

STATEMENT

by Prof. Marina Ivanova Stanilova, Ph.D.

Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences (IBER-BAS), member of the Scientific Jury, according to Order No. 31/28.05.2026 of the Director of IBER

on the dissertation thesis for the acquisition of the educational and scientific degree "Doctor" of **Rumen Velinov Denev**, a part-time doctoral student in the doctoral program "Botany", professional field "4.3. Biological Sciences", field of higher education: "4. Natural Sciences, Mathematics and Informatics", at the research group "Chemoecology and Natural Products", section "Applied Botany", department "Plant and Fungal Diversity and Resources", IBER-BAS, with scientific supervisor: Prof. Dr. Strahil Berkov and topic of the dissertation thesis: **"GC-MS analysis of silylated amaryllis alkaloids"**.

The topic of Rumen Denev's Ph.D. dissertation is notable for its relevance given the enormous interest in amaryllis alkaloids, known for their wide spectrum of biological activities, their use in pharmaceuticals, and their potential for developing new therapeutic agents. The doctoral student is well acquainted with the available scientific literature on the topic, the methods for extraction of amaryllis alkaloids and for determining their composition and content in plants, as well as the advantages and limitations of various analytical techniques related to the specifics of instrumental analysis. The goal is clearly defined, related to investigating the possibilities for optimizing the qualitative and quantitative analysis of amaryllis alkaloids through their silylation, which is expected to expand the scope and increase the efficiency of GC-MS, considered a key method in identifying these alkaloids and determining their content in extracts. The tasks and methods set to achieve the goal are adequate and realistic. The main plant species, the subject of the study, have been appropriately chosen as model species for comparative analysis of alkaloid profiles, differing in the dominant alkaloid types: *Narcissus poeticus* L., with predominant lycorine and narcyclazine derivatives, *Galanthus nivalis* L., with galantamine-type alkaloids and *Hippeastrum papilio* Van Scheepen, with homolycorine and related structures. This selection of plant species allows for a targeted evaluation of the analytical capabilities of GC-MS analysis in the separation and identification of structurally different compounds close in mass, with an assessment of the influence of derivatization on the volatility, thermal stability, and fragmentation characteristics of alkaloids.

The results of the studies show that silylation significantly improves the quality of GC-MS analysis by increasing the volatility and stability of alkaloids and increasing the sensitivity of detection, which allows for reliable separation of structurally similar compounds and detection of minor components in complex plant mixtures. The supporting material for the results contains 8 schemes, 10 tables and 18 figures, while an additional 40 pages of structural formulas and GC-MS spectra of silylated and non-derivatized alkaloids and their epimers specific to the target species, chromatograms, graphs and tables related to the quantification of galanthamine in *H. papilio*, the evaluation of its alkaloidal fractions and the statistical analysis of the data are provided in an appendix. It has been found that the silylation of amaryllis alkaloids is suitable both for various scientific studies and for controlling their content in plant raw materials and products.

Based on the results obtained, nine main conclusions have been formulated, related to the possibility of overcoming the established limitations of the GC-MS method for qualitative and quantitative analysis of alkaloids by silylating the alkaloid fractions from amaryllis plants. Five original contributions of scientific and applied importance have also been presented. The created library of EIMS spectra of unsilylated and silylated amaryllis alkaloids by parallel GC-MS analysis of standard substances and alkaloid fractions from amaryllis plants will help in the future identification of alkaloids in the numerous yet unstudied species of the Amaryllidaceae family. Seven new amaryllis alkaloids have been identified. Two GC-MS methods have been developed and validated: for the quantitative analysis of galanthamine in *Hippeastrum papilio*, proving its high content in this plant species, and for the quantitative analysis of amaryllis alkaloids in plant samples by direct injection of an acidified aqueous solution, which avoids the use of toxic organic solvents.

The doctoral student has chosen to present his dissertation in the form of related scientific publications, having complied with the structure according to Article 7 of the Regulations on the conditions and procedure for acquiring scientific degrees and for holding academic positions at IBEI. The results of the research, their interpretation and the conclusions drawn are presented in 3 publications, which have been published in prestigious international scientific journals with an impact factor (Web of Science) and impact rank (Scopus) and with quartiles: one in a journal with Q1 (J. Chromatogr. A, 2026), one with Q2 (Rapid Commun. Mass Spectrom, 2021) and one with Q3 (Rapid Commun. Mass Spectrom, 2026). Denev is the first author of one of the publications, and the other two are co-authored with Bulgarian and foreign scientists. Despite the relatively large number of co-authors in two of the

publications, it is evident from the specified contributions of each of them that the doctoral student has a major participation both in the scientific research, including validation, methodology and visualization, and in writing the initial text. Denev's participation in the publication, which is co-authored only with his scientific supervisor, on the new fast and easy method for quantitative determination of galanthamine in *H. papilio* by GC-MS and evaluation of its alkaloid fractions, reveals the doctoral student's ability to build a scientific concept, research it and prove it through appropriate application of methodology, visualization, validation, software and data processing. The publication related to the contribution to the detailed metabolic analysis of a pharmacologically active alkaloid fraction from *Narcissus* cv. *Hawera* with established antidepressant and anxiolytic effects, already has 5 citations, which shows the significance of the results; the other two papers were published in 2026, which explains the relatively long period between R. Denev's expulsion from doctoral studies at the end of the scheduled term, with the right to defend, and the beginning of the procedure for defending his dissertation. The Dissertation Abstract meets the requirements for volume and structure; I consider the unnecessarily attached incomplete list of literature used in it to be a technical oversight. The similarity report shows the absence of plagiarism. The doctoral student has also attached a Declaration of originality and authenticity of the results and contributions presented in the scientific papers with which he participates in the present procedure.

In conclusion, I believe that the doctoral student has fulfilled all the requirements for acquiring the ESD "Doctor". He has collected a total of 286 credits, which is significantly more than the required minimum of 200 credits (the required 130 for the implementation of the educational program and more than twice as many for the implementation of the scientific program: 96 from reporting results at scientific forums with a required minimum of 40 and 60 from publications on the topic of the dissertation with a mandatory minimum of 30). His dissertation meets all the requirements. The research is well planned; the results are correctly presented and adequately interpreted. All contributions are original in nature and have both scientific and applied value. Given all this, I vote in favor and I strongly recommend that the members of the esteemed scientific jury evaluate and award Rumen Denev the ESD "Doctor" in scientific field 4.3. "Biological Sciences", scientific specialty "Botany".

Sofia, 09.07.2026

Signature:
(Prof. М. Станилова)