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COMPARATIVE STUDY OF BIOLOGICAL AND TECHNOLOGICAL TRAITS IN *Bombyx mori* L. FED ARTIFICIAL FOOD AND ADDED PLANT EXTRACTS

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Abstract

Bombyx mori cultivation and cocoon production is an old and traditional industry in the country. With the creation and application of artificial nutrition, it is possible to grow them in any season of the year, regardless of the external climatic conditions and the stage of development of the mulberry. The purpose of this research is to add thyme and peppermint extracts to artificial food as plant stimulants. To follow the absorption of food and the most important biological and technological signs of the silkworm and the resulting cocoons. Larvae accept artificial food and add extracts with great willingness. In the experimental groups, exceptionally satisfactory results were observed in the larvae fed with artificial food and added thyme extract, and significantly poorer results in those with meat.

Key words: *artificial feeding, blueberry silkworm, mentha, plant extracts, Thymus vulgaris L.*

INTRODUCTION

Today, silk is considered the most valuable and healthy natural fiber (Bhattacharyya et al., 2016) Authors such as Panthee et al. (2017) and Aznar-Cervante et al. (2021) recommend the use of silkworms as laboratory animals due to their short biological development and not so expensive to raise.

The temperate continental climate in Bulgaria does not allow the cultivation of silkworms throughout the year and the production of mulberry leaves is in certain seasons. Therefore, the use of a substitute for mulberry leaves is necessary. In such cases, artificial food is a good alternative.

Researchers in Japan and China have focused on developing technologies for cultivating silkworms based on artificial food (Cui et al., 2016; Yanagawa et al., 2010; Dong et al., 2017). According to Hamamura (2001), with the introduction of artificial feeding into practice in countries such as Japan in the 1960s, the breeding of the species can be carried out at any time of the year without being dependent on the development of the mulberry. This way of feeding can solve many issues in the breeding of *Bombyx mori* L., such as maintaining a large number of mulberry trees for feeding, can reduce labor costs and those in growing and maintaining mulberry trees, collecting leaves, etc.

In Bulgaria, scientists such as Tsenov & Georgiev (Avramova et al., 2020) designed in 2010 an artificial food that could be used during the entire larval period and for all seasons.

Authors such as Sudhakar et al. (2000), Hiware (2006), Nguku et al. (2007), Kamel et al. (2016), Bhattacharyya et al. (2016) studied the effect of various plant extracts or other substances (Shankar & Rangaswamy, 1999; Rahmathulla et al., 2007) added to the food of *Bombyx mori* L., followed the growth and development of the larvae. One of the alternative ways to improve the growth of larvae and cocoon production is to enrich the food with additional nutrients.

Different breeds and hybrids of *Bombyx mori* L. have different requirements for artificial food, it must be adapted to them. Plant extracts reveal the factors that can be influenced positively or negatively in favor of silkworms (Nair & Kumar, 2004)

According to authors such as Etebari & Matindoost (2005), Etebari et al., (2004), Singh et al. (2005) and Amala Rani (2011) nutritional supplements such as vitamins, proteins, amino acids and probiotics added to the larval diet tend to enhance the nutritional adaptability and economic performance of silkworms. The way of rearing and feeding influences the growth and

development of larvae and cocoon production (Thapa & Ghimire, 2005). Enriching the diet with additional nutrients is a basic prerequisite for improving growth and development, and therefore on the yield and quality of cocoons (Buhroo et al., 2018; Jaiswal et al., 2020).

Authors such as Kumar & Balasubramanian (2014), Kumar et al. (2009), Ganeshprabu et al. (2011) and Thangapandiya & Dharanipriya (2019) added silver nanoparticles and spirulina (*Spirulina maxima*) to mulberry leaves and monitored their effect on silkworm larvae. The authors concluded that nanoparticles and spirulina had a good effect and positively affected the energetic and economic parameters of *B. mori* and could be used in commercial cocoon production.

Soliman (2021) investigated the effects of different concentrations of baker's yeast S. cerevisiae and spirulina Spirulina platensis on some biological parameters of *B. mori,* such as larval weight, pupal weight, cocoon mass, cocoon shell mass, and silk ratio. The addition of yeast and spirulina to the diet had a beneficial and significant effect on larval development and increased the yield and quality of cocoons. According to the author, the application of these two ingredients to the diet can significantly increase yields in the silkworm industry.

It has been shown that feeding without ascorbic acid has a beneficial effect on cocoon production and on slowing down the development cycle (Cappellozza et al., 2005). Ascorbic acid in the diet affects cocoon yield when feeding larvae in their first and last instars.

