Effect of optical radiation on greater wax moth \((Galleria mellonella \text{ L.})\) – pest of bee colonies

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Abstract. Wax moth is one of the main pests of bee colonies. Bee moth larvae have in their chemical composition many substances that are necessary for the preparation of drugs of the widest range of action. Beekeepers solve the problem of better control of greater wax moths using chemical, biological and other methods. The efficient methods to control \(Galleria mellonella\) include physical methods: temperature, radiation, etc. The purpose of this paper is to find the most effective optical radiation for a greater wax moth (\(Galleria mellonella\) L.), in which the productivity of bee colonies is maximally preserved, and unscathed \(G. mellonella\) larvae can be used to manufacture pharmaceutical preparations and biologically active supplements. The object of research is the Greater wax moth (eggs, larvae, pupae, and imago). We have developed a structure for determining the attractiveness of the optical radiation of different wavelengths for imago of greater wax moths. We found that the optical radiation in the wavelength range of 400…435 nm is the most attractive because it attracted a greater wax moths and therefore the number of eggs laid under the influence of this light was 1.5…2 times higher compared to radiation 491, 546 and 491 nm.

1. Introduction

In bee hives, one more insect can live except bees, namely, \(G. mellonella\) adults, and it is one of the most dangerous wreckers that makes bee colonies weaker and preventing them from getting stronger. The greater and lesser wax moths adults are gray moths, which, penetrating the beehive, lay eggs in abundance, from which larvae soon appear. The larvae is fed on wax, beebread and honey. This leads to a distortion of the honeycomb geometry, which makes it difficult to retrieve the offspring of bee colonies and collect honey [1,2].

The wax is extremely inert chemically. There are only few naturally occurring substances and compounds that can dissolve wax. Each larva is able to eat almost 2 g of wax (more than 600 cells of a honeycomb) in a short period of development (about a month) [3].

Female \(G. mellonella\) have a length of 15 to 35 mm. Their wings and body are covered with scales. The color of the front wings is violet-gray with light brown and dark spots. The color of the rear wings is gray with dark strokes on the rear edge. The shape of the wings: at the front wings – the rear edge smooth, at
the back is rounded. The head of the female is elongated and narrowed due to forward-directed tentacles; it has pubescence, short proboscis and large faceted eyes [2].

The wax moth causes damage as well with its excreta. Fecal balls contain enzyme substances. Therefore, when getting to the bottom of the cells, they disturb the final molt of bees just before their leaving. In the moth-infested bee hive, the air is different in temperature humidity, making bees to leave it. The death of bee colonies or their weakening due to the bee moth is often noted. Every year in the USA, bee moth causes damage totaling up to 4 - 4.5 million dollars. For example, in the state of Florida losses from this pest can be as high as 1 million dollars. In Bulgaria, bee moth destroys 30 tons of wax raw materials annually [3].

In turn, there are positive aspects in the processing of greater wax moth. Chitin and chitosan are present in the extract of the G.mellonella larvae. These substances regulate lipid metabolism, i.e. the extract demonstrates lipolytic activity. This means that bee moth products can be useful in atherosclerosis, diabetes, obesity. Chitosan also has antimicrobial properties and anti-tuberculosis activities [4,5].

Tincture (extract) of the bee moth is indicated for use in the following diseases or conditions: tuberculosis, oncology, cardiovascular diseases - angina pectoris, arrhythmia, ischemia, consequences of heart attacks, aortic aneurysms, broncho-pulmonary diseases, atherosclerosis, diabetes, obesity, infertility, diseases of the reproductive system (male and female), respiratory infections, prevention or improvement of adaptation possibilities of the body under stress, increased physical and emotional stress, prevention of premature aging [6].

However, there are also contraindications to the use of tincture - individual intolerance to the product, allergic reactions to bee products. The extract of G.mellonella larvae in its chemical composition has a significant amount of free amino acids, mono- and disaccharides, nucleotide bases, fatty acids, macro- and microelements (zinc and magnesium) [5]. Scientific studies of alcoholic extracts of moth extracts revealed the presence of antibacterial peptides [6]. The first mention of the use of bee moth larvae in folk medicine for the treatment of patients with tuberculosis dates back to the 17th century. In folk medicine, bee moth larvae infusions have been used in the treatment of cardiovascular and pulmonary diseases. Studies of extracts of bee moth larvae showed the presence of adaptogenic, cardioprotective, cardiotropic, hypoaglutinin properties [2]. In addition, the antioxidant properties of the bee moth larvae were determined. In therapeutic terms, a bee moth caterpillar proteinase complex is of great importance [1]. Larvae extracts have low toxicity. High doses of the drug increase the concentration of catecholamines in cardiac and aortic tissues [6]. Apolipophorin protein and other proteins isolated from bee moth endolymph have bacteriostatic and antifungal effects. Antibacterial properties are also attributable to litozin found in the bee moth endolymph [5]. Larvae of the bee moth have anti-staphylococcal and antilegionella activities [5]. A study of the hemolymph of G.mellonella larvae has demonstrated its hemolytic activity. Digestive enzymes of bee moth play an important role in protection against pathogenic bacteria. The use of the bee moth in gynecological practice is very promising, as part of suppositories, tablets, etc. [5].

