UDC 60:575,631

INVESTIGATION OF EXPRESSION OF HIGH-MOLECULAR SUBSTANCES AS THE CONSEQUENCE OF EXPOSURE TO LASER LIGHT ON THE ORGANISM OF EARTH WORM *EISENIA VENETA*

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The effect of laser light on the content of fractions of soluble and a membrane protein in tissues of worms of parental forms and generation *F1* of the species *Eisenia veneta* was estimated.

Studies have shown changes in the protein spectrum in the tissues of animals of the *F1* generation relative to the parent forms that were differently exposed to laser light.

***Key words**:* soluble proteins, membrane proteins, substrate, bottom mud, radiation exposure.

Introduction. The effect of laser radiation on living organisms, including on invertebrates, causes the researchers' unrelenting interest. However, up to the present time there is no unified theory explaining all the effects that arise when it acts. It has been established that the effect of laser radiation of low intensities on biological objects is a stimulating effect on many physiological processes in the organisms of animals and plants [1]. Red light is beginning to be acknowledged as an essential component of vermicomposting, bringing the worms to maximum effectiveness. There are many potential lighting options for large and small scale worm bins, depending on the size and depth. It was shown the benefits are connected with the colour of the light [2]. Other studies show similar results, with earthworms either being more efficient because of red light, or all congregating towards areas with red

light [3–5]. Different factors affect the rate at which worms process the compost. There are the temperature, type of feed, moisture, oxygen availability, species of worm, pH, pests and lighting. The worm castings produced by this process are a mineral rich fertiliser and soil conditioner, that enhance plant growth (germination, roots, crop yield), improve overall soil quality (aeration, microorganisms, water capacity) and even have broader environmental benefits [6]

Key structures in cells and cellular organelles that are sensitive to the effects of laser radiation are identified [7]. Laser radiation with a different exposure time can also be a promising tool for selecting and forming a new population of the *Eisenia veneta*. The new population acquires new qualities, including increased resistance to the transfer of adverse conditions. The genus *Eisenia* is the main captive breed of worms that belongs to the family *Lumbricidae* and differs from others in biological features and environmental requirements. Selection work with *Eisenia veneta* has noticeable differences from traditional farm animals and is exacerbated by the biological features of the species: the complexity of identifying phenotypes, hermaphrodites and, at the same time, the necessity of having a sexual partner for a normal reproductive process. The aim of the study was to study the dynamics of the expression of cytosolic and structural proteins of the *Eisenia veneta* population under the action of laser radiation (0.63 μm wavelength).

Materials and methods. The investigations were carried out at the Department of Biotechnology of the UDHTU and the Department of Biochemistry and Biophysics of the DNU. The tissues of invertebrate species of *E. veneta* were used for the experiment.

Animals of the species *E. veneta* from the tested population that did not reach the reproductive function in their development were presented and divided in equal amounts (20 individuals) into four experimental groups. Each group of earthworms was treated with laser radiation of the type LGN-208B (power – 1 megawatt). Exposure time for each group: 1 group – 5 minutes; 2 group – 15 minutes; 3 group – 25 minutes and 4 group – 30 minutes. These groups are the parent forms. Animals of each group in equal quantities (20 individuals each) were spread over two substrates:

substrate 1 – bottom sediments (sapropel); substrate 2 – soil. Substrates for selection of offspring were checked twice a week. Generation *F1*, consisting of youngest samples, was transplanted into similar substrates. All experimental groups of animals: parent forms; generation F1 and group control (without laser treatment) were kept at the same temperature, humidity and substrate composition.

Adult individuals were selected during the experiment. The productive indices of the groups are as follows: the average weight in the control group is 190 mg. the average weight of the special individuals, the irradiated groups contained on the sapropel substrate, is 214 mg. The average weight of the individuals of the irradiated group contained on the soil substrate is 212 mg. Individuals, the tissues of which were selected from each group, were examined according to a standard procedure [8].