According to the same author (Cappellozza et al., 2011), preparations such as Methoprene can be added to artificial foods, which have a stimulating effect on the larvae, and an increase in protein accumulation in the body and cocoons is observed.

The proportion of defatted soybean meal in today's widely used artificial feed mixtures is similar to that of mulberry leaf meal and ranges between 25% and 35% (Ito, 1980; Shinbo & Yanagaw, 1994; Cappellozza et al., 2005; Dong et al., 2017, 2018; Qin et al., 2020). Rich sources of protein are known to improve larval growth and economic performance.

For thousands of years, various herbs and spices have been used to preserve food or to protect it from microbial contamination.

According to authors such as Yanishlieva et al. (2006), Al-Turki (2007), Solomakos et al. (2008), Hoffman-Pennesi & Wu, (2010), Thyme *(Thymus vulgaris* L.) and peppermint *(Mentha piperita* L.) are medicinal and aromatic plants belonging to the Lamiaceae family and have therapeutic and pharmaceutical effects.

Thyme has strong antimicrobial and antioxidant activity due to its high content of thymol (40%), carvacrol (15%), cymene, eugenol and tetra-allylphenol.

Plant extracts are extremely valuable in traditional medicine and herbal products. They contain concentrated active substances from herbs. They are absorbed significantly faster by the body. Extracts are concentrated mixtures, with organic solvents such as water, ethanol or other solvents, of various plant parts, such as leaves, roots, stems and flowers. The final product, compared to the raw plant material, has more potent forms of the active chemicals of the plant.

The components in the volatile oil of thyme have antimicrobial activity against a wide variety of bacteria and fungi. 100 g of dried thyme contains vitamin A - 50 mg, vitamin C - 1890 mg, calcium - 124 mg, iron - 220 mg and magnesium.

All above-ground parts of *Mentha piperita* contain essential oil with menthol as a component (45-65%). The herb has a rich biochemical composition, which includes: vitamins such as C, A, B-complex, macroelements and microelements such as calcium, iron, magnesium, potassium, zinc and plant compounds: menthol, rosmarinic acid and eucalyptol. Acetic and isovaleric acid, carotene, bitter substances, tannins and mint oil. Mint oil is an indispensable raw material in the perfumery, alcohol and sugar industries (Landzhev, 2010).

These two plants have special functions such as antiseptic, antimicrobial and antioxidant effects (Sengul, 2008).

MATERIALS AND METHODS

The study was conducted at the educational and experimental base of the Faculty of Agronomy at the University of Forestry, Sofia, in March 2023. The eggs were laid for incubation on March 20, 2023. The tetrahybrid Vr35 x VB1 x

XB2 x M2 created at the Scientific Center for Sericulture - Vratsa was used.

When conducting the experiment for feeding silkworm larvae, an artificial food containing 100% dried mulberry leaf flour was used, provided by the Scientific Center for Sericulture - Vratsa. The food was purchased from the same place. The artificial food was prepared according to the method recommended by the manufacturer. Five days after winding, the cocoons were collected and weighed using an electronic balance and caliper.

Plant extracts

Extract of thyme (*Thymus vulgaris* L.) and mint (*Mentha piperita* L.) In 1 liter of water, 10 g of the herb are placed in the experimental groups. The extract is boiled for 1 hour, distilled water is added to the decoction to a liter, after which it is left to stand for 24 hours. It is filtered through filter paper, and stored at low temperature.

Method of preparing artificial food

Distilled water or herb extract is added to the dry substance. 250 g of dry substance + 700 ml, extracts of both plants. It is homogenized using a mixer and placed in a box with a layer thickness of 2 cm. The resulting mixture undergoes heat treatment and is cooled. The finished food is stored in a closed container at a temperature of 2-5˚C until the moment of feeding. The food can be stored for up to 40 days without losing its nutritional qualities. The control larvae are fed artificial food without additives, their food mixture is prepared only with distilled water.

Incubation and rearing of the larvae was carried out according to the generally accepted methods in our country (Petkov, 1982; Petkov, 1995). The eggs were stored at a temperature of 2 to 5°C, a temperature shock was carried out (increasing t to 10-12°C), which aimed to accelerate the development of the embryo in the egg. After reaching the IV stage, the eggs were placed again in storage at 2-5°C. The silkworm eggs were set for incubation under controlled parameters in an incubator. Hatching began 11 days after their setting for incubation. The experiment was conducted with one control and two experimental groups of 50 worms. The rearing and feeding of the larvae was carried out in boxes in a specialized room up to the fifth instar inclusive, at an established temperature and humidity according to Petkov, 1980, which is a universal rearing regime.

