

Review

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a member of the Scientific Evaluation Committee, appointed by Rector's Order № P-109-334/06.08.2021 giving the right to the candidate to officially defend her research

in relation with a procedure for the award of the educational and scientific degree "Doctor"

on the dissertation of Assistant Professor ***Tsonka Slavova Dimitrova***, PhD student at the Department of Biology at the Faculty of Pharmacy, Medical University - Varna

Subject of the dissertation: "***Investigations and screening methods for early diagnosis of patients with gastrointestinal disorders because of mushroom consumption***",

presented for awarding the educational and scientific degree "Doctor" in the Doctoral Programme "Medical biology", in the Field of higher education 4. Natural sciences, mathematics and informatics, Professional field 4.3. Biological sciences

Relevance of the dissertation

The research in the present dissertation is dedicated to a current scientific problem, such as the analysis of poisonings with wild mushrooms among the population in the Northern Black Sea coast of Bulgaria and the testing of easily feasible methods for their diagnosis. Early laboratory identification is important for the timely treatment of wild mushroom poisoning. In addition, the study is of high public importance, given the widespread use of wild mushrooms in Bulgaria and therefore the study of the level of awareness about these mushrooms and their use can be used as a basis for creating policies for prevention of poisoning.

Structure of the dissertation, theoretical background, and methodology

The presented dissertation contains 146 pages and it is illustrated with 71 figures and 21 tables and one appendix. The dissertation is properly structured, including the following sections in a well-balanced ratio: introduction - 1 page, literature review and conclusions - 40 pages, goal, tasks and

working hypothesis - 1 page, materials and methods - 9 pages, own results - 65 pages, summary - 1 page, conclusions - 1 page, bibliography - 14 pages, contributions to the dissertation - 1 page. The appendix presents questionnaires for collecting information on the collection and use of wild mushrooms among the population of the studied region.

219 literary sources were cited, 13 in Cyrillic and 206 in Latin. I was extremely impressed by the contemporary literature references, as the cited sources from the last year and a half only (2020 and 2021) are over 37%. The literature review contains an in-depth and informative analysis of the available scientific literature related to the general characteristics of wild mushrooms, including poisonous ones, classification, distribution and representatives of poisonous mushrooms in Bulgaria, their use, and types of toxic substances in poisonous mushrooms. Wild mushroom poisoning, morbidity and mortality, classification and clinical features of poisoning, as well as methods for modern laboratory diagnosis, treatment and prevention of wild mushroom poisoning are discussed in detail. The review shows the rich awareness of the doctoral student on the issue, as well as her ability to understand the essence of the issues and skillfully summarize them. It is illustrated with photo material, which visualizes the main representatives of poisonous mushrooms in Bulgaria. Unfortunately, the sources of the photo material are not cited or it is not indicated that these are their own photos.

The aim and tasks of the dissertation are formulated clearly and concretely, in a sequence that allows to achieve the ultimate goal - to analyze poisoning with wild mushrooms among the population in the Northern Black Sea coast of Bulgaria, to test easily feasible methods for diagnosis and to study the level of awareness of these fungi and their use as a basis for prevention of intoxications.

The results presented in the dissertation are obtained and analyzed with the skillful use of modern clinical laboratory diagnostic methods, presented informatively and clearly in the section "Material and methods". Data from all experiments were appropriately processed statistically through the competent application of the relevant statistical methods - descriptive, variational (Student-Fisher test and Fisher's precision test) and correlation analysis (Pearson's coefficient and independent χ^2). The software product SPSS, version 22.0 was used.

Results and discussion, conclusions and contributions

The results are well explained and the scientific hypothesis is clearly formulated. The results obtained include original data presented in accordance with the set tasks. Based on them, conclusions are drawn, the most important of which relate to the tested laboratory tests - gastric contents and collected mushrooms using the Meixner test and ELISA of urine for early diagnosis of poisoning with wild mushrooms. The results from these tests play an important role in the selection of conservative treatment of patients. A retrospective study of 147 patients with *Amanita phalloides* poisoning between 1991 and 2015 found a relatively high mortality rate of 17.00% (25 deaths). Therefore, early diagnosis of these intoxications using easily feasible and reliable laboratory methods is critical. New data were obtained assessing the use of edible wild mushrooms

by the population in Varna District, and about the awareness of the respondents about the edible wild mushrooms.

The conclusions are specific and accurately reflect the results obtained and their significance. It is of particular importance that some studies are conducted for the first time in Bulgaria and represent a contribution with application in clinical practice.

Contributions are clearly worded and can be accepted without remarks. Some of the significant contributions are related to the testing of the Meixner test for the detection of amatoxins in gastric contents and harvested mushrooms and the ELISA test for the detection of amatoxins in urine given the huge socio-medical significance of wild mushroom intoxications. The first survey of its kind in Bulgaria on the awareness of the population about wild mushrooms and their use is essential for the development of future policies for prevention of the population with regard to poisoning with wild mushrooms.

Assessment of publication activity

Five original full text publications summarise the results of the dissertation, three of which published in journals with impact rank. Two of the publications are in print, in which Tsonka Dimitrova is the first author and, in my opinion, they reflect the significant contribution of the doctoral student in conducting research, analysis of results and in the preparation of scientific publications.

Abstract of the dissertation

The content and quality of the proposed abstract correspond to the content of the dissertation, all requirements are met and the main results achieved are reflected.

6. Critical remarks

My overall impression of the dissertation is positive, although I have some critical remarks. For example, given that the known species of mushrooms in the world in 2018 exceed 13,500,000, and currently about 100,000 species of mushrooms are botanically described, and approximately 3% of registered wild mushrooms are poisonous, it is not clear on how were the examples of information about species included in the literature review selected, mainly related to representatives from China; species names in the scientific botanical literature should be written in Italic. And in some places I even find misunderstanding regarding the botanical meaning of the terms species and genus. There are some mistakes in citations of the scientific literature, minor technical spelling mistakes and others of this nature.

Despite the critical remarks, my general impression of the work presented by Assistant Professor Tsonka Dimitrova is that it is sufficient in volume and importance for the acquisition of the scientific degree "Doctor" and that the doctoral student in her long work on the dissertation has acquired the necessary knowledge and competencies to work independently on a scientific

problem. The scientometric indicators of the doctoral student meet the requirements for this scientific degree.

7. Final conclusion

The dissertation of Assistant Professor Tsonka Dimitrova is a completed scientific study, which obtained up-to-date original data on the use of wild poisonous mushrooms by the population in Varna region, intoxications and methods for their early diagnosis. The tasks are precisely performed, contributions of scientific-theoretical and applied nature are correctly derived from the results. The dissertation demonstrates a good theoretical and practical skills and knowledge of modern clinical laboratory diagnostic methods and approaches. I believe that the present dissertation fully complies with the requirements for the respective scientific degree, formulated by the Act for the Development of the Academic Staff in the Republic of Bulgaria and the Rules of Procedure of the Medical University of Varna thereof. With regard to the above I give my positive evaluation on the presented dissertation and I propose to the esteemed Scientific Evaluation Committee to award the educational and scientific degree "Doctor" in "Medical biology" to Assistant Professor Tsonka Slavova Dimitrova.

Reviewer:



Prof. Diana Ivanova, D.Sc.

August 30, 2021