REVIEW

by Prof. Maria Bogomilova Angelova-Dyankova, DSc
of the materials submitted for participation in the competition
for the academic position of "**professor**"
at Plovdiv University "Paisiy Hilendarski" in the field of higher education 4. Natural Sciences, Mathematics and Informatics
professional field 4.3. Biological Sciences (Phycology)

In the competition for 'professor' announced in the State Gazette, no. 98 of 19.11.2024 and on the internet page of Plovdiv University 'Paisii Hilendarski' for the needs of the Department of Botany and Biological Education at the Faculty of Biology participates Assoc. Prof. Dr. Ivanka Ivanova Teneva-Dzhambazova from the Department of Botany and Biological Education at the Faculty of Biology of PU.

1. GENERAL PRESENTATION OF THE MATERIALS

In accordance with Order No. PD-22-439 of 18.02.2025 issued by the Rector of Plovdiv University "Paisii Hilendarski" (PU), I have been designated as a member of the scientific jury for a competition pertaining to the academic position of "professor" at PU within the field of higher education 4. Natural Sciences, Mathematics and Informatics, professional field 4.3. Biological Sciences (Phycology), announced for the needs of the Department of "Botany and Biological Education" at the Faculty of Biology

Documents have been received for participation in the announced competition from one candidate, Assoc. Prof. Dr. Ivanka Ivanova Teneva-Dzambazova from the Department of "Botany and Biological Education" at the Faculty of Biology at Plovdiv University "Paisii Hilendarski". The set of materials submitted by the candidate on electronic media is in accordance with the Regulations for the Development of the Academic Staff of PU.

For the competition, the candidate Assoc. Prof. Dr. Ivanka Teneva-Dzhambazova has applied a total of 18 scientific articles, 2 book chapters, 4 textbooks and 4 teaching aids. All submitted scientific articles are in publications, referenced and indexed in Web of Science and Scopus. They are in renowned and specialized scientific journals, included in the relevant quartiles such as: International Journal of Molecular Sciences Q1 IF 5.6; Algal Research Q1 IF 5.276; Plants Q1, IF 4.5 (2 issues); Heliyon Q1 IF 4.0; Chemosphere Q2 IF 5.778; Engineering in Life Sciences Q2 IF 3.165; Applied Sciences Q2 IF 2.7; Advances in Respiratory Medicine Q2 IF 1.8; Biodiversity Data Journal Q2 IF 1.3 (2 issues); Phycological Research Q2 IF 1.342, etc. All articles are in international publications and are printed in English. The two presented book chapters (one in Bulgarian and the other in English) are in the Anniversary Collection of the University of Plovdiv. The textbooks and teaching aids have been published in Bulgarian. The candidate's results have received wide responses from the international scientific community. The articles submitted for the competition have been cited 199 times, and those outside the competition 350 times and form an h-index of 12 (Scopus, Web of Science). The total Impact Factor of the articles included in the review is 42.002.

The presented scientific production is outside the dissertation work for obtaining the ONS "doctor" and the competition for associate professor, it is in accordance with the announced competition, therefore I accept for review all submitted works, teaching aids, and research projects.

2. BRIEF BIOGRAPHICAL DATA OF THE CANDIDATE

The scientific biography of Assoc. Prof. Teneva-Dzhambazova begins at the High school "Prof. Dr. Asen Zlatarov" by teaching foreign languages, in the city of Haskovo, where her excellent knowledge of German marked the beginning of a purposeful academic career. She graduated from the Plovdiv University "Paisiy Hilendarski" in 1995 with a master's degree in "Biology and Chemistry" and qualified as a "Teacher of Biology and Chemistry". In 2007, Assoc. Prof. Teneva-Dzhambazova defended her educational and scientific degree "doctor" in the scientific specialty "Botany" at the same university on the topic "Taxonomy, phylogeny and toxic potential of some filamentous blue-green algae (*Cyanoprokaryota*)", after which she continued her studies as a Postdoc at the Medical Inflammation Research Unit, Lund University, Lund, Sweden.

After graduating, the candidate started working as a chemistry teacher at the High school "Dr. Asen Zlatarov", Haskovo, and successively passed through the positions of an expert and senior expert in chemistry at the Regional Inspectorate of the Ministry of Education, Science and Technology, Haskovo, Fellow-scientist UFZ - Centre for Environmental Research, Leipzig, Germany and EU Postdoc – Projekt assistant (Marie Curie Experienced Researcher) at the University of Lund, Sweden. From 2007 to 2013, Dr. Teneva-Dzambazova was an assistant and chief assistant in Botany at the Faculty of Botany of the "Paisiy Hilendarski" University of Plovdiv. Then (2013) she won a competition for associate professor at the same university.

