OPINION

from Tsvetelina Georgieva Batsalova, Ph.D., Assoc. Prof., Department of Developmental biology, Faculty of Biology, Paisii Hilendarski University of Plovdiv

of a dissertation to be awarded the educational and scientific degree "Doctor" in the field of higher education 4. Natural Sciences, Mathematics, and Informatics, professional field 4.3. Biological Sciences, doctoral program in Morphology.

Author: *Eleonora Tencheva Kovacheva*

Topic: „*Assessment of pollution of aquatic ecosystems with priority organic pollutants using morphofunctional biomarkers in Cyprinus carpio (Linnaeus 1785)*"

Supervisor: *Prof. Elenka Stoilova Georgieva, Ph.D.*, Department of Developmental Biology, Faculty of Biology, Paisii Hilendarski University of Plovdiv

1. General presentation of the procedure and the Ph.D. student

By order No. RD-21-519 issued on 28.02.2024 of the Rector of Paisii Hilendarski University of Plovdiv (PU) I have been appointed as a member of the scientific jury to ensure the procedure for the defense of a dissertation work on the topic "Assessment of pollution of aquatic ecosystems with priority organic pollutants using morphofunctional biomarkers in *Cyprinus carpio* (Linnaeus 1785)" for the acquisition of the educational and scientific degree "doctor of philosophy" by Eleonora Tencheva Kovacheva - a full-time Ph.D. student at the Department of Developmental biology with supervisor Prof. Elenka Stoilova Georgieva, Ph.D. from Paisii Hilendarski University of Plovdiv.

The set of documents presented by the Ph.D. student Eleonora Kovacheva is in accordance with Article 36 (1) of the Regulations for the Development of the Academic Staff of the PU (PRASPU) and includes the following documents:

– a request to the Rector of the PU to disclose a procedure for the defense of a dissertation;
– CV in European format;
– protocol from the Department of Developmental biology reporting the readiness to start the procedure and preliminary discussion of the dissertation work;
– dissertation;
– abstract;
– list of scientific publications on the topic of the dissertation;
– copies of scientific publications;
– list of noticed citations;
– declaration of originality and authenticity of the attached documents;
– reference for compliance with the minimum national requirements.

The Ph.D. student has enclosed four publications and information on participation in eight scientific forums and three scientific research projects.

Eleonora Kovacheva completed her higher education at Paisii Hilendarski University of Plovdiv. In 2018, she obtained a bachelor's degree in molecular biology, and in 2019 - a master's degree in reproductive biology. The Ph.D. candidate has three years of work experience as a laboratory technician in a histology laboratory in the period 2018-2021, after which she worked for about a year in the commercial sector. From the beginning of 2023, El. Kovacheva was appointed to the position “assistant” in the Department of Medical biology at the Medical University of Plovdiv.

2. Relevance of the topic

The intensive application of pesticides in modern agriculture carries a serious risk of environmental pollution, damage to living organisms, irreversible changes in ecosystems, and loss of biodiversity.

Pesticide pollution significantly affects aquatic ecosystems and disrupts sustainable aquaculture production. Along with this, bioaccumulation occurs which spreads the toxic impact of these priority organic substances at different levels of the food chain. The consumption of food products contaminated with pesticide residues is a threat to human health and has been associated with a worldwide increase of a number of allergies, various genetic disorders, as well as cancer. There is a need for in-depth research and assessment of the toxic potential of different types of pesticide substances. Therefore, I consider that the topic of the presented dissertation work is relevant. Its purpose is to clarify a priority question in the field of ecotoxicology by investigating the toxic impact of three commonly used worldwide, but poorly studied pesticides - pyrimiphos-methyl, propamocarb hydrochloride and 2,4-dichlorophenoxyacetic acid (2,4-D).

3. Knowledge on the problem

The Ph.D. student conducted an in-depth literature review on the scientific topic of the dissertation work. El. Kovacheva adequately interprets the results of the conducted research, as well as the results of other research teams working in the relevant scientific field. She demonstrates excellent knowledge of the state of the scientific problem, ability to analyze and apply the gathered knowledge in the implementation of the specific research aim.

