DECLARED OPINION

by Prof. Venelina Todorova Popova, DSc, Eng., Department of Technology of Tobacco, Sugar, Vegetable and Essential Oils, University of Food Technologies, Plovdiv

on a dissertation for the acquisition of the educational and scientific degree **"doctor"** (PhD)

Higher education area: 4. Natural Sciences, Mathematics and Informatics

Professional field: 4.2. Chemical Sciences

Doctoral program: Technology of animal and vegetable fats, soaps, essential oils, and perfumery and cosmetic preparations

Author of the dissertation: Liliya Stoyanova Stoyanova

Topic of the dissertation: Impact of organic production on the composition of tobacco seeds and the potential applications of the glyceride oil

Scientific supervisor: Assoc. Prof. Maria Angelova - Romova, PhD, Paisii Hilendarski University of Plovdiv

1. General presentation of the procedure and the PhD candidate

By order No. RD-21-2253 of December 5, 2024 of the Rector of Paisii Hilendarski University of Plovdiv I have been appointed a member of the scientific jury in a procedure for the defence of a dissertation on the topic "Impact of organic production on the composition of tobacco seeds and the potential applications of the glyceride oil" for the acquisition of the educational and scientific degree "doctor" (PhD) in higher education area 4. *Natural Sciences, Mathematics and Informatics*, professional field *4.2. Chemical Sciences*, doctoral program *"Technology of animal and vegetable fats, soaps, essential oils, and perfumery and cosmetic preparations".* Author of the dissertation is Liliya Stoyanova Stoyanova, a full-time doctoral student at the Department of Chemical Technology with scientific supervisor Assoc. Prof. PhD Maria Angelova – Romova. Doctoral student L. Stoyanova holds a Master's degree in "Food Analysis and Control" (2011, UFT - Plovdiv) and a Master's degree in "Organic Chemistry" (2014; P. Hilendarski University of Plovdiv). In the period 2021-2024 she is a full-time doctoral student, and has been enrolled with the right to defend a dissertation, as of 01.03.2024. She currently holds the academic position of "assistant professor" at the Tobacco and Tobacco Products Institute, Markovo, part of the Agricultural Academy.

The set of materials presented to me by PhD student L. Stoyanova is precisely prepared and includes all required documents in accordance with Art. 36, para. 1 of the Regulations on the Academic Staff Development at the Paisii Hilendarski University of Plovdiv.

2. Relevance of the topic

Tobacco (*Nicotiana tabacum* L.) is one of the most important, widely distributed and studied industrial crops in the world. In recent decades, more and more research has been focused on its

alternative use, as well as on the possibilities for valorization of the huge amount of plant waste generated by the production and processing of tobacco leaves. Tobacco has long been recognized as a "plant factory", given the distinctive feature of the species to synthesize and accumulate a wide variety of secondary metabolites. Quite logically, the most serious object of scientific research are tobacco leaves, but all other plant organs also have an undoubted potential for utilization (seeds, stems, flowers, roots). On the other hand, organically grown agricultural crops are of undisputed importance in our time, as part of programs for ecological production and circular economy. It is precisely in this direction of organic production that there is a lack of sufficient research on different ecotypes of tobacco, as well as on tobacco seeds and the obtained glyceride oil in particular.

In this context, the topic of the dissertation is extremely relevant, especially since the results obtained and the conclusions drawn provide a solid basis for practical implementation and development of the problem in the future.

3. Knowledge about the state of the problem

The choice of topic for the dissertation, the presented literature review and the parallels made with data from previous studies in the discussion of the obtained results demonstrate the PhD student's very good awareness and in-depth knowledge of the problem. The review of the state of scientific research and its analysis are based on 172 references (11 of which in Cyrillic and the rest in Latin); over half of them have been published in the last 10 years, which once again confirms the relevance of the problem under consideration and the good knowledge of the current achievements in the field. The structure and scope of the literature review are entirely in the context of the aim and objectives of the dissertation. The analytical and objective approach to the assessment of the references and data studied, as well as the summary of the scientific achievements with the highlighting of the aspects that have not been sufficiently explored so far, make a very good impression. The presented literature review and the competent discussion of the results in the following sections show that the doctoral student has the ability to synthesize and extract the essence from a large amount of information, to make critical comparisons and assessments, which reveals the achievement of the educational goals of the doctoral program, as well.

4. Research methodology

The main research objective of the dissertation is clearly formulated and justified by the conclusions from the literature review, i.e. to follow the influence of organic production on the composition of tobacco seeds and the glyceride oil extracted from them, as well as to assess the possibilities for their application. In accordance with this, seven research tasks have been set, which are specific, consistent and allow achieving the goal of the work. The methodological setting of the research is appropriately selected and adequate to the aim and objectives of the work. The materials used and the analytical methods applied are correctly and in detail described, which allows their reproduction, if necessary, and is a prerequisite for the reliability of the results obtained. In the

development of the dissertation, doctoral student L. Stoyanova has mastered and can apply various analytical methods and techniques, which is a good basis for her future research work.