Along with these data, it should be noted her excellent preparation in German and English, her skills in working with computers (Windows, MacOS, MS Word, MS Excel, MS PowerPoint, Adobe, Keynote, SigmaPlot, StatView, Canvas, UNICORN, GeneAnalysis, Sequence Analysis, PHYLIP, DNAStar, Internet) and with modern research equipment (FACS, MegaBACE, ImmunoScan, HPLC – Äkta Explorer, ELISA-readers, electrophoresis, microtomes, etc.).

Assoc. Prof. Teneva-Dzhambazova applies everything mentioned above - education, expertise, and career growth in her teaching and scientific activities. She is closely connected to botany as her main professional field, with an emphasis on phycology. The candidate's entire professional career is related to the topic of the announced competition, while at the same time reflecting current and promising directions in this field.

Assoc. Prof. Teneva-Dzhambazova has also significant administrative experience. She is a member of the Faculty Council of the Faculty of Biology at the University of Plovdiv and of the Control Council at the University of Plovdiv. She has participated in the organization of an international scientific forum, as well as in a number of committees, including the Ethics Committee of the Faculty of Biology, committees for self-assessment and accreditation of professional areas and doctoral programs at the Faculty of Biology, etc. It should also be noted her commitment as a member of the editorial board of the Journal of BioScience and Biotechnology and a member of the board of reviewers of the journal Microorganisms (Q2, IF

4.1). Her activity as a guest editor of a special issue in the Journal of Microorganisms on the topic "Genomics and Metabolomics of Cyanobacteria: Applications" is a recognition of her professionalism and corresponds to the topic of the competition.

3. GENERAL CHARACTERISTICS OF THE CANDIDATE'S ACTIVITY

Compliance with the requirements of the Law on the Development of Academic Staff in the Republic of Bulgaria (LDASRB)

- **Indicator A** - successfully defended dissertation for the National Scientific Committee and awarded the degree of "doctor", **50** point**s**
- **Indicator C4** - 6 scientific articles (6 of which are with IF) in publications referenced in Web of Science and Scopus (Q1 -2; Q2 - 4) = **130 points**, with a required score of 100 points
- I**ndicator D total - 234 points** with a required score of 200 points
  - ✓ Indicator D7 - 12 scientific articles (Q1 - 4; Q2 -1; Q3 - 2; Q4 - 2 and 3, referenced in Web of Science and Scopus without quartile) = 204 points
  - ✓ Indicator D8 - 2 book chapters x 15 p = 30 points
- **Indicator E – citations (Scopus) = 398** points with a required 100 points
- **Indicator F total – 380.9 points** with a required 150 points
  - ✓ Indicator F13 – co-supervisor of 2 successfully defended dissertations = 50 points.
  - ✓ Indicator F14 – 3 national projects x 10 points = 30 points
  - ✓ Indicator F15 – 5 international projects x 20 points = 100 points
  - ✓ Indicator F17 – 1 Leadership of the Bulgarian team in an international scientific project = 50 p
  - ✓ Indicator F18 - Funds raised for projects led by the candidate = 67 points
  - ✓ Indicator F19 – 3 textbooks in co-authorship x 5.7 points and 1 independent textbook x 40 points = 57.1 points.
  - ✓ Indicator F20 - 4 co-authored textbooks x 6.7 = 26.8 points.

Compliance with additional requirements for holding the academic position of "Professor"

- **Author and co-author of textbooks and teaching aids** in the field of the competition - required 1 textbook
  - ✓ 3 textbooks in co-authorship and 1 textbook independent;
  - ✓ 4 textbooks in co-authorship;
- Supervision of successfully defended doctoral students – 2 successfully defended diploma students with required 2;
- Teaching experience – 16.8 years with required 10;
- Participation in scientific projects
  - ✓ leader in 1 international project
  - ✓ leader in 3 national projects
  - ✓ participant in 3 national projects
  - ✓ participant in 2 international projects
- ✓ **Organizational and administrative experience (for the last 10 years) in at least one**
  - ✓ Participation in committees – 6 committees

- ✓ Participation in preparation and accreditation procedures – 5 pcs.
- ✓ Participation in the preparation and updating of educational documentation – 12 programs of the Paisiy Hilendarsky University and its branches in Kardzhali and Smolyan.
- ✓ Participation in editorial boards – 3
  Member of the Editorial Board of the Journal of BioScience and Biotechnology
  Member of the Board of Reviewers of the Journal of Microorganisms, MPDI
  Guest editor of a special edition of the Journal of Microorganisms
- ✓ Participation in the organization and conduct of international forums – 1 issue.
- ✓ Administrative activities – Member of the Faculty Council of the Faculty of Biology at the PAISIY and Member of the Control Board at the PAISIY.

As can be seen from the reference, Assoc. Prof. Dr. Ivanka Teneva-Dzhambazova meets and exceeds the requirements of the ZRASRB, as instead of the required 600 points, she presents evidence for **1192.9** points. In addition, the candidate also meets the additional criteria of the Faculty of Biology of the University of Plovdiv.

