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EFFECTS OF EXTRACT *ORIGANUM VULGARE* L. ON *BOMBYX MORI* L. ADDED TO WITH ARTIFICIAL FOOD

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Abstract

Originated in ancient times, mulberry silkworm farming is a cost-effective sub-sector of agriculture. The strong dependence on food needs and especially the seasonality and distribution area of mulberries are limiting factors in the rearing of larvae.In our country Morus alba grows well and gives high yields of foliage in certain seasons of the year due to its characteristics. The resulting leaf mass has a high nutritional value. An alternative to nutrition is artificial food. It allows for growing in any season of the year, regardless of external climatic conditions. Some plant extracts are food stimulants and improve food intake, growth and even disease resistance. The aim of the present study was to test a hybrid I1xVB1xH2xHB2 *created in Scientific Center on Sericulture, Vratsa, Bulgaria, for susceptibility to artificial food with added extract of Origanum vulgare L. as a growth stimulant. Tracking the most important biological, reproductive and technological features of silk larvae and butterflies; Artificial food was readily accepted by Bombyx mori L. Higher values were observed in the experimental groups fed with artificial food and added extract, we observed the growth intensity of the larvae and their viability.*

Key words*: artificial diet, Bombyx mori L., Morus alba, mulberry silkworm, Origanum vulgare* L.

INTRODUCTION

The silkworm (*Bombyx mori* L.) is a fully domesticated insect and mulberry leaves are its only food that is very important for proper growth and development (Legay, 1958; Kumar, 2013). Nutrition is the only factor that almost individually increases the quality and quantity of production and productivity of the silkworm cocoon (Laskar & Datta, 2000)

In their studies, Gobena & Bhaskar (2015) found that larvae fed on mulberry leaves and added plant extracts had better growth and development compared to control groups.

Mulberry leaves treated with plant extracts have different effects on growth, development and reproduction. Aqueous extracts of *Lantana camara*, *Parthenium hysterophorus* and *Tridax procumbens* (Hipparagi et al., 2001), *Tribulus terrestris* (Murugesh & 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 a beneficial effect on the species *Bombyx mori* L. Sangamithirai (2014) found that larvae fed on mulberry leaves treated with spirolin extract gave better results. All the signs related to the extraction of cocoons and their quality are significantly affected.

Bombyx mori L. can also be fed with artificial food. The introduction of technologies for the use of artificial mixtures in the practice of sericulture, testing and creation of high-yielding hybrids suitable for cultivation with artificial food, expands the area and opportunities for cultivation and experimentation 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 opportunities for growing the species *Bombyx mori* L. regardless of the season, increasing economic efficiency, reducing the cost of the final product - silk.

The creation and use of semi-synthetic compound feed makes it possible to obtain high results in the development, viability and productivity. In many ways artificial food may be more favorable than natural food (Oatmeal, 2000).

In recent years, the efforts of a number of authors have focused on the search for natural, natural and ecological products that are an alternative to the previously used growth stimulants (Grela, 2000; Meriden, 2000; Ratcliff, 2000).

It has long been known that some herbs stimulate the appetite of animals, increase metabolic and immune status and improve their general condition and productivity (Hammer et al., 1999; Close, 2000; Toncheva et al., 2004; Dimitrova et al., 2004; Dimitrova, 2009).

Plant extracts and essential oils from savory, chestnut, oregano, thyme, nettle and others have been studied (Adams, 1999; Delacon, 1999; Lyons, 2001; Spring, 2002).

There is a lot of information in the literature about the action of essential oils, especially oregano oil. It has antimicrobial (Dorman & Deans, 2000), fungicidal, antioxidant, cytostatic and antiparasitic activity (Force et al., 2000).

Oregano oil is mainly used in the pharmaceutical industry and as a spice. Carvacrol has antimicrobial, antitumor, antimutagenic, analgesic, antispasmodic, anti-inflammatory, antiparasitic, insecticidal and antihepatotoxic effects, which largely explains the in vivo mechanism of action of carvacrol (Can Baser, 2008).

One of the most widely tested and used herbs in pig farming is oregano (*Origanum vulgare*), due to its antimicrobial (Burt & Reinders, 2003; Dorman et al., 2000; Lambert et al., 2001; Sivropolou et al., 1996), anti-inflammatory (Ariza-Nieto et al., 2003), antioxidant (Lagouri et al., 1993; Milos et al., 2000; Vekiari et al., 1993), fungicidal (Adam et al., 1998; Daouk et al., 1995; Stiles et al., 1995), cytotoxic (Sivropolou et al., 1996), antiparasitic (Forse et al., 2000), insecticidal, anticoccidial and immunostimulatory effects (Park & Bilkei, 2004). In studies performed by Donev (2001) with essential oil of oregano (*Origanum vulgare*) on broiler chickens, a good nutritional effect was reported due to the biologically active substances contained in it: carvacrol, thymol, pinene, lemon and borneol.

