Short communication paper

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The influence of the *Eisenia fetida* holding environment on medicinal leech breeding

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Key words: *Eisenia fetida medical leeches, Hirudo verbana, Hirudo orientalis.*

The study used 120 medicinal leeches, including 80 Hirudo verbana and 40 Hirudo orientalis. Four groups of animals were formed: two control and two experimental. The control groups consisted of medicinal leeches of both species, which were kept using the modern jar method. Experimental scheme. Control group: a peat-soil weakly acidic environment (pH 5.5) was prepared. As fertilization belts formed in leeches, they were transplanted into 3-litre sterile containers filled with the moist peat-soil medium. Afterwards, the containers were covered with a fabric that allowed for full oxygen permeability. The containers with the animals were placed in a dark room with a temperature of +25-26 °C and diffused artificial lighting. After a month, parent leeches and soft cocoons were collected. Juveniles were then selected from the cocoons and placed in sterile 3-litre containers filled with settled tap water. Experimental groups: instead of the peat-soil environment, medical leeches were placed in an environment selected from the *Eisenia fetida* (pH 5.0). All other manipulations were performed as in the control group. In the control group, animals in the peat-soil environment exhibited good health, with low mortality rates (*Hirudo verbana* 2.2±0.2% and *Hirudo orientalis* 1.9±0.2%) and high fecundity (*Hirudo verbana* 9.2±0.3% and *Hirudo orientalis* 6.1±0.2%), indicating the suitability of the maintenance environment $p < 0.05$. The experimental group yielded negative results, reflected in significantly higher mortality rates for both species: *Hirudo verbana* 75.2±4.2% and *Hirudo orientalis* 80±3.1%, $p < 0.05$). Furthermore, in the experimental groups, most animals produced few or no cocoons and offspring (Hirudo verbana 0.9±0.05%). A significant proportion of the cocoons were defective: Hirudo verbana 55.3±3.4% ($p < 0.05$). Fertile cocoons contained a limited number of offspring.
Key words: *Eisenia fetida medical leeches, Hirudo verbana*, *Hirudo orientalis.*

Medicinal leeches (ML) are ectoparasitic animals widely used in agriculture, veterinary and medicine. ML have a wide range of therapeutic effects, as they have more than 100 biologically active substances in their body[1-11]. Due to the deterioration of the ecological state of the environment, ML has practically disappeared from many areas. Maintenance and breeding of ML take place in laboratory conditions under strictly controlled conditions[12-19]. Therefore, maintaining an optimal environment is crucial. The California red worm (*Eisenia fetida*) is widely used for producing biohumus. Biohumus is a valuable organic fertilizer, the main product of the worm's life activity. The optimal temperature for keeping and breeding *Eisenia fetida* is +20...+22°C, with a humidity of 75–88%.Their habitat consists of a special substrate saturated with organic compounds: soil, manure, composts, organic waste, leaves, and various garbage. It is used to grow many crops. According to their systematic position, *Eisenia fetida* belongs to the same superclass *Clitellata* as the ectoparasitic ML. Therefore, it is hypothetically possible to use the medium for keeping *Eisenia fetida* also for ML in laboratory conditions, which was the goal of the study.

In total, 120 medicinal leeches (ML) were used in the study: 80 *Hirudo verbana* and 40 *Hirudo orientalis* leeches. Four groups of animals were formed: two control and two experimental. The control groups consisted of ML of both species, which were kept using the modern jar method.

Experimental scheme. A peat-soil weakly acidic environment (pH 5.5) was prepared (Fig. 1). It should be noted that the soil for the medium was taken from the protected area of Khortytsia Island, Zaporizhzhia city, which eliminated the presence of various toxic substances in it. The prepared natural soil underwent visual detailed selection (for pests and other animal organisms) and disinfection (using high temperatures). Soil disinfection occurred at 100° C for one hour in a dry heat sterilizer. It is well known that this temperature and time period are sufficient for disinfection. It was then moistened with distilled water (80%), thoroughly mixed, diluted with neutral peat in a 1:3 ratio, and mixed and ground again. Subsequently, it was covered with an airtight tablecloth for at least 3 days. As fertilization belts formed in leeches, they were transplanted (2 leeches per container) into 3-litre sterile containers filled with the moist peat-soil medium. Holes were artificially created in the vessel containing the peat-soil environment, and the leeches were placed within them. The leeches were then watered with settled water. Afterwards, the containers were covered with a fabric that allowed for full oxygen permeability. The containers with the animals were placed in a dark room with a temperature of +25-26 °C and diffused artificial lighting. Strict control was maintained over environmental humidity, room temperature, and ensured constant ventilation and oxygen supply.