4. Research methodology

The chosen research methodology allows solving and adequately answering the tasks set in the dissertation work, which ensures the achievement of its goal. A laboratory experiment with common carp was conducted - a 96-hour toxicity test of the pesticides pirimiphos-methyl, propamocarb hydrochloride and 2,4-dichlorophenoxyacetic acid (2,4-D). To evaluate the negative effects of the three pesticides, a complex approach was applied, including the implementation of histopathological analyses of gills, liver and kidney of common carp based on a multifactorial system for evaluation of the studied material. Histochemical analyzes of lipid and polysaccharide content in common carp liver were performed. A biochemical study was performed on liver material isolated from pesticide-treated fish and control individuals kept for the same period of time in a pesticide-free environment. The amount of total protein was analyzed, as well as the activity of

key liver enzymes such as lactate dehydrogenase, cholinesterase, catalase, alanine aminotransferase, asparagine aminotransferase.

5. Characterization and assessment of the dissertation work and contributions

The dissertation is very well structured, balanced, its layout corresponds to the requirements of the PRASPU. The work includes 183 pages. 519 literary sources are cited, of which 10 are in Bulgarian. A comprehensive literature review on the topic of the dissertation is presented; the experiments and analyses performed, as well as the results obtained, are thoroughly described. In the Aim and tasks section the description of the main purpose of the dissertation could be expanded in order to more thoroughly reflect the specifics of the conducted research. The research presented in the dissertation clarifies the toxic potential of priority organic substances by using morphofunctional biomarkers in *Cyprinus carpio* which will allow the assessment of aquatic ecosystems pollution.

The dissertation work includes an in-depth discussion of the reported histopathological, histochemical and biochemical changes that developed under the influence of the three studied pesticides; conclusions; clearly defined contributions of the conducted research. The dissertation has significant scientific contributions but it also has contributions with practical aspect. The analyzed histopathological, histochemical and biochemical parameters can be included in a model for assessing the toxic impact of pesticides, with the aim of preparing an adequate normative base regarding the presence of organic pollutants in aquatic ecosystems. This highlights the importance of the studies conducted, both in scientific and applied aspects.

6. Evaluation of the publications and personal contribution of the candidate

The Ph.D. student has presented four scientific publications on the topic of the dissertation work. One of the articles is published in a journal with Q1, and the other three articles are published in a journal with Q4. El. Kovacheva is the first author of two of the articles. With the presented scientific publications, the doctoral student meets the minimum national requirements and even exceeds the required number of 30 points. Along with that, the results of the dissertation work were actively popularized through participation in national and international scientific forums.

7. Abstract

The abstract meets the requirements for acquiring the Ph.D. degree. It is well structured and clearly reflects the main results achieved during the work on the dissertation.

8. Recommendations for future use of the dissertation contributions and results

Eleonora Kovacheva has successfully achieved the goals and tasks of her dissertation work. Therefore, significant results have been obtained and they contribute to the development of fundamental science, but also have an applied aspect. An opportunity to develop a model to assess the pollution of aquatic ecosystems was highlighted. I recommend the doctoral student to continue her work in this direction, taking into account prospects for optimizing the experimental setup, as

well as the inclusion of other biomarkers for assessing the toxic potential of pesticides (hematobiochemical markers, immunological markers, behavioral reactions, etc.).

CONCLUSION

The dissertation contains scientific and practical approach results which represent an original contribution to science and meets all the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria (ZRASRB), the Regulations for the Implementation of ZRASRB and the relevant Regulations of Paisii Hilendarski University of Plovdiv.

The dissertation shows that Eleonora Kovacheva possesses in-depth theoretical knowledge and professional skills in the scientific specialty of Morphology, demonstrating qualities and skills for independent scientific research.

Due to the above, I confidently give my positive assessment of the conducted research and the presented dissertation work, abstract, achieved results and contributions, and I propose to the honorable scientific jury to award the educational and scientific degree “doctor of philosophy” to Eleonora Tencheva Kovacheva in the field of higher education: 4. Natural sciences, mathematics and informatics, professional field 4.3 Biological sciences, doctoral program Morphology.

25.04.2024 **Signature**: .....

/Assoc. Prof. Tsvetelina Batsalova, Ph.D./