RESULTS AND DISCUSSIONS

The results of the experiment show much better results for the larvae fed with artificial food and added thyme extract than those in the control and the variant with added mint extract. They develop better, spin larger and heavier cocoons than those in variant 2. The thyme extract has a stimulating effect on the silkworm larvae, they are more lively and take their food with a big appetite, unlike those fed with added mint extract.

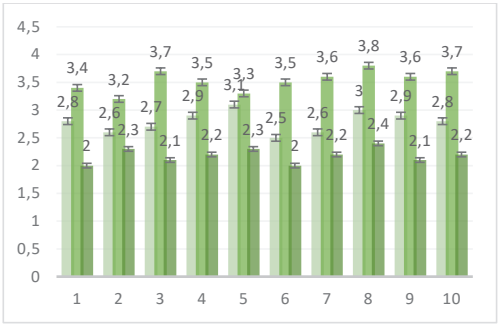
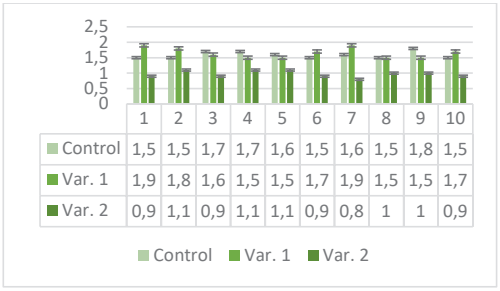


Figure 1. Larval mass in 5th instar

The results in Figure 1 show that much higher results (3.2 -3.7 g) were obtained in var.1 in larvae fed with artificial food and added thyme extract. In the control and var.2 the results obtained were significantly lower (2.0 -2.4 g).



	1	2	3	4	5	6	7	8	9	10
Control	1,5	1,5	1,7	1,7	1,6	1,5	1,6	1,5	1,8	1,5
Var. 1	1,9	1,8	1,6	1,5	1,5	1,7	1,9	1,5	1,5	1,7
Var. 2	0,9	1,1	0,9	1,1	1,1	0,9	0,8	1	1	0,9

Figure 2. Raw cocoon mass

In terms of raw cocoon mass, higher results were reported for the variant with added thyme extract (1.7 -1.9 mg). In those with added mint extract, they were low (0.9 -1.1 mg), the

resulting cocoons were small, petite and did not meet the hybrid's performance.

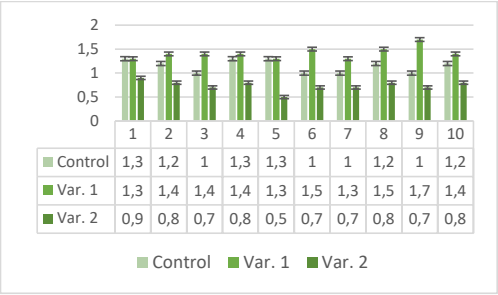


Figure 3. Dry cocoon weight

In the results of the dry cocoon mass trait, the same dependence is observed: higher results in var.1 (1.3-1.7 mg) and lower results in those fed with artificial food and added mint extract (0.5 - 0.8 mg).

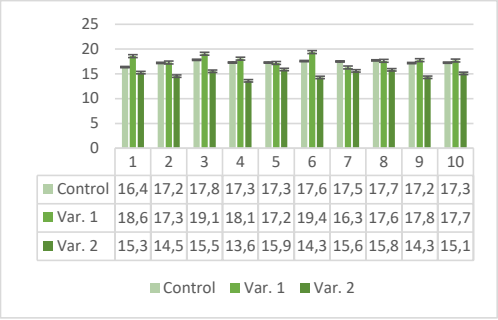


Figure 4. Cocoon width

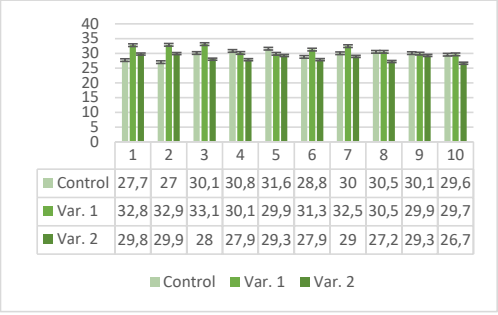


Figure 5. Cocoon height /mm

The results of Fig. 4 and Fig. 5 show higher values of the studied traits in the cocoons of variant 1, they are close to those of the control and significantly lower in variant 2. Mint has an adverse effect on silkworm larvae.