For a month after the appearance and formation of cocoons, they were checked for completeness. Very soft cocoons were mechanically opened. After a month, all offspring and mature individuals were removed. Juveniles were then selected from the cocoons and placed in sterile 3-litre containers filled with settled tap water.

Experimental groups: instead of the peat-soil environment, medical leeches were placed in an environment selected from the *Eisenia fetida* (pH 5.0). All other manipulations were performed as in the control group. The environment of all groups was tested for acidity using various methods (Fig. 1).

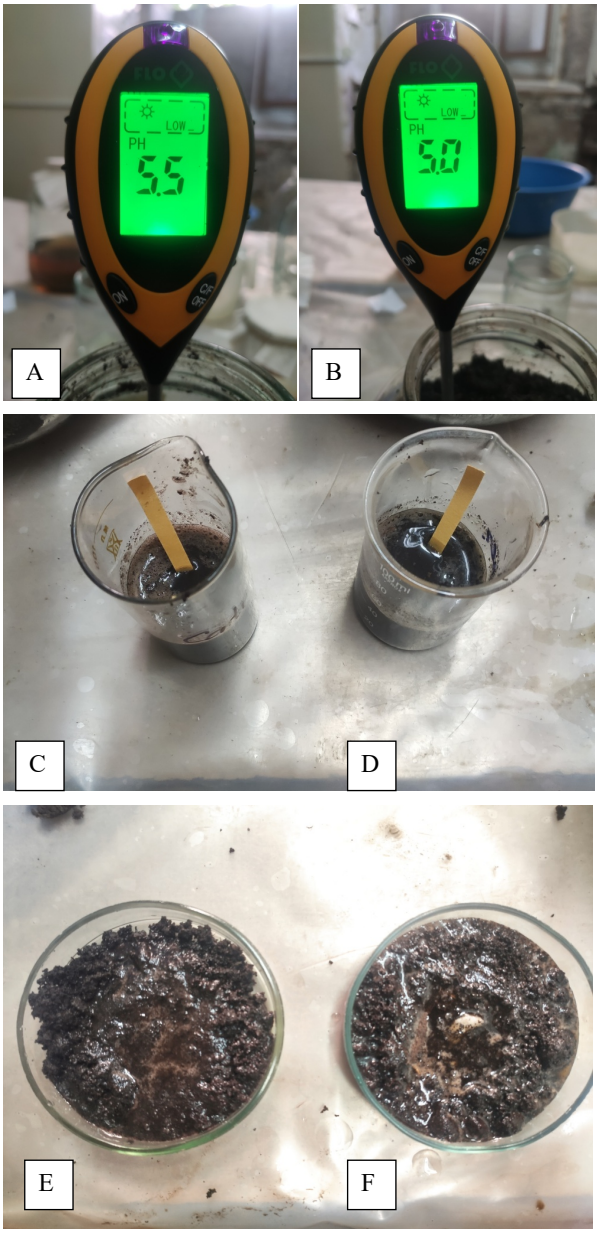

Fig. 1. Acidity test. Control group – A, E, C, Experimental group – B, D, F.

Animal manipulation was carried out in accordance with the rules and regulations for the treatment of laboratory animals: principles of bioethics, legislation, and requirements in accordance with the provisions of the „European Convention for the Protection of Vertebrate Animals Used for Research and Scientific Purposes", the Law of Ukraine „On the Protection of Animals from Animals handling".

Statistical data processing was performed using the computer program SPSS v.23,0. (IBM SPSS Statistics., USA). The selected parameters indicated in the table below have the following notation: X – the average value of the sample, SE – standard error of the average value of the sample. The significance of differences between the mean values was evaluated by the Student's criterion after checking the normal distribution[20]. Differences were considered significant at $p<0.05$.

In the control group, animals in the peat-soil environment exhibited good health, with low mortality rates (***Hirudo verbana*** 2.2±0.2% and ***Hirudo orientalis*** 1.9±0.2%) and high fecundity (***Hirudo verbana*** 9.2±0.3% and ***Hirudo orientalis*** 6.1±0.2%). The animals were not aggressive, did not release blood into the environment, their movements were active, and they intensively burrowed to lay cocoons. During the check, there were no animals on the surface of the medium. During visual observation, the animals had a normal body shape and appearance. Based on this condition of the animals, it can be concluded that the animals are healthy. These results align with findings from other researchers[7-11], indicating the suitability of the maintenance environment (Table 1, $p < 0.05$).

