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Prof. Adam Waśko,
Head of the Department of Biotechnology,
Microbiology and Human Nutrition
University of Life Sciences in Lublin
8 Skromna St., 20 -704 Lublin
e-mail: adam.wasko@up.lublin.pl

REVIEW

dissertation entitled: **"Use of natural extracts from *Lamium album* in the biological protection of cereals against *Fusarium* pathogens."**
carried out by Pascaline Aimee Uwineza, M.Sc., in the Department of Chemistry, University of Life Sciences in Poznań, Poland
under the supervision of Prof. Agnieszka Waśkiewicz

The formal basis for the realisation of the review is the Resolution of the Scientific Council of the Discipline of Food and Nutrition Technology of the University of Life Sciences in Poznań No. 3/IV/2024 signed by Ms. Chairman, Prof. UPP PhD. Dorota Cais-Sokolińska (dated 28.11.2024) and the doctoral dissertation of Pascaline Aimee Uwineza, M.Sc.

The legal basis was Article 190 of the Law on Higher Education and Science on 20 July 2018 (Dz.U. of 2023, Item 742, as amended).

Formal evaluation of the work

The dissertation submitted for review entitled: "Use of natural extracts from *Lamium album* in the biological protection of cereals against *Fusarium* pathogens". constitutes a thematically coherent series of five publications, including four original creative works and one review paper, published between 2020 and 2024 in peer-reviewed scientific journals of international scope with an IF impact index and included in Part A of the list of journals of the Ministry of Education and Science (MEiN). These are as follows:

1. **Uwineza, P.A**.; and Waśkiewicz, A. 2020. Recent Advances in Supercritical Fluid Extraction of Natural Bioactive Compounds from Natural Plant Materials. Molecules 25, 3847. https://doi.org/10.3390/molecules25173847.
2. **Uwineza, P.A.**; Gramza-Michałowska, A.; Bryła, M.; and Waśkiewicz, A. 2021. Antioxidant Activity and Bioactive Compounds of *Lamium album* Flower Extracts Obtained by Supercritical Fluid Extraction. Appl. Sci. 11, 7419. https://doi.org/10.3390/app11167419.
3. **Uwineza, P. A.**; Urbaniak, M.; Stępień, Ł.; Gramza-Michałowska, A. and Waśkiewicz, A. 2023. *Lamium album* Flower Extracts: A Novel Approach for Controlling *Fusarium* Growth and Mycotoxin Biosynthesis. Toxins, 15(11), 651. https://doi.org/10.3390/toxins15110651.
4. **Uwineza, P. A**; Urbaniak, M.; Stępień, Ł.; Gramza-Michałowska, A. and Waśkiewicz, A. 2024. Efficacy of *Lamium Album* as a Natural Fungicide: Impact on Seed Germination, Ergosterol, and Mycotoxins in *Fusarium culmorum*-Infected Wheat Seedlings. Front. Microbiol. 5, 1363204. https://doi.org/10.3389/fmicb.2024.1363204.
5. **Uwineza, P.A**.; Kwiatkowska, M.; Gwiazdowski, R.; Stępień, Ł.; Bryła, M.; Waśkiewicz, A. 2024. Field Assessment of *Lamium album* in Reducing Mycotoxin Biosynthesis in Winter Wheat Infected by *Fusarium culmorum*. Agriculture, 14, 647. https://doi.org/10.3390/agriculture14050647.

The sum of MEiN points for the mentioned publications included in the scientific achievement, according to the MEiN announcement in force in the year of publication of the works, is 540, and their summed Impact Factor according to the Journal Citation Reports (JCR) list according to the year of publication is 17.9. In all works forming the series, the doctoral student is the first author of the publication, as well as Ms. Uwinenza M.A. acted as a correspondence author.

The dissertation submitted for review consists of five chapters, including a 46-page typescript in addition to copies of published papers. The layout of this dissertation is typical of an experimental study. It includes a theoretical part containing the following: **Abstract** of the work in Polish and English; **Introduction**, which justifies the choice of the topic of the work, which includes the scientific basis and the research topic presented within the dissertation; **Hypothesis and purpose of the work**; and **Research Methods**. Next, the author presents the **most important results with discussion**, which contains selected results and their discussion from all the experimental work. **Summary, Conclusions, and Bibliography** included 108 items, as well as co-authors ' statements of participation in the development of the publication. This dissertation is supplemented by a **Table of Tables and Figures and Appendices**. The statements of the co-authors of the publication show that the Doctoral Student participated in the selection of appropriate methods to achieve the purpose of the study, performance of laboratory analyses, processing of the results and their interpretation, and preparation and editing of the manuscript. It should be noted that the entire dissertation, with the exception of the abstract, was written in English.