CONCLUSIONS

Thyme, as a plant extract added to the food of silkworm larvae, shows good results in all tested traits. The larvae fed with artificial food and thyme extract are significantly larger and spin larger and heavier cocoons.

Thyme is an alternative way to improve larval growth and cocoon production. It enriches the artificial food with additional nutrients.

Larvae fed with artificial food and mint extract are relatively smaller than the control and lime.

Mint does not give good results as a nutritional stimulant, and has a negative effect on the development of silkworm larvae and on the resulting cocoons. More in-depth studies are needed to establish this.

REFERENCES

Avramova, K., Tzenov, P., & Grekov, D. (2020). Silkworms (*Bombyx mori* L.) rearing using artificial diet during the summer. *Scientific Papers. Series D. Animal Science, LXIII* (1), 19–24.

Al-Turki A.I. (2007). Antibacterial effect of thyme, peppermint, sage, black pepper and garlic hydrosols against *Bacillus subtilis* and *Salmonella enteritidis*. *J. Food Agric. Environ.*, 5, 92-94.

Amala Rani, G., Padmalatha, C., Sorna Raj, R., & Ranjit Singh, A.J.A. (2011). Impact of supplementation of amway protein on the economic characters and energy budget of silkworm *Bombyx mori* L. *Asian Journal of Animal Sciences*, 5(3), 190-195.

Aznar-Cervantes, S.D., Monteagudo Santesteban, B., & Cenis, J.L. (2021). Products of sericulture and their hypoglycemic action evaluated by using the silkworm, *Bombyx mori* (Lepidoptera: Bombycidae), as a model. *Insects*, 12, 1059

Buhroo, Z., Bhat, M., Kamili, A.S., Ganai, N.A., Bali, G.K., Khan I.L. and Aziz, A.(2018). Trends in development and utilization of sericulture resources for diversification and value addition. *Journal of Entomology and Zoology Studies, 6*(4), 601-615.

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.

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

Dong, H.L., Sheng-Xiang Zhang, S.X., Chen, Z.H., Tao, H., Li, X., Qiu, J.F., Cui, Sima, Y.H., Cui, W.Z., & Xu, S.Q. (2018). Differences in gut microbiota between silkworms (*Bombyx mori*) reared on fresh mulberry (*Morus alba* var. *multicaulis*) leaves or an artificial diet. *RSC Advances*, 8, 26188.

Cappellozza, L., Cappellozza, S., Saviane, A., & Sbrenna, G. (2005). Artificial diet rearing system for the silkworm *Bombyx mori* (Lepidoptera: Bombycidae): effect of vitamin C deprivation on larval growth and cocoon production. *Appl. Entomol. Zool., 40* (3), 405–412.

Cappellozza, S., & Saviane, A. (2011). The effect of Methoprene treatment on V-th instar silkworm larvae reared on artificial diet. *Proceedings 5-th International conference "Sericulture for multi products – new prospects for development"*. https://www.bacsa-silk.org/user_pic/file/Dr%20Cappellozza%20Italy%20presentation.pdf

Cui, W., Zhang, S., Liu, Q., … & Mu, Z. (2016). An overview on research and commercialization of artificial diet for silkworm (*Bombyx mori*) in China. *Science of Sericulture*, 42, 3–15

Etebari, K., & Matindoost, I. (2005). Application of multivitamins as nutrients on biological and economical characteristics of silk worm, *Bombyx mori* L. *J. Asiapacific Entomol., 8*(1), 1-6.

Etebari, K., Ebadi, R., & Mantindoost, L. (2004). Effect of vitamin C on biological, biochemical and economical characteristics of the silkworm, *Bombyx mori* L. *Int. J. Indust. Entomol.,* 8, 81-87.

Ganeshprabu, G.P., Senthamarai, S.E., Selvisabhanayakam, Mathivanan, V., Pradhap, M., & Vivekananthan, T. (2011). Studies on the comparative feed efficacy of *Bombyx mori* L. fed with silver nanoparticles (AGNps) and spirulina treated MR2 mulberry leaves in relation to growth and development. *Int. J Pharm. Biosci., 2*(4), 180-189.

Hamamura, Y. (2001). Silkworm rearing on artificial diet. *Nippon Nogeikagaku Kaishi*, 37, 735–737

Hoffman-Pennesi D., & Wu, C. (2010). The effect of thymol and thyme oil feed supplementation on growth performance, serum antioxidant levels, and cecal Salmonella population in broilers. *J.Appl.Poult.Res*., 19, 432-443.

