



FORMAL OPINION

Considering the dissertation for obtaining a PhD degree based on the area of higher education, classified as code 6. Agricultural sciences and veterinary medicine, professional field code 6.1. Crop production, major "Selection and seed production of cultivated plants (Plant biotechnology)".

Author of the dissertation: Pervin Shengun Halkoglu-Hristova, full-time PhD student at the Department of Genetics and Selection at Agricultural University, Plovdiv

Dissertation topic: *In vitro* cultures of *Fabiana imbricata* Ruiz. et Pav. as technological matrices for the development of biologically active substances

Reviewed by: Associated Professor PhD Galya Stoeva Dobrevska, Department of Viticulture and Fruit Growing at the Agricultural University, Plovdiv; area of higher education, classified as code 6. Agricultural sciences and veterinary medicine, professional field code 6.1. Crop production, major "Fruit Growing", appointed as a member of the scientific jury based on issued order # RD-16-744/29.06.2021

1. Problem overview

Many plants have been studied until now and have been used by humans for hundreds of years for medical purposes and as a source of extracting and developing various chemical products and secondary metabolites for use in industries like pharmaceutical, cosmetics, food supplements and others. Today, more than half of all existing medicines worldwide are derived from plants. During the last decades, the pace of technological progress has contributed to obtaining many phytochemicals through cell cultures.

Some of the most important biologically active secondary metabolites are polyphenols and flavonoids. Polyphenols are antioxidants and protect DNA and cell membranes; thereby, preventing mutations in the human cell. Flavonoids also have antioxidant power and have low toxicity compared to other active plant compounds, such as alkaloids. This makes them safe for human use.

The plant species *Fabiana imbricata* Ruiz et Pav is rich in biologically active substances. It is a valuable medical plant which is not so well-known and researched in Europe. There are various issues with its conventional reproduction and breeding. Therefore, the development of appropriate biotechnological techniques for its cultivation would allow for its use at a widespread base. All this along with the research on the *in vitro* cultures of *Fabiana imbricata* Ruiz et Pav as technological matrices for obtaining biologically active substances, make the current dissertation extremely relevant and sufficiently important for the field of interest.

2. Purpose, objectives, hypotheses and research methods.

The main aim of the PhD study is clearly and precisely formulated, emphasising on the assessment of the potential of *in vitro* cultures of *Fabiana imbricata* Ruiz et Pav as technological matrices for the obtaining of biological substances. The study has five main objectives, which fulfil its aim. They include studies on plant material obtaining and its incorporation in *in vitro* cultures; optimising

the system of microreproduction of *Fabiana imbricata Ruiz et Pav* with varying degree of differentiation; analysis of the antioxidant activity of various extracts of *Fabiana imbricata Ruiz et Pav*; analysis to determine the polyphenolic profile of various extracts of *Fabiana imbricata Ruiz et Pav*.

An in-depth methodology section is also presented, aiming to ensure the correct realisation of pre-set aim and objectives. A large amount of workload has been completed, including precisely performed laboratory experiments. A large number of indicators have been assessed and analysed by various selected contemporary means of equipment and technology.

3. Presentation of results

The dissertation consists of 118 typewritten pages and is very well structured in 10 main sections. The standard volume and balance between different sections is complied with. The results of the analysis and research are presented in 16 tables, 31 figures, 2 appendices and an index of the abbreviations used.

4. Discussion of results and literature review.

The literature review is thorough and presents the current and contemporary state of the problem. It has been developed on the basis of 191 sources, of which 188 are in Latin and 3 are in Cyrillic. The literature review assessment also includes analysis of scientific publications related to the general characteristics of the plant species *Fabiana imbricata Ruiz et Pav*. A detailed review of the classification of chemical structures identified in extracts of *Fabiana imbricata Ruiz et Pav* has also been conducted along with assessment of the bioactivity of substances synthesized by the species and polyphenols of plant origin. Plant *in vitro* systems and their metabolic profiling have been assessed along with the methods for analysis of the biological activity of secondary metabolites of plant origin, which have been considered. The presented literature review, as well as its conclusions, demonstrated very good theoretical foundation and knowledge of the PhD student, directly relevant to the problems and concepts relevant to the study.

The discussion of the results is in-depth and based on precisely conducted assessments and their interpretation. A very good scientifically-appropriate style has been used throughout the results discussion and presentation. At the end of each chapter, specific scientific statements and conclusions are clearly made, based on the analysis of results, and then summarised in 15 main conclusions, reflecting the fulfilment of the aim and objectives of the dissertation.

5. Dissertation contribution

As a result of the conducted experiments and analysis of obtained data and results, 2 scientific and 3 scientifically applicable contributions have been developed.

Scientific contribution

1. For the first time an experiment with digital holographic microscopy (DHM) has been conducted for the purposes of measuring the size of cell clusters in suspension cultures of *Fabiana imbricata Ruiz et Pav*, through which cell division and increase in cell mass can be monitored and referred to by future experiments.

2. Spectrophotometric analysis of the antioxidant activity of *in vitro* and *in vivo* cultures of *Fabiana imbricata Ruiz et Pav* was conducted for the first time.

Scientifically applicable contributions

1. *In vitro* plant material was created from *Fabiana imbricata Ruiz et Pav* plants, which is suitable for obtaining cultures with various degree of differentiation and their use as potential biological matrices of phenolic components.

2. A system for multiplication and successful adaptation has been developed through optimisation of the nutrient environment and conditions of cultivation.

3. The polyphenolic profile of *in vitro*, *in vivo* and calluses plants, as well as plant cell suspensions obtained from *Fabiana imbricata Ruiz et Pav*, has been defined and determined with the use of HPLC analysis.

6. Critical evaluation and questions

I have only one critical remark to the PhD student which considers the scientific and scientifically applicable contributions of the paper. They are presented in the paper as a part of the conclusion of section "Results and discussion", while they had to be presented separately.

I have no questions to the PhD student.

7. Published articles and citations

Three joint publications are attached, which are directly related to the dissertation. The total number of points, according to NCID is 32.5, which fulfils the minimum scientometric requirements for obtaining the educational and scientific PhD degree, in compliance with LDASRB. There are no citations presented.

The presented abstract objectively reflects the structure and content of the dissertation.

CONCLUSION:

Based on all evidence provided by the PhD student, including the various research methods, correctly performed experiments, summaries and conclusions, I believe that the presented dissertation meets all requirements of LDASRB and the Code of Agricultural University for its implementation, which allows me to evaluate it **POSITIVE**.

I would also like to suggest to the honour scientific jury also to vote **POSITIVE** and to award Pervin Shengun Halkoglu-Hristova the educational and scientific PhD degree with major "Selection and seed production of cultivated plants (Plant biotechnology).

Date: 26.08.2021
Plovdiv

Formal opinion prepared by: _____
(Assoc. professor PhD Galya Dobrevska)