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REVIEW

by Dr Antoaneta Borissova Trendafilova,
professor in the Institute of Organic Chemistry with Centre of Phytochemistry – BAS,
a member of the Scientific Jury in the procedure for the acquisition of the educational and scientific degree "doctor" according
order РД 42/02.05.2025 г. of the Director of IBER-BAS

regarding dissertation for the acquisition of the educational and scientific degree "doctor" in higher education field: 4. Natural sciences, mathematics and informatics, professional field: 4.3. Biological Sciences, PhD Program: Botany

Author: **Gabriela Ivanova Haist**
Title: **Study of galantamine biosynthesis in *Hippeastrum papilio* (Ravenna) van Scheepen**
Research supervisor: Prof. Dr. Strahil Berkov- Institute of Biodiversity and Ecosystem Research – BAS

1. General presentation of the procedure and the PhD student

The set of materials presented by Gabriela Heist on paper and electronic media is in accordance with the Regulations for the Development of the Academic Staff of IBER - BAS, meets the criteria and includes all necessary documents. The PhD student has submitted a report on the accumulated credits, as follows: implementation of the educational program - 180 points (mandatory minimum 130 points), approbation of the implementation of the scientific program - 64 points (mandatory minimum 40 points), published scientific results - 90 points (mandatory minimum 80 points). The total number of credits for the period of doctoral studies (334 points) significantly exceeds the mandatory minimum of 250 points. The PhD student has also attached 4 publications on the topic of the dissertation, a list of noted citations of scientific works included in the dissertation, a list of participation in scientific events and the relevant evidentiary material, as well as a report on the contributions of the dissertation.

2. Brief biographical data of the PhD candidate

Gabriela Heist graduated from the Bachelor's program "Molecular Biology" (2010) and the Master's program "Molecular Biology-Biochemistry" (2013) at the Faculty of Biology of Sofia University "St. Kl. Ohridski". In the period 2021-2013, she completed a Erasmus internship at

the Faculty of Pharmacy of the University of Barcelona, where she became familiar with and mastered techniques such as GC-MS and NMR and working with a database. She was enrolled as a full-time PhD student at IBER-BAS, scientific specialty "Botany" in September 2019. After a two-year break, she was reinstated in full-time doctoral studies in April 2021. Gabriela Heist was discharged with the right to defend her thesis in December 2023.

3. General characteristics of the dissertation work

The dissertation, written on 93 pages, is structured as follows: Introduction (1 page), Literature review (16 pages), Goals and objectives (1 page), Materials and Methods, Results and Discussion, presented through copies of publications on the topic of the dissertation, according to Art. 9 of the Regulations on the conditions and procedure for acquiring scientific degrees and for occupying academic positions in IBER-BAS" (43 pages), conclusion (1 page), main conclusions (2 pages), declaration of originality and reliability (1 page), contributions (1 page) and a 20-page list of literary sources with 173 titles. The presented abstract (30 pages) is prepared according to the requirements and contains the main information from the research included in the dissertation. There is a summary of the dissertation in English (2 pages). No plagiarism is established according to the provided report.

4. Literary awareness and theoretical preparation of the candidate

A total of 173 sources are cited in the dissertation, a significant part of which are from the last 5 years. The literature review covers several areas that correspond to the formulated goals and objectives of the dissertation and includes detailed information about the representatives of the Amaryllidaceae family, the general characteristics of the genus *Hippeastrum* and *Hippeastrum papilio* (Ravenna) van Scheepen, the structure, biosynthesis, biological activity of amaryllis alkaloids, as well as the factors influencing the biosynthesis process of amaryllis alkaloids (ploidy, organ-specific biosynthesis and accumulation of alkaloids, macroelements, plant age, dynamics of alkaloid accumulation and elicitation).

The PhD candidate has mastered the specific terminology, is well versed in the issues underlying the dissertation work, and emphasizes all the main aspects of the research conducted. The presented material demonstrates the candidate's good literary awareness and theoretical preparation.

5. Relevance of the topic and appropriateness of the set goals and objectives

Alzheimer's disease is a chronic, progressive, neurodegenerative disorder affecting the brain and related functions, such as memory, thinking and behavior. According to WHO data,

this type of dementia affects more than 55 million people worldwide, with approximately 10 million new cases being diagnosed each year. The so-called acetylcholinesterase inhibitors are used for the treatment of Alzheimer's disease. One of them is the amaryllis alkaloid galantamine, approved by the US Food and Drug Administration (FDA). Sources of galantamine are *Galanthus nivalis*, *Narcissus pseudonarcissus* cv. Carlton, *Leucojum aestivum*, *Lycoris* sp., as well as *Hippeastrum papilio*, the subject of this dissertation. For industrial production, it is important that the source of biologically active substances is easily accessible and rich in the target components. This can be achieved through a thorough study of the factors influencing the biosynthesis of biologically active substances such as galantamine, as well as the dynamics of biomass accumulation. This is the goal of the dissertation. The tasks are formulated clearly and fully correspond to the topic of the dissertation. The topic is extremely relevant and interesting, both from a scientific and practical point of view.

5. Methodical approach

The study was conducted using a modern and adequate, well-developed methodology that allows achieving the set goal and solving the tasks. For morphological and karyological studies, histochemical analyses, for studying the dynamics of growth and biosynthesis of galantamine, Hippeastrum papilio plants grown in different conditions - *in vitro* and *ex vitro* plants, acclimatized on perlite as hydroponic cultures in a climate chamber or in soil, at different stages of their development, etc. were used. The effect of the elicitor on the accumulation of galantamine in *H. papilio* was studied by adding different concentrations of salicylic acid to the nutrient solution and analyzing the samples at a certain time interval. The cultivation and propagation of the plants was carried out according to established protocols. For phytochemical analyses, extracts from the respective plant material were obtained, which after silylation were analyzed by GC-MS. For the quantitative determination of galantamine and/or hemanthamine, a GC-MS method with different concentrations of galantamine and codeine as an internal standard was applied. GC-MS method was also used for the analysis of phenolic compounds obtained after hydrolysis and silylation. Statistical methods were used for processing and interpreting the obtained results.