Hiware, C.J. (2006). Effect of fortification of mulberry with homeopathic drug *Nux vomica* on *Bombyx mori.* L. *Homeopathy, 95*(3), 148-50.

Ito, T. (1980). Application of artificial diets in sericulture. *JARQ, 14* (3), 163-168.

Kumar, K., & Balasubramanian (2014). Supplymentary effects of spirulina on lipids and enzymes in silk gland of silkworm. *Journal of Entomology and Zoology Studies, 2*(4), 27282.

Kumar, V.R., Kumar, D., Kumar, A., & Dhami, S.S. (2009). Effect of blue green micro algae (spirulina) on cocoon quantitative parameters of silkworm (*Bombyx mori* L.). *J. Agri. Bio. Sci., 4*(3), 50-53.

Kamel, H. M., Abo-Laban, G., & Nabil, M.M. (2016). The effect of mulberry leaves enrichment with different nutritional supplements on biological aspects and economic traits of silkworm, *Bombyx mori* (L.). *Annals of Agric. Sci., Moshtohor, 54*(4), 977–982.

Nair, J.S., & Kumar, S.N. (2004). Artificial diet for the silkworm, *Bombyx mori* L.. A retrospection through the decades. *Indian Journal of Sericulture, 43*(1), 1-17.

Nguku, E., Muli, E. M., & Raina, S.K. (2007). Larvae, Cocoon And Post-Cocoon Characteristics of *Bombyx mori* L.(Lepidoptera: Bombycidae) Fed on mulberry leaves fortified with kenyan royal elly. *Journal of Applied Sciences and Environmental Management, 11*(4), 85-89.

Qin, D.Y., Wang, G.H., Dong, Z.M., Zia, Q.Y., & Zhao, P. (2020). Comparative fecal metabolomes of silkworms being fed mulberry leaf and artificial diet. *Insects*, 11, 851.

Panthee, S., Paudel, A., Hamamoto, H., & Sekimizu, K. (2017). Advantages of the silkworm as an animal model for developing novel antimicrobial agents. *Frontiers in Microbiology*, 8, 373

Rahmathulla, V.K., Das, P., Ramesh, M., & Rajan, R.K. (2007). Growth rate pattern and economic traits of silkworm, *Bombyx mori* L under the influence of folic acid Administration. *Journal of Applied Sciences and Environmental Management, 11*(4), 81-84.

Singh, B., Sahoo, A., Sharma, R., & Bhat, T. K. (2005). Effect of polethylene glycol on gas production parameters and nitrogen disappearance of some tree forages. *Anim. Feed Sci. Technol., 123/124*(1), 351-364.

Shankar, M., & Rangaswamy, B.T. (1999). Effect of applied nitrogen and potassium on mulberry leaf yield and quality in relation to silkworm cocoon characters. *Better Crops International, 13*(2), 21.

Sudhakar, P., Chattopadhyay, G.N., Gangwar, S.K., & Ghosh, J.K., (2000). Effect of foliar application of Azotobacter, Azospirillum and Beijerinckia on leaf yield and quality of mulberry (*Morus alba*). *The Journal of Agricultural Science, 134*(2), 227-234.

Shinbo, H., & Yanagaw, H. (1994). Low-cost artificial diets for polyphagous silkworms. *JARQ*, 28, 262-267.

Solomakos N., Govaris A, Koidis, P., & Botsoglou, N. (2008). The antimicrobial effect of thyme essential oil, nisin, and their combination against *Listeria monocytogenes* in minced beef during refrigerated storage. *Food Microbiol.,* 25, 120-127.

Thapa, R.B., & Ghimire, N.P. (2005). Performance of mulberry silkworm (*Bombyx mori* L.) under leaf and shoot feeding methods. *J. Inst. Agric. Anim. Sci.,* 26, 83-86.

Thangapandiya, S., & Dharanipriya, R. (2019). Comparative study of nutritional and economical parameters of silkworm (*Bombyx mori*) treated with silver nanoparticles and spirulina. *The Journal of Basic and Applied Zoology, 80*(21).

Yanagawa, H., Watanabe, K., & Nakamura, M. (2010). Application of the feed ingredients for livestock to an artificial diet by using polyphagous strain of the silkworm. *J. Insect Biochem. Sericult.,* 58, 401–406.

Yanishlieva, N., Marinova, E., & Pokorný, J. (2006). Natural antioxidants from herbs and spices. *Eur.J. Lipid Sci. Technol.,* 108, 776-79