terrestris* L. extract of different concentration was added. Control larvae are fed with artificial food without additives, and the food mixture is prepared with distilled water only.

The incubation and cultivation of the larvae are carried out according to the methods commonly adopted in our country (Petkov, 1982; Petkov, 1995). The eggs are stored at a temperature of 2 to 5°C, a temperature shock (an increase of t to 10–12°C) is carried out to accelerate the growth of the fetus in the egg. After reaching stage IV, eggs are re-stored at 2–5°C. Hatching started on day 11 after incubation. The experience is derived from one control and two experimental groups of 50 buddies.

Statistical analysis indexes data were analyzed bymeans of ANOVA.

The breeding and feeding of the larvae was carried out in boxes in a specialized room up to the fifth age inclusive, at the established temperature and humidity of Petkov, 1980 (Table 1), which is a universal cultivation regime.

Table 1. Module for growing silkworms 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, artificial food is cut into strips of size, consistent with the age of the larvae. The food is placed after each sleep and eating.

Some basic signs were followed; pupation rate in %, egg hatchability in % , number of normal eggs in the laying, fresh cocoon weight, (g); silk ratio (g).

Results and Discussion

Pupation rate in % is one of the most important biological indicators, with a particular contribution to the formation of productivity. It affects the extraction of cocoons and raw silk.

Figure 1 Pupation rate on larval vitality, small differences between the control and experimental groups are observed. The control values were 89.6% and higher in the test groups of 91.2 to 91.5% in Experimental Group 2 with a more concentrated extract in the feed mixture. From the results obtained, the extract of *Tribulus terrestris* L. in the diet increases insignificantly the vigor of the worms from the experimental groups.

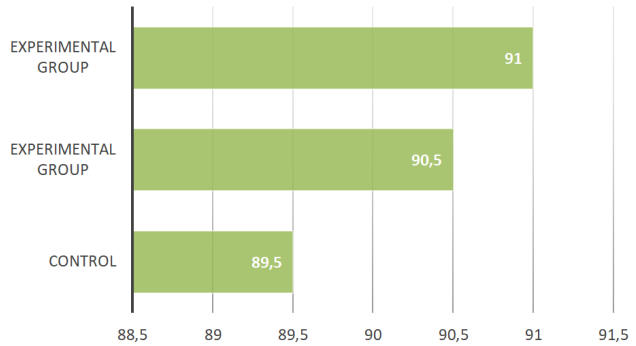

Fig. 1. Pupation rate in %

In determining the susceptibility of silkworms to artificial food, the main indicator is the number of normally developing individuals, calculated as percentage of survival.

From Figure 2, it can be seen that the survival rate of the larvae in the test groups ranges from 86.7 to 89, the susceptibility of the artificial food with added extract being high. Normal values of the attribute are observed in the control.

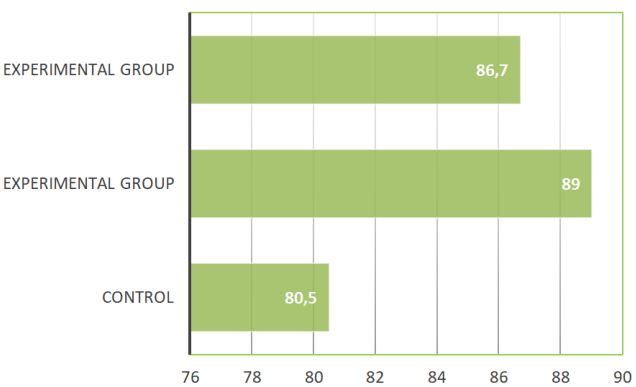

Fig. 2. Survival of larvae, %

Egg hatchability in %is largely determined by the technology of their production, storage and incubation (Petkov, 1989). There were no significant differences between control and experimental groups. High attribute values were reported from 98.6 to 98.2%. Extract added to the buffalo diet does not affect the attribute (Figure 3).