The protein was extracted by homogenization in appropriate buffers with further centrifugation at 1500 RPM. The soluble and membrane fractions were obtained. The soluble fraction was obtained by homogenizing the tissues of *E. veneta* in 50 mM Tris-HCl buffer (pH 7.4). To extract the membrane fraction of *Eisenia veneta* tissues, the same buffer was used, with the addition of SDS 1 %. The total protein content was determined in wavelength 780 nm. The sample buffer was added to the supernatant after centrifugation. Protein fractioning was performed in polyacrylamide gel with the addition of SDS. Gel plates were painted using Kumasi blue and scanned after electrophoreses with software “Alphèmager 2200”.

Results and their discussion. The basis for research in the field of population genetics is the processing and analysis of experimental data, which makes it possible to verify the hypotheses put forward and to discover new effects in the genotype of the population. At the same time, existing methods for recognizing high-molecular substances by gel electrophoresis of proteins under denaturing conditions, are based on approximate methods for comparing the contrast of electrophoregrams with a standard solution of known substances [9].

Studies of eartworml tissues on the presence of soluble and membrane proteins were carried out when they reached mature reproductive function (the presence of a girdle on the body).

The protein spectrum of the tissue samples under study is shown in Table 1.

Table 1

The proteins spectrums of the earthworm tissue samples under lazer irradiation

Studied group																	
Control (without exposure)		Parents forms								Generation F1							
Soluble proteins	Membrane proteins	Soluble proteins				Membrane proteins				Soluble proteins				Membrane proteins			
		Exposure, min.				Exposure, min.				Exposure, min.				Exposure, min.			
		5	15	25	30	5	15	25	30	5	15	25	30	5	15	25	30
A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
F	F	F	F	F	F		F		F	F	F	F	F	F	F	F	F
G	G	G	G	G					G		G	G	G	G	G	G	G
H									H		H	H	H		H	H	H
I											I	I	I		I		I
											J				J		

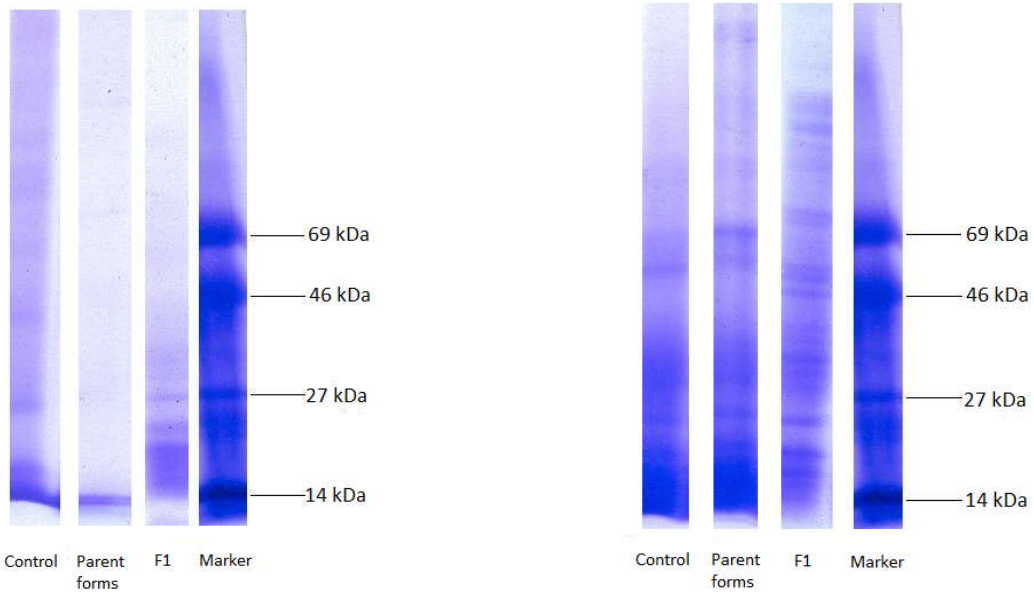
It is established that the differences in the protein spectrum of the tissue samples under study are fixed by the presence in the *FI* fractions. *F* fraction of membrane proteins does not exist after irradiation for 5 and 15 minutes. Fraction of soluble protein *G* disappears after irradiation for 30 minutes. The same fraction is absent from the electrophoregrams of membrane proteins obtained at exposures of 5–25 minutes. The fraction of membrane proteins *H* and *I* appears after irradiation with a dose of 30 minutes, respectively.

