

OPINION

by

Assoc. Prof. Anna Bogomilova Gavrilova, PhD, Faculty of Veterinary Medicine, Medical University – Pleven, appointed as a member of the scientific jury pursuant to Order No. 31/28.05.2026 of the Director of IBER-BAS

regarding

the dissertation of part-time doctoral student **Rumen Velinov Denev** for the acquisition of the educational and scientific degree “DOCTOR” in the doctoral programme “Botany”, professional field “4.3. Biological Sciences”, higher education area “4. Natural Sciences, Mathematics and Informatics”, at the research group “Chemoecology and Natural Products”, section “Applied Botany”, department “Plant and Fungal Diversity and Resources” of IBER-BAS, with scientific supervisor Prof. Strahil Hristov Berkov, PhD, and dissertation topic **“GC-MS Analysis of Silylated Amaryllidaceae Alkaloids”**.

Rumen Denev’s dissertation is a complex, in-depth work of substantial fundamental and applied scientific value, contributing to the expansion of the analytical capabilities of gas chromatography–mass spectrometry (GC-MS) in the study of Amaryllidaceae alkaloids. Representatives of the family Amaryllidaceae are a unique source of complex structural molecules with proven therapeutic effects in dementia and Alzheimer’s disease. Numerous preclinical studies demonstrate their high potential for combating various types cancer as antiproliferative agents that induce apoptosis and reduce the invasiveness of cancer cells, as well as their antiviral and antimicrobial activity. The dissertation contributes towards the solution of several key problems such as purification of alkaloid fractions from compounds of higher polarity, separation and identification of structurally related or similar alkaloids, and possible detection increase after appropriate transformation (silylation) of thermolabile alkaloids with higher polarity.

The well-planned design of the overall study is highly impressive, comprising: 1) investigation of the changes in GC-MS characteristics (separation, stability, volatility, and

fragmentation) of Amaryllidaceae alkaloids following silylation of plant samples; 2) creation of an EIMS spectral library of both non-silylated and silylated Amaryllidaceae alkaloids; and 3) development of advanced methods for qualitative and quantitative analysis of these substances. The obtained results are described clearly and precisely, supported by abundant illustrative material, including the chemical structures of the studied alkaloids, comparative GC-MS and DIP spectra before and after derivatization, MS spectra of certain tentatively identified alkaloids, fragmentation schemes, tables, and graphs. The discussion of the results is in-depth, clearly revealing the significance of the presented research, as well as its theoretical and practical contributions.

The main contributions of the dissertation are five, and they may be divided into two groups. Contributions of a fundamental nature, but also of great importance for applied science, include the identification of 7 new Amaryllidaceae alkaloids (2-oxomesembrine and 2-oxo-epi-mesembrenol in *Narcissus* cv. Hawera; 11-O-acetylhamayne, 3-(3'-O-hydroxybutanoyl)hamayne, 11-O-(3'-acetoxybutanoyl)hamayne, and 3,11-O-(2',2''-dibutanoyl)hamayne in *Galanthus nivalis* L.; 11-O-(3-hydroxybutenoyl)haemanthamine in *Narcissus poeticus* L.), the development and validation of a GC-MS method for quantitative analysis of galantamine in the promising species *Hippeastrum papilio* Van Scheepen, as well as development and validation of a “green” GC-MS method for quantitative analysis of Amaryllidaceae alkaloids in plant samples through direct injection of an acidified aqueous solution, avoiding the use of toxic organic solvents. Contributions of a hybrid nature, combining characteristics of both fundamental and applied science, include the creation of a library of EIMS spectra of non-silylated and silylated Amaryllidaceae alkaloids through parallel GC-MS analysis of standard substances and alkaloid fractions from various Amaryllidaceae species, as well as a detailed metabolic analysis of a pharmacologically active alkaloid fraction from *Narcissus* cv. Hawera with established antidepressant and anxiolytic activity.

The results of Rumen Denev's dissertation have been presented in three scientific publications in two journals with a focus on chromatographic and mass-spectral analytical techniques, indexed and referenced in the global databases of Scopus and Web of Science (WOS). All publications have an impact factor (IF) and the following indicators: Publication 1 (2021) – Q2 (Scopus), IF = 2.12 (2021); SJR 0.458 (2021); Publication 2 (2025) – Q3 (Scopus), IF = 1.66 (2024); SJR 0.362 (2025); Publication 3 (2026) – Q2 (Scopus), IF = 4.00 (2025/2026); SJR 0.668 (2025). One of the publications has 5 citations to date.

The Ph.D. candidate's thesis contains significant scientific findings that constitute an original contribution to the field and satisfy all the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria, the Regulations for its implementation, and the corresponding Regulations of IBER-BAS. The doctoral candidate possesses a profound theoretical grounding, applying sophisticated methodologies with precision. His capacity for critical data interpretation, combined with original scholarly conceptualizations, marks them as a highly capable researcher. Based on all the above, I vote with conviction in favour of awarding Rumen Velinov Denev the educational and scientific degree “DOCTOR” in the higher education area “4. Natural Sciences, Mathematics and Informatics”, professional field “4.3. Biological Sciences”, scientific specialty 01.06.03 “Botany”.

09.07.2026

Signature:

/Assoc. Prof. A. Gavrilova, PhD/