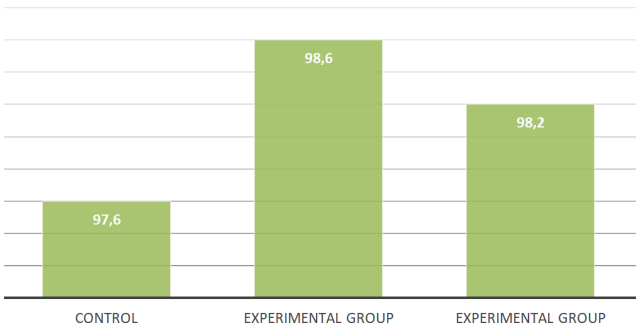

Fig. 3. Egg hatchability in %

Investigation of reproductive traits

Figure 4 shows the average values for the sign number of normal eggs in the layingreported individually for each of them.

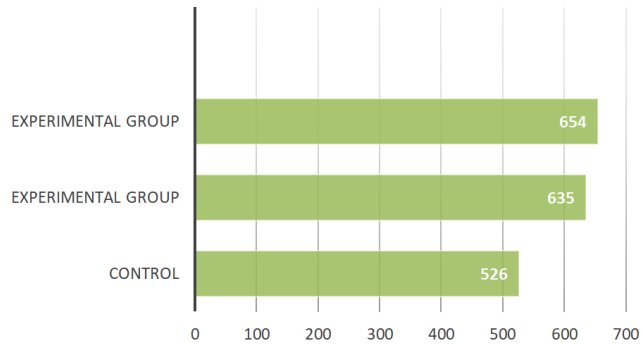

Fig. 4. Number of normal eggs in the laying

Received lying of the experimental groups was visually assessed immediately after laying. It can be seen that feeding of larvae with artificial food and added herb extract results in a difference between the numerical values of the control and experimental groups.

The number of eggs in laying with artificial food and added extract of *Tribulus terrestris* L. is greater (633 to 654) than those recorded in the control (526). The presence of *Tribulus terrestris* L. extract in the diet leads to an increase in the number of eggs in the larvae. In an attempt not found unfertilized eggs and those with non-viable embryo in them.

Both of these signs show a difference in values. In the control group, fresh cocoon weight is significantly lower 1322 g, than that in the experimental groups of 1.660 to 1.677 g. The same trend isobserved insilk ratio. Hence, it is concluded that the supplementation of *Tribulus terrestris* L. may have beneficial effect in increasing the quantity of the silk production (Table 2).

Table 2. Fresh cocoon weight (g) and silk ratio (%)

	Cocoon ± SD (g)	Silk ratio± SD (%)
Control	1.322 ± 0.133	22.50 ± 2.02
Experimental Group	1.660 ± 0.150	23.97 ± 1.83
Experimental Group	1.677 ± 0.146	24.03 ± 1.52

P < 0.001

Conclusions

On the basis of the results obtained by growing *Bombyx mori* L. with artificial foods and added extract of *Tribulus terrestris* L. the following conclusions were made:

Artificial food was perceived by the larvae with great willingness because of the high percentage of active substances of the herb and well absorbed in the form of an extract.

High values were observed at the growth rate and in pupation rate. They wrap cocoons, which do not differ from the characteristic features of the breed. As a result of the stimulating action of the extract added to the diet, the number of eggs is substantially increased. In an attempt not found unfertilized eggs and those with non-viable embryo in them.

Tribulus terrestris L. positively affects the body of the examined silkworms. The results obtained are similar to those of Valchev (2008), Dimitrov (1987) and Surdzijska (2005), that the active substances in the plant, even in minimal quantities, have a positive influence on the organism of the studied animals.

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References

Andrade, M. A. J., Morais, R. N., Spercoski, K. M., Rossi, S. C., Vechi, M. F., Golin, M., Lombardi, N. F., Greca, C. S. & Dalsenter, P. R. (2009). Effects of *Tribulus terrestris* on endocrine sensitive organs in male and female Wistar rats. *Journal of Ethnopharmacology, 127 (1),* 165-170

Avramova, K.& Grekov, D. (2013). Effect of artificial diet on the basic biological and technological parameters of some Bulgarian hybrids of mulberry silkworm (*Bombyx mori* L.). *Agricultural Science, V (14),* 259-262.