The scientific study of the medicinal properties of bee moth larvae was initiated by I.I. Mechnikov. In the late nineteenth century, in search of therapeutic agents against pulmonary tuberculosis, he drew attention to the G.mellonella larvae. His idea was that the digestive enzymes of moth larvae, which are developed by feeding on beeswax, can also destroy the wax shells of tuberculosis bacteria. The hypothesis was proved to be correct. Mechnikov’s research works were continued in Russia by his students - professor S.I. Metelnikov and microbiologist I.S. Zolotarev [2]. In XX century, a large experience in research in this area has been accumulated by doctor S. A. Mukhin. In 1961, he developed the drug "Vita" on the basis of medicinal extract of bee moth larvae and biologically active extracts from medicinal plants. According to the author, this drug helped with myocardial infarction, healed caverns in pulmonary tuberculosis, etc. [2,6]. Issues of the development of energy-efficient led light traps for bee moths with the use of programmable logic microcontrollers to control the spectrum and intensity of radiation are still not studied enough and are a relevant objective [7,8].

According [9] in the USA the losses of beekeepers from the harmful effect of Galleria mellonella exceed 8,000,000$. Since G. mellonella is evolutionary adated for living in a bee colony, its feed substrate is also located in the alveary, therefore the larva is capable of producing the cerase, which decomposes the
beeswax [10]. The greatest damage caused by G. mellonella larvae to the beekeeping is through destructing and eating the brood, bee bread and honey comb and spreading contagious diseases among bees. The bee colonies parasitized by wax moth are weakened and their productivity is reduced [11].

In addition, by eating combs and beesbread, the pest destroys the colony integrity, thus weakening it and reducing its honey productivity. Over its development cycle, the larva eats 1.2 g of wax.

The main known methods of Galleria mellonella control are divided into physical, chemical and biological. While chemical method is efficient and fast, it uses aggressive reagents which may adversely effect the bee colonies since the ecological environment becomes disturbed. In [11] there is a description of the relationship between disease and foraging in bees and highlights recent advances in this field as well as critical knowledge gaps. Diseases have important but understudied effects on bee foraging ecology. The authors examine how parasites can alter bee feeding by altering flower preferences and reducing feeding efficiency. Biological method is a softer and environmentally friendly method for greater wax moth control, however to ensure the efficient action of agent on the pest, a long time period is required [2]. Physical method is also a soft and safe way to remove the pest from alveary [3].

Most of methods are based on limiting temperature modes of the insect existence at −10...+45°C. Furthermore, these methods must be applied separately from the bee colony. The methods of insect control in the alveary itself are extremely scarce. It is known that, the γ-ray sterilisation technology is used for G. mellonella control [12,13]. In [12], laboratory male pupae of Culex pipiens were exposed to 23, 41, 74 and 128 Gy doses of gamma radiation according to the LD25, LD50, LD75 and LD90 calculation, respectively. The inherited deleterious effects of gamma radiation were observed in the F1, F2 and F3 generations. Levels of sterility index in the F1 and F2 were higher than those in unirradiated control but in the F3 generation there was a semi-sterility compared with the control. Consequently, these radiation doses are consistent with those used in the already established Sterile Insect Technique (SIT) programs against Culex pipiens. The aim of [13] was the experimental study of the delayed appearance of haploid and homozygous diploid Saccharomyces cerevisiae yeast cells of wild-type and radiosensitive mutants surviving after exposure to γ-rays and α-particles. These findings are not new for cell survival, while they are fundamentally new for genetic instability.

It has been proven that the efficient absorbed dose for sterilizing the G. mellonella imago males and pupae is 350 Gray. Similar works were performed with other animal types [14,15].

In the present study [14] compared the competitiveness, as measured by hatching rate of resulting egg batches, of irradiated males measured in small and large laboratory cages and semi-field enclosures in a greenhouse setting, when competing in a 1:1, 3:1, and 5:1 ratio with fertile males. In [15] it is stated that Aedes albopictus is a vector of several human viral diseases, including dengue, chikungunya, and Zika. New control method for Aedes albopictus is needed to replace traditional methods such as chemical insecticides which induce resistance, environmental contamination and toxicity to human. In sterile insect technique (SIT), male mosquitoes are sterilized by γ-ray or X-ray irradiation before released. In this study, the relative effectiveness of X-ray irradiation as a mosquito SIT was investigated. Induced sterility was essentially the same for both irradiated pupal and adult. At a dose of 40 Gy, 97% and 100% sterility was achieved in males and females, respectively. Overall, the results of this study showed that SIT based on X-Ray irradiation is scientific and feasible to control Aedes albopictus (Asian tiger mosquito). The sterile males were found to be equally competitive when compared to unirradiated controls, and a 5:1 ratio was sufficient to reduce, but not eliminate, the fertility of the female populations, irrespective of cage size.