In Bulgaria, Nicheva (1985), Gurgulova (1996), Malinova (2003) found that the essential oils of savory, mint, anise, eucalyptus and thyme have a relatively high activity against some pathogenic bacteria. In the treatment of suckling pigs with Bioxan-emulsum at a dose of 11.25 mg of oregano essential oil per 1 kg. t.m., Dimitrova et al. (2004) reported a reduction of about 50% in pigs with gastroenteritis and weaning until weaning.

There are studies that show the successful replacement of antibiotics, such as growth stimulants with oils, extracts or oregano-based products in suckling, growing and fattening pigs (Capms, 2005; Thomke & Elwinger, 1998; Günter & Bossow, 1998; Ingram, 1997), as well as in our country (Kanev et al., 2002; Toncheva et al., 2004).

Oregano essential oil contains monoterpenoid phenols (carvacrol and thymol), phenolic acids (rosemary acid), monoterpenes and other active ingredients. The main pharmacologically active components in its composition are first carvacrol, followed by thymol and rosemary acid.

Xu et al. (2008) tested the antibacterial activity of carvacrol and thymol against *Escherichia coli*. The author concluded that carvacrol and thymol were effective in inhibiting growth.

In a study, Lopez et al. (2007) monitored the antibacterial efficacy of essential oils of oregano, cinnamon and thyme against the bacteria *Escherichia coli*, *Yersinia enterocolitica*, *Pseudomonas aeruginosa*, *Salmonella enterica Serotype Choleraesui*s, etc., concluding that the minimum inhibitory concentrations of oil (MIC) Ritan are lower than other oils. Avtoran concluded that the carvacrol and thymol oil of oregano and cinnamaldehyde have the strongest antimicrobial activity.

In similar studies of 5 essential oils against avian and porcine strains of enterotoxigenic *Escherichia coli*, Penalver et al. (2005) found that *Origanum vulgare* showed the highest antimicrobial activity against all strains of Salmonella spp. A study conducted in Greece on the effect of Greek plant products, including oregano oil, on stomach diseases and peptic ulcer, found that some herbs, including *Origanum vulgare*, were active against one reference strain and 15 clinical isolates of *Helicobacter pylori* (Stamatis et al., 2003).

Yan et al. (2009) in an experiment conducted in Korea showed that with good nutrition of pigs at the beginning of the fattening period (24 kg) and the addition of essential oil in a

concentration of 0.01% of the ration, a significant increase in average daily growth and -good utilization of feed (P<0.01), increase in daily feed consumption and nitrogen uptake (P<0.01) and energy (P<0.05) during the first 6 weeks of weaning, compared to the group with low nutrition density and without the addition of essential oil. During the next period of experience (up to the 16th week) no significant differences were found.

MATERIALS AND METHODS

The research was conducted in the educational-experimental base of the Faculty of Agronomy at the University of Forestry, Sofia, in February 2021. The eggs were laid for incubation on 02.02.2021.

The tetrahybrid I1xVB1xH2xHB2 created in the Scientific Center for sericulture - Vratsa was used. Three variants were tested, respectively without added oregano extract and with extract. The laid eggs were in a volume of 3 g. After the third sleep, the beetles from the experimental groups were counted in 50 larvae reared until the cocoons were twisted.

During the experiment for feeding the silk larvae, artificial food containing flour from dried mulberry leaf, provided by Scientific Center on Sericulture, Vratsa, Bulgaria, was used. The artificial food is prepared according to the methodology recommended by the manufacturer.

Extract of *Origanum vulgare* L. B 1 liter of water is added/10 g of the herb in experimental group 1, in experimental group 2, 15 g/Place for 1 hour, distilled water is added to the solution in a liter, after which it is added. 24 hours. Strain through a filter cap and store at low temperature.

Method of preparation of artificial food

Distilled water or herbal extract is added to the dry substance. 1 kg dry substance + one l extract of *Origanum vulgare* L. Homogenize with a mixer and put in a box with a layer thickness of 2 cm. The resulting mixture was subjected to a heat treatment of 850 KW for 10 minutes and cooled. The prepared 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. Before giving the larvae, the prepared food is removed from the refrigerator and tempered. Cut into strips 2 cm thick

An extract of *Origanum vulgare* L. with different concentrations was added in the preparation of the artificial food for the larvae from the experimental group.

Control larvae are fed artificial food without additives, in which the nutrient mixture is prepared only with distilled water.

Incubation and rearing of larvae was performed 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 are placed again at a storage of 2-5°C.