Bhattacharyya, P., Jha, S., Mandal, P. & Ghosh, A. (2016). Artificial diet based silkworm rearing system. A review. *Int. J. Pure App. Biosci., 4 (6)*, 114-122.

Brahma, U., Subasini, P. & Bibhuti, B. (2018). Impact of vitamin C & E supplementations on 5 thinstar larvae of CSR2XCSR4 silkworm *Bombyx mori* L. *RJLBPCS, 4(6),* 414.

Dimitrov, M., Georgiev, P. & Vitanov, S. (1987). Application of tribestane in rodents with disruption of sexual activity. *Veterinary Medical Sciences, 5,* 102-109.

Dong, H. , Zhang, S. , Ta, H., Chen, Z., Li, X., Qiu, J., Cui, W., Sima, Y., Cui, W. & Xu, S. (2017). Metabolomics differences between silkworms (*Bombyx mori*) reared on fresh mulberry (*Morus*) leaves or artificial diets. *Scientific Reports, 7* (10972).

Guncheva, R. (2016). study on the receptivity of breeds silkworm, *bombyx mori* l. to artificial diet low in mulberry powder. *International Journal of Current Microbiology and Applied Sciences,* May 2016. *International Journal of Current Microbiology and Applied Sciences,* 5(5),10-18.

Hiware, C. J. & Bhalerao, R. S. (2008). Effect of some medicinal plant extracts on the economic characters of mulberry silkworm, *Bombyx mori* L. *Flora and Fauna (Jhansi), 14 (1),* 90-92.

Horie, Y. & Nakasone, S. (1968). Effects of the levels of fatty acids and carbohydrates in a diet on the biosynthesis of fatty acids in larvae of the silkworm, *Bombyx mori. Journal of Insect Physiology, 17(8)* 1441-1450.

Ito, T. (1961). Effect of dietary ascorbic acid on the silkworm. *Bombyx mori. Nature,192,* 951–952

Ito, T. (1972). Amino acid nutrition of the silkworm, *Bombyx mori*. *Proc. Japan Acad., 48,* 613–618.

Ito, T. & Kobayashi, M. (1978). Rearing of the silkworm on artificial diets. The silkworm an important laboratory tool. Kodansha Ltd., Tokyo, 88-93, 121-153.

Manimuthu, M. & Isaiarasu, L. (2010). Influence of herbal tonic alloe on the overall performance of the mulberry silkworm, *Bombyx mori* L. *Journal of Biopesticides, 3(3),* 567-572.

Murugesh, 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). Bubble of tropical subtropics and temperate climate. Academic Edition of VSI, Plovdiv, p. 220.

Petkov, M. & Penkov, M. (1980). Directory of heliports. Zemizdat, Sofia .

Saviane, A., Toso, L., Righi, C. & Pavanello, C. (2014). Rearing of monovoltine strains of *Bombyx mori* by alternating artificial diet and mulberry leaf accelerates selection for higher food conversion efficiency and silk productivity. *Bulletin of Insectology, 67(2),* 167-174.

Surdzhiyska, S. (2005). Effect of application of herbal extract of *Tribulus terrestris* on the productivity of parents for broilers. *Poultry Production, 5*, 14-15.

Thangapandiyan, S. & Dharanipriya, R. (2019). Comparative study of nutritional and economical parameters of silkworm (*Bombyx mori*) treated with silver nanoparticles and Spiruli-

na. *Journal of Basic and Applied Zoology, 80,* Article number: 21

Tzenov, P. (2012). Artificial food for bubbling. Silk Textile Cluster. https://centerofsilk.wordpress.com/tag

Tzenov, P. & Georgiev, G. (2010). Possibilities for use of soybean meal in artificial diet for rearing silkworms throughout the entire larval period. *Proceedings of Scientific-Practical Conference*, 158-165

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

Zhou, Z. (2008). Comparative proteomic analysis between the domesticated silkworm (*Bombyx mori*) reared on fresh mulberry leaves and on artificial diet. *J Proteome Res 7 (12),* 5103–5111.

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