The development of the sterile insect method (SIT) for the reductio of Aedes albopictus (Skuse) population (chikungunya and Dengue fever vector) were studied at the Reunion island [16] via a semi-field experiment conducted to evaluate the impact of sugar supply and mating activity under natural climatic conditions on wild and sterile male Ae. albopictus longevity, using large cages set up in an open clearing between trees and shrubs. In conclusion the results of this study showed that immediate availability of sugar after emergence was the most critical factor promoting longevity of sterilized males.

The purpose of this paper is to find the most effective optical radiation for a greater wax moth (Galleria mellonella L.), in which the productivity of bee colonies is maximally preserved, and
unscathed *G. mellonella* larvae can be used for the manufacture of pharmaceutical preparations and biologically active supplements.

2. Materials and methods
In our study we developed an experimental unit for determining the attractiveness of the light wavelength, experimentally established the light reaction range of imago, and determined the productivity of *G. mellonella*.

Studies of the effect of LED radiation on the total biomass growth of a large bee wax moth were conducted from March to October 2018.

The object of research was the Greater wax moth (eggs, larvae, pupae, and imago). *G.mellonella* was bred in vitro in the laboratory of the Udmurt Federal Research Centre of the Ural Branch of the Russian Academy of Sciences (the Udmurt FRC of UB RAS). Larvae of *G. mellonella* were kept in the “Motharium” 30±2°C, and a relative humidity of 60-70% under total darkness conditions. For the experiment, feed was placed in each container – apiary wax residues of 10 g and paper folded in a pleated manner for laying eggs [17].

The experimental setup consisted of five identical propylene tanks with a diameter of 110 mm and a height of 190 mm (figure 1). Tanks 1, 2, 3 and 4 were connected by propylene tubes with a diameter of 50 mm with a central tank 5.

![Figure 1. Experimental setup: 1, 2, 3, 4 and 5 are cylindrical tanks; 6 – RGB LEDs; 7 – cap; 8 – connecting tubes; 9 – construction base.](image)

On the inner walls of the cylinders 1, 2, 3 and 4 (figure 1) there are tapes with RGB LEDs of SMD 5050 brand. Each cylinder 1, 2, 3 and 4 synthesized a different radiation spectrum by means of slide resistors R1, R2 and R3, connected in series with LEDs (figure 2).

The flow of RGB LED radiation in the first tank was changed by the variable resistance R1. Similar schemes were used in tanks 2, 3 and 4 (figure 2). Therefore, the radiation with the wavelengths of 577 nm, 546 nm, 490 nm and 435 nm was studied. The wavelength obtained by emitting LEDs was measured by a spectrophotometer 'TKA-Spectrum (TKA, Russia). The radiation spectra were chosen in accordance with the recommendations on the spectral sensitivity (susceptibility) of the facetted eye of a bee moth butterfly [1,2].

In our experiments we determined the effect of the radiation spectrum on the direction of movement of 20 bee moth butterflies with a 15-min and 7 h switching on of the LEDs and on the biomass growth of butterflies with 7 h switching on of the LEDs.

In the first experiment, RGB LED strips of SMD 5050 brand were used. One meter of such strip contains 15 LEDs that consume power of 3W and create a luminous flux of 200 lumens. A 6-watt Feron LB003 static power supply unit with an output voltage of 12 V was used as the power source for LED strips. There were 5 RGB LEDs each, consuming 0.18 W at a current of 0.015 A and a voltage of 12 V. LEDs were located on the inner wall of the tanks. The experiment was carried out in three replications.
3. Results and discussion
The analysis of Table 1 shows that at 15-minute exposure of visible radiation of different colors, 45% of the adults of the bee moth moved to the tank 4, where the radiation was with the lowest wavelength equal to 435 nm.

