

OPINION

by Assoc. Prof. Dr. Yana Gancheva Gocheva, Institute of Microbiology “Stefan Angeloff”, Bulgarian Academy of Sciences (BAS), appointed as a member of the Scientific Jury by Order No. 452/27.04.2026 of the Director of the Institute of Biophysics and Biomedical Engineering, BAS, for the procedure for the defense of the PhD dissertation of Dayana Slavchova Benkova

on a dissertation: “**BIOLOGICAL ACTIVITY OF CHITOSAN-BASED NANOMATERIALS**” for the award of the educational and scientific degree “Doctor” in the field of higher education 4. Natural Sciences, Mathematics and Informatics, professional field 4.3. Biological Sciences, Doctoral Program “*BIOPHYSICS*”.

Author of the dissertation: Dayana Slavchova Benkova, PhD student at the Institute of Biophysics and Biomedical Engineering, BAS.

Dayana Benkova graduated from Sofia University “St. Kliment Ohridski” with a Bachelor's degree in Molecular Biology (2020) and a Master's degree in Biochemistry (2022). She was enrolled as a PhD student on 01.03.2023 and granted the right to defend on 01.08.2024. Pre-defense date: 07.04.2026.

The dissertation comprises 149 pages and includes: Introduction, Literature Review, Aims and Objectives, Materials and Methods, Results, Discussion, Conclusion, and Contributions. It contains 59 figures, 3 tables, and 182 references.

1. Relevance of the problem.

The relevance of the research stems from the growing scientific and practical interest in hybrid nanocomposites as functional materials with broad applications in biomedicine and agriculture. Modern nanotechnology trends focus on developing biocompatible and highly effective nanomaterials capable of controlled biological activity and minimal toxicity. Hybrid systems based on chitosan and polyethylene glycol combined with bioactive metal oxides are particularly important because of their physicochemical and functional properties. Despite numerous studies, the mechanisms governing interactions between these nanomaterials and membrane lipids remain insufficiently understood. Furthermore, increasing resistance of phytopathogens to conventional pesticides highlights the need for environmentally friendly antifungal agents.

2. Knowledge of the Problem and Literature Review

The doctoral candidate demonstrates excellent knowledge of the theoretical foundations and current advances in nanomaterials, membrane biophysics, and antifungal mechanisms. The literature review is comprehensive, current, and well selected. Critical analysis of existing studies and scientific trends is evident, contributing to the originality and focus of the dissertation.

3. Aim, Objectives, Hypotheses, and Methods

The dissertation has a clearly defined objective. The study aims to:

- Elucidate the mechanisms of interaction between chitosan-based and polyethylene glycol-based hybrid nanocomposites and membrane models reproducing lipid phase heterogeneity in eukaryotic cell membranes.

- Investigate the antifungal activity of chitosan-based and hybrid nanocomposites containing ZnO, CuO, and SiO₂ against *Alternaria solani* and *Fusarium solani*.

Six specific scientific tasks were formulated and successfully completed. The conclusions and contributions are well justified and verifiable. The methodology is appropriate and includes physicochemical, biophysical, microbiological, biochemical, and analytical approaches.

4. Visualisation and Presentation of the Results

The results concerning nanomaterial properties, membrane interactions, and antimicrobial activity are clearly presented and well structured. The figures (59) and tables (3) adequately illustrate the experimental data and enhance the quality of the presentation.

5. Discussion of Results and Literature Used

Dayana Benkova demonstrates excellent skills in interpreting and critically analyzing experimental data. Particularly impressive is the in-depth analysis of the relationship between physicochemical characteristics and biological activity, changes in membrane structures, and mechanisms related to oxidative stress generation. The discussion integrates physicochemical, biophysical, biochemical, and microbiological approaches. The discussion is thorough, scientifically sound, and supported by up-to-date literature, highlighting the author's high competence. The PhD candidate successfully fulfills the set scientific task.

6. Contributions of the Dissertation

I accept the stated contributions: four of a fundamental nature and two of a scientific-applied nature.

Fundamental contributions:- A molecular mechanism describing interactions between cationic chitosan-based and PEG-based hybrid nanocomposites and various membrane lipids and lipid phases has been proposed.- A comprehensive biophysical and physicochemical analysis identified critical parameters such as size, surface charge, and morphology affecting interactions with model and biological membranes. - Zeta potential and membrane ordering in homogeneous and heterogeneous model membranes treated with nanocomposites were quantitatively characterized for the first time. - The antifungal activity of chitosan-based hybrid nanocomposites against *Alternaria solani* and *Fusarium solani* was shown to involve oxidative stress induction and significant alterations in fungal membrane lipid organization.

Scientific-applied contributions: - Relationships between physicochemical properties, antifungal activity, membrane damage, and oxidative stress in fungal cells were established, enabling optimization of antifungal efficacy. - A scientific basis was created for the future application of chitosan-based hybrid nanocomposites as effective and environmentally friendly antifungal materials in agriculture.

7. Assessment of the Candidate's Personal Contribution.

The dissertation and accompanying publications clearly demonstrate the author's personal

contribution to the experimental work, analysis, interpretation, and publication of the scientific results.

Dayana Benkova is the first author of one publication and the co-first author of two papers indexed in Scopus. She is also the first author of five scientific communications (three oral presentations and two posters) presented at international and national scientific forums. She has participated in three research projects funded by the Bulgarian National Science Fund.

8. Remarks and Questions.

Remarks: A technical error appears on page 101 in the figure numbering: Figure 54 is followed by Figure 53.

Question: What are the main factors determining the differences in interactions between chitosan-based and polyethylene glycol-based hybrid nanocomposites and the lipid phases of model membranes?

9. Публикувани статии и цитирания.

The main results of the dissertation have been published in three scientific papers (two Q1 and one Q3 journals indexed in *Scopus*). Four citations of publications related to the dissertation topic have been identified. Two poster presentations and three conference reports have also been presented.

The abstract accurately reflects the structure and content of the dissertation.

CONCLUSION:

All formal requirements of the Bulgarian legislation and BAS regulations for awarding the educational and scientific degree “Doctor” have been fulfilled.

The dissertation demonstrates high scientific quality. The author possesses substantial theoretical knowledge, the ability to conduct independent research, interpret results, and demonstrate competence in the field. Based on my critical evaluation of the submitted documents and dissertation, **I give my positive assessment.**

I recommend that the Scientific Jury vote in favor of awarding Dayana Slavchova Benkova the educational and scientific degree “Doctor” in Biological Sciences (*Biophysics*).

Date: 29.06.2026,

Sofia

Prepared by:

Assoc. Prof. Dr. Yana Gocheva