In summary, I conclude that formally, in light of current legislation, the work meets the requirements for a doctoral thesis.

Evaluation of the choice of topic and scope of work

Plant extracts are a source of valuable components that can be used in various industries. Bioactive compounds isolated from raw materials of natural origin show, in addition to medicinal properties, the potential to promote plant growth and development, protecting them from biotic and abiotic stresses. Interest in plant extracts is constantly growing with the development of modern agriculture, for which efficiency and low cost, but above all, environmental protection is increasingly important. In recent years, much attention has been paid to preparations derived from natural substances and their possible use in plant cultivation.

Therefore, there is a need for raw materials whose extracts can act as

biostimulants and plant protection products and can be used as components of "natural" fertilisers.

Poland was rich in herbaceous plants. Therefore, it is important to study plants found in Poland, which are often underestimated and treated as "weeds", and search for bioactive compounds that can be important components of natural plant protection products. For this reason, the reviewer appreciates that the Doctoral Student in her dissertation focused on the commonly occurring species in Poland, the white buttercup *Lamium album*.

On the other hand, microbiological hazards, including the presence of pathogenic viruses, bacteria or fungi and their toxic metabolites, are a huge problem for food safety. Among the factors that significantly reduce the quality of food and feed and pose a serious threat to food safety are filamentous fungi and their toxic metabolites, so-called mycotoxins, owing to their prevalence and harmfulness. *Fusarium* fungi are among the most commonly isolated pathogens from important agricultural crops. Owing to their high pathogenicity and toxigenicity, they cause significant economic losses. In her dissertation, Ms. P. Uwinenza combined both threads in an interesting way from a scientific and practical point of view, creating a coherent dissertation.

The assessed dissertation is in the area of research from the field of rapidly developing sustainable agriculture and microbiological safety in the food industry. This problem is located in the agricultural sciences in the discipline of food and nutrition technology, and can be the basis for developing a doctoral dissertation.

Substantive evaluation of work

The title of this dissertation is consistent with its contents.

This dissertation contains the statements of the Author, Promoter and Co-authors on the independent writing of the papers and information on the financing of the research performed during the dissertation (NCN, OPUS 16, UMO-2018/31/B/NZ9/03485). The posted statements show that the individual contribution of PhD students to the creation of each dissertation was in the range of 60-80%. Uwinenza, M.D. participated in the selection of appropriate methods to achieve the purpose of the study, the performance of

laboratory analyses, the development of the results of their interpretation, and the preparation and editing of the manuscript. In the further part of the dissertation, the Doctoral Student included a list of publications included in the dissertation, taking into account the points according to the MEiN list and the total Impact Factor according to the JCR list. The doctoral student also included a List of Abbreviations, which I found necessary, and it is good that this element of the dissertation was included. The next parts of the dissertation are the abstracts in Polish and English, which include a brief introduction, a discussion of the purpose of the work and its various stages, and a characterisation of the most important results.

In the first chapter, titled Introduction, the author reviewed the current literature on the subject related to the issues addressed in the paper. The PhD student described and characterised the two species of fungi (*Fusarium culmorum* and *F. proliferatum*) on which she worked. It is noteworthy that the doctoral student treated this part of the dissertation holistically, not limiting herself only to the characterisation of the two fungal species but also taking into account the context related to the economic losses caused by these pathogens. In this part of the dissertation, Ms. Uwinenza accurately described the possibility of using various plant extracts as alternative methods to protect agriculturally important plants. Knowledge of *Fusarium* fungi and plant extracts used as alternatives to fungicides is provided in quantitative and qualitative terms. The chapter ends with a clear diagram illustrating the research methodology used in this study. In conclusion, the literature review shows the author's thoughtful approach to the subject matter and justifies the choice of the dissertation topic.

In the second chapter, the author correctly sets the main research objective complemented by five specific objectives. It can be concluded that the research objectives formulated in this way meet the requirements for doctoral dissertations by the RDN and are related to filling the knowledge gap related to the dissertation topic. The doctoral student also presented the scientific problem selected for the solution in the form of clearly described 4 research hypotheses. In the reviewer's opinion, these hypotheses were properly constructed, unambiguous, and further tested using appropriately selected methods.