4. EVALUATION OF TEACHING AND TEACHING ACTIVITIES

Teaching and teaching work is one of the main activities of the candidate and is entirely in the field of competition. It can be analyzed in several aspects – as a teacher, as a trainer of skills in laboratory practice, as a compiler of curricula and textbooks, and as a mentor of young staff. Assoc. Prof. Teneva-Dzhambazova has been a teacher for more than 16 years. She is a lecturer and leads exercises/seminars in BP /full-time and part-time education/ in Phycology, Bioresources, Medical Toxicology, Mycology, Applied Mycology, Plant Systematics, Phytopagology, and Phyto- and mycotoxins; leads field classes in Plant Systematics. Her teaching activities at the MP /full-time and part-time studies/ include the disciplines of Phyto- and mycotoxins in foods, Toxicology, Algology, Bioresources, Mycology, Phytotoxicology and Toxicology of drugs.

It should also be noted the teaching activity of the candidate in the BP in:

- ➢ the "Lyuben Karavelov" branch of the PU in the city of Kardzhali - lectures and exercises in Mycology and Plant and Fungal Resources.
- ➢ the "Paisiy Hilendarski" branch of the PU in the city of Smolyan - lectures in Bioresources

In numerical terms, the average annual workload of Assoc. Prof. Teneva-Dzhambazova for the last 5 years is 606 hours for BP, 147 hours for MP, 75 hours for the branch in Kardzhali and 15 hours for the branch in Smolyan.

Assoc. Prof. Teneva-Dzhambazova's activity in this section also includes the development of 17 curricula in the Bachelor's and Master's degree programs for students in the specialties Microbiology and Virology, Bioengineering, Pharmaceutical Biotechnologies, Biology and Natural Resources Management, Biology with Ecotourism, Biodiversity, Ecology and Conservation, Medicinal and Essential Plants (for non-biologists), Microbiological Control and Food Safety, Reproductive Biology (for non-biologists), Medical Biology and Biodiagnostics.

Simultaneously with the noted active teaching activity, Dr. Teneva-Dzhambazova is very intensively involved in the training of young professionals. After acquiring the academic position of "associate professor", she was the supervisor of 3 graduate students and co-supervisor of 2 successfully defended doctoral students. The topics of these works are in the field of phycology and present new information about objects from different regions of Bulgaria. The most important thing is that they are in accordance with the announced competition for "professor".

Here I would like to note the contributions of the candidate related to the 4 textbooks and 4 teaching aids presented. All of them are very useful for expanding the knowledge of students in universities or teachers in secondary schools. Together with this, they are proof of the candidate's responsibility as a teacher.

1. Assoc. Prof. Teneva-Dzhambazova is the author of a textbook on Phycology, in which 230 pages present historical data on the discipline, as well as modern aspects of its development. In a logical sequence, the necessary knowledge about the phylogeny and evolution of algae (modern research methods and taxonomy), their main characteristics, metabolism, mechanisms of CO_2 accumulation, and biosynthesis of valuable biologically active substances is included. Special attention was paid to important organelles – chloroplasts, and photoreceptors, to the ecology and symbiosis of algae, as well as to their biotechnological aspects. The sections on cyanobacteria, cyanophages, and pathogenic algae, with reference to medical phycology, add essential scientific information.

2. The candidate is a co-author of Pharmaceutical Botany - Part 1 (first and second revised editions), which examines in detail the sections on cytology, histology, and organography of plants and their relationship with pharmaceutical science.

3. The fourth textbook - Pharmaceutical Botany - Part 2 is a logical continuation of the above two (Pharmaceutical Botany - Part 1) and contains very well-selected and presented information on the systematics of lower and higher plants, supported by informative figures. The section "Biodiversity and Bioresources" deserves attention. I also appreciate the very useful work on the included Alphabetical Indexes.

5. EVALUATION OF THE CANDIDATE'S RESEARCH ACTIVITY

The scientific activity of Assoc. Prof. Dr. Ivanka Teneva-Dzambazova concentrated entirely on the subject of the announced competition. I assess it as topical, implemented at a modern methodological level, and for the most part with a perspective for application. The main place in her works is occupied by studies in the field of phycology - biodiversity, proving new species, toxicological assessment of plankton and cyanobacteria, their allelopathic impact, and biological activity. Her scientific interests also include questions regarding some important characteristics of higher plants (phytochemical, biological, and toxicological). In my opinion, the candidate's scientific works, which are presented for the competition, can be grouped and analyzed into 6 sections.