Comparison of the protein spectra of parental forms and the generation of *F1* indicates the appearance of fractions of *H* and *I* soluble and membrane proteins in doses of 15–30 minutes, respectively. Fraction *J* is fixed in proteins of soluble and membrane proteins after irradiation for 15 minutes.

It was found a high molecular weight protein compound that belonged to the *F1* generation with an exposure of 15 minutes.

Electrophoregrams of soluble and membrane proteins revealed by electrophoresis in animal tissues, control group, group exposed to laser light exposure

15 minutes and generation *F1* of this group are shown on Fig. 1. The graph for calculating the molecular weight of the unknown protein was constructed from electrophoretic mobility data and the logarithm of the marker protein masses. The logarithm of the protein mass, and then the mass itself, was found using the equation of a straight line. This was confirmed when processing the researched electrophoregrams with the AlphImager 2200 program.



a ***b***

Fig. 1. Electrophoregram of: *a* – soluble proteins, *b* – membrane proteins

Comparing the tracks presented on the electrophoregrams: control – proteins of non-irradiated animals with tracks *F1* – proteins of the first generation of worms irradiated by exposure for 15 minutes, identify difference in the spectrum of proteins, both soluble and membrane fractions. A new protein with a molecular weight of 16.4 kDa was found in groups of *F1* animals, whien are off spring of parental forms irradiated with a laser with an exposure time of 15 minutes

CONCLUSIONS

Comparison of the protein spectra of the parental forms and generation of *F1* indicates the appearance of fractions of soluble and membrane proteins *H* and *I* in doses of 15–30 minutes, respectively. Fraction *J* is fixed in proteins of soluble and membrane proteins after irradiation for 15 minutes.

It can be assumed that the new high-molecular protein compound, which is contained in the tissues of the *F1* generation worms (the time of exposure is

15 minutes) and was not detected by the program on the remaining electrophoregrams. This is the result of inducing a laser and manifested in the generation of *F1*, as the non-specific resistance of the studied organisms to the stress factor.

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ДОСЛІДЖЕННЯ ЕКСПРЕСІЇ ВИСОКОМОЛЕКУЛЯРНИХ РЕЧОВИН ЯК НАСЛІДОК ВПЛИВУ ЛАЗЕРНОГО ВИПРОМІНЮВАННЯ НА ОРГАНІЗМ ДОЩОВИХ ЧЕРВЯКІВ *EISENIA VENETA*

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Вивчали ефект впливу лазерного випромінювання на вміст фракцій розчинних і мембранних білків в тканинах черв'яків батьківських форм і покоління F1 виду *Eisenia veneta*.

Дослідження показали, зміни білкового спектру в тканинах тварин покоління F1 щодо батьківських форм, які безпосередньо піддавалися лазерному впливу.

***Ключові слова**:* розчинні білки, мембранні білки, субстрат, сапропель, експозиція випромінювання.

ИССЛЕДОВАНИЕ ЭКСПРЕССИИ ВЫСОКОМОЛЕКУЛЯРНЫХ ВЕЩЕСТВ КАК СЛЕДСТВИЕ ВОЗДЕЙСТВИЯ ЛАЗЕРНОГО ИЗЛУЧЕНИЯ НА ОРГАНИЗМ ДОЖДЕВЫХ ЧЕРВЕЙ *EISENIA VENETA*

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Оценено влияние лазерного излучения (длиной волны 0,63 мкм) с различным временем экспозиции (5–30 минут) на содержание растворимых и

мембранных белков в тканях червей родительских форм генерации F1 вида *Eisenia veneta*.

Установлено при воздействии лазерного излучения с временем экспозиции 15 минут у поколения червей *F1* вида *Eisenia veneta* обнаружены различия во фракциях растворимых и мембранных белков по сравнению с родительскими формами

***Ключевые слова**:* растворимые белки, мембранные белки, субстрат, сапропель, экспозиция облучения.