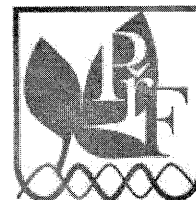




UNIVERSITY OF SOUTH BOHEMIA
IN ČESKÉ BUDĚJOVICE



Faculty of Science

STATEMENT OF THE BACHELOR/DIPLOMA* THESIS REVIEWER

Name of the student: Dieplinger Johanna

Thesis title: Profiles of beauverolide peptides isolated from entomopathogenic fungi

Supervisor: RNDr. Petr Šimek, CSc.

Reviewer: Ing. David Kahoun, Ph.D.

Reviewer's affiliation: University of South Bohemia in České Budějovice – Faculty of Science

	Point scale ¹	Points
(1) FORMAL REQUIREMENTS		
Extent of the thesis (for bachelor theses min. 18 pages, for masters theses min. 25 pages), balanced extents of the thesis divisions (recommended extent of the theoretical part is max. 1/3 of the total extent), logical structure of the thesis	0-3	3
Quality of the theoretical part (review) (number and relevancy of the references, recency of the references)	0-3	3
Accuracy in citing of the references (presence of uncited sources, uniform style of the references, use of correct journal titles and abbreviations)	0-3	2
Graphic layout of the text and of the figures/tables	0-3	2
Adequacy and clarity of the results and conclusions	0-3	2
Quality of the annotation	0-3	3
Language and stylistics, complying with the valid terminology	0-3	3
Accuracy and completeness of figures/tables legends (clarity even without reading the rest of the text, explanation of the symbols and labelling, indicating the units)	0-3	3
Formal requirements – points in total		21
(2) PRACTICAL REQUIREMENTS		
Clarity of the aims	0-3	3
Fulfilment of the aims	0-3	2
Discussion quality – interpretation of results and their discussion with the literature	0-3	2
Logic in the course of the experimental work	0-3	2
Completeness of the description of the used techniques	0-3	2

* Choose one

¹ Mark as: 0-unsatisfactory, 1-satisfactory, 2-average, 3-excellent.

Experimental difficulty of the thesis	0-3	3
Quality of experimental data presentation	0-3	2
The use of up-to-date techniques	0-3	3
Contribution of the thesis to the knowledge in the field and possibility to publish the results (after eventual supplementary experiments)	0-3	2
Formal requirements – points in total		21
POINTS IN TOTAL (MAX/AWARDED)	51	42²

Suggestions and questions, to which the student has to answer during the defense:

- 1) On page 10 of the thesis the author stated: „BVDs have been characterized by various methods including FAB, MS, NMR [4, 5].“ What does the abbreviation FAB mean? If this abbreviation means Fast Atom Bombardment (an ionization technique used in mass spectrometry), why is it stated separately from MS (mass spectrometry) methods?
- 2) How did the author find out extraction conditions and LC/MS conditions (pages 12- 14)? No optimization was done, no information source is given.
- 3) How did the author assess the suitability of the use of the quadratic equation for the calibration curve and the method working range (page 16)? Did the author perform any objective assessment (e.g. in accordance with Eurachem Guide: The Fitness for Purpose of Analytical Methods – A Laboratory Guide to Method Validation and Related Topics)?
- 4) How did the author determine LLOQ (lower limit of quantitation)?
- 5) Could the author specify which validation characteristics should be considered for validation of a quantitative analytical method?
- 6) The author stated „Figure 6.6 clearly indicates very good separation of the BVDs on the reversed phase LC column.“ (page 18), but the figure shows, that BVD E or BVD L has almost the same retention time (5.22 min) as BVD F (5.20 min). Thus, these compounds are not separated chromatographically (as the author states), but they are separated only according to their different m/z values. If a less specific detector (e.g. single channel UV detector) is used, what value is recommended as the minimum acceptable resolution for quantitative chromatographic methods?

² Enter the number of points awarded.

Eventual additional comments of the supervisor on the student and the thesis:

This part does not have to be read during the bachelor's thesis defence. These comments are made especially for the author.