5.1. Study of the taxonomic status of controversial cyanobacterial species by applying molecular biological methods and developing new taxonomic criteria

In recent years, the classification of cyanobacteria (species, genera, families, orders) has undergone extensive restructuring and revision using phylogenetic analyses based on data from

molecular genetic, morphological, ultrastructural, biochemical, and ecological approaches. Studies are published in the scientific literature that provide new information about the taxonomic status of controversial species. This trend also raises expectations for additional changes in the future. In this context, the activity of Assoc. Prof. Teneva-Dzhambazova is included in the most current areas of phycology and fully corresponds to the topic of the competition [articles B4-1, B4-4, B4-6, G7-3, G7-8, G7-9, G7-11, and G7-12]. It should also be noted that the correct determination of the taxonomic position and identification of cyanobacteria is essential for determining their biological activity, their biotechnological application, or their role in the management and monitoring of water resources.

In line with the modern taxonomy of the phylum *Cyanobacteria*, the candidate uses a polyphasic approach that combines the values of molecular genetic markers (rRNA genes, protein-coding conservative genes, phylogenetic criteria) with those of cytomorphological, ultrastructural, biochemical, and ecophysiological characteristics. Through polyphasic taxonomy, the taxonomic position of species from the genera *Microcoleus* and *Phormidium* has been revised in comparison with type species from the same genera and species of the autotrophic picoplankton in Atanasovsko Lake have been identified. It is proposed to restore the taxonomic status of the species *Microcoleus autumnalis* to the genus *Phormidium* under the name *Phormidium autumnale* (Agardh) Trevisan ex Gomont, 1892. Based on comparative genetic analysis, similarities and differences between representatives of the genus *Phormidium* have been established. An important part of the materials in this section is related to the development of new taxonomic criteria, such as genes responsible for secondary metabolites, pigment synthesis, the presence of the proteins CP43 and CP47, etc. Of interest is also the characterization of Psb27 (a small lipoprotein, 11 kDa) as a candidate for a new molecular marker for genus and species determination of cyanobacteria. In addition, the results show that the amino acid sequence of the outer membrane efflux protein (OMEP) is a more suitable marker than 16S rDNA for determining phylogenetic relationships within the genus and species level. The maximum likelihood (ML) method for phylogenetic analysis based on 16S DNA sequences in combination with morphological analysis and light microscopy identified a strain of *Anabaenopsis elenkinii*, isolated from Lake Vaya during a cyanobacterial bloom.

In my opinion, articles G7-7 and G7-10 also belong to this section, in which, based on comparative genomic analysis of strains from several genera, the role of the genes responsible for motility and chemotaxis, as well as a large part of those for secondary metabolites, is proven for correct taxonomic identification.

The theoretical contributions made can be formulated as follows:

1. The importance of the polyphasic approach for resolving taxonomic cases in cyanobacteria has been proven.

2. Based on polyphasic taxonomy, the affiliation of cyanobacteria of the species *Phormidium autumnale* to the genus *Phormidium* has been justified. Evidence has been presented that its transfer to the genus *Microcoleus* is incorrect and that the previous taxonomic status should be restored - genus *Phormidium*, species *Phormidium autumnale* (Agardh) Trevisan ex Gomont, 1892.

3. The polyphasic approach has been used to establish the species composition of autotrophic picoplankton in natural conditions - the Northern Salt Pan of Atanasovsko Lake and the phytoplankton in Lake Vaya.

4. For the first time, it has been proven that the outer membrane efflux protein (OMEP) Psb27 and the photosystem II proteins CP43 and CP47 are suitable molecular genetic markers for phylogenetic analyses in cyanobacteria at the genus and species levels.

5. Based on metabolomic analysis, 39 compounds have been selected and proposed as biochemical markers for the first time, which can serve to distinguish the cyanobacterial species *Phormidium autumnale* and *Microcoleus vaginatus*.

6. Based on an in-depth analysis at the biochemical level, the importance of the pigments phycocyanin, phycoerythrin, allophycocyanin, and phycoerythrobilin as additional biochemical markers for distinguishing strains at the genus and supergeneric levels has been proven, which contributes to solving taxonomic cases in cyanobacteria.

5.2. Clarification of the structure and phytoplankton in some Bulgarian dams, its toxicological assessment and ecological status

The rapid development of cyanobacteria and their escalating blooms have become a key issue in ecological analyses. Cyanobacteria are found in almost every terrestrial and aquatic environment. They are an important component of many biological monitoring programs for assessing water quality. This is also valid for small water bodies, such as lakes and dams. The dominance of cyanobacteria leads not only to an impoverishment of the structure and limitation of the growth of other inhabitants but also to limitation of light penetration into the water, the disappearance of macrophytes or very high biomass of dominant carp fish that feed on plants and especially zooplankton. These problems are included in the work of Assoc. Prof. Teneva-Dzambazova [articles B4-4, G7-3, G7-8, G8.1, G8.2]. Studies on the state of phytoplankton in Lake Vaya and the dams "Studen Kladenets", "Krushovitsa", "Vulchovets" and "Enitsa" are presented. The assessment of the ecological state of Lake Vaya shows that it is eutrophic with an ecological state from moderate (Vaya-east) to poor (Vaya-west). A change in the functional structure of the phytoplankton community and abundant seasonal "bloom" of cyanobacterial species, producers of toxins, have been proven. In this aspect, the results for the "Studen Kladenets" dam are also relevant, showing that the phytoplankton water area forms two zones - oligotrophic, with signs of mesotrophy and strong eutrophication with prolonged algal bloom. Conclusions have been drawn about the causes and continuous monitoring of wastewater treatment and the efficiency of treatment plants is recommended. Studies on the dams in the Pleven region (Northern Bulgaria) are of high scientific and applied value. The dominant species and those that produce toxins have been identified, and the bloom concentrations have been determined. The composition of the toxins for each studied site has been proven using modern biochemical methods.

