

REVIEW

by Stefan Antonov Philipov, PhD,

Assoc. Prof. at the Institute of Organic Chemistry with the Centre of Phytochemistry

Bulgarian Academy of Sciences

on the PhD Thesis for awarding 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: **Vladimir Metodiev Ilinkin**

Title: **Biotechnological approach for cultivation of *Tanacetum cinerariifolium* (Trevir.) Sch.Bip. (Asteraceae)**

Research supervisors: Prof. Marina Stanilova, PhD and Prof. Strahil Berkov, PhD - Institute of Biodiversity and Ecosystem Research (IBER) – BAS

1. General presentation of the procedure and the PhD student.

By order No. 35 of 11.04.2024 of the Director of IBER-BAS, I have been appointed as a member of the scientific jury in the procedure for the defense of a dissertation work on the topic "Biotechnological approach for cultivation of *Tanacetum cinerariifolium* (Trevir.) Sch.Bip. (Asteraceae)" for the acquisition of the educational and scientific degree "doctor " with author Vladimir Metodiev Ilinkin " in the field of higher education: 4. Natural sciences, mathematics and informatics, professional direction: 4.3. Biological Sciences, Doctoral Program: Botany.

The set of materials presented by land. arch. Vladimir Ilinkin 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, as according to Art. 9 of the "Regulations

on the terms and conditions for acquiring scientific degrees and holding academic positions at IBER-BAS", when presenting the dissertation in the form of bound scientific publications, the chapters "Materials and methods", "Results" and "Discussion" are replaced by copies of articles on the subject of the dissertation. The PhD student has submitted a report, certified by the scientific secretary of IBER-BAS, for the accumulated credits, as follows: completion of the educational program - 130 points (required mandatory minimum of 130 points); approval of the implementation of the scientific program - 272 points (a mandatory minimum of 40 points is required); published scientific results - 228 points (a mandatory minimum of 80 points is required). The total number of credits for the period of the doctoral studies is 630 points, exceeding by more than 2.5 times the mandatory minimum of 250 points. The PhD student has also attached 4 publications on the topic of the dissertation and a list of participations in scientific events. The presented abstract is prepared according to the requirements and it presents the results, discussions and conclusions included in the dissertation work.

2. Brief biographical data for the PhD student.

Vladimir Ilinkin graduated from the University of Forestry, Sofia (UF) with a master degree in landscape architect in 2010. He was enrolled as a full-time PhD student at IBER-BAS in 2016, scientific speciality "Botany" and was awarded with the right of defense in 2019. He worked as the Head of the "Central University Laboratory for Ecology and Environmental Protection" at UF and the Head of the "Central Laboratory for Agrochemical Analysis" at the "N. Pushkarov" Institute of Soil Science, Agrotechnology and Plant Protection (ISSAPP). He held the academic position of "assistant" at UF, IBER-BAS and ISSAPP "N. Pushkarov".

3. Relevance of the topic and appropriateness of the set goals and tasks.

The chosen topic of the dissertation work - Biotechnological approach for cultivation of *Tanacetum cinerariifolium* (Trevir.) Sch.Bip. (Asteraceae) is particularly relevant and there have been numerous scientific studies in recent years. The topic finds an intersection of three topics, namely: research of endemic and endangered plant species; applying in vitro methods and techniques, and solving the challenges of clonal propagation of plants in long-term in vitro culture; study of secondary metabolites in the plant, as potential sources of raw materials for various industrial preparations.

Formulated in this way, the dissertation work represents a well-structured multidisciplinary study, which links the topics under consideration with a logical sequence. The goal is clearly and well formulated, and the tasks set in the dissertation work are tailored to the topic of the dissertation, and their implementation requires the acquisition and application of knowledge and competences in various scientific fields.

4. General characteristics of the dissertation - volume and structure

In order to achieve the goals and objectives of the dissertation work, the PhD student, in a synthesized and well-structured way, has developed a 14-page literature review, composed of 197 literary sources (including those cited in the articles) in Bulgarian and English languages, as about half of them are from the last twenty years. The chapters "Materials and methods", "Results" and "Discussion" have been replaced by copies of the publications on the topic of the dissertation, according to Art. 9 of the "Regulations on the terms and conditions for acquiring scientific degrees and holding academic positions at the Institute of Biodiversity and Ecosystem Research at the BAS", when presenting the dissertation in the form of bound scientific publications. The presented 4 articles have been published in international journals, and in terms of volume, interpreted and discussed data fully cover the set tasks. The conclusions in the articles, as well as the conclusions and contributions in the dissertation, fully cover the three sub-topics of the dissertation work. In addition, a protocol for in vitro propagation of the species is presented, which is based on scientific research and is an indispensable solution to practical issues in in vitro propagation of the species.