The third chapter describes the Research Methods used by doctoral students in

the presented publications.

A remark that occurred to me while reading the information contained in the previous chapter:

1. In this chapter, the author accurately refers to original methodologies developed by other authors and used in her work or describes the original methodologies used by herself, such as supercritical extraction. This was not done in the case of metabolic profiling methodologies to determine whether this was intentional or overlooked.

In the fourth chapter, Uwinenza, M.D., presents the selected results included in the individual publications. In general, all of the results presented, which are the results of experiments derived from a series of thematically related publications, are very well prepared and interpreted data that fit into the discipline of sciences, such as food and nutrition technology. The first paper in the series (P1) is an extremely valuable example of a review paper describing the process of supercritical fluid extraction (SFE) of valuable compounds from various plant species. It should be emphasised that this approach, based on a well-described scientific basis and a complete review of the literature on the subject, was used for further work in the thematic series and provided a scientific basis for the subsequent experiments collected in these publications. In publication 2, the doctoral student carried out an optimization process (one factor at a time, OFAT) of supercritical fluid extraction of bioactive compounds from white buttercup flowers.As a result, the optimal parameters of the extraction process were determined: temperature at 50^{o} C, addition of methanol as a co-solvent, and pressure at 250 bar. The extracts obtained were subjected to evaluation of antioxidant properties using DPPH, ABTS, and FRAP tests, and the results confirmed that the above extraction process parameters provided the most effective isolation of phenolic compounds from white buttercup flowers. The very valuable results of this work are the determination of the profiles of phenolic compounds in the studied extracts using an ultraperformance liquid chromatography system coupled with mass spectrometry and a detector equipped with a photodiode array. The extract obtained under optimal conditions contained more than 20% more phenolic compounds than the other variants, with significantly higher concentrations of chrysin, pinostrobin, myricetin, and trans-3-hydroxycinnamic acid. In Publication 3, the M. Sc. P. Using in vitro tests on two phytopathogenic *Fusarium* species (*F. culmorum* and *F. proliferatum*), Uwinenza et al.

confirmed the antifungal properties of *Lamium album* white buttercup flower extract. In the same study, the authors showed that the tested extract not only inhibited the growth of fungi, but also affected the metabolism of the tested species by significantly reducing the biosynthesis of mycotoxins from the trichothecene group and zaralelons. It is necessary to appreciate the analytical side of the work, in which the PhD student freely uses advanced methods of microbiological analysis and instrumental analysis for the evaluation of mycotoxins and ergosterol (UPLC/MS/MS and UPLC/PDA) In the reviewer's opinion, this constitutes a complementary approach to the research topic, which should be particularly appreciated. In the discussed work, the doctoral student fills the gap in knowledge regarding the possibility of using *Lamium album* extracts as biological fungicides. This information appears in the literature for the first time and has significant scientific value. Although, as a Reviewer, I do not feel qualified to evaluate publications that have already undergone the peer-review process in high-profile journals, reading the above paper, a question arose for me regarding the species of the genus *Fusarium* used.

Question: Please explain the use of *F. culmorum* and *F. proliferatum* species (isolated from peas) which are statistically less frequently isolated from cereals under Polish conditions than, for example, F. *graminearum* or *F. poae*?

Publication 4 describes a study conducted on a plant model aimed at studying the effect of white buttercup flower extract on the germination of parsnip seeds and seedling growth. In this work, M.A. Uwinenza focused on studying the growth parameters of seedlings infected with artificially tested *Fusarium* species, in the context of mycotoxin accumulation in the roots and leaves of wheat seedlings. In the reviewer's opinion, the work was planned meticulously, and the research techniques were adequately selected for the realisation of the specified purpose and scope, which allowed us to obtain valuable results. Particularly noteworthy and praiseworthy of the work of the Doctoral Student is the very labour-intensive analysis of the identification of *Fusarium* spp. performed on a plant model by molecular methods.

The results contained in the last experimental paper of the cycle (P5) followed the general pattern of the dissertation and were obtained *in vivo* in experimental plots of winter wheat crops. The results presenting this aspect of this study are analogous to those from the fourth paper (P4), and this approach is understandable and logical in the context of

verifying the potential of white buttercup flower extract as a natural antifungal agent. This verification was successful, and the PhD student confirmed a decrease in the concentrations of mycotoxins such as deoxynivalenol (DON) and zaralelon group mycotoxins (α-ZOL, β-ZOL, and ZEN-14S) in the cultivated cereals, confirming the potential of the studied extract as a natural fungicide.