<u>This section outlines the following theoretical contributions:</u>

1. A complete ecological assessment of the current state of Lake Vaya (western and eastern parts of the lake) is proposed by applying the HPLI (Hungarian Lake Phytoplankton Index) method. An accelerated bloom of cyanobacteria has been established and their impact on

macrophytes has been studied. The need for continuous monitoring of the composition of cyanobacteria, bloom, and the presence of cyanotoxins in this water basin has been substantiated.

2. New information has been obtained on the species composition and abundance of phytoplankton in the Studen Kladenets Reservoir. 30 species belonging to six divisions - Cyanoprokaryota, Chlorophyta, Zygnemaphyta, Chrysophyta, Euglenophyta, and Bacillariophyta have been identified. The divisions Chlorophyta and Bacillariophyta are the most abundant.

3. The *Picochlorum oklahomense*/*Synechococcus* sp. community has been proven for the first time in the composition of autotrophic picoplankton (APP) of a European coastal lagoon. Through a polyphasic approach and statistical analyses, the leading abiotic factors providing an ecological optimum for the growth of APP were determined - salinity within 30 and 59‰ and the average summer temperature above 24°C.

4. The toxic potential of the dominant cyanobacterial species in Lake Vaya was assessed. The concentrations of the cyanotoxins microcystin, cylindrospermopsin, and saxitoxin were determined in both parts of the lake. The presence of cylindrospermopsin in a Bulgarian reservoir is reported for the first time.

5. For the first time, a strain of the species *Anabaenopsis elenkinii* has been proven as a producer of cyanotoxins (microcystins, cylindrospermopsin and saxitoxins). A potential danger of "cyanobacterial blooms" in which *Anabaenopsis elenkinii* is the dominant species has been identified. A cytotoxic effect on HT-29 tumor cells was demonstrated, depending on the concentration and time of exposure.

6. A toxicological analysis was performed for the presence of cyanotoxins in three dams in the Pleven region (Northern Bulgaria), the Krushovitsa, Valchovets, and Enitsa dams. Cyanobacteria of the species *Planktothrix agardhii, Anabaena spiroides*, and *Aphanizomenon flos-aquae*, repeatedly reported as producers of hepato- and neurotoxins are dominant in the studied water bodies with bloom concentrations from 1.03 mg/L to 10.5 mg/L.

7. The presence of microcystins/nodularins was reported in the water samples from the Enitsa and Krushovitsa dams, as well as saxitoxins in the sample from the Valchovets dam.

5.3. Study of the allelopathic effects of cyanotoxins produced by cyanobacteria on common algal species

Allelopathy, as a part of chemically-mediated communication, is an important biological process in many aquatic autotrophic organisms. Cyanobacteria are capable of producing and secreting compounds that exhibit allelopathic activity. Such compounds have various ecological roles, namely in algal succession and bloom acceleration, direct competition for resources, competitive response under adverse conditions, intraspecific communication, etc. Although the problems of allelopathy are of increasing interest in the field of phycology, there are a number of unresolved questions regarding the mechanisms of action. It is in this niche that the candidate is looking for the effect of the cyanotoxins microcystin-LR (MC-LR) and cylindrospermopsin (CYL) on 3 species of green algae *Chlamydomonas asymmetrica*, *Scenedesmus obtusiusculus* and *Dunaliella salina*, differing in location, motility and environmental requirements [B4-2]. The impact of the toxins on cell density, growth, development, and morphology was studied

depending on the exposure time. The results outline the role of the used cyanotoxins on ATP production and photosynthetic activity.

The following theoretical contributions are outlined:

1. New information has been obtained on the allelopathic effect of the cyanotoxins microcystin and cylindrospermopsin on the development of green algae of different species. A species-dependent effect has been proven.

2. Data are presented on the multi-target mechanism of action of these toxins through inhibition of growth and motility, as well as changes in morphology, metabolism, and photosynthetic activity.