With the help of MS Excel package the mathematical dependence of the radiation wavelength influence on the number of displaced adults \( G.mellonella \) were found, which is described by a third-order polynomial with the determination coefficient equal to \( R^2 = 0.99 \):

\[
N_1 = 0.0005\lambda^3 + 0.0545\lambda^2 – 28.009\lambda
\]

(1)

where \( N_1 \) is the number of butterflies, %; \( \lambda \) is the wavelength, 435…577 nm.
Table 1. The effect of the radiation spectrum on the direction of 20 butterflies – adult bee wax moth movement.

| Tank 1 | Tank 2 | Tank 3 | Tank 4 |
|--------|--------|--------|--------|
| λ=577nm | λ=546nm | λ=491 nm | λ=435nm |
| 2.0±0.58 | 4.0±0.58 | 4.33±0.33* | 9.67±0.33* |
| 2.0±0.58 | 4.33±0.33* | 4.67±0.33* | 9.0±0.33* |
| 2.33±0.88 | 4.0±0.88 | 4.33±0.33* | 9.33±0.33* |
| 2.5±0.5 | 4.0±0.5 | 5.0±0.58* | 9.33±0.58* |
| 2.5±1.5 | 4.0±1.5 | 5.0±0.58* | 9.33±0.58* |
| 10% | 20% | 25% | 45% |

Total number of butterflies: 20 pcs

*P≤0.005

The figure 4 shows the dependence of the influence of the radiation wavelength on the number of adult *G.mellonella* displaced. Figure 4 shows that 435 nm optical radiation is the most attractive because it attracted 45% of wax moths, which is 1.5…2 times higher compared to 491, 546 and 491 nm radiation.

Figure 4. Influence of the radiation wavelength on the number of adult *G.mellonella* displaced.

The results of the second experiment are summarized in Table 2. Table 2 shows that the long presence of adults in the fourth cylinder causes their death. At the same time, according to the results of the first experiment, it was seen that the short-term emission of this spectrum acts on the bee moth as bait or attractant.

Table 2. Distribution of adult *G.mellonella* in the second experiment after exposure for 7 days.

| The number of butterflies in the tanks, pcs. | Number of dead butterflies, pcs | Total number of butterflies, pcs. |
|---------------------------------------------|---------------------------------|-----------------------------------|
| Tank 1 | Tank 2 | Tank 3 | Tank 4 | Tank 5 |
| λ=577nm | λ=546nm | λ=491 nm | λ=435nm |          |
| 1.67±0.33 | 2.67±0.33 | 3.0±0.58 | 4.33±0.58* | 5.33±0.67 |
| 3.0±0.67 | 20 |

From Table 2 it can be seen that the largest number of bee moth butterflies chose tank 4, which once again confirmed the susceptibility of the insect's faceted eye to shortwave radiation.
Table 3. Total biomass (g and %) increase in the third experiment.

| Tank 1 $\lambda=577$ nm | Tank 2 $\lambda=546$ nm | Tank 3 $\lambda=491$ nm | Tank 4 $\lambda=400$ nm | Total   |
|------------------------|------------------------|------------------------|------------------------|--------|
| 5±0.75 g               | 7±0.84 g               | 7±1.05 g               | 9±0.99* g              | 5±0.75 g | 7±0.84 g | 7±1.05 g | 9±0.99* g              |
| 17.86%                 | 25.00%                 | 25.00%                 | 32.14%                 | 17.86% | 25.00% | 25.00% | 32.14% |

Table 3 shows that at a wavelength of 400 nm, the weight of eggs laid was 32%, which is 1.5...2 times more than in the variants 491, 546 and 491 nm.

With the help of MS Excel package, a mathematical dependence of the biomass growth of the bee moth was found, which is described by a fourth-order polynom and is given by the determination coefficient $R^2 = 0.99$

\[ N2 = 0.5417\lambda^4 + 6.0833\lambda^3 - 23.958\lambda^2 + 39.417\lambda - 16 \]  

where $N2$ – biomass weight, %; $\lambda$ – wavelength, 435…577 nm.

Studies on the effect of the optical radiation spectrum on the behavior of bee moth butterflies have shown that it is possible to correct the direction of movement of these insects. Due to this, it is possible to create special places in the hive for catching this pest without causing harm to it. The proposed experimental setup provides the possibility of controlling the spectrum and intensity of led radiation. In the future, for these purposes, it is advisable to use inexpensive and easy-to-use programmable logic microcontrollers, for example, ATmeda with Arduino UNO Board, which we have successfully used previously for similar purposes [7,17].

4. Conclusion
The relevance of our work lies in the development of a method for the deactivation of *G. mellonella* alveria while maintaining the integrity of the colony, which allows preserving the productivity of honey and using wax moth larvae for the production of pharmaceutical preparations and biologically active additives. Using the developed experimental setup, the range of the imago light reaction was determined taking into account the spectral sensitivity of the faceted eyes of butterflies. It was found that LEDs can be used as an attractant for luring insects. The radiation of the LEDs should be in the range of 400...435 nm. This ensures maximum *G. mellonella* performance, since at this wavelength, the number of eggs laid was 1.5...2 times higher compared with radiation of 491, 546 and 491 nm.

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