OPINION

from

Assoc. Prof. Dr. Radka Zapryanova Vrancheva,

University of Food Technologies – Plovdiv, Department of Analytical Chemistry and Physical Chemistry

on a dissertation for the acquisition of the educational and scientific degree "Doctor"

in the field of higher education: 5. Technical Sciences, professional field 5.11 Biotechnology, scientific specialty: Technology of biologically active substances

Author: Eng. GERGANA SPASOVA KRASTEVA

Topic of the dissertation: "Plant bioengineering systems from *Gardenia jasminoides* Ellis for obtaining active ingredients"

Scientific advisor: Assoc. Prof. Dr. Vasil Georgiev Georgiev

1. Relevance of the problem developed in the dissertation in scientific and applied terms

In the last few years, plant *in vitro* cultures have been accepted as the most promising technology for sustainable production of plant biomass and obtaining valuable biologically active substances with the lowest environmental impact. The dissertation work examined an extremely actual topic in the field of plant biotechnology with a clearly expressed scientific and scientific-applied focus. The influence of the duration of cultivation and the content of basic nutritional components on the biosynthesis of phenolic compounds and the somaclonal variability of differentiated and dedifferentiated *in vitro* systems from the plant *Gardenia jasminoides* Ellis (jasmine gardenia) was studied.

2. Degree of knowledge of the state of the problem and the literature

The dissertation is presented in 9 sections on 170 pages. It is illustrated with 35 figures and 33 tables. The literature review is properly structured and prepared in accordance with the topic being developed. The scientific articles cited in it from recent years support the relevance of the chosed topic. In the literature review, the PhD student has presented extensive information about the botanical, phytochemical characteristics and traditional application of the plant subject to analysis – *G. jasminoides*. The principles of some of the most commonly used methods for analyzing antioxidant activity are discussed in detail. The main types of undifferentiated and differentiated plant *in vitro* systems, their genetic variability and somaclonal variability, as well as the methods for their detection are described. Based on the critical review of the available scientific literature, the PhD student has logically formulated the goal and the resulting 5 main tasks that correspond to the topic of the dissertation.

3. Correspondence of the chosen research methodology with the set goal and objectives of the dissertation work

The section "Materials and Methods" covers 12 pages of the dissertation work, which describes in detail the main materials and methods used to obtain differentiated and dedifferentiated *in vitro* systems from the plant - the object of analysis. For the analysis of the primary and secondary metabolites of jasmine gardenia and *the in vitro* systems obtained from it, modern instrumental methods were used - gas chromatography with mass spectrometry and high-performance liquid chromatography. The section also contains detailed information about the methods used for the analysis of antioxidant activity, the dynamics of growth and development, the optimization of cultivation conditions, as well as molecular genetic methods for determining the occurring genetic changes in the obtained *in vitro* cultures of *G. jasminoides.*

PhD student Krasteva has mastered and used a variety of methods corresponding to the set goal and tasks, which has undoubtedly contributed to upgrading and enriching her knowledge in an educational aspect.

4. Evaluation of the abstract

The submitted abstract is well structured, prepared according to the requirements and summarizes the methodology, main results, conclusions and contributions of the dissertation work.

5. Contributions of the dissertation work

Based on the results obtained, 7 conclusions and a total of 5 scientific and scientific-applied contributions have been formulated. The results obtained contribute to enriching the scientific information regarding the *in vitro* conditions for obtaining differentiated (germination) and dedifferentiated (callus and cell suspension) *in vitro* cultures of *G. jasminoides* Ellis, as well as data on their metabolic profile and antioxidant activity. For the first time, the genetic stability of differentiated and dedifferentiated *in vitro* systems has been studied compared to that of the parent plant. For the first time, the relationships between changes in the metabolic profile and genetic variability during the cultivation of differentiated and dedifferentiated *in vitro* cultures of jasmine gardenia have been proven.

The formulated contributions clearly demonstrate the significance and innovation of the obtained scientific results, as well as the undisputed relevance of the dissertation work, both in scientific and applied aspects.

6. Evaluation of the publications on the dissertation

Four publications have been published on the topic of the dissertation, two of which have an impact factor and two - with an impact rank. In all four publications, the Phd student is the first author, which clearly proves her personal contribution to the development and analysis of the results obtained in this dissertation. The results obtained have been reported at a total of three scientific events in the country and abroad. To date, 136 citations of the publications on the dissertation have been found, which confirms the relevance and significance of the results obtained and the topic of the scientific work.

7. Opinions, recommendations, and comments

The dissertation is written in a good scientific style. The results are presented in a sufficient number of tables and figures. The conclusions logically follow from the research conducted by the candidate.

There are some minor technical errors, such as repetitions in the description of some of the literary sources used in section X. Literature used. The term "*in vitro*" is used both in Bulgarian and Latin language, and I recommend its unification.

The recommendations made and the technical errors discovered nowise decrease the scientific value of the dissertation.

8. Conclusion

The presented dissertation fully meets the requirements of the Low on the Development of the Academic Staff in the Republic of Bulgaria (LDSRB), the Regulations for the Implementation of the LDSRB and the Regulations on the Terms and Procedures for Acquiring Scientific Degrees and Holding Academic Positions at the Stefan Angelov Institute of Microbiology at the Bulgarian Academy of Sciences.

Based on the above, I confidently give my **positive** assessment of the dissertation work and propose to the esteemed members of the scientific jury to award the educational and scientific degree "Doctor**" to Gergana Spasova Krasteva** in the professional field 5.11 Biotechnologies, scientific specialty: Technology of biologically active substances.

Date: 14.11.2025 Jury member:

/Assoc. Prof. Dr. Radka Vrancheva/