

STATEMENT

of Prof. Dr. Roumiana Panayotova Metcheva, retired,
Institute of Biodiversity and Ecosystem Research, BAS

Subject: the theses of Blagovesta Dimitrova Dimitrova for the award of the educational and scientific degree "DOCTOR" in the scientific specialty "Ecology and Ecosystem Protection", Professional field 4.3. Biological Sciences, on the topic: "Impact of pesticides on amphibian species from water bodies with varying degrees of anthropogenic impact in Central Bulgaria", Scientific supervisor: Assoc. Prof. Dr. Simeon Lukanov.

The dissertation was developed within the framework of an independent doctoral study in the section "Community Ecology and Conservation Biology", Department "Ecosystem Research, Ecological Risk and Conservation Biology" at the Institute of Environmental and Biological Sciences at the Bulgarian Academy of Sciences and represents a study of the direct and indirect impact of pesticides and fertilizers on amphibian species that occur in regions of Bulgaria with intensive agricultural activity.

Overall impression of the thesis:

The dissertation is presented in 109 pages of text, structured in 8 Chapters and three Appendices. It contains 14 tables and 20 figures. The list of cited literature includes 185 titles. The aim and the specific tasks of the developed topic of Blagovesta Dimitrova's PhD are clearly formulated and determine its relevance as a problem of important fundamental and practical orientation.

The test group of amphibians was very well selected from a wide range of species from three families such as the green toad (*Bufo viridis* - Bufonidae), the tree frog (*Hyla orientalis* - Hylidae) and the great water frog (*Pelophylax ridibundus* - Ranidae). Five other target amphibian species strictly protected under the Birds of Conservation Concern, listed in Annex 2 and Annex II of EEC Directive 92/43 on the conservation of natural habitats and of wild fauna and flora, were also studied: Northern crested newt (*Triturus cristatus*), Danubian crested newt (*Triturus dobrogicus*), Bureş crested newt (*Triturus ivanbureschi*), Red-bellied booby (*Bombina bombina*) and Yellow-bellied booby (*Bombina variegata*).

It should be noted that information on the impact of pesticides and fertilizers on this class of vertebrates is very scarce. In general, the issue of the use of pesticides in agriculture is very complex due to their different chemical composition, rate of degradation by photolysis, hydrolysis or due to active degradation by microorganisms.

A study of their direct and indirect effects on the most abundant species of the relevant amphibian families in Bulgaria, which occur in diverse aquatic habitats such as rice paddies and are subjected to intensive agricultural activities, is presented.

The research has been conducted in several main areas that correspond to the chapters presented in the thesis. State-of-the-art databases and methods, acoustic determination and

laboratory experiments have been used to show the effects of commonly used pesticides and fertilizers on the larval stages of individual amphibian species. The negative effect of mass-applied ammonium nitrate on selected parameters of tadpoles of long-legged forest frog and brown toad species such as survival, growth, locomotor activity, sensitivity to chemical cues and behavioural responses was investigated.

The toxicity of the fungicide Ortiva® TOP SC was determined under acute conditions, and the LC50 and active ingredient, ethylene bis-isothio-cyanate sulfide, were determined in tadpoles of both species. Chronic experiment in four treatments showed the effects of the pesticide on larval survival, growth and locomotor activity. The results show direct toxicological effects and at extremely low concentrations and short exposure period, the subjects showed hyperactivity and accelerated growth, which are indirect negative effects on tadpoles.

Publications in scientific journals:

The abstract is formatted as required and is consistent with the results and conclusions presented in the thesis. The publication activity of Blagovesta Dimitrova consists of 5 articles, published in scientific journals of different rank. A total of 3 articles have been published on the topic of the dissertation, of which 2 with an impact factor of Q1, and 1 with an impact rank of Q4, thus fulfilling the minimum requirements for the dissertation for the award of the PhD degree. Although the presented scientific works are co-authored, in one of them the PhD student is in first place which is an indication that the research on the dissertation topic is a personal work of Blagovesta Dimitrova. One citation in a publication was noted.

During the period of her preparation, the PhD student participated as a contractor in 6 scientific research projects.

The total number of points/credits is 261, which exceeds the required minimum.

Participation in scientific forums:

8 participations in scientific forums are listed, 3 of them are on issues related to the dissertation.

Significance of contributions:

The main significance of the thesis are the contributions of an original nature:

- For the first time in Bulgaria an integrated study of the impact of pesticides and fertilizers on amphibian species in areas with different degrees of anthropogenic impact has been conducted, establishing the relationship between agricultural practices and the distribution of five protected amphibian species in Bulgaria and demonstrating a higher sensitivity of the long-legged forest frog (*Rana dalmatina*) to ammonium nitrate compared to the brown toad (*Bufo bufo*), in terms of survival, growth and locomotor activity.

- For the first time, the ability of tadpoles to exhibit avoidance behaviour towards ammonium nitrate was investigated and the direct toxicological effects of the fungicide Ortiva® TOP SC on larvae of the woodland long-legged frog were tested, with negative effects detected even at low concentrations.

The scientific and applied contributions relate to a better understanding of the ecological risks of agrochemical use and the sensitivity of different amphibian species to them. The methodology developed for laboratory experiments with tadpoles can be applied to assess the toxicity of other chemicals on aquatic organisms. These results can also be used with success as a basis for future monitoring programs and ecological assessments.

Conclusion: On the basis of the scientific research work, the results obtained and the scientific contributions presented in the dissertation present PhD student Blagovesta Dimitrova as a well-established and promising researcher, who has acquired serious scientific research experience, with in-depth knowledge in the field of genetics, has mastered and applied modern methods. In terms of its volume, content and scientific contributions, the dissertation fully meets the requirements of the Law on the Development of Academic Staff in the Republic of Bulgaria. I recommend to the esteemed members of the Scientific Jury to vote positively for the award of the scientific and educational degree "Doctor", in the scientific specialty "Ecology and Conservation of Ecosystems".

Prepared by: Prof. Dr. Roumyana Metcheva

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