- 1) Some taxonomic names are not written in italics (e.g. page 10).
- 2) Sentence "... which is shown in Table 3.2" (page 6). The Table 3.2 was not found in the thesis, only the Figure 3.2 was found.
- 3) Sentence "*Table 3.3 summarizes...*" (page 8). The Table 3.3 was not found in the thesis, only the Figure 3.3 was found.
- 4) A sentence should not begin with an abbreviation (e.g. pages 9, 10, 15 etc.).
- 5) A sentence should not begin with a numerical value (e.g. pages 12, 13, 14).
- 6) Insert one space between the numerical value and its unit (e.g. pages 12, 13, 14).
- 7) A one-letter word should not be left at the end of a line (e.g. pages 13, 17).
- 8) A numerical value should not be left at the end of a line and its unit at the beginning of the following line (e.g. pages 13, 14).
- 9) Do not use various units of full scan mass ranges – Da (page 14) vs. amu (page 17). The best choice is using m/z (mass-to-charge ratio), because multiply-charged ions with molecular weight higher than maximum of full scan mass range can be also determined.
- 10) Symbols of a protonated molecule are not written uniformly and properly e.g. $M^+ H^+$ (page 14), $[M+H]^+$ (page 17), $[MH]^+$ (page 17), MH^+ (page 17). The symbol of a protonated molecule should be written only using $[M + H]^+$ expression (in accordance with IUPAC recommendation - <http://goldbook.iupac.org/P04908.html>).
- 11) The thesis contains only a few typing errors such as „straom“ (page 12) or authors' surnames in the bibliography (page 23).
- 12) Figure 6.3 (page 16): The x-axis does not contain any name of the axis (e.g. Concentration) and the y-axis does not contain any unit (e.g. counts·min or intensity·min, because peak area unit should contain retention time unit and detector response unit).
- 13) On page 17 of the thesis the author stated two values of protonated molecule of Beauverolide I – m/z 489.3 and m/z 488.3, but according to Fig. 3.2 only m/z 488 is correct.
- 14) Bibliography:
 - a) Names of some journals are not written properly (e.g. The Journal of antibiotics,

South African journal of science, Experimental parasitology etc.).

b) Some references does not contain issue number and/or pages (e.g. reference No. 4, reference No. 28, reference No. 33).

c) Names of some authors are not written properly (e.g. Zahradničkova, Havliček, Kayčer).

d) Authors' surnames in some references are written in different fonts (e.g. reference No. 1 and reference No. 24).

Conclusion:

The bachelor thesis deals with development of a LC/MS method for the determination of the beauverolide I in mycelia extracts of four fungal strains and characterization of their beauverolide profiles.

The theoretical part of the thesis comprises an overview aimed at importance of entomopathogeneous fungi, beauverolides production and chemical structure of beauverolides. The last chapter of this part is devoted to determination of beauverolides by various analytical techniques. The experimental part describes in detail the extraction procedure, operating conditions of the LC/MS method and preparation of the calibration solutions. The results and discussion section begins with extraction of beauverolides from mycelia, what is accompanied by two illustrative photographs – before extraction and after extraction. Then, the graph of the calibration curve and its equation are stated, but without objective assessment and single method validation. Finally, MS and MS/MS mass spectra of the analytes and their contents in mycelia extracts are discussed.


The thesis is well-written and based on scientific publications, which demonstrates familiarity with the studied theme. The background theory has a logical connection to the research task, as well as to the method choice and methodological solutions. The results are presented clearly. Discussion is anchored to the main results, but this section contains some incomplete parts and inaccuracies. The structure is clear, consistent and the research process is easy to follow or repeat. I am convinced that the thesis achieves its goals and meets all requirements for this type of work.

In conclusion, I

recommend / ~~do not recommend~~*

the thesis for the defence and I suggest the grade A .³

České Budějovice, June 03, 2015.


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Ing. David Kahoun, Ph.D.

³ You can suggest a grade, which can be modified during the defense based on the presentation. However, if the reviewer is not present at the defense, the grade will not be counted.