The next part of the work is the Summary and Conclusions section. In summary, the author presented the most significant findings arising from the results of the work and provided directions for further research, but did not address where and by whom the results could be used. The doctoral student formulated five precise and well-worded conclusions summarising the entirety of the research carried out in this dissertation. At the same time, it is worth emphasising that the conclusions presented confirm both the cognitive nature and developmental potential of the dissertation presented for evaluation.

The bibliography concludes the evaluated dissertation with 108 English-language items, mostly from the last 10 years. The bibliography was prepared very carefully, with great attention paid to detail. All the journal names are given according to the same key. This chapter has no shortcomings. This dissertation still contains an index of tables and figures.

The papers included in the series have already been published and reviewed, and I rate them very highly. This statement at the same time allows the reviewer to ask the Doctoral Student to consider the following issues during the discussion:

1. Please explain the fact that when extracting by traditional methods, we get a lower content of polyphenolic compounds compared to extraction in supercritical fluids?
2. Referring to the methods used in the SFE process optimisation, please describe the RSM method using the Box - Behnken model?
3. Please specify the mechanisms of action of polyphenolic compounds on cells of fungi of the genus *Fusarium* ?

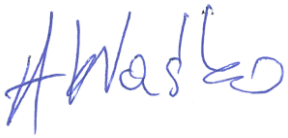
In summary, I conclude that the dissertation presented for evaluation represents an original solution to a scientific problem, proving the possibility of using the extract from the flowers of the white buttercup Lamium album as a natural fungicide. The research

hypotheses formulated in this study are positively verified. This dissertation expands the state of knowledge in the agricultural sciences in the field of food and nutrition technology. The dissertation was prepared carefully, with great attention paid to the scientific, linguistic, and editorial aspects.

Final conclusion

I conclude the doctoral dissertation submitted for review by Pascaline Aimee Uwinenza, M.Sc., entitled "**The use of natural extracts from *Lamium album* in the biological protection of cereals against Fusarium pathogens**. "Use of natural extracts from Lamium album in the biological protection of cereals against Fusarium pathogens" has a very high scientific value, and the very minor and few comments I have made do not detract from its substantive cognitive value and the importance of the research problem undertaken.

I believe that The evaluated dissertation meets the requirements for doctoral dissertations contained in the Law of 20 July 2018. Law on Higher Education and Science (Journal of Laws, 2023, Item 742, as amended). Doctoral students have demonstrated a high ability to conduct scientific research as well as to develop and interpret the obtained results based on the current literature in the field of research. Therefore, I request that the High Council of the Discipline of Food and Nutrition Technology of the University of Life Sciences in Poznań to accept the doctoral dissertation and admit Ms. Pascaline Aimee Uwinenza, M.Sc. to public defense and the further stages of doctoral proceedings.



Prof. Adam Waśko,
Head of the Department of Biotechnology,
Microbiology and Human Nutrition
University of Life Sciences in Lublin
8 Skromna St., 20 -704 Lublin
e-mail: adam.wasko@up.lublin.pl

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Justification for the application of an honourable mention of the doctoral thesis of Pascaline Aimee Uwinenza, M.Sc., entitled. "Use of natural extracts from *Lamium album* in the biological protection of cereals against *Fusarium* pathogens".

The reviewed doctoral dissertation deserves mention due to the broad and the interdisciplinary nature of this research. Ms. Pascaline Uwinenza, M.D., for the first time, obtained an effective extract of white buttercup flowers using the SFE method subjected to extensive and well-designed chemical and microbiological studies. The results obtained in both *in vitro* and in *vivo* studies have hitherto unknown antifungal activities against fungi of the genus *Fusarium*. These results have practical significance in the context of the possibility of using the developed extract as a natural fungicide with low phytotoxicity in sustainable agriculture. This study is novel, and the activity of white buttercup extracts in the context of biocontrol formulations has been described for the first time in the world literature. All the results were published in six peer-reviewed international journals.

The most important achievements presented in the reviewed work are as follows:

- Development of an extraction method in supercritical fluids and chemical characterization of the extracts obtained,
- Determination of effective extract concentrations in the context of inhibition of fungal infections in studies *in vitro* and field studies,
- Determination of the effect of the extract obtained from the flowers of *Lamium album* on germination and growth of the penetrans - phytotoxicity tests, to analyse the inhibition of biosynthesis and accumulation of mycotoxins in the tissues of

wheat produced by phytopathogenic fungi of the genus *Fusarium*.