5.4. Study of the biological activity of representatives of the phylum *Cyanobacteria*

The search for new therapeutic agents of natural origin is one of the most relevant and rapidly developing areas of science. Cyanobacteria find their important place in these studies due to the wide spectrum of biologically active metabolites synthesized by them. However, a large part of the described species has not been studied in this aspect. Section 5.4. includes articles that aim to evaluate the biological activity of previously unstudied species [articles B4-3, B4-5, B4-6, D7-6]. They are distinguished by a multidisciplinary nature, modern methodological level, and scientific style. The object of development is cyanobacteria of the species *Anabaena laxa*, *Oscillatoria limosa* and *Phormidesmis molle*, producers of polysaccharides and secondary metabolites. A detailed physicochemical characterization of biomass extracts of model cyanobacteria has been made. Modern methods have proven the content of polysaccharides (PS), phenolic compounds, fatty acids, chlorophylls, organic acids, terpenes, carotenoids, etc., as well as their biological activity. In the same context, species from the genera *Phormidium, Leptolyngbya, Microcoleus, Oscillatoria, Phormidesmis, Tolypothrix, Fisherella*, etc. have been studied. Based on modern biological and physicochemical methods, the structure of the active metabolites, the spectrum of their biological activity, and the mechanism of their action as immunomodulators, antitumor agents, and antioxidants have been established. Data on the relationship between structure and properties have been obtained.

The following theoretical contributions can be attributed to this section:

1. The chemical composition of extracts from *Anabaena laxa, Oscillatoria limosa,* and *Phormidesmis molle* has been established and their broad spectrum of biological activity (immunomodulatory, antitumor, and antioxidant) has been proven.

2. The biological activity of extracts from cyanobacteria belonging to poorly studied genera has been proven.

3. A new approach for rapid screening of immunomodulatory properties of cyanobacterial extracts has been developed.

4. New information has been obtained about the active components of extracts from the *Phormidium papyraceum* strain, which have a clearly expressed modulatory effect on the main types of immune cells. Their activity against Gram+ and Gram- bacteria is also of interest.

5. A characterization of the chemical profile of the non-polar fraction of *P. papyraceum* extract with potential for future developments has been presented.

6. For the first time, the antioxidant activity and toxic potential of extracts from *Fischerella major* have been proven, which creates a perspective for further research.

7. New knowledge has been obtained about the biological activity of a strain of the species *Fischerella major*. It has been established that 45 components of the extract exhibit a wide spectrum of biological activity.

8. It has been established that the used strain of the species *Fischerella major* is a producer of cyanotoxins (microcystins and saxitoxins), which adds to the ecological problems of its spread.

5.5. Study of the phytochemical, biological, and toxicological characteristics of higher plants

The development of new products from plants related to human health is a direction that has been developing extremely intensively in the last 10 years. Its relevance and prospects are undeniable. A large part of modern drugs, including antitumor preparations, are obtained on the basis of plant extracts. This section includes the candidate's activity in this direction. The presented articles are a continuation of the previous section with a change of the object from cyanobacteria to plants [articles G7-1, G7-4, G7-5]. I assess these developments as being carried out at a high scientific level, with precise experiments and an academic style of analysis. The studies included endemic species known for their medicinal properties - *Marrubium friwaldskyanum* and *Betonica bulgarica*. Extracts from aerial parts of *M. friwaldskyanum* exhibit antitumor activity against the HT-29 colon adenocarcinoma cell line, the HeLa cervical adenoma cell line, and the A549 lung carcinoma cell line. Reduced HT-29 tumor spheroid growth and viability were found. The extracts demonstrated antimicrobial activity against *Escherichia coli* and *Bacillus cereus*, as well as an immunomodulatory effect against specific leukocyte populations.

The antitumor effect of *Betonica bulgarica* inflorescence extract against human cervical adenocarcinoma cells has been studied in great detail. Interestingly, the same extract does not exhibit cytotoxicity against non-tumor human cells and various types of mouse cells, which outlines a good prospect for further studies. Its immunomodulatory activity in a situation of increased lymphocyte levels should also be added here.

Studies on the antitumor activity of commercial preparations containing xylooligosaccharides (XOS) have been carried out at a high methodological level. Despite everything known about this preparation, the article provides new data on the mechanism of action. I would like to emphasize that the design of the study is characterized by a logical connection between the experiments, which provides new data on the mechanism of action of XOS. Given the known causality between oxidative stress and carcinogenesis, studies on the effect of XOS on the redox status of cancer cells, the GSH/GSSG ratio and the activity of some antioxidant enzymes prove the participation of the glutathione antioxidant system in the strategy for cancer treatment. This, in turn, defines XOS as a promising antitumor agent. The results obtained are a serious basis for further developments.

The following important contributions have been made in this section:

1. For the first time, a complex biological effect of extracts of *Marrubium friwaldskyanum* (stems, leave, and flowers) has been established with the potential for the development of valuable pharmaceutical products.

2. New information has been obtained on the biological activity of extracts from *Betonica bulgarica* inflorescences. The proven antitumor activity and their strong cytotoxic effect on tumor cells outline the obtained extracts as potential objects of further development.