5. Literary awareness and theoretical preparation of the candidate

The literature review includes detailed information that is concise and informatively structured, written at a high scientific level in several subsections: Botanical characteristics and distribution of *Tanacetum cinerariifolium*; Ecological requirements and cultivation of the species; Main biologically active substances; Content of pyrethrins in natural populations and in agricultural crops; Pyrethrin products; Application of pyrethrin products; Synthetic analogues of natural pyrethrins; Seed propagation; Vegetative and *in vitro* propagation. It is noteworthy that the PhD student has mastered and is well-versed with the specific terminology in several scientific fields, namely botany, soil science, plant biotechnology and phytochemistry, as well as being able to systematize and carry out a thorough and critical analysis of information from literary sources. It makes a particularly good impression that the PhD student is well-acquainted with the problems

and scientific developments related to the species in a local for Bulgaria and global aspect, bearing in mind the scientific developments in the past and to date by scientists from Croatia, where the species is naturally distributed. Vladimir Ilinkin has conducted a specialization at the University of Zagreb, Croatia under the guidance of Assoc. Prof. Martina Grdiša, PhD, a scientist with numerous scientific studies related to *Tanacetum cinerariifolium* (Trevir.) Sch.Bip. This has undoubtedly had an extremely beneficial impact on the development of the dissertation under consideration.

The presented dissertation material shows the very good and thorough literary awareness and considerable theoretical training of land. arch. Vladimir Ilinkin.

6. Research methodology.

In carrying out the research of the dissertation, the doctoral student used plant material - seeds originating from a private *ex situ* collection from Bulgaria (Bogdan village) and from a natural population of the species in Croatia. In all studies, the representativeness and reliability of the results is ensured through a sufficient number of repetitions, according to the specifics of the specific studies. Germination and seed viability experiments followed a standard of four replicates of 100 seeds using a classic tetrazole seed viability test. A significant volume of soil characteristics of 6 soil types from Bulgaria were analyzed using classical, nationally adapted and international methods, and the influence of soil characteristics on seed germination was evaluated. Observations and studies in the *in vitro* cultivation of the species are based on several hundred individuals of the species, in different nutrient media with the addition of an antibiotic and modification of nutrient media with the addition of Ca.

For the phytochemical analyses, extracts were obtained from plant materials - ponies, *in vitro* cultivated plants and flower baskets of *in vitro* propagated, acclimatized and planted in the *ex situ* collection of IBER-BAS plants. The extracts were analyzed with modern laboratory equipment, namely a gas chromatograph with a mass spectral detector and a methodology corresponding to the specifics of the studied secondary metabolites - pyrethrins.

In all studies, appropriate statistical methods were used to analyze the obtained results. The methods applied in the research fully correspond to the goals and tasks set in the dissertation work.

7. Significance and persuasiveness of the obtained results, interpretations and conclusions.

The dissertation work represents a significant complex study of seed germination, *in vitro* specificities in the propagation of *Tanacetum cinerariifolium* (Trevir.) Sch.Bip. and the pyrethrin content. The presented results are based on long-term and large-scale analyses, applying statistical methods, which allowed the PhD student to make in-depth discussions and draw significant conclusions in terms of quality and quantity.

8. Critical notes and recommendations

I have no objections to the thesis work, except some technical ones which do not detract from its value. For example, in the "Literature Review" and "Materials and Methods" the chemical structures are marked with their names in English, but they should be in Bulgarian. I recommend to the land. arch. V. Ilinkin the literature review of the dissertation work, which is very informative to be published as a review article.

9. Evaluation of scientific contributions

The presented total of five contributions are a logical consequence of the empirical data obtained and analyzed by the PhD student, and the conclusions drawn on them. The contributions are well structured and articulated, covering the three sub-themes of the dissertation. The contributions presented are both new to science, and of a confirmatory nature, and with an applied aspect.

10. Evaluation of the quality of the scientific works reflecting the research on the dissertation work

There are four published articles related to the PhD studies, three of which are referenced in world famous databases (Scopus or Web of Science), and one article is published in a scientific journal with an international editorial board. According to these criteria, the PhD student fulfills the requirements of the IBER-BAS regulations for the publication of at least two scientific articles, one of which is published in a journal referenced in the Scopus or Web of Science databases. The PhD student is the first and corresponding author, and one article has been published with IF/SJR on each of the sub-topics of the thesis and several poster reports have been presented at

international and national scientific forums. The presented articles are written at a high scientific level with a literature review well balanced in terms of volume and informativeness, with a thorough and critical analysis of the data and clear and conditioned conclusions.

11. Authorship of the dissertation work

The chapters "Materials and methods", "Results" and "Discussion" are presented in the format of a total of 4 publications for the three sub-topics of the dissertation work. Each of the sub-topics has a published article in a journal with IF/SJR, in a different collective, but land. arch. V. Ilinkin is leading and corresponding author. In the second article from the subtopic "Vitality and germination of seeds", the PhD student is the sole author. Undoubtedly, the dissertation is the personal work of the PhD student.

CONCLUSION

The presented PhD thesis contains original scientific and scientifically applied results, which represent an original contribution to science and in terms of quantity and quality meet all the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria (LDASRB), the Regulations for its Implementation and the Regulations for the Implementation of LDASRB of BAS, as well as fully comply with the specific requirements of the Regulations of IBER-BAS for the application of LDASRB.

The PhD student Vladimir Ilinkin shows excellent theoretical and professional training, demonstrating qualities and skills for independent research work, distinguished by precision and thoroughness.

Due to the above, I confidently propose to the honorable scientific jury to award the educational and scientific degree "doctor" to land. arch. Vladimir Ilinkin in the field of higher education: 4. Natural sciences, mathematics and informatics, professional field 4.3. Biological Sciences, scientific specialty Botany.

Sofia, 13.05.2024

Reviewer:
(Assoc. Prof. Stefan Philipov, PhD)