The environment was thoroughly utilized, and the initial deposition of cocoons was observed within it (Fig. 2A).

The experimental group yielded negative results, reflected in significantly higher mortality rates for both species: ***Hirudo verbana*** 75.2±4.2% and ***Hirudo orientalis*** 80±3.1% (Table 1, $p < 0.05$). Furthermore, in the experimental groups, most animals produced few or no cocoons and offspring (***Hirudo verbana*** 0.9±0.05%). A significant proportion of the cocoons were defective: ***Hirudo verbana*** 55.3±3.4% ($p < 0.05$). Fertile cocoons contained a limited number of offspring (Table 1).

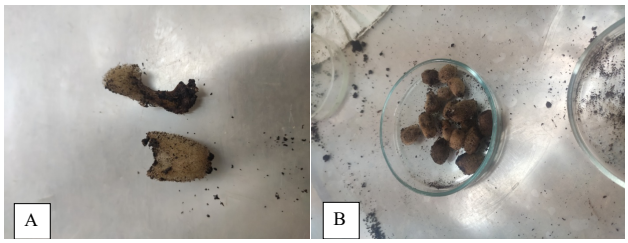

Fig. 2. Cocoons. Control group – A, Experimental group – B.

It is noteworthy that animals died while still bearing fertility belts on their bodies (Fig. 3).


Fig.3. A dead leech with a fertility belt

Based on the obtained results, a preliminary conclusion can be made regarding the possible toxic effect of the medium derived from Eisenia fetida, which is significantly toxic to ML.

It should be noted that the soil for the medium was taken from a protected area, which eliminated the possibility of various negative factors. For example, the accumulation of toxic substances, but the possibility of accumulation remains. Since the soil for the medium was the same in both the control and experimental groups, *Eisenia fetida* may make the medium toxic to other animals. Therefore, a detailed analysis of the soil for the presence of various toxic substances that could have affected the vital functions of the animals will be carried out in future studies.

Table 1. Indicators of the experimental group in comparison with the control group (X± SE; n=120)

Group of animals	Average number of juveniles obtained from a cocoon per 1 parent leech	Mortality of parent leeches during breeding, %	Defective and non-fertile cocoons, %
Control group (modern banking method) *Hirudo verbana* (n=40)	9,2±0,3	2,5±0,2	5,0±0,3
Experimental group *Hirudo verbana* (n=40)	0,9±0.05*	80,0±4,2*	50,0±3,4*
Control group (modern banking method) *Hirudo orientalis* (n=20)	6,1±0,2	5,0±0,2	10±0,2
Experimental group *Hirudo orientalis* (n=20)	–	80±3,1*	–

Note: * – $p < 0.05$ compared to the control group.

References

(1) Ojo, P.O.; Babayi, H.; Olayemi, I.K.; Peter, O.O.; Fadipe, L.A.; Baba, E.; Izebe, K., Anti-Tubercular Activities and Molecular Characterization of Salivary Extract of Leech (Hirudo medicinalis) against Mycobacterium tuberculosis. *Journal of Tuberculosis Research*, **2018**, 6, pp. 1-9. URL: https://doi.org/10.4236/jtr.2018.61001.

(2) Krashenyuk, A.I. "Neurotrophic (Neural Stimulating) and Neuromediator Effects of *Hirudo medicinalis*. Pathogenetic Mechanism of Treatment of Diseases of the Nervous System of the Human". *Acta Scientific Medical Sciences*, **2020,** *4*(3), pp. 4-9. URL: https://doi.org/10.31080/ASMS.2020.04.0552.

(3) Nowicki, A.; Jaworska, J.; Baranski, W. Leech therapy in the treatment of a penile haematoma in a stallion. *Veterinární Medicína*, **2021**, *66*(6), pp. 266–271. URL: https://doi.org/10.17221/163/2020-vetmed.

(4) Trenholme, H.N.; Masseau, I.; Reinero, C.R. Hirudotherapy (medicinal leeches) for treatment of upper airway obstruction in a dog. *Journal of Veterinary Emergency and Critical Care*, **2021**, *31*(5), pp. 661-667. URL: https://doi.org/10.1111/vec.13094.

(5) Dudhrejiya, A.V.; Pithadiya, S.B.; Patel, A.B. Medicinal Leech Therapy and Related Case Study: Overview in Current Medical Field. *J Pharmacogn Phytochem,* **2023**, *12*(1), pp. 21-31. URL: https://doi.org/10.22271/phyto.2023.v12.i1a.14543.