3. New evidence has been obtained on the mechanism of the antitumor action of a preparation of xylooligosaccharides, which identifies the glutathione antioxidant system as an important target of the therapeutic effect.

5.6. Studying the possibility of creating a vaccine against pollen allergens from grass group 1

The separate section, which at first glance is a new direction in the activities of Assoc. Prof. Teneva-Dzhambazova is actually in unison with the publication activity presented above. Here the emphasis is on developing a protective strategy against the action of plant allergens. This type of allergy is a global health problem, for which a number of pharmaceutical preparations have been created. The shortcomings of passive immunotherapy, however, lead to the need to develop new active forms of immunotherapeutic strategies, such as vaccines. They prepare the immune system for action against its own IgE antibody, which is neutralized and the allergic disease is terminated. The study presented by the candidate is focused on the development of an effective epitope-based vaccine against grass group 1 allergens [article G7-2]. The target sequence of a suitable epitope was subjected to immunoinformatics analyses to predict antigenic T-cell and B-cell epitopes. By combining various modern methods such as population coverage analysis, validated by molecular docking analysis and affinity determination, CD8+ and CD4+ T-cell epitopes with very high binding affinity to major histocompatibility complex class I and class II molecules were created. Based on the results of the in silico and ex vivo analyses, a functional peptide with the potential to induce T-cell immune response and not recognized by grass-specific IgE antibodies was proposed.

The theoretical contributions made can be formulated as follows:

1. An *in silico* peptide consisting of 20 amino acids was designed as a vaccine against grass pollen allergy using an immunoinformatics approach.

2. The epitope-based vaccine was validated by molecular docking and *ex vivo* T-cell stimulation test.

6. EVALUATION OF THE CANDIDATE'S SCIENTIFIC AND APPLIED ACTIVITIES

In the overall activity of Assoc. Prof. Teneva-Dzhambazova, a tendency to combine new scientific knowledge with its application in medicine and pharmacology stands out (articles B4-6, G7-2, G7-3, G7-4, G7-5, G7-6, G7-8, G7-12].

Scientific and applied contributions

1. The potential of 18 cyanobacterial strains for the production of phycobiliproteins has been assessed. The strains *Microcoleus autumnalis* (PACC 5505, PACC 5522, and PACC 5527) and the strain *Leptolyngbya boryana* (CCALA 084) produce phycobiliproteins in appropriate concentrations for their biotechnological production. There is a real possibility for mass cultivation and exploitation of the above-mentioned cyanobacterial strains in order to obtain these pigments.

2. The molecular genetic marker OMER has been validated for practical use in phylogenetic analyses and polyphasic taxonomy in cyanobacteria.

3. Cyanobacterial strains of the species *Anabaena laxa* and *Phormidesmis molle* have been proposed as effective producers of compounds with immunomodulatory and antioxidant effects with potential application in dietetics and medicine.

4. A strain of the species *Betonica bulgarica* has been selected as a promising producer of compounds with antitumor effects in cervical carcinoma.

5. New evidence for the antitumor effect of commercial xylooligosaccharide preparations has been provided and the possibility of their use in the pharmaceutical and food industries has been confirmed.

6. After additional clinical trials, the immunogenic peptide, designed as a vaccine against grass pollen allergy, can be used to treat patients with grass pollen allergy by activating the T-cell response and producing competitive IgE antibodies.

I positively assess the scientific research and scientific-applied activity of Assoc. Prof. Dr. Ivanka Teneva-Dzhambazova in terms of subject matter, methodological approaches, and achievements. I would like to emphasize that the research is complex, involving specialists with different qualifications, as required by today's science.

I evaluate the contributions of Assoc. Prof. Teneva-Dzhambazova such as innovative and significant, both original scientific and those with a pronounced applied significance. There are achievements of an obvious theoretical and methodological nature, including obtaining new information and confirming known data in the main directions of phycology and other basic scientific fields related to it in these developments. It should be emphasized that in a large part of the works, a mechanism of action is sought, which gives depth to the research. Simultaneously with the achievements in the theoretical aspect, applied contributions have been formulated for use in taxonomy, medicine, pharmaceutical, and food industries. Undoubtedly, the achieved results are a solid scientific basis for future research.

In my opinion, the candidate has her own place in the activities of the teams she works with, her qualifications and experience contribute greatly to the realization of ideas and achievement of goals. This also determines her share in the contributions as significant. Here we must add the fact that out of all 20 scientific works (18 articles and 2 book chapters), Assoc. Prof. Teneva-Dzhambazova is in first place in 6 of them and in 6 she is a corresponding author, which is proof of her significant contribution to the presented works.