6. Significance and persuasiveness of results, interpretations and conclusions

The results obtained are described, analyzed and interpreted in detail in the attached publications. Based on the analysis of the obtained results and the processing of the data with statistical methods, the most suitable conditions for the biosynthesis and accumulation of

galantamine and hemantamine in hydroponically grown *Hippeastrum papilio* have been established. The most significant results can be mentioned as:

-the positive influence of polyploidy on alkaloid biosynthesis, while in the accumulation of biomass this effect is negative;

-establishment of the organ and cellular localization of alkaloids in *Hippeastrum papilio*, namely the accumulation of galantamine and hemantamine mainly in the bulbs - especially in their central part (53-61%), while the detection of alkaloids in the conducting bundles and other tissues indicates that active transport of these important secondary metabolites occurs between the different organs;

- monitoring the dynamics of alkaloids and biomass accumulation in the different organs of *Hippeastrum papilio* during a vegetative period and the effect of adding nitrogen, potassium and calcium to nutrient solutions of hydroponically grown plants.

- establishing that the amount of galantamine per sq. m. of annual *Hippeastrum papilio* plants grown as hydroponic cultures is comparable to that for biennial plants.

- increasing the biosynthesis of galantamine in *Hippeastrum papilio* upon elicitation with different concentrations of salicylic acid.

The significance of the established dependencies in the study of the process of biosynthesis and accumulation of galantamine and hemantamine under the influence of biotic and abiotic factors in different organs and ages of hydroponically grown *H. papilio* plants is undeniable. The conclusions are precisely and clearly formulated.

7. Critical notes on the dissertation

I have no significant critical remarks on the dissertation. I would like to make one recommendation to the doctoral student for her future scientific research. The GC-MS technique is not suitable for the study of polar compounds in methanol extracts even after their silylation, because compounds with a higher molecular mass and those that are more difficult to volatile and cannot be registered are missed. HPLC is a more sensitive and reliable method for the analysis of polar compounds, especially phenolic ones. It is not correct to use the term "metabolic profile" when studying extracts after preliminary hydrolysis, since information is obtained about the hydrolyzed products, and not about the real metabolites in the plants. Under the conditions of hydrolysis, other transformations may occur, leading to products (artifacts) that are not contained in the plants.

8. Nature of scientific contributions

The scientific contributions are formulated briefly, clearly and correctly reflect the results obtained by the doctoral student. All scientific contributions are original, as they reflect the results of research on *Hippeastrum papilio*, conducted for the first time, namely the study of the chromosome morphology of diploid plants *H. papilio*, comparative analysis of primary and secondary metabolites in diploid and autopolyploid plants, histological studies to establish the tissue localization of alkaloids, quantitative determination of the content of galantamine and hemantamine in various plant organs and parts of *Hippeastrum papilio* and the influence of nutrient solutions on their accumulation, the influence of elicitors in hydroponic cultures of amaryllis plants to stimulate the biosynthesis of biologically active substances and analysis of the productivity of *Hippeastrum papilio* as a source of galantamine.

The results described in the dissertation provide new information and contribute to expanding knowledge in the field of botany, plant biotechnology and phytochemistry of *Hippeastrum papilio*.

The results obtained also have a scientific-applied nature with potential for practical application, namely the hydroponic cultivation of H. papilio as an alternative for the production of galantamine. Additional studies are needed on plant density (vertical farming), nutrient solution and electrical conductivity, lighting, humidity, etc., to determine the appropriate conditions for the accumulation of biomass and galantamine of economic importance.

I accept the statement of scientific contributions formulated by the doctoral student

9. Assessment of the PhD student's publications and personal contribution

The research on the dissertation work is summarized in 4 publications, of which 3 in journals in quartile Q1 (Planta, Journal of Plant Physiology and Agronomy) and 1 in a journal in quartile Q3 (Reports of the Bulgarian Academy of Sciences), which have been evaluated by reviewers. I believe that the experimental results, as well as their presentation and analysis, are largely the personal work of the doctoral student. This is also confirmed by the fact that Gabriela Heist is the first author in the presented publications. Six citations were noted, which is a confirmation of the relevance of the topic and the interest in the conducted research. The results of the research included in the dissertation work have been presented as poster reports at two international conferences.

CONCLUSION

The dissertation work contains scientific and applied scientific results that represent an original contribution to science and meet all the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria (LDASRB), the Regulations for the

Implementation of the LDASRB and the Regulations for the Implementation of the LDASRB of the Bulgarian Academy of Sciences. The presented materials and results fully comply with the specific requirements of the Regulations of IBER -BAS for the Implementation of the LDASRB. The dissertation work shows that the PhD student Gabriela Heist possesses in-depth theoretical knowledge and professional skills in the scientific specialty "Botany" by demonstrating qualities and skills for independent conduct of scientific research.

Due to the above, I confidently give my positive assessment of the conducted research and propose to the esteemed scientific jury to award the educational and scientific degree "doctor" to Gabriela Heist in the field of higher education: 4. Natural Sciences, Mathematics and Informatics, professional field 4.3. Biological Sciences, scientific specialty "Botany".

11.06.2025 г. Reviewer: .....

(Prof. Antoaneta Trendafilova)