(6) Sonani, S.R.; Dudhamal, T.S. Leech therapy and adjuvant Ayurveda treatment in the management of diabetic foot ulcer with atherosclerosis. *BLDE Univ J Health Sci,* **2023**, *8*, pp. 192-196.

(7) Zaidi S.A. Unani treatment and leech therapy saved the diabetic foot of a patient from amputation. *International wound journal*, **2016**, *13*(2), pp. 263-264.

(8) Sharma P.; Kajaria D. Management of non-healing venous ulcer in systemic sclerosis with leech therapy—A case report. *Journal of Family Medicine and Primary Care*, **2020**, *9*(4), pp.2114.

(9) Lari A, Zaid Iqbal Mt, Ali M. Management Of Ghangrana (Dry Gangrene) By Irsal-E-Alaq (Leech Therapy)-A Case Study. Indian Journal Of Unani Medicine Vol Xiv, **2021**, (1), pp. 56-60.

(10) Balasooriya D.; Karunarathna C.; Uluwaduge I. Wound healing potential of bark paste of Pongamia pinnata along with hirudotherapy: A case report. *Journal of Ayurveda and Integrative Medicine,* **2021**, *12(*2), pp. 384-388.

(11) Shakouri A.; Adljouy N.; Abdolalizadeh J. Anti-Cancer Activity of liposomal Medical leech saliva extract (LSE). Proceedings of the 3rd World Congress on Recent Advances in Nanotechnology (RAN'18), April, Budapest, Hungary, **2018,** Paper No. NDDTE XXX. https://doi.org/10.11159/nddte18.102.

(12) Ceylan M.; Küçükkara R.; Karataş E. Effects of cocoon incubation angle on hatching success of medicinal leeches (*Hirudo* spp.). *Invertebrate Reproduction & Development*, **2023.** https://doi.org/10.1080/07924259.2023.2241418

(13) Bidmal H.R.; Sudagar M.; Shakouri, M. The effect of different blood (goat and sheep) on sexual maturity, survival and the production of cocoons and larvae in oriental leech (*Hirudo orientalis*). *Journal of Animal Environment (AEJ),* **2022**, *13*(4), pp. 301-306. https://doi.org/10.22034/AEJ.2020.232247.2268.

(14) Ceylan M. Effects of maternal age on reproductive performance of the southern medicinal leech, *Hirudo verbana* Carena, 1820. *Animal Reproduction Science*, **2020**, 218, pp. 106507. https://doi.org/10.1016/j.anireprosci.2020.106507.

(15) Malek, M.; Jafarifar, F.; Aminjan, A.R.; Salehi, H.; Parsa, H. Culture of a new medicinal leech: growth, survival and reproduction of *Hirudo orientalis* Utevsky and Trontelj, 2005 under laboratory conditions. *Journal of Natural History,* **2019**, *53*(11-12), pp. 627-637. URL: https://doi.org/10.1080/00222933.2019.1597200.

(16) Ceylan, M.; Çetinkaya, O.; Küçükkara, R.; Akçimen, U. Reproduction efficiency of the medicinal leech *Hirudo verbana* Carena, 1820. *Turk. J. Fish. Aquat. Sc.*, **2015**, *15*, pp. 411–418. URL: https://doi.org/10.4194/1303-2712-v15_2_27.

(17) Ceylan, M.; Küçükkara, R.; Akçimen, U. Effects of broodstock density on reproduction efficiency and survival of southern medicinal leech, *Hirudo verbana* Carena, 1820. Aquaculture, **2019**, *498,* pp. 279-284. URL: doi: 10.1016/j.aquaculture.2018.08.016.

(18) Zhang, B.; Lin, Q.; Lin, J.; Chu, X.; Lu, J. Effects of broodstock density and diet on reproduction and juvenile culture of the leech, Hirudinaria manillensis Lesson, 1842. *Aquaculture,* **2008**, *276*, pp. 198–204. URL: https//doi.org/10.1016/j.aquaculture.2008.02.00

(19) Manav, M.; Ceylan, M.; Büyükçapar, H.M. Investigation of reproductive efficiency, growth performance and survival of the southern medicinal leech, *Hirudo verbena* Carena, 1820 fed with mammalian and poultry blood. *Animal Reproduction Science*, **2019**, *206*, pp. 27-37. URL: https://doi.org/10.1016/j.anireprosci.2019.05.004

(20) Aminov, R. "Average number of juveniles obtained from a cocoon per 1 parent leech, mortality of parent leeches during breeding, defective and non-fertile cocoons ", Mendeley Data, **2025**, V1, URL: doi: 10.17632/p7k3m9472k.1