The results presented for the competition are available to the international scientific community, as evidenced by the number of cited articles and the number of citations. This is also a recognition of the level and importance of her activity. Some of the articles have been cited 12 or more times (e.g. 19, 27, 37, 63 times) in such renowned journals as Phytochemistry Journal of Phycology, Aquatic Sciences and Engineering, Plants, Toxicon, Water Research, Toxins, Journal of Experimental Botany, Chemosphere, Life, Algal Research - Biomass Biofuels and Bioproducts and many others. It should be noted that the list of citations does not include self-citations, and the citing articles have been published in international journals.

No plagiarism was found in the articles submitted for the competition.

7. PARTICIPATION IN RESEARCH PROJECTS

Assoc. Prof. Ivanka Teneva-Dzhambazova presents information about participation in 9 research projects (3 international and 6 national), in 4 of them (1 international and 3 national) she is a leader. Two of the international projects are financed under the Bilateral Projects Program of the National Science Foundation (with Austria and Russia) and 1 - under the BS-ERA-NET Program, co-financed through the Seventh Framework Program of the EC. Of the national projects, 2 are financed respectively by the National Science Foundation at the Ministry of Education and Science and the National Science Foundation at the University of Sofia, 1 is under the OP "Environment 2007-2013" co-financed by the ERDF, and the Cohesion Fund of the European Community and 1 under the Operational Program NOIR - Center of Competence "Personalized Innovative Medicine". All of them are in the field of the announced competition and concern the main areas of research and teaching of the candidate.

8. PARTICIPATION IN SCIENTIFIC FORUMS

Assoc. Prof. Ivanka Teneva-Dzhambazova has participated in 29 scientific forums, 15 of which are international. All presented titles are related to the candidate's activity as a teacher and scientist in the field of the announced competition.

9. CRITICAL REMARKS AND RECOMMENDATIONS

I have no critical remarks about the materials included for the competition by Assoc. Prof. Teneva-Dzhambazova. They correspond to the topic of the competition, both in volume and in quality, with which the recommended indicators for occupying the academic position of "Professor" are met and even exceeded.

Questions:

One of the main achievements in the work of Assoc. Prof. Teneva-Dzhambazova is the restoration of the taxonomic status of the species *Microcoleus autumnalis* as *Phormidium autumnale* (Agardh) Trevisan ex Gomont, 1892. Has this change been recognized by the scientific community and what were your steps in this direction?

Recommendation:

The presented scientific works outline serious scientific and scientific-applied contributions, most of which are very promising and are the basis for further developments. I recommend that some of them be continued until a final product is obtained.

10. PERSONAL IMPRESSIONS

I do not know Assoc. Prof. Ivanka Teneva-Dzhambazova well, but from the review of her educational and research activities I got the impression that she is an active teacher and scientist, competent and highly erudite in the field of modern directions of phycology, a sought-after partner for project development. This impression was reinforced by the materials presented for the competition, prepared at an academic level, in detail, and precisely.

CONCLUSION

The documents and materials presented by Assoc. Prof. Dr. Ivanka Teneva-Dzhambazova meet all the requirements of the Law on the Development of Academic Staff in the Republic of Bulgaria, the Regulations for the Implementation of the LDASRB, and the relevant Regulations

of Paisiy Hilendarski University. The candidate has presented a sufficient number of scientific papers in the competition, published after the materials used in the defense of the ONS "Doctor" and the academic position "Associate Professor". The scientific and teaching qualifications of Assoc. Teneva-Dzhambazova is undoubted. The achieved results in the educational and scientific research activities fully comply with the minimum national and additional requirements of the Faculty of Biology, adopted in connection with the Regulations of the University of Sofia for the implementation of the LDASRB.

I would like to emphasize that Assoc. Prof. Dr. Ivanka Teneva-Dzhambazova is an established and promising scientist in the field of this competition, distinguished by her own scientific profile and modern research approach. Her teaching activities correspond to the current requirements of higher education. She is the author and co-author of curricula, textbooks, and teaching aids, and actively works with graduate and doctoral students. She is a sought-after partner in the development of scientific projects and an active member of the teams she works with. The presented scientific works define her as a professionally competent specialist. They have been published in reputable publications and have become known to our and the international scientific community. The formulated scientific and applied contributions are the basis for further developments. Her participation in applied tasks is evidence of a responsible attitude to practical issues.

After reviewing the materials and scientific papers presented in the competition, after analyzing their significance and the scientific and applied scientific contributions contained in them, I find it reasonable to give my **positive assessment and recommend** to the Scientific Jury to prepare a report-proposal to the Faculty Council of the Faculty of Biology for the election of Associate Professor Dr. Ivanka Ivanova Teneva-Dzhambazova to the academic position of "**PROFESSOR**" at the "Paisiy Hilendarski" University in the field of higher education 4. Natural Sciences, Mathematics, and Informatics, professional field 4.3. Biological Sciences (Phycology).

09.04.2025
Sofia

Reviewer:……
/Prof. Maria Angelova-Dyankova, DSc/