Hatching began 11-12 days after their incubation. Growing parameters: first and second age t-30°C and relative humidity 85%, in the third age t-27°C and relative humidity 80%, fourth age t-26°C and relative humidity 75%, in the fifth age and when turning the cocoons t-25°C and relative humidity 70%.

Studied were the most important productive features, the data were processed by the conventional methods.

Table 1. Larvae fed on artificial diet

Instar	Temperature, °C	Relative humidity
I	29–30	90
II	29–30	90
III	27–28	80
IV	26	70-75
V	24	70
Cocooning	25–27	55–60

RESULTS AND DISCUSSIONS

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

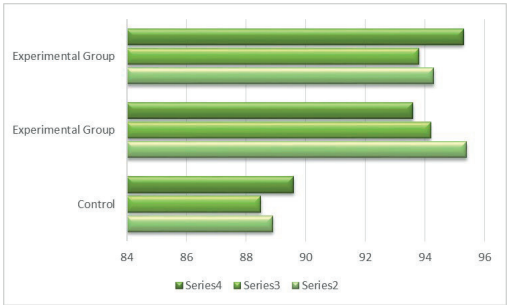

Figure 1. Pupation rate, %

Figure 1 presents the data for Pupation rate, there are small differences between the control and experimental groups. In the control, the values were 88.5 to 89.6% and higher in the experimental groups from 93.6 to 95.54% in experimental group 2 with a more concentrated extract in the food mixture. From the obtained results it can be said that the extract of *Origanum vulgare* L. in the diet slightly increases the viability of the beetles from the experimental groups.

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

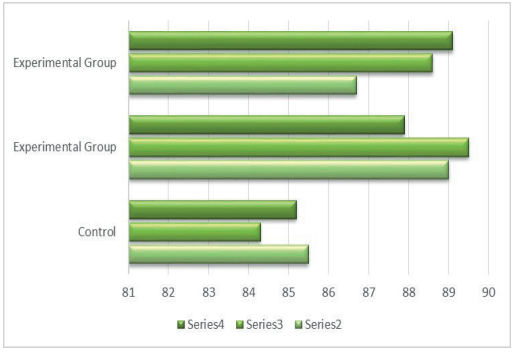

Figure 2. Survival of larvae, %

Figure 2 shows that the survival of larvae in the experimental groups is from 86.7 to 89.5, the susceptibility of artificial food with added extract is high. During the control, lower but normal values of the symptom are observed.

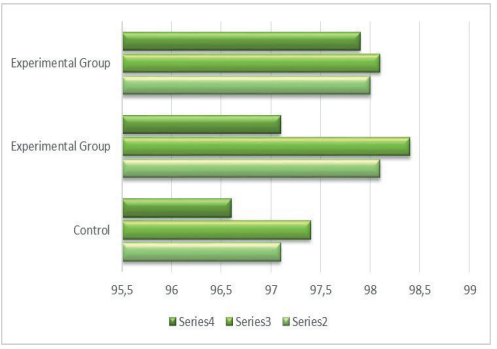

Figure 3. Hatching of the beetle seed (%), average for the period

The hatchability of eggs is largely determined by the technology of their production, storage and incubation. (Petkov, 1989). Figure 3 shows the average values of the sign of hatchability of the beetle seed in percent. No significant differences were observed between the control and the experimental groups. High values of the trait from 96.6 to 98.4% were reported. The extract added to the food of the beetles does not affect the trait.

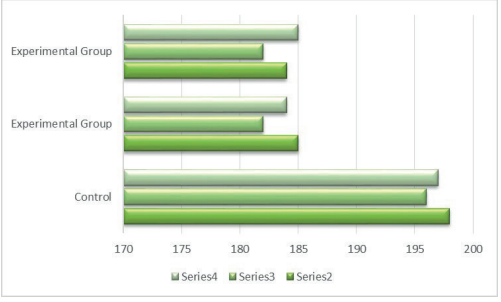

Figure 4. Vth instar duration, h

In Vth instar duration higher values were observed in the control group with values from 196 to 198 h (Figure 4). In the experimental groups, the duration of V-age is shorter, the beetles reach maturity after 182 to 185 h. From the obtained results it can be said that the extract of *Origanum vulgare* L. in the diet has a positive effect on this trait.

CONCLUSIONS

Artificial food was readily accepted by *Bombyx mori* L. Higher values were observed in the experimental groups fed artificial food and added extract, we observed the growth intensity of the larvae and Pupation rate.

The effect of the extract is most clearly manifested at V^{th} instar duration. The effect of the extract is most clearly manifested at duration of V-age.

The results obtained by us are close to those of Gobena & Bhaskar (2015). The larvae fed with artificial food and added plant extracts have better growth and development compared to the control groups. The extract of *Origanum vulgare* L. in food improves the nutritional intake and growth of the beetles from the experimental groups. It has a beneficial effect on the species *Bombyx mori* L.

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