5. Characterization and evaluation of the dissertation and its contributions

The dissertation, presented on a total of 170 pages with 2 schemes, 35 tables and 42 figures, is very well structured, with a balance achieved between the individual sections. Reasonably, the Results and Discussion section occupies the largest part of the work (79 pages, 47%). The research conducted is consistent and covers all important aspects of the problem under consideration, as follows:

– Monitoring and comparative assessment of the main chemical indices of tobacco seeds of one and the same variety, obtained under organic and conventional production conditions, and their stability in two consecutive crop years. Investigation was further continued with a detailed characterization of the lipid fraction of tobacco seeds, by determining the yield of glyceride oil, its physicochemical indicators, fatty acid composition, lipid indices, content of total and individual phospholipids, unsaponifiables, sterols, and tocopherols.

– Application and assessment of the efficiency of different techniques for extraction of glyceride oil from tobacco seeds – by using solvents of different polarity (*n*-hexane, ethyl acetate, *n*-hexane:acetone) and different methods (classical Soxhlet extraction, maceration, ultrasonic extraction).

– Determination of phenolic compounds and antioxidant activity (by three methods with different mechanisms) in aqueous, ethanolic and methanol extracts from tobacco seeds and the remaining seedcakes, as well as in the extracted glyceride oil.

– Assessment of the potential of waste (unsuitable for sowing) tobacco seeds as a source of valuable nutrients – by determining the content of primary metabolites, the fatty acid composition of the glyceride oil and its lipid indices, the content of biologically active substances in the lipid fraction, and the antioxidant activity of waste seed extracts.

– Application of glyceride oil from organic tobacco seeds in a cosmetic product – by comparative assessment of the composition and properties of tobacco seed oil against the more popular grape seed oil and the development of a formulation for a cosmetic emulsion (O/W) based on natural ingredients.

An extremely good impression is made by the in-depth discussion of each of the results obtained, as well as the critical and objective comparison with data from previous research. After each separate part of the investigation, a summary of the results and the observed trends is made, and in many cases, guidelines for conducting future research are indicated. Based on the data obtained, seven summarized conclusions have been formulated, which reflect the most important achievements of the dissertation.

Three applied scientific and three applied contributions of the dissertation are indicated, which

I accept as fully reasoned and substantiated by the conducted research and the obtained results. Contributions of an applied scientific nature are the new data obtained on the chemical composition of the seeds of organically grown Bulgarian varieties of Oriental tobacco, on the content of phenolic compounds and the antioxidant activity of the obtained seed and seedcake extracts and the glyceride oil, as well as the data on the chemical and lipid composition and the antioxidant activity of waste tobacco seeds and the extracts obtained from them. The applied contributions are related to: *i)* the identification of a fast and affordable method for extracting glyceride oil from tobacco seeds (maceration with *n*-hexane:acetone and ultrasound), as an alternative to the classic Soxhlet extraction; *ii)* the presentation of a method for extracting tobacco glyceride oil with a high content of biologically active tocopherols (maceration and ultrasound extraction with ethyl acetate); *iii)* the development of a formulation for a cosmetic emulsion cream (O/W) based on natural ingredients, containing tobacco seed oil and lemongrass essential oil.

6. Assessment of the publications and the personal contribution of the doctoral student

Four publications related to the dissertation have been presented, which reflect the main results of the research. Three of the publications are in indexed journals (Scopus/Web of Science – one from quartile *Q3* with *SJR=0.245* and two from quartile *Q4*, with *SJR=0.213* and *SJR=0.148*), and one is in an edition from the National Reference List of NACID. They ensure the achievement of 39 points in group of indicators D, with required 30 points. The doctoral student is the first author in all publications, which suggests her significant role in conducting the experimental part and manuscript preparation. Results obtained from the development of the dissertation have also been reported at five scientific forums in the country. An additional certificate of the quality of the publications is the detected positive citation of one of them (Scopus, 2024), which shows that the doctoral student's work is of interest to the international scientific community.

7. Abstract

The abstract is prepared according to the requirements, and it reflects in a concise form the essence of the research conducted, the results and contributions of the dissertation, as well as their popularization through publications, participation in scientific forums and citations.

8. Recommendations for future use of the dissertation contributions and results

The dissertation presents in an excellent manner the research conducted by the doctoral student and the new knowledge generated by them, which have scientific and practical significance. I do not personally know doctoral student Liliya Stoyanova, but the relevance of the topic being investigated, the high level of the dissertation and the related publications are an indication that she is an ambitious, inquisitive and capable young scientist, with established skills for conducting quality scientific research. I can only wish her to continue and expand her research in the future, because it is undoubtedly valuable for everyone interested in tobacco.

CONCLUSION

The dissertation contains scientific, applied scientific and applied results that represent an original contribution to science and meet all the requirements of the Academic Staff Development Act in the Republic of Bulgaria (ASDARB), the Regulations on the Implementation of the ASDARB (RIASDARB) and the Regulations on the Academic Staff Development at the Paisii Hilendarski University of Plovdiv.

The dissertation demonstrates that doctoral student Liliya Stoyanova Stoyanova has in-depth theoretical knowledge and professional skills in the scientific area, and demonstrates qualities and skills for independent conduct of scientific research.

Due to the above, I confidently give my positive assessment for the conducted research, which has been presented by the above-reviewed dissertation, abstract, achieved results and contributions, and I propose to the esteemed scientific jury to award the educational and scientific degree “doctor” to Liliya Stoyanova Stoyanova in higher education area 4. Natural Sciences, Mathematics and Informatics, professional field 4.2. Chemical Sciences, doctoral program “Technology of animal and vegetable fats, soaps, essential oils, and perfumery and cosmetic preparations”.

03.02.2025 Opinion was executed by:

Plovdiv Prof. Venelina Popova, DSc